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Spinal dis-inhibition in inflammatory pain

Hanns Ulrich Zeilhofer[a,b,*], Ulrike B. Zeilhofer[c]

[a] Institute of Pharmacology and Toxicology, University of Zurich, Winterthurerstrasse 190, CH-8057 Zurich, Switzerland
[b] Institute of Pharmaceutical Sciences, ETH Zurich, Switzerland
[c] Children's Hospital, University of Zurich, Switzerland

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ABSTRACT

Inflammatory diseases and neuropathic insults trigger signaling cascades, which frequently lead to intense and long-lasting pain syndromes in affected patients. Such pain syndromes are characterized not only by an increased sensitivity to painful stimuli (hyperalgesia), but also by a qualitative change in the sensory perception of other, tactile stimuli (allodynia) and the occurrence of spontaneous pain in the absence of any sensory input. Long-term potentiation (LTP)-like changes in synaptic transmission between nociceptive C-fibers and spino-periaqueductal grey projection neurons as well as a loss of inhibitory control by GABAergic and glycinergic spinal dorsal horn neurons have repeatedly been proposed as underlying principles. While considerable evidence supports a significant contribution of C-fiber LTP to hyperalgesia, such monosynaptic plasticity cannot explain the occurrence of allodynia and spontaneous pain. In this review, we focus on mechanisms of synaptic dis-inhibition in inflammatory pain and propose that pathologically heightened pain sensitivity can be reversed by restoring synaptic inhibition with drugs that target specific spinal $GABA_A$ receptor subtypes.

More than 40 years ago, the gate control theory of pain [29] has attributed a critical role to inhibitory neurotransmission in the substantia gelatinosa of the spinal dorsal horn. Although several details of this theory could not be experimentally verified [14,52], the basic principles withstood the challenge by experimental pain research. Not only has it been shown that pharmacological blockage of GABAergic and/or glycinergic neurotransmission in the dorsal horn mimic many symptoms of inflammatory and neuropathic pain [39,40], but more importantly, it could be demonstrated that a loss of synaptic inhibition occurs naturally in the course of inflammatory [16,33,35] and neuropathic diseases [8,9]. In the original model, the activity of inhibitory substantia gelatinosa neurons was controlled by electrical input from low threshold mechano-sensitive fibers and from high threshold nociceptors, which would increase or reduce inhibition, respectively. Today, we know that chemical mediators released in response to inflammation or neuropathy are the dominant factors mediating the loss of synaptic inhibition in pathological pain states. Two such endogenous mediators, prostaglandin E2 (PGE_2) and brain-derived neurotrophic factor (BDNF), were identified recently that reduce synaptic inhibition through distinct and well-defined pathways when produced in response to inflammation [1,16] or nerve injury [8].

Although a great variety mediators diminish inhibitory neurotransmission in the spinal dorsal horn (for a review see [47]), PGE_2 plays a pivotal role in inflammation-induced dis-inhibition [46]. For a long time, it has been thought that PGE_2 sensitizes the nociceptive system only through a local action on primary nociceptors. Several lines of evidence have changed this view. It was demonstrated that intrathecal injection of prostaglandins, mainly of PGE_2, causes a sensitization to heat and mechanical stimulation (for a review see [44]). Conversely, it was found that inhibitors of postaglandin production induce analgesia not only after systemic administration but also after local injection into the spinal canal [28]. Finally, it became apparent that cyclooxygenase-2 (COX-2) [4,36] and microsomal prostaglandin E synthase 1 (mPGES1) [15], two key enzymes of inflammation-induced PGE_2 synthesis, become induced in the spinal cord after peripheral inflammation.

Without a detailed knowledge of the signaling pathways activated by spinal PGE_2 it proved however difficult to determine their contribution to inflammatory pain. In contrast to what was generally believed at that time, we [1] and others [3] found no evidence for a direct facilitating effect of PGE_2 on excitatory synaptic transmission between primary afferent nerve fibers and intrinsic spinal dorsal horn neurons. What we found instead was a selective block of inhibitory (strychnine-sensitive) glycine receptors in the superficial dorsal horn [1]. We could subsequently show that this inhibition occurred through a reduced responsiveness of postsynaptically

* Corresponding author at: Institute of Pharmacology and Toxicology, University of Zurich, Winterthurerstrasse 190, CH-8057 Zurich, Switzerland.
Tel.: +41 44 6355912; fax: +41 44 6355988.
E-mail address: zeilhofer@pharma.uzh.ch (H.U. Zeilhofer).

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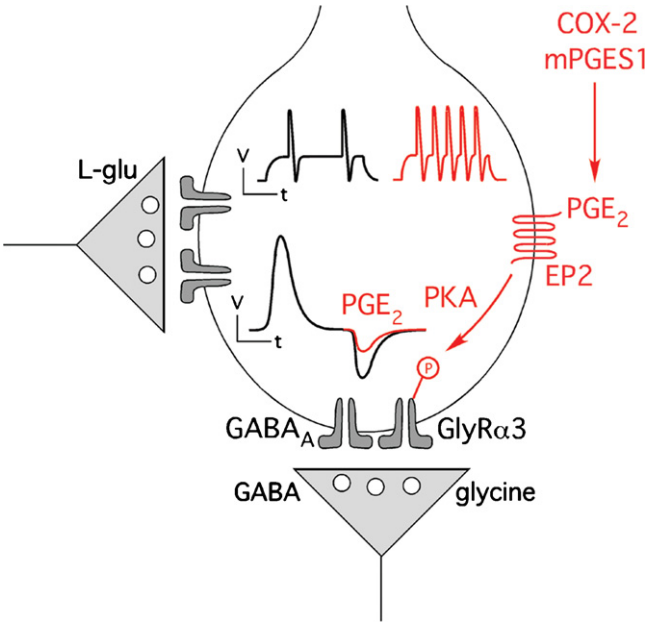

Fig. 1. Dis-inhibition in inflammatory pain states. Cyclooxygenase-2 (COX-2) and microsomal prostaglandin E synthase (mPGES1) become induced in the spinal cord in response to inflammation in peripheral tissues and produce PGE_2. PGE_2 binds to PGE_2 receptors of the EP2 subtype, which increase cAMP levels and activate protein kinase A (PKA). PKA then phosphorylates and inhibits a specific subtype of glycine receptors containing the α3 subunit, which normally control the excitability of superficial dorsal horn neurons. This dis-inhibition facilitates the firing of these neurons and promotes transmission of nociceptive signals through the spinal cord to higher brain areas where pain becomes conscious.

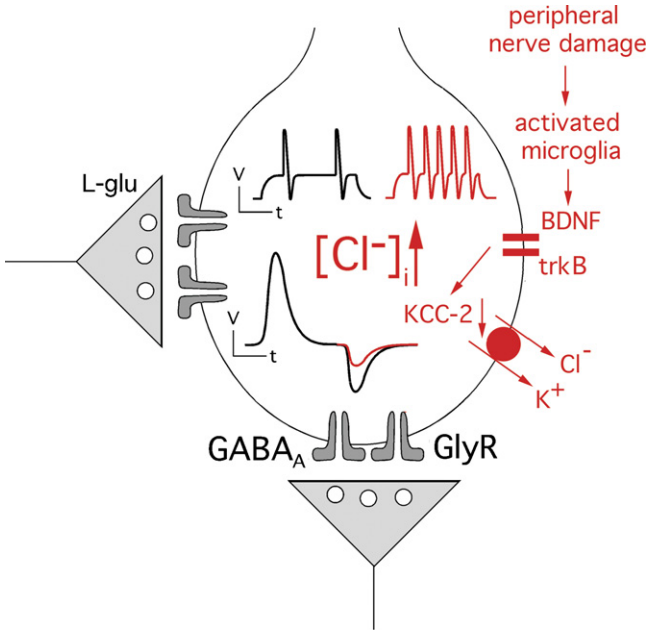

Fig. 2. Dis-inhibition in neuropathic pain states. Spinal microglia activated in response to peripheral nerve damage, releases brain-derived neurotrophic factor (BDNF). BDNF subsequently down-regulates the potassium-chloride cotransporter (KCC-2), which normally keeps intracellular chloride concentration ($[Cl^-]_i$) low. This down-regulation reduces the inhibitory action of $GABA_A$ and glycine receptors. In some neurons GABAergic and glycinergic inhibition may become even depolarizing and excitatory. This dis-inhibition promotes the firing of dorsal horn neurons and the transmission of nociceptive signals [8,22].

located glycine receptors, which was mediated by PGE_2 receptors of the EP2 subtype [1,35]. EP2 receptor activation increased intracellular cAMP and activated protein kinase A (PKA), which in turn phosphorylated and blocked a distinct glycine receptor isoform containing the α3 subunit [16]. This subunit is in the spinal cord exclusively expressed in the superficial dorsal horn, where primary nociceptive afferents terminate and where PGE_2 inhibits glycinergic neurotransmission (Fig. 1).

The identification of two key signaling proteins (GlyRα3 and EP2 receptors) in this pathway and the availability of respective gene-deficient mice ($EP2^{-/-}$ mice and $GlyR\alpha3^{-/-}$ mice) allowed us determining the contribution of this pathway to different forms of pain. Both types of knock-out mice did not differ from wild-type mice in their baseline sensitivity to thermal or mechanical stimuli. In the case of the $EP2^{-/-}$ mice this is not surprising, however the lack of a baseline phenotype in the $GlyR\alpha3^{-/-}$ mice requires an explanation. Most likely was the deletion of the GlyRα3 subunit compensated by increased expression of the GlyRα1 subunit, which lacks the strong PKA consensus site present in the GlyRα3 subunit. Such a compensatory up-regulation of GlyR α1 also explains our observation that baseline glycinergic neurotransmission was not altered in $GlyR\alpha3^{-/-}$ mice [16].

$EP2^{-/-}$ and $GlyR\alpha3^{-/-}$ mice were then tested in models of inflammatory and neuropathic pain. In most of these tests, $EP2^{-/-}$ and $GlyR\alpha3^{-/-}$ mice showed nearly identical phenotypes. When injected intrathecally with PGE_2, both types of mice were almost completely protected against mechanical and thermal hyperalgesia. When inflammatory pain was investigated following subcutaneous injection of zymosan A, both types of mice initially developed thermal and mechanical sensitization, but recovered quickly (within 24 h) from this sensitization, while in wild-type mice sensitization lasted for more than 7 days [48]. These results not only imply that an EP2 receptor-mediated suppression of glycinergic inhibition (i.e. a dis-inhibition of spinal nociception) is the most important mechanism of spinal pain sensitization by PGE_2 but also that spinal mechanisms of inflammatory pain sensitization dominate over peripheral ones during prolonged disease states.

A dominant contribution of spinal prostaglandins to inflammatory pain sensitization is also supported by a recent study from Clifford Woolf's group, who employed the cre-loxP system to generate mice lacking COX-2 specifically in neurons and glial cells of the central nervous system. These mice developed significantly less mechanical sensitization after peripheral inflammation-induced by subcutaneous injection of complete Freund's adjuvant, while thermal sensitization was less affected [45]. Indeed, several lines of evidence suggest that sensitization to mechanical stimuli is mainly, if not exclusively of central origin, while peripheral and central mechanisms act probably in concert to induce COX-dependent heat hyperalgesia [46,49]. The relative contributions of peripheral versus central mechanisms may very well depend on the nature and the intensity of the peripheral inflammatory stimulus.

Do prostaglandins also contribute to dis-inhibition after peripheral nerve damage? It is well established that the pathology of peripheral nerve damage depends on complex signaling cascades involving proinflammatory cytokines such as tumor necrosis factor α (TNFα), which might well subsequently activate prostanoid synthesis in the injured nerve or in the spinal cord (e.g. [26]). At present, a possible contribution of prostanoids to neuropathic pain is controversial. Several reports have suggested that prostaglandins might be involved in neuropathic pain [17,51]. On the other hand, COX inhibitors exhibit, if at all, only modest activity against neuropathic pain [11,38]. Consistent with this lack of efficacy, neuropathic pain develops similarly in wild-type mice and in $EP2^{-/-}$ and $GlyR\alpha3^{-/-}$ mice [18] and increases in spinal COX-2 expression [6] and PGE_2 formation [38] remain insignificant after nerve damage.

Although, PGE_2-mediated inhibition of dorsal horn glycine receptors does apparently not contribute to neuropathic pain following peripheral nerve injury, synaptic dis-inhibition is still a

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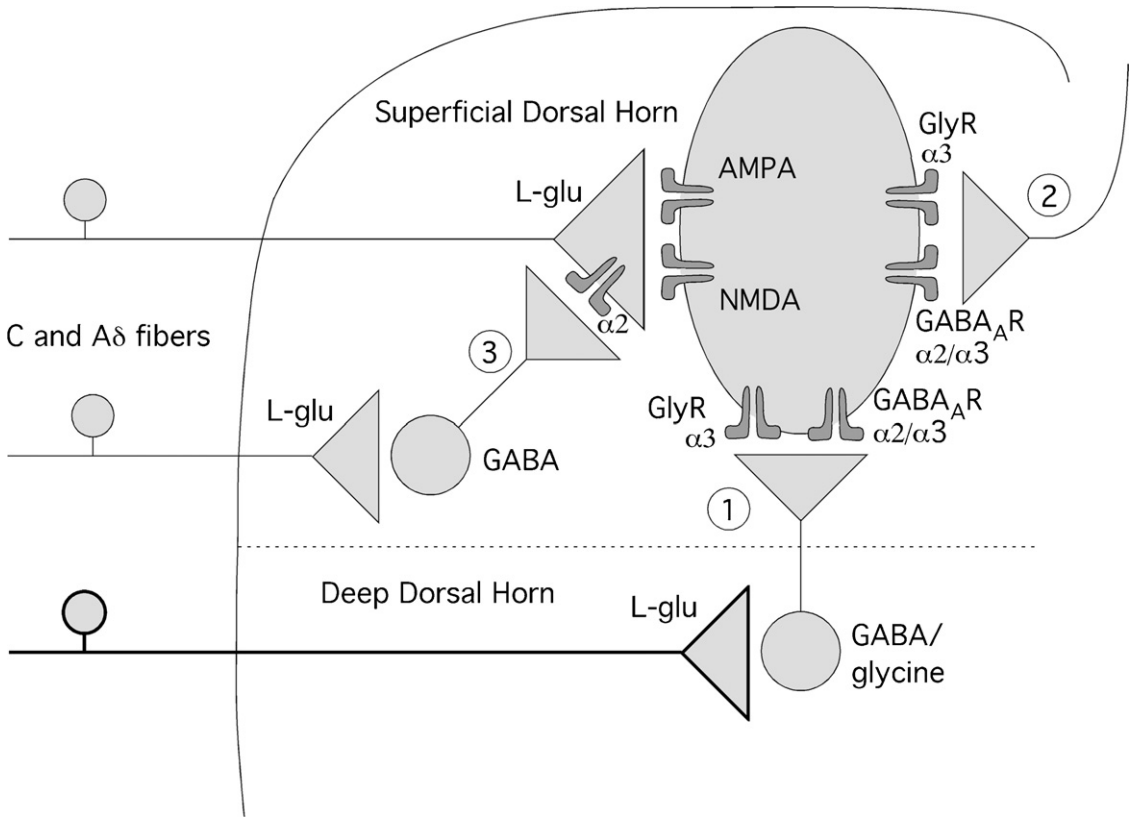

Fig. 3. Inhibitory synapses in the spinal dorsal horn circuitry. At least three populations of neurons contribute to the synaptic inhibition in the spinal dorsal horn. (1) GABAergic and glycinergic neurons, which are mainly located in the deeper dorsal horn, are activated by mechano-sensitive Aβ or Aδ fibers. Many of these neurons release both GABA and glycine simultaneously, but fast synaptic inhibition appears to be mainly mediated by activation glycine receptors [23]. The dominant glycine receptor isoform at these synapses contains the α3 subunit, while $GABA_A$ receptors at this site probably mainly contain α2 and/or α3 subunits in addition to a β and a γ2 subunit. (2) GABAergic and glycinergic inhibition also comes from inhibitory fiber tracts descending from the rostral ventromedial medulla. (3) Inhibitory interneurons located in the superficial dorsal horn probably form mainly axo-axonic synapses with the spinal terminals primary afferent nerve fibers, which express mainly the α2 subunit.

major factor contributing to neuropathic pain—albeit through different mechanisms. In neuropathic pain, a microglia-triggered cascade results in a shift in the neuronal chloride gradient caused by down-regulation of the potassium-chloride co-exporter KCC-2 rendering GABAergic and glycinergic input less inhibitory [8,9,22] (Fig. 2). Possible changes in KCC-2 expression have also been studied under inflammatory conditions in arthritic rats. Yet, under these inflammatory conditions an up-regulation was found [32]. Available data hence suggest that a loss of synaptic inhibition occurs both in inflammatory and neuropathic diseases but through very distinct mechanisms.

A pertinent question at this point is the organization of the inhibitory circuitry in the spinal dorsal horn. Although we are still far from a comprehensive model of the dorsal horn circuitry, significant new insights have come during recent years from electrophysiological experiments performed in slices and *in vivo* as well as from morphological studies in wild-type and transgenic animals, summarized in Fig. 3. *In vivo* patch clamp studies from Megumu Yoshimura's group have shown that inhibitory postsynaptic potentials can be elicited in superficial dorsal horn neurons by light (low threshold) mechanical stimulation of the skin, but not by noxious heat stimulation [34]. This observation fits nicely with earlier morphological studies, which have shown that glycinergic neurons in the deep dorsal horn are postsynaptic to low threshold mechano-sensitive primary afferent nerve fibers [7], which terminate in the deep dorsal horn. In this region, glycinergic neurons are densely packed as seen in transgenic mice expressing enhanced green fluorescent protein (EGFP) under the transcriptional control of the neuronal glycine transporter (GlyT2) gene [50], i.e. in glycinergic neurons, and in *in situ* hybridization experiments [19]. Many of these glycinergic neurons apparently project to the superficial dorsal horn and normally prevent the excitation of nociceptive specific neurons by innocuous stimuli. The behavioral correlate of dis-inhibition at this site is a painful sensation elicited by light mechanical stimuli, called allodynia [22,31]. Reduced synaptic strength at these inhibitory synapses most likely also accounts for mechanical hypersensitivity observed after strong C-fiber stimulation in a skin area surrounding the stimulation site (secondary hyperalgesia) [30]. On the other hand, Aβ-fiber driven activation of glycinergic synapses probably contributes to the beneficial effects on pain of transcutaneous electrical stimulation (TENS) [27].

At this point two questions remain. First, diminished glycinergic inhibition not only leads to mechanical sensitization but also to thermal hyperalgesia. Hence, inhibitory glycinergic tone should not exclusively be dependent on input from mechano-sensitive fibers. In fact, glycinergic fiber tracts have been described which descend from the rostral ventromedial medulla to the superficial spinal dorsal horn [2,21], where they probably inhibit nociceptive transmission tonically.

Another open question is the role of inhibitory interneurons located in the *superficial* dorsal horn, mainly in lamina II. These neurons are mainly GABAergic and apparently receive synaptic input from C-fibers as shown in recordings made from EGFP-tagged GABAergic neurons [10], yet, as shown in the experiments by Yoshimura's group described above, they do not contribute significantly to classical postsynaptic inhibition [21]. Interestingly, a very recent study by Santos et al. [37] found that the vast majority of

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synaptic connections between pairs of substantia gelatinosa neurons is excitatory despite the fact that 30% of these neurons should be inhibitory [41]. An appealing, yet so far unproven hypothesis is that these neurons mainly form axo-axonic synapses with primary afferent nerve terminals in the superficial dorsal horn. For two reasons these axo-axonic synapses are probably not subject to the dis-inhibitory processes described above. First, PGE_2-mediated blockade of inhibitory synapses is specific for glycine receptors, which are not expressed by primary afferent neurons [43]. Second, KCC-2 is expressed in DRG neurons only at very low levels and as a consequence and in contrast to central neurons, the intracellular chloride concentration is high in most DRG neurons already under normal conditions [13]. Whether or not this axo-axonic inhibition is modulated in neuropathic or inflammatory pain through other mechanisms is not known at present.

As outlined above, both inflammatory and neuropathic pain pathologies converge at a loss of inhibitory pain control, which probably accounts for the most debilitating symptoms of chronic pain including spontaneous pain and pain evoked by even slightest cutaneous stimulation. This should have tremendous implications for the treatment of chronic pain. Instead of interfering with signal transduction pathways specific to either inflammatory pain or neuropathic pain, restoring synaptic inhibition in the spinal dorsal horn should be an effective means against a variety of pain syndromes. Given the distinct expression of GlyRα3 in the superficial dorsal horn [16], this subunit should be an almost ideal target for such pharmacological interventions. Yet, so far no compounds are available which would act as specific positive allosteric modulators of inhibitory glycine receptors [25]. Another more realistic approach might be targeting dorsal horn $GABA_A$ receptors [20]. Indeed pharmacological facilitation of $GABA_A$ receptor activation with spinally applied benzodiazepines produces significant analgesia in animal models of inflammatory and neuropathic pain [12] and in human patients [42]. Yet, systemic analgesic treatment with classical benzodiazepines is precluded by their sedative action and their liability to tolerance development and addiction, side effects, which mainly originate from supraspinal sites. In a recent study employing genetically modified mice and subtype-selective benzodiazepine site ligands, we now could show that specifically targeting spinal $GABA_A$ receptor subtypes provides significant analgesia (or strictly speaking anti-hyperalgesia) against inflammatory and neuropathic pain without causing sedation, motor impairment or tolerance [24]. Drugs suitable for this approach should be agonists at benzodiazepine binding sites of $GABA_A$ receptors containing the α2 or α3 subunit, which are the dominating $GABA_A$ receptor α subunits in the spinal dorsal horn [5]. In addition, they should spare the α1 subunit, which is responsible for the sedative action of benzodiazepines. Such compounds are already in development as so called anxio-selective or non-sedative benzodiazepines. It will be extremely interesting to test, whether they are also active against chronic pain in human patients.

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