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Chapter 9 The dorsal horn and hyperalgesia

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9.1. Definition of hyperalgesia

Both peripheral and central mechanisms play a role in the processing of primary afferent input that makes the transition from the normal signaling of unpleasant sensory experience to a hyperalgesic state. In 1927, Lewis suggested that primary and secondary hyperalgesia at the site of peripheral injury have a common neurochemical cause. He hypothesized that pain threshold is lowered through the release of a “stable pain substance” in the tissues in close proximity to primary afferent fiber terminal endings in the periphery. This was the purported “substance P”. Activation of collateral nerve terminal fibers of the same nerves, he explained, resulted in hyperalgesia in the surrounding tissues. Since that time, we have learned a great deal not only about substance P but also about nociceptive responses evoked by glutamate and other neuromodulators that result in hyperalgesic states upon release not only at peripheral sites but also at central termination sites of afferent nerve fibers. While the local neuronal reflex likely contributes particularly to the flare reaction seen at peripheral injury sites, Hardy suggested that a neurochemical communication network located in the spinal cord dorsal horn was a major factor in nociceptive hyperalgesia and ultimately shapes the physiological and behavioral responses (Hardy et al., 1967). Hardy more clearly defined the two general classifications of hyperalgesia as intensification of pain sensation that is associated with tissue damage (1) occurring at the site of injury (primary hyperalgesia) and (2) occurring in undamaged tissue adjacent to and extending some distance from the site of injury (secondary hyperalgesia) (Hardy et al., 1967). No clearer pictorial depiction of the development of hyperalgesia has been provided than his original schematic diagrams (Figs. 9.1 and 9.2).

Secondary hyperalgesia, Hardy explained, arises because the primary input begins to involve the interconnected network of neurons in the dorsal horn typically receiving input from adjacent cutaneous areas and the underlying deep tissue. The intensification of the pain sensation in the primary damage zone and the spread of the pain sensation to regions adjacent to the site where the noxious event originated come about through facilitation of impulses above the pain threshold (Fig. 9.1; Hardy et al., 1967).

The extent of the sensitization is paralleled by involvement of expanding populations of primary afferents, interneurons, projection neurons and higher brain centers. Not only are a greater number of neurons participating in the state of increased excitation, but the primary input intensifies the subsequent output to higher brain sites through prolonged, sustained activation of the spinal neurons involved through cellular mechanisms that we are only beginning to define. Noxious inputs derived from the skin, shown to the left in Fig. 9.2 (Hardy et al., 1967), enter the spinal cord to impact the dorsal horn neuronal pool to synapse. In addition to primary and secondary neurons receiving the noxious input, a

connected interneuronal network may become involved in establishing and maintaining an excitatory state in the neuronal pool in response to the intensity of the input from the periphery.

With incredible insight, Hardy proposed that similar activation mechanisms come into play when damage involves peripheral nerves, superficial or deep tissues, visceral structures or spinal and brain regions such as the thalamus. Amplification of pain, he supposed, could arise regardless of whether the clinical syndrome was initiated by tissue, nerve, circulatory or even mental injury.

Subsequently, a multitude of studies have sought a clearer understanding of the pain amplification processes. Hyperalgesia is the amplification and/or persistence of the normal transmission of pain signals that occurs under conditions such as injury. While there are peripheral and central components of the hyperalgesic experience, the spinal component is equated as a major contributor of the central sensitization state defining pathological pain. The integration of abnormal or distorted signaling of nociceptive input at the spinal cord level may lead to an increase in the resulting unpleasantness of the sensory experience not only at the primary site of injury (primary hyperalgesia), but also at sites at some distance from the injury (secondary hyperalgesia).

The focus of this review will be dorsal horn mechanisms and input that contribute to sensitization of nociceptive events. Both peripheral and central events that impact dorsal horn sensitization will be considered. While the Hardy figure (Fig. 9.2; Hardy et al., 1967) depicts with stippling the “states of excitation” surrounding sensitized neurons, we now know that the facilitated activation state that can be recorded from neurons in the dorsal horn is initiated and maintained by clouds of extracellular neurotransmitter/neuromodulators surrounding the excited neurons (Fig. 9.3), adaptable populations of membrane receptors and malleable intracellular events responding to the changing neurochemical milieu of the dorsal horn. The involvement of glia in this process is also now recognized.

Multidisciplinary experimental approaches have led to key findings enhancing the Hardy model. Time-related comparisons of information gathered using a variety of methodologies have included anatomical, pharmacological, physiological and behavioral studies. Anatomical studies have revealed additional features of the dorsal horn circuitry, as well as neurotransmitter and receptor changes unique for specific models under study. Controlled pharmacological manipulation of receptor mechanisms at peripheral and central primary afferent terminal sites increased our knowledge of the specific dorsal horn neuronal processes and receptors involved. Pharmacological manipulations at dorsal horn sites with intrathecal or microdialysis administration have had the advantage of affecting larger populations of descending, intrinsic and/or primary afferent endings simultaneously, though not in isolation as can be studied with single-unit electrophysiological recordings from pharmacologically perturbed, identified spinal cord neurons and from primary afferent fibers.

Hyperalgesic or pain-related behavioral responses to noxious stimuli are revealed with characteristic biphasic time courses in a variety of experimental animal models (Fig. 9.4). After an initial insult, a slowly evolving and pretracted period of increased sensitization may arise. Increased sensitivity to noxious stimuli (hyperalgesia) have been reported in animal models of inflammation in various behavioral tests (Millan et al., 1987; Hargreaves et al., 1988; Stein et al., 1988; Kawamura et al., 1989; Kayser et al., 1991; Ren et al., 1992; Sluka and Westlund, 1993a; Lawand et al., 1997b). Studies with inflammatory models demonstrate both mechanical and heat hyperalgesia, i.e. a decrease in the paw pressure withdrawal threshold (WT) and paw withdrawal latency (PWL) to radiant heat, respectively.

While the overall length of the time course varies, characteristic features of the hyperalgesic state are shared in common among experimental models. A rapid onset, first-phase response to the insult or injury occurs with short duration. This is followed by a slowly rising second phase of increased sensitization with a longer duration. For more acute models of hyperalgesia such as with cutaneous injections of capsaicin and formalin, the time course may proceed over hours (Abbott et al., 1995), while it may be prolonged over weeks for more chronic models, such as complete Freund's adjuvant (CFA) inflammation. The time course of the hyperalgesic state parallels that of glutamate increases in the dorsal horn in animals with inflammatory pain (Sluka and Westlund, 1993b). Thus, this experimental model will be heavily referred to in this review of dorsal horn events that produce pain-related behavior in animals indicative of hyperalgesia. Hyperalgesic mechanisms in the dorsal horn for neuropathic pain remain more elusive at this time. The measurement of pain perception in animals is limited to measurable behavioral and physiological responses to sensory stimuli. The relevance of equating quantitative measures of behavior in animals to pain perception reported through the pain assessments used clinically, such as the *Daily Living Inventory*, is open for debate. However, pain researchers believe measurements of events evoked by application of noxious stimuli producing alterations of predictable behaviors are reliable, consistent and relevant measures of pain in animals (Yaksh, 1997; Paul-Murphy et al., 2004). Pharmacological modulation of physiological and behavioral responses to sensory stimuli provide further support.

9.2. Neurochemistry of hyperalgesia

9.2.1. Primary afferent neuromodulators contributing to hyperalgesia

Hypersensitive responses of dorsal horn neurons are driven by repetitive activation of primary afferent endings by mechanical, thermal and chemical stimuli. Nerve endings can be influenced through autoreceptors by the neurotransmitters/neuromodulators released into the periphery by the nerve fibers themselves. In particular, substantial evidence exists in support of a role for peripheral glutamate receptors in the amplification of nociceptive events. Local intraplantar injections of L-glutamate have been shown to initiate thermal nociception in awake animals, and glutamate receptor antagonists attenuate hindpaw carrageenan-induced inflammation (Jackson et al., 1995). Based on behavioral and electrophysiological studies, compelling evidence suggests that different glutamate receptor subtypes act in concert in the development of pain-related behaviors in rats (Neugebauer et al., 1993, 1994; Sluka and Westlund, 1993a,b, 1994a,b; Zhang et al., 2002). Direct intra-articular injection of a combination of glutamate and aspartate (GLU/ASP) or GLU/ASP combined with arginine increases blood flow and produces a persistent hyperalgesic state while injection of single amino acids in the same manner and concentration has no effect (Lawand et al., 1997a, 2004). A significant decrease in PWL is noted when radiant heat is applied to the hindpaw, and WT to repetitive innocuous mechanical stimulation on the experimental side is decreased. GLU antagonist intervention in the periphery has been shown to attenuate secondary hyperalgesia in several models of peripheral experimentally induced nociceptive behaviors (Jackson et al., 1995; Zhou et al., 1996; Davidson et al., 1997; Lawand et al., 1997a). Administration of either *N*-methyl-D-aspartate (NMDA) or non-NMDA glutamate receptor antagonists (AP7, CNQX or ketamine) directly into the knee joint attenuates hyperalgesic responses induced by either the acute kaolin/carrageenan model or the chronic CFA model (Lawand et al., 1997a). Likewise, hindpaw heat hyperalgesia and mechanical allodynia induced by intraarticular injection of combined amino acids, GLU/ASP/ARG, can be blocked by either NMDA or non-NMDA glutamate receptor antagonists administered to the knee joint (AP7 or CNQX, respectively). Antagonists of NMDA and non-NMDA GLU receptors injected either in the hindpaw (Zhou et al., 1996) or in the knee joint (Lawand et al., 1997a) reverse hyperalgesia if the hindlimb has been previously inflamed. Peptide receptors may also be involved based on anti-nociceptive effects of

neurokinin antagonists when applied directly into the joint (Sluka et al., 1997) through peptide receptors such as those that have been localized in the skin (Carlton and Coggeshall, 1997).

Neuroactivators, including calcitonin gene-related peptide (CGRP), substance P (SP), neurokinin A and neuropeptide Y, are increased during inflammation in subcutaneous or knee joint afferent nerves and in the joint space where they activate receptors on the peripheral vasculature and contribute to neurogenic vasodilation and plasma/protein extravasation (Brain and Williams, 1985; Levine et al., 1985a; Kuraishi et al., 1989; Lam and Ferrell, 1989; Larsson et al., 1989, 1991; Bileviciute et al., 1993). There are related neurogenic contributions to vasodilation described for nitric oxide and NMDA (Ortega and Amaya, 2000; Lawand et al., 2004). Substance P is a well known contributor to inflammatory edema through promotion of protein/plasma extravasation (Green et al, 1992). Substance P has been reported to increase in acutely inflamed knee joints in rats (Bileviciute et al, 1993, 1994a) and can increase the severity of arthritis if added to the joint by increasing protein/plasma extravasation (Levine et al, 1985b) in experimentally induced models of inflammation. Direct knee joint administration of L-NAME, a nitric oxide synthase inhibitor, reduces knee joint swelling and temperature changes in addition to inflammation-induced secondary hyperalgesia in an arthritis model (Lawand et al., 1997a). The dorsal horn activation state enabling the peripheral release of neurotransmitters is described below.

9.2.2. Dorsal horn neurochemical profiles of hyperalgesia

Unique profiles of neurochemical changes are reported in the spinal cord dorsal horn and primary afferent nerves in experimental models depending on whether it is inflammatory, neuropathic or cancer-type pain (Honore et al, 2000) (Fig. 9.3). Various lines of evidence have particularly implicated glutamate, CGRP and SP and their receptors in nociceptive events. While an overabundance of these nociceptive substances predominate in the dorsal horn neurochemical microenvironment reported for inflammatory pain models, in general, decreases in these same transmitters characterize neuropathic changes at the level of injury. Neurotransmitter increases are consistent with increased activity and responsiveness of neurons in the spinal cord recorded during inflammatory pain (Schaible et al., 1987; Neugebauer and Schaible, 1990; Dougherty et al., 1992a). The reduction in dorsal horn neurotransmitters likely reflects the retraction/absence of primary afferent fiber endings in portions of the dorsal horn that are observed after primary afferent nerve transection and ligation (Chung et al, 1988, 1989). With neuropathic pain, large increases in neurotransmitter content are evident only selectively, as seen with the neurotransmitter agents dynorphin, galanin and neuropeptide Y (Villar et al., 1991) and the NK-1 SP receptor (Abbadie et al., 1996). While changes for these same substances are not evident in a sarcoma cancer model examined, the content of glial markers are dramatically increased bilaterally in most cases (Honore et al., 2000). The emerging awareness of the importance of the role of glia, glial products and inflammatory mediators in the activation of spinal neurons will be discussed below.

9.2.2.1. Inflammatory pain—In models of inflammatory pain, large increases in neurotransmitter content in the dorsal horn have been measured by microdialysis and antibody microprobes. Localization of the increases in cellular structures can be visualized by immunohistochemistry. There are no significant changes in the neurotransmitter profiles in dorsal root ganglia in inflammatory models. Released glutamate, SP and CGRP have been followed in the dorsal horn over time in the dorsal horn (Schaible et al., 1990, 1994; Sorkin et al., 1992; Sluka and Westlund, 1993c; Duggan, 1995) (Fig. 9.4). As an example, after an initial spiking release of glutamate simultaneous with the initial injury, glutamate begins to

increase within 15 min, ramps upward for 4 h, remains elevated for over 8 h and is highly correlated with the time course of hyperalgesic behaviors observed in a hindlimb (knee joint) kaolin/carrageenan inflammatory model (Sluka and Westlund, 1993b,c). With inflammatory type pain models, immunocytochemical localization reveals that γ -aminobutyric acid (GABA), serotonin and dynorphin are also greatly increased in the dorsal horn (Marlier et al, 1991; Castro-Lopes et al., 1994; Honore et al., 2000; Lai et al., 2001) (Fig. 9.3). Studies indicate that there are initial large increases in neuropeptide release in the dorsal horn measurable with antibody microprobes (Schaible et al., 1990) resulting in neuropeptide depletions by 4 h that are then followed by increases in dorsal horn content 3 days after injury. This is true for SP and CGRP, as well as for SP receptor (NK1), nitric oxide synthase (nNOS) and protein kinase C gamma (Sluka and Westlund, 1993b; Wu et al., 1998; Honore et al., 2000; Vera-Portocarrero et al., 2003; Vera-Portocarrero and Westlund, 2004). While NK1 receptor knock-out animals do display hyperalgesic behavioral changes with inflammatory pain models, the increasing levels of staining for NK1 receptor implicate its involvement. Arthritic CGRP knock-out mice have reduced hypersensitivity with no significant changes in the dorsal horn content of SP (Zhang et al., 2001).

The time course and amount of glutamate release and hyperalgesia is variable depending on the agent used to produce the injury, but an identical pattern of glutamate release is noted in the inflammatory pain models examined. There is an initial spiking release of glutamate at the time of injury followed by a slow sustained wave of release (Fig. 9.4). Injection of glutamate antagonists directly into the knee joint attenuates hyperalgesic behavioral responses to joint inflammation (Lawand et al., 1997a). Cutaneous administration of capsaicin or formalin produces a greatly compressed time frame for the release of neurotransmitters and the development of hyperalgesic responses over minutes. Hindpaw or knee joint injections of the irritants, kaolin and carrageenan, produce sensitization over several hours. The time course for development of hyperalgesia due to CFA (and likely release of glutamate in the spinal cord) is greatly protracted over days to weeks. Using the hindpaw inflammation model, biphasic release of NO was shown to parallel the glutamate release with the glutamate peak preceding that of NO (Vetter et al., 2001). It is likely that the two glutamate surges produce the “first pain” and “second pain” events reported in the literature by some investigators and that the second plateau phase of glutamate release is synonymous with the sustained sensitization during the hyperalgesic state. Thus, the importance of plastic changes in the neurochemical microenvironment in activating and maintaining the sensitized state in the dorsal horn should not be underestimated.

9.2.2.2. Neuropathic pain—Neuropathic pain can originate from peripheral nervous system damage or in the absence of any apparent damage. Neuropathic pain is associated with several distinct sensory characteristics, including abnormal, unfamiliar or unpleasant sensations called dysesthesias. It may also involve more debilitating symptoms such as shooting, lacerating or burning pains in the absence of tissue damage (Dickinson et al., 2003). Numerous studies have examined the dorsal horn changes in animals after various nerve injuries and design experiments to measure hypersensitive behavioral responses. In models of neuropathic pain, lesions and constrictions of primary afferent nerves result in significant decreases in neuropeptides, SP and CGRP, in both the dorsal horn and in the dorsal root ganglia (Villar et al., 1991; see Honore et al., 2000). While neurotransmitter levels in the dorsal horn are initially decreased in nerve injury models, elevations of several neurotransmitter receptor levels have been reported. Most notably, dynorphin increases in fos-labeled cells scattered throughout the spinal cord after nerve lesions (Noguchi et al., 1991). The number of dorsal root ganglia expressing mRNAs for the P2X(2) purinoceptor subtypes triples to 75% after nerve injury (Kim et al., 2003). Changes in expression of three subtypes of tetrodotoxin (TTX) sensitive sodium channels in sensory neurons are also noted after spinal nerve ligation (Kim et al., 2001).

The up-regulated expression of $\alpha(1\text{ b})$ -adrenergic receptor mRNA in axotomized DRG neurons may play an important role in the development of adrenergic sensitivity and ectopic firing in injured sensory neurons and thus contribute to the sympathetically maintained pain in spinal nerve ligated neuropathic Lewis rats (Xie et al., 2001). Increases are also noted for galanin and neuropeptide Y in both peripheral nerves and the spinal cord dorsal horn. These neurotransmitters of the sympathetic nerves likely increase since alterations in the sympathetic innervation occurs in rat models of neuropathic pain (Dickinson et al., 2003). In this model, cross-talk between nociceptive and non-nociceptive neurons is also occurring, contributing to the dysesthesias. Increases in glia and white cells are also noted. Subsequently, at time points later than 21 days to allow for sprouting as the nerves attempt to regenerate, there are increases in the numbers of primary afferent terminal endings staining for CGRP in the dorsal horn after nerve injury (McNeill et al, 1990) and for sympathetic nerves sprouting into the dorsal root ganglion (DRG) (Chung et al., 1996).

9.2.2.3. Cancer pain—In contrast to the many neurotransmitter changes reported for inflammatory, neurotrauma and neuropathic pain models, no detectable changes in any of these markers are observed in either primary afferent neurons or the spinal cord in a model of cancer pain induced by injection of osteolytic sarcoma cells into the femur (Honore et al., 2000). In this cancer pain model, the changes that were noted included massive hypertrophy of the astrocytes without significant neuronal loss. A significant increase in the neuronal expression of *c-Fos* is noted, and there is an increase in the number of neurons immunoreactive for dynorphin in the spinal cord on the side ipsilateral to the limb with cancer (Shimoyama et al., 2005), as has been reported for both neuropathic and inflammatory pain models (Noguchi et al., 1991; Lai et al., 2001).

9.2.3. Dorsal horn neurotransmitters contributing to hyperalgesia Glutamate

The importance of glutamate in hyperalgesic states is reflected in the finding that 50% of the axonal contacts surrounding identified spinothalamic tract (STT) cells contain glutamate (Westlund et al., 1992a). Dorsal horn glutamate receptor subtypes have been reported in axonal terminals contacting STT neurons (Ye and Westlund, 1996). The glutamate receptor subtypes *N*-methyl-D-aspartate R1 (NMDAR1), α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) GluR1 and GluR2/3 have been localized postsynaptically, and the NMDAR1 and AMPA GluR2/3 receptor subunits have been identified as autoreceptors on the presynaptic side of terminals contacting STT cells. NMDA receptors have also been observed on primary afferent terminals in the dorsal horn (Liu et al., 1994).

In functional studies, the excitatory amino acids (EAAs), aspartate (ASP) and glutamate (GLU), and their receptors have consistently been shown to play a key role in nociception and the hyperalgesia associated with the acute inflammatory response (Schaible et al., 1991a; Dougherty et al., 1992a; Ren et al, 1992; Sluka and Westlund, 1993a,b,c). Previous research has extensively characterized the role of GLU receptors and the effect of excessive GLU, both to the normal functioning and pathological activation of neuronal cells. The use of indwelling microdialysis fibers for simultaneous spinal sampling and GLU receptor antagonist administration has proved to be an effective method for monitoring the rising dorsal horn neurotransmitter levels and for study of the effects of blockade of individual receptors (Skilling et al., 1988; Sorkin et al., 1988, 1992, 1993; Sluka and Westlund, 1993a, 1993c). Nociceptive hyperalgesia levels indicated by behavioral responses as well as inflammation are impacted by receptor antagonists applied regionally in the termination zone of primary afferent fibers in the dorsal horn of the spinal cord in ways that iontophoretic applications to inhibit responses of individual dorsal horn neurons in electrophysiological studies cannot reveal. For comparisons of concentrations of amino acids and measurable nitric oxide metabolites (nitrites/nitrates), the intrathecal and

intraspinal microdialysis administration methods have been used to allow comparative measurement of chemical changes in the dorsal horn when challenged with physiological and pharmacological manipulations. For example, after the induction of arthritis, microdialysis monitoring indicates that there is a consistent pattern of release in dorsal horn EAA content measurable by HPLC, specifically increases of GLU and ASP near the neurotoxic range (Sluka and Westlund, 1992; Sorkin et al., 1992). The increases can be visualized with immunocytochemistry in structures such as terminal endings, interneurons and glia through one week (Sluka et al., 1992; Sluka and Westlund, 1993b). The amino acid concentration increases that occur as a result of intraarticular injection of kaolin and carrageenan can be divided into two phases: (1) an early brief release that occurs at the time of injection when concentrations of all amino acids rise, and (2) a late or prolonged release phase for ASP, GLU and GLN beginning at 3.5 h after knee joint injection and continuing through and likely beyond the 8h measurement period (Sluka and Westlund, 1992, 1993c). The ASP and GLU are good candidates for pharmacological manipulation of events associated with acute arthritis since the enhanced responsiveness of dorsal horn neurons is reduced by glutamate NMDA, non-NMDA and metabotropic receptor antagonists in the arthritic cat (Schaible et al., 1991a; Neugebauer et al., 1993) and rat (Ren et al., 1992; Neugebauer et al., 1994a,b).

Dorsal horn receptor involvement in both the nociceptive and the inflammatory events are successfully attenuated using intrathecal or more restricted spinal microdialysis administration of pharmacological agents while simultaneously performing behavioral testing and/or monitoring amino acid concentration changes. Specific antagonists of non-*N*-methyl-D-aspartate (non-NMDA) glutamate or GABA_A receptors, when infused through a semipermeable microdialysis fiber directly into the dorsal horn, greatly reduce both the inflammation and the nociceptive behaviors typically induced in the arthritis model. For example, infusion of agents such as CNQX and bicuculline directly into the dorsal horn of the spinal cord prior to induction of arthritis significantly reduces the extent of the joint inflammation (Ren et al., 1992; Sluka and Westlund, 1993a,c; Sluka et al., 1994a). Pretreatment of the spinal cord with bicuculline or CNQX also prevents the increase in joint temperature typical of knee joint inflammation while AP7 does not. The pain related behaviors indicating mechanical and thermal hyperalgesia, limping and guarding, are also greatly decreased by pretreatment with the non-NMDA receptor antagonist and not by treatment with the NMDA receptor antagonist, which is consistent with a decrease in the degree of peripheral inflammation caused by CNQX. Thus far, studies have shown that CNQX is also effective in *blocking* the inflammation in posttreatment studies, suggesting clinical relevance for agents blocking the non-NMDA receptors. Infusion of a GABA_B receptor antagonist had no effect on the inflammation or the nociception. Infusion of neurokinin and NMDA receptor antagonists while greatly reducing the hyperalgesia, do not reduce the inflammation itself. Further studies with selective metabotropic antagonists has shown a reduction in hyperalgesic behaviors (Zhang et al., 2002) and increased responsiveness of dorsal horn neurons (Neugebauer et al., 1999). The results of many studies indicate that after a hyperalgesic state is established, reversing the inflammation is difficult and that the role of NMDA receptors become foremost in reversal of the hyperalgesic state.

Neurotransmitter GLU and EAA receptors are important to the functioning of most neurons. Many of the intracellular events initiated by GLU receptor activation in the central nervous system are well known. Previous research has characterized the role of GLU receptors and the effect of excessive GLU both on normal and activated neuronal function most extensively in the hippocampus. Increased Ca²⁺ fluxes and translocation of PKC to the cell membrane have been described after binding of glutamate to its receptors. Gene transcription occurs in the nucleus within 30 min of GLU administration to neurons

affecting neuronal responses such as prolonged Ca^{2+} gradients in response to GLU stimulation (Woodman and Connor, 1992; Bading et al., 1993). One event shown to be involved in sustained pain transmission is translocation of GLU receptor subunit GluR1 from the cytosol to the plasma membrane mobilized by the nociceptive stimulus (Galan et al., 2004). In the same study, Glu2/3 subunits were shown to only be constitutively recycled as during states of normal neuronal transmission, indicating specificity of the GluR1 involvement in hyperalgesic responsivity. Nitric oxide synthase is also upregulated following GLU administration (Dawson et al., 1993; Sorkin, 1993; Garry et al., 1994). In the case of excessive GLU, cells can become overactivated by the excitotoxic levels and may ultimately sustain enough damage to die as in spinal cord injury. In the case of animal models of hyperalgesia, dorsal horn cells overactivated by excessive GLU typically do not show indications of excitotoxicity as have been noted in spinal cord injury but do express Fos and pPKC. Some as yet unknown protective processes are likely called into action in the overactivated state when high concentrations of GLU are present. When GLU receptors are transfected into COS-7 cells, the cells display increased *c-fos* and PKC translocation to the cell membrane confirming that GLU receptors on peripheral cells can also behave in the same manner as that seen in the central nervous system (Sakai et al., 1997). While GLU may affect many cellular types, the intracellular processes that are initiated by GLU under specific conditions as in the dorsal horn during hyperalgesic states are likely specific. For example, no previous reports of presynaptic NMDA receptors are found for other regions of the brain as has been shown on primary afferent endings (Liu et al., 1994). There are compelling data for a similar scenario of overactivation orchestrated for peripheral neurogenic interactions in inflammatory conditions since GLU receptors have been described peripherally on T-lymphocytes (Kostanyan et al., 1997), on Schwann cells and fibroblasts (Dememes et al., 1995) and on nerve endings in the skin (Carlton et al., 1995).

Thus, clear involvement of GLU receptor events on both central and peripheral neuronal elements is evidence that can lead to facilitated spinal dorsal horn activity and hyperalgesic responses. Signal transduction processes initiated by GLU receptors in the dorsal horn or at the peripheral nociceptive endings may provide an avenue for more specific pharmacological intervention albeit incumbent on pharmacological regimens to leave GLU receptors at hippocampal and other sites unaffected.

9.2.3.1. Substance P—The neuropeptide substance P (SP) activates neurokinin-1 (NK-1) receptors and has been shown to potentiate the release of glutamate in the spinal cord dorsal horn (Kangra et al., 1990). This result is substantiated by co-localization of SP and glutamate NMDAR1 receptor subunit staining in the same nerve terminal endings in the superficial dorsal horn with dual immunocytochemical electron microscopic localization (Aicher et al., 1997). The majority of SP terminals make presynaptic contact with NMDAR1 labeled dendrites. Lamina I NK-1-expressing and lamina IV–V glutamate labeled neurons are spinothalamic and spinoparabrachial neurons, which are involved in the ascending conduction of nociceptive input (Todd et al., 2002; Westlund et al., 1992a). The plasma membrane SP receptors on spinothalamic tract neurons undergo internalization after noxious stimulation (Mantyh et al., 1995). The NK-1 receptors have been shown to increase in inflammatory models (Abbadie et al., 1996; Vera-Portocarrero et al., 2003). Interactions of SP with EAA receptors at central and peripheral nerve endings enhance and increase the duration of glutamate-induced behavioral responses (Bileviciute et al., 1994b; Dougherty and Willis, 1994; Rees et al., 1996).

9.2.3.2. Calcitonin gene-related peptide—CGRP is a good marker for primary afferent activity in these studies since it is exclusively of primary afferent fiber origin in the dorsal horn of the spinal cord (Chung et al., 1988). Increased CGRP mRNA is observed in

the DRG during inflammation (Donaldson et al., 1992). The CGRP receptors are localized in the superficial dorsal horn as well as in the dorsal roots and ganglia (Ye et al., 1999). The CGRP receptor alpha has been localized ultrastructurally in postsynaptic membrane specializations on target cells and presynaptically on axon terminals in the dorsal horn, suggesting the presence of autoreceptors.

9.2.3.3. Dynorphin—Dorsal horn increases in the pro-nociceptive neuropeptide, dynorphin, stand out in commonality among several of the pain models. Dynorphin is notably increased in fos positive dorsal horn interneuron inflammatory pain models (Iadorola et al., 1988; Draisci et al., 1991; Noguchi et al., 1991; Pohl et al., 1997), in nerve injury models (Cho and Basbaum, 1988; Malan et al., 2000) and in cancer pain models (Honore et al., 2000). In various inflammatory pain models, the increases in dynorphin occur within 2 h and can increase three- to four-fold after 4h (Iadorola et al., 1988). Preprodynorphin mRNA then increases approximately three-fold within 24 h after CFA injection and reaches eight- to nine-fold 5 days after induction of inflammation. The preprodynorphin increases parallel the time course of the thermal hyperalgesia measured in animals with inflamed hindpaws. Studies have shown that dynorphin A (1–8) immunoreactivity in rat ventral horn neurons is initially depleted upon stimulation of unmyelinated primary afferent fiber (Klein et al., 1991), while its localization is greatly increased at later time points in dorsal horn interneurons in inflammatory pain models (Noguchi et al., 1991). The functional significance of this finding has recently been elucidated. Dynorphin has been shown to release CGRP in the dorsal horn just after injury, and the evoked CGRP release is blocked by anti-dynorphin (Gardell et al., 2002). The sustained production of dynorphin throughout the hyperalgesic state is a major factor in the increased nociceptive state.

9.2.3.4 Protein kinase C, inflammatory mediators, prostaglandins and nitric oxide

The repetitive activation of primary afferents also upregulates protein kinase C gamma (PKC γ). Three days after CFA hindpaw inflammation, PKC γ expression is doubled and is translocated to the plasma membrane. The increase parallels the behavioral hypersensitivity (Martin et al., 1999) and inhibitors of PKC significantly reduce nociceptive hyperalgesic responses (Yashpal et al., 1995). Glutamate (GLU), other neurotransmitters and pro-inflammatory cytokines (including IL-1 β , nerve growth factor, and protein kinase C) can activate nuclear factor kappa B (NF κ B) in glia which in turn can regulate expression of pro-inflammatory cytokines, inducible nitric oxide synthase (NOS) and cyclooxygenase-2 (COX-2) (O'Neill and Kaltschmidt, 1997). While many second messenger systems are undoubtedly involved in dorsal horn hyperalgesia, glutamate receptor-mediated events have a common pivotal convergence point through rapid activation of NF κ B for coordinated activation of target genes promoting tumor necrosis factor alpha (TNF α) and the expression of COX-2 (Guerrini et al., 1995; for reviews see Barnes and Adcock, 1997; Barnes and Karin, 1997; O'Neill and Kaltschmidt, 1997). Activation of NF κ B occurs in many pathogenic settings including exposure to glutamate, kainate and high potassium, as well as exposure to a variety of immune and inflammatory activators (Kaltschmidt et al., 1995; Kopp and Ghosh, 1995). The NF κ B in turn regulates the expression of many immune and inflammatory activators forming an intracellular amplification loop involving glutamate, NF κ B and inflammatory activators. Inhibition of NF κ B action using competition for NF κ B binding with an NF κ B specific oligonucleotide decoy potently reduces CFA-induced mechanical allodynia and thermal hyperalgesia (Lee et al., 2004). Inflammation is also amplified by blood-borne inflammatory mediators including TNF α and IL1 β . The TNF α

induces mechanical allodynia after spinal nerve ligation by activation of p38 mitogen-activated protein kinase (MAPK) in primary sensory neurons (Schafers et al., 2003).

NMDA-induced thermal hyperalgesia is mediated by nitric oxide and protein kinase C (Meller et al., 1996). Systemic treatment with L-NAME which reduces nitric oxide by inhibiting inducible nitric oxide synthase (iNOS) has been found to reduce edema formation and c-fos expression in the spinal cord after hindpaw inflammation with carrageenan (Honore et al., 1995). Experiments also demonstrate that formalin-induced hyperalgesia in rats can be blocked by intrathecal administration of an agent which disrupts synthesis of nitric oxide and cytokines in monocyte-derived cells (Watkins et al., 1997). Thermal but not mechanical hyperalgesia following peripheral zymosan injury can also be dose-dependently reduced by iNOS inhibition with aminoguanidine (Malmborg and Yaksh, 1993; Meller et al., 1994).

Inflammatory mediators play a critical role in the initiation and persistence of hyperalgesic states. Intrathecal injection of substance P or NMDA produces an immediate increase in prostaglandin (PG) or cyclooxygenase-2 (COX-2) release, respectively (Dirig et al., 1997; Ghilardi et al., 2004). Most neurons and radial glia in the rat spinal cord constitutively express high levels of COX-2 protein (Goppelt-Strube and Beiche, 1997). COX-2 is also a major contributor to prostaglandin E₂ (PGE₂) synthesis and release in the spinal cord after injury (Barnes and Adcock, 1997; O'Neill and Kaltschmidt, 1997; Ghilardi et al., 2004). Prostaglandins E₂ sensitize nociceptors to mechanical, thermal and chemical stimuli to produce hyperalgesia (Evans et al., 2000). Prostaglandins modulate TTX-R currents in nociceptive afferent fibers by increasing the magnitude, the rates of activation and inactivation, and lowers the threshold of excitation (England et al., 1996). Increases in PGE₂ activate phospholipase A₂ secondary to increased intracellular calcium and the liberation of arachidonic acid. Arachidonic acid acts on the COX-2 isozyme that is constitutively present, resulting in PG release.

9.3. Glial involvement in hyperalgesia

The role of glia as major participants in hyperalgesia has been underappreciated to date. Beyond the role of glia in maintenance of dorsal horn homeostasis, evidence is mounting that neuroactive substances activate glia, such as excitatory amino acids, substance P, nitric oxide, prostaglandins and ATP. One neuronally associated surface protein responsible for glial activation is the chemokine, fractalkine (Milligan et al., 2004).

Under normal circumstances, neuropeptide transmitters are rapidly degraded by the endopeptidases produced by glia and a significant role is played by glia in reuptake of major amino acid neurotransmitters. Glia remove excess neurotransmitter glutamate with excitatory amino acid neurotransmitter transporter proteins EAAT1 and EAAT2. Transporter proteins GAT-1 and GAT-3 remove GABA. During activation states, glia upregulate these proteins in an effort to restore the neurochemical environment and equilibrate the dorsal horn activation state (Ng and Ong, 2001; Vera-Portocarrero et al., 2002; Sung et al., 2003).

Upon activation, glia release a variety of neuroactive substances known to be critical for the mediation of subcutaneous formalin-induced hyperalgesia including glutamate, aspartate, nitric oxide, arachidonic acid and cyclooxygenase products such as prostaglandins. Activated glia assume numerous other functions critical for responses to plastic changes occurring in the nervous system, as reviewed recently by Hansson and Ronnback (2003). This is particularly true of neuroactive substances promoting Ca²⁺ and ATP signaling between astrocytes, microglia, oligodendrocytes and neurons, with an array of molecules acting as signaling substances. Glial activation includes not only upregulation of glial

markers but upregulation of cytokine (interleukin-1 β ; TNF α) expression since glia are immunocompetent cells. These cytokines have been shown to play a role in producing hyperalgesia following either peripheral inflammation or peripheral nerve injury (Sweitzer et al., 1999; DeLeo et al., 2000; Milligan et al., 2004).

Thus, data support the importance of neuronal–glial communication and major participation by astrocytes and other types of glial cells in the sequelae occurring in the dorsal horn during hypersensitive states. In pain-related studies, marked but reversible reduction of both thermal and mechanical hyperalgesia can be accomplished with intrathecal administration of glial metabolic inhibitor, fluorocitrate (Meller et al., 1994; Watkins et al., 1997). Glial staining increases of the glial fibrillary acidic protein (GFAP) have been noted following constriction injury of a peripheral nerve (Garrison et al., 1991). The magnitude of the increase in GFAP staining correlates with the degree of hyperalgesia. The microglial marker, OX-42, is also upregulated and microglial activation parallels the time course of the hyperalgesic behaviors after hindpaw formalin injection (Fu et al., 1999, 2000). Spinal glial activation has been shown to parallel the development and maintenance of both neuropathic and inflammation-induced mechanical allodynia (Sweitzer et al., 1999; Hashizume et al., 2000). However, in the case of neuropathic pain, the presence of activated astrocytes does not correlate with any behavioral measure tested (Stuesse et al., 2001).

Concentrations of reactive oxygen species, such as nitric oxide, are likely to increase during hyperalgesic activation states in other oxidative stress conditions. Leakage of reactive oxygen species or “free radicals” from mitochondria are signaling events highly relevant for neuronal–glial communication and production of pro-inflammatory cytokines during hyperalgesic states. Increase in the synthetic enzyme, inducible nitric oxide synthase, significantly increases in the glial ependymal cells lining the central canal through all levels of the spinal cord following peripheral Freund’s adjuvant inflammation (Wu et al., 1998). While the neurochemical changes in the spinal cord reported with inflammatory pain, typically are confined to the level and side of the spinal cord corresponding to the peripheral injury, the broad distribution of those that are precipitated by glial activation increase bilaterally. This suggests that a glial cell communication network exists independent of neurotransmitter actions. The ependymal cell-to-cell communication network that upregulates iNOS content through the length of the spinal cord after peripheral inflammation may facilitate development of other processes contributing to hyperalgesic states. The ependymal cell which is capable of undergoing immune and inflammatory reactions itself may assist in rapid migration of the other CNS inflammatory cells, such as the meningeal macrophage/microglial cells, into the dorsal horn region via the cerebrospinal fluid.

9.4. Neurotransmitter actions during inflammatory pain contributing to hyperalgesia

9.4.1. Dorsal horn primary afferent input

It has been known for a century that activity in primary afferent fibers supplying the skin can cause “antidromic vasodilatation” (Bayliss, 1901) and it has been assumed that the activity is generated locally (axon reflex). It is well known that neurotransmitter substance P, CGRP and glutamate released at peripheral nerve endings participate in peripheral vasodilatory and cutaneous extravasation processes (Brain and Williams, 1985; Levine et al., 1985b; Lawand et al., 2004). They also activate autoreceptors initiating ectopic activity generated at injury sites at the terminals, along nerves and/or in dorsal root ganglia. They are likely to release aberrant quantities of neurotransmitter substances at these sites that may be contributory to hyperalgesic states as well as peripheral inflammatory processes in pathological pain states.

These same chemical mediators in the dorsal horn, glutamate (GLU), GABA, CGRP, substance P (SP) and nitric oxide (NO), have been studied extensively in efforts to understand their role in nociceptive processes. The content of both the neuropeptides, CGRP and SP, increases significantly in the dorsal horn during the development of inflammation (Takahashi et al., 1988, 1990; Sluka et al., 1992; Sluka and Westlund, 1993b). These mediators “kindle” events that result in hyperalgesic behaviors (Brain and Williams, 1985; Ferrell and Russell, 1986; Ruda et al., 1986; Haley et al., 1990, 1992; Schaible et al., 1990;Coderre and Melzack, 1991; Dougherty and Willis, 1991; Woolf and Thompson, 1991; Meller et al., 1994; Evans, 1995). In the adjuvant arthritis model in rats, which causes a more chronic inflammation, SP and CGRP are increased in the dorsal horn as well as in the periphery (Oku et al., 1987; Takahashi et al., 1988; Weihe et al., 1988; Kuraishi et al., 1989; Minami et al., 1989). In addition, increased mRNA levels for preprotachykinin A, the precursor to SP, are observed in both dorsal root ganglia (DRG) and the spinal cord dorsal horn (Minami et al., 1989; Donaldson et al., 1992; Dubner and Ruda, 1992; Noguchi and Ruda, 1992). Iontophoretic application of the CGRP receptor antagonist reduces the hyperexcitability of dorsal horn neurons driven by joint inflammation (Neugebauer et al., 1996). Antibodies to CGRP administered intrathecally reduce the hyperalgesia associated with inflammation (Kuraishi et al., 1988). Studies have shown differential effects of NK2 on behavioral hyperalgesia when administered spinally prior to the induction of acute arthritis while NK1 is effective after the inflammation has developed (Sluka et al., 1997). Both are effective when administered systemically or directly into the joint.

9.4.2. Presynaptic inhibition

It has long been known that presynaptic inhibition of incoming primary afferent fiber input is one mechanism by which the dorsal horn attempts to limit its overactivation in the face of a barrage of incoming nociceptive input (Barron and Matthews, 1938; Lloyd and McIntyre, 1949; Eccles, 1964; Schmidt, 1971). In this mechanism the activated dorsal horn interneurons release neurotransmitter substances that depolarize the central terminals of the primary afferent fibers. In addition to the well known effect of this mechanism in limiting the further release of neurotransmitters and thus dampening the impact of the incoming afferent activity that is signaling the adverse nociceptive event, presynaptic inhibition can have a direct effect on the primary afferent ending itself, leading to primary afferent depolarization. Primary afferent depolarization can be recorded as dorsal root potentials (DRPs) or demonstrated by excitability testing of the primary afferent terminals (Eccles, 1964). The DRPs can be evoked following stimulation of either large or fine primary afferent fibers (Eccles et al., 1962; Zimmermann, 1968; Burke et al., 1971), including joint afferents (Quevedo et al., 1993). Primary afferent depolarization is most readily investigated when it occurs in large axons including muscle afferents, but several studies have demonstrated that primary afferent depolarization also occurs in fine myelinated afferent fibers following noxious stimuli (Jänig et al., 1968; Whitehorn and Burgess, 1973), as well as in C-fibers (Hentall and Fields, 1979). Small joint and visceral afferents are also subject to primary afferent depolarization (Jankowska et al., 1993).

9.4.3. Dorsal root reflex

Dorsal horn primary afferent depolarization events can also result in the generation of action potentials that have been shown to propagate outward toward the periphery under certain pathophysiological conditions and were termed dorsal root reflexes (DRRs) (Gotch and Horseley, 1891; Bayliss, 1902; Barron and Matthews, 1938; Toennies, 1938; Brooks and Koizumi, 1956; Eccles et al., 1961). This phenomenon was noted previously under certain conditions, as when the body temperature is lowered (Brooks et al., 1955). Depolarization of the central end of primary afferent fibers occurring in response to excessive afferent nerve stimulation after deep tissue insult produces increased activity of glutamate and GABA

interneurons. The increasing amount of GABA in the dorsal horn activates chloride channels on the primary afferent endings through an action on GABA_A receptors (Eccles et al., 1963; Eccles, 1964; Schmidt, 1971; Levy, 1977; Duchen, 1986). This unusual circumstance occurs with the assistance of a special Na⁺-K⁺-Cl⁻ co-transporter found on primary afferent neurons. The pump transports two chloride ions into primary afferents along with each sodium and potassium ion on a routine basis (Alvarez-Leefmans et al., 1998). The resulting three-fold concentrated intracellular chloride typically present in primary afferent endings in the dorsal horn allows the chloride resting potential to reside near its depolarization potential. Thus, the excessive GABA released when interneurons are overactivated readily causes depolarization of the primary afferent terminal ending, rather than a hyperpolarization as is the usual case with GABAergic postsynaptic inhibition in most other neural systems. The DRRs propagate out of the dorsal root toward the periphery, presumably invading the peripheral primary afferent nerve receptive ending. There they may antidromically activate the primary afferent fibers supplying the skin to cause “antidromic vasodilatation” (Bayliss, 1901). This can be amplified peripherally through release of vasodilatory neurotransmitter substances via local axon reflexes. More recently, it has been shown that under pathophysiological conditions such as surgery or peripheral inflammation, primary afferent depolarization can be exceed the threshold for the generation of action potentials in primary afferent fibers and the DRR generated contributes to peripheral inflammation (Sluka et al, 1995b).

The development of DRRs can contribute to pathological events in the periphery through the release of inflammatory mediators, glutamate and other neuromodulators in the dorsal horn of the spinal cord. It has been demonstrated that DRRs generated in the spinal cord dorsal horn traveling back out in articular primary afferent fibers contribute to the increased blood flow, vasodilatation and neurogenic edema occurring peripherally in the knee joint in an animal model of arthritis (Sluka et al., 1994b, 1995b; Lawand et al., 2004). The DRR activity has been recorded ipsilaterally in articular afferent fibers after induction of both acute and chronic models of inflammation (Rees et al., 1994, 1995, 1996). In the chronic arthritis model induced with complete Freund’s adjuvant (CFA), the DRRs can be recorded from both sides, providing a mechanistic explanation for “mirror” effects in arthritis and indicating that the dorsal horn events are also bilateral. The DRRs can be recorded from the medial articular nerve (MAN) (Rees et al., 1994) and from dorsal root filaments (Sluka et al., 1995a) following mechanical and electrical stimulation in animals with knee joint inflammation producing a barrage of output with various delays (3–40 ms) suggesting that DRRs can travel in fiber types of several conduction velocities. The recordings could be made 4 h after inflammation in the case of acute arthritis model or persisting after the joint was inflamed with CFA for one week. The recordings were made from the proximal stump of cut nerves using a platinum hook electrode and thus were generated centrally. Furthermore, DRRs have been recorded in the central end of cut dorsal roots of arthritic animals in response to peripheral input arriving over adjacent nerves, demonstrating that the DRRs are generated centrally in the spinal cord rather than in the dorsal root ganglia. The presence of DRRs in cut dorsal roots and nerves following peripheral stimulation in animals with inflamed knee joints provides clear evidence of a mechanism by which centrally activated events can further amplify peripheral events.

Another expected result of the development of DRRs is further increase in the activity of the dorsal horn neuronal circuits. The DRRs are also known to further release neurotransmitters from the central terminals of primary afferent fibers (Eccles et al., 1961, 1962; Duchen, 1986; Sluka et al., 1992, 1995b). It has been demonstrated that generation of DRR activity is mediated by the same mechanisms that produce dorsal horn sensitization, i.e. the release of neurotransmitters from the *central* terminals of the primary afferent fibers (Eccles et al., 1963; Duchen, 1986; Sluka et al., 1994a, 1995b).

Studies have shown that administration of non-NMDA (CNQX) and GABA_A (bicuculline) receptor antagonists directly into the dorsal horn through a microdialysis fiber eliminate the DRRs, as does dorsal rhizotomy, peripheral application of lidocaine or crushing the medial articular nerve proximal to the recording site (Rees et al., 1994, 1995, 1996). The DRRs were unaffected by administration of NMDA (AP7) and GABA_B (CGP35348) receptor antagonists. It was determined that all fiber types (types II, III and IV) contribute to the DRR in conduction velocity experiments in cats and monkeys (Sluka et al., 1995a). This previously unrecognized positive feedback mechanism impacting dorsal horn circuitry and activation plays a major contributory role in initiation, amplification and maintenance of spinally driven neurogenic inflammation and the associated nociceptive changes. Perpetuated release in the periphery prolongs the neurogenic vasodilation and plasma/protein extravasation (Kuraishi et al., 1989; Larsson et al., 1989, 1991; Bileviciute et al., 1993; Lawand et al., 2000).

9.4.4. Neurogenic amplification loop

Although early studies have suggested a neurogenic component for peripheral inflammation (Lewis, 1927), very little evidence was accumulated for neurogenic involvement in the inflammatory process, however, prior to studies with experimental arthritis models in rats (Levine et al., 1985a,b; Sluka et al., 1995b; Coderre and Katz, 1997). These and more recent experimental studies have shown that both the peripheral inflammatory and the central hyperalgesic mechanisms described above are potentiated and perpetuated through activation of a neurogenic amplification loop (Fig. 9.5). Neurogenic amplification can result in (1) the persisting release of excessive amounts of neurotransmitters both centrally and peripherally, (2) activation of pathological responses of glia, (3) initiation and maintenance of central hyperalgesic responses and (4) peripheral inflammation. The “wind-up” of overexcited dorsal horn neurons is known to be NMDA mediated (Davies and Lodge, 1987). Excessive afferent nerve fiber activity over a prolonged period, occurring in response to a severe peripheral insult, releases the neurotransmitters that overactivate the dorsal horn circuitry and overpower the clearance mechanisms normally active, such as glial uptake of excitatory amino acids through specific transporter proteins. Near-toxic measurable glutamate (7 ng/μg is well above metabolic levels) and stainable glutamate content increases (30%) are found in the spinal cord (Sluka and Westlund, 1993b,c). Glutamate is found in twice as many small myelinated and unmyelinated fibers in a peripheral nerve innervating an inflamed knee joint compared to the control side (Westlund et al., 1992b). The resulting development of DRRs contributes to the pathological events in both the peripheral and central nervous systems through further release of neurogenic mediators, glutamate and other modulators. The dorsal root reflexes release EAAs and vasoactive peptides in the periphery that provide additional positive feedback to what becomes a reverberating amplification loop and a vicious cycle of hyperalgesic pain and inflammation (Sluka et al., 1994c, 1995b). Neurotransmitter substances, including glutamate, CGRP and substance P, released in central sites by sensitized afferent nerve fibers, activate the central intrinsic and peripheral afferent terminals through autoreceptors further amplifying and perpetuating the central hyperalgesic mechanisms.

Thus, when afferent input is persistent and intense, dorsal horn protective mechanisms give way. Plastic, adverse events develop and can persist with a resulting higher nociceptive set-point. This includes increased neuronal background activity and responsivity. Centrally, high-frequency firing (100 Hz) of primary afferents excites interneuronal release of glutamate and GABA from dorsal horn interneurons. Synaptic transmission between primary afferent terminals and second-order neurons is primarily mediated by AMPA/kainate receptors (Yoshimura and Jessell, 1990; Randic et al., 1993). Both glutamate and GABA interneurons are present in the dorsal horn and are activated. Cervero and Laird

(1996) have proposed that, during central sensitization states, touch-evoked pain is generated when low-threshold mechanoreceptive A-fiber afferent input activates dorsal horn excitatory interneurons. Excitatory interneuronal connectivity in turn presynaptically further activates the high-threshold nociceptive C-fiber endings that are already providing nociceptive input to spinothalamic tract neurons and are generating dorsal root reflexes. Alvarez-Leefmans has proposed that activation of the GABA_A receptors on primary afferent endings produces Cl⁻ outflow and presynaptic depolarization since the Na⁺-K⁺-2Cl⁻ co-transporter maintains Cl⁻ levels inside primary afferent terminals as described above (Alvarez-Leefmans et al., 1998). This increases the effectiveness of action potentials opening voltage-gated calcium channels. The Ca²⁺ entry triggers increased release of glutamate and substance P from primary afferent endings, activating many dorsal horn elements. This would include dorsal horn interneurons, terminations of descending facilitatory pathways and projection neurons. The increased depolarization opens more NMDA receptor channels and likely promotes insertion of NMDA receptor subtypes with increased open time that are more efficacious. The activation produced may in this manner persist long term and may be likened to long-term potentiation (LTP) in the hippocampus that contributes to memory of events, though the specific receptor mechanisms involved may vary. Abundant glutamate would increase the probability of the involvement of metabotropic glutamate receptors in the generation of dorsal root reflex activity (Zhang et al., 2000). Gereau and colleagues have found that Group I metabotropic glutamate receptors and their downstream signaling pathways involving the extracellular signal-regulated kinases (ERKs) are implicated as mediators of the plastic events contributing to the long-term enhancement of nociceptive behavioral responses (Adwanikar et al., 2004). It is likely that all of these scenarios contribute to dorsal horn hyperalgesia, generation of dorsal root reflexes, the vicious cycle of pain and inflammation, and the LTP-like persistence of pain. The rising levels of neurochemical transmitters, glutamate and GABA in particular, are the commonality in activation of all of these mechanisms.

Centrally, excessive amounts of glutamate and other neuromodulators are found through several segments of the dorsal horn and would thus involve other dorsal horn cells at these levels that also become overactivated as in the Hardy model (Hardy et al, 1967). For example, sensitization of wide dynamic range dorsal horn neurons (Menetrey and Besson, 1982; Schaible et al., 1990; Dougherty et al., 1992b) to cutaneous and joint stimuli occurs after induction of arthritis and is accompanied by a concurrent increase in descending inhibition (Schaible et al., 1991a). Dorsal horn neurons with afferent input from an inflamed joint have an increased receptive field size in spinalized rats (Neugebauer and Schaible, 1990). The overactivation measurable as behavioral hyperalgesia persists through the time course of the excessive concentrations of glutamate and aspartate measurable in the dorsal horn in the case of knee joint inflammation (at least 8h) (Sluka and Westlund, 1993a,b). The bottom line is an NMDA-mediated sensitization of spinothalamic tract projection neuron activity relayed to higher centers results in hyperalgesic behavioral responses. Thus, the amplification loop provides the neurogenic drive underlying the *initiation* and the *persistence* of both the altered nociceptive state centrally and the peripheral inflammation for up to several weeks.

Increased GABA_A receptor activation has also been shown to facilitate neurokinin release (Lao and Marvizón, 2005). While the selective GABA_A receptor agonists, muscimol and isoguvacine, can substantially increase the amount of neurokinin-1 receptor (NK1R) internalization evoked by dorsal root stimulation at 1 Hz, pathophysiological activation during inflammation increases the distribution of dorsal horn neurons that internalize the NK1R response to both noxious and non-noxious stimulation (Abbadie et al., 1997; Marvizón et al., 1997) The increase in NK1R internalization is mediated by NMDA receptor activation and facilitated by neurokinin release evoked by primary afferent firing. The

sensitized responses of spinothalamic tract neurons amplify nociceptive information transmitted by the spinothalamic tract.

The DRRs contribute to the persistent nociceptive changes, peripheral vasodilatation and neurogenic edema that are characteristic of hyperalgesic states that develop after inflammatory events through measurable release of excitatory amino acids (EAAs) and vasoactive peptides from afferent nerve endings in the joint that act in concert with the blood-borne or local factors that had previously been considered to be the primary players in the development of inflammation. Pharmacological manipulations using microdialysis reveals that blocking ionotropic non-NMDA glutamate receptor transmission at the first synapse as well as blocking interneuronal GABA_A-type transmission at the second synapse recurrent to the primary afferent terminal can block the dorsal root potential and initiation of dorsal root reflexes traveling back down afferent nerves to the periphery. The DRR activity recorded in the medial articular nerve of inflamed joints is reduced or eliminated with spinal application of antagonists of non-NMDA glutamate and GABA_A receptors and thus this neurogenic amplification process acts independently of other neurogenic amines (Rees et al., 1995). Similarly, dorsal rhizotomy, peripheral application of lidocaine or crushing the medial articular nerve proximal to the recording site also eliminates DRR activity.

While hypersensitive responses in the short term are reversed by antagonism of the actions described above, the circumstances which allow chronic pain conditions to develop and persist are not known at this time but rely more heavily on NMDA and metabotropic glutamate receptor mechanisms. Isolated clinical reports indicate that damage to nervous pathways and peripheral nerves due to neurological insults, such as stroke and peripheral nerve damage, can provide a *protective effect* against development of arthritis on the affected side, proportional to the magnitude of the neurologic insult. Thus, evidence is increasing that the peripherally released glutamate has effects on inflammatory cascades. The development of DRR activity contributes to pathological events through which the release of glutamate and other neuromodulators affecting inflammatory mediator cascades become a vicious cycle. Thus, the dorsal root reflex is an important mechanism for the neurogenic amplification loop and persistence of both inflammation and the enhanced hyperalgesic nociception.

9.5. Neuropathic pain-related pathology contributing to hyperalgesia

As discussed above, in the case of nerve injury a significant dorsal horn change noted at the spinal level of interest is a decrease in neurotransmitter content. This is due to a reduction in the number of primary afferent fiber endings as damaged fibers retract after injury (Chung et al., 1988, 1989). However, it is the major changes that occur in the adjacent uninjured nerves which allow the development of neuropathic pain hyperalgesic conditions. In particular, the C-fiber-type high-threshold nociceptive primary afferent neurons become hypersensitive to mechanical, heat and chemical stimuli (Blumberg and Jänig, 1984; Koschorke et al., 1991; Koltzenburg et al., 1994). This is in part due to increases in Na_v1.8 sodium channels (TTX-resistant) on the high-threshold nociceptors and development of spontaneous ectopic firing, i.e. aberrant firing at sites that are out of place such as the ganglia, the peripheral nerve and the central terminals (Kajander et al., 1990; Liu et al., 2000; Wu et al., 2001, 2002; see Gold, 2000). Other consequences related to the hyper-responsiveness include increased release of neurotransmitters at the central terminals of the intact primary afferent fibers, including ATP-mediated glutamate release (Gu and MacDermott, 1997). Antisense knockdown of Na_v1.8 sodium channels reduces both immunoreactivity and TTX-resistant current that correlates with reductions in both mechanical and thermal hyperalgesia (Gold et al., 2003).

9.6. Descending facilitation influences spinal hyperalgesic mechanisms

Descending brainstem pathways have been studied extensively and shown to play a major role in spinal modulation of nociception. The spinal cord not only receives and modifies nociceptive information generated in response to nociceptive conditions, but it is clear that the information relayed to higher brain centers returns to modulate nociception at the spinal cord level with both inhibitory and excitatory inputs (Fig. 9.6). Evidence of these descending facilitatory and inhibitory mechanisms are reported for both neuropathic and inflammatory pain syndromes (Ren and Dubner, 1996; Urban et al., 1996; Ossipov et al., 2000; Porreca et al., 2002; see Vanegas and Schaible, 2004). As an example, the sensitization of dorsal horn neurons (Menetrey and Besson, 1982; Schaible et al., 1990; Dougherty et al., 1992b) to cutaneous and joint stimuli that occurs after induction of arthritis is accompanied by a concurrent increase in descending inhibition and facilitation (see Vanegas and Schaible, 2004). The generalized effects of descending controls on the hyperexcitability of dorsal horn neurons were demonstrated by an increase in neuronal activity with a cold block of the spinal cord after knee joint inflammation (Schaible et al., 1991a,b). In addition to the well-studied descending inhibitory controls exerted by higher centers (Fields and Basbaum, 1978; Gebhart, 1986; Besson and Chaouch, 1987), descending facilitatory pathways are also involved (Urban et al., 1996; Guan et al., 2002; Gardell et al., 2003). While the transmitters involved still remain under study, noradrenergic systems in the pons and serotonergic systems in the rostral ventromedial medulla (RVM) are known to play a major role and have been studied primarily in the context of descending inhibition. In the rat, α_2 -adrenoreceptor *agonists* have no effect in normal animals but are effective in reducing behavioral responses in animals with inflammation (Hylden et al., 1991; Stanfa and Dickenson, 1994). The α_2 -adrenoreceptor *antagonists* are pronociceptive in animals with acute inflammation (Stanfa and Dickenson, 1994). Serotonin increases excitability of primary afferents through cAMP-coupled TTX-resistant sodium channels (Cardenas et al., 2001). It is clear that both serotonergic and cholecystokinin systems in the RVM are essential to the descending facilitation and send descending axonal fibers into the spinal dorsolateral quadrant (Urban et al., 1996; Vanegas et al., 2004; Xie et al., 2005). In fact, dorsolateral funiculus lesions negate the exaggerated release of CGRP evoked by capsaicin in nerve-injured animals implying that brainstem descending facilitatory pathways in the dorsolateral funiculus of the spinal cord are a major contributor to spinal cord mechanisms resulting in hyperalgesia (Gardell et al., 2003).

Experiments are underway to unravel roles played by each specific neuroactivator and the hyperalgesic mechanisms that evolve through ascending and descending brainstem connections activated after tissue injury. The balance between inhibitory and excitatory descending influences activated by nociceptive input play a significant role in the development, persistence and perceived intensity of the hyperalgesic state. There is little doubt that the descending inhibitory pathways play a major role and that without them the hyperalgesic state would be even more pronounced. In fact, patients with chronic arthropathies have simultaneous hypoalgesia in response to von Frey fibers in the innocuous range along with allodynia to higher-strength von Frey fibers in the noxious range, suggesting that their pain level would be higher without descending inhibition (Hendiani et al., 2003). Two recent excellent reviews are available discussing the facilitatory and inhibitory influences of the parabrachial and RVM regions on spinal hyperalgesia in animal studies (Millan, 2002; Vanegas and Schaible, 2004).

9.7. Summary

The actions of neurotransmitters, neuromodulators, glial activation products, nitric oxide and inflammatory mediators that are imposed on the spinal cord from peripheral, intrinsic and

central descending facilitatory sources have been shown to play a critical role in the initiation and persistence of dorsal horn hyperalgesic responses to nociceptive input. Consideration of the role of glial and neurogenic amplification factors in persistent hyperalgesic nociceptive states will be key in reducing on-going chronic pain. Studies continue to examine the pharmacological, physiological and intracellular interactions of neurotransmitters, receptors, glial activators and intracellular messengers utilized by neurons and glia in the dorsal horn in the plastic cellular events that form the basis of hyperalgesic responses. Researchers will be specifically addressing development of strategies to abrogate more effectively the response to neuromodulators in the dorsal horn for improved treatment of chronic pain conditions. Challenges remain in our understanding of the hyperalgesic mechanisms through which the nociceptive response process is initiated, amplified, maintained and reinitiated.

A better understanding of dorsal horn mechanisms is a laudable goal toward translation into novel strategies for stopping the disease progression and debilitation for patients with chronic pain syndromes. Clinically relevant studies are being designed to disrupt activation of cellular circuitry and intracellular cascades that are creating the amplification and persistent “memory” of nociceptive events that allow hyperalgesic conditions to establish themselves as chronic pain conditions. Pharmacological, molecular and gene therapeutic interventions are being sought to disrupt actions of neurogenic and glial initiators that potentiate neurotransmitter receptor mechanisms preventing return to a normal set-point for nociception. Yet elusive is identification of mechanisms that allow “wind-down” of on-going processes in some cases that when absent uniquely contribute to the persistence of each chronic pain condition. It is this difficult goal that will provide clinical relevance.

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Fig. 9.1.

Hardy and colleagues proposed that in the case of sustained noxious stimulation, primary and secondary hyperalgesia were the result of the involvement of increasingly larger pools of neurons which become activated by released neuroactive substances. (From Hardy et al., 1967.)

Hardy and colleagues proposed that primary afferents activate interneuronal networks in the dorsal horn which activate other neurons in the spinal cord and provide the secondary hyperalgesia sensation in the adjacent cutaneous areas. (From Hardy et al., 1967.)

Dorsal Horn Neurochemical Microenvironment

Fig. 9.3.

The dorsal horn neurochemical microenvironment is characterized by an overabundance of neurochemical mediators and neurotransmitters which are not successfully inactivated or taken back up in prolonged states of activation. Neurochemical profiles demonstrated in various animal models vary but all have in common an increase in dorsal horn dynorphin believed to contribute to the sensitized nociceptive state. (GLU: glutamate; Sub P: substance P; CGRP: calcitonin gene-related peptide; PKCγ: protein kinase C gamma; NOS: nitric oxide synthase; GABA: gamma-aminobutyric acid; SHT: S-hydroxytryptamine.)

Fig. 9.4.

After an initial insult or injury, there is an initial spiking release of specific neurotransmitters at the time of injury followed by a slow sustained wave of release. During this same time there is a slowly evolving and protracted period of increased sensitization that arises and persists for a variable amount of time dependent on factors such as the increased release of glutamate into the neurochemical microenvironment of the dorsal horn varying with the type of inflammatory injury. Cutaneous administration of capsaicin or formalin produces a greatly compressed time frame for the release of neurotransmitters and the development of hyperalgesic responses over minutes. Hindpaw and knee joint injections of the irritants, kaolin and carrageenan, produce sensitization over several hours. The time course for development of hyperalgesia due to complete Freund's adjuvant is greatly protracted over days to weeks.

Fig. 9.5.

Sluka and Westlund proposed a neurogenic amplification loop that increases afferent nerve input to the dorsal horn and hyperalgesic responses of dorsal horn neurons, and triggers dorsal root reflex (DRR) firing back out of the nerves to the periphery. The self-perpetuating vicious circle produces persistence of hyperalgesic pain states and maintains inflammation in the periphery. The dorsal horn circuitry involves non-NMDA and GABA_A receptor activation and primary afferent depolarization, and impacts NMDA-mediated ascending spinothalamic tract output. (Modified from Sluka et al., 1995b.)

Fig. 9.6.

Descending systems impact the nociceptive processes in the spinal cord. Activation of brainstem regions by ascending nociceptive input from the spinal cord produces complex interactive events in the brainstem that result in a shift in the balance of the integrated excitatory and inhibitory output that arrives in descending projections to the spinal cord dorsal horn. Descending inhibition serves to damp the dorsal horn activation state while descending facilitation will shift the balance to a higher activation state. During sustained nociceptive states, descending facilitation shifts the balance of the spinal activation, conveying sensitivity to previously innocuous stimuli. The more diffuse nature of the descending system innervation of the spinal cord participates in the expansion of the sensitization to additional spinal levels bilaterally. Descending facilitation impacts dorsal horn hyperalgesia through dorsolateral funicular axonal pathways carrying serotonin (5HT) and cholecystokinin (CCK). Descending facilitatory pathways originate in the periaqueductal gray (PAG) and the rostral ventromedial medulla (RVM) and travel in the dorsolateral funiculus of the spinal cord through its length. Descending noradrenergic (NA) inhibitory pathways that impact alpha 2-type receptors in the spinal cord originate from noradrenergic cell groups A5–A7 in the pons and are situated ventrolaterally through the length of the spinal cord.