J Anesth (2001) 15:39–48

Journal of
Anesthesia

Review

A possible role for glutamate receptor-mediated excitotoxicity in chronic pain

Andrei B. Petrenko and Koki Shimoji

Department of Anesthesiology, Niigata University School of Medicine, 1-757 Asahimachi, Niigata 951-8510, Japan

Key words Glutamate receptors · Excitotoxicity · Chronic pain

Introduction

The gate-control theory of pain, advocated by Melzack and Wall in 1965 [1], deeply stimulated further research in the field of pain and its treatment. The precision of its formulation enabled scientists to design experiments to test the hypothesis, and clinicians to devise novel forms of pain-relieving strategies. More recent work, however, has changed the emphasis of this theory. Although Melzack and Wall's theory makes allusion to a descending inhibitory control system of pain transmission in the dorsal horn, contemporary studies suggest that the activation of afferent axons initiates a powerful and long-lasting inhibitory modulation of pain transmission that descends from the brain [2]. In a chronic pain state, the spinal relay of central inhibitory systems is damaged, thus precluding effective pain management [3].

In this review, an important role of glutamate and its receptors in the neuronal degeneration of superficial dorsal horn neurons in the chronic-pain setting is discussed. Prevention of neuronal damage in pain-relaying structures, as a result of prolonged injury, is suggested to be critical in terms of pain relief and improving the efficiency of currently used therapies.

Spinal mechanisms of descending modulation

During the 1970s and 1980s, evidence accumulated for an endogenous antinociceptive system originating in the brainstem and targeting pain transmission within the dorsal horn. Significant progress has been made in the study of pain-modulating networks. However, little is understood regarding the spinal circuits through which descending pain modulatory neurons exert their effects. With recent insight into the pharmacology of different neural circuits, the importance of spinal mechanisms of descending modulation in the response of the nervous system to persistent pain after injury is being reevaluated.

Evidence that descending systems can selectively modulate pain was first provided by the discovery of stimulation-produced analgesia, which was first elicited by electrical stimulation of the midbrain periaqueductal gray (PAG) [4]. The PAG integrates inputs from the limbic forebrain and diencephalon with ascending nociceptive input from the dorsal horn [5]. The pain-modulating action of the PAG upon the spinal cord is relayed through the rostral ventromedial medulla (RVM). Thus, the PAG–RVM connection is critical for pain modulation.

The RVM is the major brainstem source of axons that project to the spinal cord dorsal horn. The spinal terminals of RVM descending axons are most dense in dorsal horn laminae I, II (the substantia gelatinosa), and V [6]. These laminae are targets of nociceptive primary afferents (Aδ- and C-fibers), and their consistent neurons respond maximally to stimuli that are noxious [7]. Lamina II is of particular interest, as the sensory input to this area is almost entirely C-fiber in nature. Most lamina II neurons are glutamatergic excitatory interneurons that relay inputs from primary afferents to lamina I projection neurons [8,9]. Other interneurons in laminae I and II contain inhibitory neurotransmitters gamma-amino butyric acid (GABA) [10], glycine, and enkephalin [10,11] and are a likely source of the inhibitory transmitters in terminals on projection neurons. Recent insights into synaptic transmission and plasticity in the superficial dorsal horn and its role in the develop-

Address correspondence to: A.B. Petrenko
Received: January 17, 2000 / Accepted: September 18, 2000

ment and maintenance of pain in response to various forms of tissue injury have been reviewed [12].

A number of studies indicate that brainstem activation directly inhibits lamina I projection neurons in the dorsal horn. In fact, electrical stimulation in the RVM evokes the release of norepinephrine (NE) [13] and serotonin (5-HT) [14] in the spinal cord cerebrospinal fluid (CSF), and produces a monosynaptic inhibitory postsynaptic potential in spinothalamic tract (STT) neurons [15]. The demonstration that STT neurons receive a direct serotoninergic [16] and catecholaminergic [17] input provides a substrate for direct postsynaptic control by brainstem neurons.

Furthermore, antinociceptive PAG and RVM stimulation activates inhibitory neurons in the superficial laminae of the dorsal horn [18,19]. Inhibitory amino acids that are released from dorsal horn interneurons contribute to antinociception. The inhibition of dorsal horn neurons produced by 5-HT agonists acting at the excitatory 5-HT3 receptor is blocked by a GABA receptor antagonist, suggesting that the 5-HT inhibition is mediated via a GABAergic inhibitory interneuron [20]. 5-HT and NE can facilitate inhibitory neurotransmission—as demonstrated in trigeminal lamina II neurons—via activation of GABA- and glycine-containing interneurons [21]. It should be noted that both GABAergic and glycinergic interneurons can be activated by primary afferents, as two distinct types of polysynaptic inhibitory postsynaptic potentials (IPSP) can be elicited by Aδ-fiber stimulation [22,23].

The vast majority of opioid terminals in the dorsal horn derive from local interneurons [24]. Enkephalin terminals and cells are present in superficial dorsal horn [11], as are dense concentrations of μ-opioid receptor [25]. Spinal application of opioids produces analgesia and opioid iontophoresis inhibits dorsal horn nociceptive neurons [26]. Intrathecal naloxone reduces the antinociceptive action of electrical stimulation of RVM [27]. Opioids suppress excitatory synaptic transmission—as shown in adult rat spinal cord lamina II neurons—possibly through the presynaptic activation of μ- and δ-opioid receptors [28]. As a result, the amplitude of Aδ-fiber-evoked excitatory postsynaptic currents (EPSCs) in lamina II neurons is diminished by μ- and δ-opioid agonists. Importantly, the activation of PAG neurons inhibits the response of sacral dorsal horn neurons to noxious heat, an effect that is mediated, at least in part, by a presynaptic inhibition of afferents carrying noxious thermal information to dorsal horn neurons [29]. This presynaptic inhibition is mediated by μ-opioid and α2-adrenergic receptors and possibly by other receptors as well [29,30].

It is well known that lamina II interneurons provide a major excitatory input from C fibers to lamina I projection cells. On the other hand, some lamina II interneurons make asymmetric, presumably excitatory, synapses onto neurons in deeper laminae of the dorsal horn. Opioids have also been shown to directly inhibit lamina II interneurons [31]. Furthermore, in vitro studies provided evidence that μ-opioid agonists hyperpolarize neurons in lamina II that are excited by dorsal root stimulation [32]. Thus, in addition to direct inhibition of the release of neurotransmitters from primary afferents, as described above, opioid ligands reduce excitatory drive onto marginal projection neurons and neurons in deeper laminae of the dorsal horn.

Consistent with a role in pain modulation for supraspinal inhibition of lamina II excitatory interneurons, a subset of nociceptive lamina II cells which is inhibited by RVM stimulation has been described [33]. The inhibition of spinal nociceptive transmission produced by stimulation of the RVM is due, in part, to the activation of RVM neurons that terminate in the spinal cord and release 5-HT [19]. 5-HT suppresses the response to N-methyl-D-aspartate (NMDA) in acutely isolated spinal dorsal horn neurons of the rat [34]. Excitatory synaptic transmission in the superficial dorsal horn is also inhibited by NE activation of postsynaptic α2-adrenergic receptors which are coupled to a K^+ conductance that directly hyperpolarizes the majority of lamina II neurons [35].

In summary, there is evidence that the spinal cord dorsal horn is a critical site of action for descending pain-modulatory neurons inhibiting nociceptive transmission by several mechanisms: direct inhibition of projection neurons, inhibition of transmitter release from primary afferents, excitation of inhibitory interneurons, and inhibition of excitatory interneurons.

Role of glutamate receptors in the mechanism of chronic pain

Glutamate, the major excitatory neurotransmitter in the central nervous system (CNS), has a crucial role in nociceptive transmission in the spinal cord dorsal horn [36]. It is released from the terminals of small nociceptive primary afferent fibers ending in the superficial layers and lamina V of the dorsal horn. In the rat, capsaicin-evoked release of glutamate from primary afferents is reduced by μ- and δ- but not κ-opioid agonists, suggesting that μ- and δ-opioid receptors modulate pain transmission in the spinal cord dorsal horn [37]. Consistent with this idea, opioids were found to suppress glutamatergic excitatory transmission in the substantia gelatinosa through the presynaptic activation of μ- and δ- but not κ-receptors [28].

Glutamate is also known to be neurotoxic to cells. Neurons exposed to high concentrations of glutamate degenerate and die. In the human brain and spinal

cord, neurons degenerate after acute insults (e.g., stroke, cardiac arrest, and trauma) and during progressive, adult-onset diseases (e.g., amyotrophic lateral sclerosis (ALS), Alzheimer's disease, and, possibly, chronic pain). Glutamate-receptor-mediated neurodegeneration—defined as excitotoxicity—has been implicated in all of these neurological conditions. Moreover, neuronal damage appears to be due to the activation of different subtypes of glutamate receptors that directly gate ion channels; namely, receptors for NMDA, α-amino-3-hydroxy-5-methyl isoxozole propionic acid (AMPA), and kainate—a structural analog of glutamate.

Role of N-methyl-D-aspartate (NMDA) receptors

The ionotropic NMDA receptor seems to be particularly involved in neuronal damage, since its channel is permeable to Ca^{2+} and an increase in the cytoplasmic concentration of this cation promotes a chain of events that lead to cell death. In cortical cell cultures, glutamate-receptor-induced $^{45}Ca^{2+}$ accumulation correlated with subsequent neuronal degeneration [38].

In an animal model of neuropathic pain [39], chronic constrictive nerve-injury-induced hyperalgesia was shown to be associated with an increase in glutamate content and subsequent activation of NMDA receptors, followed by an increase in intracellular Ca^{2+} ($[Ca^{2+}]i$) within the dorsal horn. Spinal NMDA receptors have also been shown to be critical for the induction of hyperalgesia by tail amputation in mice [40].

NMDA receptor activation plays an important role in both excitatory neurotransmission and synaptic plasticity in the CNS [41]. NMDA receptor function is highly regulated by various modulating sites located on the receptor, including the redox modulatory site that is suggested to act as a gain control of NMDA receptor activity. Sulfhydryl-reducing agents, such as dithiothreitol (DTT), potentiate NMDA-receptor-evoked currents in vitro, whereas oxidizing agents, such as 5,5-dithio-bis-(2-nitrobenzoic acid) (DTNB), attenuate these currents through the redox modulatory site [42]. Consistent with glutamate receptor antagonism, extracellular acidity reduced neuronal death in murine cortical cultures that were deprived of oxygen and glucose [43]. The same authors suggest that extracellular acidity has beneficial effects beyond the attenuation of ionotropic glutamate receptor activation. Nonetheless, NMDA receptors contribute to neuronal death under acidic conditions; thus, NMDA antagonists may retain therapeutic value at low pH conditions, such as in hypoxic injury.

Reducing agents have also been shown to potentiate the NMDA-receptor-induced increase in $[Ca^{2+}]i$ [44], neurotransmitter release [45], and neuronal cell death [46]. All these effects have been shown to be reversed by DTNB.

Studies in which single-channel measurements have been performed have demonstrated that DTT increases the frequency of NMDA-receptor-induced channel openings as well as the number of open channels [47]. This process of increasing the frequency of channel opening and the number of active channels is one potential mechanism by which spinal cord neurons could become hypersensitive to endogenous glutamate.

Modulating NMDA function with exogenously added glycine has been shown to alter nociceptive transmission in the spinal cord [48], suggesting that modification of the various modulating sites on the NMDA receptor can alter nociceptive transmission. A study examining the role of the NMDA receptor redox modulatory site in nociceptive spinal cord signaling in mice [49] showed that reduction of the NMDA receptor by endogenous reducing agents may contribute to augmented pain transmission in response to activation by endogenous glutamate. Moreover, blockade of in vivo NMDA receptor reduction or oxidation of the NMDA receptor redox site may prove therapeutically useful in the treatment of chronic pain.

The recent advances in our understanding of the pharmacological properties of NMDA receptor channels have been summarized by Yamakura and Shimoji [50]. The authors emphasize that the development of subunit- and site-specific drugs is necessary for effective therapeutic intervention in a variety of glutamate-mediated pathological states, including neuropathic pain.

Role of non-NMDA receptors

Ca^{2+}-permeable AMPA receptors may also be implicated in glutamate-mediated excitotoxicity [51]. An increase in AMPA receptors in the spinal dorsal horn has been reported after nerve injury [52]. Subpopulations of AMPA receptors that are either permeable or impermeable to Ca^{2+} are expressed on dorsal horn neurons in culture. While both types of receptors mediate synaptic transmission, the Ca^{2+}-permeable AMPA receptors provide a Ca^{2+} signal that may result in a transient change in synaptic strength [53].

Neuronal populations in laminae I–III of the dorsal horn have a characteristic pattern of expression of the AMPA receptor subunits: GluR1 is particularly associated with inhibitory neurons, and GluR2 with excitatory neurons [54]. This pattern makes it likely that some of the AMPA receptors present on the inhibitory interneurons lack the GluR2 subunit, and may therefore have significant Ca^{2+}-permeabilty—and susceptibility to excitotoxic action. In addition, AMPA receptors show selective expression in identified subpopulations of dorsal horn neurons. GABAergic dorsal horn neurons are

important in local inhibition as well as in the regulation of transmitter release from primary afferent terminals. Dorsal horn neurons expressing the substance P receptor, NK1, include many of the projection neurons in the nociceptive spinothalamic pathway. A majority of GABA and NK1 receptor-expressing neurons, representing important components of dorsal horn function, express Ca^{2+}-permeable AMPA receptors. Furthermore, several subpopulations of putative excitatory interneurons do not express these receptors [55]. Other investigators have also shown the localization of Ca^{2+}-permeable AMPA receptors on neurons in lamina I and outer lamina II, a region of the dorsal horn strongly innervated by nociceptive primary afferents [56].

Recent observations indicate that plasticity in AMPA receptor subunits in the dorsal horn may contribute to pain following deafferentation [57]. Three days after dorsal rhizotomy, immunostaining for GluR1 and GluR2/3 subunits of AMPA receptor significantly decreases in laminae I and II of the dorsal horn. Given the permeability of AMPA receptors lacking the GluR2 subunit to Ca^{2+}, down-regulation of GluR2 subunits in the superficial dorsal horn would mean that glutamate activation of these receptors would initiate or potentiate second messenger cascades that could well contribute to neuronal changes documented in the dorsal horn of deafferented animals.

Acromelic acid, a kainate derivative of natural origin, markedly increased $[Ca^{2+}]i$ in cultured rat spinal neurons in a concentration-dependent manner, and was more potent in increasing $[Ca^{2+}]i$ than any other glutamate receptor agonist tested. The rank order of the activity was as follows: acromelic acid > AMPA > NMDA > L-glutamate. The acromelic acid-induced $[Ca^{2+}]i$ increase was due to Ca^{2+} influx mediated by the activation of non-NMDA receptors [58].

In the superficial dorsal horn of the spinal cord, kainate receptors may be restricted to synapses formed by high-threshold nociceptive and thermoreceptive primary afferent fibers. Consistent with this possibility, kainate-receptor-mediated EPSCs are blocked by the analgesic μ-opioid-receptor agonist Damgo, and spinal blockade of both kainate and AMPA receptors produces antinociception [59].

Role of metabotropic glutamate receptors

Metabotropic glutamate receptors (mGluRs) also mediate the effects of glutamate. Evidence is now accumulating that individual mGluR subtypes mediate distinct, facilitatory (group I) or inhibitory (groups II and III) actions on neurodegenerative processes. A study using cortical cells in culture demonstrated that activation of group I mGluRs enhances NMDA-receptor-mediated neuronal toxicity [60].

The group I selective mGluR agonist, (RS)-3,5-dihydroxyphenylglycine ((RS)-3,5-DHPG), potentiated the responses of rat spinal neurons to ionotropic excitatory amino acid (EAA) agonists NMDA, AMPA, and kainate in vivo. This potentiation was considered to be secondary to an enhancement of cell excitability rather than due to a specific interaction with ionotropic EAA receptors [61].

Activation of group II metabotropic glutamate receptors is sufficient, per se, to protect neurons against excitotoxic degeneration [62]. This finding encourages the search for potent, selective, and systemically active mGluR2/3 agonists as neuroprotective drugs. Similarly, the activation of group III metabotropic glutamate receptors exerts neuroprotective activity against excitotoxic neuronal death, as shown in cortical cultures [63].

The difference in cellular effects among mGluR subtypes is probably related to their mechanism of action. Subtypes in group I, mGluR1 and mGluR5, are coupled to stimulation of phosphatidylinositol (PI) hydrolysis and the subsequent mobilization of Ca^{2+} from intracellular stores. Subtypes in groups II, mGluR2 and mGluR3, and III, mGluR4, mGluR6, and mGluR7, are linked to the inhibition of the cyclic adenosinomonophosphate (cAMP) cascade [64].

Group I mGluRs, in particular mGluR1, play a critical role in mediating nociception, particularly following sustained noxious input [65]—apparently resulting in neuronal degeneration in the superficial dorsal horn.

Metabotropic glutamate receptors are also differentially localized in neuronal elements of the dorsal horn [66]. Interestingly, immunoreactivity for mGluR1 was detected in laminae I–III of the dorsal horn, while mGluR2/3 immunoreactivity was detected primarily in lamina III. Another study of the distribution of mGluRs in laminae I and II of the spinal cord—a critical site of nociceptive processing in acute and chronic pain states—indicated that mGlu2/3 receptors were mainly in the inner part of lamina II, and immunostaining for mGluR5 was mainly in laminae I and II [67]. The same investigators also showed presynaptic and postsynaptic localization of mGluRs in both inhibitory and excitatory interneurons. Taken together with the fact that selective agonists of mGluRs may modulate GABA release [68], this demonstration suggests that facilitatory effects may involve a mechanism of disinhibition.

Drugs interacting with mGluRs are expected to influence both the induction and progression of neuronal degeneration, without hampering the efficiency of rapid excitatory synaptic transmission. Thus, mGluRs can be considered as promising drug targets in the experimental therapy of acute or chronic neurodegenerative diseases [69]—potentially including chronic pain.

In summary, the ability of glutamate to kill neurons seems to be mediated, in most cases, by an interaction with NMDA receptors, leading to an uncontrollable rise in $[Ca^{2+}]i$ concentrations and, thence, cell lysis and death. The establishment of glutamatergic loops seems to be a key process in the maintenance, spread, and amplification of neurodegenerative foci [70]. Glutamate may be involved in the regulation of neuronal cytoarchitecture. The amino acid was found to specifically affect the cytoarchitecture of hippocampal pyramidal neuron dendrites in a graded manner, suggesting that it may be involved in establishing hippocampal circuitry during brain development, maintaining and modifying circuitry in the adult, and inducing neurodegeneration in several disorders including epilepsy, Alzheimer's disease, and stroke [71]. Similarly, glutamate and its receptors may well play a role in dorsal horn neuroplasticity during chronic pain states.

Nerve injuries and pain

AMPA/kainate receptor activation may play a significant role in excitotoxic injury to spinal cord neurons. Compared with cortical neurons, spinal neurons are less vulnerable to NMDA and more vulnerable to AMPA and kainate. Neurons with Ca^{2+}-permeable AMPA/kainate channels are resistant to NMDA; these cells are significantly more prevalent in spinal cord cultures [72].

During high levels of neuronal discharge activity, such as occur during the induction of lasting changes in synaptic efficacy, there is a large influx of Na^+, leading to substantial increases in its intracellular concentration [73]. Neurons express a diversity of Na^+-permeable channels, e.g., ionotropic glutamate receptors (AMPA, kainate) and voltage-gated Na^+ channels, and it was found that Na^+ influx through these various channels may up-regulate neighboring NMDA receptors [74–76]. It appears that following peripheral nerve injury, non-NMDA glutamate receptors are up-regulated in the superficial laminae of the spinal cord [52]. Taken together, the up-regulation of NMDA receptors by sodium and increased expression of glutamate receptors permeable to this ion are likely to operate in concert to enhance glutamate excitotoxicity.

Results of in vivo studies in which specific agonists for non-NMDA receptors were infused into the adult rat spinal subarachnoid space indicate that these receptors are heterogeneous, mediating neuronal damage with different selectivity [77]. The authors also propose that chronic activation of glutamate receptors is capable of inducing slowly progressive neuronal death, which is relevant to the pathogenesis of ALS. Furthermore, intrathecal infusion of acromelic acid, a specific agonist of non-NMDA receptors, causes selective degeneration of inhibitory interneurons in the rat spinal cord. However, acromelic acid appears to exert its unique pharmacological actions by activating a subclass of non-NMDA receptors distinct from those activated by kainate and AMPA [78].

A study performed to investigate the role of apoptosis in epileptic brain damage induced by intra-amygdaloid injection of kainate showed that apoptotic cell death contributes to the local and distant damage induced by kainate [79].

NMDA- and non-NMDA-receptor-mediated excitotoxic injury results in neurodegeneration along an apoptosis–necrosis continuum, in which neuronal death (appearing as apoptotic, necrotic, or intermediate between the two extremes) is influenced by the degree of brain maturity and the subtype of glutamate receptor that is stimulated [80]. Other findings [81] support the concept that degenerative phenotypes of excitotoxically injured neurons are influenced by the degree of brain maturity and GluR subtype stimulation, independent of the severity of the excitotoxic insult, and run along a morphological continuum ranging from apoptosis to necrosis.

Nociceptor-driven excitotoxic insults (constriction nerve injury or surgical incision)—presumably involving NMDA receptor activation—have been shown to result in the formation of pyknotic and hyperchromatic neurons (dark neurons, DNs) in the superficial lamina of the dorsal horn [82]. It has been proposed that at least some DNs are inhibitory interneurons whose functional impairment or death contributes to a central state of hyperexcitability that underlies neuropathic hyperalgesia.

Animal studies have shown that peripheral nerve lesions can induce secondary changes in the dorsal horn of the spinal cord. Histological changes include degeneration of both primary afferent terminals (transganglionic degeneration) and second-order neurons (transsynaptic degeneration). Functional changes suggesting a sensitization of spinal neurons have been reported. Such a central sensitization might depend on a reduction of segmental inhibitory controls and/or intracellular changes induced by the activation of NMDA receptors by glutamate released by primary afferents [83].

Peripheral nerve injury causes abnormal sensory processing, possibly due to neuroplastic changes in the CNS. Following the constriction injury of the sciatic nerve, transsynaptic degeneration is suggested by the presence of DNs found in the superficial lamina of the dorsal horn [84].

In a rat model of chronic constriction injury of the sciatic nerve, the incidence of neurons with signs of transsynaptic degeneration was significantly increased in the lumbar dorsal horn on both sides, with the ipsilat-

eral increase being significantly greater than that on the contralateral side. The majority of the DNs were found in the sciatic nerve's territory in laminae I–II [85]. Interestingly, in a group of rats with unilateral sciatic nerve transection, no increase in the incidence of DNs was noted. The earlier results also suggest that a peripheral nerve injury that produces neuropathic pain induces morphological alterations of the intraspinal somatosensory circuitry [86].

Studies in vivo and in neuronal cell cultures investigating the effect of extracellular glutamate accumulation on excitotoxicity showed that, at least under the experimental conditions used, an increase of endogenous extracellular glutamate, induced by either enhancing its release or inhibiting its transport, is not sufficient to cause neuronal death [87,88].

NMDA glutamate receptor is known to be the key regulator of synaptic efficacy within the dorsal horn. At normal resting membrane potentials it contributes relatively little to the primary afferent evoked synaptic currents in dorsal horn neurons, which are mostly dependent on glutamate acting on AMPA receptors [89]. This is because, at resting membrane potentials, the NMDA receptor ion channel is blocked by Mg^{2+} ion, and therefore when glutamate binds to NMDA receptor at resting membrane potentials, no response is elicited because no current can be carried by the passage of Na^{+} or Ca^{2+} ions through the channel. Membrane depolarization relieves the voltage-sensitive Mg^{2+} block of NMDA channels.

Membrane depolarization produced by corticostriatal and/or thalamostriatal innervation may be required for maturation of glutamate receptors on striatal neurons, and such maturation may be important for expression of NMDA or non-NMDA receptor-mediated excitotoxicity by striatal neurons [90]. Chronic depolarization produces changes in glutamate receptors that may contribute to the potentiation of excitotoxicity. Similarly, sustained noxious stimulation (and resultant depolarization) may potentiate the excitotoxic effect of glutamate in the dorsal horn.

The above-mentioned data are in contrast with an earlier investigation that indicates that chronic depolarization may be neuroprotective by the induction of a fundamental alteration in intracellular Ca^{2+} handling [91]. When challenged with lethal concentrations of NMDA or kainate, spinal neurons cultured in high (25 mM) extracellular potassium exhibited markedly attenuated Ca^{2+} currents and $[Ca^{2+}]i$ responses, and survived more readily than controls. Surprisingly, NMDA and kainate currents remained comparable between neurons grown in the presence of high- and low-K^{+} concentrations.

Modulation of synaptic efficacy in the dorsal horn is of fundamental importance for its operation. Some synapses between primary afferent fibers and spinal dorsal horn neurons may be inefficient or silent. It has been shown that 5-HT, an important neurotransmitter of the raphe-spinal projecting pathway, transforms silent glutamatergic synapses into functional ones [92]. Recruitment of silent synapses by activation of postsynaptic 5-HT receptors could contribute to enhancement of synaptic transmission induced by 5-HT. Moreover, silent synapses may be involved in persistent pain. Recruitment of silent synapses on wide dynamic range cells could enhance their response to sensory stimuli, particularly nonnoxious stimuli. For nociceptive-specific cells, activation of silent glutamatergic synapses might even change their phenotype and cause them to respond to nonnoxious stimuli. These synaptic mechanisms could contribute to plastic changes in nociception after tissue or nerve injury [93].

In the case of inhibitory interneurons in the lamina II of the dorsal horn, recruitment of silent synapses could predispose them to glutamate excitotoxicity and cause at least some degree of functional impairment that would result in a decrease of the inhibitory processes that are normally triggered by primary afferent input. Electrophysiological evidence for such disinhibition has been obtained in animal models [94].

It has become apparent that NMDA receptor is located in both pre- and postsynaptic components of synapses in the spinal cord, and many small-diameter primary afferent fibers in the spinal cord dorsal horn express presynaptic NMDA autoreceptors that facilitate and prolong the transmission of nociceptive messages through the release of substance P and glutamate [95,96]. Activation of these presynaptic receptors enhances the postsynaptic effects exerted by glutamate in the production of pain [96]. Indeed, glutamate released from C-fibers following tissue or nerve injury would not only depolarize postsynaptic neurons, but could also act on presynaptic NMDA autoreceptors to maintain the depolarization of the presynaptic terminal, increasing intracellular Ca^{2+} and prolonging its own release and postsynaptic effects. This circuit may provide prolonged exposure to glutamate and result in disinhibitory processes associated with excitotoxic consequences, including neuronal death within the spinal cord dorsal horn.

As described above, there is histological evidence showing that peripheral nerve injury induces excitotoxic transsynaptic morphological changes of superficial dorsal horn (laminae I–II) neurons (dark neurons), which have been proposed to be inhibitory interneurons [85]. These morphological changes may reflect a pathological process in which injury-induced central responses result in a persistent imbalance of the excitatory–inhibitory circuitry within the spinal cord dorsal horn with the predominant loss of function of

inhibitory interneurons. Although it is reasonable to think that intracellular events initiated by excessive NMDA receptor activation may lead to excitotoxic changes in excitatory interneurons, it is unclear how this is related to control over pain transmission. The loss of function of spinal cord inhibitory interneurons seems to be more relevant to altered nociceptive processing in chronic pain. The rat neuropathic pain models support this possibility by the demonstration of the antiallodynic effects produced by GABA receptor agonists [97] and the demonstration that pharmacological blockade of the spinal cord GABA and glycine inhibitory system augments thermal hyperalgesia [98].

Thus, over the past several years, evidence has accumulated that peripheral nerve injury leads to neuronal death of spinal cord inhibitory interneurons and disihibitory processes, which are likely to contribute to the development of hyperalgesia [85,99–102]. Whether excitotoxic processes are involved in mechanisms of pain syndromes will depend on the nature of noxious input to the spinal cord. This input, in turn, may result from peripheral nerve injury or tissue inflammation. Although the phenomenon of disinhibition may contribute to hyperalgesia associated with nerve injury, this may not be the case for inflammatory pain. Indeed, an increase in number of GABA-immunoreactive cells in the superficial dorsal horn following peripheral inflammation was reported [103,104]. The up-regulation of the dorsal horn GABAergic system by an inflammatory process suggests the involvement of regulatory mechanisms distinct from those subserving neuropathic pain.

Summary

There is an extensive body of evidence demonstrating the development of an excitotoxic insult in certain neurons following peripheral nerve injury. The neuronal damage is detected anatomically by the appearance of transsynaptic degenerative changes in small- to medium-sized spinal cells named "dark neurons" in the dorsal horn laminae I–II. It is unclear whether dark neurons die or they completely, or partially, recover. If dark neurons that appear secondary to exaggerated discharge in injured primary afferent neurons include inhibitory interneurons, one would expect to see a decrease in the inhibitory processes that are normally triggered by primary afferent input. A rapidly growing body of evidence from experiments in animals with neuropathic pain supports these predictions.

In the case of sustained injury, CNS structures responsible for nociception–antinociception are gradually involved in the pathological processes. At first, spinal inhibitory neurons are injured by an excitotoxic mechanism triggered by nociceptive barrages. The excitotoxic damage contributes to a state of spinal hyperexcitability due to disinhibition. An abnormality in spinal cord neurons generates an abnormality in the responsiveness of thalamic neurons, which, in turn, may generate dysfunction in the cerebral cortex. The lines of evidence suggest that with time, these high-level abnormalities may become independent of the lower-level abnormalities that generated them [105].

It is suggested that the pattern of expression and distribution of glutamate receptors in the superficial dorsal horn neurons renders them particularly vulnerable to glutamate excitotoxicity and leads to substantial neuroplastic and degenerative changes following the onset of sustained injury. Considering the pivotal role of superficial laminae of the dorsal horn in mediating antinociception, the importance of preventing or minimizing changes in spinal inhibitory neurons cannot be underestimated. Therapeutic intervention aimed specifically at glutamate receptors of inhibitory neurons may prove beneficial in neuropathic pain control and prevention of its progression. Further studies are needed to characterize glutamate receptor properties of inhibitory interneurons of the spinal cord dorsal horn.

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