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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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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].

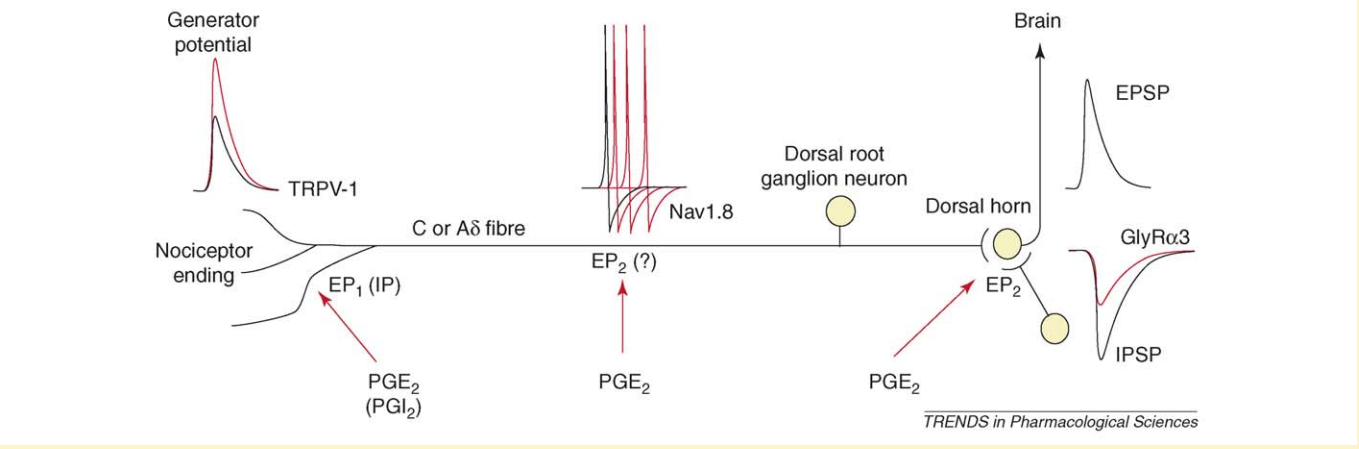

Figure I. PGE_2 modulates nociceptive signals at multiple sites in the pain pathway. At the peripheral endings of primary nociceptors, PGE_2 and EP_1 receptors potentiate TRPV-1 activation in concert with PGI_2 and IP receptors [44]. The generation and propagation of action potentials are facilitated 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