Review

No NO, no pain? The role of nitric oxide and cGMP in spinal pain processing

Achim Schmidtko, Irmgard Tegeder and Gerd Geisslinger

Pharmazentrum frankfurt/ZAFES, Institut für Klinische Pharmakologie, Klinikum der Goethe-Universität, Theodor-Stern-Kai 7, 60590 Frankfurt/Main, Germany

A large body of evidence indicates that nitric oxide (NO) and cyclic guanosine monophosphate (cGMP) essentially contribute to the processing of nociceptive signals in the spinal cord. Many animal studies have unanimously shown that inhibition of NO or cGMP synthesis can considerably reduce both inflammatory and neuropathic pain. However, experiments with NO donors and cGMP analogs also caused conflicting results because dual pronociceptive and antinociceptive effects of these molecules have been observed. Here, we summarize the most recent advances in the understanding of NO- and cGMP-dependent signaling pathways in the spinal cord and further unravel the role of NO and cGMP in pain processing.

Introduction

The sensory experience of acute pain is initiated by activation of specialized primary afferent sensory neurons, so-called nociceptors. The cell bodies of these first-order neurons are localized in dorsal root ganglia (DRGs), and their thinly myelinated or unmyelinated axons (Aδ and C fibers, respectively) form synapses with neurons in the superficial dorsal horn of the spinal cord (Figure 1). Upon nociceptor activation, excitatory transmitters such as glutamate are released in the dorsal horn and spinal projection neurons are stimulated, which then convey the information to supraspinal brain areas. In addition, spinal excitatory and inhibitory interneurons are activated, thereby modulating the nociceptive processing in a complex manner. Thus, the dorsal horn of the spinal cord acts as a filter at which millions of peripheral signals arrive and are sorted before being sent to supraspinal sites that determine the final pain response and add an affective and emotional context to nociception [1,2] (Figure 1).

In case of persistent pain in response to tissue damage and inflammation (inflammatory pain) or lesions to the peripheral or central nervous system (neuropathic pain), the nociceptive system develops a state of hyperexcitability (Box 1). This sensitization clinically manifests as pain in response to normally innocuous stimuli (allodynia), increased response to noxious stimuli (hyperalgesia) or spontaneous pain in the absence of a stimulus, and it can persist long after the initial injury is resolved. Although the management and treatment of acute pain is reasonably good, the needs of chronic pain sufferers are largely unmet. In fact, most of the currently available analgesic drugs are only partially effective in the treatment of chronic pain and often cause distressing side effects or have abuse potential. Hence, unraveling the molecular mechanisms underlying pain sensitization is a crucial prerequisite for the development of novel analgesics [3,4].

Intense research over the last decades has revealed that several signaling pathways in the dorsal horn essentially contribute to the pain sensitization [5–7]. In particular, nitric oxide (NO) and cyclic guanosine monophosphate (cGMP) are among the important mediators in nociceptive processing. Numerous animal studies have unambiguously shown that NO and cGMP essentially contribute to the sensitization during both inflammatory and neuropathic pain. They are, however, not involved in basal pain perception, which serves as an essential early warning device [5]. Therefore, blocking the strong pain-sensitizing effects of NO- and cGMP-dependent signaling pathways could be potentially useful for the management of inflammatory and neuropathic pain. In this review we highlight our current understanding of NO- and cGMP-dependent signaling pathways that mediate the pain sensitization in the spinal cord, taking account of the substantial recent progress made in this field.

Evidence for a role of NO in pain sensitization

Our knowledge for a role of NO in spinal pain processing is mainly based on work with inhibitors of NO synthases (NOS; Box 2). Many studies conducted in the 1990s demonstrated that local inhibition of NO synthesis in the spinal cord by intrathecally (i.t.) administered NOS inhibitors such as L-NAME and L-NMMA, which inhibit all three NOS isoforms in a non-specific manner, led to a reduction of the nociceptive behavior in several animal models of inflammatory and neuropathic pain (for a review, see Refs [8,9]). More recent experiments with selective NOS inhibitors and in NOS-deficient mice revealed the neuronal NOS isoform (nNOS) to be the most important NO-producing enzyme in the spinal cord during the development and maintenance of inflammatory and neuropathic pain [10–13]. The inducible NOS isoform (iNOS) might also contribute to processing of inflammatory pain, whereas eNOS is obviously not involved in pain processing [13–15]. However, it should be noted that the expression of other NOS isoforms is upregulated in a compensatory manner in the spinal cords of nNOS- and iNOS-deficient mice, which might explain that in some models the pain phenotype in knockout mice was less pronounced than after injection of NOS inhibitors or even not present [10,14].

Corresponding author: Schmidtko, A. (schmidtko@em.uni-frankfurt.de).

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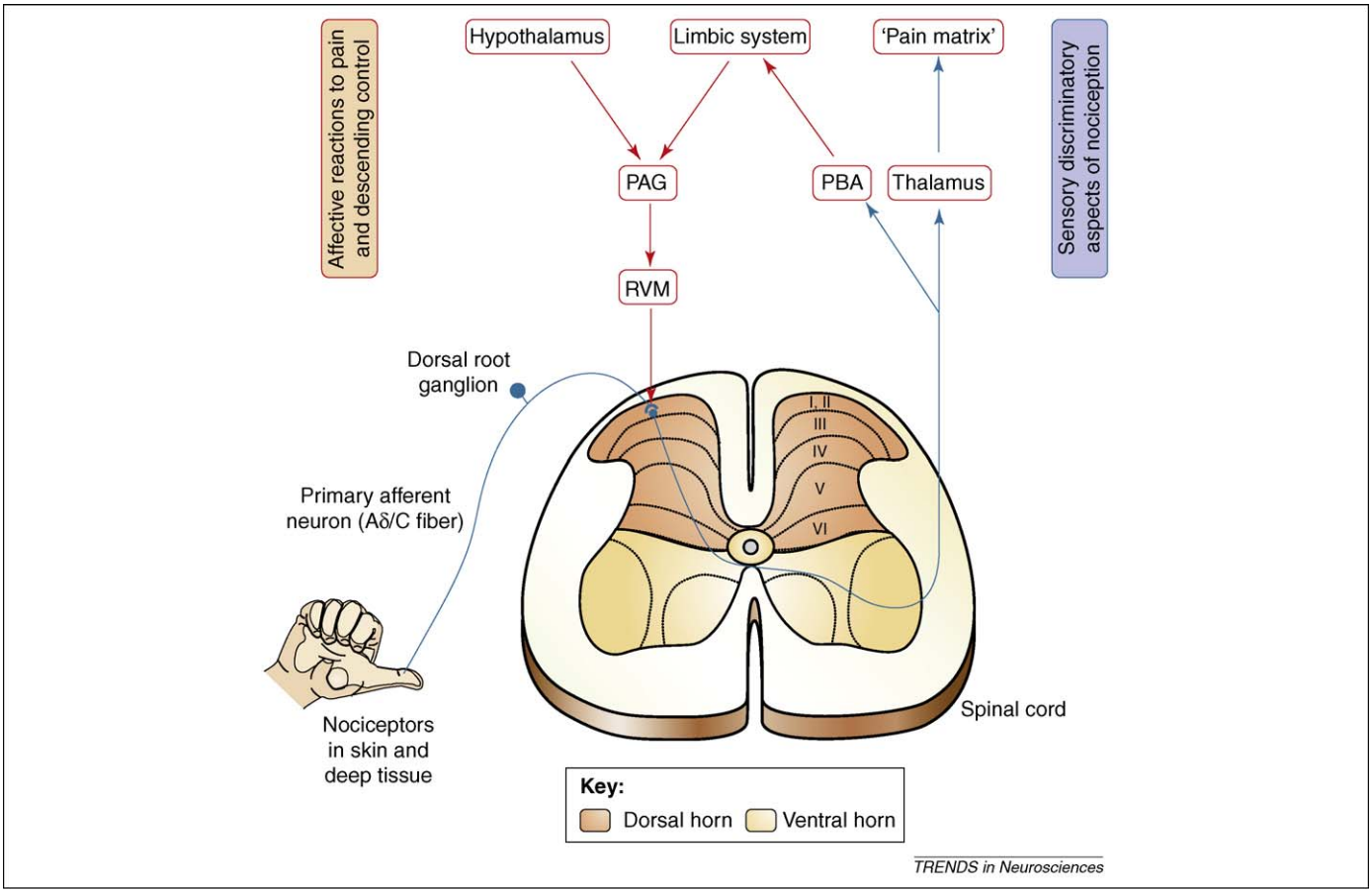

Figure 1. Important pain processing areas. Stimulation of nociceptors by noxious stimuli leads to excitation of Aδ and C fibers, which transmit action potentials to their terminals in the superficial dorsal horn of the spinal cord. There the first synaptic transmission occurs leading to activation of spinal cord neurons. Projection neurons of the spinal cord forward the excitation to the thalamus and to the parabrachial area (PBA). From the thalamus, cortical regions forming the 'pain matrix' (somatosensory, insular, anterior cingulate and prefrontal cortices) are activated, which detect the sensory discriminatory aspects of nociception. The processing of pain also initiates affective reactions such as the immediate feelings of unpleasantness and negative emotions. In addition, descending pathways are activated that control the spinal processing. Affective reactions and descending control are mediated by a network of brain structures including the PBA, the limbic system, the hypothalamus, the periaqueductal grey (PAG) and the rostral ventromedial medulla (RVM) [7]. Thus, the dorsal horn of the spinal cord integrates peripheral, spinal and supraspinal components of pain processing. Abbrevations: I-VI, laminae of the dorsal horn.

The essential contribution of NO to pain sensitization was nicely confirmed by recent studies revealing that inhibiting the *de novo* synthesis of tetrahydrobiopterin (BH4), an essential cofactor for NO production by NOS, attenuated inflammatory and neuropathic pain in rodents. Moreover, polymorphisms in the gene encoding GTP cyclohydrolase (GCH1), the rate-limiting enzyme for BH4 synthesis, are protective against persistent neuropathic pain and are associated with reduced sensitivity to experimental inflammatory hyperalgesia in humans [16].

Where does NO come from?

In which cells NO is produced during spinal pain processing was a matter of debate for some time. Immunohistochemical analyses revealed that nNOS is constitutively expressed in a subset of neurons with somata in the inner lamina II and fiber plexi in lamina I–III of the spinal cord dorsal horn but only in a few (<5%) lumbar DRG neurons [17–20]. Double-labeling studies demonstrated that the vast majority of nNOS-expressing neurons in the dorsal horn are GABAergic inhibitory interneurons [17,21–23]. By contrast, the nNOS-positive primary afferent DRG neurons are excitatory peptidergic neurons containing calcitonin gene-related peptide or substance P [24]. Of note, nNOS expression is considerably upregulated during the processing of persistent pain, but the affected neurons differ depending on the pain type. During inflammatory pain evoked by injection of proinflammatory agents such as formalin, zymosan or Complete Freund's Adjuvant into a hindpaw, nNOS is upregulated in inhibitory dorsal horn interneurons. By contrast, during neuropathic pain in response to peripheral nerve injury, nNOS is not upregulated in spinal cord neurons but the number of nNOS-positive DRG neurons and the nNOS content in their central terminals entering the dorsal horn considerably increases [11,12,24–30]. This increase in NO production seems to be important for the survival of DRG neurons after peripheral nerve injury because the injury-induced cell death of DRG neurons is increased in nNOS-deficient mice [31].

Data about the distribution and regulation of other NOS isoforms in the dorsal horn and in DRGs are less consistent. In naive animals, iNOS is, if at all, only weakly expressed [12,19,20,26,30–33]. Whether or not peripheral noxious stimulation leads to induction of iNOS in the dorsal horn or DRGs remains controversial. Whereas some studies indicate that iNOS is not induced [11,12,31,34], other studies demonstrate increased iNOS mRNA or