Prostaglandins in labor – a translational approach

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1. ABSTRACT

The mechanisms involved in the initiation of human labor are largely unknown. Understanding the molecular pathways is fundamental in both the development of effective therapeutic strategies and intervention to prevent preterm labor. Prostaglandins are bioactive lipids and members of the eicosanoids family, derived from arachidonic acid, which act in a paracrine or autocrine manner and function via binding to specific G-protein-coupled receptors, activating intracellular signaling and gene transcription. Prostaglandins have a central role in the maintenance of pregnancy and initiation of labor, with the change from uterine quiescence to a contractile state facilitated by differential expression of prostaglandin receptors within the myometrium and fetal membranes. Clinical evidence for the key role of prostaglandins in human parturition is evident from their successful exploitation as exogenous agents for the induction of labor and the role of prostaglandin synthase inhibitors as a preventative therapy for preterm labor. This review aims to focus on prostaglandin synthesis and metabolism and how differential regulation of prostaglandins and their receptors in gestational tissues interact in the initiation of labor.

2. INTRODUCTION

Prostaglandins were first extracted from semen, the prostate gland and seminal vesicles by Goldblatt and Von Euler in the 1930s, with purification of the first prostaglandin isomers in the 1950s and 60s by Bergström and colleagues (1). Further characterization has demonstrated that prostaglandins are 20 carbon chain unsaturated fatty acids (eicosanoids) synthesized from arachidonic acid (AA; 5, 8,11,14-eicosatetraenoic acid) via the enzymatic action of phospholipases and cyclooxygenase (COX). Prostaglandin synthesis and activation in gestational tissues is now recognized as one of the fundamental signals responsible for labor (2). In this review, we will discuss recent developments in our understanding of the synthesis of prostaglandins, their metabolism and transport, the interaction with receptors and the downstream signaling pathways with respect to the physiology of labor.

3. PROSTAGLANDIN BIOSYNTHESIS AND TRANSPORT

3.1. Biosynthesis of prostaglandins

Prostaglandin synthesis can be broadly divided into three main steps. In the first step, AA is liberated from
Figure 1. Prostaglandin biosynthesis pathways. Arachidonic acid liberated from cellular membrane phospholipids by the action of phospholipases A2 and cyclooxygenase are the rate-limiting steps in the biosynthesis of PGs. COX-1 and COX-2 convert arachidonic acid to PGH2, with further conversion to specific PGs depending on the specific PG synthases. Prostaglandin dehydrogenase (PGDH) metabolizes bioactive PGs into inactive keto-metabolites. The PG receptors for PGD2, PGF2α, PGI2, TXA2 and PGE2 are DP, FP, IP, TP and EP respectively. There are four receptor-subtypes for PGE2 (EP1-4). Their actions are mediated via increases in intracellular calcium level (FP, TP, EP1), increases in cAMP level (DP, IP, EP2, EP4) and increases or decreases in intracellular cAMP levels.

In addition to the PLA2 pathway, phospholipase C (PLC) enzyme forms diacylglycerol that can be cleaved to generate AA via a mono- or diglycerol lipase (20). The action of PLC coupled to diacylglycerol lipase, could provide a further indirect mechanism for the release of arachidonic acid for prostaglandin biosynthesis during parturition (21, 22).

The next step is the oxidation of AA and subsequent reduction to an intermediate endoperoxide prostaglandin H2 (PGH2). Production of PGH2 is the rate limiting step in the synthesis of prostaglandins, with this reaction catalyzed by the prostaglandin H synthase (PGHS) enzyme, a 72-kD heme protein containing both cyclooxygenase (COX) and peroxidase activity. PGHS is also known as COX and PTGS (Prostaglandin-endoperoxide synthase), with the latter representing the most recent consensus nomenclature. To date, three isoforms of PGHS enzyme have been characterized; PGHS-1, PGHS-2 and PGHS-3, also known as cyclooxygenase COX-1 (PTGS1), COX-2 (PTGS2) and COX-3 (PTGS3) respectively, are responsible for PG synthesis, however, to date only COX-1 and COX-2 have been implicated in human parturition (23, 24).

COX-1 and -2 are both capable of catalyzing a cyclooxygenase and a peroxidase reaction, in which AA...
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and two molecules of oxygen are converted to PGG2, with subsequent reduction to PGH2 by the addition of two electrons (Figure 1). The cyclooxygenase reaction occurs in a hydrophobic channel in the core of the COX enzyme and the peroxidase reaction occurs at a heme-containing active site located near the protein surface (25). The two isoforms of human COX-1 and -2, although having approximately the same number of amino acids; 599 and 604 respectively (26), only demonstrate 60% homology at the nucleic and amino acid levels. Moreover, COX-1 contains a sequence of 17 amino acids in its amino terminus that is absent in COX-2, whereas COX-2 has an additional insert of 18 amino acids in its carboxy terminus (27). It is these alterations in COX structure that facilitate differential regulation. Indeed COX-1 is constitutively expressed in all cell types and functions as a housekeeping enzyme, and may therefore considered to be ‘constitutive’, whereas COX-2 is an ‘inducible’ isofrom, found at variable levels in tissues, with induction of activity by cytokines, growth factors and by a variety of other agonists (28, 29). COX-2 is also activated at an approximately ten fold lower concentration of hydroperoxide than that of COX-1 (30, 31), further facilitating COX-2 to function independently of COX-1 in cells expressing both isoforms. Despite COX-1 and -2 having significant similarities in terms of cyclooxygenase active site structures, catalytic mechanisms and kinetics, there are two major structural differences. First, the cyclooxygenase active site of COX-2 is larger and more accommodating than COX-1, with this difference in size exploited to facilitate the development of selective COX-2 inhibitors. Second, although COX-1 and -2 have similar $K_m$ and $V_{max}$ values with arachidonic acid, COX-1 at low AA concentrations exhibits negative allosterism; this difference may facilitate COX-2 to compete effectively for newly released AA when both the isozymes are expressed in the same cell (25). For example, AA at 1µM concentration stimulates prostaglandin synthesis by human embryonic kidney 293 cells expressing COX-2, whereas concentrations of more than 10µM are required in order to stimulate prostaglandin synthesis in cells expressing only COX-1 (32).

The transport and post-transcriptional regulation of COX-2 mRNA is of critical importance in controlling the expression of the COX-2 gene and consequently may have significant functional impact. The nuclear export receptor chromosome maintenance region 1 (CRM1) is required to transport the COX-2 mRNA from the nucleus to the rough endoplasmic reticulum (33, 34), and a specific inhibitor of this pathway, leptomycin B, inhibits the transport and decreases the expression of COX-2 gene in mammary cancer cells (35). Although these data suggest that exploitation of inhibition of the CRM1 receptor may be useful in controlling the pathogenic expression of COX-2, to date the role of this receptor in human parturition has not been explored.

The third and final step in the prostaglandin synthesis pathway is the conversion of the PG intermediate, PGH2 to one of the biologically active prostaglandins, namely; D (PGD2), E (PGE2), F (PGF2α), thromboxane (TXA2), and prostacyclin (I2) series with these reactions catalyzed by prostaglandin D synthase (PGDS), prostaglandin E synthase (PGES), prostaglandin F synthase (PGFS), thromboxane synthase (TXS) and prostacyclin synthase (PGIS) (36) respectively.

With respect to human parturition the role of these enzymes, the final step in prostaglandin synthesis continue to be elucidated, with multiple isoforms for most now being recognized. Indeed two distinct isoforms of PGDS have been characterized and cloned (37, 38); lipocallin-type PGDS (L-PGDS) and a cytosolic protein hemopoietic PGDS (H-PGDS) enzyme. In pregnancy H-PGDS was localized in human placenta; in contrast, L-PGDS was detected in amniotic fluid with further up-regulation with the onset of human labor (39). Changes in the L-PGDS level in human cervico-vaginal fluid has been suggested as a possible indicator of membrane rupture during pregnancy (40).

To date, four isoforms of PGES have been characterized; two are membrane bound PGES (mPGES-1 and mPGES2), one cytosolic (cPGES) and one is glutathione S-transferase (GST-µ) (41). cPGES primarily converts COX-1 derived PGE2 to PGE1 and is responsible for immediate PG biosynthesis. In contrast, mPGES-1 may be induced in response to pro-inflammatory stimuli and is primarily associated with COX-2 when there is limited availability of AA, however, it can also couple to COX-1 where AA is available, thereby facilitating the delayed biosynthesis of PGE2. (42). Relative to mPGES-1, mPGES-2 is structurally different and can couple with both COX-1 and COX-2. Lastly although the GST-µ isoform has been implicated in PG production; whether it is coupled with either COX-1 or COX-2 (41), and has a role in human labor has yet to be established.

Two isoforms of PGFS have been characterized and reported; one known as lung-type PGFS (or PGFS 1) (43, 44) and the other liver-type PGFS (or PGFS II) (45, 46). Data on PGFS regulation have been limited to in vitro studies mostly in bovine species (47, 48), with a decrease in PGFS mRNA expression in amnion (49) was noted after dexamethasone induced labor, but in contrast no changes were observed in either the myometrium or endometrium of sheep after induced and spontaneous labor (49). In contrast PGIS (50) and TXS (51-53), membrane-bound hemoproteins and members of the cytochrome P450 family have been implicated in human labor with PGIS shown to be expressed by human myometrial smooth muscle (54) with additional localization to vascular smooth muscle and endothelial cells within the lower uterine segment, although expression decreased with advancing gestation (55). Expression of TXS has been shown in the human endometrial and myometrial cells as well as in uterine blood vessels (56) and also in placental villi, fetal membranes, umbilical cord and myometrium (57).

3.2. Prostaglandin transport

Synthesized prostaglandins are organic anions with a pKa value of about 5 and exist primarily as charged species at a physiological pH (58, 59). After biosynthesis PGs can cross the plasma membrane and this rapid
Figure 2. Prostaglandins with target receptor subtypes and signaling mechanisms. 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 D2 (PGD2), PGE2, PGF\textsubscript{2\alpha}, PGI2 and thromboxane A2 (TXA2), 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, EP\textsubscript{1}–EP\textsubscript{4}, FP, IP and TP, to activate second messengers, such as cyclic AMP (cAMP) and inositol (1,4,5)-trisphosphate (IP\textsubscript{3}), and intracellular signaling cascades. Reproduced with permission from (193).

3.3. Concentrations of prostaglandins in tissues

Due to the labile nature of prostaglandins they are difficult to measure, however, concentration of 15d-PGJ\textsubscript{2} and \Delta^{12}\textsubscript{1}-PGJ\textsubscript{2} have been reported in the picomolar-to-nanomolar range in the medium of 3T3-L1 adipocytes and in human urine respectively (69-71), with plasma PGE and PGF concentrations of 0.7nM (72). Maternal human plasma levels of PGFM, a PGF\textsubscript{2\alpha} metabolite, increase considerably with advancing gestation with further increases associated with labor at term. In contrast, no significant difference were found in plasma levels in preterm labor when compared with term not in labor (73, 74), suggesting a gestational rise in addition to the observed increases with labor. Human amniotic fluid PGE\textsubscript{2} concentrations have also been shown to increase 10 fold, with a concomitant 3-fold rise in PGF\textsubscript{2\alpha} (75). Similarly the concentration of \( \alpha,11\beta\textsubscript{1}-\text{PGF}\textsubscript{2 Instituto de Investigación} \), a PGD\textsubscript{2} derivative with equal myometrial contractile potency as PGF\textsubscript{2\alpha}, (76, 77)
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demonstrated a two fold increase in amniotic fluid concentrations with human labor (78). Lastly some studies have reported increased decidual cells PG concentrations with the onset of labor at term (79, 80), with others reporting no change (81, 82). These widespread changes in concentrations of PG with human labor, would appear to be consistent with high concentrations having a contractile effect on myometrial smooth muscle. Certainly PGs appear to exhibit dose-dependent effects with potentially relaxatory effects at lower dose ranges and contractile effects at higher concentrations (83). Consistent with this, non-pregnant human myometrium, PGE2 appears to have a variable effect on contractility (84) with a biphasic response in pregnant human myometrium (85). These concentration dependent responses in conjunction with alterations in receptor levels may facilitate the tight regulation of contractile responses and enable the dynamic transition of myometrium from a quiescent to an active phenotype prerequisite for labor.

4. PROSTAGLANDIN RECEPTORS IN PREGNANCY AND LABOR

The action of prostaglandins are predominantly mediated via specific receptors, including the four prostaglandin E receptor subtypes EP1a, the DP receptor for prostaglandin D2, and the FP receptor for prostaglandin F2alpha. It is based on their historical effects on smooth muscle cells and on their second messenger system (Figure 1), that these prostaglandin receptors have been divided into relaxatory (EP2, EP3, DP, IP) and contractile (EP1, EP3, FP, TP) groups. This differential response is mediated via differential coupling of PG receptors to either Gs or Gi proteins (Figure 2). Activation of Gi stimulates adenylate cyclase resulting in an increase in cAMP levels, with subsequent relaxation of smooth muscle. Conversely, activation of Gs coupled PG receptors inhibits adenylate cyclase, reduces cAMP production resulting in an increase in intracellular calcium with initiation of an action potential and contraction of smooth muscle (86). Notably although the effects of PGs are predominantly mediated through G-protein coupled plasma membrane receptors, data on neonatal porcine brain and adult rat liver has shown that PGE2 may also modulate gene transcription via perinuclear envelope expression of EP3, EP1 and EP2 receptors (87).

To date the role of the various forms of PG receptors continues to be elucidated with increasing recognition that for any given type additional splice variants may also exist. For example several isoform of EP1 receptor subtypes have been reported (88, 89), with generation of subtypes by alternative splicing at the carboxy-terminal tail of nine encoded transcripts; EP1alpha, EP1beta, EP1gamma, EP1delta, EP1epsilon, EP1delta, EP1gamma, EP1epsilon, and EP1delta. Notably in PG receptors, it is the carboxy-terminal tail that is important in mediating G protein coupling, and thus, changes in this region seems to alter the coupling affinity of particular receptor subtypes. Specifically, EP1 is coupled to Gi and hence can mediate a contractile effect; however, splice variants (EP1gamma and EP1delta) may lead to different cellular responses depending on coupling to Gi and/or Gq respectively (90, 91).

Prostaglandin E receptor isoforms also show differential spatial expression in myometrium with significantly increased EP1 expression in laboring human lower segment myometrium at term (92), with an up-regulation of EP3 in fundal tissue. In contrast EP2 mRNA expression was significantly increased in the lower segment of non laboring preterm human myometrium as compared to term samples, potentially reflecting the role of EP3 in the maintenance of uterine quiescence (93). Furthermore mRNA expression of the four non-contractile EP3 splice variants (EP3alpha, EP3beta, EP3gamma and EP3delta) was down-regulated with increasing gestation in both upper segment and the lower segment of human myometrium (92). Given the well established paradigm that labor involves the switch from myometrial quiescence to an active contractile state, this orchestrated up-regulation of contractile receptors, with concurrent loss of the relaxatory receptors and a decrease in intracellular cAMP levels would be consistent. Anatomical differences in location of the prostaglandin receptors may also be important, with studies in baboons demonstrating an abundance of the contractile EP3 receptors in fundal tissue, in contrast to the predilection for the relaxatory EP2 and EP3 receptors for the lower uterine segment (94), where they may be partly responsible for relaxation of the uterus to allow smooth passage of the fetus and help in delivery of the baby.

Although there are dramatic changes in PG receptor expression with labor, substantive changes also occur with the initial transition to a pregnant state with maintenance of pro-quiescent changes across gestation (92). Indeed during human pregnancy, a decrease in the number of contractile PG receptors have been reported (95-97), with EP2 expression in pregnant human myometrium 40% less than in non-pregnant samples. FP expression is also decreased during human pregnancy by approximately 45%, although these changes are not sustained as expression increases with labor (98, 99). In rats similar increases with labor were noted, although a decrease with pregnancy per se was not noted (99). Certainly these gestational and labor associated changes suggest a role for FP receptors in maintaining and then switching the myometrial phenotype. The noted concomitant increase in relaxatory EP2 receptor responses during pregnancy would also facilitate uterine quiescence (96). In addition increased coupling of Ga to adenylate cyclase with subsequent increases in relaxatory cAMP formation throughout gestation as compared to the reduction in coupling observed with labor, may also potentially act as a triggering mechanism for the initiation of labor (100).

At present there is a limited amount of published data available on the regulation of prostaglandin receptors as related to uterine physiology. Certainly steroids have been implicated in PG receptor regulation in rats. With increased FP and decreased EP2 mRNA expression noted after progesterone receptor antagonism by RU486 (99). Similarly, estrogen antagonism decreased FP mRNA, in contrast to the enhanced FP expression with administration of exogenous estradiol (99). Conversely supplementation with progesterone alone increased EP2 expression, and when this was combined with estradiol, the increase was...
even greater (99). These studies suggest that steroids have a role in the regulation of prostaglandin receptors, at least in rats, and these effects may underlie some of the observed effects of progesterone on preterm birth (101).

With respect to additional mechanisms of regulation of prostaglandin receptors, in vitro myometrial cell cultures are known to express EP2 receptors, with stimulation by IL-1β inducing EP2 expression (102), suggesting that pro-inflammatory cytokines which have been implicated in the pathophysiology of labor may partly regulate myometrial activity via prostaglandin receptor up-regulation. PG receptors may also exhibit auto-regulation via their own ligands, with PGE2 shown to decrease the number of binding sites in the rat myometrium, consistent with ligand-induced receptor down regulation (103). Our own recent gene array studies of human parturition also showed a 10 fold down-regulation in EP1 receptors expression in lower segment laboring human myometrium compared to non laboring samples (104), consistent with the published mRNA and immunohistochemistry data (92).

5. ROLE OF PROSTAGLANDINS AND ITS REGULATION IN LABOR

5.1. Prostaglandins and labor at term

It is clear that prostaglandins have an important role to play in parturition, with exogenous prostaglandins used for abortion (105), cervical ripening (101) and induction of labor (106, 107). Conversely inhibition of prostaglandin synthesis can delay the onset of labor, reduces uterine contractions, and prolongs the process of labor (108, 109). Certainly endogenous prostaglandin production has been implicated in human parturition with levels in maternal plasma, amniotic fluid and urine all known to rise prior to the onset of labor, with considerable elevations and subsequent plateauing of levels with parturition (101, 110-112). The primary sites of this PG synthesis are the fetal membranes and decidua (80, 113), however, myometrial and cervical contributions may also be important with prostaglandin E and prostaglandin F2α increasing in a time-dependent manner during late gestation. Altered expression of the synthetic enzymes has been noted with labor, with lower segment human myometrium PGF synthase expression decreased by 3-fold (P<0.02), in contrast to a 6-fold increase in cervical PGES expression (104). The c-PGES and m-PGES-1 isoforms have also been identified in human fetal membranes and placenta to show differential expression with labor (114-116), with similar up-regulation of expression of m-PGES1 and m-PGES-2 in human myometrium (55, 117), (118). Notably m-PGES activity may be further enhanced via the positive feedback of PGE2 enhancing IL-1β-induced mPGES-1 expression (119), this loop will also enable continuous production of PGE2 by invading monocytes with the downstream effect of myometrial stimulation. In addition, human amnion, chorion and decidua primarily produce PGE2 further propagating this cycle in contrast to the decidua which mainly produces PGF2α. This production of PGE2, PGF2α, and other prostanoids by the fetoplacental unit would appear to relate to the observed increased contractile activity during labor. Further, any stimulus (hypoxia, infection, exposure to oxytocin, hypertonic solutions, prostaglandins, or arachidonic acid) facilitating PGE2 synthesis in the fetal membrane is likely to induce the formation of PGF2α in decidua and myometrium and clinically translate to a stimulated myometrial phenotype. Notably however, since naturally occurring prostaglandins in the human body are rapidly metabolized, induction of labor will require a sustained presence of PGs (120).

5.2. Cyclooxygenase (COX) enzymes and gene expression in labor

Despite the apparent central role for PGs in the process of parturition, analysis of gene expression profiles of fundal myometrium from pregnant women before and after the onset of labor have not demonstrated an alteration in fundal COX-2 expression in association with spontaneous labor (121, 122), with human microarray data similarly not demonstrating any change in COX-1 or COX-2 expression in lower segment myometrial samples (123). Furthermore quantitative RT-PCR and protein studies have also not shown any change in COX-1 or COX-2 expression in human laboring myometrium both at term and preterm (124). However, studies are not consistent with COX-2 mRNA expression reported as increased in human fundal myometrium prior to the onset of term labor (121). These inconsistencies in results may be partially attributable to infection, as myometrial COX-2 expression was noted to be increased in women with chorioamnionitis (121), and IL-1β stimulates COX-2 transcription via activation of NF-κB in human myometrial cells (125). Moreover in a mouse model of inflammation-mediated preterm labor, there was a significant increase in uterine COX-2, but not COX-1 gene expression, with inhibition of COX-2 abolishing LPS-induced preterm labor (126).

In addition to these changes within the myometrium, COX-2 has also been implicated in cervical ripening. We observed an 11-fold (P<0.01) increase in COX-2 expression in laboring human cervix (104), with others noting differential regulation of COX-2 in endocervix mucosa as compared with cervical stroma (121). Immunohistochemical studies suggest that it is this differential localization to the endocervical glandular epithelial cells that are responsible for the observed up-regulation of COX-2 in the endocervix during human labor at term (121). In addition to these local cervical changes COX-2 protein expression also appears to be spatially regulated within the uterus with a distinct positive concentration gradient from fundus to cervix (127). Given that COX-2 expression is predominantly localized to macrophages, the major cell subtype responsible for the cervical inflammatory influx, this may underlie the observed gradient. Further increases within the cervix are possible due to additional localization primarily within cervical epithelial cells, activated endothelial cells and stromal fibroblasts, with numbers of positive COX-2-stromal fibroblasts per tissue cross-section increased in cervical tissues in labor (121).

Several studies have examined the relative contributions of COX-1 and -2 in the process of labor, hypothesizing that COX-2 is predominantly responsible for
the observed labor associated increases in PG production. Admittedly although expression of COX-1 enzyme has previously been reported to be constant throughout gestation in the lower segment human myometrium (122), recent analysis of mRNA expression suggests that COX-1 levels decline precipitously towards term in fetal membranes (128). Conversely, the mRNA level of COX-1 is reported to increase about three to four fold in late pregnancy, starting at approximately 35 weeks of gestation. The level of COX-1 reaches a peak at term, with no observed increase with the onset of labor. Conversely, mRNA levels of COX-2 are very low throughout pregnancy, but increase dramatically about six fold at the same time as COX-1. However, COX-2 mRNA level in amnion continues to rise during labor (129, 130). Similarly, COX-2 activity in the fetal membranes, chorio-decidua and myometrium significantly increases with advancing gestation (124, 131).

It would appear that these increases in COX-2 mRNA are tissue specific and mediated by transcriptional control in amnion and post-transcriptional control in chorion (128). Certainly COX-2 activation is NF-κB dependent, with two NF-κB response elements located within the COX-2 promoter, whereas, COX-1 has none (132). It is this NF-κB dependent pathway that interleukin-1β (IL-1β) could potentially exploit as IL-1β increases NF-κB activity in human amnion (133), the main site of COX-2 activity, and myometrium, while also repressing progesterone receptor activity. Indeed IL-1β induced COX-2 expression in lower segment human fibroblast cells is suppressed by progesterone (134), with this effect mediated by reduced recruitment of NF-κB p65 to both proximal and distal NF-κB elements within the COX-2 promoter and concurrent increased expression of the NF-κB inhibitor IκBα (135). Clear evidence for the role of NF-κB in COX-2 regulation in human myometrial cells, has been achieved by inhibition of IκBα degradation with the result of impaired NF-κB nuclear translocation, suppression of COX-2 mRNA and protein expression, and inhibition of PGE₂ and PGF₂α synthesis (125). Collectively these data supports a concept whereby the pro-inflammatory state associated with both term and preterm labor potentially facilitates the induction of labor in a COX-2 and prostaglandin-dependent manner. With these changes being principally brought about by increased NF-κB activation and a decline in progesterone receptor coactivators (136), with the overall effect of overcoming the quiescent state that is maintained by progesterone throughout gestation (137).

5.3. Factors affecting prostaglandin production during labor

5.3.1. Prostaglandin metabolism

Prostaglandin dehydrogenase (PGDH) has a key role to play in the physiology of labor, as PGDH determines the concentration of biologically active PGs within tissues, due to its role as a key metabolizer of prostaglandins. This metabolic activity of PGDH is dependent on an oxidized form of nicotinamide adenine dinucleotide (NAD+), to transform PGs into biologically inactive 15-keto compounds. These molecules are subsequently converted to 13,14-dihydro-15-keto derivatives (Figure 1) by Δ13,14-reductase, which are the stable inactive PG metabolites (138). Consequently the distribution of PGDH within gestational tissues may directly affect the concentration of PGs that reach the myometrium.

Chorion is the principal site of PGDH activity, with subsequent degradation of PGE₂ and PGF₂α into their inactive metabolites (139). Thus, the chorion may act as an in-built protective barrier minimizing the free transfer of bioactive PGs produced by the amnion or indeed even the chorion from passing to the underlying decidua and myometrium (49, 140, 141). Consistent with this, increases in PGE₂ concentration in amnion after labor do not result in an increase in PGE₂ concentration in myometrium as assessed in vitro (142). By contrast, a decrease in the metabolic activity of PGDH, and consequently, an increase in the concentration of bioactive PGs at the onset of labor both at term and preterm could allow PGE₂ to reach myometrium (143, 144), enabling contractions and thereby inducing preterm labor (145). Despite this clear evidence for a potentially significant role for PGDH in regulating PG trafficking, assessment of the permeability of intact-amnion-chorion-deciduala revealed that 72% of PGE₂ remained in the active form and was not metabolized after passage across this barrier, with no alteration in the barrier characteristics after the onset of spontaneous labor (146).

PGDH expression is regulated by additional signals with pro-inflammatory cytokines and infectious agents including IL-1β and LPS inducing a reduction in PGDH expression. This regulation of PGDH may be responsible for the increased availability of bioactive prostaglandins which have been implicated in infection-induced preterm labor (147). In addition, PGDH may be regulated at a transcriptional level by the competing activities of progesterone and cortisol (148). A potential model whereby during pregnancy, fetal membrane PGDH activity is maintained by progesterone acting either through low level of progesterone receptors or via glucocorticoid receptors (GR) is subsequently reversed at term by elevated levels of cortisol competing with progesterone for GR with subsequent inhibition of PGDH transcription and activity is attractive. This would also enable local withdrawal of progesterone action within human intrauterine tissues, without requiring changes in systemic and circulating levels of progesterone. Notably glucocorticoids also induce the expression of prostaglandin synthesizing enzymes within the amnion and chorion directly, by up-regulating COX-2 expression and also indirectly via intermediary actions of paracrine effectors including CRH (148).

In addition to these effects within myometrium we have also shown that PGDH expression was down-regulated 11-fold (P<0.01) in the laboring human cervix at term (104). This cervical decrease in PGDH enzyme expression in association with active labor may act to increase the concentration of bioactive PGs within the human cervix, thereby facilitating cervical remodeling and dilation. Potentially localized reductions in PGDH activity may be present in those patients with premature cervical
softening, or conversely PGDH activity may be particularly high in those patients with an unfavorable cervix, presenting with a low Bishop score and poor progression at the onset of labor (149). If indeed PGDH crucially determines the passage and availability of biologically active prostaglandins from amnion and chorion to underlying cervix, then pharmacological manipulation of PGDH activity may represent a novel therapeutic intervention for not only preterm labor, but also cervical incompetence and induction of labor (149).

5.3.2. Stretch
It is well recognized that the uterus is subject to stretch throughout pregnancy, with mechanical stretch inducing myometrial smooth muscle hypertrophy and increases in tensile strength. Mechanical stretch, in addition to protein synthesis, can also activate the transcription of specific genes in particular; Connexin 43, oxytocin receptor, ion channels and FP receptors thereby rendering the myometrium responsive to uterotonic - a key step in the initiation of labor (150). Indeed stretching of human myometrium in-vitro has been reported to release prostaglandins (151). Furthermore, mechanical stretching of human lower uterine segment and cervix, with a rubber balloon, has shown induction of cervical softening and parturition at term, due to increases in the levels of PGF$_{2 \alpha}$ in amniotic fluid (152) and plasma (153). Indeed COX-2 appears to be one of the target genes activated by mechanical stress, with myometrial COX-2 and PGE$_2$ expression increased with twins or polyhydramnios (154) and COX-2 and downstream PGE$_2$ expression increased after tonic stretch of primary human uterine myocytes (155). Pathway analysis to determine the principal players has suggested that this stretch related induction of COX-2 activity is via activation of the activator protein-1 (AP-1) system (155). Similarly stretch of primary human amnion epithelial cells is also associated with an increase in COX-2 expression and PGE$_2$ synthesis, again via activation of AP-1 and NF-$\kappa$B transcription factors (156). The regulation of these stretch induced responses is unclear, however, data from animal studies suggest that progesterone may partially contribute by blocking stretch-induced responses, with little induction of gene expression until after functional progesterone withdrawal has occurred (150).

5.3.3. Oxytocin
Prostaglandins also have a close functional interaction with oxytocin, an important modulator of myometrial function (157). Myometrial oxytocin receptor activation is coupled with the activation of the phosphoinositide cycle, producing diacylglycerol (DAG) and inositol trisphosphate (IP$_3$). DAG is a source of arachidonic acid, furthermore IP$_3$ via an influx of extracellular calcium by receptor operated channels increases intracellular $[Ca^{2+}]$, activating PLA$_2$, thereby further increasing AA release. In addition to these immediate changes infusion of oxytocin for longer periods to augment or induce labor may also initiate the myometrial transcription of COX-2 gene via the MAPK signaling pathway, ensuring continuous prostaglandin production (158). Furthermore although oxytocin and PGs can both independently stimulate myometrium (159), the response of myometrial strips to oxytocin is significantly increased after pre-treatment with prostaglandins (160).

5.3.4. Cytokines
Pro-inflammatory cytokines including IL-1$\beta$ have been widely implicated in the initiation and progression of human labor both at term and preterm (161). This may be potentially through interactions with PGs as IL-1$\beta$ is a potent agonist of prostaglandin synthesis in human myometrial cells, primarily via the induction of PLA$_2$ and COX-2 enzymes, with IL-1$\beta$ stimulated cultured human myometrial cells exhibiting a dose- and time-dependent increase in PGI$_2$ and PGE$_2$ production, to the order of 15- to 23-fold (162). Furthermore mRNA level of PGE$_2$ synthesizing enzymes are associated with IL-1$\beta$ in human labor supporting this pathway as a key factor (117).

6. TOCOLYTIC STRATEGIES

6.1. Clinical use of enzyme inhibitors in preterm labor
Given these critical roles for PGs in labor, the obvious clinical intervention has been the prevention of preterm labor by inhibition of COX enzymes. Certainly non-specific COX inhibitors, such as indomethacin, have reduced preterm birth, and increased gestational age at birth and birthweight (163). However, the major problem with such agents is due to their low molecular weight and high lipid solubility with the inevitable consequence that they readily cross the placenta. Due to their non-specific nature once in the fetal circulation they may cause premature closure of the fetal ductus arteriosus or due to the role of PGs regulating renal blood vessels may induce fetal renal failure. The use of specific COX-2 inhibitors to block the pro-inflammatory effects of PGs, but allow the constitutive production of PGs for homeostatic mechanisms would appear to have been a logical step forward. However trials with specific COX-2 inhibitors have reported serious fetal side-effects even though they have delayed labor to a certain extent. A meta-analysis of two trials found that COX-2 inhibitors did not show any improved maternal or neonatal outcome compared to non-specific COX inhibitors (163-165). Consistent with meta-analysis a randomized controlled trial of rofecoxib (a specific COX-2 inhibitor) found a significant, but reversible, effect on fetal urine production rate and on the ductus arteriosus. Due to their non-specific nature once in the fetal circulation they may cause premature closure of the fetal ductus arteriosus or due to the role of PGs regulating renal blood vessels may induce fetal renal failure. The use of specific COX-2 inhibitors to block the pro-inflammatory effects of PGs, but allow the constitutive production of PGs for homeostatic mechanisms would appear to have been a logical step forward. However trials with specific COX-2 inhibitors have reported serious fetal side-effects even though they have delayed labor to a certain extent. A meta-analysis of two trials found that COX-2 inhibitors did not show any improved maternal or neonatal outcome compared to non-specific COX inhibitors (163-165). Consistent with meta-analysis a randomized controlled trial of rofecoxib (a specific COX-2 inhibitor) found a significant, but reversible, effect on fetal urine production rate and on the ductus arteriosus, with no reduction in the incidence of early preterm birth (166). In contrast a prospective observational study by the same group of the use of nimesulide in the prevention of preterm labor suggested that it had a significant benefit for high risk cases with no long-term side-effects on the fetus (167). Although in patients with preterm prelabor rupture of membranes the actual incidence of preterm birth was increased by the use of COX-2 inhibitors (168). Therefore at present COX-2 inhibitors are not fulfilling their promise as therapeutic agents for preterm birth. However, it is not clear whether the side-effects of COX-2 inhibitors are due to COX-2-dependent mechanisms or to COX-1 inhibition and their limited ability to differentiate between prostaglandins for maternal and fetal physiological and developmental processes and those that are responsible for labor (169). Secondly selective COX-2 inhibitors also only
block the cyclooxygenase activities while leaving peroxidise activities intact (170). Notably the formation of a COX-1 and COX-2 heterodimer has recently been implicated in the closure of the ductus arteriosus in mice (171), which may explain the observed differential tissue sensitivity to non-steroidal anti-inflammatory drugs (NSAIDs) and potentially offers a new approach to therapeutically exploit the formation of prostaglandins. Future exploration of the role of COX inhibition has been facilitated by the development of a novel selective COX-2 inhibition mouse model (171). These COX-2 Y385F mice in combination with COX-2 knock-out mice, may allow precise assignment of the respective roles of both cyclooxygenase and peroxidise activities in physiological as well as pathophysiological conditions, and help in separating out non-COX NSAID-related activities (170).

6.2. Prostaglandin F receptor antagonists: THG113 and THG113.31

PGF\textsubscript{2\alpha} plays a key role in parturition, and it is an effective smooth muscle contractile agent (172), having a significant stimulatory effect on myometrium contractility. THG113 is a selective, non-competitive, reversible and potent FP receptor antagonist (173, 174), which works by blocking the interaction of FP receptor with G\textsubscript{q}, consequently inhibiting the further increase in intracellular calcium level (175). A variant of the FP receptor antagonist, THG113.31 has been reported to delay significantly endotoxin-induced preterm labor in mouse (174) and in RU486-induced preterm labor in sheep (49). The mechanism by which THG113.31 inhibits contractions in the uterus remains to be fully elucidated, however, recently, it has been reported that THG113.31 induced a rapid concentration-dependent relaxation on spontaneous human uterine contractions, suggesting that the activation of BK\textsubscript{Ca} channels, the predominant K\textsuperscript{+} channel found in the human myometrium (176-180), and not FP receptors were responsible for mediating the relaxatory effect of THG113.31 in the human myometrium (181). At present clinical studies are urgently required to determine whether these agents will play a role in the pharmacological armamentarium for the prevention of preterm birth.

7. COX AND PG RECEPTOR KNOCK-OUT MOUSE MODELS

Despite the structural similarities in COX-1 and COX-2, they are encoded differently with COX-1 encoded by a >28 kb gene, in contrast to ~8 kb for COX-2(182, 183), although the organization of exons and introns is similar for the two with the COX-1 locus containing an extra exon, intron-1, that gives rise to COX-3 by alternative splicing (23). To date manipulation of these loci for COX-1 and COX-2 has resulted in knock-out mice being developed with COX-2 knock-out mice exhibiting a major disruption of reproductive capacity and are left infertile (184). In contrast COX-1 knock-out mice although fertile, have delayed spontaneous parturition at term (185), but respond normally to preterm labor induced with LPS, suggesting that the mechanisms of inflammation-induced preterm and term labor differ in mice (126). In a microsomal PGES-1 knock-out mouse LPS induced preterm labor was also not delayed, possibly due to compensation by microsomal PGES-2 (186). cPLA\textsubscript{2} knock-out mice have been reported to have small litters and usually dead pups (187, 188), furthermore, cPLA\textsubscript{2} knock-out mice do not undergo labor at term, although labor can be achieved/rescued by administering the progesterone receptor antagonist, RU486 (189). This delayed onset of labor in cPLA\textsubscript{2} knock-out female mice is supportive of cPLA\textsubscript{2} being an important component in the initial signaling cascade of murine luteolysis and thereby labor, consistent with cPLA\textsubscript{2} acting as a direct catalyzing enzyme for AA release with coupling of COX to PGF\textsubscript{2\alpha} synthesis, luteolysis and labor induction (188), however the direct relevance to humans may be limited due to luteolysis in humans not being attributable to PGs.

Mice lacking each type and subtype of the PG receptors have now been produced with research ongoing to elucidate their respective roles (190). EP\textsubscript{4} knock-out mice demonstrate failure of closure of the ductus arteriosus as neonates, suggesting a crucial involvement of this receptor sub-type (190), however, they do not appear to demonstrate any defect in parturition. In contrast mice lacking the FP receptor exhibit a complete failure of parturition at term, although the fetuses are phenotypically normal (191). Much like cPLA\textsubscript{2} knock-out mice, both COX-1 and FP receptor knock-out mice fail to initiate spontaneous labor (191, 192), with studies ongoing on the other PG receptor null mice.

8. SUMMARY AND PERSPECTIVE

Despite significant research in elucidating the role of PGs in labor, we have yet to fully understand their contribution to the physiology of term and preterm labor. Continued focus on receptor regulation and activation is essential as prostaglandins may be a final common pathway in human parturition. Notably however human labor is a multistep process, with significant redundancy in molecular pathways, therefore the role of other important mediators like cytokines should not be overlooked, and indeed combined therapeutic interventions may be the key to preventing preterm delivery.

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