Review TRENDS in Pharmacological Sciences Vol.27 No.9

Analgesic strategies beyond the inhibition of cyclooxygenases

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Blocking the formation of prostaglandins with cyclooxygenase (COX) inhibitors has been the treatment of choice for inflammatory pain for more than a century. Although these agents provide profound pain relief, their long-term use is hampered by severe side-effects, mainly ulceration of the upper gastrointestinal tract. The development of COX-2-selective inhibitors ('coxibs') has significantly reduced gastrointestinal toxicity, but evidence from controlled clinical trials and experimental studies indicates that the use of coxibs has a significant cardiovascular risk. Recently, signalling elements downstream of COX-2 inhibition have been identified, which offer a great diversity of possible targets. This review focuses on prostaglandin E synthases, prostaglandin receptors and downstream effectors of prostaglandins in the PNS and CNS, including transient receptor potential channels, tetrodotoxin-resistant Na^+ channels and inhibitory glycine receptors. These novel targets should enable inflammatory pain to be treated with improved specificity and, possibly, fewer side-effects.

Pharmacology of the prostanoid pathway

Nonsteroidal anti-inflammatory drugs (NSAIDs) are the most frequently used drugs worldwide. Their most prominent indication is the treatment of inflammatory pain, which constitutes a major medical problem in patients suffering from prevalent diseases including rheumatoid arthritis, osteoarthritis and, perhaps, migraine. Although inhibition of prostaglandin (PG) production by blocking the activity of cyclooxygenase (COX) is the main mechanism of their analgesic action [1], some of these drugs might also have COX-independent effects [2]. The two COX isoforms, constitutively expressed COX-1 and inducible COX-2, mediate the conversion of arachidonic acid into the prostaglandin precursors PGG_2 and PGH_2. These precursors are then processed by tissue-specific isomerases or (terminal) prostaglandin synthases into biologically active prostaglandins (PGD_2, PGE_2, $PGF_{2\alpha}$ and PGI_2) and thromboxane (collectively called prostanoids), which act on rhodopsin-like G-protein-coupled receptors (GPCRs).

It is not surprising that COX inhibitors, which discriminate only poorly between different prostanoids, not only interfere with pain but also disturb many body functions. Gastrointestinal toxicity, renal failure and cardiovascular risks (which have been the focus of recent research) [3] are well known side-effects of COX inhibitors. In most therapeutic areas, pharmacological intervention relies largely on drugs that target individual types or subtypes of receptor to gain significant specificity. Despite the success of this approach in other fields, inhibition of COX activity is the only strategy that is used routinely to interfere with the prostanoid pathway at present. However, considering terminal prostaglandin synthases rather than COXs increases the diversity of potential targets: whereas COXs are encoded by two genes, at least ten terminal prostaglandin synthases produce five biologically active prostanoids that, in turn, act on at least nine receptors, not including the various splice variants of the receptors. The physiological roles of many of these effectors have been identified and some might constitute potential targets for novel analgesics.

PGE_2 and PGI_2 are the prostanoids most relevant for the induction of inflammatory pain, and evidence indicates that the renal and cardiovascular toxicities of classical NSAIDs result predominantly from the inhibition of PGI_2 synthesis. This review focuses on synthesizing enzymes and receptors of PGE_2 and PGI_2, and their downstream targets in the nociceptive system.

Prostaglandins and prostaglandin receptors in pain sensitization

Peripheral inflammation induces the production of prostanoids in inflamed tissues and the CNS (mainly in the spinal cord). At both sites these act as pronociceptive and hyperalgesic mediators by either increasing the responsiveness of primary nociceptors or by changing the spinal processing of nociceptive input. Changes in the spinal processing of nociceptive signals induce central pain sensitization [4] and a phenomenon called allodynia in which stimuli that are normally not painful evoke pain (Box 1).

Evidence for the role of individual prostanoids to pain came first from *in vivo* experiments employing local injections of prostanoids. Most of these studies observed a strong pronociceptive effect of PGE_2 after either local subcutaneous injection or intrathecal injection into the spinal canal. There is also strong support for a pronociceptive role of PGI_2, mainly in the PNS, whereas results with PGD_2 and $PGF_{2\alpha}$ are ambiguous, with some reports describing pro-allodynic effects of these two prostaglandins after

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Available online 31 July 2006.

 0165-6147/$ – see front matter doi:10.1016/j.tips.2006.07.007

Box 1. Neuronal mechanisms of inflammatory pain

Stimuli that have the potential to cause tissue damage, including noxious heat and intense mechanical stimuli, are sensed by nociceptors. These are either thinly myelinated or unmyelinated, slow-conducting C fibres or Aδ fibres that transmit nociceptive ('pain') signals to the dorsal horn of the spinal cord. Nociceptive input is processed mainly in the superficial layers of the dorsal horn, where nociceptive fibres make synaptic connections with intrinsic spinal cord neurons. Projection neurons integrate excitatory postsynaptic potentials (EPSPs) that originate from primary nociceptive afferents, and local excitatory interneurons and inhibitory postsynaptic potentials (IPSPs) from GABA- and/or glycine-containing interneurons. Pain sensitization can occur at the level of the primary nociceptor, called primary hyperalgesia, and through changes in the central processing of nociceptive input, called secondary hyperalgesia [56]. Changes in central processing can also lead to a phenomenon called allodynia, which describes the painful sensation of normally innocuous stimuli. There is substantial evidence that PGE_2 contributes to all three forms of pain sensitization.

Traditionally it was thought that prostaglandins sensitize the nociceptive system only at the level of the primary nociceptor (i.e. in the periphery). Such peripheral mechanisms seem to be important primarily in the early phases of inflammatory pain whereas spinal processes probably prevail at later stages. TRPV-1 channels and TTX-resistant Na^+ channels are important targets of peripherally produced PGE_2 [44,49]. PGE_2 facilitates the activation of nociceptors by increasing receptor potentials in their peripheral endings (Figure I) and promotes the generation of action potentials by facilitating the activation of TTX-resistant Na^+ channels (Figure I). More recently, the significant contribution of spinal PGE_2 produced by inducible COX-2 [59] and mPGES-1 [9,25] has become apparent. Both enzymes are expressed in the spinal cord in response to peripheral inflammation via proinflammatory cytokines including interleukin 1β [11,22,59,60]. The relief of spinal nociceptive processing from glycine-mediated inhibition (disinhibition) (Figure I) appears as a major mechanism of PGE_2-mediated, central pain sensitization [8,54,55].

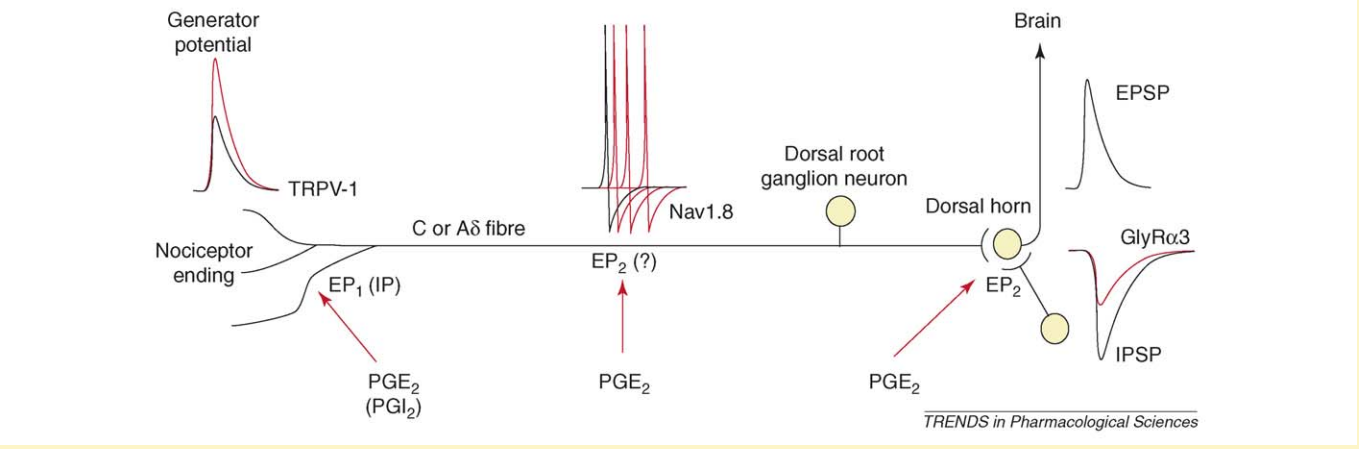

Figure I. PGE_2 modulates nociceptive signals at multiple sites in the pain pathway. At the peripheral endings of primary nociceptors, PGE_2 and EP_1 receptors potentiate TRPV-1 activation in concert with PGI_2 and IP receptors [44]. The generation and propagation of action potentials are facilitated by PGE_2 acting on Nav1.8 channels through EP_2 receptors [61]. In the spinal cord, EP_2 receptors seem to be the most relevant ones for PGE_2-mediated pain sensitization. Red traces denote changes elicited by PGE_2.

intrathecal injection (reviewed in Ref. [5]). These studies have several limitations. For example, the respective prostanoid receptors might be expressed only after sensitization, which would lead to false-negative results. In addition, the prostaglandin concentration is unknown after local bolus injection and the effect might be nonspecific. Finally, even if a positive result is obtained it is not known whether the concentrations reached are comparable to those achieved endogenously. Therefore, results obtained with either prostanoid receptor antagonists or with genetically modified mice that are deficient in either prostanoid receptors or prostanoid synthases are likely to be more indicative. Such studies have identified nociceptive phenotypes in mice with deficits in PGE_2 and PGI_2 pathways [6–9] but not in mice deficient in receptors for PGD_2 and $PGF_{2\alpha}$ (DP and FP receptors, respectively). Some evidence for a modulatory role of PGD_2 in pain pathways comes from experiments using mice that are deficient in neuronal (lipocalin-type) L-PGD-synthase [10].

Strong support for the dominant role of PGE_2 in central inflammatory sensitization also comes from a recent comprehensive analysis by Guay *et al.* [11], who demonstrated that PGE_2 is the most prevalent prostaglandin in cerebrospinal fluid and spinal cord tissue after peripheral, carrageenan-induced inflammation.

IP and EP receptors

Given the pivotal role of PGE_2 and PGI_2, specific inhibition of their production, receptors and downstream targets should be pursued. The cellular effects of both prostanoids are mediated by GPCRs: PGI_2 acts via the IP receptor, and PGE_2 acts through four receptors, termed EP_1–EP_4 receptors. Thus, in principle, pharmacological intervention should discriminate between different actions of PGE_2. Understanding of the *in vivo* function of individual prostanoid receptors has advanced by analysis of the prostanoid-receptor-deficient mice that have been generated by several groups [12,13] (Table 1). The pivotal role of prostanoids in pain sensitization has promoted the characterization of the nociceptive phenotypes of these mice. In many cases, initial screening includes tests of acute thermal nociception in either tail-flick or hot-plate tests, and of acute inflammatory pain in the, so-called, mouse writhing test. In most cases, acute thermal nociception was unchanged in prostanoid-receptor-deficient mice, indicating that prostanoids are not necessary for acute

Table 1. Nociceptive phenotypes in mice with targeted mutations in prostanoid pathways

Gene encoding	Nociceptive phenotype	Anti-inflammatory phenotypes	Other phenotypes relevant to possible side-effects of COX inhibitors
mPGES-1	Reduced nociception in the acetic acid writhing test, normal sensitivity in the hot-plate test [25], reduced neuropathic pain after spinal-nerve ligation [62]	No increase in PGE_2 production from macrophages after LPS treatment [9], reduced type II collagen-induced arthritis [9], reduced collagen antibody-induced arthritis [25]	Unknown
mPGES-2	Unknown	Unknown	Unknown
cPGES	Unknown	Unknown	Unknown
EP_1 receptor	Reduced nociception in the acetic acid writhing test [6], unchanged nociception in the formalin test [63], reduced peripheral pain sensitization by PGE_2 and mustard oil [44]	Unknown	Reduced systolic blood pressure and increased renin–angiotensin activity [6]
EP_2 receptor	No hyperalgesia after intrathecal PGE_2 [8], fast recovery from inflammatory hyperalgesia [8], unchanged neuropathic pain in the chronic constriction-injury model [64]	Unchanged inflammatory paw swelling after subcutaneous PGE_2 injection [8]	Reduced female fertility [40,65], salt-sensitive hypertension [40], hypotension [39], increase in blood pressure instead of fall in response to PGE_2 [40], lack of PGE_2-induced bronchodilatation [66]
EP_3 receptor	Reduced nociception in the acetic acid writhing test only after pretreatment with LPS [14], no change in nociceptive sensitization following intrathecal or subcutaneous injection of PGE_2 [8], unchanged nociception in the formalin test [63]	Reduced arachidonic acid-induced oedema [67]	Increase in urine osmolarity after indomethacin treatment [68], loss of PGE_2 and IL1β-induced febrile responses [69]
EP_4 receptor	Unknown	Decreased severity of collagen- or collagen antibody-induced arthritis [70,71]	Patent ductus arteriosus (dependent on genetic background) [72]
PGIS	Unknown	Unknown	Renal ischaemia, infarction and nephrosclerosis [37]
IP receptor	Reduced nociception in the acetic acid writhing test [7], no peripheral pain sensitization by PGI_2 [44]	Reduced oedema formation after carrageenan injection [7]	Increased susceptibility to thrombosis [7], decreased susceptibility to renovascular hypertension [73]

nociception but, rather, mediate nociceptive sensitization (hyperalgesia and allodynia) triggered by inflammatory processes. The latter can be assessed, for example, in the writhing test, in which a chemical irritant (most often acetic acid) is injected into the peritoneal cavity and the number of dorsoventral flexions ('writhes') is counted, usually for 30 min. In $IP^{-/-}$ mice and $EP_1{}^{-/-}$ mice, the number of writhes in this test is reduced to the level obtained with complete pharmacological blockade of prostaglandin production [6,7]. A third study employing the same test has identified a significant antinociceptive phenotype in $EP_3{}^{-/-}$ mice, but only after COX-2 is induced by pre-treatment with lipopolysaccharide (LPS) [14]. This and a similar study by Doi *et al.* [15] demonstrate the limitations of such acute tests that last for $\leq$1 h, which is not long enough to cover many relevant processes, including the induction of COX-2.

Few studies have analyzed nociceptive phenotypes systematically in more physiological, clinically relevant models of pain. Suitable models include subcutaneous injection of complete Freund's adjuvant, carrageenan, and the yeast extract zymosan A, and arthritis induced by injection of collagen or collagen antibodies. These models involve an inflammatory response that is accompanied by pain sensitization that lasts for days to weeks. Such a systematic evaluation of pain phenotypes is important because the contribution of different prostanoids and prostanoid receptors probably depends on the kind of pain and its origin (e.g. inflammatory versus neuropathic), the site of sensitization (peripheral versus central) and the duration of the underlying disease (acute versus chronic).

A distinct role of EP_2 receptors in central inflammatory pain sensitization is apparent from the zymosan A model of peripheral inflammation. The initial pain sensitization (until 6 h) of $EP_2{}^{-/-}$ mice is similar that of wild-type mice but, after this initial phase, $EP_2{}^{-/-}$ mice recover from sensitization almost completely within 24 h, whereas hyperalgesia in wild-type mice lasts for approximately seven days. Interestingly, $EP_2{}^{-/-}$ mice respond normally to peripheral PGE_2 but do not experience the pronociceptive effects of intrathecally injected PGE_2 [8]. Studies using prostaglandin-receptor-deficient mice also show that the prostaglandins and prostaglandin receptors responsible for inflammatory oedema and inflammatory hyperalgesia overlap but are not identical (Table 1).

Another tool with which to identify the contribution of individual EP-receptor subtypes are subtype-selective EP-receptor antagonists but few are available at present. Many studies of pain have investigated the role EP_1 receptors using the EP_1-receptor antagonist ONO8711 (see Chemical names) [16]. Experiments employing local, peripheral injection of ONO8711 support the contribution of EP_1 receptors to peripheral pain sensitization [17]. ONO8711 is also active after intrathecal injection in a

model of inflammatory pain [18] with a time-course that mimics the phenotype of $EP_2^{-/-}$ mice [8]. The relevance of these latter results is questionable because of the high concentration (≥2.5 mM) of ONO8711 needed to reverse inflammatory pain: although the antagonist has 1000-fold selectivity for EP_1 receptors compared with EP_2 receptors [16], this might not be sufficient to prevent nonspecific activity at this concentration. No data are available on the analgesic properties of the more recent, more specific EP_1-receptor antagonist ONO8713 [19]. Furthermore, $EP_1^{-/-}$ mice have not been tested in more prolonged models of inflammatory pain and for the pain-sensitizing effects of spinal PGE_2.

Together, the studies of prostaglandin-receptor-deficient mice indicate that PGI_2 contributes significantly to pain sensitization via peripheral IP receptors and that PGE_2 probably acts through different EP receptor subtypes, including peripheral EP_1 and spinal EP_2 receptors. The results of the experiments by Reinold *et al.* [8] indicate that the central (spinal) component of pain sensitization quickly becomes dominant in the course of inflammation, which implies that EP_2 receptors might be a favourable target for centrally acting, non-opioid analgesics. However, because pain sensitization does not seem to occur through a single prostaglandin receptor, it must be kept in mind that increased specificity might be achieved at the expense of reduced efficacy.

PGE synthases

Recently, PGE_2 synthases have gained much attention. At least three enzymes are known to produce PGE_2 from PGH_2: two membrane-bound forms, called microsomal PGE synthase-1 (mPGES-1) and mPGES-2; and one cytosolic form, called cPGES [20] (Figure 1). In addition, the glutathione-*S*-transferases GST-μ2 and GST-μ3 might also produce PGE_2 from PGH_2 [21]. In pain studies, mPGES-1 has attracted most interest. Like COX-2, mPGES-1 is induced in peripheral tissues and in the spinal cord in response to inflammation [22–24]. mPGES-1 is associated closely with COX-2 in many tissues [22] and, thus, it primarily metabolizes PGH_2 that is generated by COX-2. Indeed, mPGES-$1^{-/-}$ mice almost completely lack arachidonic acid-induced PGE_2 release after COX-2 induction with LPS, and acetic acid-induced writhing is reduced by ~50% [9,25]. More importantly, collagen (and collagen-antibody)-induced arthritis, which are models of human rheumatoid arthritis, develop in a much milder fashion in mPGES-$1^{-/-}$ than in wild-type mice, which indicates that this enzyme is also crucial for prolonged inflammatory responses.

Unlike mPGES-1, cPGES and mPGES-2 are expressed constitutively in most tissues and are not coupled closely to COX-2. cPGES mainly metabolizes PGH_2 generated by COX-1 [26], whereas mPGES-2 shows no preferential coupling to either COX-1 or COX-2. Although the contributions of cPGES and mPGES-2 have not been assessed *in vivo*, the available data indicate that most of the COX-2-dependent rise in PGE_2 in both the periphery and the spinal cord originates from mPGES-1.

When discussing mPGES-1 as an alternative drug target, one should also consider that mPGES-1 might

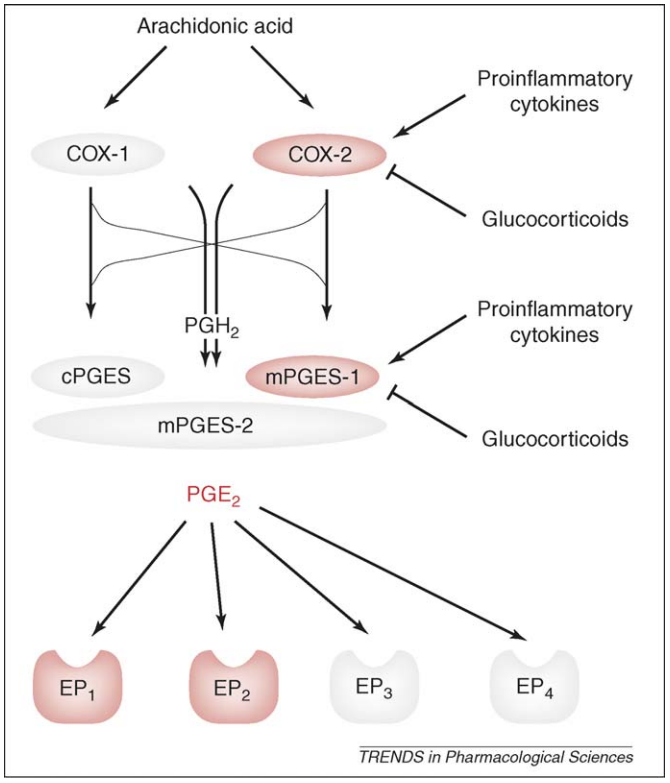

Figure 1. Biosynthesis of PGE_2. Arachidonic acid, which is released from the cell membrane by phospholipase A_2 in response to tissue damage and inflammatory stimuli, is the starting point of several biosynthetic pathways that give rise to different lipid messengers including prostaglandins, leukotrienes and endocannabinoids. Constitutively expressed COX-1 and inducible COX-2 metabolize arachidonic acid into the two prostaglandin precursors PGG_2 and PGH_2. At least three PGE synthases, mPGES-1 mPGES-2 and cPGES, produce PGE_2 from these precursors. mPGES-1 and COX-2 are coupled functionally, spatially and temporally: they colocalize at cellular and subcellular levels, and are induced by proinflammatory cytokines and suppressed by glucocorticoids. PGE_2 exerts its cellular effects through four subtypes of GPCRs called EP_1–EP_4. Enzymes and receptors highlighted in pink indicate those that contribute the most to pain sensitization.

contribute to constitutive PGE_2 formation in certain organs, including kidney, CNS and arterial walls, in the same way as does COX-2. Another potential problem of inhibitors of terminal prostaglandin synthases arises from a possible shift of PGH_2 to prostaglandin synthases remaining unblocked or from an accumulation of PGH_2, which can become pharmacologically active in the absence of the physiological end-product. Such problems have significantly hampered the development of thromboxane synthase inhibitors as antiplatelet agents [27]. Specific inhibitors of mPGES-1 are not yet available but a recent study demonstrated that a series of derivatives based on the 5-lipoxygenase-activating protein (FLAP) inhibitor MK-886 specifically inhibits mPGES-1, with potencies in the low nanomolar range [28].

Prostaglandin receptors involved in the side-effects of COX inhibitors

When evaluating the potential benefits of alternative, more specific targets, it is important to determine whether they contribute to the generation of unwanted effects. Again, work in genetically modified mice has helped to identify relevant prostaglandin receptors (Table 1). According to these studies, the mucosa-protective effect of PGE_2 is mediated by EP_1 receptors in the stomach

[29,30] and by EP_3 receptors in the intestine [31]. However, these findings must be interpreted with caution because they could not all be fully reproduced in studies employing selective agonists or antagonists. For example, PGE_2-mediated inhibition of acid secretion was mimicked by sulprostone (a mixed EP_1–EP_3 receptor agonist) but was not blocked by the EP_1 receptor antagonist ONO8711 [32]. This and another study also attribute a role in the gastrointestinal tract to EP_4 receptors. EP_4 receptor agonists (e.g. ONOAE1329 and 1-OH PGE1) stimulate PGE_2-evoked acid secretion in the stomach [32] and bicarbonate secretion in the duodenum [33]. Cardiovascular toxicity and impairments of kidney function have gained much attention following the results of the VIGOR [34] and APPROVe [3] studies. The contribution of the reduced synthesis of PGI_2 to the cardiovascular risk of COX inhibitors is still not universally accepted [35] but experimental data, in particular from $IP^{-/-}$ mice intercrossed with $ApoE^{-/-}$ mice, indicate that the disruption of PGI_2 signalling and a disturbance of the PGI_2–thromboxane balance greatly increase the progression of atherosclerosis and the risk of thrombosis [36]. Furthermore, among the different prostaglandin receptor and prostaglandin synthase-deficient mice, significant disturbances in kidney function have been observed only in PGI_2 synthase $(PGIS)^{-/-}$ mice (in addition to $COX\text{-}2^{-/-}$ mice) [37] (for review, see Ref. [38]).

In contrast to $PGIS^{-/-}$ mice, EP-receptor-deficient mice develop normally and have normal life-expectancies, with the exception of $EP_4{}^{-/-}$ mice, which on several genetic backgrounds die from a patent ductus arteriosus. Nevertheless, vascular function and regulation of blood pressure are also regulated by EP receptors. $EP_1{}^{-/-}$ mice have lowered blood pressure [6], which would, if relevant, probably be beneficial. Blood pressure in $EP_2{}^{-/-}$ mice is more sensitive to changes in salt intake than in wild-type mice [39,40], and vasodepressor responses after infusion of PGE_2 are diminished in $EP_2{}^{-/-}$ and $EP_4{}^{-/-}$ mice [41]. At present, it is unclear what these effects of PGE_2 contribute to the cardiovascular risks of COX inhibitors or whether they pose a potential risk for users of future mPGES-1 inhibitors and EP receptor antagonists.

Taken together, the studies outlined indicate that inhibiting mPGES-1 is a rational alternative to COX inhibitors as analgesics. Blockade of mPGES-1 should interfere selectively with inducible PGE_2 production and, hence retain, the reduction in gastrointestinal tract toxicity of COX-2 selective inhibitors. Inhibitors of mPGES-1 should also spare PGI_2 and, probably, exert less cardiovascular and renal toxicity. Of the EP-receptor subtypes, EP_1 and EP_2 seem most relevant for pain sensitization. The dominant role of EP_2 receptors during prolonged inflammatory pain and the contribution of EP_1 receptors to PGE_2-mediated mucosal protection favour the use of antagonists of EP_2 receptors rather than EP_1-receptor antagonists, providing the results in knock-out mice hold true in humans.

Downstream targets of PGE_2 in nociception

Alternative strategies might go beyond the inhibition of either prostanoid synthesis or prostanoid receptors. Pain sensitization requires a change in either neuronal excitability or synaptic transmission in the pain pathways [42] (Box 1). Identifying these downstream targets provides a better understanding of the generation of pathological pain, in addition to supporting the descriptive studies on nociceptive phenotypes in genetically modified mice. During the last couple of years, several possible downstream effectors of prostaglandins in pain pathways have been identified. Three of these, all ion channels, deserve particular attention.

Transient receptor potential vanilloid 1 (TRPV-1) channels (also known as capsaicin receptors) have been identified recently as important targets of PGE_2. TRPV-1 channels are expressed by primary nociceptors and function as integrators of different nociceptive stimuli [43]. In the absence of inflammation, they are activated by temperatures $\geq 43\,^{\circ}C$. However PGE_2 and PGI_2, acting through EP_1 receptors and IP receptors, can lower the threshold of activation to temperatures as low as $35\,^{\circ}C$ [44]. Thus, after sensitization, TRPV-1 channels become spontaneously active, which might contribute significantly to inflammatory pain. Reduced inflammatory hyperalgesia in $TRPV\text{-}1^{-/-}$ mice [45,46] supports a crucial role for this ion channel in inflammatory pain sensitization.

Pharmaceutical companies now run programs to develop TRPV-1 antagonists as analgesics. Several such blockers have been developed (e.g. SB366791, SB452533, A425619 and JYL1421), some of which (e.g. A425619) exhibit promising antinociceptive activity in animal models of inflammatory pain [47].

Another peripheral target is the tetrodotoxin (TTX)-resistant Na^+ channel Nav1.8, which is expressed exclusively by primary nociceptors [48]. PGE_2 facilitates activation of this channel by shifting the voltage-dependence of activation to more hyperpolarized values and by increasing the magnitude of the current [49]. The role of Nav1.8 as a downstream effector of PGE_2 has not been demonstrated directly *in vivo* in $Nav1.8^{-/-}$ mice, but its involvement in PGE_2–PGI_2 pathways is consistent with the delayed development of inflammatory hyperalgesia that is observed in these mice [48,50]. Na^+-channel blockers are used widely as local anaesthetics but none of them exhibits specificity for Nav1.8. Specific block should, however, be possible. It has recently been reported that a toxin from the marine snail *Conus striatus* (μ-SIIIA) selectively blocks rat TTX-resistant Na^+ channels [51].

As outlined above spinal sensitization might be the dominant source of pain during prolonged inflammation. Although a possible contribution of PGE_2 to central pain sensitization was suggested soon after the discovery of prostaglandins, the cellular and molecular targets of spinal PGE_2 have long been elusive. *In vitro* work indicates that applying PGE_2 to slices of spinal cord increases the release of glutamate [52], directly depolarizes deep dorsal-horn neurons [53] and disinhibits nociceptive neurons in the superficial dorsal horn by blocking inhibitory glycine receptors [54]. The latter mechanism occurs at low PGE_2 concentrations (IC_{50} ~16 nM) and involves the activation of postsynaptic EP_2 receptors and the subsequent protein kinase A-dependent phosphorylation and inhibition of strychnine-sensitive glycine receptors that contain the α3 subunit (GlyRα3). This subunit is expressed in the superficial layers of the dorsal horn of the spinal cord,

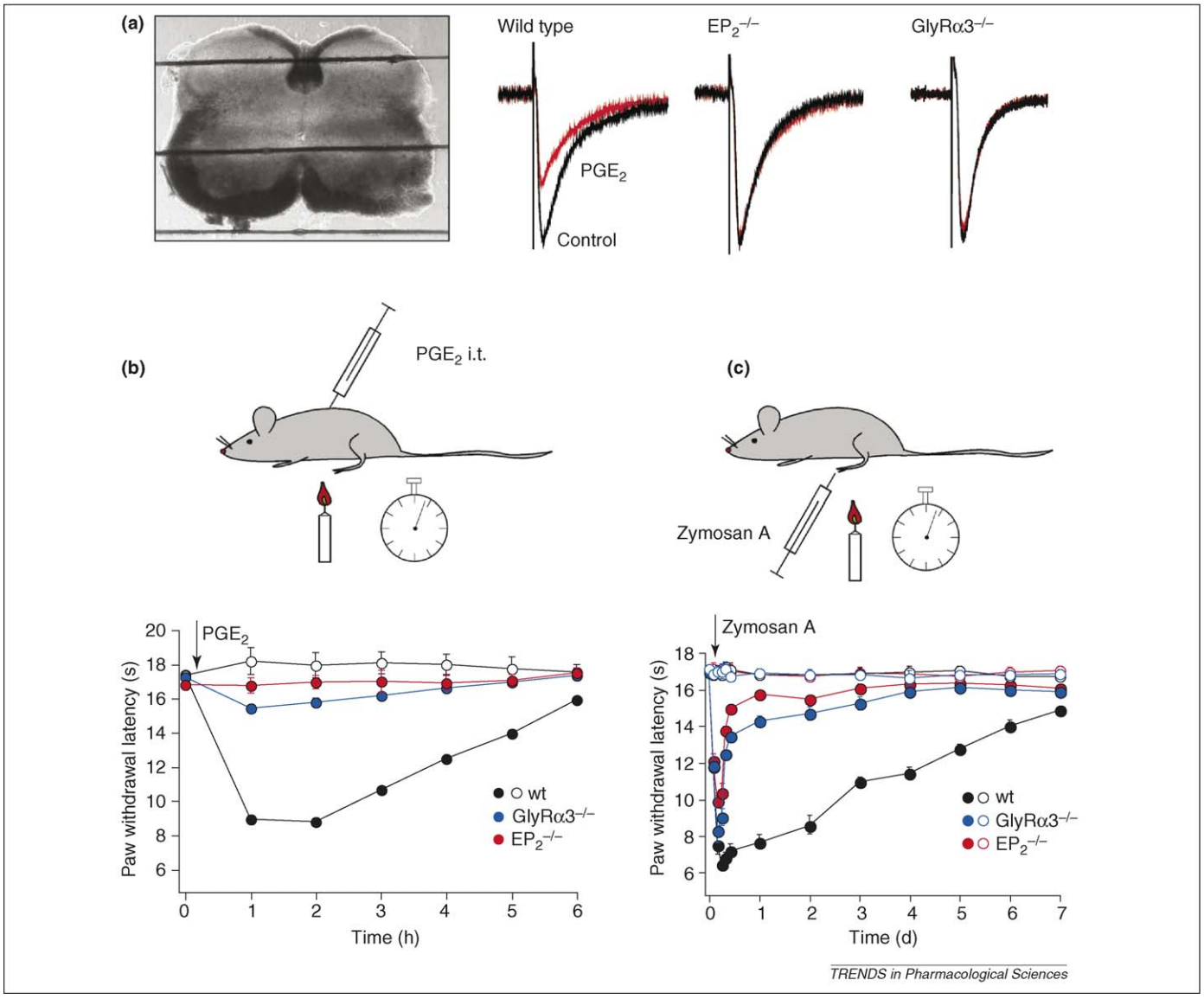

Figure 2. Spinal pain sensitization by PGE_2. **(a)** PGE_2 inhibits evoked, inhibitory, glycine-mediated neurotransmission in the superficial dorsal horn. The current traces depict the inhibitory postsynaptic currents evoked by synaptically released glycine in transverse slices of the superficial dorsal horn of the spinal cord in control conditions (black) and in the presence of PGE_2 (10 μM, red) in wild-type, $EP_2^{-/-}$ and $GlyR\alpha3^{-/-}$ mice. PGE_2 does not inhibit glycine-mediated neurotransmission in mice that lack either EP_2 receptors or GlyRα3. **(b,c)** The contribution made by EP_2-receptor-mediated inhibition of glycine receptors to the hyperalgesia induced by either spinal PGE_2 or peripheral inflammation has been tested in $EP_2^{-/-}$ and $GlyR\alpha3^{-/-}$ mice. Intrathecal PGE_2 injection (b) induces pronounced sensitization to stimulation with noxious heat in wild-type mice (black) but not in $EP_2^{-/-}$ mice (red) and $GlyR\alpha3^{-/-}$ mice (blue). Graphs show paw-withdrawal latencies following exposure to a defined, radiant heat stimulus. White circles in (b) denote vehicle. Injection of the yeast extract zymosan A into the hind paw (c) induces an inflammatory response that is accompanied by pronounced thermal hyperalgesia that lasts >1 week in wild-type mice (black). $EP_2^{-/-}$ mice (red) and $GlyR\alpha3^{-/-}$ mice (blue) develop early thermal hyperalgesia that is similar to wild-type mice, but recover more quickly from pain sensitization. Open symbols: contralateral, non-inflamed paw. Modified, with permission, from Refs [8,42,55].

which is the first site of synaptic integration in the pain pathway. The availability of $EP_2^{-/-}$ and $GlyR\alpha3^{-/-}$ mice has enabled the contribution of this pathway to pain sensitization to be determined *in vivo* (Figure 2). Both types of knockout mice have similar nociceptive phenotypes in which the pronociceptive action of intrathecally injected PGE_2 is almost absent. In addition, early pain sensitization after the induction of peripheral inflammation is almost normal, but animals recover much quicker from hyperalgesia than the corresponding wild-type mice. These observations indicate that both EP_2 receptors and GlyRα3 contribute to the same pain-sensitizing pathway. Furthermore, electrophysiological experiments in slices of the dorsal horn show that PGE_2 does not block glycine-mediated neurotransmission in the superficial layers of the dorsal horn in either type of knockout mice [8,55]. This adds to the growing evidence that loss of synaptic inhibition in the dorsal horn is a major factor in the generation of pathological pain [55,56].

Accordingly, the specific facilitation of glycine and GABA receptors at central sites of nociceptive processing (e.g. in the dorsal horn) should reverse pathological pain. Unlike their closely related cousins, the ionotropic $GABA_A$ receptors, glycine receptors have been largely neglected as promising targets for drug development [57]. Nevertheless, recent evidence indicates that, similar to $GABA_A$ receptors, glycine receptors have a binding site for positive allosteric modulation [58].

Chemical names

A425619: 1-isoquinolin-5-yl-3-(4-trifluoromethyl-benzyl)-urea.
AE1329: 16-(3-methoxymethyl)phenyl-omega-tetra-nor-3,7-dithio prostaglandin E_1.
JYL1421: *N*-(4-tert-butylbenzyl)-*N*′-[3-fluoro-4-(methylsulfonylamino)benzyl]thiourea.
ONO8711: 6-{(2*S*,3*S*)-3-(4-chloro-2-methylphenylsulfonylaminomethyl)-bicyclo[2.2.2]octan-2-yl}-5Z-hexenoic acid.
ONO8713: -{2-[*N*-isobutyl-*N*-(2-furylsulfonyl)amino]-5-trifluoromethylphenoxymethyl} cinnamic acid.
SB366791: *N*-(3-methoxyphenyl)-4-chlorocinnamide.
SB452533: *N*-(2-bromophenyl)-*N*′-{2-[ethyl(3-methylphenyl)amino]ethyl}-urea.

Concluding remarks and future perspectives

The discovery of at least three PGE synthases and four subtypes of EP receptor has identified a hitherto unforeseen diversity in the PGE_2 pathway. Recent studies in genetically modified mice have helped to unravel the role of these proteins in the generation of pathological pain. From these studies, inducible mPGES-1 and EP_1 receptors and/or EP_2 receptors seem to be the most promising targets.

The identification of downstream effectors of PGE_2 both in the central and peripheral nociceptive system adds another level of complexity to the PGE_2 system. An intriguing feature of these downstream effectors is that they are often expressed mainly in the nociceptive system. Mice that lack these ion channels have no major abnormalities. Antagonists of either TRPV-1 channels or TTX-resistant Na^+ channels might, thus, be peripherally acting antihyperalgesic agents. Similarly, facilitating inhibitory neurotransmission in the dorsal horn might reverse the disinhibition of spinal nociception that occurs in inflammatory and neuropathic diseases. Therefore, subtype-selective agonists of glycine receptors and $GABA_A$ receptors provide an intriguing future avenue for the development of centrally acting non-opioid antihyperalgesic agents.

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