

Review

The glycinergic control of spinal pain processing

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Abstract. Alterations in synaptic transmission within the spinal cord dorsal horn play a key role in the development of pathological pain. While N-methyl-D-aspartate (NMDA) receptors and activity-dependent synaptic plasticity have been the focus of research for many years, recent evidence attributes very specific functions to inhibitory glycinergic and γ -aminobutyric acid (GABA)-ergic neurotransmission in the generation of inflammatory and neuropathic pain. The central component of inflammatory pain originates from a disinhibition of dorsal

horn neurons, which are relieved from glycinergic neurotransmission by the inflammatory mediator prostaglandin E_2 (PGE₂). PGE₂ activates prostaglandin E receptors of the EP2 subtype and leads to a protein kinase A-dependent phosphorylation and inhibition of glycine receptors containing the $\alpha 3$ subunit (GlyR $\alpha 3$). This GlyR $\alpha 3$ is distinctly expressed in the superficial dorsal horn, where nociceptive afferents terminate. Other but probably very similar disinhibitory mechanisms may well contribute to abnormal pain occurring after peripheral nerve injury.

Key words. Pain; glycine; prostaglandin; spinal cord; dorsal horn; synapse; protein kinase A; synaptic inhibition.

Introduction

Our body senses potentially tissue-damaging (or noxious) stimuli by so-called nociceptors [1]. These are unmyelinated or thinly myelinated primary afferent nerve fibers which connect the peripheral tissues with the central nervous system (CNS) (fig. 1). Whenever a potentially tissue-damaging stimulus such as noxious heat or mechanical or chemical stress hits a peripheral nociceptor ending, a generator potential is elicited which travels along the peripheral nerve to the spinal cord dorsal horn. Here, nociceptors make synaptic contacts with local excitatory and inhibitory interneurons and central projection neurons, which convey nociceptive information to higher CNS areas. The proper functioning of this nociceptive system is essential to protect our body from tissue damage. However, in inflammatory or neuropathic diseases nociception can become sensitized, and the physiological sensation of pain can then turn into a disease.

The spinal cord dorsal horn is the first site of synaptic integration in the pain pathway. At this site, nociceptive afferents coming from the periphery make synaptic connections with spinal projection neurons, which are mainly located in lamina I and lamina V of the spinal cord. While projection neurons located in lamina I receive primarily nociceptive input, those in lamina V integrate input both from nociceptors and from low-threshold mechanoreceptors ('touch receptors'). Plenty of information indicates that the activation of these projection neurons not only depends on primary afferent input but is also under the control of a local network of excitatory and inhibitory interneurons and of descending pain-modulating tracts, which tune the nociceptive system to states of increased or decreased pain sensitivity. The neuronal network of the dorsal horn hence serves as a 'gate' controlling propagation of nociceptive signals to higher brain areas, where pain becomes conscious. Glutamatergic, in particular N-methyl-D-aspartate (NMDA) receptor-mediated, synaptic transmission from primary nociceptive afferents to

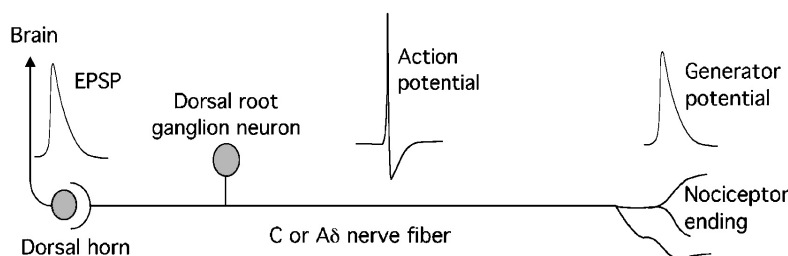


Figure 1. Schematic representation of nociceptive transmission from the peripheral nociceptor ending to the spinal cord dorsal horn. Painful stimuli elicit generator potentials in the fine peripheral endings of C and A δ nerve fibers. If these generator potentials become suprathreshold, action potentials are evoked which travel along the peripheral nerve and enter the spinal cord dorsal horn via the dorsal roots. In the superficial layers of the dorsal horn, nociceptive afferents make synaptic contact with central projection neurons. Glutamate released from these primary afferent nociceptors evokes excitatory postsynaptic potentials (EPSPs), which in turn can activate the projection neurons, which then transmit the nociceptive signals to higher brain areas where pain becomes conscious.

spinal projection neurons has long been the focus of pain research, while inhibitory synaptic transmission within this network has in the past drawn much less attention. The present review will focus on recent discoveries revealing very specific roles for glycine and GABA receptors in the development of spinal pain sensitization.

Inhibitory synaptic transmission in the spinal cord dorsal horn

Inhibitory neurons in the spinal cord dorsal horn use GABA and glycine as their principle fast neurotransmitters. Both transmitters open ligand-gated ion channels, which permit the permeation of chloride and to a lesser extent also of bicarbonate ions through the plasma membrane. In most neurons both transmitters inhibit neuronal activation by hyperpolarizing the cell membrane and by activation of a shunting conductance, which impairs propagation of excitatory postsynaptic potentials along the dendrite of neurons. The majority of inhibitory neurons in the spinal cord contain both glycine and GABA in their presynaptic terminals [2–4]. Accordingly, at least early in development, inhibitory postsynaptic signals are composed of glycinergic and GABAergic components [5]. During maturation, however, mixed GABAergic and glycinergic postsynaptic events become less frequent [6]. In the adult dorsal horn, the contribution of glycine to fast inhibitory postsynaptic transmission dominates, at least in the superficial layers, whereas GABA activates in addition extrasynaptic GABA_A receptors [7]. Because glycine and GABA are still colocalized in the terminals of mature inhibitory neurons, these results suggest a postsynaptic mechanism of specialization, implying that during maturation glycine receptors become or remain clustered at subsynaptic sites, whereas GABA_A receptors are located more diffusely.

Inhibitory (strychnine-sensitive) glycine receptors belong to the large family of nicotinic acetylcholine-like receptors. Glycine receptors are pentameric ion channels com-

posed of α and β subunits. Four genes, called *glra1–4*, are known which encode the ligand binding α subunits GlyR α 1– α 4. One gene encodes for the so-called structural β subunit, which permits postsynaptic clustering of glycine receptors through an interaction with the postsynaptic density protein gephyrine. GlyR α 3 is another adult glycine receptor subunit which is much less abundant than GlyR α 1. GlyR α 2 is believed to be an embryonic and juvenile subunit, and GlyR α 4 is probably not expressed in humans because of a premature stop codon in the *GLRA4* gene. For detailed descriptions of the molecular and structural biology of glycine receptors readers are referred to recent reviews by Lynch [8] and Legendre [9].

Immunofluorescence studies have shown that markers of glycinergic innervation are abundant in the spinal cord dorsal horn. The Mab4a antibody, which recognizes glycine receptor α and β subunits [10], shows intense staining throughout the dorsal horn. Glycinergic boutons characterized by the expression of the neuronal glycine transporter isoform GlyT2 and glycinergic cell bodies characterized by their immunoreactivity against glycine are widely distributed in the dorsal horn. Recently, mice expressing enhanced green fluorescent protein (EGFP) under the control of the GlyT2 promoter have been generated, which allow detailed morphological and functional analysis of glycinergic neurons [11]. In the dorsal horn of these mice numerous glycinergic (EGFP/GlyT2-positive) neurons are found in the deeper dorsal horn (laminae III–V) and in lamina I, whereas relatively few glycinergic neurons are seen in lamina II (fig. 2). Comparison of the localization of GlyT2-EGFP positive cell bodies and GlyT2-immunoreactive axon terminals suggests that the axons of glycinergic neurons extend into lamina II.

Ionotropic GABA_A receptors and markers of GABAergic boutons, characterized by the presence of the GABA-synthesizing enzymes glutamate decarboxylase (GAD) 65 or GAD 67 [12], are found throughout the spinal cord. Interestingly, Bohlhalter et al. [13] could demonstrate a laminar specialization among the benzodiazepine-sensi-

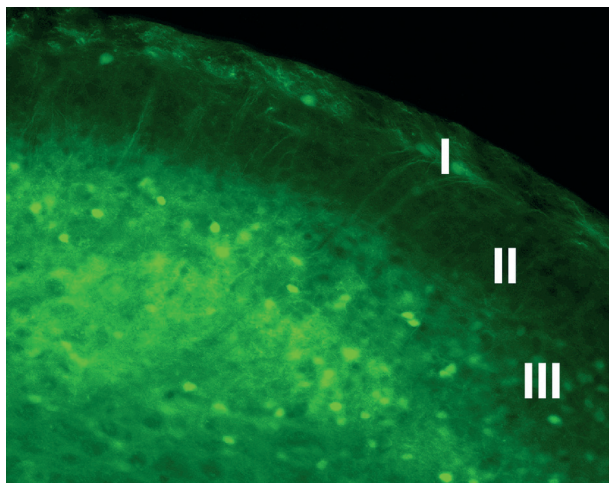


Figure 2. Localization of glycinergic neurons in the superficial layers (I, II and III) of the spinal cord dorsal horn visualized in a slice obtained from a mouse expressing EGFP under the transcriptional control of the neuronal glycine transporter GlyT2 gene. In these mice glycinergic neurons, including their fine axonal and dendritic processes, can easily be identified by green fluorescence.

tive GABA_A receptor subunits, with $\alpha 2$ and $\alpha 3$ being most prominent in lamina II of the dorsal horn, possibly suggesting a specific function of these subunits in nociceptive processing. The distribution of GABA and glycine has been analyzed in detail in lamina III of the dorsal horn [14]. Of 52 neurons studied, 30 were GABAergic. Most of these (25/30) were also positive for glycine and received synaptic input from low-threshold mechanoreceptors, suggesting that these neurons are particularly important for the proper processing of tactile input. It is generally assumed that inhibition of glycinergic transmission at these synapses would lead to a painful sensation of tactile stimuli, a phenomenon called allodynia which frequently occurs in patients suffering from neuropathic pain. Similar synaptic connections probably exist in the superficial dorsal horn, as Narikawa et al. [15] have found that stimulation of rat skin with brushing and pinching elicited a barrage of glycinergic IPSCs at this site.

Substantial information meanwhile indicates that relief from GABAergic and glycinergic inhibition by blockers of GABA_A and glycine receptors in the dorsal horn can elicit and exaggerate nociceptive responses [16–20]. It was therefore intriguing to speculate that pain sensitization in inflammation and neuropathy originates from a decrease in the endogenous inhibitory control of pain by glycine and/or GABA. Indeed several reports have demonstrated that both inflammatory pain and pain originating from peripheral nerve injury are due at least in part to disinhibition of spinal nociception. The underlying mechanisms, however, turned out to be rather different.

Relief from inhibition as a common source of inflammatory and neuropathic pain

Inflammatory pain

It is well established that inflammatory pain originates to a large extent from prostanoids, which are produced in response to inflammation and tissue damage both in peripheral inflamed or injured tissue and in the spinal cord. Phospholipase A2 activation releases arachidonic acid from the lipids of cell membranes, which is then transformed into the prostaglandin precursors PGG₂ and PGH₂ by constitutively expressed cyclooxygenase-1 (COX-1) or inducible cyclooxygenase-2 (COX-2) [21]. Tissue-specific isomerases or prostaglandin synthases further convert these precursors into the different biologically active prostaglandins and thromboxane, which primarily act on G-protein-coupled rhodopsin-like receptors [22]. The pivotal role of prostaglandins in pain sensitization is obvious from our everyday experience that profound analgesia can be achieved through inhibition of prostanoid formation either by the classical non-specific cyclooxygenase inhibitors (aspirin and related drugs) or by the more recently developed COX-2-specific inhibitors.

For a long time, it has generally been accepted that during inflammatory diseases prostaglandins sensitize nociception primarily by increasing the responsiveness of peripheral nociceptors in inflamed tissue. Conversely, it was believed that cyclooxygenase inhibitors exert their analgesic action primarily through inhibition of peripheral prostaglandin formation. Pain researchers have indeed identified molecular targets for prostaglandins in the periphery. Most important among these is probably a facilitating action of PGE₂ on the TRPV1 channels [23], formerly called capsaicin receptors [24]. These non-specific ion channels integrate multiple nociceptive stimuli including noxious heat and tissue acidosis [25]. Work by Lopshire and Nicol [26] demonstrated that PGE₂ can increase the current flowing through these channels severalfold. Furthermore, PGE₂ facilitates the activation of so-called tetrodotoxin-resistant Na⁺ channels [27, 28], which are specifically expressed in nociceptive afferent nerve fibers [29]. Both actions probably act in concert to increase the excitability of peripheral nociceptors and to facilitate the propagation of nociceptive signals along the peripheral nerve.

Spinal mechanisms of inflammatory pain

The view outlined above of an exclusively peripheral pain-sensitizing action of prostaglandins has changed significantly during the last 10 years. In the mid-1990s several groups demonstrated that inflammation induces COX-2 expression not only in peripheral inflamed tissue but also in the CNS, in particular in the spinal cord dor-

sal horn [30, 31]. Many behavioral studies have shown that intrathecal injections of prostaglandins induce hyperalgesia and allodynia in mice and rats. However, the mechanism of prostaglandin-induced spinal hyperalgesia has remained elusive. In order to understand how prostaglandins sensitize spinal nociception, it was necessary to identify the mechanisms by which prostaglandins increase the excitability of spinal nociceptive neurons. It has long been speculated that such an increase in excitability originated from a facilitating effect of prostaglandins, in particular of PGE₂, on glutamatergic transmission between primary afferent nerve fibers and central projection neurons. Such facilitation could be accomplished either by an increased amount of glutamate released from the primary afferent nerve endings or by an increased responsiveness of postsynaptically located excitatory glutamate receptors of the α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and NMDA type [32]. Experimental evidence for direct action of prostaglandins on glutamatergic neurons or terminals has remained anecdotal [33], and most studies found no evidence for such direct action [34, 35].

PGE₂-mediated inhibition of glycinergic neurotransmission underlies central inflammatory hyperalgesia

When we undertook a systematic approach to study the effect of PGE₂ on synaptic transmission in the superficial layers of the spinal cord dorsal horn, we found that PGE₂ selectively interfered with inhibitory (strychnine-sensitive) glycinergic neurotransmission (fig. 3 and [35]). We further found that this inhibitory effect of PGE₂ was largely restricted to the superficial layers of the dorsal horn, where mainly nociceptive afferents terminate. Other prostaglandins were without effect. It turned out that inhibition of glycinergic neurotransmission by PGE₂ in the dorsal horn was due to a postsynaptic mechanism involving prostaglandin E receptors of the EP2 subtype and protein kinase A (PKA). The involvement of PKA in this pathway fits nicely to earlier observations by Malmberg et al. [36], who found that thermal hyperalgesia induced by intrathecal injection of PGE₂ was strongly diminished in mice lacking neuronal PKA.

In order to determine the relevance of glycine receptor inhibition for inflammatory hyperalgesia *in vivo*, it was necessary to identify the glycine receptor subunit inhibited by EP2 receptor activation. Heterologous expression experiments indicated that the inhibitory action of PGE₂ is restricted to glycine receptors containing the GlyR α 3 subunit and occurs through PKA-dependent phosphorylation of serine 346 in the long intracellular loop between transmembrane regions 3 and 4 [37]. The lack of inhibition of GlyR α 1 homomers and GlyR α 1/ β heteromeric

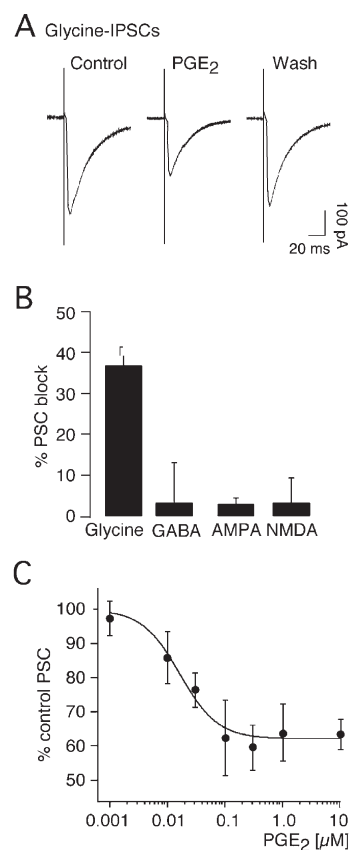


Figure 3. (A) Glycinergic inhibitory postsynaptic currents (IPSCs) were evoked by extracellular electrical stimulation and recorded from rat substantia gelatinosa neurons in the whole-cell configuration of the patch-clamp technique. Traces are averages of 10 consecutive stimulations recorded under control conditions, in the presence of 10 μ M PGE₂ and after its removal. (B) Significant inhibition was obtained for glycinergic IPSCs, but not for GABAergic IPSCs or AMPA or NMDA receptor-mediated excitatory postsynaptic currents (EPSCs). (C) Inhibition of glycinergic IPSCs occurred with an IC₅₀ (inhibitory concentration 50%) of 16 nM. Modified from [35].

channels corresponds well with a report by Findlay et al. [38], who found no nociceptive phenotype in mice carrying a loss of function mutation in the GlyR α 1 subunit. Figure 4A shows a schematic representation of the pathway leading to PGE₂-mediated inhibition of glycinergic neurotransmission in the superficial layers of the spinal cord dorsal horn. Interestingly, GlyR α 3 in the spinal cord is distinctly expressed in the superficial layers (fig. 4B), exactly where the nociceptive afferents terminate and where inhibition of glycinergic neurotransmission by PGE₂ has been observed [35]. Surprisingly, mice deficient in GlyR α 3 exhibited 'normal' baseline sensitivities to both thermal and mechanical stimulation and largely unchanged glycinergic transmission in the superficial layers of the dorsal horn, indicating that the lack of GlyR α 3 was probably compensated by another glycine receptor subunit. Nevertheless, GlyR α 3^{-/-} mice differed

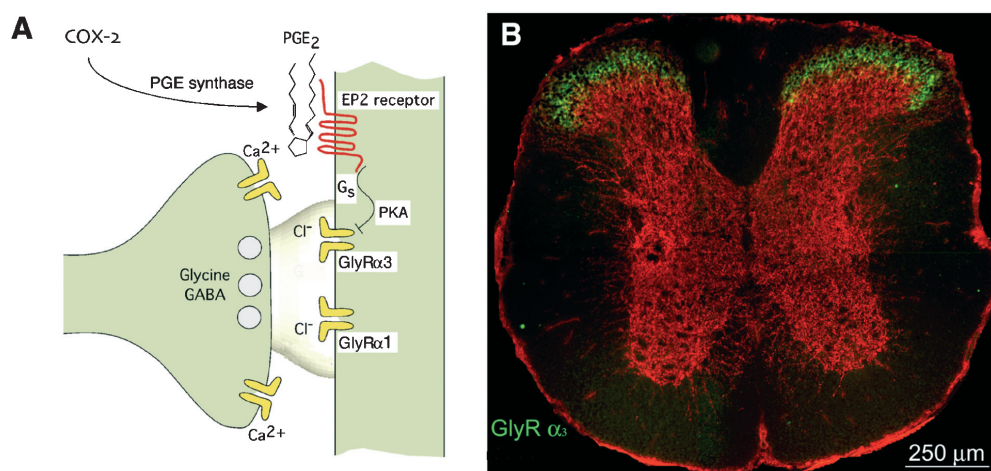


Figure 4. (A) Schematic illustration of the pathway leading to PGE₂-mediated inhibition of glycinergic neurotransmission in the superficial layers of the spinal cord dorsal horn. Peripheral inflammation induces the expression of COX-2 and microsomal prostaglandin E synthase in the spinal cord. PGE₂ produced by these two enzymes activates prostaglandin receptors of the EP2 subtype, which couple to a stimulatory G-protein (G_s) and increase intracellular cAMP. Subsequently activated protein kinase A (PKA) causes phosphorylation and inhibition of glycine receptors containing the α3 subunit. (B) Transverse spinal cord slice stained with an antiserum against GlyRα3 (yellow) and gephyrine (red). Modified from [37].

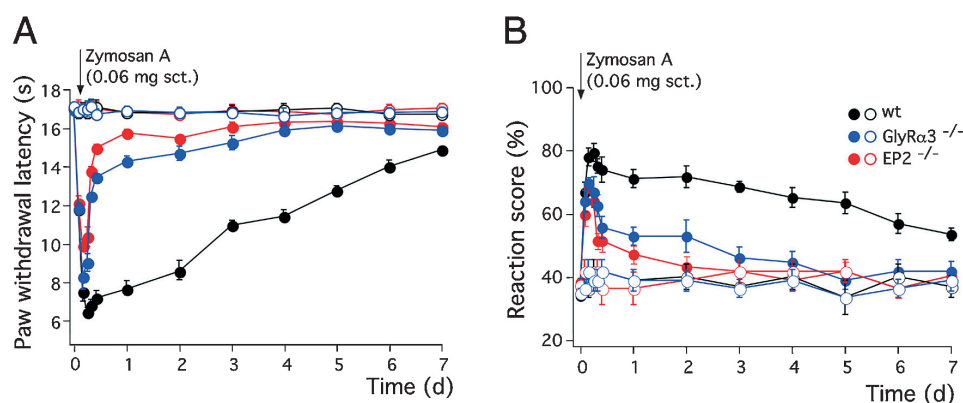


Figure 5. Thermal and mechanical hyperalgesia in EP2- and GlyRα3-deficient mice. A small amount of the yeast extract zymosan A was injected subcutaneously into the plantar side of the left hind paw of wild-type mice and of mice lacking either the EP2 receptor or GlyRα3. Thermal hyperalgesia was assessed as a reduction in paw withdrawal latency in response to stimulation with a defined radiant heat stimulus, and mechanical hyperalgesia was determined by scoring the reaction of the mice upon stimulation with calibrated von-Frey-filaments (for details see [40, 52]). Wild-type mice and the two strains of knockout mice initially developed similar thermal (A) and mechanical (B) hyperalgesia. While hyperalgesia lasted for more than a week in wild-type mice, both types of knockout mice recovered quickly from inflammatory pain sensitization within about 24 h. Subsequent experiments [40] have shown that the remaining early hyperalgesia was most likely peripheral in origin. Filled and open circles represent zymosan A-injected (inflamed) and non-injected (control) paws, respectively.

in one important respect from their wild-type littermates. Inhibition by PGE₂ of glycinergic neurotransmission was completely absent in GlyRα3^{-/-} mice. These mice therefore allowed us to assess how important the inhibition of glycinergic neurotransmission by PGE₂ was for the spinal pain-sensitizing action of PGE₂ and for inflammatory pain sensitization in general. It turned out that pain sensitization by intrathecal PGE₂ was nearly completely abolished in these mice. In addition, we found that GlyRα3^{-/-} and EP2^{-/-} mice displayed nearly identical phenotypes in the zymosan A model [39] of peripheral inflammation ([37,

40] and fig. 5). In wild-type mice subcutaneous injection of the yeast extract zymosan A induced inflammation and subsequent thermal and mechanical hyperalgesia lasting for about a week. Both types of knockout mice initially developed thermal and mechanical sensitization similar to wild-type mice, but recovered much more quickly from hyperalgesia. These findings indicate that inhibition of glycinergic neurotransmission by PGE₂ is a key event in the generation of inflammatory pain. A small but reproducible difference was observed in nociceptive sensitization between EP2^{-/-} and GlyRα3^{-/-} mice, suggesting

the presence of EP2 receptor-dependent but GlyR α 3-independent mechanisms. Such processes might include the direct activation of deep dorsal horn neurons by PGE₂, as described by Baba et al. [41].

A somewhat unexpected finding was that inhibition of glycinergic neurotransmission in the superficial dorsal horn contributed both to mechanical and thermal hyperalgesia. Because of the localization of glycinergic neurons between axons from low-threshold mechanoreceptive afferents and central projection neurons [14], the contribution of glycinergic transmission to the processing of mechanical input is widely accepted. Less well established is the role of glycine in the processing of input originating from noxious thermal stimuli.

An obvious question that arises at this point is the origin of the glycinergic input and the conditions of its activation. The available data suggest at least three possible sources (fig. 6). First, *in vivo* recording experiments have shown that glycinergic and GABAergic input to superficial dorsal horn neurons is directly activated by input from mechanoreceptors, suggesting a local inhibitory circuit in the dorsal horn [15]. Second, local GABAergic and glycinergic interneurons are probably also activated

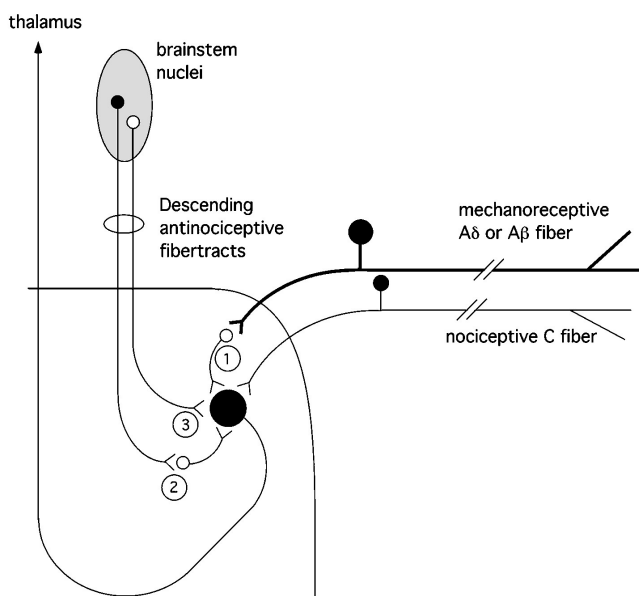


Figure 6. Schematic illustration of three possible sources of inhibitory glycinergic innervation of the spinal cord dorsal horn. Inhibitory glycinergic and excitatory neurons are represented by open and filled circles, respectively. First, spinal glycinergic interneurons can be directly activated by input from mechanosensitive primary afferent nerve fibers. Second, descending antinociceptive fibers can activate local glycinergic interneurons. Third, glycinergic input to the dorsal horn can originate directly from descending glycinergic neurons originating from the ventral rostromedial medulla. The available evidence suggests that the glycinergic control of noxious thermal input most likely comes from descending fibers, while that controlling mechanosensation may be activated directly by primary mechanosensitive primary afferent fibers.

by descending antinociceptive pathways, which mediate heterosegmental antinociception during intense nociceptive activation [42, 43]. Third, inhibitory input to dorsal horn neurons also originates from GABAergic and glycinergic neurons descending from the rostral ventromedial medulla and contacting nociceptive spinothalamic projection neurons [11, 44]. The latter two pathways suggest a critical role of GABAergic and glycinergic neurons not only in local segmental but also in supraspinal control of spinal nociceptive processing.

As outlined above, *in vivo* patch-clamp recordings have clearly demonstrated that mechanical stimulation of the skin evokes polysynaptic inhibitory transmission onto superficial dorsal horn neurons [15]. Since similar activation of glycinergic input has not been observed in response to thermal stimulation, either activation of glycinergic interneurons by thermal stimulation may require stronger excitatory input, e.g. from fibers sensitized by inflammation, or, alternatively, glycinergic input might derive from descending glycinergic fiber tracts originating e.g. from the rostral ventromedial medulla [44] or from spinal glycinergic interneurons activated by descending antinociceptive pathways [43].

Finally, it should be mentioned that the inhibitory action of PGE₂ on glycinergic neurotransmission is mainly restricted to excitatory interneurons and projection neurons [40]. It is thus tempting to speculate that reduction in the inhibitory tone might promote the induction of long-term potentiation (LTP)-like phenomena in dorsal horn projection neurons through depolarization and subsequent relief of NMDA receptors from voltage-dependent Mg²⁺ block. Relief from inhibition during inflammation may thus also promote the development of chronic pain.

Neuropathic pain

A role for cyclooxygenase products has also been proposed in neuropathic pain originating from peripheral nerve injury [45, 46]. However, a significant contribution of COX-2 to neuropathic pain appears rather unlikely (e.g. [47]). Other mechanisms that induce similar relief from inhibition may underlie neuropathic pain. Possible actions include inhibition of glycine release from the terminals of glycinergic interneurons, reduction in the transmembrane chloride gradient rendering the inhibitory tone of GABAergic and glycinergic synaptic input less efficient, and loss of inhibitory innervation due to selective death of GABAergic or glycinergic interneurons.

There is indeed experimental evidence for all three possibilities, but their contribution to pain sensitization is less clear. The neuropeptide nocistatin [48] specifically inhibits GABA and glycine release in the dorsal part of the spinal cord but has no effect on glutamate release [49].

After intrathecal injection (i.e. into the spinal canal) nocistatin increases nociceptive responses in the rat formalin test [49] and in the chronic constriction injury of neuropathic pain [50]. Although nocistatin has been found in the cerebrospinal fluid (CSF) of humans and animals [51], a contribution of endogenous nocistatin to thermal hyperalgesia evoked by inflammatory stimuli (zymosan A) or to nociceptive responses in the formalin test could not be detected [52]. Another example of an endogenous inhibitor of glycine and GABA release is adenosine [53, 54].

Reduction in the transmembrane chloride gradient in dorsal horn neurons following peripheral nerve injury was recently reported [55]. Peripheral nerve trauma induces a transsynaptic reduction in the expression of the potassium chloride exporter KCC2 in dorsal horn neurons and thereby shifts the chloride equilibrium potential to more depolarized values. Such a shift might even cause glycinergic or GABAergic input to become excitatory. Another extensively discussed report suggests that peripheral nerve injury induces specific loss of spinal inhibitory GABAergic neurotransmission in the dorsal horn of rats in the chronic constriction injury model and the spared nerve injury model of neuropathic pain [56]. This original report suggests that the loss of GABAergic input was due to the selective apoptotic death of GABAergic interneurons. Subsequent studies have, however, shown that such loss is at least not necessary for the development of thermal hyperalgesia in the chronic nerve injury model of neuropathic pain [57, 58].

Given that relief from the inhibitory actions of glycine and GABA underlies inflammatory and neuropathic pain, one might speculate that facilitation of inhibitory transmission might contribute to endogenous antinociception and pain control. Several transmitters, including ATP [59, 60] and norepinephrine [61, 62], indeed facilitate glycine release through the activation of P2X receptors and $\alpha 2$ adrenoceptors in the spinal cord, respectively. Glycinergic membrane currents in rat sacral commissural neurons (lamina X) are potentiated by norepinephrine acting on $\alpha 2$ adrenoceptors [63]. This potentiation is mediated by a decrease in cyclic AMP (cAMP), inhibition of PKA and prevented by pretreatment with pertussis toxin, clearly indicating that the potentiation was due to a reversal of PKA-mediated inhibition. Although GlyR $\alpha 3$ is not expressed in lamina X (at least not in mice), these features are very reminiscent of the inhibition of $\alpha 3$ -containing glycine receptors by PGE₂, discussed above.

Therapeutic implications

From a therapeutic point of view the question arises whether glycine or GABA_A receptors might be suitable targets for the development of novel analgesics. Given the

specific expression of GlyR $\alpha 3$ in the ‘pain-processing’ superficial layers of the spinal cord and the absence of a disabling phenotype in mice lacking these receptors, GlyR $\alpha 3$ should be an attractive target. Bearing in mind that reduction in the responsiveness of these receptors to glycine underlies inflammatory pain, a selective agonist at GlyR $\alpha 3$ or, even better, a positive allosteric modulator would be desirable. Unfortunately, so far no specific agonists for GlyR $\alpha 3$ have been identified. Only a few agents are known to potentiate GlyR-mediated currents, and most of them lack receptor specificity [64,65].

Unlike glycine receptors, GABA_A receptors are extensively used as therapeutic targets. The classical benzodiazepines, which enhance GABA responses at benzodiazepine-sensitive GABA_A receptors, cause sedation, anxiolysis, central muscle relaxation and are anticonvulsive. Antinociceptive actions of benzodiazepines have been repeatedly described in animal models of pain, in particular after spinal injection, and a few studies have reported analgesic effects of agonists at GABA_A receptors in human patients [66, 67]. However, classical benzodiazepines are not routinely used as analgesics. Underlying reasons may include the accompanying sedation, which probably occurs at lower doses than that needed for analgesia, and possible pronociceptive effects of benzodiazepines at supraspinal sites [68], which might counteract spinal antinociception. The generation of ‘knock-in’ mice carrying point mutations at benzodiazepine binding sites in the different GABA_A receptor subunits has proven that the different actions of classical benzodiazepines can be assigned to different GABA_A receptor subunits [69]. This has already led to the discovery of non-sedative anxiolytic ‘benzodiazepines’ [70, 71]. Future research will determine whether drugs targeting glycine or GABA_A receptors can be used to prevent or reverse the loss of neuronal inhibition occurring in inflammatory and neuropathic pain.

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