

Corticotrophin-releasing hormone and human parturition

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Corticotrophin-releasing hormone (CRH), the hypothalamic peptide that controls function of the pituitary–adrenal axis in response to stress, is expressed in abundance in the human placenta and is present in high concentrations in maternal and fetal plasma during late pregnancy. A number of lines of evidence now imply a role for this hormone in the control of parturition and fetal maturation in humans. It has been proposed that CRH, through interactions with oestrogen, adrenal steroids, prostaglandins and oxytocin, establishes positive-feedback loops that initiate parturition and labour. Excessive production of placental CRH has also been linked to preterm labour. However, there are a number of significant gaps in our knowledge of the function of this peptide in pregnancy. This review examines current evidence regarding the putative role of CRH in human parturition.

Corticotrophin-releasing hormone (CRH) is a 41 amino acid peptide hormone first identified in the hypothalamus of mammals, where it acts as the main physiological regulator of corticotrophin (ACTH) secretion from corticotropes of the anterior pituitary (Vale *et al.*, 1981). Hypothalamic secretion of CRH is stimulated by many forms of physical or psychological duress and CRH plays a critical role in co-ordination of the neuroendocrine response to stress. However, in addition to its function in the hypothalamic–pituitary–adrenal axis, expression of the gene encoding CRH has now been recognized at a number of other sites, where it serves disparate roles. CRH acts as a neurotransmitter in some regions of the central nervous system (CNS), particularly those responsible for the neuropsychological responses to stress (Lenz *et al.*, 1987), and CRH is also produced in a variety of tissues outside the CNS, including the gut, ovary and testis (Shibasaki *et al.*, 1982; Suda *et al.*, 1984). During human pregnancy, the placenta and fetal membranes produce large amounts of CRH. Synthesis of CRH in these tissues increases exponentially with advancing gestation and, by term, it is present in high concentrations in the maternal and fetal blood, and in amniotic fluid. A considerable body of evidence now indicates a role for placenta-derived CRH in the physiology of human pregnancy, particularly in the process of parturition.

Corticotrophin-releasing hormone and the placenta

Placental corticotrophin-releasing hormone production

Shortly after the identification of CRH in the hypothalamus, CRH immunoreactivity and bioactivity were recognized

in the human placenta (Shibasaki *et al.*, 1982). The placenta also expresses the gene encoding CRH (Grino *et al.*, 1987) and the concentration of the peptide and its mRNA in the placenta increases with advancing gestation, paralleling an exponential increase in maternal plasma CRH concentrations. Immunohistochemistry and *in situ* hybridization studies have localized CRH production to the syncytiotrophoblast of placental villi, the amniotic epithelium, trophoblast cells of the chorion, and the decidual stroma (Riley *et al.*, 1991; Petraglia *et al.*, 1992; Challis *et al.*, 1995). Gel filtration studies show that most CRH present in placental extracts is the same 41-amino acid peptide that is expressed in the hypothalamus. In addition, the placenta contains a larger molecular weight precursor, pre-pro-CRH, which is also biologically active (Chan *et al.*, 1988).

Control of placental corticotrophin-releasing hormone secretion

Placental CRH output increases exponentially as pregnancy advances owing to specific upregulation of placental CRH gene expression (Frim *et al.*, 1988). Although CRH release from the hypothalamus is predominantly under neural control, there is no nervous tissue in the placenta and placental CRH secretion is probably regulated exclusively by circulating and locally produced humoral factors. Several studies that used either cultured placental trophoblasts or perfused placental villous tissue showed secretion of CRH *in vitro* (Grino *et al.*, 1987; Saijonmaa *et al.*, 1988) that can be stimulated by a number of neurotransmitters, cytokines, and polypeptide or steroid hormones. In particular, prostaglandins E₂ and F_{2α}, noradrenalin, interleukin 1 (IL-1) and oxytocin all increase CRH release from cultured human syncytiotrophoblasts (Petraglia *et al.*, 1989, 1990). In addition,

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glucocorticoids potently increase production of CRH mRNA and peptide from placental cells (Robinson *et al.*, 1988; Jones *et al.*, 1989) in marked contrast to their effect in the hypothalamus, where cortisol inhibits CRH synthesis and release. The positive feedback effect of glucocorticoids on placental CRH may be of great significance in parturition (see below). Progesterone inhibits placental CRH secretion *in vitro* (Petraglia *et al.*, 1989), as do nitric oxide donors such as sodium nitroprusside (Sun *et al.*, 1994).

Corticotrophin-releasing hormone in the maternal circulation

Hypothalamic CRH has a local effect on the anterior pituitary after passage through the hypothalamic–pituitary portal blood vessels but, in non-pregnant women, CRH is virtually undetectable in the peripheral blood, at concentrations of about 10–20 pg ml⁻¹. As a consequence of the exponential increase in placental CRH output during pregnancy, maternal plasma CRH concentrations increase progressively to peak values at term and in labour of 1000–10 000 pg ml⁻¹, which is similar to the concentration found in the hypothalamic–pituitary portal blood during stress (Goland *et al.*, 1986; Sasaki *et al.*, 1987; Wolfe *et al.*, 1988a). This high maternal plasma CRH concentration is constantly maintained (unlike the episodic CRH secretion from the hypothalamus), is not subject to diurnal variation, and does not fluctuate in response to stress (Petraglia *et al.*, 1994; Magiakou *et al.*, 1996).

Biological actions of corticotrophin-releasing hormone in pregnancy

Maternal pituitary–adrenal axis

If a non-pregnant woman is given an intravenous bolus injection of CRH to achieve a plasma CRH concentration equivalent to that of a term pregnant woman, there is a prompt release of ACTH from the pituitary, and thence cortisol from the adrenal cortex (Schurmeyer *et al.*, 1984). It might be expected that the very high concentration of circulating CRH in pregnancy would produce sustained stimulation of the maternal pituitary–adrenal axis, but this is not the case. Although pregnancy is characterized by mild hypercortisolism, this is predominantly a result of an oestrogen-induced increase in cortisol-binding globulin, and the circadian rhythm of ACTH and cortisol secretion is retained throughout pregnancy (Nolten and Rueckert, 1981; Scott *et al.*, 1990). There is also a normal cortisol response to stress, for example, during surgery, and a marked increase in plasma cortisol concentrations at the time of labour (Namba *et al.*, 1980; Nolten *et al.*, 1980). It seems that the physiological function of placental CRH is not related to the maternal pituitary–adrenal axis and recent research has addressed other possible roles for this peptide in pregnancy. In view of the particularly high concentrations of CRH in late pregnancy, much work has focused on a possible role of CRH in parturition.

Fetal pituitary–adrenal axis

The human fetus has a functioning pituitary–adrenal axis by mid-trimester. Placental CRH is released into the fetal circulation where, although present in concentrations about tenfold lower than in the maternal blood, it is capable of stimulating ACTH secretion from the fetal pituitary. Further ACTH is synthesized within the placenta under the influence of paracrine stimulation by CRH (Petraglia *et al.*, 1987; Margioris *et al.*, 1988). In the fetus, ACTH derived from the pituitary and the placenta stimulates steroidogenesis in the fetal adrenal. The principal products of the fetal adrenal gland are cortisol and the androgenic steroids dehydroepiandrosterone (DHEA) and its sulphate (DHEA-S). Cortisol secretion is potently induced by ACTH and an increase in fetal cortisol production in late pregnancy is an important factor in the promotion of fetal organ maturation before delivery (Fencl *et al.*, 1980). DHEA and DHEA-S are synthesized in the specialized fetal zone of the adrenal, which forms more than 50% of the adrenal mass in late fetal life, but which involutes completely shortly after delivery. The fetal zone is responsive to stimulation directly by CRH, independently of ACTH, with CRH stimulating secretion of DHEA and DHEA-S from the fetal adrenal in a dose-dependent manner (Smith *et al.*, 1998). The significance of these findings *in vitro* for the physiology of the fetus is still uncertain. In particular there are few data regarding the normal concentrations of CRH in the fetal plasma before labour and therefore it is not proven that the observed actions of CRH on the fetal pituitary and adrenal occur at physiological concentrations. The influence of CRH-binding protein (CRH-BP, see below) or other inhibitors of CRH action in the fetus are also unknown. However, placental CRH may act as a promoter of hypertrophy of the fetal zone during pregnancy, and the sudden withdrawal of CRH at delivery may be the event that leads to its post-natal disappearance. In humans, production of DHEA and DHEA-S in the fetal zone is a significant link in the events leading to initiation of parturition. These androgenic steroids are taken up by the placenta and form 80–90% of the substrate for placental synthesis of oestrogen (Challis and Olson, 1988), which in turn acts in the maternal compartment to promote the onset of labour through its actions on the myometrium, cervix and fetal membranes.

The actions of CRH in sheep fetuses have been demonstrated elegantly in two sets of experiments by different researchers. Wintour *et al.* (1986) showed that long-term infusion of CRH into the fetus resulted in premature activation of the fetal pituitary–adrenal axis, accelerated maturation of a number of fetal organs and spontaneous preterm delivery of viable lambs. The converse experiment, prolonged infusion of a CRH antagonist into sheep fetuses, resulted in significant prolongation of pregnancy beyond term (Chan *et al.*, 1998). However, these data cannot necessarily be extrapolated to human pregnancy since there are a number of important differences

between the species, including the absence of a circulating CRH-BP or a fetal adrenal zone in sheep, and significantly lower expression of CRH in the ovine compared with the human placenta.

Fetal membranes and amniotic fluid

CRH is expressed in all layers of the fetal membranes and is present in amniotic fluid (Okamoto *et al.*, 1990a,b; Riley *et al.*, 1991). The CRH concentration of amniotic fluid increases with advancing gestational age, but less steeply than the maternal plasma concentrations. In early pregnancy, the amniotic fluid concentration is approximately equal to that of the maternal plasma but in late pregnancy and at term, it is similar to that of fetal blood and considerably lower than in the maternal circulation (Laatikainen *et al.*, 1988; Sasaki *et al.*, 1990). There is an important potential interaction between CRH and prostaglandin (PG) production in the amniotic compartment. Synthesis of PGE₂ and PGF_{2α}, potent promoters of cervical maturation and uterine contractility, is induced by CRH in preparations at physiological concentrations *in vitro* (Jones and Challis, 1989). These prostaglandins are capable of promoting further CRH secretion by the placenta and fetal membranes (Petraglia *et al.*, 1989, 1996), resulting in the formation of a positive feedback loop. CRH also stimulates output of interleukin 6 from peripheral blood mononuclear cells, which infiltrate the fetal membranes, placenta and cervix in increasing numbers during parturition and intrauterine infection (Angioni *et al.*, 1993). IL-6 and IL-1, in turn, have been shown to stimulate CRH release from cultured human placental trophoblasts *in vitro* (Petraglia *et al.*, 1990).

Myometrium

During parturition, a number of important cellular changes occur in the uterine smooth muscle in preparation for labour, including the development of gap junctions between adjacent muscle cells, activation of pacemaker cells that initiate spontaneous contractions, and expression of receptors for uterotonins such as oxytocin and prostaglandins. The most important stimulus to these changes in the myometrium is oestrogen, the formation of which is linked indirectly to CRH in the placenta and fetus as outlined above. A possible direct action of CRH on uterine smooth muscle has also been the subject of considerable research. Quatero and Fry (1989) used strips of human myometrium obtained at Caesarean section to study the effects of CRH on electrically stimulated myometrial contractility. Although CRH alone had no influence on smooth muscle contraction, the presence of CRH (or pre-incubation with CRH) resulted in a greatly enhanced response of the myometrial strips to the uterotonic effect of oxytocin (Quatero and Fry, 1989). A similar enhancement by CRH of PGE₂-mediated myometrial contractility has also been demonstrated (Benedetto *et al.*, 1994). Two clinical studies have found an inverse relationship between the maternal plasma CRH concentration and the duration of

labour during either spontaneous or induced labour in women (Stalla *et al.*, 1989; McLean *et al.*, 1994).

CRH receptors (CRH-r) are expressed in the human myometrium and their numbers and binding affinity increase greatly during pregnancy (Hillhouse *et al.*, 1993). A variety of different molecular species of CRH-r are present in different tissues, with differing second messenger systems. The pituitary CRH-r is coupled through a stimulatory G-protein (Gs) to the adenylate cyclase–cAMP–protein kinase A (PKA) signalling pathway. If this receptor is present in the myometrium, CRH would promote muscle relaxation. However, in non-pituitary tissues, other CRH-r isoforms have been identified which couple to the phospholipase C–inositol phosphate–protein kinase C (PKC) second messenger system (Ulisse *et al.*, 1990; Chakravorty *et al.*, 1999). In the myometrium, this receptor would lead to a CRH-stimulated increase in intracellular calcium and promote contraction. Using a radio-receptor assay, isoelectric focusing and autoradiography, Grammatopoulos *et al.* (1995) identified five isoforms of CRH-r in human myometrium with different physicochemical characteristics. The functional significance and second messenger coupling of the different subtypes expressed in pregnant myometrium is not known. However, during pregnancy, the human myometrium appears to express different types of CRH-r at different stages of gestation. Before term, CRH signalling in the myometrium is predominantly via Gs-PKA (that is, it is pro-relaxation), but there is a significant decrease in the coupling of the human myometrial CRH-r to adenylate cyclase at term (Grammatopoulos *et al.*, 1996), possibly as a result of oxytocin-induced phosphorylation of these CRH-r isoforms (Grammatopoulos and Hillhouse, 1999), leading to withdrawal of CRH-mediated relaxation of uterine muscle at term. However, currently, there is no evidence demonstrating linkage of myometrial CRH-r to cellular processes that favour contraction.

Positive-feedback circuits in human parturition

Challis and Hooper (1989) outlined the novel concept of a series of positive-feedback circuits promoting parturition. In this model, placental CRH promotes fetal cortisol and DHEA-S production, and these steroids return via the umbilical circulation to the placenta, where cortisol promotes further CRH secretion (Fig. 1). Once established, this positive feedback loop would be progressively amplified and drive the fetal–placental unit inexorably towards the final outcome of fetal maturation and delivery. In cases of fetal distress or compromise (for example, from intrauterine infection or hypoxia), activation of the fetal pituitary–adrenal axis in response to stress may accelerate fetal organ maturation and feedback to the placenta to increase CRH secretion and promote the onset of parturition, providing a means of escape from a threat to fetal survival *in utero*. A second feed-forward circuit is established in the amniotic compartment where CRH and prostaglandins promote production of each other. Positive

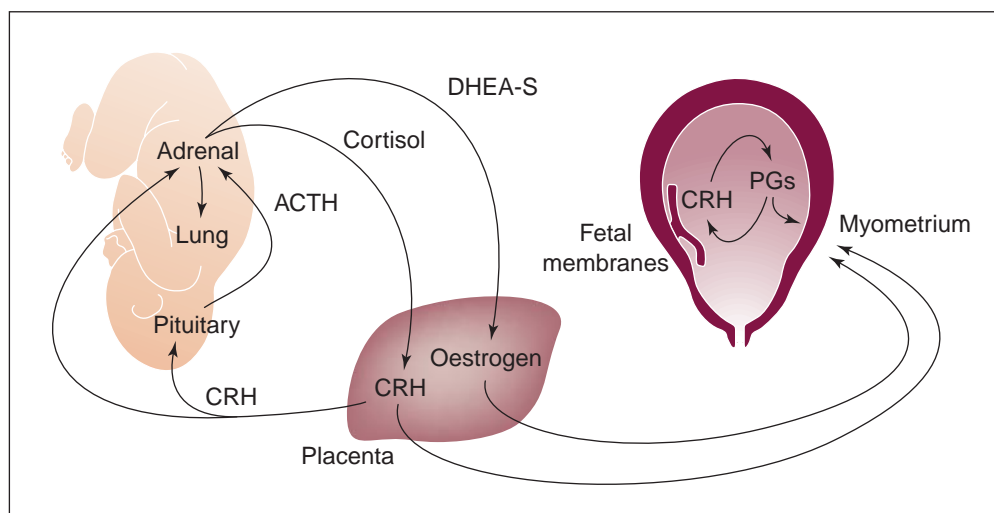


Fig. 1. Interacting positive-feedback hormonal loops in the fetal, amniotic and maternal compartments that may promote the progression of fetal maturation and initiation of parturition in humans. Each of the arrows represents a stimulatory action of the indicated hormone. ACTH: adrenocorticotrophin; CRH: corticotrophin-releasing hormone; DHEA-S: dehydroepiandrosterone sulphate; PGs: prostaglandins.

feedback loops are very uncommon in physiological systems. However, in the initiation of labour, a positive feedback loop has distinct theoretical advantages. The hormonal signals promoting parturition will be progressively amplified until labour and delivery occur and the only way these amplification loops can be broken is by delivery of the fetus and placenta (Fig. 1). The onset of parturition may occur as a result of a shift from a hormonal system promoting uterine quiescence (a negative feedback system) to the positive feedback circuit outlined above. However, the mechanism of such a 'switch' is unknown. Although this model is consistent with many of the events known to occur during human parturition, it is not necessarily preferable to, or exclusive of, other hypotheses. Parturition is a complex process in which many other hormonal factors play a part.

Corticotrophin-releasing hormone-binding protein

Human plasma contains a binding protein for CRH (CRH-BP) that couples to the peptide with high affinity in a 1:1 molecular interaction and prevents its recognition and activation of the CRH receptor. Therefore, CRH-BP blocks the bioactivity of CRH in the circulation. CRH-BP is the only known specific binding protein for a neuropeptide and, while numerous hormone-carrying proteins have been recognized, including some for peptide hormones (for example, insulin-like growth factors), CRH-BP has an important unique feature. Other binding proteins serve to prolong the circulating half-life of their ligands but CRH-BP appears to do the opposite (Saphier *et al.*, 1992). Association of CRH-BP with its ligand triggers dimerization of the complex, which is then rapidly cleared from the circulation (Woods *et al.*, 1994a). Infusion of CRH into non-pregnant

women induces a rapid decrease in the circulating CRH-BP concentration (Woods *et al.*, 1994b). A radioimmunoassay for CRH-BP was developed by Linton and Lowry (Linton *et al.*, 1993) and has been used by the present authors and others to investigate changes in plasma CRH-BP concentrations during pregnancy (Perkins *et al.*, 1993; McLean *et al.*, 1995). During most of pregnancy, CRH-BP is present in the maternal plasma at a concentration that greatly exceeds that of CRH, thereby making circulating CRH largely biologically inactive. However, in the final few weeks of pregnancy, co-incident with the rapid increase in plasma CRH, there is a simultaneous decrease in the plasma CRH-BP concentration of approximately 50% to a nadir at delivery (Linton *et al.*, 1993) (Fig. 2). CRH-BP returns to normal non-pregnant concentrations within 48 h after delivery. Only in the final 3 weeks of gestation is CRH present in sufficient concentration in maternal plasma to fully saturate CRH-BP, resulting in the sudden appearance at that time of free CRH, which is biologically active (Behan *et al.*, 1996). This is the stage of gestation at which most of the cellular changes of parturition are initiated in the myometrium, fetal membranes, cervix and fetus and we have suggested that the saturation of circulating CRH-BP and the consequent availability of free CRH in late pregnancy may be a mechanism contributing to the triggering of parturition in humans (McLean *et al.*, 1995).

Corticotrophin-releasing hormone and the timing of labour

Corticotrophin-releasing hormone in preterm labour

An association between increased maternal plasma CRH and preterm labour was first noted in a cross-sectional study

of 11 women admitted to hospital with preterm labour, whose plasma CRH concentrations were significantly higher than 80 gestational age-matched controls who were not in labour (Campbell *et al.*, 1987). In a subsequent small longitudinal study, the same investigators noted above normal plasma CRH for up to 11 weeks before the onset of preterm labour, raising the possibility that it could be a useful predictive test (Wolfe *et al.*, 1988b). Other cross-sectional studies reported similar findings (Kurki *et al.*, 1991; Warren *et al.*, 1992). A more substantial longitudinal study by Wolfe *et al.* (1990) calculated the slope of the increase in plasma CRH for 168 individual subjects who had sequential plasma CRH estimations at 2–4 week intervals from 24 weeks of gestation until delivery. Women who delivered before 37 weeks ($n = 15$) had a steeper increase in CRH than women who delivered at term; the slope of the CRH curve was about 25% higher in affected pregnancies. However, the small number of preterm deliveries in this study meant that the observed difference did not achieve statistical significance.

Our own longitudinal study, first reported in 1995 (485 pregnancies, 24 spontaneous preterm deliveries) and updated in 1999 (860 pregnancies, 60 preterm deliveries), was designed to test the association between early pregnancy maternal plasma CRH and the later timing of labour (McLean *et al.*, 1995, 1999). When expressed as multiples of the median (MoM) value for normal pregnancies at the same gestational age, plasma CRH was threefold higher in women delivering preterm (median MoM 3.25, 95% CI 1.99–4.26, $P < 0.00005$), and 40% lower in women delivering post-term (median MoM 0.61, 95% CI 0.48–0.82, $P < 0.005$) than in women delivering at term (median MoM 1.00, 95% CI 0.91–1.08). These differences were evident from 16–18 weeks of gestation, which is the earliest stage of pregnancy at which CRH can be measured in our assay. The diverging patterns of exponential increase in plasma CRH with gestation are shown in Figure 3. There was no difference in CRH-BP concentrations among women with premature, term or post-term deliveries. Two further longitudinal studies, in different study populations, have confirmed the association of high second trimester maternal plasma CRH concentrations with subsequent preterm labour and delivery (Hobel *et al.*, 1999; Leung *et al.*, 1999). A third study found a trend towards the same association but the effect was not significant (Berkowitz *et al.*, 1996).

'Placental clock' hypothesis

It was surprising to find that different patterns of plasma CRH could be recognized by mid-trimester in women who subsequently experienced early, normal or late timing of delivery. These data indicate the existence of a progressive maturational process in the placenta that is established early in pregnancy, proceeds in a predictable fashion throughout gestation in most pregnancies, and is linked to the eventual timing of parturition. This phenomenon may be analogous

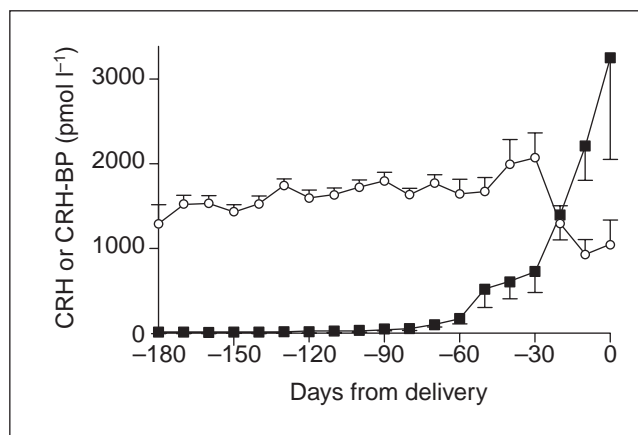


Fig. 2. Mean (\pm SEM) concentrations of corticotrophin-releasing hormone (CRH; ■) and CRH-binding protein (CRH-BP; ○) in maternal plasma in normal pregnancies ending in spontaneous term labour ($n = 308$). As pregnancy advances, maternal plasma CRH increases exponentially, but full saturation of the binding capacity of CRH-BP does not occur until about 20 days before delivery, when the CRH-BP concentration also decreases by about 50%. Therefore, free CRH is available at the time of parturition. (Reproduced, with permission, from McLean *et al.*, 1995.)

to the 'placental clock' that triggers the onset of parturition after a predetermined duration of gestation, and appears to progress at different rates in individual pregnancies with some pregnancies 'programmed' for early or late delivery. Measurement of maternal plasma CRH provides a marker of the rate of this process in an individual (Fig. 3). However, CRH is not the only marker of this phenomenon since maternal plasma alpha-fetoprotein (AFP) concentrations are also associated with the timing of delivery, although less strongly than CRH (McLean *et al.*, 1999). These findings challenge the previously held notion that the duration of human gestation is determined solely by events in late pregnancy and indicate that conditions leading to preterm or post-term delivery can be established early in gestation.

In the placental clock hypothesis, the rate of increase in maternal plasma CRH through pregnancy influences the timing of labour by determining when saturation of CRH-BP will be achieved and therefore when free CRH becomes available to act as a parturition trigger. This would occur prematurely if acceleration of the placental clock resulted in a shift of the CRH curve to the left, and would be delayed in women whose slower clock results in low CRH concentrations. Other placental processes unrelated to CRH may also be linked to a 'maturational timetable' leading to the onset of parturition. The important concept contained in the placental clock hypothesis is that, whether it be through the action of CRH or by other mechanisms, parturition results from a process begun very early in pregnancy and the rate of this process can be inferred from observation of specific biochemical markers. Many factors may affect the speed of the placental clock in an individual pregnancy, including genetic predisposition and pathological events in the

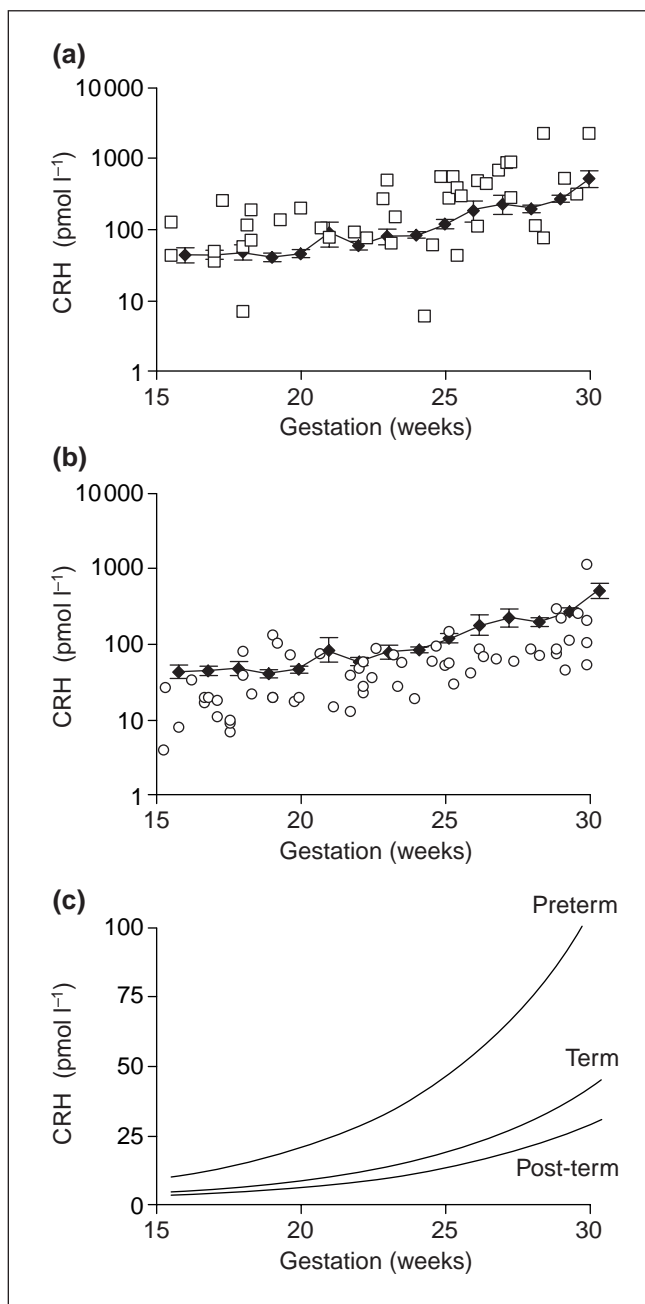


Fig. 3. Mean (\pm SEM) plasma corticotrophin-releasing hormone (CRH) (on log scale) in women with spontaneous term delivery (\blacklozenge ; $n = 308$) compared with individual samples from women with (a) spontaneous preterm delivery (\square , $n = 24$) or (b) post-term delivery (\circ , $n = 29$). (c) Regression curves of maternal plasma CRH (on linear scale) versus gestation fitted to data from the three groups. (Reproduced, with permission, from McLean *et al.*, 1995.)

mother or fetus. Individual women have a marked tendency to have the same gestational duration in subsequent pregnancies (Mittendorf *et al.*, 1993; Mongelli and Opatola, 1995), indicating a predisposition which may have a genetic basis, although environmental factors also seem to

be important in some women who experience repeated preterm deliveries.

Clearly, the placental clock would not be the sole determinant of the timing of delivery in women. Premature delivery does not necessarily represent an acceleration of the normal parturition process and pathological events such as infection may intervene to precipitate labour through alternative mechanisms. It is a consistent finding from a number of studies that preterm labour associated with intrauterine infection is characterized by low or normal maternal CRH concentrations while 'idiopathic' preterm labour is usually accompanied by increased plasma CRH concentrations (Warren *et al.*, 1992; Korebrits *et al.*, 1998; McLean *et al.*, 1999).

Inconsistencies and deficiencies in the data

Although a considerable body of circumstantial evidence now links CRH with the process of parturition in humans, there are some significant gaps in the model. There is a paucity of data relating to the direct cellular and molecular actions of CRH in the putative target tissues such as the myometrium, fetal membranes and placenta. CRH receptors are expressed in these tissues but their characteristics are not yet well defined. A particular problem is the action of CRH in the myometrium, in which the only CRH receptors identified to date appear to be linked to the Gs-adenylate cyclase system. Therefore, CRH would be expected to promote muscle relaxation through an increase in intracellular cAMP: an action inconsistent with a role as a promoter of parturition. Alternative forms of CRH receptor have been shown in other human tissues to act through alternative second messenger systems, including some that increase intracellular calcium or activate the inositol phosphate-protein kinase C pathway (Ulisse *et al.*, 1990; Chakravorty *et al.*, 1999). If present in uterine smooth muscle, these receptors could theoretically mediate an action by CRH to promote contraction. Therefore, it has been hypothesized that the influence of CRH on the myometrium at term alternates from promotion of relaxation to favouring contraction through a change in CRH isoforms (McLean and Smith, 1999). However, this suggestion is highly speculative and the absence of a proven link between CRH action and the onset of uterine contraction is the major flaw in the theory that CRH is a significant promoter of parturition.

There is very wide variability of plasma CRH concentration among individual women, even among those with normal pregnancies, which limits the accuracy with which plasma CRH measurements can be used to discriminate between normal and abnormal pregnancies. Preterm labour can occur as the final outcome of a diverse range of pathological processes, many of which appear to be independent of CRH, indicating that high maternal plasma CRH concentrations are not requisite for initiation of labour.

The cellular mechanism of the putative placental clock is unknown. Factors regulating gene expression for the CRH

precursor in the placenta are also poorly understood. Many other hormone systems have been implicated in human parturition, including oxytocin, relaxin, endothelins, nitric oxide, prostaglandins, and oestrogen–progesterone. There are many points of possible interaction of these hormones with each other and with CRH, and few of these have been explored in detail.

Species differences

A significant difficulty in providing experimental data in this area is the wide variation in the physiology of pregnancy in different species. Although parturition may well be precipitated by the production of CRH by the fetal hypothalamus in many species, only in primates does significant production of CRH occur in the placenta (Robinson *et al.*, 1989). Initial studies using northern blot analysis of primate placenta CRH mRNA and CRH mRNA from the placentas of a variety of other mammals demonstrated expression only in New and Old World monkeys and apes. Several studies in pregnant baboons have revealed that maternal plasma CRH increases rapidly in early pregnancy in this species and reaches peak values in the second third of pregnancy (60–80 days) before decreasing to concentrations that remain stable until delivery. This pattern is quite different from the exponential increase observed throughout human pregnancy (Goland *et al.*, 1990; Smith *et al.*, 1993). In limited studies in rhesus monkeys, increases in maternal plasma CRH appear to occur in the later part of pregnancy but detailed analysis of earlier stages of pregnancy are not available, and concentrations are generally lower than those in baboons. Studies in chimpanzees and gorillas have revealed patterns of CRH change in maternal blood indistinguishable from those in humans (Smith *et al.*, 1999). No circulating binding protein for CRH is observed in baboons, but CRH-BP is found in gorillas, in which the gestational age-related changes in this protein are virtually identical to those of humans. It appears that only in our closest relatives are the patterns of CRH production very similar to that seen in our own species.

Clinical implications

Plasma corticotrophin-releasing hormone as a predictor of preterm delivery

If plasma CRH is high in women destined to experience preterm delivery, can this be used to identify high-risk pregnancies and allow preventive intervention? In a prospective study of 860 women, plasma CRH and AFP were measured on one occasion between 16 and 24 weeks of gestation to assess their predictive accuracy for delivery before 37 weeks of gestation and this was compared with a clinical risk-factor score (McLean *et al.*, 1999). Sixty women (7%) delivered before 37 weeks of gestation (37 women after spontaneous preterm labour). If the upper limit of normal CRH and AFP concentrations is set at the 95th percentile for normal pregnancies (that is, allowing a 5% false positive

rate), the detection rate (sensitivity) of plasma CRH and AFP measurements were low at 24 and 25%, respectively, but both tests predicted more cases of preterm delivery than the risk-factor score. The best predictive information was obtained from an algorithm using all three results that predicted 37% of cases of preterm delivery with a false positive rate of 5%. The odds of having a preterm delivery for women with a positive triple test was 1:1.7 (positive predictive value 37%). This is the best test yet described for prediction of preterm delivery, but the detection rate is probably still too low for effective use as a routine clinical test.

Corticotrophin-releasing hormone antagonists as inhibitors of parturition

Peptide and non-peptide antagonists of the CRH receptor have now been developed and have shown activity in animal experiments. As yet, no studies have been performed in humans. A theoretical application of these drugs is the inhibition of CRH-mediated effects in parturition. In the only study reported in pregnant animals, a CRH antagonist, antalarmin, was continuously infused directly into sheep fetuses during the final 5–10 days of gestation (term in sheep = 140 days), resulting in a significant delay in spontaneous delivery of 7 days compared with placebo-treated animals (Chan *et al.*, 1998). Administration directly into the fetus would be impractical in humans, and it is not known if initiation of treatment after the onset of parturition (for example, to a woman presenting in preterm labour) would have any effect on the progression of labour.

Conclusions

CRH has emerged as a leading contender for a role in the initiation and promotion of human parturition. Recent discoveries of the endocrinology of placental CRH have led to a revision of some theories concerning the control of labour. Much is still unknown about the mechanism of its actions in pregnancy but exciting theories regarding receptor and second messenger actions in the myometrium are now being tested. Clinical applications of CRH research, although remote at present, may lead to improvements in our ability to prevent preterm birth, which is the leading cause of perinatal morbidity and mortality in developed countries.

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