Lipid Mediators and Pain Signaling

Prostaglandin E₂ and Pain—An Update

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Prostaglandin E₂ (PGE₂), a cyclooxygenase (COX) product, is the best known lipid mediator that contributes to inflammatory pain. Nonsteroidal anti-inflammatory drugs (NSAIDs), inhibitors of COX-1 and/or COX-2, suppress inflammatory pain by reducing generation of prostanoids, mainly PGE₂, while they exhibit gastrointestinal, renal and cardiovascular toxicities. Selective inhibitors of microsomal PGE synthase-1 and subtype-selective antagonists of PGE₂ receptors, particularly EP₁ and EP₄, may be useful as analgesics with minimized side-effects. Protein kinase C (PKC) and PKA downstream of EP₁ and EP₄, respectively, sensitize/activate multiple molecules including transient receptor potential vanilloid-1 (TRPV1) channels, purinergic P2X3 receptors, and voltage-gated calcium or sodium channels in nociceptors, leading to hyperalgesia. PGE₂ is also implicated in neuropathic and visceral pain and in migraine. Thus, PGE₂ has a great impact on pain signals, and pharmacological intervention in upstream and downstream signals of PGE₂ may serve as novel therapeutic strategies for the treatment of intractable pain.

Key words prostaglandin E₂; pain; protein kinase A; protein kinase C; analgesia

1. INTRODUCTION

Prostanoids, metabolites of arachidonic acid through the cyclooxygenase (COX) pathway, are the best known lipid mediators that contribute to inflammatory pain. Among the prostanoids, prostaglandin E₂ (PGE₂) and, perhaps, PG₁₂ have the greatest impact on processing of pain signals. Nonsteroidal anti-inflammatory drugs (NSAIDs), the most frequently used analgesics, reduce production of prostanoids including PGE₂ by inhibiting COX-1 and/or COX-2, and thereby suppress inflammatory pain in patients suffering from rheumatoid arthritis, osteoarthritis, gout and, probably, migraine. The side-effects of COX inhibitors include gastrointestinal and renal toxicity, and the use of selective inhibitors of COX-2 is associated with an increased cardiovascular risk. Inhibitors of PGE synthases are thus considered clinically beneficial in order to avoid the cardiovascular and renal side-effects, since PG₁₂ plays major protective roles in the kidney and cardiovascular system. Blockade of PGE₂ receptors is another approach for the development of analgesics with few side-effects. Four subtypes of PGE₂ receptors, EP₁, EP₂, EP₃ and EP₄, have been identified. It is likely that subtype-selective antagonists of PGE₂ receptors may have a reduced gastrointestinal toxicity. This review focuses on recent advances in the studies on molecular mechanisms for pain modulation by PGE₂ and in the development of isozyme-selective inhibitors of PGE synthases or subtype-selective antagonists of PGE₂ receptors as novel analgesics.

2. MICROSOMAL PROSTAGLANDIN E SYNTHASE-1 AS A THERAPEUTIC TARGET FOR TREATMENT OF INFLAMMATORY PAIN

PGE₂ is generated from PGH₂, a COX product, by at least three different isomerases, a cytosolic PGE synthase (cPGES), and two membrane-bound PGE synthases, called microsomal PGE synthase-1 (mPGES-1) and mPGES-2. Of these PGE synthases, cPGES and mPGES-2 are constitutively expressed in various organs/tissues, whereas mPGES-1, like COX-2, is up-regulated in response to various inflammatory stimuli (Fig. 1). Although selective COX-2 inhibitors including SC-236 suppress generation of not only PGE₂ but also other PGs including PG₁₂ and PGF₂α, selective inhibitors of mPGES-1, such as MF63, PF-9184 and AF3442, reduce the formation of PGE₂, but not the other PGs. Interestingly, MF63 relieves inflammatory pain, but does not cause NSAID-like gastrointestinal toxic effects in an animal model. In addition, selective inhibition of mPGES-1 might also have some beneficial effect on the cardiovascular system, at least in part due to compensatively augmented formation of PG₁₂.

3. SUBTYPES OF PGE₂ RECEPTORS AND THE DOWNSTREAM SIGNALS

All four subtypes of PGE₂ receptors belong to a large superfamily of G-protein-coupled, seven-trans-membrane domain receptors (GPCRs). EP₁ receptors are coupled to heterotrimeric Gₛ/₁₁ protein, and the agonist stimulation of EP₁ receptors causes activation of phospholipase C, which in turn produces inositol trisphosphate (IP₃) and diacylglycerol (DG), followed by cytosolic calcium mobilization and activation of protein kinase C (PKC). EP₂ and EP₄ receptors are coupled to Gₛ protein, and the agonist stimulation of those receptors activates adenylyl cyclase, which generates cyclic AMP (cAMP), followed by activation of protein kinase A (PKA) and, perhaps, exchange protein activated by cAMP (Epac). EP₃ receptors are alternatively spliced, yielding at least three variants, EP₃α, EP₃β and EP₃γ. EP₃α and EP₃β receptors are coupled to Gₛ protein, and the activation of...
those receptors inhibits adenylyl cyclase. In contrast, EP3 receptors have both stimulatory and inhibitory effects on adenylyl cyclase via activation of Gq and Gi proteins, respectively. Surprisingly, EP2 receptors may also be capable of causing cytosolic calcium mobilization.[14,15] Any or all of the EP1, EP2, EP3, and EP4 isoforms may be expressed in sensory neurons[16] and may play roles in peripheral and/or spinal nociceptive processing.[17]

4. PROCESSING OF SOMATIC PAIN BY PGE2 RECEPTORS IN PERIPHERAL NOCICEPTORS

Studies using PG receptor-knockout (KO) mice have not necessarily provided simple explanations of the roles of each of PGE2 receptor subtypes in nociceptive processing. There is evidence that deletion of EP1 or EP4 does not affect the formalin-induced nociception in mice,[18,19] and that acute heat pain is suppressed by deletion of EP4, but enhanced by deletion of EP1, while acute mechanical pain is not affected by deletion of either receptor.[18] Thus, compensatory and/or developmental adaptations, and site-specific roles of EP3 or EP2 receptors might have led to the inconsistent results from studies using mice lacking a specific subtype of PGE2 receptor. In this context, pharmacological intervention with a specific subtype of PGE2 receptor appears to have more potential to identify the impact of EP1 or EP4 on pain signals in health and disease. Actually, the development of highly selective antagonists of EP1 and EP3 receptors has contributed to our understanding of the roles played by those receptors in peripheral nociceptors.[19–27] In particular, increasing evidence demonstrates the effectiveness of selective EP3 receptor antagonists as analgesics in a variety of inflammatory pain models. AH23848, MF766, CJ-032423 and CJ-042794, selective EP3 receptor antagonists, strongly suppress the inflammatory hyperalgesia induced by complete Freund’s adjuvant (CFA) or by carrageenan,[23,25–27] MF498, another EP4 antagonist, relieves joint pain in rodent models of rheumatoid and osteoarthritis, without showing the gastrointestinal toxicity.[21]

Involvement of PKA and/or PKC has been described in the hyperalgesia induced by intraplantar administration of PGE2. PKA and PKC mediate the hyperalgesia following activation of EP4 and EP1 receptors, respectively, in peripheral nociceptors.[19,28] Studies analyzing the downstream signals of PKA and PKC in nociceptors suggest that PGE2-evoked thermal hyperalgesia involves sensitization of transient receptor potential vanilloid-1 (TRPV1) channels by PKA and PKC,[19] and that the phosphorylation of TRPV1 by either kinase is dependent on A-kinase anchoring protein 150 (AKAP150) and AKAP79, respectively the rodent and human homologues of a scaffolding protein.[29] The sensitization of TRPV1 through the PGE2/EP4/PKC and PGE2/EP1/PKA cascades is thus considered to be involved in inflammatory thermal hyperalgesia (Fig. 1), but does not appear to be responsible for the mechanical hyperalgesia induced by PGE2. The tetrodotoxin-resistant (TTX-R) voltage-gated sodium channel Na1.9 is expressed in TRPV1-positive nociceptor sensory neurons, and might contribute to the peripheral PGE2-evoked hyperalgesia. Genetic deletion of Na1.9 results in complete and partial inhibition of thermal and hyperalgesia, respectively, following intraplantar administration of PGE2.[30] Another TTX-R voltage-gated sodium channel, Na1.8, expressed in nociceptors appears to contribute to the maintenance of chronic mechanical hyperalgesia induced by repeated intraplantar administration of PGE2.[31] T-type calcium channels, particularly Ca3.2 isoform, are abundantly expressed in nociceptors,[21] and can be phosphorylated and sensitized by PKA.[32] Our latest study implies that PKA-dependent sensitization of T-type calcium channels mediates the mechanical hyperalgesia caused by intraplantar administration of PGE2.[34] Similarly, PKA-dependent sensitization of purinergic P2X3 receptors in nociceptors may also be associated with peripheral PGE2-induced mechanical and thermal hyperalgesia.[10,11] Based on this body of evidence, multiple molecules including TTX-R sodium channels, T-type channels and P2X3 receptors might be downstream of PKA and/or PKC, contributing to the mechanical and thermal hyperalgesia evoked by peripheral PGE2 (Fig. 1).

There is evidence that PKA is essential in the early phase of hyperalgesia following intraplantar administration of PGE2, while PKCe is downstream of PKA, contributing to the later phase of the PGE2-induced hyperalgesia.[28] During prolonged inflammation and hyperalgesia, EP3 receptor (i.e. EP3C) and EP2 mediate the PGE2-induced production of cAMP, which in turn activates Epac in addition to PKA. Epac then activates PKCe via the Rap pathway, leading to sensitization of P2X3 receptors.[9,11,16,35] (Fig. 1). Activation of the Epac/Rap pathway by PGE2 via EP2 and EP4 might also occur in synovial and gingival fibroblasts, playing a role in the pathologies of rheumatoid arthritis and inflammatory periodontal disease.[2,13]
5. INVOLVEMENT OF SPINAL PGE\textsubscript{2} RECEPTORS IN PROCESSING OF SOMATIC PAIN

The pro-nociceptive role of EP\textsubscript{1} receptors in the spinal cord might be greater than that in the peripheral nociceptors. The mechanical allodynia and hyperalgesia following intrathecal administration of PGE\textsubscript{2} disappear in EP\textsubscript{1} receptor KO mice, and are prevented by selective EP\textsubscript{1} receptor antagonists.\textsuperscript{17,36} The activation of PGE\textsubscript{2}/EP\textsubscript{1} pathway and subsequent cytosolic calcium mobilization in the spinal dorsal horn are considered to be involved in the late phase of carrageenan-induced inflammatory pain.\textsuperscript{37} GSK345931A, a selective EP\textsubscript{1} receptor antagonist that is capable of penetrating the central nervous system (CNS), exhibits potent analgesic activity in rats with CFA-induced sub-chronic pain, most probably through blockade of both spinal and peripheral EP\textsubscript{1} receptors.\textsuperscript{38} Spinal EP\textsubscript{2} receptors also contribute to PGE\textsubscript{2}-evoked facilitation of nociceptive input from the spinal cord to higher brain areas where pain becomes conscious, and the underlying mechanisms involve suppression of inhibitory glycinergic neurotransmission through PKA-dependent blocking of GlyR\textsubscript{α}, a glycine receptor subtype.\textsuperscript{39,40} The spinal EP\textsubscript{2}-mediated inhibition of GlyR\textsubscript{α} appears to play an important role in a second sustained hyperalgesic phase of spinal origin following intraplantar administration of zymosan A,\textsuperscript{40,41} but does not contribute to the formalin-induced acute pain.\textsuperscript{42} Spinal EP\textsubscript{3} and EP\textsubscript{4} receptors are not greatly involved in facilitation of pain by spinal PGE\textsubscript{2},\textsuperscript{8,41,43} although the hyperalgesia following intrathecal administration of PGE\textsubscript{2} at low doses might involve spinal EP\textsubscript{3} receptors.\textsuperscript{17} Interestingly, the EP\textsubscript{3} subtype, a G\textsubscript{i} protein-coupled inhibitory splice variant of the EP\textsubscript{3} receptors, is activated in inflammatory pain states, thereby limiting the pro-nociceptive effects of spinal PGE\textsubscript{2}.\textsuperscript{43}

6. IS PGE\textsubscript{2} INVOLVED IN NEUROPATHIC PAIN?

There is evidence that COX-2 and PGE\textsubscript{2} receptors, particularly EP\textsubscript{1} and EP\textsubscript{4}, are up-regulated in injured nerves and/or the surrounding tissues 2—4 weeks after partial sciatic nerve ligation (PSNL) in rats, and that the PSNL-induced neuropathic pain is reversed by ketorolac, a non-selective COX inhibitor, NS-398, a selective COX-2 inhibitor, and ONO-8711, a selective EP\textsubscript{1} receptor antagonist.\textsuperscript{44—48} Most interestingly, increased expression of EP\textsubscript{1} receptors has been reported in human injured brachial plexus nerves, painful neuromas and avulsion injured dorsal root ganglion (DRG).\textsuperscript{49} A recent report shows that spinal PGE\textsubscript{2} formed by mPGES-1 participates in the maintenance of PSNL-evoked neuropathic pain by activating EP\textsubscript{1} and EP\textsubscript{4} receptors in the central terminals of primary afferent fibers.\textsuperscript{50} On the other hand, it is to be noted that spinal PGs, derived from COX-1 but not COX-2, may be involved in early allodynia, 24 h after L5—L6 spinal nerve ligation in rats.\textsuperscript{51} Taken together, in the peripheral and central terminals of nociceptors, PGE\textsubscript{2} appears to play a role in the maintenance of neuropathic pain, and selective EP\textsubscript{1} receptor antagonists, COX inhibitors and selective mPGES-1 inhibitors might be available for the treatment of neuropathic pain.

7. APPLICATION OF PGE\textsubscript{2} RECEPTOR ANTAGONISTS TO TREATMENT OF VISCERAL PAIN AND HEADACHE

A human study has shown that acid infusion into the lower esophagus caused a decrease in upper esophageal pain thresholds in response to electrical stimulation, and this secondary esophageal hyperalgesia was attenuated by ZD6416, an EP\textsubscript{1} receptor antagonist, suggesting a role of PGE\textsubscript{2}/EP\textsubscript{1} in human visceral pain hypersensitivity.\textsuperscript{52} EP\textsubscript{1} receptors are not only expressed in the primary afferents, but also in the urothelium, contributing to PGE\textsubscript{2}-induced facilitation of the micturition reflex and to enhanced afferent nerve activity during urinary bladder inflammation.\textsuperscript{53,54} We have shown that ONO-8130, a selective EP\textsubscript{1} receptor antagonist, reverses bladder pain-like behavior and referred hyperalgesia in mice with cyclophosphamide-induced cystitis, and prevents prompt phosphorylation of ERK in the spinal dorsal horn following intravesical administration of PGE\textsubscript{2}.\textsuperscript{55} There is also evidence for involvement of peripheral EP\textsubscript{2} receptors in the regulation of bladder micturition and bladder nociception.\textsuperscript{56} Thus, selective antagonists of EP\textsubscript{1} receptors and, perhaps, EP\textsubscript{3} receptors may be useful for treatment of visceral pain including esophageal and/or bladder pain.

EP\textsubscript{3} receptors in the peripheral nociceptors mediate peripheral PGE\textsubscript{2}-evoked hyperalgesia, as described above, and also contribute to PGE\textsubscript{2}-induced vasodilation in human cerebral artery rings.\textsuperscript{22} In this context, it is proposed that BGC20-1531, an EP\textsubscript{3} receptor antagonist, may be useful as a putative new treatment for migraine headache that results from cerebral vasodilation and sensitization of the trigeminal nerves followed by neurogenic inflammation.\textsuperscript{22}

8. CONCLUSION

The pro-nociceptive role of PGE\textsubscript{2} has long been known, and NSAIDs including selective COX-2 inhibitors are widely used for the treatment of pain. Recent studies imply that selective mPGES-1 inhibitors and selective antagonists of specific subtypes of PGE\textsubscript{2} receptors, particularly EP\textsubscript{1} and EP\textsubscript{4}, may be useful in order to avoid gastrointestinal, renal and cardiovascular side-effects. EP\textsubscript{4} receptor antagonists are considered to suppress inflammatory pain by inhibiting actions of peripheral PGE\textsubscript{2}, while the analgesic effect of EP\textsubscript{1} receptor antagonists may result from inhibition of actions of PGE\textsubscript{2} mainly in the spinal cord and secondarily in the peripheral tissues. Further, it is also likely that PGE\textsubscript{2} is implicated in neuropathic and visceral pain and in migraine headache. Thus, PGE\textsubscript{2} has a great impact on pain signals in the peripheral tissue and spinal cord, and pharmacological intervention in the upstream and downstream signals of PGE\textsubscript{2} may serve as novel therapeutic strategies for treatment of neuropathic pain, visceral pain and migraine, in addition to inflammatory pain.

REFERENCES
