Introduction

Pre-term birth occurs in 5% to 10% of all pregnancies in North America. This figure may be higher in certain population groups and has not decreased over the past 20 to 30 years. Although some pre-term births may be elective, approximately 30% occur in association with an underlying infectious process, and about 50% are idiopathic pre-term births of unknown cause. Pre-term birth is associated with a 70% rate of neonatal death and up to 75% of neonatal morbidity. Infants born before term have an increased rate of cerebral palsy, neurologic handicap and pulmonary disorders. The costs of caring for pre-term babies in the United States has been estimated at $8 billion annually. At present, there are no effective diagnostic indicators of pre-term birth, and there are no effective treatments for this condition. Thus, the current direction of research remains to understand the underlying biochemistry of the birth process and to utilize that information to develop better diagnostic indicators and improved methods of therapy.

Phases of parturition

Uterine contractility during pregnancy and parturition can be divided into different phases. Phase 0
corresponds to pregnancy, a time of relative uterine quiescence. Phase 1 is associated with activation of uterine function, wherein mechanical stretch or uterotrophic priming leads to upregulation of a cassette of genes required for contractions. These contraction-associated proteins (CAP) genes include connexin–43 (Cx43) the major protein gap junctions, agonist receptors and proteins encoding ion channels. In phase 2, the uterus can then be stimulated by uterotonins, including prostaglandins, oxytocin and corticotropin-releasing hormone (CRH). Phase 3 includes expulsion of the placenta and the involution process and has been attributed primarily to the effects of oxytocin.

Regulation of uterine quiescence during pregnancy (phase 0) has been discussed in several recent reviews. Major effectors of myometrial relaxation, acting in a paracrine or endocrine fashion, include progesterone, relaxin, prostacyclin (PGI2), parathyroid hormone-related peptide (PTH-rP), nitric oxide and CRH, which may inhibit and stimulate uterine contractility. These agents act in different ways but in general result in increased intracellular levels of cyclic adenosine monophosphate (cAMP) or cyclic guanosine monophosphate. These nucleotides inhibit intracellular calcium release and reduce the activity of the enzyme myosin light-chain kinase (MLCK) that is required for shortening of the myofilaments. Several current strategies for managing pre-term labour are directed at increasing intracellular cAMP or reducing the availability of calcium.

It is clear now that activation of myometrial function (phase 1) is driven through the fetal genotype and effected through 2 separate but interdependent pathways. One involves precocious activation of the fetal hypothalamic-pituitary-adrenal (HPA) axis. The second involves mechanical distension of the uterus, leading to stretch-related upregulation of CAP gene expression. Activation of the fetal HPA axis occurs in the presence of an adverse intrauterine environment, for example with compromised uteroplacental blood flow or conditions of fetal hypoxemia. Mechanical stretch likely contributes to the greater incidence of pre-term birth in pregnancies with multiple fetuses. In addition, stretch may account for the higher incidence of pre-term birth in pregnancies where the fetal size is large for gestational age.

During pregnancy the steroid hormone progesterone is required for uterine growth, but it simultaneously suppresses expression of CAP genes. At term in most animals species, the influence of progesterone on the myometrium declines, uterine stretch no longer stimulates uterine growth and the increase in wall tension caused by continued fetal growth becomes translated into increased expression of CAP genes and myometrial activation. In women, there does not appear to be a decline in circulating progesterone concentrations prepartum. We have argued recently that this represents a mechanism to maintain relaxation of the lower uterine segment at the time of birth, while local antagonism of progesterone action in the fundal region of the uterus facilitates development of uterine contractions predominantly in that region.

**Prostaglandins and parturition**

There is now compelling evidence that increased output of intrauterine prostaglandins contributes to the drive to myometrial contractility at term and pre-term. The stimulus to increased prostaglandin synthesis may be a consequence of fetal HPA activation or uterine mechanical stretch, or both (see below). It is clear, however, that the role of these substances is primarily in phase 2 rather than phase 1 of parturition. The evidence implicating prostaglandins in the parturition processes is overwhelming. Concentrations of prostaglandins or their metabolites in maternal plasma or amniotic fluid increase at the time of labour before the onset of coordinated uterine contractility. Drugs that block prostaglandin synthesis are known to promote myometrial quiescence and may prolong the length of gestation. Furthermore, pregnant mice in which the prostaglandin synthase type 1 (PGHS-1) gene has been inactivated by mutation have prolonged gestations.

In women, prostaglandin production is discreetly compartmentalized within the tissues of the pregnant uterus. Prostaglandin E2 (PGE2) is formed predominantly in amnion, and its output increases at the time of labour. Chorion and decidua also produce PGE2. The presence in chorion of the enzyme 15-hydroxy prostaglandin dehydrogenase (PGDH) is presumed to cause metabolism of PGs generated in amnion.
and chorion, preventing their passage to the underlying tissues. Thus, in normal pregnancy those PGs that stimulate myometrial contractions, are likely generated either within decidua or in the myometrium itself. In some cases of pre-term birth, however, PGDH activity in chorion is diminished, the metabolic barrier is reduced and PGs generated within amnion or chorion could then provide the stimulus to myometrial contractions. Studies on prostaglandin production from myometrium collected from women at the time of labour have led to divergent findings. Although some investigators have reported increased PG output, most reports have failed to demonstrate increased PG synthesis or PGHS activity in myometrium collected from women in labour at term or pre-term.

Primary prostaglandins are formed from arachidonic acid through the activity of the prostaglandin synthase (PGHS) enzyme complex. There are 2 forms of PGHS — PGHS-1 and PGHS-2. These are separate gene products. PGHS-1 has been described as constitutive, PGHS-2 as inducible by growth factors, cytokines and, paradoxically, in human fetal membranes by glucocorticoids. The expression and activity of PGHS-2 in human amnion and chorion increase at the time of labour; PGHS-2 is also upregulated in amnion collected from patients in pre-term labour. Thus, new strategies for inhibiting the myometrium in pre-term labour have included development of specific inhibitors of PGHS-2 activity. It is anticipated that these drugs should be more efficacious than the nonspecific PGHS-1 inhibitors used previously and will have fewer cardiovascular and renal side effects in the fetus since they will exert minimal inhibition on PGHS-1.

Prostaglandin action is mediated through specific receptor binding. There are 4 main subtypes of receptors: EP1, EP2, EP3 and EP4 for PGE2 and FP for prostaglandin F2α (PGF2α). EP1 and EP3 receptors mediate contractions by increasing intracellular calcium mobilization and inhibiting intracellular cAMP generation. Conversely, PGE2 acting through EP2 and EP4 receptors increases adenylate cyclase and relaxes smooth muscle. Theoretically, inhibition of uterine activity might be achieved through antagonism of EP1, EP3 or FP action, or through activation of the inhibitory EP2 and EP4 receptors. At present, however, there is little information concerning the regulation and regional changes in any prostaglandin receptor subtype in human myometrial tissue at the time of labour.

Increased expression of PGHS-2 occurs in response to a variety of growth factors including epidermal growth factor (EGF), cytokines (interleukin-1 [IL-1]; tumour necrosis factor [TNF], and interleukin-6 [IL-6]). The action of cytokines appears to be mediated through a nuclear factor kappa beta (NF-κB) consensus sequence in the promoter region of PGHS-2. This promoter also contains a cAMP response element and a glucocorticoid response element (GRE) at approximately 760 base pairs from the PGHS-2 transcription start sign. Several years ago Gibb and colleagues showed that human amnion cells maintained in monolayer tissue culture produced increased amounts of PGE2 in a dose-dependent fashion in response to treatment with a synthetic glucocorticoid, dexamethasone. It remains unclear whether this action is on amniotic epithelium or fibroblast cells, since both contain glucocorticoid receptors (GRs) and both have been reported to increase expression of PGHS-2 on glucocorticoid stimulation. However, in mixed cell cultures, the action of glucocorticoids clearly depends on interaction with GR and apparently requires activation of protein kinase-C (PKC).

Proinflammatory cytokines also increase PGHS-2 gene expression, messenger ribonucleic acid (mRNA) and protein levels and PG output by cultured amnion and chorion cells. Interestingly, anti-inflammatory cytokines such as IL-10 attenuate the stimulatory effect of IL-1β on PGHS-2 gene expression and activity. Thus, in vivo, it is apparent that the relative amounts of eicosanoids and cytokines produced from an interactive cytokine–eicosanoid cascade will be critical in regulating the final response of the tissue and the level of prostaglandin produced. These results also raise the interesting possibility that anti-inflammatory cytokines might be utilized therapeutically to modulate the action of compounds such as IL-1.

It is possible that cytokines may contribute to the stimulus for uterine contractility in patients at normal term with a subclinical infection. Cytokines are very clearly involved, however, in precipitating...
prostaglandin synthesis in association with an infective process. Infection-driven pre-term labour occurs in the presence of increased concentrations of IL-1, IL-6 and TNF in amniotic fluid. Experimentally, the administration of IL-1 or of bacterial endotoxin to pregnant mice provokes premature delivery, and this is associated with increased prostaglandin output by intrauterine tissues. Cytokines increase phospholipase A2 and PGHS-2 gene expression in a time- and dose-dependent fashion by amnion and choriodecidual tissue maintained in vitro. Thus, it is generally accepted that in the presence of an ascending bacterial infection, organisms pass between the fetal membranes and later reach the amniotic cavity. Bacterial organisms release phospholipases, which may in turn stimulate prostaglandin synthesis. They also release endotoxins, such as lipopolysaccharide, which provoke prostaglandin output directly or release cytokines from macrophages. These cytokines in turn stimulate PGHS-2 expression and prostaglandin output from amnion and decidual cells. In addition, IL-1 stimulates output of other cytokines including IL-6 and IL-8 from decidua, thereby establishing a positive feed-forward cascade.

**Prostaglandin metabolism**

Recently it has become apparent that the biologically active levels of PG depend not only on rates of synthesis but also on the rates of metabolism. Normally, high levels of PGDH expressed in chorion trophoblasts would be expected to metabolize effectively PG generated within amnion or chorion. However, patients in pre-term labour with an underlying infective process have markedly reduced numbers of trophoblasts in the chorion layer and dramatically reduced levels of PGDH activity. In addition, approximately 15% of patients with idiopathic pre-term labour had diminished expression of PGDH but normal presence of trophoblasts. PGDH activity is reduced modestly in chorion from patients at term but is markedly diminished in myometrium and the cervix of patients presenting in pre-term labour. Thus, reduced prostaglandin metabolism appears to be an effective way of increasing prostaglandin levels that may then reach agonist PG receptors in a paracrine fashion. Furthermore, levels of matrix metalloproteinase-9 (MMP-9) in chorion are increased with pre-term labour. Since this gelatinase enzyme contributes to the controlled degradation of collagen within the fetal membranes and MMP-9 activity is increased by PGE2, this feed-forward cascade may also help explain the mechanism of pre-term premature rupture of the membranes with MMP-9 as the predominant gelatinolytic activity.

Recent studies have been directed toward understanding the mechanism by which steroid hormones might regulate PGDH. Surprisingly, these studies have also revealed a mechanism for local progesterone withdrawal within the human fetal membranes. Patel and colleagues have shown that human chorionic PGDH-gene expression and activity is inhibited by glucocorticoids, (cortisol, betamethasone and dexamethasone) and maintained in a tonic fashion by progesterone. Chorion trophoblasts express the enzyme 3β-hydroxysteroid dehydrogenase (3β-HSD) and have the capacity to produce their own progesterone from pregnenolone. Inhibition of 3β-HSD enzyme inhibited progesterone output from chorion trophoblast cells and reduced PGDH mRNA levels. Replacement of progesterone or adding a synthetic progestagen restored PGDH activity. This effect could be blocked, in part, by a progesterone receptor antagonist. However, the action of progesterone to restore PGDH could also be blocked by a specific GR antagonist. This observation suggested that progesterone, produced locally within chorion acts throughout pregnancy to maintain chorionic PGDH activity. It does so, however, by interacting with GR. At term, increased availability of endogenous cortisol would displace progesterone from GR, resulting in loss of the stimulation to PGDH and a direct inhibitory effect on PGDH expression. This interaction, whereby the effects of progesterone are mediated through GR but can be opposed by increased output of glucocorticoid, may provide a mechanism for producing local progesterone withdrawal in the human uterus. We have suggested elsewhere that this activity may be greater in the fundal area, thereby contributing to regionalized changes in uterine contractions. Regulation of PGDH, however, is clearly multifactorial. The enzyme is also down-regulated by
cytokines, cAMP and CRH. It is unclear whether the CRH effect on PGDH is mediated through cAMP since the CRH receptor in human fetal membrane does not appear to be coupled through the Gas protein. Anti-inflammatory cytokines such as IL-10 appear to increase PGDH gene expression.

Surprisingly, we found that the biologically inactive corticosteroid, cortisone, was almost as effective as cortisol in inhibiting PGDH in chorion cells, but not in placental trophoblast cells. In chorion, the action of cortisone could be blocked by a GR antagonist and was completely attenuated in the presence of the drug carbonexolone. This drug, an active ingredient of liquorice, inhibits the enzyme 11βHSD-1. 11βHSD-1 is abundantly expressed in chorion trophoblasts, and predominantly converts cortisone to cortisol. Thus, these cells have the potential to form cortisol locally from cortisone, in addition to forming progesterone locally from pregnenolone. Therapeutic regulation of PGDH, theoretically, could be accomplished by steroid hormones or by drugs that alter the levels of 11βHSD-1.

**CRH and pre-term labour**

It is now well established that the concentrations of CRH in maternal blood rise progressively during human pregnancy. This rise correlates with increased levels of CRH mRNA and CRH peptide in placental tissue. In the circulation, CRH is largely associated with a high-affinity circulating CRH-binding protein (CRH-BP) produced in the liver, placenta and at other sites, including the brain. CRH-BP effectively blocks the action of placental CRH on the maternal pituitary gland and on the myometrium. Near term, and in association with pre-term labour, CRH-BP concentrations fall, coincident with the increase in circulating CRH. Thus, it has been suggested that there is a substantial increase in free CRH concentrations in systemic plasma as a component of the trigger to the labour process.

Regulation of placental CRH output is multifactorial and has been reviewed extensively. Briefly, CRH gene expression and CRH output by placental trophoblast cells is paradoxically increased by glucocorticoids. CRH output from placenta and fetal membranes also increases in response to prostaglandins, cytokines and catecholamines, and is decreased by nitric oxide and progesterone. Karalis and Majzoub have suggested that the inhibitory effect of progesterone is exerted through binding to GR in trophoblast cells. At term, increased levels of cortisol displace progesterone bound to GR, and this is reflected as an increase in CRH output. Thus, the mechanism of interaction between progesterone and cortisol in the regulation of CRH is similar to that proposed for the regulation of PGDH.

The action of CRH on the intrauterine tissues and myometrium is effected through an extensive network of high-affinity CRH receptors with different specificities. There are 2 main classes of CRH receptor, CRH-R1 and CRH-R2. In myometrium, CRH acts by binding to CRH-R1, which is coupled to Gas, leading to stimulation of cAMP output. Thus, the primary effect of CRH throughout pregnancy is likely to be one of uterine relaxation. The binding affinity of the CRH receptor in human myometrium increases during pregnancy, but then decreases before parturition. Studies by Grammatopoulos and Hillhouse have suggested that oxytocin effects this change by upregulating a protein kinase C which phosphorylates the CRH-receptor protein resulting in desensitisation and loss of the inhibitory influence of CRH on myometrium. Therefore, the peptide CRH may act as an inhibitor or stimulant to the myometrium, depending upon the affinity and second messenger of the different receptor species.

The differential effects of CRH on the myometrium may also contribute to the regionalization of myometrial activity at term and in the pre-term period. Stevens and colleagues showed that the expression of CRH-R1 in myometrium collected from the lower uterine segment was higher in patients in labour than in those not in labour. Furthermore, expression of CRH-R1 was substantially higher in lower segment than in fundal myometrium when paired samples of tissue from individual patients were examined. Thus, at the time of labour, CRH may promote relaxation of the lower segment but stimulate activity in the body of the uterus. This stimulatory action could be direct; it could also be indirect, since CRH stimulates output of prostaglandins by upregulating PGHS-2 and down-regulating PGDH in human fetal membranes.
There has been much interest recently in the possibility that elevations of maternal plasma CRH concentration may be used to predict women destined to enter pre-term labour. McLean and colleagues demonstrated elevated maternal plasma concentrations of CRH as early as 14 to 16 weeks’ gestation in women who subsequently delivered pre-term, and lower concentrations of CRH in the plasma of women who delivered post-term. Korebrits and associates found that maternal plasma CRH concentrations were elevated in patients at 28 to 32 weeks’ gestation having an initial diagnosis of threatened pre-term labour, who delivered within 48 hours. However, CRH concentrations were within the normal range in patients with the same initial diagnosis who proceeded to delivery at term. At present, it seems unlikely that a single measurement of maternal plasma CRH will provide an adequate means of predicting the patient who is at risk of pre-term labour. However, we and others have suggested that a combination of biochemical tests including CRH and salivary estriol, combined with measurements of fibronectin, may be of sufficient sensitivity and specificity to be clinically useful.

Birth — an integrated cascade?

From the preceding discussion it should be apparent that birth, at term and pre-term, results from processes leading to increased prostaglandin output. Glucocorticoids have a central role in those processes. Glucocorticoids also stimulate CRH output within placenta and fetal membranes and CRH similarly upregulates PGHS-2 and down-regulates PGDH. The effects of CRH may be modulated by the state of the CRH receptor. Oxytocin appears to play a key role in changing the affinity of CRH-receptor interaction. Oxytocin could be derived from the systemic circulation, but also could be derived locally from chorion or decidua.

Fig. 1: Cortisol and prostaglandin (PG) interactions in the fetal membranes. (For abbreviations see box on page 60.)
Increased levels of cortisol could be derived from the maternal circulation, for example in association with a maternal stress response, or from the fetus after precocious activation of the fetal HPA axis. In addition, cortisol can be formed locally within chorion trophoblast cells from the inactive precursor cortisone. The expression of 11β-HSD-1 enzyme that effects this conversion, increases in chorion trophoblasts progressively during human gestation. More recently, it has been shown (N. Alfaidy, J.R.G. Challis: unpublished results) that the activity of 11β-HSD1 is increased significantly by prostaglandins through a mechanism that involves increased release of intracellular calcium and phosphorylation of the enzyme. In this way, increased production of prostaglandins (PGE₂, PGF₂α) increased 11β-HSD1 activity, leading to more cortisol formation from cortisone (Fig. 1). Cortisol in turn increases further PG production. It is evident that with infection other agents such as cytokines can intercede in this series of loops by stimulating PGHS-2 and down-regulating PGDH expression. It is also apparent that the mechanisms predisposing to pre-term labour almost certainly vary at different stages of gestation. The incidence of pre-term birth in association with chorioamnionitis is higher earlier in pregnancy. Later in gestation the fetal stress response may predominate. In this situation, fetal HPA activation increases fetal cortisol output, which in turn upregulates placental CRH expression. This is consistent with elevated concentrations of CRH in the umbilical cord plasma of fetuses with intrauterine growth restriction. Recently, it has been shown that placental CRH also drives fetal adrenal steroidogenesis. This leads to increased production of dehydroepiandrosterone from the fetal zone of the fetal adrenal. Dehydroepiandrosterone in turn is aromatized in the placenta to estrogen, thereby contributing to myometrial activation.

There are several caveats in using this information to develop better diagnostic tests for the patient at risk of pre-term birth. First, one must recognize that prevention of pre-term delivery may not always be in the best interests of the fetus. Second, the causes of pre-term birth likely vary at different times of gestation and need to be recognized as such, and treated appropriately. It is unlikely that a single test or management strategy will be efficacious for all patients. Hence, many groups of investigators are now turning their attention to micro-chip gene array techniques for the prediction of premature birth. Ongoing studies in rats have shown that distinct gene clusters are altered at the time of birth and that there is a greater tendency to decrease rather than increase gene expression at this time (H. Zingg: unpublished results). Future studies will need to examine the applicability of this information to human pregnancy. It is possible that gene array techniques can be applied to maternal peripheral blood samples for diagnostic purposes.

The ability to predict or diagnose the patient in pre-term labour will be invaluable in selecting those women for whom prenatal corticosteroids should be administered to help promote fetal pulmonary maturity. There is increasing concern with the use of repeated corticosteroid administration at regular intervals to women who may not actually be at risk of pre-term birth. Animal and human studies have now demonstrated detrimental effects of glucocorticoids on fetal growth, glucose homeostasis, cardiovascular function and neural development. Clinically, the aim should be to restrict the use of corticosteroids and tocolytic treatment to patients in whom pre-term labour has been diagnosed. The purpose of continuing studies in this area will be to achieve those objectives.

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**References**


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