Prostaglandins and Bone

Carol Pilbeam

Contents

Abstract

Prostaglandins (PGs) are highly bioactive fatty acids. PGs, especially prostaglandin E_2 (PGE_2), are abundantly produced by cells of both the bone-forming (osteoblast) lineage and the bone-resorbing (osteoclast) lineage. The inducible cyclooxygenase, COX-2, is largely responsible for most PGE_2 production in bone, and once released, PGE_2 is rapidly degraded in vivo. COX-2 is induced by multiple agonists – hormones, growth factors, and proinflammatory factors – and the resulting PGE_2 may mediate, amplify, or, as we have recently shown for parathyroid hormone (PTH), inhibit responses to these agonists. In vitro, PGE_2 can directly stimulate osteoblast differentiation and, indirectly via stimulation of RANKL in osteoblastic cells, stimulate the differentiation of osteoclasts. The net balance of these two effects of PGE_2 in vivo on bone formation and bone resorption has been hard to predict and, as expected for such a widespread local factor, hard to study. Some of the complexity of PGE_2 actions on bone can be explained by the fact that there are four receptors for PGE_2 (EP1–4). Some of the

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major actions of PGE_2 in vitro occur via EP2 and EP4, both of which can stimulate cAMP signaling, but there are other distinct signaling pathways, important in other tissues, which have not yet been fully elucidated in bone cells. Giving PGE_2 or agonists of EP2 and EP4 to accelerate bone repair has been examined with positive results. Further studies to clarify the pathways of PGE_2 action in bone may allow us to identify new and more effective ways to deliver the therapeutic benefits of PGE_2 in skeletal disorders.

Keywords
Bone formation · Bone resorption · Cyclooxygenase · EP receptors · NSAIDs · Prostaglandin

1 Introduction

Prostaglandins (PGs) are highly bioactive unsaturated fatty acids. PGs are not stored but are synthesized and released, as needed, and rapidly metabolized. This chapter will focus on prostaglandin E_2 (PGE_2), which is abundantly produced by cells of both the bone-forming and bone-resorbing lineages. Production of PGs depends on the availability of substrate; one of two cyclooxygenases (COXs), constitutively expressed COX-1 or inducible COX-2; and a specific downstream synthase. Most PGE_2 in bone is produced by COX-2. COX-2 is induced by multiple hormones and proinflammatory factors, and the resulting PGE_2 can mediate or modify responses to the agonist, sometimes in very unexpected ways, as we discuss for the COX-2 agonist, parathyroid hormone (PTH). PGE_2 can act on four G-protein-coupled receptors whose signaling pathways have not been fully elucidated in bone cells. It has been 50 years since PGE_2 in bone was first shown to stimulate cyclic AMP (cAMP) production and resorption in bone organ cultures (Klein and Raisz 1970). The early studies in cell and organ cultures led to the conclusion that PGE_2 could increase both bone formation and resorption, and this has led to continuing interest in the potential for therapeutic manipulation of PGE_2 or its receptors.

2 PGE_2 Production

Eicosanoids are bioactive lipids derived from arachidonic acid (AA) and other 20-carbon polyunsaturated fatty acids (PUFAs) (Buczynski et al. 2009; Smith et al. 2011). The term "prostanoid" refers to products of the COX pathway: PGE_2, prostaglandin D_2 (PGD_2), prostaglandin F_{2a} (PGF_{2a}), prostacyclin (PGI_2), and thromboxane (TXA_2). PGs are 20-carbon fatty acids with a cyclopentane ring. Although TXAs have an oxane ring, they are generally discussed under the "PG" heading. The subscript for prostanoids denotes the number of double bonds (e.g., PGE_1, PGE_2, and PGE_3). The two series of prostanoids are the most abundant and the best characterized.

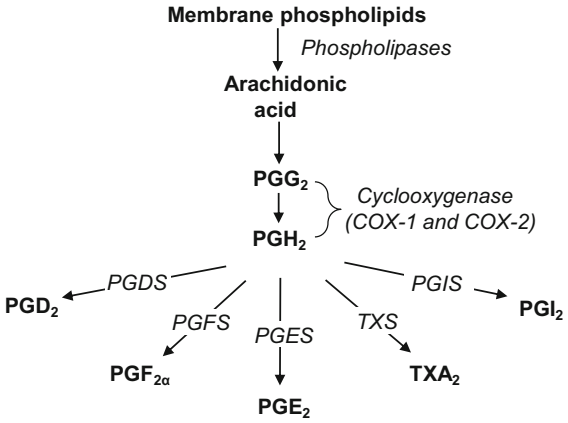

Fig. 1 Major prostanoids generated from arachidonic acid. Free arachidonic acid, released from membrane phospholipids by phospholipases, is converted by a bifunctional enzyme, called cyclooxygenase, to prostaglandin G_2 (PGG_2) in a cyclooxygenase reaction followed by reduction of PGG_2 to prostaglandin H_2 (PGH_2) in a peroxidase reaction. PGH_2 is then converted to specific prostanoids by the terminal synthases

There are three steps in the production of PGs (Fig. 1). The first is the mobilization of AA from membranes by phospholipases. The second step is catalyzed by a bifunctional enzyme that converts free AA to prostaglandin G_2 (PGG_2), first by a cyclooxygenase (hence, the name COX) reaction and then a peroxidase reaction, to prostaglandin H_2 (PGH_2). PGH_2 is then converted by terminal synthases to the various prostanoids.

AA Release Phospholipase A_2 (PLA_2) enzymes catalyze the hydrolysis of membrane phospholipids, from membrane glycerophospholipids, releasing free fatty acids, such as AA. The PLA_2 superfamily has 16 groups and many subgroups (Dennis et al. 2011; Murakami et al. 2011, 2015; Vasquez et al. 2017). The most important PLAs for PG production are probably the Ca^{2+}-independent PLA_2s ($iPLA_2$), the Ca^{2+}-dependent cytosolic PLA_2s ($cPLA_2$), and the secreted PLA_2s ($sPLA_2$). As a general summary, $iPLA_2$ is the primary PLA_2 in cells, producing low levels of free fatty acids, some of which may be AA, needed for daily cellular functions; $cPLA_2$ is the major inducible enzyme hydrolyzing AA-containing phospholipids during infection or inflammation; $sPLA_2$ is also inducible and augments $cPLA_2$ function (Dennis and Norris 2015).

COX Enzymes The bifunctional enzyme is formally named prostaglandin endoperoxide H synthase or prostaglandin G/H synthase (PGHS), and the gene name is *ptgs*. However, because the COX reaction site is inhibited by nonsteroidal anti-inflammatory drugs (NSAIDs), the interest in developing new inhibitors selective for COX-2 led to calling PGHS simply COX and the inhibitors coxibs. The two

enzymes for COX, COX-1 and COX-2, are encoded by separate genes (Herschman 1994; Smith et al. 2000). Both COX-1 and COX-2 are N-glycosylated dimeric proteins inserted into the luminal face of the ER and the contiguous inner membrane of the nuclear envelope (Smith et al. 2011).

Despite having similar catalytic mechanisms, COX-1 and COX-2 are independently functioning pathways (Simmons et al. 2004; Smith and Langenbach 2001). COX-2 is rapidly and transiently inducible by multiple factors in many tissues (Kang et al. 2007). COX-1 is expressed at relatively low, stable levels in most tissues and is considered to be "constitutive." The half-life of COX-2 protein is reported to be 2–7 h in various tissues, while the half-life of COX-1 protein is much longer (Kang et al. 2007; Mbonye et al. 2006). However, it is now evident that COX-2 mRNA is also expressed constitutively at low levels in many tissues and cells. A recent study examined signaling pathways underlying constitutive expression of COX-2 in the kidney, gastrointestinal tract, and brain (Kirkby et al. 2016). It is still unclear how much this expression contributes to PG production. Another difference is that COX-2 is much more efficient at using low AA concentrations (below 5 μM) than COX-1 (Swinney et al. 1997). This difference may explain why osteoblasts from COX-2 KO mice make little or no measurable PGE_2 in culture despite the constitutive expression of COX-1 (Chikazu et al. 2005; Choudhary et al. 2003; Okada et al. 2000a; Xu et al. 2007).

It was initially hypothesized that COX-2 was responsible for acute pathological PG responses, while COX-1 produced prostanoids for ongoing "housekeeping" functions, such as maintenance of renal blood flow, platelet aggregation, and gastric cytoprotection. Once highly selective inhibitors of COX-2 activity were available, it became apparent that COX-2 also has physiologic functions and COX-2 selective NSAIDs turned out to have serious adverse side effects, especially on the cardiovascular system, that would limit their use (Grosser et al. 2017a, b).

PG Synthases PGH_2 is converted to each PG by specific PG terminal synthases (Fig. 1), and the synthases may determine the major PG synthesized in a tissue. Prostaglandin E synthase (PGES), which converts PGH_2 to PGE_2, occurs in multiple forms (Hara et al. 2010; Hara 2017). The predominant PGES, mPGES-1, is inducible, located in ER and perinuclear membranes, and regulated similarly to COX-2. mPGES-1 may also be located in the Golgi apparatus, and because COX-2 and $cPLA_2$ may also be located there, the Golgi apparatus may be a dedicated PGE_2 synthesis site (Leslie 2015; Yuan and Smith 2015). mPGES-2 is constitutively expressed and functionally coupled with both COX-1 and COX-2. A third form, cytosolic PGES (cPGES), is preferentially coupled to COX-1 and thought to maintain PGE_2 production for cellular homeostasis (Tanioka et al. 2000). Knockout (KO) mice for the synthases are reviewed in Hara et al. (2010) and Hara (2017). Mice deficient for mPGES-1 have reduced inflammatory and pain responses, and potential inhibitory drugs have been developed (Psarra et al. 2017). Mice deficient for mPGES-2 have no specific phenotype, and deficiency of cPGES in mice is perinatal lethal.

PGE_2 Degradation Degradation of circulating PGE_2 occurs rapidly (Ferreira and Vane 1967), and measurement of PGE_2 metabolites in the urine may be the preferred way to track changes in PGE_2 production in vivo. The first step in degrading PGE_2 is mediated by the enzyme 15-hydroxyprostaglandin dehydrogenase (HPGD, also known as 15-PGDH), which generates metabolites that are orders of magnitude less potent than PGE_2 itself. Many tissues make HPGD, but it is not clear how quickly bone cells degrade PGE_2. It is common practice to follow PGE_2 production in vitro by measuring PGE_2 accumulation in osteoblastic or marrow stromal cell cultures over 2–3 days between medium changes, suggesting shared knowledge of some stability in culture.

In humans, mutations in HPGD are associated with a rare genetic disorder called idiopathic hypertrophic osteoarthropathy, characterized by chronically elevated PGE_2, coarse or thickened skin, and periostosis in bone (Uppal et al. 2008). In mice, inhibition of HPGD increases PGE_2 and potentiates recovery in marrow transplant models and accelerates tissue generation in models of colon and liver injury (Antczak et al. 2017; Desai et al. 2018; Zhang et al. 2015b). Although the known associations of elevated PGE_2 with increased inflammation and tumorigenesis might be thought to limit the usefulness of inhibiting HPGD, initial studies in animal models suggest that a small molecule inhibitor can promote transplant recovery without limiting side effects (Desai et al. 2018).

3 PGE_2 Receptors

There are four G-protein-coupled receptors (GPCRs), called EP1, EP2, EP3, and EP4, mediating actions of PGE_2 (Fig. 1) (Woodward et al. 2011). The EP1 receptor is known to increase Ca^{2+} and may couple to $G\alpha_q$ because studies have reported involvement of the PLC/PKC pathway (Tang et al. 2005). The major signaling pathway for EP3 receptors is $G\alpha_i$-induced adenylate cyclase inhibition. EP2 and EP4 are the receptors most extensively studied in bone. Mice deficient in each EP receptor subtype have been generated, and highly selective agonists for the receptors have been developed (Sugimoto and Narumiya 2007; Woodward et al. 2011).

The first major pathway for PGE_2 signaling identified in bone was cAMP (Klein and Raisz 1970). Both EP2 and EP4 can stimulate $G\alpha_s$ to activate adenylyl cyclases (ACs) and produce cAMP, which can then activate protein kinase A (PKA) or a PKA-independent pathway mediated by EPAC (exchange protein directly activated by cAMP). The PKA pathway is able to crosstalk with other pathways that regulate cell growth, motility, migration, and apoptosis, including the Wnt/β-catenin signaling pathway (Buchanan and DuBois 2006; Castellone et al. 2006; Estus et al. 2016; Hino et al. 2005; Shao et al. 2005). EP2 and EP4 may also transactivate the epidermal growth factor receptor (EGFR) signaling pathway, leading to multiple signaling pathways, including PI3K/Akt/β-catenin. The recruitment of β-arrestin by EP receptors may also activate c-Src, resulting in EGFR transactivation (O'Callaghan and Houston 2015). There was much interest in studying EP receptors in bone in the early days because of the possibility of developing drugs that could

target a specific receptor to increase bone mass and fracture healing without the side effect of inhibiting COX. In more recent years, most of the data on EP signaling has come from studies on other tissues.

4 Bone Remodeling and PGE_2

Bone remodeling, the cycle of bone resorption followed by bone formation, occurs throughout our adult life. The net bone balance of these cycles determines whether bone is lost, gained, or maintained. Agents used to prevent or treat osteoporosis, that is, skeletal fragility associated with a high risk of fracture, are aimed at manipulating this cycle. Exogenously applied PGE_2 has been shown to stimulate both resorption and formation in bone, but the role of endogenous PGE_2 in remodeling is still being defined (Blackwell et al. 2010). The potential involvement of endogenous PGE_2 is shown in Fig. 2.

Bone Resorption Early work adding PGs in organ culture showed that exogenous PGs of the E series were potent activators of resorption (Klein and Raisz 1970). Studies with inhibitors of PG production showed that the resorptive effects of many agonists were mediated in part by PGs (Pilbeam et al. 2008). In the early 1990s, the inducible COX-2 was identified (O'Banion et al. 1991; Kujubu et al. 1991; Xie et al. 1991). Subsequently, many resorption agonists were shown to induce COX-2 expression and PGE_2 production in osteoblastic cells that contributed to increasing osteoclasts, including interleukin-1 (IL-1) (Kawaguchi et al. 1994; Sato et al. 1996; Lader and Flanagan 1998; Min et al. 1998), IL-6 (Tai et al. 1997), tumor necrosis

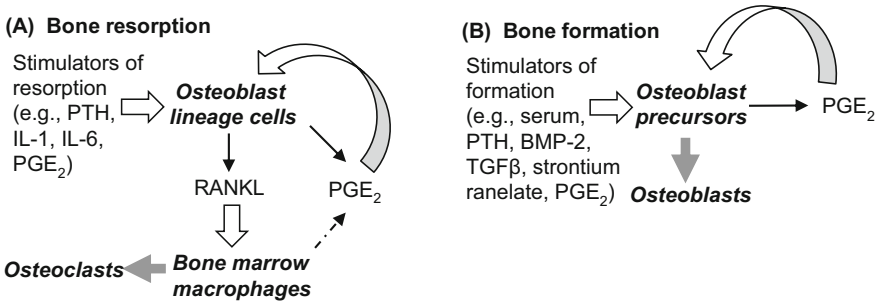

Fig. 2 Potential roles of PGE_2 in bone resorption and formation. (**a**) Agonists of resorption, including PGE_2, act on osteoblastic cells to induce RANKL, which then binds with its receptor, RANK, on bone marrow macrophages (BMMs) and drives them to differentiate into osteoclasts. These agonists are often also inducers of COX-2 expression in osteoblastic cells, as well. The PGE_2 can act back on osteoblasts to induce more RANKL. (RANKL can also induce COX-2 in BMMs.) (**b**) Some stimulators of osteoblastic differentiation also induce COX-2 and produce PGE_2. PGE_2 can then act on the osteoblastic precursors to stimulate osteoblastic differentiation. In some cases, such as serum and strontium ranelate (see text), the major stimulatory effect in cell culture is due to PGE_2

factor-α (TNF-α) (Lader and Flanagan 1998), parathyroid hormone (PTH) (Kawaguchi et al. 1994; Inoue et al. 1995; Okada et al. 2000a), and $1,25(OH)_2D_3$ (Okada et al. 2000a). PGE_2 can amplify its own production by inducing COX-2 (Pilbeam et al. 1994). PGE_2 has receptors on both osteoblastic and osteoclastic lineage cells, but stimulates osteoclast differentiation largely indirectly via upregulation of expression of receptor activator of NFκB ligand (RANKL) and inhibition of expression of the decoy RANKL receptor, osteoprotegerin (OPG), in osteoblastic lineage cells (Li et al. 2000) (Fig. 2a). Although both EP2 and EP4 may be involved in PGE_2-stimulated resorption, a number of in vitro studies have suggested that EP4 is the more important receptor for resorption (Miyaura et al. 2000; Suzawa et al. 2000; Raisz and Woodiel 2003; Zhan et al. 2005).

Bone Formation Many studies have shown that PGE_2 stimulates osteoblastic differentiation in murine or rat bone marrow stromal cell and primary osteoblast/calvarial cell cultures (Choudhary et al. 2013; Flanagan and Chambers 1992). In cultured marrow stromal cells or primary osteoblasts from mice with deletion of *ptgs2* or treated with NSAIDs to inhibit COX-2 activity, osteoblastic differentiation is decreased (Choudhary et al. 2013, Okada et al. 2000b, Xu et al. 2007, Zhang et al. 2002). Systemic injections of PGE_2 can increase both periosteal and endosteal bone formation in the rat and produce substantial increases in bone mass (Jee and Ma 1997; Lin et al. 1994; Suponitzky and Weinreb 1998). Systemic administration of PGE_2 in humans (Faye-Petersen et al. 1996; Ueda et al. 1980) and dogs (Norrdin and Shih 1988) has also been shown to increase cortical and cancellous bone mass. Local infusion of PGE_2 has been shown to increase bone in mice (Yoshida et al. 2002).

Similar to resorption agonists, many osteogenic factors induce COX-2 (Fig. 2b), including transforming growth factor β (TGFβ) (Pilbeam et al. 1997), basic fibroblast growth factor (FGF-2) (Kawaguchi et al. 1995), bone morphogenetic protein (BMP-2) (Chikazu et al. 2005), strontium (Choudhary et al. 2007), and fluid shear stress or mechanical loading (Klein-Nulend et al. 1997; Pavalko et al. 1998; Wadhwa et al. 2002). Serum is also a potent inducer of COX-2 expression and PGE_2 production in cultured osteoblasts (Pilbeam et al. 1993). For some osteogenic factors, such as serum, BMP-2, and strontium ranelate, their ability to stimulate osteoblast differentiation in vitro is due largely to their induction of COX-2 produced PGs (Pilbeam et al. 1993; Chikazu et al. 2005; Choudhary et al. 2007).

Both EP2 and EP4 receptors have been positively implicated in the osteogenic and anabolic effects of PGE_2 (Alander and Raisz 2006; Choudhary et al. 2008; Li et al. 2007), while mice deficient in EP1 receptors have enhanced fracture healing, higher trabecular bone volume, increased bone formation, and accelerated osteoblastic differentiation compared to WT mice (Zhang et al. 2011, 2015a). It has been difficult to study EP4 receptors in vivo because EP4 KO murine neonates in a pure C57Bl/6 background die shortly after birth due to patent ductus arteriosus (Segi et al. 1998). Reduced bone mass and impaired fracture healing were found in aged EP4 receptor KO mice compared to WT mice (Li et al. 2005) in contrast to another study which did not find any difference in bone formation between aged WT and EP4 KO mice (Gao et al. 2009). Both of these studies were done in mice with mixed

backgrounds to circumvent the patent ductus arteriosus problem, and the difference in phenotypes might be due to variability in backgrounds.

Agonists of EP2 and EP4 can increase fracture healing in animals. Local application of an EP2 agonist and local and systemic application of EP4 agonists have been shown to accelerate bone repair (Li et al. 2003; Paralkar et al. 2003; Tanaka et al. 2004; Yoshida et al. 2002). Clinical studies have not been done with these agonists, perhaps because increased PG can have adverse effects (Markovic et al. 2017).

5 Skeletal Phenotypes of Mice with COX-2 Deficiency

Disruption of genes for PG production has given us some indications of the role of endogenous PGs in vivo. It was clear in early studies that COX-1 KO mice were healthy and survived normally, while COX-2 KO mice had more profound effects (Dinchuk et al. 1995; Langenbach et al. 1995, 1999; Morham et al. 1995). However, one study reported 35% of neonatal COX-2 KO mice died with a patent ductus arteriosus and the mortality increased to 100% when both genes for COX-1 were inactivated, indicating the dependence of COX-2 effects on levels of COX-1 expression (Loftin et al. 2001). Other studies found 20% of COX-2 KO mice dying between 7 and 23 weeks of age secondary to renal dysplasia, despite normal renal development at birth (Morham et al. 1995; Norwood et al. 2000). COX-2 KO female mice were infertile, with multiple failures in female reproductive processes, including ovulation, fertilization, and implantation (Lim et al. 1997). Initial studies of skeletal phenotypes in the C57Bl/6,129 background also reported early death in COX-2 KO mice, gave variable phenotypic results, and suggested that older COX-2 KO mice might have primary hyperparathyroidism (HPTH) (Alam et al. 2005; Xu et al. 2005; Robertson et al. 2006).

Effects of Background Strain Mice for all these initial studies were in the C57Bl/6 inbred strain or mixed C57Bl/6 and 129 inbred strains. Both C57Bl/6 and 129sv mice have been shown to have a natural mutation that results in lack of the gene for the serum phospholipase (GIIA $sPLA_2$) that is important for releasing AA (Kennedy et al. 1995; MacPhee et al. 1995). Hence, COX-2 KOs in these backgrounds are really double KOs, and this may impair the ability of COX-1 to produce PGs and compensate for absent COX-2. The MC3T3-E1 osteoblastic cell line, commonly used to study osteoblastic cells in vitro, was derived from C57Bl/6 mice and may also lack GIIA PLA_2.

Mice in the outbred strain, CD-1, were reported to be heterozygous for the GIIA $sPLA_2$ mutation (Kennedy et al. 1995). We bred COX-2 KO mice into the CD-1 background and found that COX-2 KO mice had no increased mortality and no renal dysfunction and that COX-2 KO females were fertile (Xu et al. 2010). Despite being healthy, 5-month-old male COX-2 KO mice had twofold elevated serum PTH compared to WT mice. COX-2 KO mice also had increased serum markers of bone formation and resorption, decreased femoral BMD by DXA (dual-energy X-ray absorptiometry) and cortical bone thickness by μCT, and small but nonsignificant

decreases in trabecular bone volume by μCT or dynamic histomorphometry. We concluded that this bone phenotype could be due to hyperparathyroidism, COX-2 deficiency, or both.

PGE_2 and Sympathetic Activity Recently it was reported that PGE_2 secreted by osteoblastic cells activated EP4 in sensory nerves to increase bone formation by inhibiting sympathetic activity through the central nervous system (Chen et al. 2019). They found that EP4 KO targeted to sensory nerves or COX-2 KO targeted to mature osteoblasts in 3-month-old mice significantly reduced bone volume in adult mice. They postulate that low bone mineral density is sensed by osteoblasts, perhaps because of increased mechanical stresses and stimulated PGE_2 production by osteoblasts. PGE_2 then acts via EP4 on sensory nerves to downregulate sympathetic tone, which then leads osteoblast to increase bone formation. Because so many different genetically engineered mice were subjected to so many different protocols, it is difficult to assess reproducibility. If these results are confirmed, it would mean that targeted COX-2 KO in mature osteoblasts has a larger effect than global COX-2 KO on bone phenotype in mice. Part of the argument for doing this study was the skeletal effects in congenital disorders with insensitivity to pain, disorders caused by several mutations (Nahorski et al. 2015). However, it seems likely that most skeletal problems involving fractures and joint disorders in these patients initially arise from repeated injury due to the lack of pain sensitivity and not from bone loss (Zhang and Haga 2014; Phatarakijnirund et al. 2016; Kayani et al. 2017).

6 COX-2 and PTH: A Special Relationship

PTH is the major hormone responsible for maintenance of calcium homeostasis. It is a major stimulator of bone resorption, acting via a GPCR, which is highly expressed by osteoblast lineage cells and activates both $G\alpha_s$ and $G\alpha_q$ signaling pathways (Vilardaga et al. 2011; Mahon 2012). PTH stimulates bone resorption by increasing RANKL and decreasing osteoprotegerin (OPG) (Boyce and Xing 2008). When PTH is injected intermittently, bone formation is increased more than resorption resulting in bone gain. Intermittent PTH was the first anabolic agent approved for osteoporosis therapy in the USA (Potts and Gardella 2007; Augustine and Horwitz 2013).

Intermittent PTH Both PTH and PGE_2 stimulate cAMP signaling, and both can induce both resorption and formation. We hypothesized that PGE_2 might mediate some of the anabolic effects of PTH but found instead that the anabolic effects of intermittent PTH were increased in COX-2 KO mice (Xu et al. 2010). This led us to consider that PGE_2 might inhibit the osteogenic effects of PTH in vitro. We found that continuous PTH inhibited or had no effect on osteoblastic differentiation in WT marrow stromal cultures but stimulated osteoblastic differentiation in COX-2 KO cultures (Choudhary et al. 2013). The COX-2-dependent inhibition of the osteogenic effects of PTH was shown to be due to a factor secreted by the hematopoietic lineage (bone marrow macrophage) cells in the cultures in response to a combination of

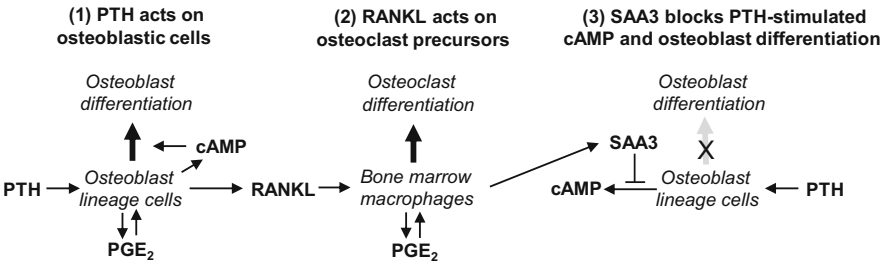

Fig. 3 Role of PGE_2-dependent SAA3 in blocking the osteoblastic response to continuous PTH. (1) PTH acts on osteoblastic precursors to induce cAMP, which causes them to differentiate into mature, bone-forming osteoblasts. PTH also induces COX-2/PGE_2 and RANKL. (2) RANKL acts on bone marrow macrophages (BMMs) to induce them to become osteoclast precursors. RANKL also induces COX-2/PGE_2. RANKL combined with PGE_2 causes the osteoclastic precursors to produce and secrete SAA3. (3) SAA3 acts on the osteoblastic cells to block PTH-stimulated cAMP and suppress osteoblastic differentiation

RANKL, from osteoblastic lineage cells, and PGE_2, produced by either osteoblastic or hematopoietic lineage cells, acting via the EP4 receptor (Choudhary et al. 2013). We subsequently identified the COX-2-dependent secreted inhibitor in vitro as serum amyloid A3 (SAA3) (Choudhary et al. 2016) and showed that secreted SAA3 acted back on osteoblastic cells to inhibit PTH-stimulated cAMP signaling.

Continuous PTH In contrast to intermittent PTH, continuous PTH infusion causes bone loss (Iida-Klein et al. 2005; Robling et al. 2011). It was generally thought that this difference was due to increased resorption when PTH was given continuously. We examined effects of continuous PTH infusion in COX-2 KO mice, which did not express SAA3 in bone marrow macrophage cells when stimulated by PTH-induced RANKL, and in SAA3 KO mice, which have a normal COX-2 response to PTH (Choudhary et al. 2015, 2018). Continuous PTH increased bone formation in both COX-2 KO mice and SAA3 KO mice but suppressed bone formation in WT mice. There was no effect of COX-2 KO or SAA3 KO on PTH-stimulated bone resorption. Because the PTH stimulated increase in bone formation was greater than the PTH stimulated increase in bone resorption, continuous PTH was anabolic in both COX-2 KO and SAA3 KO mice. Hence, our data suggest that the effects of continuous PTH on bone are due to the PGE_2-dependent secretion of SAA3, which suppresses bone formation, and not due to increased bone resorption. Our working hypothesis is shown in Fig. 3.

7 Effects of NSAIDS on Bone

Early studies in animals suggested that NSAIDs impaired fracture healing (Einhorn 2003; Brown et al. 2004; Simon and O'Connor 2007). However, other studies proposed that the effects of NSAIDs on fracture healing were dose and duration

dependent and reversible after discontinuation of brief treatment (Gerstenfeld et al. 2007). Recent reviews of animal studies indicate that loss of COX-2 activity primarily affects fracture healing via callus chondrogenesis or endochondral ossification (Geusens et al. 2013; O'Connor et al. 2014; Janssen et al. 2017). Recent reviews of fracture healing in humans conclude that short-term use of NSAIDs does not impair fracture healing (Kurmis et al. 2012; Marquez-Lara et al. 2016; Pountos et al. 2012) or spinal fusion surgery (Sivaganesan et al. 2017). On the other hand, 6 weeks of indomethacin increased risk for non-union after acetabular fracture surgery (Sagi et al. 2014), and chronic use of NSAIDs increased risk for a second hip fracture after hip fracture surgery (Huang et al. 2015). Hence, NSAIDs should probably be used at low dose and for a short duration in situations of bone repair.

It is difficult to obtain data in humans on the effects of NSAIDs on bone loss, given the wide range of NSAID dose, drug adherence, physical activity, inflammatory conditions, and considerable variability at an individual level in the degree of COX-2 inhibition and selectivity attained by selective COX-2 inhibitors (Fries et al. 2006). In a study of men age 65 and older, daily COX-2 inhibitor use in men was associated with lower hip and spine bone mineral density (BMD) compared to nonusers (Richards et al. 2006). In postmenopausal women not on estrogen replacement therapy, it was associated with a higher BMD. There was no effect of COX-2 inhibitor in women on estrogen replacement. A review of the literature on controlled randomized clinical trials with bone remodeling outcomes found some evidence for increased BMD and decreased rate of resorption and no evidence for increased bone formation in NSAID users, but the data were too limited for firm conclusions (Konstantinidis et al. 2013).

8 Summary

PGs are highly bioactive fatty acids, produced by most cells in the body and rapidly released and rapidly degraded. PGE_2 is abundantly produced by both the mesenchymal lineage cells and the hematopoietic cell lineages, which give rise to the bone-forming osteoblasts and the bone-resorbing osteoclasts, respectively, as well as multiple other cells in the bone environment. The production of PGE_2 in bone is highly regulated by multiple factors that induce COX-2. Cell and organ culture studies, as well as in vivo studies of animals given exogenous PGE_2, have demonstrated that PGE_2 can stimulate both bone resorption and bone formation. However, studies of mice with globally absent COX-2 have not shown a major skeletal phenotype. PGE_2 acts at four G-protein-coupled receptors, EP1–4, with distinct signaling pathways. Many of the actions of PGE_2 in bone have been attributed to increasing cAMP via the EP2 and EP4 receptors, and agonists of the EP2 and EP4 receptors have been investigated for their ability to stimulate bone formation and enhance fracture repair. Because COX-2 is induced by multiple hormones, growth factors, and proinflammatory factors, PGE_2 may integrate, amplify, or actually mediate, the responses to these factors, a possibility that is often overlooked. Recent studies have shown that when PTH is given continuously,

PTH-stimulated COX-2/PGE_2 leads to the production of a factor that blocks the osteogenic effects of PTH in vitro and the anabolic effects of PTH in vivo.

As might be expected for a local factor with widespread production, regulated by many factors, and acting at multiple receptors, it has been difficult to define specific functions of PGE_2 in bone as therapeutic targets. As the signaling pathways in bone are better characterized in physiologic and pathologic conditions, it may be possible to identify therapeutic applications of manipulating PGs in skeletal disorders.

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