

Prostanoids and pain: unraveling mechanisms and revealing therapeutic targets

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Advances in our understanding of the synthesis, regulation and function of prostanoids have led to a new appreciation of their actions in health and disease. Prostanoid synthesis is essential for the generation of inflammatory pain and this depends not only on prostanoid production at the site of inflammation, but also on the actions of prostanoids synthesized within the central nervous system (CNS). Moreover, central prostanoid synthesis is controlled both by neural and humoral signals, the latter being a novel form of input to the CNS. Diverse compounds that act along the pathway of prostanoid synthesis and action, both in the periphery and in the CNS, might provide increased benefit for treating inflammatory pain hypersensitivity and its associated sickness syndrome, with a reduced risk of adverse effects.

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Tissue injury and inflammation are associated with increased prostanoid synthesis and pain hypersensitivity. Soon after their initial isolation, prostanoids were shown to influence inflammation and immune responses, and their administration was found to reproduce the major signs of inflammation, including augmented pain sensitivity [1]. Peripheral inflammation increases prostanoid levels at the site of inflammation, and this local release contributes directly to inflammation and pain. More recently, it has been revealed that peripheral inflammation also increases central prostanoid levels [2,3], and this mediates more-widespread changes in pain perception as well as eliciting a sickness syndrome comprising fever, anorexia, altered mood and changed sleep patterns [4].

Basal levels of prostanoids are important for homeostatic functions in many tissues, particularly in the kidney, gastric mucosa and platelets [5]. In other tissues, constitutive production of prostanoids is low, but can be increased within minutes by inflammatory stimuli acting on constitutively expressed prostanoid synthetic enzymes [5]. Pro-inflammatory signals trigger multiple transcriptional and post-translational changes that alter the synthetic enzyme levels and activity, and this leads to early, massive and sustained increases in prostanoid levels.

Advances have been made in our understanding of the molecular biology of the phospholipase A₂ (PLA₂), cyclooxygenase (COX) and prostaglandin (PG) synthase enzymes. This knowledge, together with the development of new pharmacological tools that inhibit these enzymes, the signals that regulate them

and the prostanoid receptors through which prostanoids act, promise to expand rapidly our understanding and treatment of clinical inflammatory pain states.

Prostanoid synthesis

Prostanoids derive from arachidonic acid liberated from phospholipids in the cell membrane by the action of PLA₂ enzymes. COX catalyzes the first two reactions of the PG pathway, leading to the formation of PGH₂, an intermediate metabolite. Tissue-specific isomerases (PG synthases) metabolize PGH₂ into the PG isoforms PGE₂, PGD₂ and PGF₂, prostacyclin (PGI₂) or thromboxane A₂ (TXA₂) [6,7], which then exert their biological actions through seven-transmembrane-domain, G-protein-coupled receptors, classified as DP, EP, FP, IP and TP receptors, according to their respective prostanoid ligand [8–11] (Fig. 1). Differential tissue expression and induction patterns of PLA₂, COX, isomerases and prostanoid receptors play a major role in determining and modulating the cellular effects of the prostanoids.

PLA₂

Membrane phospholipids are enriched with arachidonic acid. The PLA₂ enzymes liberate free fatty acid from membrane phospholipid [12] and thereby control the flow of cellular arachidonic acid. The PLA₂s are a diverse enzyme superfamily with more than 13 different enzymatically active isoforms [13]. These isoenzymes have different substrate specificity, pH sensitivity, expression patterns, regulation and subcellular localization, and are classified according to their structure and calcium requirements. The levels of secretory (s)PLA₂s are rapidly increased by experimental inflammation and in human inflammatory diseases, and their pathophysiological contribution to several inflammatory diseases is well reported [14]. There are also intracellular, nonsecreted forms of both calcium-dependent and -independent PLA₂s. These can be cytosolic, associated with membrane compartments, or integral membrane proteins. There are several forms of calcium-independent PLA₂, including the 80-kDa Group VI enzyme commonly referred to as iPLA₂ [15]. Although iPLA₂ is the predominant form of PLA₂ in rodent brain, biochemical studies suggest that it serves a membrane remodeling and homeostasis function, and

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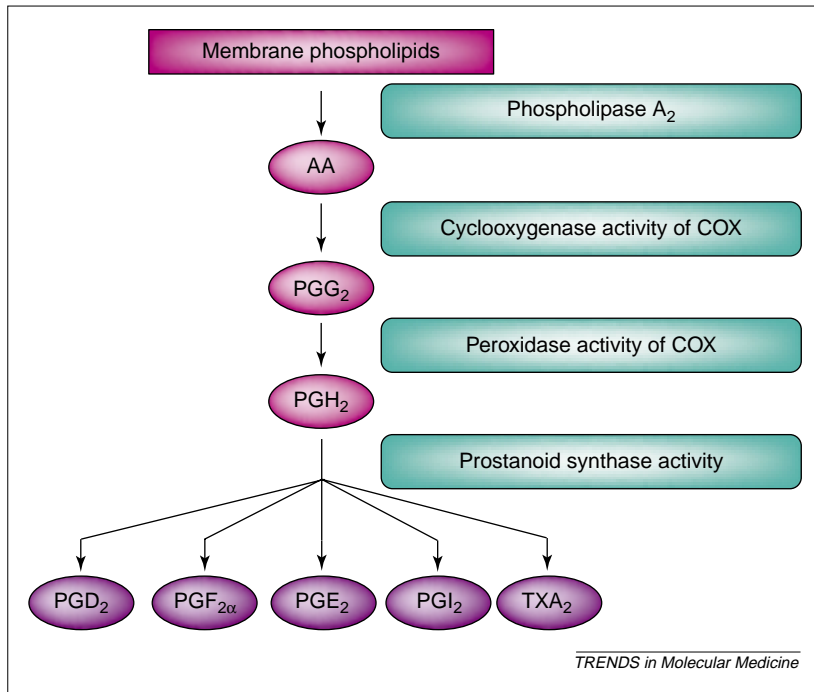


Fig. 1. Prostanoid biosynthetic pathway. Following the release of arachidonic acid (AA) from cell membranes by phospholipase A₂ (PLA₂), it is converted in a two-step reaction, first to prostaglandin G₂ (PGG₂), then to PGH₂, by the action of cyclooxygenase (COX) enzyme. PGH₂ is then converted to various prostaglandins and thromboxanes by the action of multiple tissue-specific synthases.

is not involved in the production of arachidonic acid for the synthesis of prostanoids [16].

The best-characterized intracellular calcium-dependent PLA₂ is the Group IVA cytosolic (c)PLA₂. cPLA₂ is a large (85.2-kDa) protein [17] that has specificity for substrates containing arachidonic acid, and its activity is regulated by phosphorylation. cPLA₂ is constitutively expressed in a wide variety of tissues and cells. In quiescent cells, cPLA₂ resides in the cytosol but, in response to an increase in intracellular calcium, it translocates to cellular membranes [18,19], bringing it into close proximity both with phospholipids and the prostanoid synthetic enzymes COX and 5-lipoxygenase [20]. These characteristics strongly suggest that cPLA₂ regulates the release of arachidonic acid for prostanoid production and might have a role in inflammation and pain.

The function of sPLA₂s depends upon their ability to bind to heparan sulfate proteoglycans on cell surfaces. sPLA₂s that bind to glypican have an enhanced ability to release arachidonic acid to a pool that is coupled to COX-2 [21]. sPLA₂ appears to be internalized by neurons and contributes to increased eicosanoid synthesis and inflammatory pain [22].

COX-1 and COX-2

The existence of a COX isoform that is positively and negatively regulated by cytokines and glucocorticoids, respectively, was long suspected but it was only in the early 1990s that an inducible COX isoenzyme (COX-2) was successfully cloned [23,24,25]. The identification of two COX isoforms, COX-1 and COX-2, has led to intense efforts to characterize the relative contribution of each isoform to prostanoid production in specific situations. The development of isoform-specific antagonists has been extremely useful, first as experimental tools and, more recently, for clinical therapy.

COX-1 and COX-2 are membrane-associated enzymes with a 60% amino acid sequence homology [6]. The major sequence differences occur in the membrane-binding domains [6]. Structure–function studies of the COX isoforms reveal a homodimer profile and both isozymes are composed of three independent folding units: an epidermal growth factor-like domain, a membrane-binding motif and an active enzymatic domain that consists of a long hydrophobic channel [26]. In spite of their remarkable structural similarity, the two COX isoforms have different gene expression profiles, distinct kinetic properties, and different interactions with PLA₂s and synthases [6,27].

COX-1 is expressed constitutively and produces prostanoids that fine-tune physiological processes requiring instantaneous or continuous regulation (e.g. hemostasis) [6,28]. COX-2 expression is usually low but can be induced by numerous factors including neurotransmitters, growth factors, proinflammatory cytokines, lipopolysaccharide (LPS), calcium, phorbol esters and small peptide hormones [28]. However, there are exceptions to the original 'constitutive versus inducible' theory of COX expression. COX-1 expression can be induced in some stress conditions, such as nerve injury [29–33] and many tissues, including the central nervous system (CNS) and the kidney, constitutively express COX-2 [3,28]. In the spinal cord, there are detectable basal levels of both COX-1 and COX-2 and this might enable immediate reactions to transmitter release that result in prostanoid production [34,35].

Peripheral and central induction of COX-2

Inflammatory diseases such as rheumatoid arthritis (RA) are characterized by an exudative and proliferative inflammatory response that is accompanied both by spontaneous pain and by excessive pain sensitivity. Movement of an affected joint within a normal range becomes painful, and systemic changes can be observed in mood and behavior that constitute a sickness syndrome.

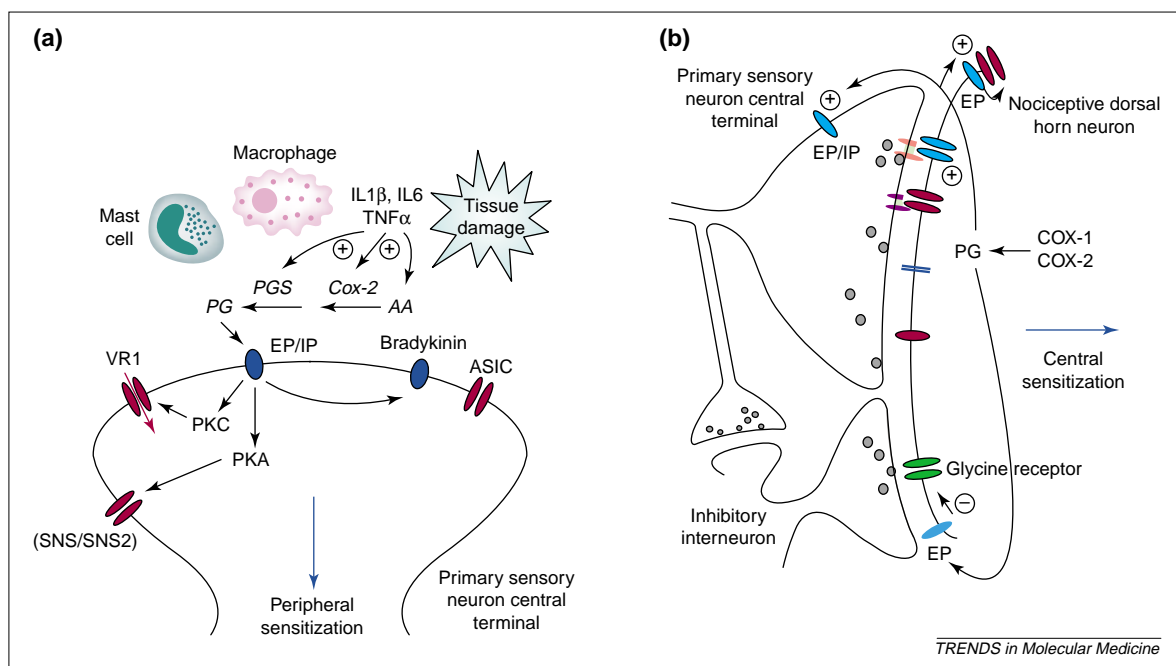
Marked increases in PLA₂ and COX-2 expression occur at the site of local inflammation. The original hypothesis formulated by John Vane in his Nobel prize-winning work for the mechanism of action of nonsteroidal anti-inflammatory drugs (NSAIDs) was that they inhibited prostanoid production in the periphery, preventing a sensitizing action of PGE₂ on the peripheral terminals of sensory fibers. More recently, peripheral inflammation has been shown also to induce a widespread increase in COX-2 and PGE synthase (PGES) expression in the CNS. The pro-inflammatory cytokine interleukin 1β (IL-1β) is upregulated at the site of inflammation and plays a major role in inducing COX-2 in local inflammatory cells by activating the transcription factor NF-κB. IL-1β is also responsible for the induction of COX-2 in the CNS in response to peripheral inflammation, but this interestingly is not the consequence either of

Fig. 2. Schematic representation of the peripheral and central action of prostanoids.

(a) The peripheral terminal of a nociceptor neighboring the site of injury/inflammation where prostanoids are massively produced and released to act on their specific receptors [EP for prostaglandin E_2 (PGE_2); IP for prostacyclin (PGI_2)] expressed on the nociceptor. Prostanoid signaling is mediated by second messengers such as protein kinase A (PKA) and PKC, which regulate the activity of many receptors and ion channels and contribute to the potentiation of primary sensory neurons leading to peripheral sensitization.

(b) The central terminal of a nociceptor where neurotransmitters/neuro modulators are released and act on receptors and ion channels in the dorsal horn neurons to activate intracellular signaling and induce cyclooxygenase 2 (COX-2) gene expression and prostaglandin synthesis. Prostaglandins in turn, act on pre- and post-synaptic receptors. This signaling mechanism in which prostanoids constitute a key mediator contributes to the regulation of the functional properties and membrane excitability of dorsal horn neurons leading to central sensitization.

Abbreviations: AA, arachidonic acid; IL, interleukin; $TNF\alpha$, tumor necrosis factor α .



neural activity arising from the sensory fibers innervating the inflamed tissue or of systemic IL-1 β in the plasma. Instead, peripheral inflammation produces some other signal molecule that enters the circulation, crosses the blood-brain barrier and acts to elevate IL-1 β , leading to COX-2 expression in neurons and non-neuronal cells in many different areas of the spinal cord [36]. An elevation of COX-2 also occurs at many levels in the brain, mainly in the endothelial cells of the brain vasculature [37].

Thus, there appear to be two forms of input from peripheral inflamed tissue to the CNS. The first is mediated by electrical activity in sensitized nerve fibers innervating the inflamed area, which signals the location of the inflamed tissue as well as the onset, duration and nature of any stimuli applied to this tissue. This input will be sensitive to peripherally acting COX-2 inhibitors and to neural blockade with local anesthetics, as with epidural anesthesia. The second is a humoral signal originating from the inflamed tissue, which acts to produce a widespread induction of COX-2 in the CNS. This input will not be affected by regional anesthesia and will only be blocked by centrally acting COX-2 inhibitors. One implication of this is that patients who have an epidural anesthesia for surgery might also need a centrally acting COX-2 inhibitor to reduce optimally their postoperative pain and the postoperative stress response.

Pain hypersensitivity generated by peripheral inflammation (Fig. 2) is reduced by direct application of NSAIDs to the CNS [38–40], and administration of inhibitors of either COX-2 or IL-1 β synthesis into the subarachnoid space of the spinal cord reduces inflammation-induced central PGE_2 levels and mechanical hyperalgesia [41,36]. At present, the permeability of the blood-brain barrier to currently used NSAIDs and COX-2 inhibitors has not been fully

elucidated. Inhibitors of COX-2 that better penetrate the blood-brain barrier might represent both more-efficient pain killers and could act to reduce many of the more-diffuse aspects of inflammatory pain, such as generalized aches and pains, depression and loss of appetite, which are key aspects in determining the 'quality of life' response to treatment [41]. The contribution of centrally induced COX-2 to the postoperative stress response needs to be actively explored.

All current clinical trials using COX-2 inhibitors for the management of pain have looked at efficacy only in the immediate postoperative period. A substantial number of patients continue to suffer from pain after discharge from hospital and some of these go on to develop chronic persistent pain. It is not yet clear whether aggressive sustained treatment with COX-2 inhibitors, until all signals arising from inflamed tissue that would be expected to induce central and peripheral COX-2 have resolved, would ameliorate the longer lasting elements of postoperative pain, and prevent the transformation of acute pain into chronic pain. This important issue needs to be addressed by large, carefully controlled clinical trials.

PGES: expression and regulation

Although the isomerization of PGH_2 into PGE_2 has been well-characterized biochemically and pharmacologically, the enzyme responsible, PGES, was only purified and cloned very recently [42,43]. This enzyme is a member of the MAPEG ('membrane-associated proteins in eicosanoid and glutathione metabolism') superfamily, which consists of six proteins with divergent functions [44]. Intermediate-to-low basal levels of human PGES mRNA expression are present in many tissues, including placenta, prostate, testis, mammary

gland and bladder. However, like PLA_2 and COX-2, PGES is an inducible enzyme and is upregulated by IL-1 β , LPS or adjuvant administration, suggesting a regulatory role in inflammation, pain and fever [43]. Cerebral vascular endothelial cells respond to circulating cytokines, such as IL-1 β , by increasing COX-2 and PGES expression levels to produce PGE₂. The small size and lipophilic properties of PGE₂ allow it to diffuse into the brain parenchyma, where it might contribute to the systemic responses to inflammation such as fever, fatigue and pain hypersensitivity, along with the PGE₂ produced by COX-2 induced in the brain [36].

Prostanoid receptors: expression, regulation and function

The first prostanoid receptor to be isolated and cloned was the TXA₂ receptor (TP) [45,46] a G-protein-coupled receptor with seven transmembrane domains. Homology screening of cDNA libraries resulted in the isolation and identification of seven other prostanoid receptors that had been predicted pharmacologically: the PGD receptor (DP), four PGE receptors (EP1, EP2, EP3, EP4), the PGF receptor (FP) and the PGI receptor (IP). These receptors are highly conserved in mammals, with several splice variants of the EP3, FP and TP receptors occurring [47]. PGD₂ actually acts both through DP and the chemoattractant receptor CRTH2, which is preferentially expressed in T helper 2 (Th2) cells, eosinophils and basophils in humans [48]. Prostanoid receptors are expressed in many tissues and cell types. Among the EP receptors, EP3 and EP4 are the most widely distributed, whereas EP1 and EP2 distribution is restricted to the kidney, stomach and uterus, as well as to neuronal and non-neuronal cells in the nervous system [49]. EP1, EP3 and EP4 mRNAs are expressed in primary sensory neurons, in the dorsal root and trigeminal ganglia [50,51], suggesting involvement in PGE₂-mediated peripheral sensitization. Inflammatory signals affect the expression levels of many prostanoid receptor subtypes [1].

The function of the prostanoid receptors varies according to cell type, ligand concentration and structure. Generally: EP2, EP4, one isoform of EP3, DP and IP increase intracellular cyclic (c)AMP; EP1, other isoforms of EP3, FP and TP induce intracellular calcium mobilization and release; and one EP3 isoform, TP and CRTH2 induce a decrease in cAMP levels. Although the limited number of available specific EP receptor agonists and antagonists, as well as the complex tissue distribution pattern and cellular signaling of the receptors, has restricted elucidation of their function, transgenic mice deficient in each prostanoid receptor are proving to be a very useful tool [47].

Prostanoids in Inflammation

Although PGs and thromboxanes function as both pro- and anti-inflammatory molecules, the specific response is determined by: (1) the prostanoid

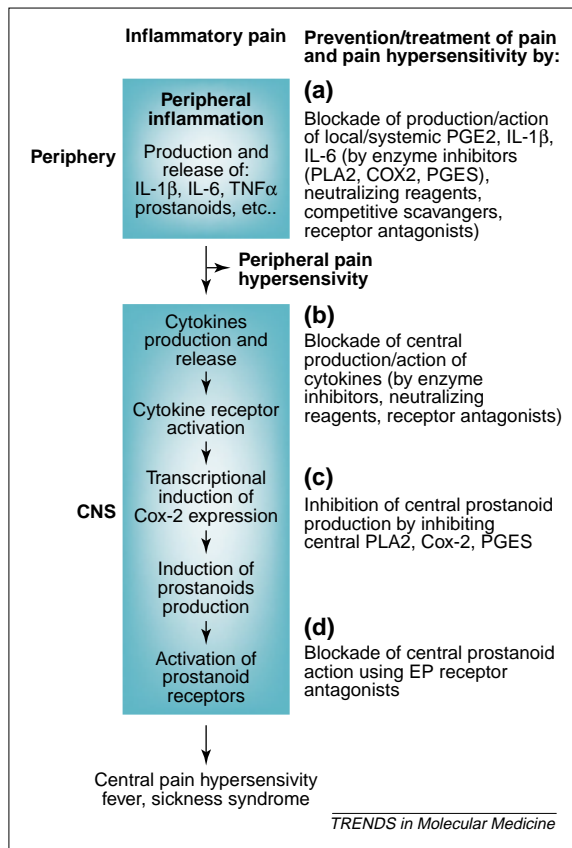
species, (2) the specific prostanoid receptor, (3) the cell type and (4) the time course of inflammation. For example, while PGE₂ is usually considered as a pro-inflammatory molecule that causes vasodilatation and chemotaxis by activating the EP2 receptor and increasing intracellular cAMP, in certain circumstances, it suppresses T-cell proliferation and inhibits cytokine release from monocytes [52]. Aspirin-triggered lipoxins, which are synthesized by COX-2, have been demonstrated to be potent direct-acting anti-inflammatory agents [53], but their role in pain signaling has not yet been examined. Prostanoid products bind to the peroxisome proliferator-activated receptor (PPAR) γ nuclear hormone receptor and might modulate inflammation through this non-receptor pathway [54].

Prostanoids contribute to the development of pain by acting both peripherally and centrally (Fig. 2). Peripherally, they play a major role in generating peripheral sensitization by increasing the sensitivity of the peripheral terminals of high-threshold pain fibers (nociceptors). They do this by producing a protein kinase A (PKA)-mediated phosphorylation of sodium channels and other receptors in the nociceptor terminals after EP receptor activation, thereby increasing excitability, reducing the pain threshold and potentiating the action of pain-producing stimuli such as heat or irritant molecules like bradykinin [55,56]. Some NSAIDs have been shown to mediate a COX-independent inhibition of dorsal root acid-sensing ion channels (DRASIC), which are expressed on sensory terminals that might contribute to their peripheral analgesic actions [57]. Centrally administered PGs produce marked alterations in pain behavior, including exaggerated responses to noxious stimuli as well as pain responses to normally innocuous stimuli [58]. At a cellular level, PGE₂ can increase excitability in pain-transmission neuronal pathways in the spinal cord in multiple ways. These include: an increase in transmitter release from the central terminals of pain fibers [59]; a direct depolarization of spinal cord neurons by activating a nonselective cation channel [60]; and, most intriguingly, a reduction in glycine-mediated inhibition in the spinal cord [61]. Glycine is one of the major inhibitory transmitters and reducing its action (i.e. inhibiting an inhibitor) will markedly increase excitability.

COX and PLA_2 : a two-component system

There are two distinct phases of immune- and inflammatory-mediated PG release. An immediate phase of arachidonic acid and eicosanoid release that occurs within minutes of stimulation, and a delayed phase seen hours after the initial exposure, which depends upon the synthesis of new proteins such as COX-2 and PGES. Stimulation initially activates cPLA₂ to produce arachidonic acid [62], which is metabolized by constitutively expressed COX-1/COX-2 and PG isomerases to form PGs. The activity of cPLA₂ also serves as a signal for both the induction and

Fig. 3. Schematic representation of the peripheral and central contributors to inflammatory pain, fever and the sickness syndrome. The possibility of altering the peripheral and/or central inflammatory cascade offers new alternatives for the prevention and treatment of pain and pain hypersensitivity. Abbreviations: IL, interleukin; TNF α , tumor necrosis factor α .



activation of sPLA₂s and COX-2. Arachidonic acid generated by sPLA₂ then becomes the substrate for COX-2 in the late-phase response.

In the spinal cord, there is also an immediate (within minutes) and delayed (within hours) release of PGE₂, the former produced by COX-1, and the latter by COX-2 [35]. However, the relative contribution of constitutive COX-1 and COX-2 in the spinal cord to the immediate response to peripheral injury is controversial, with data pointing to a role for either enzyme [34,35]. Nevertheless, there is no question that there is a very substantial increase in COX-2 levels in the spinal cord some hours after peripheral injury [36]. This indicates that pain very soon after an injury, such as in the immediate postoperative period, may not be as sensitive to COX-2 inhibitors as the pain experienced some time later. It might be beneficial to ensure clinically that the enzyme is inhibited immediately, thereby reducing prostanoid production and initiating intracellular changes in neurons that might contribute to persistent pain. The optimal time for treatment, pre- or postoperatively, needs to be determined and could depend on the presence of pre-existing pain, the particular surgery undergone, its location and duration, as well as any risks of side effects.

Therapeutic possibilities

The aim of therapy targeted at prostanoid production during inflammation is to reduce the pathological accumulation of prostanooids without affecting normal

prostanoid-dependent homeostatic function, unless that is an indication, as with aspirin and prevention of myocardial infarction. There are several potential ways of achieving this: directly inhibiting COX, PLA₂ or PG synthases; blocking their induction or activation; or antagonizing the action of prostanooids on their receptors (Fig. 3). COX inhibitors are the most readily available means of reducing prostanoid synthesis and can be classified into four categories: selective COX-1 inhibitors (of which there are none clinically available); nonselective COX inhibitors (comprising most conventional NSAIDs); preferential COX-2 inhibitors (e.g. meloxicam and diclofenac); and specific COX-2 inhibitors (e.g. rofecoxib, celecoxib and valdecoxib) [33].

Selective COX-2 inhibitors seem to have comparable anti-inflammatory, anti-pyretic and analgesic activities to that of classical NSAIDs, with less gastrointestinal side effects [63], but there is concern about an increased risk of myocardial infarction, edema and delayed wound healing, at least for some COX-2 inhibitors [64]. Whether these are drug or class effects awaits extensive clinical investigation. A reduction of the risk of bleeding and damage to the gastric mucosa might enable more-aggressive and early treatment for postoperative pain or trauma management.

PLA₂ provides an interesting target; however, there is a question as to which enzyme to inhibit. cPLA₂ is an obvious candidate, and several selective inhibitors with no activity on sPLA₂ or phospholipase have been produced and have been shown to have anti-inflammatory actions; to date, analgesic effects have not been described.

Prevention of COX-2 induction is an exciting future prospect for therapeutic intervention. The advantage here is that this should leave constitutive activity of COX-2 and COX-1 intact and could also prevent production of other effector molecules that participate in inflammation and pain, such as PGES. The most obvious way of achieving this is to block IL-1 β production with inhibitors of interleukin-1 β -converting enzyme (ICE), or to block IL-1 β action with the endogenous IL-1 receptor antagonist, or to block exogenous antagonists of the type 1 IL-1 receptor. Two further possibilities are PG synthase inhibitors and EP receptor antagonists. For the latter, a downside of the presence of multiple subtypes is that a single highly specific drug might have a limited indication, an upside is that side effects could be more limited. Finally, there is the issue of acetaminophen (paracetamol), which, either alone or in combination, is the most widely used over-the-counter analgesic. Unlike the NSAIDs, it has no anti-inflammatory action, although it does reduce prostanoid production in the CNS [65]. It has only very weak effects on COX-1 and COX-2, and claims that it inhibits another COX (COX-3) remain unsupported by the failure to find such an enzyme in spite of a major search by many laboratories [66]. Arguments have also been made that it might act on a variant of COX-2 [65].

Concluding remarks

Production of prostanoids is highly regulated, resulting in complex patterns of constitutive and induced synthesis in different tissues. Both peripheral and central prostanoids contribute to inflammatory pain and treatment should be targeted against both sources. Selective COX-2 inhibitors have provided a major advance for the treatment of arthritic pain, although their central penetration has not been investigated and the full class side effect profile remains to be established. Nevertheless, it is likely these drugs will have a major role in managing many acute and chronic inflammatory pain conditions. The analgesic profile of PLA₂ inhibitors

should prove interesting, as will the exploitation of techniques to prevent the central induction of COX-2 or PGES. Central COX-2 induction might be responsible for the expression of a broad and diffuse response of the CNS to tissue injury and trauma, including many poorly defined symptoms that, together, constitute the sickness syndrome, and which substantially impair quality of life. Treatment of inflammatory conditions requires a therapeutic strategy that targets the inflammation, pain and general well-being of the patient. Since prostanoids appear to be involved in all, the major question now is what is the most effective way to block prostanoid production with the least side effects.

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Reciprocal products of chromosomal translocations in human cancer pathogenesis: key players or innocent bystanders?

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Chromosomal translocations are frequently involved in the pathogenesis of leukemias, lymphomas and sarcomas. They can lead to aberrant expression of oncogenes or the generation of chimeric proteins. Classically, one of the products is thought to be oncogenic. For example, in acute promyelocytic leukaemia (APL), reciprocal chromosomal translocations involving the retinoic acid receptor α (*RAR α*) gene lead to the formation of two fusion genes: *X-RAR α* and *RAR α -X* (where *X* is the alternative *RAR α* fusion partner: *PML*, *PLZF*, *NPM*, *NuMA* and *STAT 5b*). The *X-RAR α* fusion protein is indeed oncogenic. However, recent data indicate that the *RAR α -X* product is also critical in determining the biological features of this leukemia. Here, we review the current knowledge on the role of reciprocal products in cancer pathogenesis, and highlight how their expression might impact on the biology of their respective tumour types.

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Recurring chromosomal abnormalities have been identified in a variety of cancers, but are most frequently associated with leukaemias, lymphomas and

sarcomas [1,2]. At present, more than 500 recurring cytogenetic abnormalities have been reported in hematological malignancies, a frequency several times higher than that reported in mesenchymal and epithelial cancers, according to the Cancer Genome Anatomy Project/Cancer Chromosome Aberration Project of the National Cancer Institute (<http://cgap.nci.nih.gov/Chromosomes/RecurrentAberrations>). Whether the observed differences in frequency of associated chromosomal abnormalities in hematological and non-hematological cancers are related to distinct mechanisms governing genomic plasticity (e.g. VDJ recombination in lymphoid cells) and/or stability in different tissues/cells, or, merely reflect technical limitations in their detection in solid tumours is still unclear.

Three main cytogenetic changes have been detected in cancer cells: chromosomal deletions, inversions