Minireview

Cyclooxygenase-dependent signalling: molecular events and consequences

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Abstract Non-steroidal anti-inflammatory drugs (NSAIDs) currently attract large interest. Next to pain relief, NSAIDs have important anti-thrombotic and anti-oncogenic effects. NSAIDs exert their action by inhibition of cyclooxygenase, the enzyme responsible for the production of prostanoids. Prostanoid signal transduction is still poorly understood, but it has become clear that these inflammatory lipids influence cellular physiology at three different levels: (1) activation of a 7× transmembrane receptor coupled to heterotrimeric G proteins, (2) the inhibition of inflammation by activating corticosteroid-like receptors, (3) participation in receptor protein tyrosine kinase signal transduction. In this review prostanoid signalling at these three different levels will be reviewed and the relevance in (patho)physiological processes will be evaluated.

Key words: Prostaglandin; Non-steroidal anti-inflammatory drug; G protein-coupled receptor; Peroxisome proliferator; Activated receptor; Signal transduction

1. Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs), such as aspirin, have been used with great therapeutic success for over a century. The mechanism of action of these drugs has long remained a mystery. In 1982, however, the Nobel Prize in Physiology or Medicine was awarded to Sune Bergstrom, Bengt Samuelson and John Vane for their work on prostaglandins and biochemically related substances (now designated the prostanoid type of eicosanoids [1]), and the demonstration that NSAIDs act by interfering with the synthesis of these inflammatory lipids. Since then it has become clear that prostanoids act in a broad range of biological and (patho)-physiological processes, such as inflammation, thrombosis, and cancer. An important role for prostanoid signalling is further supported by the evolutionary conservation of this signalling: also plants and yeasts are capable of prostaglandin synthesis (e.g. [2,3]), and the prostanoid-generating system probably dates from before 3.0 billion years ago [4]. As will become clear from this review, however, prostanoid signalling and its relation to these pathophysiological effects are still poorly understood, but seem to be mediated via effects on both plasma membrane receptors and intracellular receptors, as well as by action downstream of receptor activation.

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2. Synthesis and structure of prostanoids

Prostanoids were first discovered in the 1930s, when Von Euler showed that semen compounds are able to lower blood pressure in experimental animals [1]. Synthesis of prostanoids is rate limited by the release of the precursor lipid arachidonic acid from plasma membrane phospholipids. Either phospholipase A2 or the combined action of phospholipase C, a diglyceride lipase and a monoglyceride lipase, produces arachidonic acid. Upon its release, arachidonic acid is converted by cyclooxygenase (COX) to PGG_2 and subsequently peroxidised to PGH_2 by the same enzyme, which acts as a precursor for further prostanoid synthesis by specific prostaglandin synthetases which are often expressed in a cell type-specific fashion. All prostanoids are composed of oxygenated, 20 carbon fatty acids, which contain a cyclic ring, a C-13→C-14 *trans* double bond and a hydroxyl group at C-15. Prostanoids can be divided into prostaglandins (PG), which contain a cyclopentane ring, and thromboxanes (TX), which contain a cyclohexane ring. The first group include the prostaglandins of the D, E, F, and I series, the latter indicating the locations of the oxygen group(s) in the cyclopentane ring. Likewise, thromboxanes are subdivided into TXA and TXB. Commonly, these abbreviations are followed by an index (for instance PGE_2), which indicates the number of double bonds present in the side chains attached to the cyclopentane ring. Five primary prostanoids are distinguished: PGD_2, PGE_2, PGI_2, $PGF_{2\alpha}$ and TXA_2 (Fig. 1, [5]), the exact metabolites synthesised being dependent on the tissue type and stimulus. For instance, TXs, PGD_2, PGI_2, $PGF_{2\alpha}$ are major metabolites in platelets, mast cells, endothelial cells, and kidney glomerulus cells respectively. In contrast, PGE_2 is synthesised by a broad range of cell types (for references, e.g. [5]).

3. Cyclooxygenase I and II

As stated above, prostanoid synthesis critically depends on the action of cyclooxygenase (Fig. 2). Two isoforms of this enzyme exist (COX-1 and COX-2) which share 60% amino acid homology. The most striking difference between COX-1 and -2 is the ultrastructural localisation. Whereas COX-2 acts at the nuclear envelope [6], COX-1 functions predominantly in the endoplasmic reticulum and close to the plasma membrane. This differential localisation of the COX-isoforms is the consequence of differences occurring in the signal peptides and membrane binding domains near the N- and C-terminal ends

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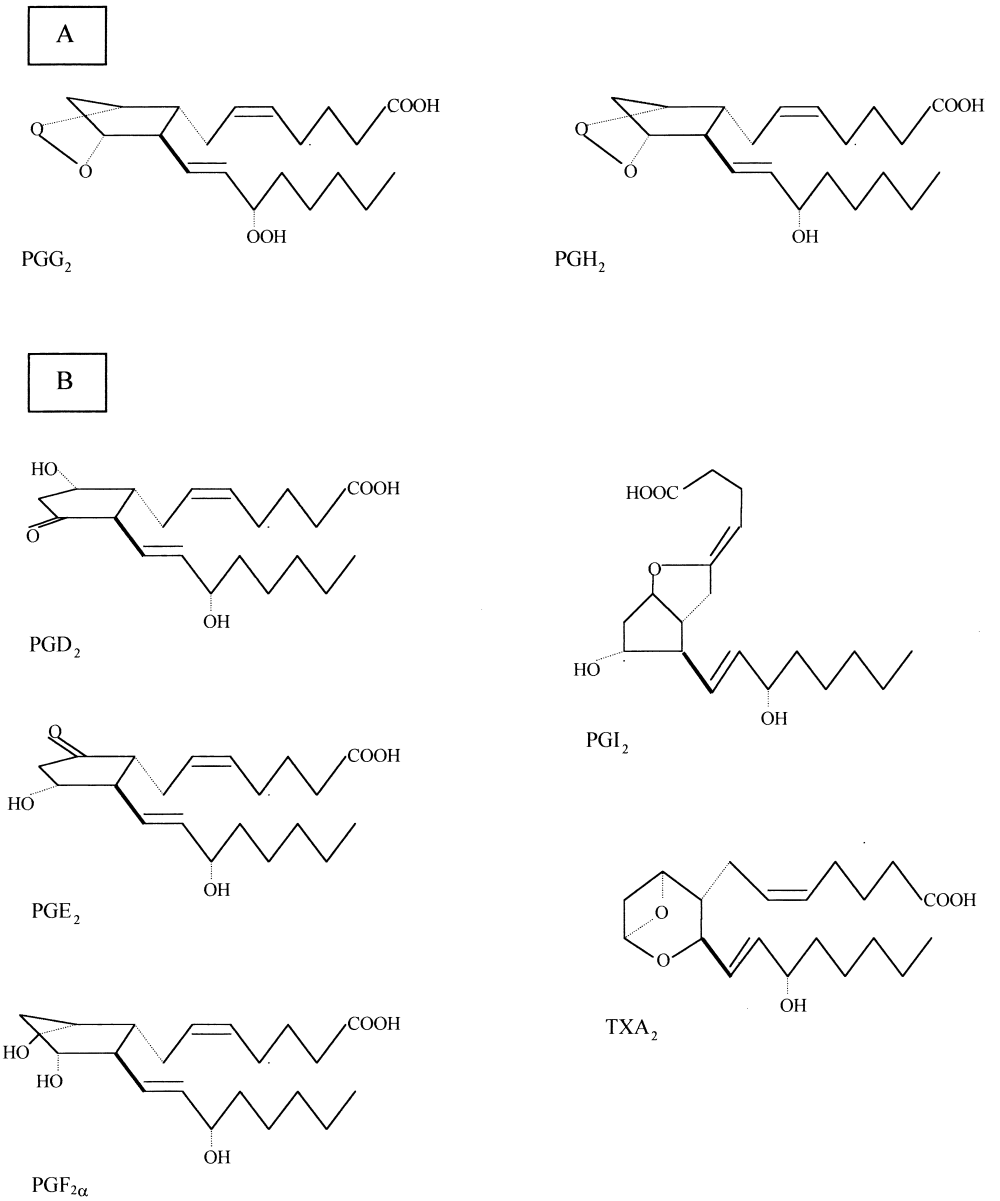

Fig. 1. Structure of the primary prostanoids and their precursors. A: The subsequent products of PGH synthase (COX), PGG_2 and PGH_2 are depicted. B: The structure of the five primary prostanoids PGD_2, PGE_2, $PGF_{2\alpha}$, PGI_2 and TXA_2.

[7–9]. The functional importance, if any, of this differential localisation is unclear.

COX-1 is constitutively expressed in many mammalian tissues [10], albeit at different levels. The level of COX-1 expression remains considerably constant and is only increased after stimulation of the cells with growth factors, cytokines or tumour promoting agents [11–14]. From this, it was concluded that COX-1 is essential for common though primary functions, such as the aggregation of platelets and regulation of renal blood flow [15,16]. Although COX-1 was not thought to be a major target of NSAIDs during inflammation, COX-1-negative mice displayed reduced experimental inflammation and gastric ulceration [17]. In addition, these mice exhibit strongly diminished fertility, although the physiological basis for this effect remains unexplained.

COX-2 is not detected in most tissues, but levels of expression are induced rapidly upon stimulation of cells with various substances including growth factors [18], cytokines [19], and endotoxins [20]. Although it is generally assumed that COX-2 is the important NSAID target during inflammation, gene disruption of COX-2 in mice induces nephropathy but does not alter inflammatory responses [21]. Together with the support for a role of COX-1 in inflammation from knockout experiments, the exact functioning of both enzymes in this process is probably more subtle than previously thought.

COX-2 overexpression in rat intestinal epithelial cells resulted in increased adhesion to the extracellular matrix, diminished E-cadherin expression, and enhanced levels of Bcl2 [22]. In addition, these cells became more resistant to butyrate-induced apoptosis. Interestingly, knockout of COX-2 decreased the size and number of polyps in mice suffering from intestinal polyposis and COX-2 overexpression is associated with tumorigenesis [23]. Together, these observations indicate that COX-2 inhibition may be important in chemoprevention of

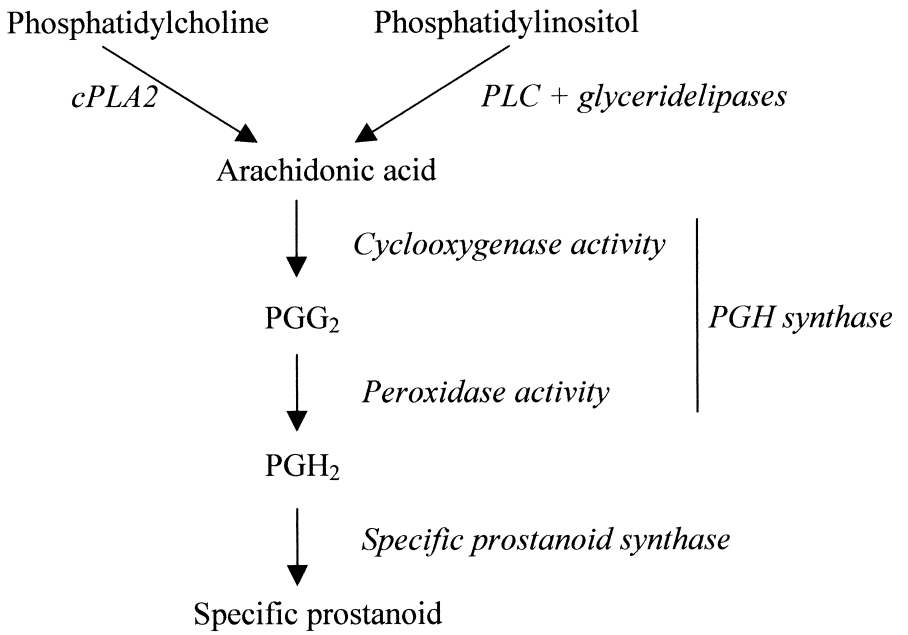

Fig. 2. Schematic representation of the prostanoid synthesis pathway.

cancer by NSAIDs [24]. A recent study by DuBois et al. [25], however, demonstrated that both COX-1 and COX-2 inhibition are important targets for the chemoprevention of cancer by NSAIDs through inhibition of neo-angiogenesis. Whereas COX-1 was essential for the formation of the endothelial tube, COX-2 was essential for the production of angiogenic factors, again demonstrating that NSAID actions in pathophysiology are not easily attributed to effects on a single COX isoform.

4. Prostanoid receptors

In general, prostanoid receptors belong to either the superfamily of G protein-coupled rhodopsin type of receptors or to the superfamily of nuclear steroid/thyroid hormone receptors. Within the family of G protein-coupled receptors, the prostanoid receptors constitute a subfamily, with remarkably low overall homology ranging from 24 to 44%. These prostanoid receptors are named after the ligand they bind: PD which binds PGD, TP which binds TX, PI which binds PGI, TF which binds PGF and PE which binds PGE. Of the PE receptor four subtypes exist named EP1, EP2, EP3 and EP4 [26]. These receptors display considerable cell type-specific expression. The TP receptor, for instance, is mainly found in platelets, EP1 in fibroblasts, EP2 and EP4 in smooth muscle cells, FP in kidney cells and astrocytes. Therefore, apart from localised production of the different prostanoids, their action is further restricted by localised receptor expression. This localised action may account for the highly specific effect seen in the EP4 knockout mouse: only the perinatal closure of the ductus arteriosus is affected [27].

In general, these plasma membrane-localised prostanoid receptors can be grouped into three categories, depending on the heterotrimeric G proteins to which they couple and thus to the cellular response these receptors elicit via their C-terminal regions. The first category consists of IP, EP2, EP4 and DP ([28–30] and references therein, [31]) which lead to the activation of adenylate cyclase via G_s. The second category encompasses the TP, EP1 and FP type of prostanoid receptors [32–35] and are coupled to the mobilisation of Ca^{2+} via G_q and phosphatidylinositol turnover. In addition, TP also activates G_{i2}, G_{12}, G_{13} and an unidentified G protein of 85 kDa [36–38], functions may be related to the action of TP in haemostasis. It is currently not known to which G protein EP1 is coupled, but it is generally implicated in calcium channel gating.

The EP3 type receptor is an exceptional case ([39] and references therein): in general it activates G_i [40,41] but due to its alternative splicing forms, differing only in the carboxy-terminal tails, it is capable of inducing a range of effects. Murine EP3α, β and γ, for instance, are all activators of G_i, but with different efficiencies [42]. Bovine EP3A activates G_i, whereas EP3B activates G_s. Bovine EP3D stimulates all three main G proteins, whereas EP3C couples to G_0 next to G_s [43]. Coupling of bovine EP3C to G_0 results in its inhibition, enhancing its affinity for GTP dissociation factor [44]. Action of the different EP3 receptor subtypes therefore displays considerable species specificity.

In addition to these plasma membrane receptors, also the peroxisome proliferator-activated receptor γ (PPARγ) [45], a member of the nuclear hormone receptor superfamily, has been identified as a prostanoid receptor. This interaction is not as specific as is prostanoid binding, to the transmembrane receptor, the K_d for prostanoid binding to this intracellular receptor (2 μM) being much larger than that of transmembrane receptors (varying over a range of 1.2–40 nM). PPARγ has been shown to bind several ligands including 15-deoxy-Δ^{12-14}prostaglandin J_2 (15-d-PGJ2) which is a metabolite of PGD_2, and interestingly, also NSAIDs. Upon activation, PPARγ heterodimerises with the retinoic acid receptor (RXR) [46] and binds to specific DNA sequences (peroxisome proliferator responsive elements), activating target genes. Like PPARα, an intracellular leukotriene receptor, PPARγ can

bind and inactivate NF-κB, thus knocking out inflammatory responses [47]. In agreement, stimulation of PPARγ with 15-d-PGJ2 results in reduced production of inflammatory products [47,48]. Since inhibition of inflammation only occurs in the presence of high intracellular concentrations of 15-d-PGJ2, it is plausible that this inhibition acts as a feedback mechanism on prostaglandin inflammation. Furthermore, as PPARγ is also activated by NSAIDs these compounds not only interfere with inflammation by inhibition of COX, but also directly, via stimulation of this class of receptors. A role in NF-κB regulation has recently also been described for some but not all NSAIDs acting through the inhibition of the NF-κB activator IKKβ [49], suggesting a threefold anti-inflammatory role for NSAIDs by regulating both inhibition of COX and NF-κB as well as activation of PPARγ.

Interestingly, very recently new effects mediated by PPARγ have been described. It has been suggested that PPARγ may be important for atherogenesis (e.g. [50]). Furthermore, they have also been associated with colon cancer [51,52]. Since high intracellular concentrations of 15-d-PGJ2 have been shown to serve as PPARγ ligands, this correlates with the effects seen after excessive prostaglandin production. In contrast, activation of PPARγ has also been shown to result in reversal of malignant changes in colon cancer [53], again demonstrating the complicity of prostanoid signalling.

5. Prostanoids as second messengers

A number of observations indicate that prostanoids also act downstream of receptors, as second messengers. We have shown that growth factor-dependent actin reorganisation requires PG synthesis [54]. Furthermore, in smooth muscle cells, cPLA2 activity and PGE_2 were required for a PDGF-induced rise in cAMP and PKA activation [55]. HGF-induced [56] and EGF-induced [57] DNA synthesis was also demonstrated to be dependent on COX activity. NSAID effects on cancer may therefore relate to inhibition of receptor tyrosine kinase-induced mitogenesis. Also, Muthalif et al. [58] presented evidence for a prostanoid-dependent activation of MAPK by angiotensin in rabbit aortic smooth muscle cells. Whether these effects are mediated via autocrine stimulation of prostanoid receptors or represent a genuine action of these lipids as second messengers is unknown. Nevertheless it is clear that prostanoids act in cellular physiology at multiple levels, and that the actions of NSAIDs in pathophysiology may only be fully explained if the exact functioning of prostanoids at these different levels is understood.

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