Cyclooxygenase enzymes and prostaglandins in pathology of the endometrium

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Prostaglandins are bioactive lipids produced from arachidonic acid by cyclooxygenase (COX) enzymes and specific terminal prostanoid synthase enzymes. After biosynthesis, prostaglandins exert an autocrine–paracrine function by coupling to specific prostanoid G protein-coupled receptors to activate intracellular signalling and gene transcription. For many years, prostaglandins have been recognized as key molecules in reproductive biology by regulating ovulation, endometrial physiology and proliferation of endometrial glands and menstruation. More recently, a role for COX enzymes and prostaglandins has been ascertained in reproductive tract pathology, including carcinomas, menorrhagia, dysmenorrhea and endometriosis. Although the mechanism by which prostaglandins modulate these pathologies is still unclear, a large body of evidence supports a role for COX enzymes, prostaglandins and prostaglandin receptor signalling pathways in angiogenesis, apoptosis and proliferation, tissue invasion and metastases and immunosuppression. Here, an overview is provided of some of the findings from these studies with specific emphasis on the role of COX enzymes, prostaglandin E2 and PGF2\alpha in disorders of endometrial proliferation and menstruation in non-pregnant women.

The arachidonic acid cascade generates a family of bioactive lipids, including prostaglandins, thromboxanes and leukotrienes that modulate diverse physiological and pathophysiological responses in the reproductive tract. There has been a great deal of interest over the past few years in the involvement of arachidonic acid metabolites in pathology. However, there is still much uncertainty about the manner in which they contribute to specific phenotypic cellular changes that may precipitate pathophysiology. The focus of this review is to provide some insight into the role of cyclooxygenase (COX) enzymes, prostaglandin E2 (PGE2) and PGF2\alpha and their respective receptors in reproductive pathophysiology, with specific regard to disorders of endometrial proliferation and dysfunctional menstruation.

The kinetics of the prostaglandin biosynthetic pathway is driven by the availability of free arachidonic acid. When released from plasma membrane phospholipids or dietary fatty acids, after activation of phospholipase A2 (PLA2) or PLC, arachidonic acid is cyclized and oxygenated by COX enzymes by addition of the 15-hydroperoxy group to form prostaglandin G2 (PGG2). The hydroperoxy group of PGG2 is reduced to the hydroxy group of PGH2 (Marnett, 1992) (Fig. 1). This intermediate serves as the substrate for terminal prostanoid synthase enzymes. These enzymes are named according to the prostaglandin D2 is synthesized by prostaglandin-D-synthase (PGDS), prostaglandin E2 (PGE2) by prostaglandin-E-synthase (PGES); prostaglandin F2\alpha by prostaglandin-F-synthase (PGFS), prostacyclin by prostaglandin-I-synthase (PGIS) and thromboxane by thromboxane synthase (TXS) (Narumiya et al., 1999) (Fig. 1). The physiological response to oxygenation of arachidonic acid is determined by the expression of terminal synthase enzymes in specific cells and tissues, as each prostaglandin has its own range of biological activities that may be cell-type specific.

Three isoforms of COX enzyme (COX-1, COX-2 and COX-3) catalyse the committed step in prostanoid biosynthesis (Chandrasekharan et al., 2002; Morita, 2002). The structures of COX-1 and COX-2 from various species have been well characterized and several functional roles for COX-1 and COX-2 in physiology and pathology have now been ascertained (Morita, 2002). A functional role for COX-3 in human physiology and pathophysiology remains to be established.

COX-1 is generally considered to be involved in performing normal physiological functions. However, COX-2 is an immediate early gene which is rapidly
Fig. 1. Schematic representation of the cyclooxygenase–prostanoid biosynthetic and signalling pathway. Arachidonic acid (AA) is released from plasma membrane phospholipids by phospholipase A2 (PLA2) and utilized by cyclooxygenases (COX) and specific synthase enzymes to form prostaglandin (PG), PGD2, PGE2, PGF2α, PGI2 and thromboxane (TX) A2. These products are actively transported out of the cell by a prostanoid transporter (PGT), on which they exert an autocrine–paracrine effect by coupling to heptahelical transmembrane receptors to activate intracellular signalling. Inhibitors of COX enzyme function such as non-steroidal anti-inflammatory drugs (NSAIDs) can block PG biosynthesis and subsequent downstream events. IP3: inositol trisphosphate; MAPK: mitogen-associated protein kinase; PGDS: prostaglandin-D-synthase; PGES: prostaglandin-E-synthase; PGFS: prostaglandin-F-synthase; PGIS: prostaglandin-I-synthase; TXS: thromboxane synthase.

Prostaglandins and their receptors

After biosynthesis, prostanoids are transported out of the cell by a prostaglandin transporter (PGT) (Chan et al., 1998), where they exert their biological function through G-protein receptor (GPRC)-mediated interaction (Fig. 1). There are eight types and subtypes of prostanoid receptor, which are encoded by different genes. Separate receptors, showing selective ligand binding specificity, have
been described for PGD₂, PGE₂, PGF₂α, thromboxane A₂ (TxA₂) and PGI₁ (Narumiya et al., 1999). The focus of this review is mainly on the E and F series of prostaglandins in the endometrium, which exert their biological functions via EP and FP receptors respectively.

EP and FP receptors

PGE₂ elicits its autocrine–paracrine effects on target cells after interaction with four sub-types of PGE₂ receptor (Coleman et al., 1994), which have been pharmacologically divided into EP₁, EP₂, EP₃ and EP₄. These receptors use alternate and in some cases opposing intracellular pathways (Ashby, 1998) (Fig. 1). Interaction of PGE₂ with the EP₁ receptor mobilizes intracellular calcium and inositol trisphosphate (IP₃) via Gαq (Fig. 1). Activation of the EP₂ and EP₄ receptors results in an increase in cAMP accumulation via Gαs (Sugimoto et al., 1993; Narumiya et al., 1999). There are several splice variants for the EP₃ receptor, which are coupled to different signalling pathways that result in either a positive or negative cAMP response to PGE₂ administration or increase in intracellular calcium mobilization and accumulation of IP₃, depending on the splice variant and type of cell (Sugimoto et al., 1993; Narumiya et al., 1999) (Fig. 1).

PGF₂α exerts its action via FP receptors and alternative mRNA splice variants designated FPₐ and FP₈ have been reported. These splice variants are identical except for their intracellular carboxyl-terminal domain, which is truncated in the FP₈ isoform and lacks the last 46 carboxyl-terminal amino acids (Fujino et al., 2002). Both isoforms of FP receptor are coupled to Gαq and can activate IP₃ via activation of phospholipase C, intracellular calcium flux and activation of protein kinase C. In addition, both isoforms can activate Rho, a member of the Ras family of small GTPases, which in turn leads to phosphorylation and activation of focal adhesion kinase associated with cell morphology (Narumiya et al., 1999).

In general, it has been assumed that signal transduction cascades are initiated after ligand–receptor binding at the plasma membrane. However, Bhattacharya et al. (1998, 1999) have ascertained a nuclear location for EP receptors, indicating that PGE₂ directly regulates the transcription of target genes after the release of calcium from nuclear calcium pools or by activation of calcium channels. In addition, it is possible that PGE₂ influences transcription of target genes by interacting with nuclear peroxisome proliferator-activated receptors (PPAR). Although no role for PGE₂, PGF₂α, and PPAR in regulation of gene transcription has been described, prostanoids such as PGI₁ and PGI₂ have been reported as ligands for PPARα and PPARβ, which alter transcription of target genes involved in lipid metabolism and homeostasis (Forman et al., 1997). This diversity of receptors with opposing functions may confer a homeostatic control of an autocoid, which is released in high concentrations close to its site of synthesis (Ashby, 1998).

Functional roles for the prostanoid receptors have been determined by studies in knockout mouse model systems, deficient for each of the receptors. The most startling observations arising from these studies have been derived from the EP₂ and FP receptor knockouts, which have shown that the EP₂ and FP receptors are indispensable in female reproduction (Hizaki et al., 1999; Kennedy et al., 1999; Tilley et al., 1999; Narumiya and FitzGerald, 2001). Loss of EP₂ receptor function by gene ablation in mouse model systems results in impaired ovulation and marked reduction in litter size (Hizaki et al., 1999; Kennedy et al., 1999; Tilley et al., 1999), whereas ablation of the FP receptor in mice results in loss of parturition, although the pups can be successfully rescued by Caesarean section (Narumiya and FitzGerald, 2001). In FP null mice, there is loss of induction of the oxytocin receptor and maintenance of serum progesterone (both of which can be restored following ovariectomy). This finding reinforces the proposal for an essential role for PGF₂α in parturition and regulation of expression of the oxytocin receptor (Sugimoto et al., 1998).

COX enzymes and prostaglandins in endometrial pathology

Over the past decade, many epidemiological, pharmacological and laboratory studies using gene disruption and gene overexpression systems have provided conclusive evidence to support a role for COX enzymes and prostanoids in pathophysiology (Chulada et al., 2000). In this section, some of the functions for COX enzymes and prostaglandins in endometrial dysfunction and pathology are highlighted.

Carcinomas

Recent studies have indicated that there is a correlation between upregulated expression of COX enzymes and uterine carcinomas. Expression of COX-1 is unaltered in endometrial carcinomas (Tong et al., 2000), but is upregulated in carcinomas of the uterine cervix (Sales et al., 2002a). By contrast, COX-2 expression is upregulated in endometrial (Tong et al., 2000; Jabbour et al., 2001; Ferrandina et al., 2002) and cervical carcinomas (Ryu et al., 2000; Kulkarni et al., 2001; Sales et al., 2001) and localized in both neoplastic epithelial and endothelial cells. These findings have led to the observation that COX enzyme inhibitors may be of potential benefit as therapeutic regimens for uterine–cervical carcinomas, as has been suggested for other types of carcinoma highly expressing COX enzyme (Subbaramaiah et al., 1997).

Upregulated expression of COX enzymes in uterine and cervical carcinomas coincides with an increase in
Possible mechanisms involved in the autocrine–paracrine–intracrine regulation of cyclooxygenase (COX) enzyme expression in epithelial and endothelial cells. A positive feedback loop is formed by prostaglandins (PG) to promote intracellular signalling via the cAMP, inositol trisphosphate (IP3), mitogen-associated protein kinase (MAPK) and phosphatidylinositol 3 kinase–protein kinase B (PI3K/Akt) pathways, and induction of COX enzyme expression. This positive feedback loop, in turn, results in activation of transcription of target genes involved in angiogenesis, inhibition of apoptosis, proliferation and tissue invasion and metastasis. In addition, the COX–prostanoid biosynthetic and signalling pathway, especially in neoplastically transformed endocervical cells, may also be regulated by immune function, cytokines, chemokines and seminal plasma prostanoids.

the synthesis and secretion of PGE2, expression and signalling of EP receptors and an increase in PPAR (Ryu et al., 2000; Tong et al., 2000; Jabbour et al., 2001; Kulkarni et al., 2001; Sales et al., 2001). These findings indicate an autocrine–paracrine–intracrine regulation of neoplastic cell function by prostanoids in response to coupling with either transmembrane or nuclear EP receptors, or nuclear PPAR. A role for PGE2 and EP receptor function in tumorigenesis has been established using several in vitro and in vivo model systems (Watanabe et al., 1999, 2000; Sheng et al., 2001). Therefore, it is possible that interaction of PGE2 with target receptors in uterine and cervical carcinomas potentiates tumorigenesis by activating various signal transduction cascades and consequently transcription of target genes involved in enhancing or maintaining the neoplastic state. A model for autocrine–paracrine–intracrine regulation of neoplastic cell function is depicted schematically (Fig. 2).

Menorrhagia

Menorrhagia, with the absence of uterine pathology, is a clinical definition for dysfunctional uterine bleeding with excessive menstrual blood loss (defined as > 80 ml of blood lost per menstrual cycle). Menorrhagia affects 10–30% of women of reproductive age and up to 50% of perimenopausal women (Haynes et al., 1977; Fraser, 1992; Prentice, 2000). Although the aetiology of aberrant menstruation, including menorrhagia, is poorly
understood, several studies have associated heavy menstrual blood loss with abnormalities in vasodilatory prostanooid production and secretion from the uterus (Buttram and Reiter, 1981; Smith et al., 1981a,b).

In women diagnosed with menorrhagia, PGE2 synthesis and the number of PGE binding sites are greater in uterine tissues compared with those of normal women and correlate directly with menstrual blood loss (Smith et al., 1981a; Hofmann et al., 1983; Rees et al., 1984a; Adelantado et al., 1988a,b). Synthesis of PGl2 and nitric oxide are also high in menstrual blood collected from menorrhagic endometrium of women presenting with excessive menstrual bleeding (Smith et al., 1981b; Makarainen and Ylikorkala, 1986). This finding indicates that the degree or duration of menstrual bleeding in women diagnosed with menorrhagia may be augmented after an increase in vasodilatory factors. The increase in these vasodilatory factors may further enhance menstrual bleeding and vascular dysfunction by upregulating the COX–PG biosynthetic pathway via a positive feedback loop, and promote an autocrine–paracrine regulation of growth factors specific for vascular function (vascular endothelial growth factors, VEGFs) (Chiarugi et al., 1998; Clancy et al., 2000). Prostaglandins are known to increase COX enzyme expression, arachidonic acid metabolism and prostanooid biosynthesis in several model systems (Maldve et al., 2000; Sales et al., 2002b).

The increased expression of prostanooids present in the menorrhagic endometrium has led to the administration of COX enzyme inhibitors as a means of therapy (Stirrat, 1999). COX enzyme inhibitors such as ibuprofen reduce menstrual blood loss (Makarainen and Ylikorkala, 1986). A dual mode of action has been demonstrated for fenamates such as sodium meclofenamate and mefenamic acid which reduce prostaglandin synthesis and inhibit binding of PGE2 to its receptor (Rees et al., 1988).

**Dysmenorrhoea**

Primary dysmenorrhoea is a frequent occurrence in ovulating women and is often preceded by premenstrual tension and intense painful menstrual cramps occurring in the absence of a pelvic abnormality (Rees, 1980; Rees and Turnbull, 1987). Secondary dysmenorrhoea is clearly differentiated from primary dysmenorrhoea, because it is a symptom of uterine abnormalities or adnexal diseases such as endometriosis, pelvic inflammatory disease, or submucous leiomyomas (Rees, 1980; Rees and Turnbull, 1989). Primary dysmenorrhoea is associated with spastic uterine hypercontractility. During contractions, endometrial blood flow decreases and there is now good correlation between minimal blood flow and maximal pain, indicating that ischaemia due to hypercontractility is the primary cause (Rees, 1980; Rees and Turnbull, 1989). In addition, PGE2 and PGF2α concentrations are higher in the menstrual fluid of women with dysmenorrhoea than in women with painless periods (Rees et al., 1984b; Rees, 1989). Studies in vitro have demonstrated that endometrial explants from women with dysmenorrhoea produce more PGF2α in response to arachidonic acid compared with endometrium from pain-free women (Lundstrom and Green, 1978). This finding has prompted the use of COX enzyme inhibitors such as mfenamic acid, ibuprofen and naproxen as therapeutic regimens for management of this disorder; treatment being administered during menstruation, or before the onset of menses (Rees, 1989). More recently selective COX-2 inhibitors have proven to be even more efficacious in treatment of dysmenorrhoea and the pain associated with it (Daniels et al., 2002), making it a potential choice for treatment of women with primary dysmenorrhoea.

**Endometriosis**

Endometriosis is a chronic disease of unknown aetiology manifested by pelvic pain, infertility and abnormal uterine bleeding. Endometriosis is defined as the presence of endometrial glands and stroma within the pelvic peritoneum and other extra-uterine sites. Surgical findings in patients with endometriosis range from microscopic disease to frozen pelvis with extensive pelvic adhesions, complete cul-de-sac obliteration and large ovarian endometriomas (Canis et al., 1993). Sampson's (1927) theory of transplantation of endometrial tissue on the pelvic peritoneum via retrograde menstruation is one of the most widely recognized explanations for the development of pelvic endometriosis. Retrograde menstruation is observed in nearly all cyclic women, and endometriosis is considered to develop as a result of the coexistence of a defect in clearance of the menstrual efflux from the pelvic peritoneal surfaces (Halme et al., 1988).

Endometriosis is an oestrogen-dependent disorder and immunohistochemical studies have localized oestrogen receptor expression and increased expression of aromatase in epithelial and stromal cells of endometriotic tissues and peritoneum (Matsuzaki et al., 2001; Bulun et al., 2002b). Other factors including aberrant expression of cytokines, matrix metalloproteinases (MMPs) and reduction in progesterone concentrations are contributing factors important in the pathophysiology of the disease (Bulun et al., 2000a). Aromatase activity is absent in normal endometrium, but high in patients with endometriosis giving rise to local biosynthesis of oestrogen. Oestrogen promotes PGE2 synthesis, establishing a positive feedback loop to induce transcription of COX-2, synthesis of PGE2 and expression of aromatase (Bulun et al., 2000b, 2002a,b). This positive feedback cycle is partially mediated by cAMP after ligand binding to the cAMP-linked EP receptors and induction of COX-2 and favours accumulation of oestrogen and synthesis of prostaglandins to potentiate inflammation.
In various model systems of epithelial cell lines that overexpress COX-1 or COX-2, a role for COX enzymes in cell proliferation and inhibition of apoptosis in response to growth factor, mitogen and cytokine stimuli has been ascertained. Selective inhibition of COX-2 in these model systems results in a decrease in cell proliferation and restoration of the apoptotic rate (Erickson et al., 1999). Epithelial cell lines stably transfected to overexpress COX-2 exhibit a higher proliferation rate and an inhibition of apoptosis by prolongation of the G1 phase of the cell cycle through effects on cyclin D. Administration of selective COX-2 inhibitors to these cells results in a reduction in proliferative potential and an increase in the apoptotic rate (Tsujii and DuBois, 1995; DuBois et al., 1996). These effects have also been observed in vivo after administration of COX-2 inhibitors to rats with chemically induced colon cancers, in which an increase in the apoptotic rate coincides with administration of
selective COX-2 inhibitors (Samaha et al., 1997). In reproductive tract carcinomas, similar mechanisms may regulate tumorigenesis, as overexpression of COX-2 in cervical tumours correlates also with a reduction in apoptosis at the site of tumour invasion (Ryu et al., 2000). PGE$_2$ and PGF$_{2\alpha}$ have been shown to directly enhance the proliferation rate of glandular epithelial cells (Milne and Jabbour, 2003), indicating a role for COX enzyme products in pathologies of the uterus that are associated with proliferation and apoptosis, such as endometriosis and cancer.

**Tissue invasion and metastasis**

A number of endometrial pathologies, such as endometriosis and cancer, involve local invasion of surrounding tissues. This invasion is accomplished via the degradation of the extracellular matrix by matrix metalloproteinases (MMPs). A correlation between COX-2 and MMP expression and invasiveness has been established by the observation that overexpression of COX-2 in epithelial cells upregulates production of PGE$_2$ and expression of MMP-2. The overexpression of COX-2 in these cells promoted the invasion of surrounding matrix in *in vitro* model systems, which was reversed by cotreatment with COX-2 inhibitors. These data indicate that COX enzymes and prostaglandins can promote invasion of surrounding tissues and may be important in diseases such as endometriosis (Tsujii et al., 1997).

In order for cells to become mobile to invade distant parts of the body where space and nutrients are not limiting, changes in cell-surface adhesion molecules (CAMs) are needed. The most widely observed alteration in CAMs is that involving E-cadherin (Aplin et al., 1998). Model systems overexpressing E-cadherin have demonstrated a reduction in cell invasiveness and metastasis (Christofori and Semb, 1999). A link between COX enzymes and loss of cell surface adhesion molecules has been identified: COX-2 overexpressing cell lines downregulate E-cadherin coincident with increased PGE$_2$ production compared with wild-type and COX-2 antisense cell lines (Tsujii and Dubois, 1995). In addition, expression of E-cadherin is downregulated in a number of solid tumours and is closely and inversely related to enhanced invasion of neoplastically transformed cells (Mayer et al., 1993). Thus, COX-2 upregulation promotes cell invasion and metastases by downregulating cell adhesion molecules (Tsujii and Dubois, 1995). Whether COX-2 overexpression induces similar cellular mechanisms in solid uterine and cervical tumours and endometriosis remains to be established.

**Immunosuppression**

Several uterine pathologies, such as endometriosis and cancer, may also be regulated by mechanisms that allow them to bypass normal immune function. For example, the high concentrations of PGE$_2$ present in uterine carcinomas and endometriotic tissues may inhibit B- and T-cell proliferation and accessory monocyte–macrophage function, thereby allowing defective cells to proliferate undetected by the immune system (DeWitt, 1991). Cytokines such as IL-1 are known to induce COX-2 expression *in vitro* (Huang et al., 1998), and may act in a similar way *in situ* when secreted from immune cells and macrophages in endometriotic or neoplastic uterine tissues (Halme et al., 1988). This mechanism could augment COX-2 expression and PGE$_2$ biosynthesis and sustain immune evasion. Furthermore, in sexually active women with neoplastic utero-cervical lesion, the immunosuppressive effects of seminal plasma may further enhance any effect derived by exposure of uterine–cervical cells to endogenous PGE$_2$ (Kelly, 1995).

**Conclusion**

This review has highlighted several aspects of the COX–PG biosynthetic pathway and its function in endometrial dysfunction and pathology. It is now clear that both COX enzymes may contribute to pathophysiology by upregulating cellular machinery involved in signal transduction and gene transcription. This finding poses a dilemma for the use of selective COX inhibitors, as these may be only of partial therapeutic benefit in reproductive tract pathologies in which both COX isoforms are upregulated. In light of the evidence for a role of the EP receptors in regulating reproductive dysfunction, the use of selective receptor antagonists and modulators of signal transduction may be more effective therapeutic regimens for treatment of reproductive tract dysfunction. These regimens would selectively block transcription of target genes involved in vascular growth, cell adhesion, proliferation and survival. This is of particular relevance and importance for sexually active women in whom the potential regulation of uterine–cervical cell function by seminal plasma must be taken into account.

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