**Prostaglandin receptor signalling and function in human endometrial pathology**

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Prostaglandins are bioactive lipids that exert an autocrine or paracrine function by binding to specific G-protein-coupled receptors (GPCRs) to activate intracellular signalling and gene transcription. Prostaglandins are key regulators of reproductive processes, including ovulation, implantation and menstruation. Prostaglandins have been ascertained to have a role in various pathological changes of the reproductive tract including menorrhagia, dysmenorrhea, endometriosis and cancer. Although the mechanism by which prostaglandins modulate these changes remains unclear, much evidence suggests that prostaglandins and their receptors and downstream signalling pathways are involved in angiogenesis and in alterations in cell adhesion, morphology, motility, invasion and metastases. The potential role of prostaglandin receptors in pathological changes of the endometrium has significance for the future development of therapeutic interventions.

Prostaglandins, thromboxanes and leukotrienes, collectively referred to as ‘eicosanoids’, are the cyclooxygenase (COX) and lipoxygenase metabolites of arachidonic acid (AA). Over the past decade, much effort has gone into elucidating the roles of the COX enzymes in health and disease, and into creating selective COX enzyme inhibitors as a means of therapy. Only recently has the focus turned towards elucidating the function of specific prostanoid receptors and their signalling pathways in pathology.

In this short review, we outline the role of prostaglandins, their receptors and signalling pathways in pathological changes of the endometrium and explore the potential phenotypic effects that they might mediate in target cells.

**Cyclooxygenase enzymes**

So far, three isoforms of the COX enzyme (COX-1, COX-2 and COX-3) have been reported to catalyse the committed step in the biosynthesis of prostaglandin and thromboxane (collectively termed ‘prostanoids’) [1,2]. After the activation of phospholipase A2 (PLA2), AA is released from plasma membrane phospholipids or dietary fats, and is cyclized, oxygenated and reduced to the intermediary prostaglandin, prostaglandin H2 (PGH2), by COX enzymes. This intermediate serves as the substrate for terminal enzymes in the prostanoid biosynthetic pathway. These enzymes are named according to the prostanoid that they produce, such that PGD2 is synthesized by prostaglandin D synthase (PGDS), PGE2 by PGFS, PGI2 (also known as prostacyclin) by PGIS, thromboxane (TXA2) by thromboxane synthase (TXS), and PGE2 by PGES [3] (Figure 1).

At least four isoforms of PGES have been described: two membrane-bound isoforms, mPGES-1 and mPGES-2; a cytosolic isoform, cPGES; and a glutathione S-transferase isoform, GST-μ [4]. cPGES preferentially converts COX-1-derived PGH2 to PGE2 and is associated with immediate prostaglandin biosynthesis. The inducible membrane-associated form, mPGES-1, is preferentially associated with COX-2 under conditions of limited AA supply (but can couple to COX-1 under conditions where AA is available) and is associated with delayed biosynthesis of PGE2 [5]. mPGES-2 is structurally distinct from mPGES-1 and seems to be expressed in tissues where biosynthesis of mPGES-1 is low. mPGES-2 can couple with both COX-1 and COX-2. The GST-μ isoform of PGES has been described recently; however, its specificity for coupling with either COX isoform remains to be determined [4].

COX-1 has been long considered to be a constitutively expressed enzyme involved in normal physiological functions, but more recently it has been shown to be upregulated in various carcinomas [6–9] and to have a central role in tumorigenesis [10–12]. COX-2 is the product of an immediate early gene that is rapidly induced by growth factors, oncogenes, carcinogens and tumour-promoting phorbol esters, and its involvement in rheumatic disease, inflammation and tumorigenesis has been demonstrated [1]. In vitro model systems of cell lines overexpressing COX-2 have shown that the upregulation of this enzyme, coupled with the consequent increase in PGE2 biosynthesis, promotes angiogenesis [13], inhibits apoptosis [14] and increases the proliferation and metastatic potential of epithelial cells [15]. A role for a functional COX-3 isoform in human physiology and pathophysiology remains to be established.

The importance of COX-1 and COX-2 in reproduction has been observed from studies in knockout mice. Mice deficient
in COX-1 show longer gestation periods and protracted parturition and deliver fewer live young as compared with wild-type mice\[16,17\]. This effect is predominantly caused by abolition of the luteolytic role of PGF$_2\alpha$ in parturition.

Ablation of the gene encoding COX-2 in mice results in multiple reproductive failures, including in ovulation, fertilization, implantation and decidualization, confirming that the prostaglandins produced by COX-2 have a crucial role in these processes\[18,19\].

Prostaglandin receptors

After biosynthesis, prostaglandins are rapidly transported out of the cell by means of a prostaglandin transporter (PGT), a protein belonging to a superfamily of 12-transmembrane organic anion-transporting polypeptides\[20,21\]. PGT is responsible for the efflux of newly synthesized prostaglandins\[20\]. Expression of PGT has not been verified in the human endometrium, but PGT has been shown to be involved in the transport of various prostaglandins in the bovine endometrium\[22\].

Once released outside the cell, prostaglandins act in an autocrine or paracrine manner on their cognate heptahelical transmembrane GPCRs in the vicinity of their sites of production. PGD$_2$, PGE$_2$, PGF$_2\alpha$, PGI$_2$ and TXA$_2$ exert their biological function through interactions with, respectively, the DP, EP, FP, IP and TP prostaglandin receptors. There are four subtypes of EP receptor (EP1–EP4), which are encoded by four separate genes\[3\] (Figure 1). In addition, there are several splice variants of the EP3, FP and TP receptors, which differ only in their carboxy-terminal tails. In general, prostaglandin receptor isoforms show similar ligand binding but differ in their signalling pathways, their sensitivity to agonist-induced desensitization, and their tendency towards constitutive activity.

Phylogenetic analyses indicate that receptors sharing a common signal pathway have higher sequence homology than do receptors sharing a common prostanoid as their preferential ligand. Among the different receptors, the IP, DP, EP2 and EP4 receptors increase the accumulation of intracellular cyclic (cAMP) via G$_\alpha$s and have been termed ‘relaxant’ receptors because they induce smooth muscle relaxation. The TP, FP and EP1 receptors induce Ca$^{2+}$ mobilization via G$_\alpha$q and constitute a ‘contractile’ receptor group because they cause smooth muscle contraction. The remaining receptor, EP3, is generally associated with a decline in cAMP. This ‘inhibitory’ receptor usually stimulates smooth muscle contraction; depending on the splice variant and cell type, however, the EP3 receptor can also increase intracellular cAMP and mobilize Ca$^{2+}$\[3\].

Figure 1. The cyclooxygenase (COX) and prostanoid biosynthetic and signalling pathways. Arachidonic acid (AA) is released from plasma membrane phospholipids by phospholipase A2 (PLA2) and used by COX enzymes and specific synthase enzymes, such as prostaglandin D synthase (PGDS), PGES, PGFS, PGIS thromboxane synthase (TXS), to form prostaglandin D$_2$ (PGD$_2$), PGE$_2$, PGF$_{2\alpha}$, PGI$_2$ and thromboxane A$_2$ (TXA$_2$), respectively. These molecules are actively transported out of the cell by means of a prostaglandin transporter (PGT), where they exert an autocrine or paracrine effect by coupling to their respective heptahelical transmembrane receptors, DP, EP1–EP4, FP, IP and TP, to activate second messengers, such as cyclic AMP (cAMP) and inositol (1,4,5)-trisphosphate (IP$_3$), and intracellular signalling cascades.
Prostaglandin receptor cross-communication

Recent studies have shown that prostaglandin receptor signalling (via EP2 and FP) to downstream signalling pathways involves productive cross-communication with the epidermal growth factor receptor (EGFR) [23,24]. These data are supported by several studies providing evidence that prostaglandin GPCRs activate receptor tyrosine kinases (RTKs) [23–28]. This cross-communication results in increased autophosphorylation and dimerization of RTKs such as the EGFR and platelet-derived growth factor receptor, culminating in the activation of mitogen-activated protein kinase (MAPK) or phosphatidylinositol 3-kinase (PI3K) signalling [23–28] (Figure 2). The diversity of RTK activation by prostaglandin receptors, the exact intracellular mechanisms of the activation, and the physiological or pathological significance of this cross-communication are not, however, fully elucidated.

Several mechanisms have been proposed for the transactivation of EGFR by GPCRs [29–31]. One of these mechanisms involves activation of transmembrane matrix metalloproteinases and extracellular release of heparin-binding EGF (HB–EGF) from its latent membrane-spanning precursor in the plasma membrane. Once cleaved, the HB–EGF ligand can associate with and activate the EGFR, and thereby induce downstream signalling events such as phosphorylation of the MAPK extracellular-signal-regulated kinases 1 and 2 (ERK1/2). In addition, several studies have shown that activation of the c-Src family of non-receptor tyrosine kinases is involved in GPCR-mediated transactivation of the EGFR [29,30,32]. The latter observation is supported by our recent findings in endometrial epithelial cells, in which PGE2 activation of the EP2 receptor increases ERK1/2 signalling via c-Src-mediated transactivation of the EGFR [24] (Figure 2).

Prostaglandin synthesis and receptor expression in endometrial pathology

Over the past decade, many epidemiological, pharmacological and laboratory studies using gene disruption and overexpression systems have provided conclusive evidence in support of a role for COX enzymes and prostaglandins in benign and neoplastic pathologies of the endometrium including menorrhagia (heavy menstrual blood loss), endometriosis, dysmenorrhea (painful periods) and cancer [33]. Collectively, these disorders place a heavy burden on health service resources and are major contributors to morbidity in reproductive health.

The potential role of COX enzymes, their prostaglandins and their receptors in benign uterine pathologies such as menorrhagia, endometriosis and dysmenorrhea is well recognized. Synthesis of PGE2 and the number of PGE-binding sites are greater in the uterine tissues of women with menorrhagia than in the tissues of normal women and correlate directly with menstrual blood loss. Levels of PGL2 and nitric oxide are also increased in menstrual blood collected from women presenting with excessive menstrual bleeding. This suggests that the degree or duration of menstrual bleeding in women diagnosed with menorrhagia might be augmented after an increase in vasodilatory factors [33]. The increased biosynthesis of prostaglandins present in the endometrium of women with menorrhagia has led to the administration of COX enzyme inhibitors as a means of therapy. Inhibitors of COX have been shown to reduce menstrual blood loss in women with menorrhagia [33].

As with heavy menses, recent evidence suggests that the pathology of endometriosis is associated with aberrant biosynthesis of COX enzymes and prostaglandins. Immunohistochemical studies have shown that COX-2 protein is upregulated in endometriotic endometrium [34]. Moreover, increases in prostaglandins have been reported in the peritoneal fluid of infertile women with endometriosis, suggesting that ectopic endometrium directly synthesizes and releases prostaglandins into the peritoneal fluid [35]. This release could have an adverse impact on tubal function and on spermatozoa, oocyte and embryo transport, thereby reducing the likelihood of conception [35]. Prostaglandins in peritoneal fluid might also act in a paracrine manner on surrounding tissues to sustain the
state of endometriosis or to facilitate further dysfunction of the reproductive tract.

Little is known about the endocrine and local factors that might result in primary dysmenorrhea (painful periods in the absence of an anatomical cause); however, many researchers have suggested that dysmenorrhea is associated with local disturbances in the expression and synthesis of inflammatory mediators. Primary candidates that have been described include COX enzymes and their prostanoid products. The hyperalgesic effects of PGE2 and PGF2α have been described in several inflammatory models of nociception (reviewed in Ref. [36]). An increase in the synthesis of prostanoids such as PGE2 and PGF2α has been demonstrated in the menstrual fluid of women with dysmenorrhea as compared with the menstrual fluid of women with painless periods [33]. In vitro studies have shown that endometrial explants from dysmenorrheic women produce more prostanoids in response to AA than do endometrial explants from pain-free women, suggesting that they express a higher level of COX enzymes and specific prostanoid synthase enzymes. This observation has prompted the use of COX enzyme inhibitors in therapeutic regimens for managing this disorder, with treatment being administered either during menstruation or before its onset [33].

The biosynthesis of COX-1 and/or COX-2 is also upregulated in cancers of the endometrium [6,37–39]. This finding has led to the suggestion that COX enzyme inhibitors might be of potential benefit in the treatment of uterine carcinomas, as has been recommended for other types of carcinoma that express high levels of COX enzymes [40]. Upregulated expression of COX enzymes in endometrial carcinomas coincides with increased synthesis and secretion of PGE2 and increased expression and signalling of EP receptors [37,38]. Although much emphasis has been placed on the potential role of PGE2 in regulating neoplastic cell function [41–43], other prostanoids using the same or alternative signal transduction pathways might similarly enhance or contribute towards the neoplastic state. Recent data have outlined a marked upregulation in synthesis and signalling of the FP receptor in endometrial adenocarcinomas [23], and its potential role in enhancing the proliferation of epithelial cells [44].

Interestingly, many of the phenotypic changes that are required for promoting the above-named pathologies, such as cell adhesion, cell invasion, cellular proliferation, vascular permeability and angiogenesis, have been associated with COX enzymes and their prostanoid products [33]. However, the potential role of the prostanoid receptors and their signalling pathways in benign and neoplastic endometrial pathologies remain largely unexplored.

**Prostaglandin receptor signalling in human endometrium**

Agonist stimulation of prostanoid GPCRs leads to receptor activation and dissociation of the heterotrimeric G-protein complex. This action generates various soluble second messengers (cAMP, inositol 1,4,5-trisphosphate and Ca2+) by effector enzymes, depending on the heterotrimeric subtypes (Gαi, Gαo or Gαq; Figure 1), leading to the activation of divergent signalling cascades. In the human endometrium, the E and F series of prostanoids are the principal eicosanoids produced, and PGD2, TXA2 and PGI2 are present in lesser amounts. The effect of the divergence of intracellular signalling via the respective prostanoid receptors on target gene transcription and regulation of reproductive function remains to be fully elucidated.

In the normal human endometrium, PGE2 activation of the EP receptors [45] and PGI2 activation of the IP receptor [46] result in an increase in the accumulation of intracellular cAMP, whereas PGF2α–FP receptor coupling mobilizes inositol trisphosphates and intracellular Ca2+ [44]. Activation of these second messenger systems is greatest during the middle-to-late proliferative phase of the menstrual cycle, when levels of the prostanoid receptors are highest, and is increased even further in endometrial adenocarcinomas as compared with normal endometrium [23,38]. The second messenger systems and cell signalling pathways that are activated in other uterine disorders remain to be fully elucidated.

Because the number of PGE2-binding sites is higher in the endometrium of women with menorrhagia than in normal endometrium [33], it is possible that the activation of second messenger systems in the menorrhagic endometrium might be also increased as compared with normal endometrium. Increases in the biosynthesis and signalling of other prostanoid receptors might similarly exacerbate the prevailing endometrial pathology. Once activated, second messenger systems can mediate downstream signalling, including the activation of MAPK cascades such as ERK, Jun amino-terminal kinase (JNK), p38 and ERK5 (also known as BMK) [23,44,47,48]. Further investigation into the diversity of signalling events triggered by one or multiple prostanoid receptors is needed to define the roles of prostanoid receptor signalling pathways in reproductive function and pathology.

**Activation of prostaglandin receptors by seminal plasma**

In addition to their endogenously synthesized prostanoids, sexually active women come into contact with PGE2 that is present in seminal plasma. Prostaglandin levels are 10 000 times higher in seminal plasma than at a site of inflammation, and PGE2 is one of the predominant types of prostaglandin detected in seminal plasma [49]. Little is known about the effect of seminal plasma prostaglandins on the physiology and pathology of the uterus; however, recent evidence suggests that seminal plasma is transported into the uterine cavity in humans. Research using labelled albumin macrospheres deposited at the external cervical opening has shown that these spheres can travel into the uterine cavity within minutes of deposition. The proportion of spheres entering the uterine cavity goes up with the increasing intensity of uterine contractions during the menstrual cycle [50].

More recently, seminal plasma has been shown to influence the expression of genes in endometrial epithelial and stromal cells. The synthesis of several proinflammatory mediators such as interleukins (IL-1β and IL-8) is increased after treatment with seminal plasma [51]. Research in our laboratory has demonstrated that seminal plasma prostaglandins can activate the intracellular
signalling and biosynthesis of proinflammatory mediators via specific prostanoid receptors such as EP2 and EP4 [52]. Thus, it is envisaged that seminal plasma prostaglandins might exacerbate endometrial pathologies that are associated with an increase in prostaglandin receptors in the plasma membrane compartment, especially those of the prostaglandin E series.

**Uterine effects mediated by prostanoid receptors**

Many of the phenotypic effects associated with the expression and activation of prostanoid receptors have been derived from studies involving the genetic or pharmacological manipulation of prostanoid receptors in animal models or in vitro cell culture. Some of the known effects of prostanoid signalling include angiogenesis, proliferation, adhesion, alterations in cellular morphology, motility, invasion [33,48] and pain perception [53,54].

In the human endometrium, the COX–prostanoid biosynthetic pathway plays a role in vascular function–dysfunction by promoting the transcription of angiogenic factors or by reducing the expression of anti-angiogenic factors [55,56]. Vascular endothelial growth factor (VEGF) is known to be the progenitor angiogenic factor in the formation of new blood vessels [57], and it promotes angiogenesis by recruiting endothelial cells from the pre-existing vasculature to form vascular tubes [57]. Other angiogenic factors have been proposed to act in parallel or synergistically with VEGF to stabilize endothelial cells and to promote their proliferation and arrangement into tubular structures to form new blood vessels. This not only serves to provide a blood supply and nutrients to encourage tissue repair, growth and differentiation, but can also promote receptivity in the uterus that prepares the endometrium for implantation in the next menstrual cycle.

Recent in vivo work in knockout mice and in vitro studies manipulating the expression and signalling of prostanoid receptors have shown that prostanoids promote angiogenesis by coupling to target receptors that might be specific to the cell or tissue type. For example, PGE₂ coupling to the EP2 receptor induces the expression of VEGF in pancreatic cancer cells and endometrial adenocarcinoma cells [24,58], whereas the EP3 receptor regulates vascular function–dysfunction in ocular tissues and promotes vitreal neovascular diseases such as ischaemic retinopathies [59]. Although little is known of the angiogenic potential of other prostanoid receptors, increased levels of the EP4 and FP receptor have been reported in perivascular cells in endometrial adenocarcinomas [23,38]. It is therefore predicted that dysregulated biosynthesis and signalling of prostanoid receptors can promote aberrant neovascularization in endometrial pathologies, thereby promoting dysfunctional uterine bleeding and the growth of endometrial cells in pathologies such as cancer and endometriosis.

In addition to the role of prostanoids in promoting vascular function–dysfunction and vascular tone, in vitro model systems have shown that prostaglandins can downregulate cell-surface adhesion molecules and stimulate cell motility, invasiveness and metastasis [15,26] (Figure 2). In vivo model systems in which cancer cell xenografts have been implanted subcutaneously in nude mice have demonstrated that reduced tumour proliferation, invasiveness and metastatic nodules occur coincidently with the inhibition of prostanoid production with COX inhibitors [60,61]. More recent findings have shown that alterations in cellular adhesion, morphology and proliferation can occur after the binding of ligands to specific prostanoid receptors (namely, the EP4, FP and TP receptors) and the activation of downstream signalling pathways such as the MAPK and PI3K pathways [23,43,62,63] (Figure 2). Thus, it is predicted that the aberrant biosynthesis and signalling of prostanoid receptors in the endometrium [23,38] might promote uterine pathologies such as carcinoma and endometriosis by stimulating unregulated cellular proliferation and differentiation and downregulating cell-surface adhesion molecules, such as E-cadherin [14,64], to facilitate cell motility, invasion and metastases.

Prostanoid receptors are also involved in the pathology of dysmenorrhoea. Current data investigating pain perception in mice lacking individual prostanoid receptor strongly suggest that EP3 and IP are the principal receptors that mediate pain perception [53,54]. In an in vivo model system, IP and EP3 receptor knockout mice that were treated with lipopolysaccharide to induce COX enzyme and prostanooids biosynthesis showed a significant reduction in pain perception; by contrast, the response in mice deficient in EP1, EP2 or EP4 was similar to that in wild-type mice [53]. Together with the significant increase in synthesis and signalling of the IP receptor observed during menstruation [46], these findings indicate that this receptor has a potential role in regulating pain perception in menstrual disorders associated with intensely painful menstrual cramps, such as dysmenorrhoea.

**Conclusion**

Over the past few years, much effort has gone into the discovery and implementation of specific COX enzyme inhibitors as a therapeutic regimen for diseases associated with increased synthesis of endogenous COX enzymes and prostaglandins. In these disorders, treatment with COX enzyme inhibitors will suppress only the production of endogenous prostaglandins. In sexually active women, however, any increase in prostanoid receptor activation can be further enhanced by seminal plasma prostaglandins. Furthermore, from the observations reported herein, it is envisaged that an understanding of the role of the specific prostanoid receptors, their signalling pathways and their phenotypic effects in the female reproductive tract might result ultimately in the development and implementation of more efficacious interventions in the clinic.

Of particular significance is the involvement of RTKs in transducing the signal from the prostanoid receptor. The trans-phosphorylation of RTKs by prostanoid receptors might have a crucial role in endometrial function–dysfunction and consequently have important implications for drug development. Recent data suggest that combinational therapies such as a COX enzyme inhibitor coupled with a selective inhibitor of an RTK, such as the EGFR, can be more effective therapeutically than either
compound administered alone [65]. In light of these studies, the combination of a prostanoid receptor antagonist and an RTK inhibitor might be an efficacious therapy for endometrial pathologies that are regulated by prostaglandins and their respective receptors. Further studies are needed to evaluate the therapeutic use of such combinatorial approaches targeted at the prostanooid and growth factor receptor signalling pathways.

Current research has focused on the creation of cell model systems in which single receptors are targeted. This focus has opened a new vista into the understanding of the complexity of signalling networks and cross-communication that exist between various prostaglandin receptors, and between these receptors and RTKs. Unravelling these networks might lead to a better understanding of the role of the various prostanooid receptors in endometrial pathologies and might outline further novel therapeutic directions in the clinic.

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The WHO and six medical journal publishers have launched the Access to Research Initiative, which enables nearly 70 of the world’s poorest countries to gain free access to biomedical literature through the Internet.

The science publishers, Blackwell, Elsevier, the Harcourt Worldwide STM group, Wolters Kluwer International Health and Science, Springer-Verlag and John Wiley, were approached by the WHO and the British Medical Journal in 2001. Initially, more than 1000 journals will be available for free or at significantly reduced prices to universities, medical schools, research and public institutions in developing countries. The second stage involves extending this initiative to institutions in other countries.

Gro Harlem Brundtland, director-general for the WHO, said that this initiative was ‘perhaps the biggest step ever taken towards reducing the health information gap between rich and poor countries’.

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