BIOCHEMICAL PHARMACOLOGY 73 (2007) 165–174

available at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/biochempharm

Commentary

Prostanoids in nociception and pain

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ARTICLE INFO

Keywords:
Pain
Nociception
Prostaglandin
Analgesia
Mouse mutant
NSAID

ABSTRACT

Prostaglandins are lipid mediators produced by cyclooxygenases from arachidonic acid, which serve pivotal functions in inflammation and pain. Inhibition of their production is the major analgesic mechanism of action of non-steroidal anti-inflammatory drugs (NSAIDs)—but also the source of most of their unwanted effects. While the development of selective inhibitors of inducible cyclooxygenase (COX)-2 (so called coxibs) has greatly reduced gastrointestinal side effects, the recent disappointment about a potential cardiovascular toxicity of COX-2-selective inhibitors has boosted interest in alternative targets. The discovery of several prostaglandin synthases and of distinct prostaglandin receptors has unraveled an unforeseen diversity within the prostanoid synthetic pathway. Behavioral and electrophysiological work in particular with genetically engineered mice meanwhile provides new clues to the role of different prostaglandins, prostaglandin synthases and prostaglandin receptors in pain pathways.

1. Introduction

Thirty-five years ago, the pioneering work of Sir John Vane and colleagues [1] has identified inhibition of prostaglandin synthesis as the dominant mechanism of action of acetylsalicylic acid (aspirin) and related non-steroidal anti-inflammatory drugs (NSAIDs). This inhibition occurs through the blockade of the two cyclooxygenases (or prostaglandin G/H synthases, EC 1.14.99.1), constitutively expressed cyclooxygenase (COX)-1 and inducible COX-2. Both enzymes convert arachidonic acid, which is released upon tissue damage and inflammation from cell membranes through activation of phospholipases A2, into the prostanoid precursors PGG_2 and PGH_2. Tissue-specific terminal prostaglandin synthases or isomerases then convert PGH_2 into the different biologically active prostaglandins (PGD_2, PGE_2, $PGF_{2\alpha}$, PGI_2 [prostacyclin]) and thromboxane A2 (TXA_2), collectively called prostanoids. These prostanoids exert most of their actions via rhodopsin-like G-protein coupled receptors, which differ in their agonist selectivity, tissue distribution and signal transduction pathways. It is meanwhile generally accepted that both the desired effects (analgesia, antipyresis and the anti-inflammatory

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Abbreviations: CFA, complete Freund's adjuvant; COX, cyclooxygenase (EC 1.14.99.1); CSF, cerebrospinal fluid; DRG, dorsal root ganglion; GlyRα3, glycine receptor α3 subunit; GST, glutathione S transferases; LPS, lipopolysaccharide; mPGES, microsomal prostaglandin E synthase (EC 5.3.99.3); NSAID, non-steroidal anti-inflammatory drug; PG, prostaglandin; PGIS, prostaglandin I synthase (EC 5.3.99.4); PKA, cAMP-dependent protein kinase; PKC, protein kinase C; PPAR, peroxisome proliferator activated receptor; RT-PCR, reverse transcriptase-polymerase chain reaction; TRPV1 channel, vanilloid type 1 transient receptor potential channel; TTX, tetrodotoxin; TXA2, thromboxane A2

0006-2952/$ – see front matter
doi:10.1016/j.bcp.2006.07.037

actions) and the unwanted effects (e.g. gastrointestinal toxicity and nephrotoxicity) of COX inhibitors, originate from inhibition of prostaglandin formation. Enormous efforts have been made throughout the last 30 years both in basic and therapy-oriented research to better understand the various biological functions of COX products aiming at the development of better-tolerated analgesic drugs.

With the arrival of COX-2-selective agents this issue seemed largely resolved as COX-2 selective blockers exhibited much less gastrointestinal side effects than conventional COX inhibitors. This euphoria however was significantly blunted when work from experimental pharmacology indicated that COX-2 was present in several organs already in the absence of inflammation and served important physiological functions in addition to its pro-inflammatory effects. Such constitutive expression of COX-2 has meanwhile been reported for many organs including kidney [2,3], spinal cord [4] and brain [5]. It was therefore not totally unexpected, when in 2000 the VIGOR study group reported unforeseen potential side effects of a COX-2 selective inhibitor, in this case a higher incidence of cardiovascular events in rofecoxib-treated patients as compared to those on naproxen [6]. Later, skepticism reached a maximum when Merck withdrew its rofecoxib (VIOXX^{TM}) from the market following results from a controlled clinical trial (APPROVe [7]) that suggested that the risk of cardiovascular events was nearly twice as high in patients who had taken rofecoxib for $\geq$18 months than in placebo-treated control patients.

These events have boosted research in alternative targets in the prostanoid synthetic pathway. At least 10 different terminal prostaglandin synthases produce the different biologically active prostanoids and more than 8 receptors exists that mediate their effects. Such targets should in principle allow an interaction with the prostanoid pathway with much higher specificity than achievable with the available drugs.

In the process of searching for suitable novel targets within the prostanoid pathway the obvious tasks are to identify the prostanoids and prostanoid receptors responsible for a specific function and the respective terminal prostaglandin synthases. Because pain is by far the most frequent indication of COX inhibitors, this review will focus on the role of different prostanoids, prostanoid receptors and prostanoid synthases in pain.

2. Acute-inflammatory-neuropathic pain

Pain is not a single entity, but differs in its underlying causes, symptoms and neurobiological mechanisms. On a coarse scale, three major forms of pain can be distinguished. Nociceptive pain originates from the acute activation of primary nociceptive nerve fibers. It serves an important physiological function as it protects the body from tissue damage. The optimal analgesic would spare this pain form. Inflammatory pain originates from all forms of inflammation. It may still serve some physiological function as it makes us take special care of, e.g. an inflamed joint and may thus promote healing. It can nevertheless significantly reduce the wellbeing of patients. Finally, neuropathic pain originates from the damage of peripheral or central nerves and neurons. It is certainly the form of pain most difficult to treat. It is often accompanied by intense spontaneous pain and by pain evoked by even modest stimulation. Inflammatory and neuropathic pain may outlast the duration of the primary cause of pain. They can thus turn into chronic pain syndromes, in which plastic changes in nociceptive processing have occurred that are no longer readily reversible by pharmacological treatment.

3. Prostaglandins involved in pain sensitization

An obvious way to determine which prostanoids are involved in pain sensitization is to inject different prostanoids locally at various sites of the pain pathway. Such an approach has been used already in the very early times of prostanoid research. Ferreira [8] found that subcutaneous infusion of small amounts of PGE_1 in human volunteers caused pronounced hyperalgesia, a state of increased pain sensitivity. Later, the same author described similar hyperalgesic effects of PGE_2 and PGI_2 in rats and dogs [9]. Since then, it has been a long-held view that prostaglandins sensitize the nociceptive system only at the level of the peripheral nociceptor through prostanoid receptors located on the peripheral terminals of primary sensory (dorsal root ganglion, DRG) neurons. Accordingly, COX inhibitors were erroneously classified as ''peripheral'' analgesics. During the 1990s however it was progressively realized that the prostaglandins exerted at least part of their pronociceptive effects also in the CNS mainly at the level of the spinal cord. Behavioral experiments demonstrated that prostaglandins, mainly PGE_2, injected intrathecally into the spinal canal caused profound pain sensitization [10–13]. Furthermore, RT-PCR studies and Western blot analyses showed that peripheral inflammation induced COX-2 mRNA and protein expression not only at the site of (peripheral) inflammation but also in the spinal cord [4,14]. Accordingly, local injection of both non-specific and COX-2-selective inhibitors into the spinal canal produced analgesia [15,16].

While a substantial role of prostaglandins in central pain sensitization is well accepted, the contribution of different prostanoids and prostanoid receptors to centrally mediated hyperalgesia is still debated. Reinold et al. [17] found pronounced pronociceptive effects of intrathecally injected PGE_2, but not of PGD_2, PGI_2, or $PGF_{2\alpha}$. However, other groups have reported nociceptive sensitization by $PGF_{2\alpha}$ [18,19], PGD_2 [10] and PGI_2 [20]. The physiological meaning of these results is difficult to judge. Guay et al. [21], who have analyzed concentrations of different prostaglandins in the cerebrospinal fluid (CSF), found that PGE_2 was the most prevalent prostanoid in the CSF and exhibited the highest increase after peripheral carrageenan-induced inflammation. In almost all studies employing local injections of prostanoids into the spinal canal, the actual prostaglandin concentration after distribution in the CSF is unknown, yet the concentrations in the injection volume were usually in the millimolar range (e.g. 0.1–100 nmol/5–10 μl). Binding assays performed in heterologous expression systems indicate that at these concentrations prostaglandins sometimes lose their specificity and can

interact with receptors different from the ''primary'' target [22,23].

4. Prostaglandin receptors

As outlined in Section 1, prostanoids exert most of their action through G-protein coupled receptors. At least in vitro they also bind to nuclear peroxisome proliferator activated receptors (PPARs). So far, there is no evidence for an involvement of PPARs in pronociceptive effects of prostanoids in vivo. Because highly specific prostanoid receptor antagonists useful for in vivo testing have been lacking for a long time and are in many cases still not available most studies addressing the contribution of individual prostanoid receptors to different functions in physiology or pathophysiology have relied on genetically modified mice. Such mice have been generated by several groups [24,25] and – forced by the pivotal role of prostaglandins in pain – already the initial screening of these mice often included analysis of pain phenotypes (Table 1). Unfortunately, most of these early studies have concentrated on only one, at best on a few models and systematic analyses in different models are still missing in many cases. Often, prostaglandins have been injected locally at peripheral or central sites and either spontaneous nociceptive reactions or changes in sensitivity of thermal or mechanical stimulation were monitored. Because inflammation and neuropathy could potentially induce prostaglandin receptors not present in naïve animals, these studies may produce false negative results. An alternative and in terms of therapeutic relevance probably more predictive approach should be the analysis of nociceptive phenotypes in models involving endogenous prostaglandin production. This can be achieved in models of chemically induced pain (e.g. formalin-evoked pain, writhing test), in tests of inflammatory pain elicited by, e.g. subcutaneous injections of complete Freund's adjuvant (CFA), carrageenan or zymosan A, or in neuropathic pain, e.g. following peripheral nerve damage. Meanwhile, an overwhelming number of reports has been published on nociceptive phenotypes of different prostaglandin receptor-deficient mice, yet probably due to the small number of systematic analyses it is nearly impossible to reconcile the published data without obvious inconsistencies.

A potential limitation of these studies is that they relied so far exclusively on non-conditional mouse mutants. The absence of the respective enzyme or receptor already throughout development can sometimes trigger the up-regulation of other receptors and enzymes compensating this loss. With respect to the nociceptice phenotypes of these mice this may not be a big problem, but the arrival of inducible mutants and their analysis may nevertheless foster unexpected results.

5. Peripheral mechanisms

A consistent finding throughout most studies is that prostanoid receptor-deficient mice exhibit normal baseline nociceptive behavior. Responses to acute thermal and mechanical stimulation were in nearly all cases indistinguishable from those obtained for the corresponding wild-type mice, indicating that prostaglandins are not required for acute nociception and that they do not exert a tonic influence on the nociceptive system [17,26,27].

Consistent with the existence of only a single IP receptor, the pronociceptive effects of PGI_2 were completely absent in $IP^{-/-}$ mice tested in the mouse writhing test [27] or after subcutaneous injection [26]. More importantly, $IP^{-/-}$ mice also showed reduced writhing responses after intraperitoneal injection of acetic acid to an extent similar to the one obtained with the COX blocker indomethacine [27], supporting an important contribution of PGI_2 to nociceptive sensitization. Because the writhing test usually lasts for less than an hour, it is too short to cover central prostaglandin-dependent sensitization, which requires spinal COX-2 induction. These results therefore probably reflect changes in peripheral rather than central (spinal) pain sensitizing mechanisms.

Similar studies have been conducted with mice deficient in EP receptors. These receptors show considerably more

Table 1 – Prostanoid receptors involved in pain sensitization: G-protein coupling, preferential expression in the nociceptive system and phenotypes of the genetically modified mice

Receptor	Coupling [22]	Preferential expression relevant to nociception	Phenotypes of mice deficient in the respective prostanoid receptor in tests of nociception and inflammation
EP1	$G_{q/11}$	Primary sensory neurons [28], including their spinal terminals	Reduced nociception in the acetic acid writhing test [30]
EP2	G_s	Intrinsic spinal cord neurons [52], possibly also primary sensory neurons [29]	No hyperalgesic effect of spinal PGE_2 and reduced delayed inflammatory hyperalgesia [17]; unchanged neuropathic pain in the chronic constriction injury model [94]
EP3	A: G_i; B: G_s; C: G_s; D: G_i, G_s, G_q	Primary sensory neurons, including their spinal terminals [28,53,54]	Reduced nociception in the acetic acid writhing test only after pretreatment with LPS [43]; no change in nociceptive sensitization upon intrathecal or subcutaneous PGE_2 injection [17]
EP4	G_s	Primary sensory neurons [28]	Decreased severity of collagen- or collagen antibody-induced arthritis [50,51]
IP	G_s	Primary sensory neurons [28]	Reduced nociception in the acetic acid writhing test and reduced edema formation after carrageenan injection [27], no pain sensitization after peripheral PGI_2 injection [26]

diversity. Unlike PGI_2, which acts at a single receptor, PGE_2 exerts its effects through four different G-protein coupled receptors encoded by different genes and called EP1 through EP4. In situ hybridization and RT-PCR studies suggest that all four EP receptor subtypes are expressed in subsets of primary sensory (dorsal root ganglion; DRG) neurons [28,29]. It is obvious that EP receptor-deficient mice could not only be used to clarify the role of PGE_2 in pain sensitization, but also to identify the contribution of individual EP receptors. In tests of peripheral pain sensitization, $EP1^{-/-}$ mice exhibited a phenotype very similar to that of $IP^{-/-}$ mice. They show reduced responses in the writhing test [30] and reduced peripheral pain sensitization after subcutaneous injection of mustard oil [26].

A dominant role for IP and EP1 receptors in the peripheral nociceptive system is strongly backed by studies elucidating the mechanistic basis of peripheral pain sensitization. If one accepts that prostanoids sensitize nociception this process must finally occur through an increase in either neuronal excitability or a change in synaptic communication. Both of these processes would finally involve ion channels as targets of prostanoids. Over the years numerous ion channel targets of prostaglandins have indeed been identified in various tissues [31], however relatively few studies have addressed such downstream targets in the nociceptive system. Nevertheless these studies appear extremely valuable, as they can put behavioral studies, which are much more sensitive to differences in genetic background, etc., onto a mechanistic basis.

One such target in the periphery is the vanilloid type transient receptor potential channel (TRPV1) also known as capsaicin receptor. This receptor is a non-selective cation channel mainly expressed on primary nociceptive nerve endings, where it integrates multiple noxious stimuli including heat above 43 °C and tissue acidosis [32,33]. It is specifically activated by capsaicin an ingredient of hot pepper plants. Responses of this channel to capsaicin are potentiated several fold by PGE_2 and PGI_2 in DRG neurons [26,34]. This potentiation could be reconstituted in HEK 293 cells after cotransfection of TRPV1 with EP1 or IP receptors. Activation of EP1 and IP receptors by PGE_2 and PGI_2 shifted the threshold of activation by heat from 43 °C to temperatures as low as 31 °C. Hyperalgesia induced by PGE_2 and more importantly, because it indicates activity of endogenous PGE_2, by mustard oil was reduced by about 50% in EP1 receptor-deficient mice and PGI_2-induced thermal hyperalgesia was completely abolished in TRPV1 or IP receptor-deficient mice. Because TRPV1 receptor activation can be potentiated through protein kinase A (PKA)-dependent and protein kinase C (PKC)-dependent phosphorylation [26,35], the coupling of IP and EP1 receptors to different G-proteins (G_s and $G_{q/11}$) does not contradict this dual modulation. Facilitation of TRPV1 by PGE_2 may also link increases in cAMP to stimulation of neuropeptide (CGRP and substance P) release [35].

Other potential targets of peripheral prostanoids are tetrodotoxin (TTX) resistant Na^+ channels (Nav1.8), which are specifically expressed in primary nociceptors [36]. PGE_2 shifts the voltage-dependence of this channel (and also of Nav1.9 [37]) to more hyperpolarized values [38,39]. The physiological meaning of this process has not been studied in great detail, but Nav1.8-deficient mice develop inflammatory hyperalgesia with a certain delay [40], which would be consistent with a contribution of this channel to PGE_2 induced inflammatory pain. It is not known which prostaglandin receptors mediate this potentiation, but according to the role of PKA in this process, EP receptors different from EP1 (which are G_q-coupled) should be involved. Matsumoto et al. [41] demonstrated that EP2 and EP4 receptor activation potentiates TTX-resistant currents in rat nodose ganglion neurons, a process that, if it is also present in primary sensory neurons of the DRG, could explain a small reduction in mechanical sensitization seen in $EP2^{-/-}$ mice upon subcutaneous injection of PGE_2 [17].

In general, a possible contribution of EP receptor subtypes different from EP1 to peripheral pain sensitization is less well established. Best studied among those EP receptors is the EP3 subtype. RT-PCR results suggest that the EP3C splice variant, which couples to stimulatory G-proteins, is predominantly expressed in DRG neurons [42]. In naïve $EP3^{-/-}$ mice subcutaneous PGE_2 evoked hyperalgesia indistinguishable from that seen in wild-type mice [17]. However, Ueno et al. [43] reported that EP3 receptors did contribute to peripheral hyperalgesia after a conditioning stimulus with LPS suggesting that pro-inflammatory agents would induce not only the expression of COX-2 but also of EP3 receptors. Yet, although such an up-regulation was observed for COX-2, it was not observed for any of the EP receptors [44] and another study reported that $EP3^{-/-}$ mice developed normal thermal and mechanical sensitization lasting for 1 week after subcutaneous injection of the yeast extract zymosan A into one hind paw [17]. There is even evidence for an antinociceptive role of EP3C receptors on the spinal terminals of primary afferent nociceptive fibers [45].

The situation is similarly complex for the EP4 receptor. In isolated DRG neurons, the EP4 receptor selective agonist ONO-AE1-329 and PGE_2 increased cAMP production [46], an action, which is blocked by pretreatment with EP4 receptor antisense oligonucleotides [42,47]. $EP4^{-/-}$ mice are in general less well characterized, probably because they die on several genetic backgrounds from a patent ductus arteriosus [48,49]. A contribution of this receptor to chronic inflammation has recently been demonstrated [48,50,51], but in vivo evidence for a direct involvement in pain sensitization is still missing.

6. Central mechanisms

While peripheral pain sensitization is generally believed to occur through a direct sensitization of specialized primary nociceptors, central mechanisms appear to involve more complex processes. They can not only sensitize specific nociceptive pathways, which leads to central or secondary hyperalgesia, but can also elicit mechanical allodynia, which means that input from low threshold mechanosensitive fibers can evoked pain.

As outlined above PGE_2 is the dominant prostaglandin in the spinal nociceptive system. Among the different EP receptor subtypes, expression in intrinsic spinal cord neurons is best documented for the EP2 receptor [52], while EP1 and EP3 receptors, which are also found in the spinal cord dorsal horn,

appear to be localized at the central terminals of the primary afferent nerve fibers [28,53,54].

In the spinal cord a number of studies have reported effects of PGE_2 on neuronal excitability [55,56] or synaptic transmission [57,58]. These effects include an increased release of glutamate [58], a direct depolarization of deep dorsal horn neurons [56] and an inhibition of strychnine-sensitive glycine receptors [57]. For the latter process a detail analysis of the underlying signal transduction mechanisms has been performed which allowed determination the relevance of this process for pain sensitization in vivo. Inhibition of strychnine-sensitive (inhibitory) glycinergic neurotransmission occurs through a postsynaptic mechanism involving the activation of EP2 receptors, which in turn stimulate adenylylcyclase, trigger increases in cAMP, and lead to a PKA-dependent phosphorylation of a specific glycine receptor isoform. This isoform contains the glycine receptor α3 subunit (GlyRα3), which is in the spinal cord distinctly expressed in the superficial layers, where primary nociceptive nerve fibers terminate [57,59]. As expected from these results, $EP2^{-/-}$ mice and $GlyR\alpha3^{-/-}$ mice do not only lack PGE_2-induced inhibition of glycinergic neurotransmission, but also pain sensitization induced by intrathecal injection of PGE_2. Noteworthy, both types of knockout mice exhibit almost identical phenotypes in models of peripheral inflammation, where they recover much faster from inflammation-induced hyperalgesia than wild-type mice [17,59,60]. Importantly, $GlyR\alpha3^{-/-}$ mice behave normally in tests of acute nociception probably because the loss of GlyRα3 is compensated by another GlyRα subunit, which is not inhibited by PKA. These results show that disinhibition of spinal nociception constitutes the major pronociceptive mechanism of spinally produced PGE_2. They further suggest that antagonists at the EP2 receptor might be useful as centrally acting non-opioidergic anti-hyperalgesic agents.

The $GlyR\alpha3^{-/-}$ mice also allowed the assessment of the contribution of spinal PGE_2-dependent sensitization to inflammatory pain [59]. In the zymosan A model of peripheral inflammation it turned out that PGE_2-dependent sensitization was particularly important for later stages of inflammatory pain. It is also interesting to note that GlyRα3-deficient mice exhibited no significant phenotype in a model of neuropathic pain, the so called chronic constriction injury model suggesting that this pathway does not play a significant role in neuropathic pain (compare Table 1)—a result that would fit with both experimental studies and clinical observations that neuropathic pain only poorly responds to COX inhibitors [61–64].

Other groups, who have employed intrathecal injections of PGE_2 in EP receptor-deficient mice, report a prominent contribution of EP1 receptors. Minami et al. [65] tested the effect of intrathecally injected PGE_2 (about 0.04 nmol in 5 μl saline) on allodynia (tested with a paintbrush) and hyperalgesia (assessed in the hotplate test). PGE_2-induced allodynia was nearly absent in $EP1^{-/-}$ mice. A significant contribution of EP1 receptors has also been suggested by Namiki and coworkers [66], who injected ONO-8711, a rather specific EP1 receptor antagonist, either peripherally or spinally. While an inhibitory effect on inflammatory pain after peripheral injection fits with the expectations, its effectiveness after intrathecal injection in particular in neuropathic pain models comes as a surprise. Interestingly, the authors attribute the antinociceptive action of ONO-8711 to an antagonism at EP1 receptors located on the central terminals of primary afferent nociceptive fibers [67], where PGE_2 might facilitate the release of glutamate as previously suggested [58]. However, neither Baba et al. [56] and Ahmadi et al. [57] have found evidence for a potentiating effect of PGE_2 on glutamate release in the dorsal horn. In this context it is interesting to note that the antinociceptive effect of ONO-8711 was negligible in the early phase of inflammatory pain (3 h after carrageenan injection) and maximal at 15 h. This time course very much reflects the phenotype of $EP2^{-/-}$ and $GlyR\alpha3^{-/-}$ mice. It cannot be excluded that non-specific effects on EP receptors different from EP1 occurred at the doses of ONO-8711 used in these studies (10–100 μg in 10 μl, equivalent to 2.5–25 mM). Unfortunately, so far no pain studies are available that used the more specific EP1 receptor antagonist ONO-8713 [68] and nor has a comprehensive analysis of $EP1^{-/-}$ mice in different mouse models of pain been published yet.

Taken together, our present knowledge indicates that there is not a single prostanoid or prostanoid receptor responsible for pain sensitization by COX products. Most relevant among them however seem to be PGI_2 acting on IP receptors and PGE_2 acting on EP1 and EP2 receptors. IP and EP1 may turn out to be most relevant during early stages of inflammatory pain, while EP2 may be more relevant at chronic stages of inflammation. Although not yet known, such a scenario indicates that the blockade of a single prostanoid receptor might not be sufficient for adequate analgesic therapy (Fig. 1).

7. Prostaglandin synthases

Inhibition of a prostanoid synthase might be considered as an alternative strategy that would fall in between global blockade by COX inhibition and blockade of a single receptor. In total more than 10 enzymes have been discovered that convert prostaglandin precursors into biologically active prostaglandins. According to the pivotal role of PGI_2 and PGE_2 in pain, this section will focus on PGI_2 and PGE_2 synthases (Table 2). As already seen during the discussion of prostaglandin receptors, the PGE_2 pathway displays much greater diversity than the PGI_2 system also in terms of prostaglandin synthases. While the conversion of PGH_2 to PGI_2 (EC 5.3.99.4) occurs through a single terminal prostaglandin synthase (PGIS) [69], several isoenzymes mediate the PGE_2 synthase reaction (EC 5.3.99.3) [70].

Mice deficient in PGIS have been generated, but published data on their nociceptive phenotype is not available, although their phenotype should be very similar to the one obtained in $IP^{-/-}$ mice. Much more interesting is the situation in case of the PGE_2 synthases.

PGE_2 can be produced by several enzymes: two membrane bound forms of PGE synthases, called mPGES-1 and mPGES-2, and one cytosolic form, termed cPGES. In addition, GST-μ2 and GST-μ3, two glutathione S transferases might also contribute to the formation of PGE_2 from PGH_2 [71]. Best studied are the two mPGES and cPGES. Although all three enzymes convert PGH_2 into PGE_2, they differ considerably in their spatial and temporal pattern of expression and their coupling to either COX-1 or COX-2.

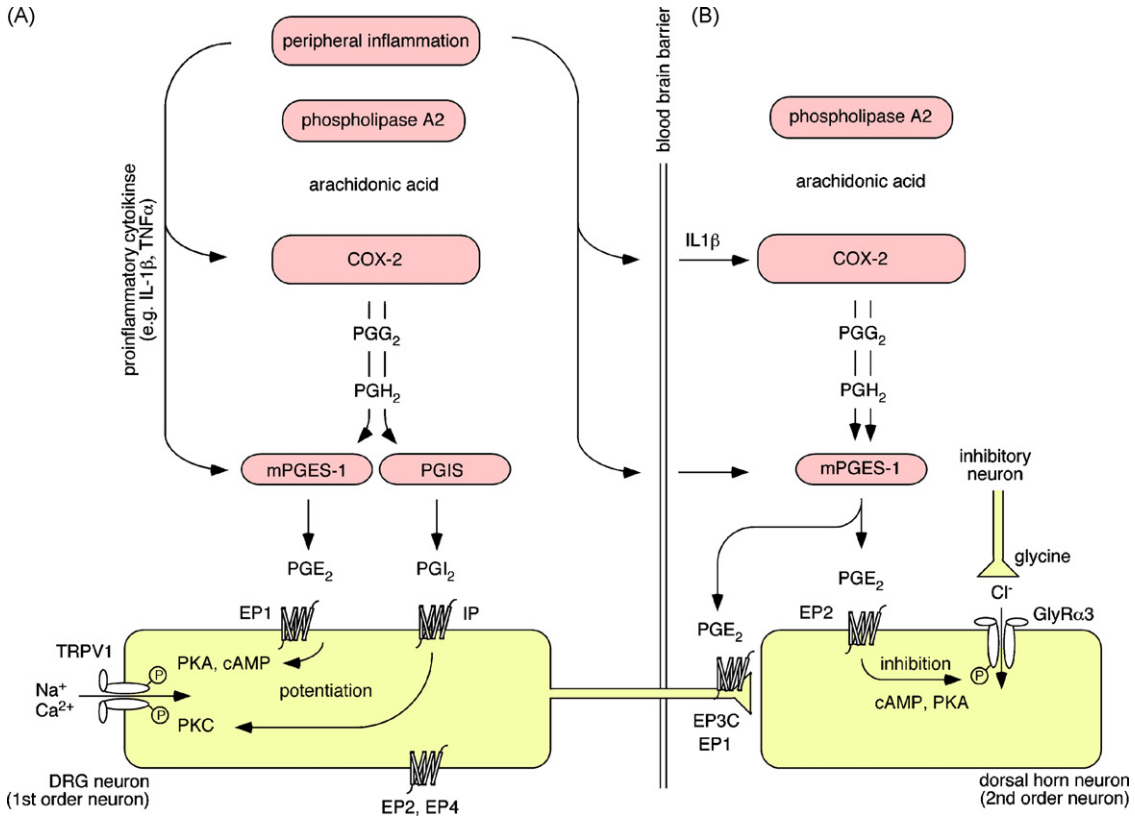

Fig. 1 – Schematic representation of major signal transduction pathways responsible for pain sensitization and activated by prostanoids. (A) Peripheral inflammation induces COX-2 and mPGES-1 both in the periphery and in the CNS through the action pro-inflammatory cytokines including IL-1β and TNFα. It is not exactly known what stimuli trigger COX-2 expression in the CNS, but apart from IL-1β, other cytokines and increased neuronal activity may be involved there. Arachidonic acid released from phospholipids of the cell membrane by phospholipases A2 serves as substrate for COX-2, which generates first PGG_2 through COX activity and then PGH_2 though peroxidase activity. PGH_2 is the substrate for most terminal prostaglandin synthases including mPGES-1 and PGIS. Pain sensitization by members of the prostanoid family occurs both in the periphery and in the CNS, in particular in the spinal cord. PGE_2 and PGI_2 sensitize TRPV1 channels located on primary nociceptive nerve fibers (DRG neurons, first order neurons) through an interaction with EP1 and IP receptors. The activation of TRPV1 is facilitated through PKA and PKC-dependent phosphorylation. There is good evidence for the expression of other EP receptor subtypes (EP2, EP3C and EP4) in primary nociceptive neurons. (B) In the spinal cord dorsal horn, PGE_2 acts on EP2 receptors located on intrinsic spinal cord neurons, where it leads to a PKA-dependent phosphorylation and inhibition of glycine receptors containing the α3 subunit. This subunit is distinctly expressed in the superficial layers of the dorsal horn, where primary nociceptive afferents terminate. PGE_2 produced in the spinal cord may also act on the EP1 and EP3C receptors expressed on central terminal of primary nociceptive afferents. The physiological consequences of their activations are largely unknown.

Table 2 – Terminal prostaglandin synthases involved in pain sensitization: coupling to COX isoforms, temporal expression pattern and phenotypes of genetically modified mice

Terminal prostaglandin synthase	Coupling	Temporal expression pattern	Phenotypes of mice deficient in the respective synthase in tests of nociception and inflammation	References
mPGES-1	COX-2	Inducible	Reduced nociception in the acetic acid writhing test; normal responses in the hot plate test; reduced type II collagen induced arthritis	[72–76]
mPGES-2	No preferential coupling to either COX-1 and COX-2	Constitutive	Not yet known	[70]
cPGES	COX-1	Constitutive in most organs, inducible in the brain	Not yet known	[93]
PGIS	COX-2	Constitutive	Not yet known	[69]

mPGES-1 is in many tissues closely associated with COX-2, primarily metabolizes PGH_2 produced by COX-2 and is, similar to COX-2, induced by pro-inflammatory stimuli both in peripheral tissues as well as in the spinal cord [72–74].

In contrast to mPGES-1, mPGES-2 and cPGES are constitutively expressed in most organs and show no strict coupling to COX-2. In fact, cPGES seem to be closely associated with COX-1 and mPGES-2 shows no preferential coupling to either COX-1 or COX-2. This differential association of PGES isoforms to COX-1 or COX-2 is reflected in the phenotypes of genetically targeted mice. Mice deficient in mPGES-1 almost completely lack arachidonic acid-induced PGE_2 increases after induction of COX-2 with LPS [75,76]. In the mouse writhing test they show a reduction in acetic acid-evoked responses by about 50%. Collagen-induced and collagen antibody-induced arthritis develop in a much milder fashion in mPGES-$1^{-/-}$ mice than in wild-type mice.

Although mPGES-$2^{-/-}$ mice and cPGES$^{-/-}$ mice have not yet been tested in such models, available evidence indicates that most of the COX-2-dependent rise in PGE_2 both in the peripheral tissue and in the CNS originates from mPGES-1.

8. Implications for drug discovery

It is obvious that the prostanoid receptors and synthases discussed above qualify as potential targets for novel and potentially better-tolerated analgesics. All of them are in principle ''drugable'' targets and for many of them more or less specific ligands have already been developed [24].

In this context it is important to discuss which prostanoids and prostanoid receptors should not be blocked in order to avoid the side effects of present COX inhibitors. Two such side effects shall be discussed here in more detail: gastrointestinal toxicity and cardiovascular side effects.

Work from EP receptor-deficient mice suggests that reduced activation of EP1 and EP3 receptors underlies the ulcerogenic effects of classical COX inhibitors [77–79], yet pharmacological studies on human tissue have suggested a contribution also for EP4 receptors [80].

The situation with the cardiovascular side effects appears even more complex. Although a comprehensive analysis of the available data and hypotheses would go far beyond the scope of this review, the actual basic ideas shall briefly be discussed here. Fitzgerald's group [81,82] and others [83] have suggested that the cardiovascular risk of COX-2-selective agents might come from the imbalance of vasoconstrictive and proaggregatory thromboxane and vasodilating and anti-aggregatory PGI_2. According to this hypothesis, coxibs would reduce endothelium- and COX-2-derived protective PGI_2, but would in contrast to classical NSAIDs leave COX-1-derived thromboxane unchanged. Others have argued that this view might be too simplistic. Indeed, it is not known whether COX-2 expressed in arteriosclerotic plaques is protective or harmful [84]. Furthermore, individual COX-2 selective agents may have pharmacological effects different from COX inhibition [85]. Finally, it cannot be excluded that all COX inhibitors (except for low-dose acetylsalicylic acid) bear a significant cardiovascular risk due to the increase in blood pressure that is seen with nearly all COX inhibitors.

According to the PGI_2/TXA_2 imbalance hypothesis, drugs targeting EP receptors or PGE synthases should be devoid of cardiovascular effects. However, if cardiovascular risks result from the rise in blood pressure or from inhibition of prostanoid production in the arteriosclerotic plaque, the situation becomes more complicated. Although major abnormalities in renal function have primarily been observed in PGIS deficient mice [86,87], EP2 and EP4 receptor-deficient mice also show subtle abnormalities in blood pressure regulation [88–90], which may become important during prolonged treatment of predisposed patients.

Finally it cannot be excluded that new unwanted effects could arise from the targeting of a single (EP) receptor. For example, EP2 receptors exert an inhibitory control on the proliferation of lymphocytes [91]. Blockade of EP2 receptors might hence promote the progression of autoimmune diseases (e.g. rheumatoid arthritis). On the other hand EP2 receptors have also been implicated in positive feedback loops leading to PGE_2 mediated increases in COX-2 expression [92]. Whether an inhibition of this feedback loop would be beneficial or harmful is difficult to predict.

9. Conclusions

In the outline of this review, the question was raised whether suitable analgesic targets in the prostanoid pathway exist that are different from the two COX isoforms. This question is still far from being solved, but work mainly performed in genetically modified mice has put corner stones for future direction. Such studies indicate that PGI_2 and PGE_2 are not only the dominant mediators for inflammatory pain, but that their inhibition is also responsible for the gastrointestinal and cardiovascular toxicity of conventional NSAIDs. The majority of available evidence suggests that sparing PGI_2 synthesis and IP receptors should reduce the risk of unwanted cardiovascular effects. Targeting of a single EP receptor could potentially further reduce unwanted effects including gastrointestinal toxicity, but this might be achieved on the expense of reduced analgesic efficacy. Blockade of inducible mPGES-1 might turn out as a favorable choice, which could keep the balance between reduced unwanted effects and sufficient efficacy. Finally, in the future drugs acting on downstream targets of PGE_2 such as peripheral TRPV1 receptors (already in development), TTX-resistant Na^+ channels or dorsal horn glycine receptors might constitute completely new classes of analgesic drugs.

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