

Prostaglandin E₂ at new glance: Novel insights in functional diversity offer therapeutic chances

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A B S T R A C T

Prostaglandin E₂ (PGE₂) is the most abundant eicosanoid and a very potent lipid mediator. PGE₂ is produced predominantly from arachidonic acid by its tightly regulated cyclooxygenases (COX) and prostaglandin E synthases (PGES). Secreted PGE₂ acts in an autocrine or paracrine manner through its four cognate G protein coupled receptors EP1 to EP4. Under physiological conditions, PGE₂ is key in many biological functions, such as regulation of immune responses, blood pressure, gastrointestinal integrity, and fertility. Deregulated PGE₂ synthesis or degradation is associated with severe pathological conditions like chronic inflammation, Alzheimer's disease, or tumorigenesis. Therefore, pharmacological inhibition of COX enzymes and PGE₂ receptor antagonism is of great therapeutic interest.

Keywords:

Prostaglandin E₂
Cyclooxygenase
Eicosanoid
Aspirin

1. Introduction

Prostaglandins (PGs) are short-lived potent bioactive lipid messengers belonging to the family of eicosanoids (Funk, 2001; Harris et al., 2002; Simmons et al., 2004; Smith et al., 2000). The first prostaglandin was independently isolated by Maurice W. Goldblatt and Ulf S. von Euler from the prostate gland and seminal fluid back in 1935, and was shown to induce smooth muscle contraction and to reduce blood pressure. PGs derive from 20-carbon fatty acid precursors, mainly arachidonic acid (AA). Most cells synthesize almost undetectable or basal levels of PGs. PGs are de novo synthesized rapidly upon cell activation by most cells of the body and act in an autocrine and paracrine fashion. A variety of stimuli regulate the synthesis of PGs, which have an extraordinary broad spectrum of action (Funk, 2001; Harris et al., 2002). Prostaglandin E₂ (PGE₂; IUPAC: 7-[3-hydroxy-2-(3-hydroxyoct-1-enyl)-5-oxocyclopentyl]hept-5-enoic acid), also known as dinoprostone, is the most abundant prostanoid in humans and involved in regulating many different fundamental biological functions including normal physiology and pathophysiology (Dey et al., 2006; Park et al., 2006; Wang et al., 2007).

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2. Structure

PGE₂ is an unsaturated carboxylic acid based on a 20-carbon skeleton containing a cyclopentane ring and its structure is depicted in the center of Fig. 1A and B. Its molecular mass is 352.465 g/mol. The two double bonds in the carbon chains designate the numerical subscript in PG nomenclature also termed series-2 prostaglandins. PGE₂ can be distinguished from other series-2 PGs, i.e. by its degree of oxidation.

3. Expression, activation and turnover

The synthesis of PGs is initiated by the liberation of AA (Fig. 1A and B) from plasma membrane phospholipids by members of the phospholipase A₂ (PLA₂) family, of which the Ca²⁺-dependent cytosolic PLA₂ (cPLA₂) plays a dominant role (Park et al., 2006; Simmons et al., 2004; Smith et al., 2000). The amount of liberated AA designates the outcome of PG synthesis (Park et al., 2006). AA is immediately metabolized at the luminal side of nuclear and ER-membranes into the intermediate PGH₂ by cyclooxygenases (COX) and converted into different PGs by cell- and tissue-specific prostaglandin synthases (PGS) (Park et al., 2006; Samuelsson et al., 2007; Simmons et al., 2004).

COX exists in three isoforms (Dey et al., 2006; Park et al., 2006; Simmons et al., 2004; Smith et al., 2000): The constitutively expressed COX-1 is responsible for basal, and upon stimulation, for immediate PG synthesis, which also occurs at high AA concentrations. COX-2 is induced by cytokines and growth factors and

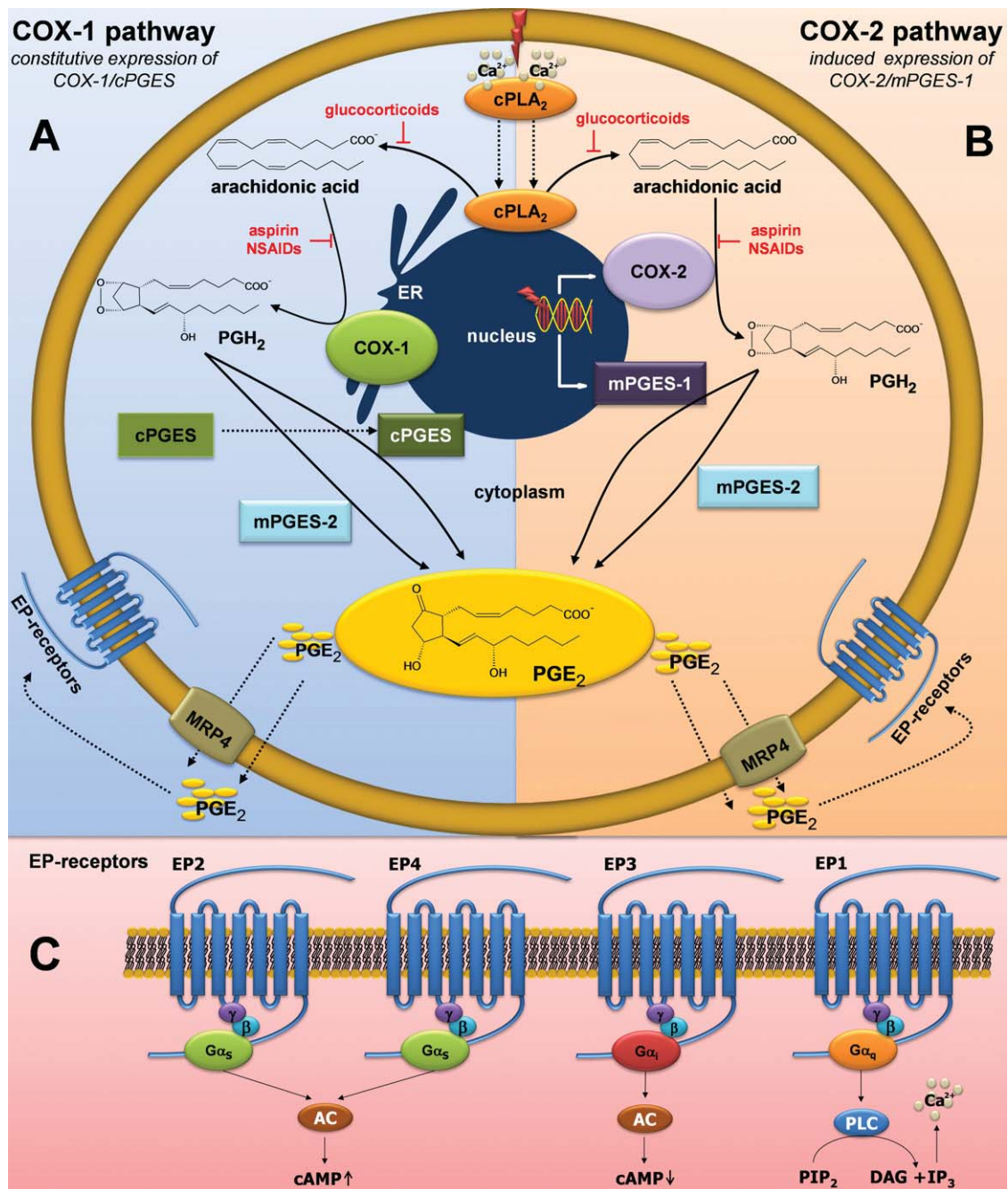


Fig. 1. Biosynthesis of PGE₂ and its interaction with its cognate receptors EP1–EP4 and main routes of pharmacological inhibition. PGE₂ is synthesized by cyclooxygenases (COX) and prostaglandin synthetases (PGES) from arachidonic acid. After release from the producing cell via passive diffusion through the plasma membrane or active transport by the multidrug resistance protein 4 (MRP4), PGE₂ binds to and signals through a family of specific E-prostanoid (EP) receptors. (A) COX-1 pathway of basal or stimulus-induced immediate PGE₂ biosynthesis. After membrane interaction of cytosolic phospholipase A₂ (cPLA₂) in response to transient calcium increases, arachidonic acid is liberated from phospholipids of cellular membranes. At the luminal side of nuclear and ER-membranes, COX-1 converts arachidonic acid into its transient metabolite prostaglandin H₂ (PGH₂) which is then metabolized into PGE₂ via the membrane-tethered cytosolic prostaglandin E synthase (cPGES) or, alternatively, via cytosolic residing, microsomal prostaglandin E synthase (mPGES)-2. (B) COX-2-mediated PGE₂ biosynthetic pathway. At sites of inflammation, cytokine- and growth factor-inducible COX-2 oxidizes arachidonic acid to form PGH₂ which is subsequently converted into PGE₂ by mPGES-1 or mPGES-2. Black lines with arrow indicate conversion; dotted lines, translocation. The white right-angled arrows indicate transcription/translocation. Red letters indicate sites of inhibition of PG synthesis; non-steroidal anti-inflammatory drugs (NSAIDs). (C) PGE₂ signaling through the EP receptor family of seven-transmembrane G-protein-coupled receptors; PGE₂ acts through four different receptor subtypes, EP1 to EP4. EP1 couples to Gα_i protein and signals through the phospholipase C (PLC)/inositol-1,4,5-trisphosphate (IP₃) pathway resulting in the formation of the second messengers diacylglycerol (DAG) and IP₃, with the latter rapidly liberating Ca²⁺ ions from intracellular stores. EP3 couples to Gα_i for signaling and inhibits adenylyl cyclase (AC) activation resulting in decreased cAMP concentrations. In contrast, EP2 and EP4 receptor subtypes couple to Gα_s and its activation leads to increased cAMP production.

primarily involved in the regulation of inflammatory responses. COX-3 is a splice variant of COX-1 predominantly expressed in brain and heart. PGE₂ is synthesized from PGH₂ by cytosolic cPGES or by membrane-associated/microsomal mPGES-1 and mPGES-2 (Park et al., 2006; Samuelsson et al., 2007). cPGES is constitutively and

abundantly expressed and preferentially couples with COX-1. The expression of mPGES-1 is induced by cytokines and growth factors similar to COX-2, with which it couples. This suggests a coordinated regulation of COX-2 and mPGES-1 by common signaling pathways, such as NF-κB. However, constitutive expression of mPGES-1 in

certain tissues and cell types was also reported. The widely and constitutively expressed mPGE₂ was shown to be further induced under pathological conditions (i.e. cancer) and interacts with COX enzymes (Park et al., 2006; Samuelsson et al., 2007).

Finally, de novo synthesized PGE₂ is actively transported through the membrane by the ATP-dependent multidrug resistance protein-4 (MRP4) or diffuses across the plasma membrane (Park et al., 2006) to act at or nearby its site of secretion. PGE₂ then acts locally through binding of one or more of its four cognate receptors, termed EP1–EP4 (Sugimoto and Narumiya, 2007). EP receptors belong to the large family of seven transmembrane domain receptors coupled to specific G proteins with different second messenger signaling pathways (Fig. 1C). EP1 couples most probably to G α_q , and PGE₂ binding leads to an elevation of cytosolic free calcium concentration. G α_s -mediated EP2 and EP4 signaling increases intracellular cAMP. EP3 is regarded as an “inhibitory” receptor that couples to G α_i proteins and decreases cAMP formation.

As is the rule for locally acting lipid mediators, PGE₂ is not stored but rapidly metabolized. The major enzymes responsible for rapid (within minutes) inactivation of PGE₂ are the cytosolic enzymes 15-ketoprostaglandin Δ^{13} -reductase and 15-hydroxyprostaglandin dehydrogenase, of which the latter is deregulated in some forms of cancer (Tai et al., 2006).

4. Biological functions

Since PGE₂ can be produced by virtually any cell of the human body, either constitutively or upon stimulation, and signals through different receptors, its biological effects are diverse and of an astounding complexity, depending on the amount of PGE₂ available within the microenvironment of diverse tissues and on the subtype of EP receptors expressed on target cells (Funk, 2001; Harris et al., 2002; Sugimoto and Narumiya, 2007).

Besides other prostanoids, PGE₂ has been described as a regulator of numerous physiological functions ranging from reproduction to neuronal, metabolic and immune functions. In the central nervous system, PGE₂ has been implied in the regulation of body temperature and sleep–wake activity, and is involved in hyperalgesic responses as part of sickness behavior. It has been described as a regulating factor for bone formation and bone healing. One of the most important features of PGE₂, which makes it a key player in the control of multiple physiological processes, is its vasodilatory activity, through which PGE₂ participates for example in embryo implantation and modulation of haemodynamics in the kidney (Fortier et al., 2008). Moreover, the effect of PGE₂ on contraction and relaxation of smooth muscle cells are not only evident in childbirth and blood pressure control, but also in gastrointestinal motility, where it plays a major role in coordination of peristaltic movement. Distinct expression and distribution of EP receptors in the gastrointestinal tract determine additional functions of PGE₂ in the gut (Dey et al., 2006). Besides motility, PGE₂ plays a role in gastrointestinal secretion and mucosal barrier functions. The first line of defense of the intestinal immune system is the secretion of mucins, glycoprotein polymers that protect the mucosa. Secretion of mucin from gastric epithelial cells can be induced by PGE₂. Moreover, in a mouse injury model, PGE₂ was demonstrated to protect small intestinal epithelial cells from radiation-induced apoptosis (Dey et al., 2006).

In inflammation, PGE₂ is of particular interest because it is involved in all processes leading to the classic signs of inflammation: redness, swelling and pain (Funk, 2001; Harris et al., 2002). Redness and edema result from increased blood flow into the inflamed tissue through PGE₂-mediated augmentation of arterial dilatation and increased microvascular permeability. Hyperalgesia is mediated by PGE₂ through EP1 receptor signaling and acts on peripheral sensory neurons at the site of inflammation, as well

as on central neuronal sites. Because of its role in these basic inflammatory processes, PGE₂ has been referred to as a classical pro-inflammatory mediator. The relevance of prostaglandins during the promotion of inflammation is emphasized by the effectiveness of non-steroidal anti-inflammatory drugs (NSAIDs) acting as COX-inhibitors (Simmons et al., 2004). However, the role of PGE₂ in the regulation of immune responses is even more complex. Studies on knock-out mice deficient for individual EP receptors clearly revealed that PGE₂ not only acts as a pro-inflammatory mediator, but also exerts anti-inflammatory responses (Sugimoto and Narumiya, 2007). The environment, in which dendritic cells (DCs) take up antigens and undergo maturation, shapes the outcome of the induced adaptive immune response. As pro-inflammatory mediator, PGE₂ contributes to the regulation of the cytokine expression profile of DCs and has been reported to bias T cell differentiation towards a T helper (Th) 1 or Th2 response. A recent study showed that PGE₂-EP4 signaling in DCs and T cells facilitates Th1 and IL-23-dependent Th17 differentiation (Yao et al., 2009). Additionally, PGE₂ is fundamental to induce a migratory DC phenotype permitting their homing to draining lymph nodes (Kabashima et al., 2003; Legler et al., 2006). Simultaneously, PGE₂ stimulation early during maturation induced the expression of co-stimulatory molecules of the TNF superfamily on DCs resulting in an enhanced T cell activation (Krause et al., 2009). In contrast, PGE₂ has also been demonstrated to suppress Th1 differentiation, B cell functions and allergic reactions (Harris et al., 2002; Kunikata et al., 2005). Moreover, PGE₂ can exert anti-inflammatory actions on innate immune cells like neutrophils, monocytes and NK cells (Harris et al., 2002).

Deregulation of COX has been described in the pathogenesis of various diseases and a number of different tumor types (Greenhough et al., 2009; Wang et al., 2007). COX-2 over-expression leads to increased levels of PGE₂ and has been associated particularly with colorectal, pancreatic, lung and breast cancer (Wang et al., 2007), albeit a recent study found reduced expression of COX-2 in primary breast cancer compared to surrounding healthy tissue (Boneberg et al., 2008). Moreover, PGE₂ has been implicated in various tumorigenic processes, and the involvement of specific EP receptors and signaling pathways has been elucidated (Greenhough et al., 2009; Wang et al., 2007). For example, PGE₂ facilitates tumor progression through stimulation of angiogenesis via EP2, mediates cell invasion and metastasis formation via EP4 and promotes cell survival by inhibiting apoptosis via numerous signaling pathways. Moreover, tumor cell-produced PGE₂ has been implicated in strategies of tumors for evasion of immune-surveillance (Ahmadi et al., 2008). The mechanisms by which PGE₂ participates in suppression of anti-tumor immune responses could be multifaceted and are not yet fully understood. It has been demonstrated that PGE₂-secreting lung cancer cells can induce human CD4⁺ T cells to express Foxp3 and develop a regulatory phenotype. Furthermore, the presence of PGE₂ can enhance the inhibitory function of human regulatory T cells (Baratelli et al., 2005). Additionally, PGE₂ in the tumor environment can effect DCs by altering their cytokine expression profile, resulting in reduction of anti-tumor-specific cytotoxic T cell activation (Ahmadi et al., 2008; Muthuswamy et al., 2008). However, PGE₂ has also been described as tumor-suppressive, which seems to be contradictory, but could be explained by different expression levels of PGE₂ and co-occurrence of other factors leading to an opposing outcome (Greenhough et al., 2009; Muthuswamy et al., 2008). This fact emphasizes the complexity of the regulatory system of prostanoids, but also offers exciting and promising targets for therapeutic intervention. Targeting PGE₂ levels during tumor therapies could be beneficial, as the administration of antibodies against PGE₂ has been shown to delay tumor growth in mice (Greenhough et al., 2009).

5. Pharmaceutical targeting of PGE₂ synthesis and antagonizing specific EP receptors

Beside the clinical use of PGE₂ to induce childbirth or abortion, and as vasodilator in severe ischemia or pulmonary hypertension, the main pharmaceutical focus lies in the inhibition of PGE₂ synthesis (Fig. 1) or in the specific blockage of selected EP receptors. NSAIDs act as COX inhibitors although through different mechanisms and belong to the most utilized pharmaceutical drugs worldwide (Simmons et al., 2004). Its most prominent representative is acetylsalicylic acid (aspirin), which was first marketed in 1898. One unique feature of aspirin is that it covalently modifies COX-1 and, with lesser efficiency, COX-2 by acetylating a serine residue at the active site of the enzyme. Other NSAIDs predominantly compete for binding with arachidonic acid in the active site of COX. Well-known NSAIDs include the synthetic COX inhibitors indomethacin, NS398, celecoxib (Celebrex), rofecoxib (Vioxx), valdecoxib, flurbiprofen, or etoricoxib. Their modes of action and known side effects are precisely described (Simmons et al., 2004). PGE₂ synthesis may also be blocked by glucocorticoids which inhibit PLA₂. Recent studies with gene targeted mice, in which single EP receptors were deleted, gave new insights on the various actions of PGE₂ (Sugimoto and Narumiya, 2007). This, in combination with the development of specific EP receptor agonists and antagonists, will boost novel therapeutic approaches both in physiology and pathology.

Acknowledgments

We are grateful to current and past members of the BITg. We received research funding from Swiss National Science Foundation, Vontobel Stiftung, Thurgauische Stiftung für Wissenschaft und Forschung, and Swiss State Secretariat for Education and Research. DFL is a recipient of a career development award from the Prof. Dr. Max Cloëtta Foundation.

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