Inflammatory Hyperalgesia: The Role of the Prostaglandin System in the Spinal Cord

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Abstract. Hyperalgesia is a symptom often associated with inflammation. The essential role of prostaglandins (PG) in hyperalgesia has long been known since the early 1970 s. Molecular identification of PG receptors and PG-synthesizing enzymes in the late 1980 s/early 1990 s has greatly advanced the understanding of inflammatory hyperalgesia. In this review, we provide an overview of the PG system in the spinal cord, focusing on its relevance to inflammatory hyperalgesia.

Keywords: Inflammation, hyperalgesia, cytokine, prostaglandin, cyclooxygenase-2, spinal cord

INTRODUCTION

Inflammation, the innate immune response, produces a condition known as hyperalgesia, which is characterized by enhanced pain sensation and reduced pain threshold. Accumulating evidence has shown that prostaglandins (PGs) are involved in hyperalgesia under inflammatory conditions. PGs are lipid mediators derived from unsaturated fatty acids of membrane phospholipids, and play a variety of physiological roles throughout the body [1–3]. Inflammatory hyperalgesia, in particular, involves the PG system in the spinal cord. In this review, we overview the functions and location of the spinal PG system involved in inflammatory hyperalgesia. In the first part, biochemical aspects of PG system are summarized. The second part surveys the literature concerning the hyperalgesic mechanisms of PGs in the spinal cord. The third part reviews how PGs are produced in the spinal cord under inflammatory conditions. In the last part, recent reports on novel lipid mediators having anti-nociceptive action are shortly summarized.

BIOCHEMISTRY OF THE PROSTAGLANDIN SYSTEM

Prostaglandins (PGs)

All PGs are unsaturated fatty acids with 20 carbon chains. There are three possible precursors for PGs including gamma-linolenic acid, arachidonic acid and eicosapentaenoic acid. Among them, arachidonic acid is the major fatty acid from which PGs having two carbon-carbon double-bonds are biosynthesized. During the 1960 s and 1970 s, most of the bioactive PGs were identified and named in alphabetical order from PGA to PGI and thromboxane A (TXA). The naturally occurring bioactive ones are PGs D_2, E_2, $F_{2\alpha}$, I_2, and TXA_2, the numeral "2" indicating the number of carbon-carbon double-bonds in the compound (Fig. 1). Enzymes for PG biosynthesis [4] and receptors for their actions [5] are widely distributed in various tissues throughout the body, and, therefore, the biological actions of PGs are extremely diverse.

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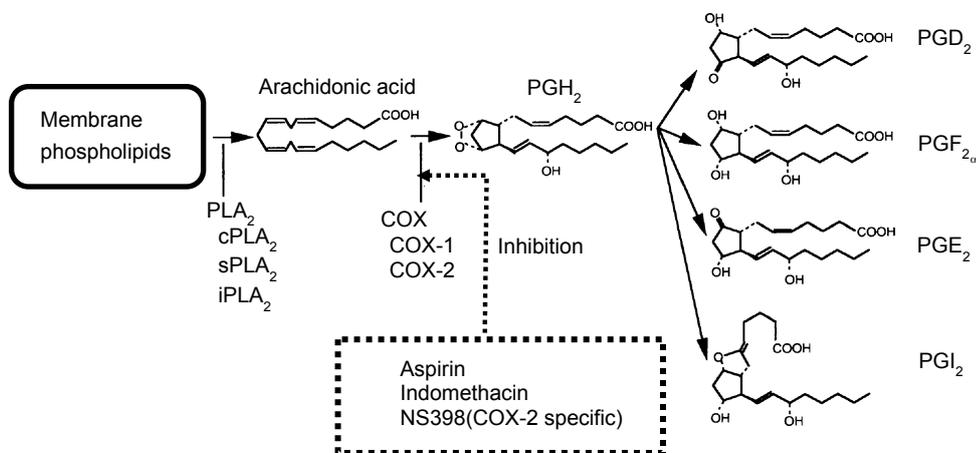
Fig. 1. Overview of the arachidonic acid cascade. PLA_2 phospholipase A_2; $cPLA_2$ cytosolic PLA_2; $sPLA_2$ secretory PLA_2; $iPLA_2$ Ca^{2+}-independent PLA_2; COX cyclooxygenase.

Enzymes for prostaglandin biosynthesis

PGs are biosynthesized from phospholipids of the cell membrane through three enzymatic steps (Fig. 1). First, phospholipase A_2 (PLA_2) cleaves arachidonic acid from the membrane phospholipids. Arachidonic acid is then converted to PGH_2 by cyclooxygenase (COX). PGH_2 is converted to bioactive PGs by PG isomerases. In this enzymatic cascade, PLA_2 and COX are common to all PGs and are considered to play rate-limiting roles in PG biosynthesis. There are approximately 20 proteins having PLA_2 activity in mammals [6]. They are classified into three general categories, i.e., cytosolic PLA_2 ($cPLA_2$), secretory PLA_2 ($sPLA_2$) and Ca^{2+}-independent PLA_2 ($iPLA_2$). $cPLA_2$s are activated within cells by a nanomolar range of intracellular Ca^{2+}. $sPLA_2$s are secreted from cells and activated by a millimolar range of Ca^{2+}. $iPLA_2$s are cytosolic enzymes and do not require Ca^{2+} for activation. The second enzyme, COX, a prostaglandin endoperoxide G/H synthase, catalyzes two chemical reactions; the first bis-lipoxygenase reaction to synthesize PGG_2 from arachidonic acid and, the second peroxidase reaction from PGG_2 to PGH_2. There are two types of COX, i.e., COX-1 and COX-2. COX-1 is constitutively expressed and seems to be involved in physiological functions under normal conditions. COX-2 is also constitutively expressed in some tissues but promptly induced by various types of stimuli, such as inflammatory cytokines, and is thought to act under both physiological and pathological conditions [7]. PG synthases, which biochemically act as isomerases, have been identified as the third step enzyme including

PGE synthase (PGES) [8], PGI synthase [9], PGD synthase, and PGF synthase. So far 4 proteins possessing PGES activity have been identified including microsomal PGES (mPGES)-1, mPGES-2, cytosolic PGES (cPGES), and the mu class glutathione -S-transferase.

PG membrane receptors

Based on the results of bioassays, the existence of specific receptors for each PG was postulated and designated DP, EP, FP, IP, and TP. The EP receptor was further classified into four subtypes (EP1, EP2, EP3, and EP4) based on their preferences for synthetic agonists and antagonists and on the pattern of second-messenger recruitment [5] (Table 1). Molecular cloning of cDNAs for PG receptors was first reported in 1991, revealing the structure of the human thromboxane A_2 receptor [10]. Based on the homology with the nucleotide sequence of TX receptor cDNA, cDNAs for other PG receptors have been cloned and expressed *in vitro* in cell lines for further characterization [11]. The receptor proteins are all coupled to G proteins and have seven transmembrane domains, a common feature of G-protein-coupled receptors. PG signals at the receptor sites are, thus, converted to second messenger molecules, including cAMP, Ca^{2+} and inositol triphosphate [3, 12].

ROLE OF THE SPINAL PG SYSTEM IN NOCICEPTION

Nonsteroidal anti-inflammatory drugs (NSAIDs), which are potent and specific inhibitors of COX and

Table 1
Classification and nomenclature of PG receptors

Receptor type	Endogenous ligand	Subtype	G-proteins	Second messengers	Histological evidence
TP	TXA_2/PGH_2		Gs, Gq, Gi	Pl turn over ↑	DRG, PNE
EP	PGE_2	EP1	?	Ca^{2+} ↑	DRG
		EP2	Gs	cAMP ↑	
		EP3	Gi, Gs	cAMP ↓↑	DRG, DH
			Gq	Pl turn over ↑	
		EP4	Gs	cAMP ↑	DRG
FP	$PGF_{2\alpha}$		Gq	Pl turn over ↑	
IP	PGI_2		Gs	cAMP ↑	DRG, DH
			Gq	Pl turn over ↑	
DP	PGD_2		Gs	cAMP ↑	
CRTH2	PGD_2		Gi	cAMP ↓	

PI: inositol-3-phosphate, ?: not identified, DRG: dorsal root ganglia, PNE: peripheral nerve endings, DH: dorsal horn.

inhibiting the production of proinflammatory chemical mediators, are also potent analgesics [13–15]. This fact strongly suggests that endogenous PGs enhance nociceptive function. Indeed, PGE_2, PGI_2, $PGF_{2\alpha}$ and PGD_2 induce hyperalgesia or allodynia [16] when injected centrally or peripherally. Among these PGs, PGE_2 and PGI_2 are best studied in various models of inflammatory hyperalgesia. Although there seem to be multiple sites within the nervous system where PGs act to modulate pain signal transmission, accumulating evidence indicates that the primary sensory neurons are the major targets for the hyperalgesic actions of PGs. The primary sensory neurons are bipolar in shape, sending one process to peripheral tissue and the other to the dorsal horn of the spinal cord with the cell bodies located in the dorsal root ganglia. PGs are likely to act on both terminals of these neuronal processes to induce hyperalgesia.

Location of PG receptors in the spinal cord

Studies in mice showed an abundant expression of mRNAs for EP and IP receptors in the neurons of sensory ganglia [17, 18]. An immunohistochemical study of EP3 revealed its expression in the neurons of sensory ganglia in rats [19]. These results indicate that PG receptors are produced in the primary sensory neurons.

Distribution of PG receptors was also investigated by binding of radio-labeled receptor ligands to frozen sections [20]. This approach can demonstrate the sites of receptor functioning rather than the sites of receptor production. An autoradiographical study using ^{3}H-labeled PGE_2 or ^{3}H-labeled iloprost, a stable PGI_2

analog, showed that a high density of iloprost binding sites and PGE_2 binding sites was present in the superficial layers of the dorsal horn (Fig. 2a, c) and the spinal trigeminal nucleus. An immunohistochemical study also localized EP3-like immunoreactivity in the superficial layers of rat dorsal horn [19]. The dorsal horn and the spinal trigeminal nucleus receive somatosensory primary afferent inputs, the former from the dorsal root ganglia and the latter from the trigeminal ganglia. Importantly the superficial layers of the dorsal horn and the spinal trigeminal nucleus receive nociceptive and thermosensitive inputs via unmyelinated fibers (C-fiber) and fine myelinated fibers (Aδ-fiber).

As all the above mentioned regions receive primary sensory afferents, there arose a possibility that these PG receptors are located in the central terminals of the primary sensory afferent but not in the postsynaptic neurons in the dorsal horn or in the medulla. This possibility was examined in rats in which some of the dorsal roots were unilaterally sectioned between the dorsal root ganglia and the spinal cord. Eight days after the dorsal roots had been sectioned, the binding sites for PGE_2 and iloprost were significantly decreased, almost disappeared, ipsilateral to the lesion [20] (Fig. 2b, d). These results clearly demonstrate that the receptors for PGs are biosynthesized in the sensory ganglia and function after being transported to the central sensory terminals in the spinal cord and medulla oblongata. In contrast, there is no histological study that localizes EP or IP receptors in the peripheral terminals of sensory neurons. However, the physiological studies described below clearly indicate the presence of EP and IP receptors in the peripheral terminals of sensory neurons.
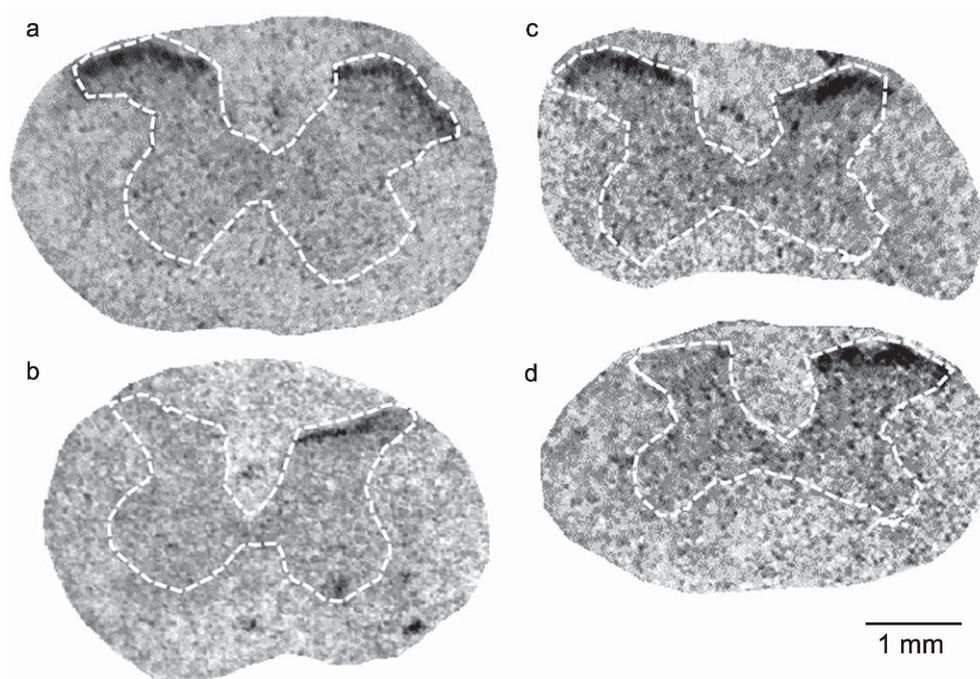
Fig. 2. Receptor autoradiography of PG receptors in the rat spinal cord. (a) ^{3}H-PGE$_2$ binding sites (b) ^{3}H-PGE$_2$ binding sites 8 days after the dorsal root had been sectioned unilaterally (c) ^{3}H-iloprost (a stable analogue of PGI$_2$) binding sites (d) ^{3}H-iloprost binding sites 8 days after the dorsal root had been sectioned unilaterally. Dotted lines delineate the gray matter.

Action of PGs in the peripheral terminals of the primary nociceptive neurons

PGs when injected into peripheral tissues evoke hyperalgesia. This was first demonstrated in humans in 1972 [21] and was confirmed by a large number of studies using behavioral pain models in experimental animals, such as the paw-withdrawal reflex to a mechanical or heat stimulus. The results are largely consistent with PGE$_2$ and PGI$_2$ being the most potent PGs in augmenting behavioral pain when injected into peripheral tissues [22, 23] and that these PGs themselves do not evoke pain but instead lower the threshold for pain evoked by other stimuli, a phenomenon called sensitization.

Hyperalgesia is often associated with peripheral inflammation. Application of NSAIDs, inhibitors of cyclooxygenase, to the inflamed tissue suppresses hyperalgesia. Thus, endogenous PGs seem to work as a mediator of hyperalgesia during inflammation. Consistent with these findings from behavioral studies, neurophysiological experiments also showed that application of PGE$_2$, PGI$_2$ or an analog of PGI$_2$ to peripheral tissues increased the number of afferent nociceptive fiber action potentials [24–27]. These results clearly indicate that the peripheral terminals of the primary nociceptive neurons are the sites of hyperalgesic action of PGE$_2$ and PGI$_2$. The ionic mechanism by which PGs lower the pain threshold remains to be clarified *in vivo*. This is mainly due to the difficulty in conducting electrophysiological experiments in the peripheral terminals of primary sensory neurons. Instead, electrophysiological studies employing cultured sensory neurons *in vitro* have provided important information on the mechanism of PG-induced sensitization. A long-lasting hyperpolarization, which follows an action potential, was present in a subpopulation of primary sensory neurons. This hyperpolarization was driven by a Ca^{2+}-dependent K$^+$ current and thought to reduce the excitability of the neurons. PGE$_2$ suppressed this current and therefore, increased the excitability of the neurons [28, 29]. This idea is in agreement with the *in vivo* observation that PGE$_2$ itself does not induce pain but instead lowers the threshold for pain elicited by other stimuli.

Another possible mechanism has also been proposed. A subpopulation of primary sensory neurons in culture was shown to possess a tetrodotoxin-resistant

Na^+ current. PGE_2 augmented this current in a cAMP-dependent manner, thus increasing neuronal excitability [30, 31].

Another intriguing hypothesis involves the noxious heat receptor TRPV1, which is a cation channel activated by capsaicin, low pH, and noxious heat above $42°C$. TRPV1 is highly expressed in small- to medium-sized primary sensory neurons in both the central and peripheral terminals of their axons. Activation of TRPV1 induces cation inflow and consequently, elevates the excitability of neurons [32]. TRPV1-expressing neurons are therefore, considered to be involved in nociception. Moriyama et al. [33] demonstrated that PGE_2 and PGI_2 phosphorylate TRPV1 in a cAMP-dependent manner and lowers the temperature threshold for activation to normal body temperature. Thus, in inflamed tissues, the excitability of TRPV1-expressing sensory terminals should be elevated by PGE_2 and PGI_2, resulting in hyperalgesia.

Action of PGs in the central terminals of primary sensory neurons

As in the case of peripheral application of PGs, intrathecal infusions of PGs also modulate (mostly facilitate) nociception. PGE_2, PGD_2, and $PGF_{2\alpha}$ have been reported to induce hyperalgesia when infused intrathecally [34–37]. Effects of intrathecal injection of PGs are classified into two categories: allodynia and hyperalgesia. Allodynia is a state of discomfort and pain evoked by innocuous stimuli such as a gentle touch or brushing, whereas hyperalgesia is a state of lowered threshold for pain to a eventually noxious stimulus. Minami et al. [34, 35] showed that PGE_2 and $PGF_{2\alpha}$ induced allodynia and that PGE_2 also induced hyperalgesia. Using subtype-specific agonists for PGE_2 receptors, they showed that PGE_2 induced allodynia through the EP1 receptor whereas hyperalgesia occurred through the EP3 receptor [34].

Again, intrathecal injection of NSAIDs in rats suppressed the hyperalgesia elicited either by injection of formalin in peripheral tissue [38] or by subcutaneous injection of carrageenan in the hind paw [39]. The central terminals of the primary sensory neurons principally release glutamate to transmit the pain signals. Neuropeptides, such as substance P (SP) and calcitonin gene-related peptide (CGRP) are also released and considered to be involved in the modulation of pain signals. Using an intrathecal microdialysis probe, Malmberg and Yaksh [40] showed that intradermal injection of formaldehyde into a rat hind paw, a treatment which provokes biphasic behavioral pain, increased the levels

of excitatory amino acids and PGE_2 in the cerebrospinal fluid of the lumbar cord. Intrathecal as well as intraperitoneal administration of an NSAID lowered the levels of PGE_2, excitatory amino acids, and behavioral pain.

Similarly, using carrageenan/kaoline-induced knee joint inflammation as an experimental model, Yang et al. [41] demonstrated increases in the levels of excitatory amino acids, PGE_2, and behavioral pain. Intrathecal infusion of capsaicin evoked behavioral pain and amino acid release, and co-administration of PGE_2 enhanced these responses [42].

The effects of PGs on neuropeptide release were mainly studied in cell culture systems. PGE_2 as well as PGI_2 and carbacyclin, a stable PGI_2 analog, enhanced the release of SP and CGRP by bradykinin from rat sensory neurons in culture [43, 44]. These results imply that PGs also act on the central terminals of sensory neurons to enhance pain transmission by increasing the release of excitatory amino acids and/or neuropeptides.

Postsynaptic action of PGE_2 in the spinal cord

PGE_2 also acts on the spinal cord neurons postsynaptically. Glycine is an inhibitory neurotransmitter in the spinal cord pain pathway. Alpha3 glycine receptors are located in lamina II neurons of spinal dorsal horn. Electrophysiological study showed that PGE_2 acts on lamina II neurons and reduces the amplitude of glycine-induced inhibitory postsynaptic current [45]. This should result in the enhancement of neuronal excitability of the pain pathway. The action of PGE_2 on the glycine receptor is mediated by EP2 PGE_2 receptor and subsequent cAMP-protein kinase A signaling [46]. Consistently with the electrophysiological results, behavioral study using gene-deleted mice showed that EP2 PGE_2 receptor and alpha3 glycine receptor are involved in the inflammatory hyperalgesia [46]. Thus, PGE_2 acts both pre- and post-synaptic sites of the dorsal horn neurons to enhance pain signal transmission.

Inflammatory hyperalgesia in PG receptor-deficient mice

Inflammatory hyperalgesia have been studied in mice genetically lacking PG receptors (Table 2). Although the models of inflammation were limited, these studies clearly demonstrated the involvement of EP and IP receptors in inflammatory hyperalgesia. It should be also noted that hyperalgesia was enhanced in mice lacking some of the PG receptors.

Table 2
Inflammatory hyperalgesia in PG receptor-deficient mice

Genotype	Inflammatory model (route of application)	Type of hyperalgesia	Changes in (−/−) mice	Ref
EP1(−/−)	PGE_2 (s.c.)	Thermal	↓	[33]
	PGE_2 (i.t.)	Mechanical allodynia	↓	[68]
	Mustard oil (skin)	Thermal	↓	[33]
EP2(−/−)	PGE_2 (i.t.)	Thermal/mechanical	↓	[46]
	PGE_2 (s.c.)	Mechanical	↓	[46]
	Zymosan A (s.c.)	Thermal/mechanical	↓	[46]
	Formalin (s.c.)	Flinching	↑	[50]
EP3(−/−)	Formalin (s.c.)	Licking	↓	[50]
	LPS (i.p.)	Writhing to acetic acid (i.p.)	↓	[69]
IP(−/−)	Acetic acid (i.p.)	writhing	↓	[70]
	PGI_2 (s.c.)	Thermal	↓	[33]
DP1	Formalin (s.c.)	Flinching	↑	[33]

↓: reduced, ↑: enhanced, s.c.: subcutaneous, i.t.: intrathecal, i.p.: intraperitoneal.

This fact suggests that some PG receptors may have anti-nociceptive function.

PG SYNTHESIS IN THE SPINAL CORD UNDER INFLAMMATORY CONDITIONS

Although the hyperalgesic actions of PGs were reported in the early 1970 s, it continued to be unclear for a long time how PGs are produced in the spinal cord under inflammatory conditions. The discovery of COX-2, the inducible type of COX, opened the way to answer this question. A number of studies in both human and experimental animals have shown that COX-2 inhibitors are quite potent in suppressing hyperalgesia under inflammatory conditions [47, 48]. COX-2-deficient mice showed reduced hyperalgesia in acute and chronic inflammation models [49, 50]. These facts strongly suggest that PGE_2 and/or PGI_2 produced by COX-2 act on the peripheral or central terminals of nociceptive neurons, which seems to be the primary targets of hyperalgesic PGs.

We and other groups have succeeded in identifying COX-2-expressing cells in the spinal cord under different inflammatory conditions. These conditions include subcutaneous injection of carrageenan, burn injury and subcutaneous injection of complete Freund's adjuvant (CFA), the former two being models for acute peripheral inflammation, and the latter being a model for chronic peripheral inflammation. The results obtained in these three models are summarized below.

Carrageenan model

Carrageenan-induced inflammation evoked thermal hyperalgesia. This hyperalgesia was suppressed by systemic or intrathecal injection of NS398, a COX-2-specific inhibitor [39]. Carrageenan injection induced expression of COX-2-like immunoreactivity in vascular endothelial cells throughout the central nervous system including the spinal cord (Fig. 3a). This response became evident by 3 h, and was most prominent at 6 h after carrageenan injection. The COX-2 induction was associated with an elevation of PGE_2 in the cerebrospinal fluid, being evident at 3 h, larger at 6 h, and alleviated by a COX-2 selective inhibitor [39]. Thus, in this model, hyperalgesia seems to be brought about by PGE_2, and possibly PGI_2, that are produced in and released from endothelial cells in the spinal cord, and sensitize the central terminals of primary nociceptive neurons.

The next question is how peripheral inflammation induced COX-2 in the spinal cord. Our subsequent study indicated the involvement of a cytokine, interleukin-6 (IL-6). Levels of IL-6 but not IL-1β or tumor necrosis factor α were significantly elevated in the circulating blood 3 h after carrageenan injection. A prior intraperitoneal injection of IL-6 antiserum attenuated COX-2 expression, PGE_2 levels in the cerebrospinal fluid (CSF), and thermal hyperalgesia [51]. IL-6 seemed to directly act on endothelial cells because signal transducer and activator of transcription 3 (STAT3), an intracellular messenger downstream of IL-6 receptor activation, translocated to the nucleus in endothelial cells, which also expressed COX-2. These results suggest that circulating IL-6 could act as a messenger of inflammatory information from peripheral inflammatory sites to the CNS via the brain endothelial cells. In addition, this study showed that carrageenan injection also induced mPGES-1 (Fig. 3b), which converts PGH_2 to PGE_2 in endothelial cells, where
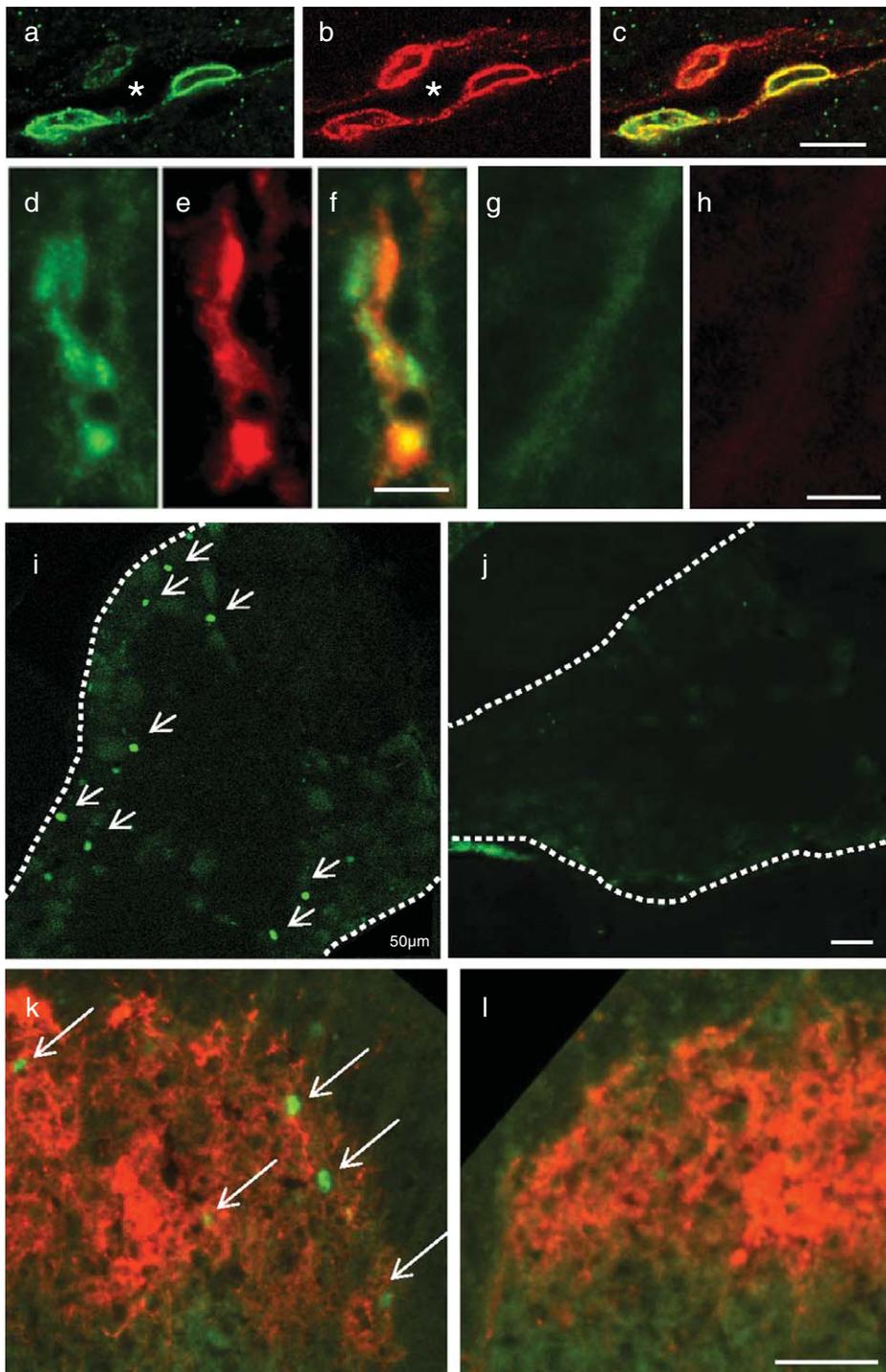
Fig. 3. (a–c) Double immunostaining of COX-2 (a) and mPGES-1 (b) in rat vascular endothelial cells 6 h after carrageenan injection in the hind paw. Asterisks indicate the lumen of blood vessel. (c) merged view, (d–h) double immunostaining of pNFkB (d, g) and COX-2 (e, h) in the rat spinal cord 3 h after carrageenan injection (d–f) or saline (g-h) in the hind paw. (f) merged view, (i-j) pNFkB in the right L4 dorsal root ganglion (i) and left L4 dorsal root ganglion (j) 20 min after formalin injection to the right hind paw in a mouse. Arrows indicate pNFkB-positive cells. Dotted lines delineate the border of dorsal root ganglion (k-l) double immunostaining of pNFkB (green) and TRPV1 (red) in the right dorsal horn (k) and the left dorsal horn (l) of a rat 3 h after carrageenan injection in the right hind paw. Arrows indicate pNFkB-positive cells. Scale bars in (c), (f), (h) indicate 10 μm, and those in (j) and (l) indicate 50 μm.

mPGES-1 was colocalized with COX-2 (Fig. 3c). Both COX-2 and mPGES-1 were negative in rats injected saline (data not shown).

Burn injury model

Burn injury often accompanies hyperalgesia [52, 53]. Thirty six hours after approximately 25% full thickness burn injury in rats, PGE_2 in the CSF was significantly elevated and this elevation was suppressed by a COX-2 specific inhibitor, NS398. In burn-injured rats but not in intact ones, COX-2 and mPGES-1 proteins were detected in vascular endothelial cells throughout the CNS including the spinal cord. COX-2 and mPGES-1 are colocalized in the perinuclear region of the endothelial cells [54]. These enzymes likely elevate the CSF concentration of PGE_2, a prostanoid that, in turn, activates PGE_2 receptors on the spinal and higher order neurons involved in hyperalgesia following burn injury.

CFA model

Intraplanter injection of CFA also evokes inflammation and hyperalgesia in rats. This hyperalgesia is dependent on COX-2 in the spinal cord [55–57], as in the case of hyperalgesia by carrageenan. In contrast, however, several studies showed that cells expressing COX-2 in the spinal cord were neurons but not endothelial cells [58–60]. Furthermore, some studies indicated the involvement of IL-1β and TNF-α in the induction of COX-2 [59, 61]. The differences in the type of COX-2 expressing cells and in the type of cytokines involved suggest distinct mechanisms underlying signal transmission from the peripheral inflammatory loci to the CNS between the acute (carrageenan) and chronic (CFA) inflammation models. It is possible that long lasting neuronal activation in chronic inflammation may induce COX-2 in neurons as discussed below.

Possible role of nuclear factor kappa B in inflammatory hyperalgesia

Nuclear factor kappa B (NFkB) is a transcription factor, activation of which induces a series of proteins involved in inflammation. COX-2 induction is under the control of NFkB in various cell types. Spinal NFkB activation induced COX-2 upregulation and contributed to inflammatory pain hypersensitivity [62]. We found nuclear localization of phosphorylated NFkB (pNFkB), an activated form of NFkB,

in endothelial cells in the spinal cord 3 h after carrageenan injection in the hind paw (Fig. 3d). In these endothelial cells, pNFkB was colocalized with COX-2 (Fig. 3e, f) suggesting that pNFkB in endothelial cells activates induction of COX-2 cooperatively with STAT3. Both pNFkB and COX-2 were negative in the spinal cord 3 h after saline injection in the hind paw (Fig. 3g, h).

We also found intense sensory stimuli to the skin resulted in nuclear translocation of pNFkB in primary sensory neurons [63]. Nuclear translocation of pNFkB occurred in the right L4 dorsal root ganglia (Fig. 3i) but not in the left L4 dorsal root ganglia (Fig. 3j) 20 min after formalin injection in the right hind paw. Furthermore, nuclear translocation of pNFkB was also observed in the dorsal horn neurons, possibly the second order sensory neurons, 3 h after carrageenan injection in the hind paw (Fig. 3h). The response was restricted to the injection side since contralateral dorsal horn did not show any pNFkB-positive neurons (Fig. 3i). This result suggests that painful stimuli activate NFkB in the primary and second order sensory neurons, and facilitate the induction of inflammation-related genes. If painful stimuli continue for a certain period under chronic inflammatory states, the activation of NFkB may induce COX-2 in neurons and contribute to the maintenance of the hyperalgesic state.

LIPID MEDIATORS HAVING ANTI-NOCICEPTIVE ACTION

Studies in the late 1990 s to 2000 s identified a series of lipid mediators having anti-inflammatory and proresolving activities and designated them as lipoxins, resolvins and protectins [64]. Lipoxins are derived from omega-6 polyunsaturated fatty acid, arachidonic acid, while resolvins and protectins are from omega-3 polyunsaturated fatty acid, i.e., eicosapentaenoic acid and docosahexaenoic acid. Recent studies demonstrated that some of these lipid mediators exert potent anti-nociceptive action. Resolvin E1 (RvE1), RvD1 and RvD2 attenuate inflammatory hyperalgesia when applied to the inflamed loci at the dose of 1 ng or less [65, 66]. RvE1 and RvD2 are also effective when applied intrathecally. The anti-nociceptive mechanism of Rvs seems to involve their inhibitory action on the sensory TRP channels including TRPV1, TRPA1, TRPV3 and TRPV4 [65, 67]. The effective concentrations (IC_{50}) are quite low ranging from 0.1 nM to 9 nM. The actions of Rvs on the TRP channels are indirect via the G-protein coupled receptors. These results suggest

that inflammatory hyperalgesia is controlled by the balance between hyperalgesic PGs and anti-nociceptive Rvs.

PERSPECTIVES

Molecular identification of PG receptors and PG-synthesizing enzymes in the 1990s has greatly advanced the understanding of inflammatory hyperalgesia. There are, however, a number of issues to be answered. Among them, it is of special importance to identify the PLA_2 isoform that supplies arachidonic acid to COX-2 to induce inflammatory hyperalgesia. Another issue would be how COX-2 is differentially regulated in endothelial cells and neurons in acute and chronic inflammatory states. Furthermore, recent findings of anti-nociceptive Rvs lead us to reconsider inflammatory hyperalgesia on the basis of its initiation and resolution. It is of importance to understand how the biosynthesis of hyperalgesic PGs and anti-nociceptive Rvs are differentially regulated in the course of inflammation and its resolution.

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