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Loss of glycinergic and GABAergic inhibition in chronic pain—contributions of inflammation and microglia

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Abstract

Tissue trauma, inflammation and neuropathy can under unfortunate condition progress into chronic pain syndromes. It is meanwhile generally accepted that chronic pain, i.e. pain, which persists beyond the resolution of tissue traumata and inflammation, is due to plastic changes in the neuronal processing of sensory stimuli in the CNS. A loss of synaptic inhibition (i.e. disinhibition) in the spinal cord dorsal horn has been increasingly recognized as an important process in the development and maintenance of chronic pain of both inflammatory and neuropathic origin. Although inflammation and neuropathy involve distinct mechanisms of synaptic disinhibition, the production of inflammatory mediators and/or the activation of immune cells, two events that have once been thought to be normally excluded from the CNS, appear to be critical for both conditions.

In the intact organism, pain serves an extremely important function as it forces the body to withdraw from physical or chemical stimuli that threaten its integrity. It is hence not surprising that a nociceptive system has evolved in all higher organisms and that children born with genetic defects in the nociceptive system suffer from repeated injuries and experience significantly reduced life spans. Despite this important warning function most of us associate pain with discomfort rather than with protection. This is particularly true for patients who experience severe traumata or suffer from chronic painful disorders such as rheumatoid arthritis. During acute inflammation, this form of pain may, however, still serve an important physiological function as it can promote heeling by making us look after inflamed joints or injured limbs. Under unfortunate conditions, however, pain can persist beyond the resolution of inflammation or the healing of injury. It can then turn into a disease of its own right.

It is meanwhile generally accepted that such chronic pain states are caused by plastic changes in the processing of nociceptive signals mainly in the central nervous system. Such chronic pain manifests itself through different phenomena: hyperalgesia means that already painful stimuli are sensed as more painful, while allodynia means that stimuli normally not sensed as painful (such as light touch) can evoke pain. Both terms reflect not only different phenomena but

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also involve different pathomechanisms. Hyperalgesia occurs when either peripheral nociceptive nerve fibers become more sensitive to noxious stimuli or when the signals arriving from nociceptive fibers in the CNS are processed differently presumably leading to increased excitation of the so called pain matrix in the CNS. Like hyperalgesia, allodynia can occur for both thermal (heat and cold) and mechanical stimuli. The best-studied form of alloadynia is probably mechanical allodynia or touch-evoked pain, which is mainly, if not exclusively, of central origin: light mechanical stimuli are sensed by low threshold mechanoreceptive Aβ fibers, but mechanical allodynia is not accompanied by an increase excitability of Aβ fibers. Central changes in the processing of mechanically evoked input must therefore account for mechanical allodynia (see also below). This form of pain sensitization is particular important, as allodynia and spontaneous pain are the most typical and disabling features of chronic pain in patients.

Neuroplastic changes occur probably in many CNS areas during the development of chronic pain syndromes, but the spinal cord dorsal horn appears to be of particular relevance. At this site primary nociceptive afferent nerve fibers

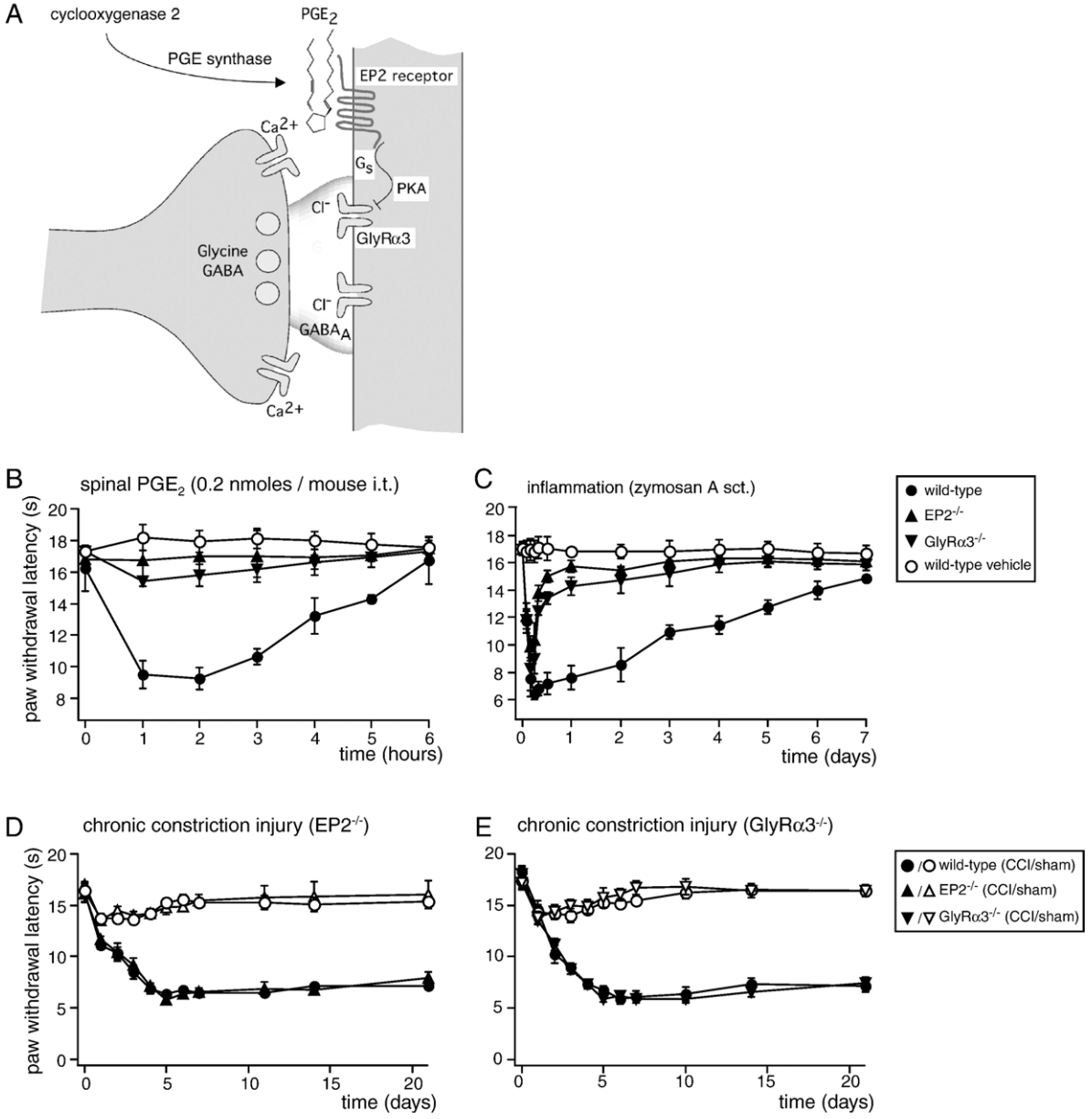

Figure 1 The central component of inflammatory pain sensitization is mainly caused by PGE_2-mediated reduction in glycinergic neurotransmission. (A) Signal transduction pathway in the superficial dorsal horn activated by cyclooxygenase-2 derived PGE_2. (B) Mice deficient in EP2 receptors ($EP2^{-/-}$ mice) or in the GlyRα3 subunit ($GlyR\alpha3^{-/-}$ mice) are largely protected from the pronociceptive effects of intrathecally injected PGE_2. (C) Central inflammatory hyperalgesia is dramatically reduced in $EP2^{-/-}$ mice and in $GlyR\alpha3^{-/-}$ mice. (D, E) Neuropathic pain sensitization develops normally in $EP2^{-/-}$ and $GlyR\alpha3^{-/-}$ mice as compared to wild type mice. Modified from [17–20].

terminate and make synaptic connections with projection neurons and local excitatory and inhibitory interneurons. This CNS area hence represents the first site of temporal and spatial synaptic integration in the pain pathway. For many years, pain researchers have largely concentrated on changes in the excitatory synaptic transmission between primary nociceptive afferent nerve fibers and spinal projection neurons, which transmit nociceptive signals to higher brain areas where pain becomes conscious. This focus is not at all surprising. Long lasting changes in synaptic strength, namely long term potentiation (LTP), at excitatory hippocampal synapses have extensively been studied as cellular correlates for learning and memory. At this site intensive stimulation of excitatory synapses induces a long lasting increase in postsynaptic excitation. Such LTP is also found in the superficial layers of the spinal cord dorsal horn between nociceptive C-fibers and spinal projection neurons [1,2]. Although difficult to prove experimentally, clinical evidence supports that it contributes to the development of hyperalgesia and possibly also of chronic pain in patients.

Alternative explanations of the development of chronic pain focus on the role of inhibitory interneurons and on their synaptic connections. More than 40 years ago, Ronald Melzack and Patrick Wall have proposed a model, in which inhibitory GABAergic and glycinergic interneurons in the superficial spinal dorsal horn, the substantia gelatinosa, play a pivotal role in the control of nociceptive transmission from the periphery through the dorsal horn to higher brain areas [3]. According to their theory, which has become famous as the so-called gate control theory of pain, inhibitory interneurons in the superficial spinal dorsal horn are activated by low-threshold touch-sensitive Aβ fibers. These inhibitory interneurons make in turn synaptic connections with second order neurons in the deep as well as in the superficial dorsal horn. According to this theory innocuous input, e.g. from touch-sensitive fibers is felt as non-painful only as long as synaptic inhibition is intact. Under normal conditions, excitatory (touch-evoked) input and input from inhibitory interneurons would cancel out. However, when inhibition is lost, even light touch could activate these neurons, which under normal condition only become activated by input from nociceptors. Interestingly, synaptic inhibition in the dorsal horn is not only mediated by local (segmental) interneurons, but also by GABAergic and glycinergic neurons descending from the rostral ventromedial medulla as demonstrated by morphological studies and *in vivo* electrophysiological recordings [4,5].

Although the gate-control theory has been criticized intensively in the past, their fundamental conclusions have been corroborated very nicely during the recent years. Not only has it been shown that inhibitory synaptic currents can be recorded in the dorsal horn in *in vivo* recordings in intact rats upon light touch [6], but it has also been shown that both peripheral inflammation and nerve injury induce a loss of synaptic inhibition in the superficial dorsal horn. Both actions involve completely different mechanisms but finally converge at similar synaptic effects.

Inflammatory pain involves both peripheral and central mechanisms. In the periphery, it is well established that PGE_2 facilitates the activation of ion channels involved in nociception, namely that of the capsaicin receptors TRPV1 [7,8] and of tetrodotoxin-resistant Na^+ channels [9,10]. This peripheral action is generally accepted and its suppression was believed to fully explain the analgesic (or antihyperalgesic) action of cyclooxygenase inhibitors, including aspirin. Later, it has been increasingly recognized that peripheral inflammation also induces changes in the central in particular spinal processing of sensory input. Like in the periphery, PGE_2 has again been recognized as a key messenger. When injected into the spinal canal of naive mice PGE_2 but not other prostaglandins induces a sensitization to thermal and mechanical stimuli. Enzymes necessary for the spinal production of PGE_2, cyclooxygenase-2 [11,12] and a specific prostaglandin E synthase isoform (mPTGES1) [13] become expressed in the spinal cord within a few hours after induction of peripheral inflammation. Electrophysiological work in spinal cord slices has identified several possible mechanisms for the central pro-nociceptive effects of PGE_2. These findings include an increase in the synaptic release of the excitatory neurotransmitter L-glutamate from the presynaptic terminals of primary nociceptive afferent nerve fibers [14], a direct depolarization of deep dorsal horn neurons [15] and a blockade of inhibitory glycine receptors in the superficial dorsal horn [16–18]. While contribution of the first two effects to pain sensitization *in vivo* has been difficult to assess, the role of PGE_2-mediated inhibition of glycine receptors in the development of inflammatory pain has been proven experimentally (Fig. 1). This inhibition involves the activation of postsynaptically located EP2 receptors, which subsequently activate a stimulatory G-protein and trigger increases in cAMP. cAMP-dependent proteinkinase (PKA) then phosphorylates and inhibits a glycine receptor isoform which contains the α3 subunit (GlyRα3) and, which is in the spinal cord specifically expressed in the superficial dorsal horn where the nociceptive afferents terminate. Inhibition of glycine receptors could be reconstituted in HEK293 cells transfected with EP2 receptors and with the glycine receptor α3 subunit. Furthermore, inhibition of glycinergic neurotransmission by

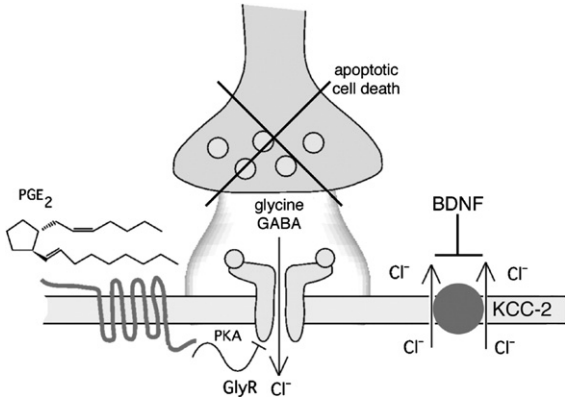

Figure 2 Different mechanisms contribute to synaptic disinhibition after peripheral inflammation or following peripheral nerve damage. PGE_2-mediated inhibition of glycinergic inhibition [16–18], apoptotic cell death of GABAergic interneurons [21,22] and BDNF-mediated down-regulation of KCC-2 [26,28] converge on a diminished synaptic inhibition in the spinal dorsal horn.

PGE_2 was absent in mice lacking either the EP2 receptor or the glycine receptor $\alpha 3$ subunit. The use of these genetically modified mice allowed us to examine the contribution of EP2-mediated inhibition of glycinergic neurotransmission to pain sensitization *in vivo*. In a first series of experiments, we found that PGE_2 injected intrathecally into the lumbar spinal canal almost completely failed to induce thermal or mechanical hyperalgesia in EP2 and GlyR$\alpha 3$ deficient mice, indicating that inhibition of GlyR$\alpha 3$ was the dominant mechanism of the spinal pain sensitizing action of PGE_2 (Fig. 1B). In a second series of experiments we addressed two questions: what was the contribution of PGE_2 to spinal inflammatory pain sensitization compared to other spinal mediators (e.g. substance P, calcitonin gene related peptide [CGRP], etc.) and, secondly, what was the contribution of spinal versus peripheral mechanisms of pain sensitization. The latter question could be addressed in these mice, because GlyR$\alpha 3$ is only expressed in the central nervous system and hence peripheral pain sensitization should be completely normal in these mice. In these experiments we induced a peripheral inflammation by injecting zymosan A into one hindpaw. In wild type mice this induces an inflammation and an accompanying pain sensitization, which last for about a week. Initially (within the first few hours) EP2 and GlyR$\alpha 3$ knock-out mice developed similar thermal and mechanical hyperalgesia as the wild type mice, but then recovered completely from inflammatory hyperalgesia within 2 days [19] (Fig. 1C). Interestingly, inflammatory paw swelling was unchanged in both knock-outs indicating that EP2 receptors do not contribute to edema formation and that spinal pain sensitization did not feed back to the peripheral inflamed tissue [18]. In an additional study we used these mice also to test whether an EP2 receptor-dependent inhibition of glycinergic neurotransmission would also contribute to neuropathic pain following peripheral nerve injury [20]. Although clinical evidence, in particular the lack of effectiveness of cyclooxygenase inhibitors in neuropathic pain, argues against an involvement of spinal prostaglandins some experimental studies have suggested such a contribution.

In our experiments mice lacking the EP2 receptor or the GlyR$\alpha 3$ subunit developed thermal and mechanical hyperalgesia indistinguishable from that of wild type mice (Fig. 1D and E), suggesting that central pain sensitization originating from inflammatory and neuropathic conditions involve clearly distinct mechanisms.

Work from other groups indicates that a loss of synaptic inhibition occurs also under neuropathic conditions albeit through completely different mechanisms (Fig. 2). Clifford Woolf's group has shown that synaptic inhibition and immunoreactivity against GAD65 are severely reduced after peripheral nerve injury, a finding which they attribute to an apoptotic cell death of GABAergic interneurons in the superficial dorsal horn [21]. This study is supported by a second publication from the same group in which it was shown that inhibition of caspases could prevent neuropathic pain sensitization [22]. Others, however, did not find a clear association of GABAergic cell loss and neuropathic pain [23–25].

A second hypothesis supporting the contribution of synaptic dis-inhibition to neuropathic pain comes from the group of Yves de Koninck. They have demonstrated that GABAergic and glycinergic input becomes less inhibitory after peripheral nerve injury due to a diminished transmembrane chloride gradient [26] (Fig. 3). GABAergic and glycinergic input is inhibitory only as long as the opening of

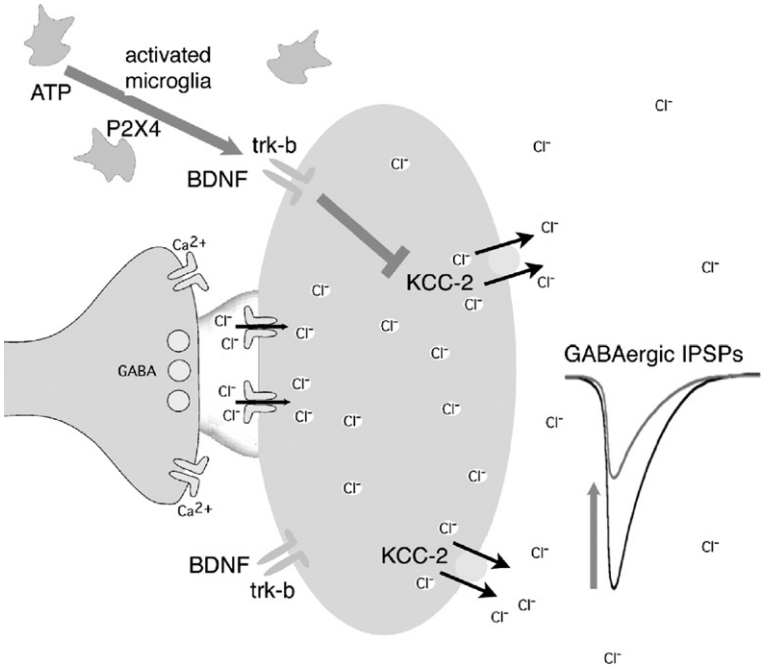

Figure 3 Microglia-induced dis-inhibition in neuropathic pain states. Peripheral nerve damage activates dorsal horn microglia, which upon stimulation with ATP releases BDNF to down-regulate KCC-2 expression. The resulting shift in the transmembrane chloride gradient renders GABAergic and glycinergic input less inhibitory (or even excitatory). For details, see [26–28].

GABA and glycine gated ion channels induces an inward flow of chloride ions. Under physiological conditions this is permitted through a low intracellular chloride concentration, which is achieved by continuous outward transport of chloride ions by the activity of the potassium/chloride exporter KCC-2. At about the same time the group of Kazuhide Inoue has proposed that activated microglia would serve a pivotal role in the generation of central sensitization induced by a peripheral nerve injury [27]. A very elegant collaboration of both groups then provided a clue as to how peripheral nerve injury can reduce the transmembrane chloride gradient of neurons located in the central nervous system [28]. According to their hypothesis, peripheral nerve injury activates microglia cells in the spinal cord. Part of this activation process involves the novel expression P2X4 receptors on the surface of the activated microglia cells. Activation of these receptors would then trigger the release of brain derived neurotrophic factor (BDNF) and the subsequent down-regulation of KCC-2.

Central pain sensitization and synaptic dis-inhibition in the dorsal horn can also be induced by intense activation of C-fibers alone, in the absence of any inflammation or nerve damage [29]. This form of pain sensitization, which shall be referred to here as activity-dependent central sensitization, has been extensively studied in pain research. Injection of the C fiber stimulant capsaicin or stimulation of skin with noxious heat elicits intensive pain sensation at the site of injection or stimulation through the activation of cutaneous C fibers (for a comprehensive review, see [30]). Following this stimulation, an area of secondary mechanical hypersensitivity develops which extends far beyond the site of injection. Many studies have convincingly shown that the increased mechanical sensitivity at this site is due to changes in the central processing of sensory input. The responsiveness of sensory fibers in the secondary hyperalgesic area is not increased [31] and the secondary hyperalgesic area does not correlate with the receptive fields of the stimulated C fibers. Although a loss of synaptic inhibition by glycine and/or GABA has been proposed as an underlying mechanism already in 1994 [29], the responsible mediator linking intense C-fiber activation to reduced synaptic inhibition has remained elusive. As expected from the absence of any tissue damage or inflammation, prostaglandins are most likely not involved as cyclooxygenase inhibition has been proven to be largely ineffective in preventing activity-dependent secondary hyperalgesia. Activation of microglia might in theory be involved but the time course and the absence of cytokine production, which could activate microglial cells, argue against this possibility. At present the nature of the mediator responsible for this form of central sensitization remains elusive.

To this end several studies point to a loss of synaptic inhibition as a major form of synaptic plasticity different from well-studied LTP and as an important source of pathological pain. If this concept holds true in the future, it might also have important implications for the therapy of pain. Although neuropathic and inflammatory pain can be clearly distinguished in experimental models of pain, patients will often suffer from mixed pain syndromes. Tumor pain most likely involves an inflammatory component arising from the immune response and a neuropathic component originating for example from nerve compression. If dis-inhibition is indeed a mechanism common to several forms of pathological pain, a compensatory facilitation of inhibitory neurotransmission in the spinal dorsal horn might be a promising new strategy for the treatment of chronic pain, which is very often resistant to conventional analgesic drugs.

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