

Placental corticotrophin-releasing hormone: activator of the pituitary–adrenal axis in human pregnancy?

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During pregnancy, the placenta develops into a highly active endocrine organ capable of synthesizing both steroid and protein hormones. Besides pregnancy-specific proteins such as placental lactogen and chorionic gonadotrophin, the placenta contains several hypothalamic hormones (thyrotrophin-releasing hormone (TRH), growth hormone-releasing hormone (GRH), gonadotrophin-releasing hormone (GnRH), corticotrophin-releasing hormone (CRH)) and also pituitary hormones (adrenocorticotrophin (ACTH), β -endorphin and β -lipotrophin), although the placental concentration of these proteins is several orders of magnitude lower than those described for their original sites of production (Krieger, 1982). Of the brain peptides common to the human placenta, CRH is unusual in that it is the most abundant (with 20–30 $\mu\text{g}/\text{placenta}$ commonplace) as well as being the only neuropeptide that is secreted into the maternal circulation where substantial concentrations are maintained, in contrast to the very low CRH levels found in non-pregnant humans (Linton *et al.* 1987*b*). This has led to much speculation as to the physiological significance of placental CRH, especially with regard to its possible role in parturition.

Stress-induced activation of the central nervous system involves the release of CRH, along with other ACTH-secretagogues such as vasopressin, from the hypothalamus. This results in the coordinated control of the hypothalamo–pituitary–adrenocortical (HPA) axis. CRH is a forty-one amino acid peptide (Vale *et al.* 1981) which, like many neuropeptides and hormones is synthesized as part of a precursor protein, the gene for which has now been identified in the hypothalamus (Shibahara *et al.* 1983). CRH induces the pituitary corticotrophs to release ACTH, which regulates glucocorticoid synthesis in the cells of the zona fasciculata of the adrenal cortex. The glucocorticoids, among which cortisol predominates in humans, exert a wide range of effects throughout the body, including a negative feedback influence of the synthesis and release of both CRH and ACTH. With the advent of sensitive and specific immunoassays for CRH, ACTH and total and free cortisol in the 1980s, it became possible to investigate these stress hormones in both mother and fetus during pregnancy, in an attempt to assess whether placental CRH can modulate the HPA axis.

THE MATERNAL STRESS AXIS

Following the demonstration of CRH bioactivity (Shibasaki *et al.* 1982) and immunoreactivity (Sasaki *et al.* 1984) in extracts of human placentas, several groups have reported the presence of elevated levels of the peptide in maternal peripheral blood during the third trimester of pregnancy (Stalla *et al.* 1984; Goland *et al.* 1986; Campbell *et al.* 1987). In these various studies, a wide range of CRH concentrations has been

reported and it is thought that the different plasma extraction and immunoassay methods used may account for this discrepancy, together with the demonstration that considerable variation exists in CRH profiles in different women (Campbell *et al.* 1987). The CRH concentrations measured in late gestational maternal plasma are similar to the levels reported in rat hypothalamic portal blood and to those capable of stimulating ACTH *in vitro*. Using an immunoradiometric assay (Linton *et al.* 1987a), CRH levels were measured and found to rise from a median of 20 pg/ml at 28 weeks to 1320 pg/ml at 40 weeks and 1732 pg/ml during labour. There was a strong correlation ($r\ 0.81$; $P<0.001$) between gestational age and CRH levels. The rate of rise of CRH (pg/ml) per week was associated with weight gain ($r\ 0.36$, $P<0.05$) but with no other obstetric variable (Wolfe *et al.* 1988a). The sharp rise in maternal plasma CRH levels up to term is similar to that found for pregnancy-associated plasma protein A (PAPP-A) (Smith *et al.* 1979) but is unlike most other placental proteins, which plateau in the late third trimester (Bischof *et al.* 1982). The small but significant rise in CRH levels between the end of pregnancy and labour is of interest with regard to the possible role of CRH in the process of parturition. A marked rise of CRH in labour has been reported by Sasaki *et al.* (1987) but this has not been substantiated by other groups (Laatikainen *et al.* 1987; Wolfe *et al.* 1988a). More recently, Petraglia *et al.* (1990) have demonstrated that plasma CRH increased progressively throughout labour, whilst women undergoing elective caesarean section showed no further change in plasma CRH, suggesting that the increase reflects the stress of labour.

We have also measured maternal plasma CRH in a range of abnormal pregnancy states (Wolfe *et al.* 1988b) and significantly elevated levels associated with pregnancy-induced hypertension and preterm labour were found. Levels were raised up to 11 weeks before the onset of preterm labour, again indicating a possible link between CRH and parturition. Furthermore, CRH infusion in fetal sheep has been shown to cause maturation of fetal organs and preterm delivery of the lamb (Wintour *et al.* 1986). There is also evidence that interleukins which have a possible role in the aetiology of preterm labour (Romero *et al.* 1989) stimulate placental CRH release *in vitro* (Petraglia *et al.* 1987).

Several groups have shown that maternal plasma CRH levels fall precipitously after delivery, returning to normal values within 24 h (Goland *et al.* 1986, 1990; Campbell *et al.* 1987; Laatikainen *et al.* 1987; Sasaki *et al.* 1987). The rapid clearance of the peptide after delivery would suggest that the placenta is the source of plasma CRH. Further evidence in support of this came with observations that placental CRH, like extracted maternal plasma CRH (Linton *et al.* 1987b) was biologically and physicochemically identical to synthetic human CRH (Stalla *et al.* 1986) and that its concentration in the placenta increases with gestational age (Frim *et al.* 1988). It is now known that the CRH gene is expressed in the human placenta (Grino *et al.* 1987) and the production of the peptide has been localized to the cytotrophoblast layer (Petraglia *et al.* 1987; Saijonmaa *et al.* 1988).

In contrast to the high levels of maternal plasma CRH in the third trimester of pregnancy, plasma ACTH levels remain within the normal non-pregnant range, although increasing slightly with advancing gestation (Rees *et al.* 1975; Laatikainen *et al.* 1987; Wolfe & Linton, 1990) and it is thought that this ACTH also originates from the placenta. A well-documented sharp rise in plasma ACTH then occurs with the stress of labour (Genazzani *et al.* 1975; Rees *et al.* 1975; Carr *et al.* 1981).

Maternal plasma cortisol levels in pregnancy follow a different pattern still: total

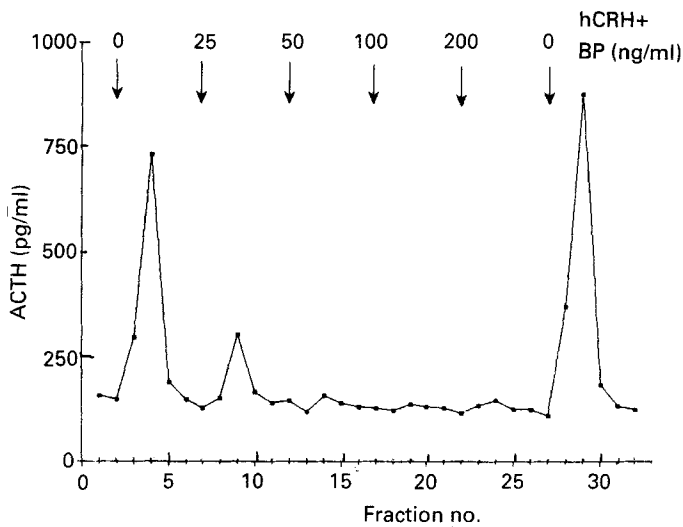


Fig. 1. Effect of corticotrophin-releasing hormone (CRH)-binding protein (BP) on the adrenocorticotrophic hormone (ACTH)-releasing activity of human(h) CRH after their pre-incubation for 15 min at 37°. ACTH release in the isolated pituitary cell bioassay is increasingly reduced following exposure to increased concentrations of BP (25, 50, 100, 200 ng/ml) premixed with a fixed concentration of CRH (1.5 ng/ml).

cortisol increases mainly as a result of the increased cortisol-binding globulin (CBG), a doubling of the cortisol concentration occurring in the first trimester with a two- to threefold increase at term (Carr *et al.* 1981). Plasma free cortisol is the biologically active fraction and several groups have demonstrated a progressive threefold rise from the first to third trimester, although others have shown a significant rise to occur only late in gestation (Rosenthal *et al.* 1969; Nolten & Rueckert, 1981; Demey-Ponsart *et al.* 1982; Allolio *et al.* 1990).

How then can these changes in CRH, ACTH and cortisol throughout pregnancy be explained? It has been shown that most of the CRH in the maternal circulation is carried by specific CRH-binding protein (CRH.BP) (Linton & Lowry, 1986; Orth & Mount, 1987; Linton *et al.* 1988) and that the CRH-CRH.BP complex has reduced ACTH-releasing activity (see Fig. 1) (Linton *et al.* 1988, 1990; Suda *et al.* 1988) which may account for the coexistence of high plasma CRH alongside marginally increased plasma ACTH. Since cortisol is a well-established negative feedback inhibitor of pituitary ACTH synthesis and secretion and maternal plasma levels of free cortisol are high in the third trimester, it is surprising that ACTH does not remain very low as gestation proceeds. The small rise in plasma ACTH that occurs throughout pregnancy could, therefore, be caused by the small proportion of placental CRH in maternal plasma that remains free of binding protein. However, *in vitro* studies with adrenocorticotroph cells have shown that high levels of CRH are necessary to overcome the negative feedback effect of glucocorticoids (Vale *et al.* 1983). Alternatively, the small rise in plasma ACTH could result from increasing quantities of placental ACTH which may be released into the peripheral circulation under the paracrine influence of CRH, which has been shown not to be subject to negative feedback by cortisol (Robinson *et al.* 1988; Jones *et al.*

1989). Whether the CRH involved in paracrine ACTH stimulation is protein bound has yet to be ascertained, however. Although the CRH.BP may, in part, explain how elevated plasma CRH coexists with normal plasma ACTH, recent experiments where bolus CRH injections in late pregnancy resulted in a lack of ACTH and cortisol responses in humans (Schulte *et al.* 1990) or blunted responses in baboons (Goland *et al.* 1990) point to the existence of additional mechanisms. It is unlikely that bolus administration provides sufficient time for the circulating CRH.BP to complex with the injected CRH, and thus to neutralize its ACTH-releasing activity, especially when one considers that the CRH.BP is also found in non-pregnant women and men (Linton *et al.* 1988) who react normally to bolus CRH. Additionally, the Goland *et al.* (1990) study used the ovine CRH molecule, which differs from the human in seven amino acids and, critically, is not bound by the CRH.BP. However, it is not at present known whether the CRH.BP occurs in the baboon circulation. At least two alternatives have been proposed to explain the lack of response to CRH injection in late pregnancy. First, high levels of placental CRH may result in desensitization of the CRH receptors on pituitary corticotrophs, and second, the negative feedback effect of the elevated free cortisol may well be sufficient to blunt the ACTH response to CRH. If indeed this maternal pituitary refractoriness does occur, hypothalamic substances other than CRH (e.g. vasopressin) must be responsible for maintaining the stress response during pregnancy. Evidence that CRH is not the only mediator of the pituitary–adrenal axis in late pregnancy is also provided by the maintenance of a circadian rhythm in plasma cortisol despite high circulating CRH, which does not itself show any diurnal variation (Schulte *et al.* 1990).

In summary, then, it is quite possible that all three mechanisms (the CRH.BP, desensitization and down-regulation) operate together in humans to provide a failsafe method whereby placental CRH release throughout the third trimester is prevented from activating maternal pituitary ACTH secretion. The manner of placental CRH release has not yet been studied, although the gradual increase in plasma CRH in the absence of any major fluctuations (Wolfe *et al.* 1989) may suggest either a slow, constant or low-level pulsatile release which could allow sufficient time for complex-formation with the CRH.BP to occur. In this case, it is unlikely that placental CRH has any major role in modulating the maternal hypothalamic–pituitary–adrenal axis. However, if the rise in plasma CRH observed during labour occurs as a result of a sudden increase in the pattern of placental CRH release, secretion of the peptide could well be greater than the rate of CRH–CRH.BP complex formation, leaving sufficient free CRH to activate the pituitary–adrenal axis at parturition. In the light of the existence of the binding protein, then, an endocrine function for placental CRH (in terms of ACTH modulation) would depend on the rate of peptide secretion. It is also possible that placental CRH has other, as yet unknown, endocrine functions if one considers the possibility that the CRH.BP could act as a delivery system transporting placental CRH to some peripheral CRH receptor, e.g. such as has been shown to exist in the immune system (Webster *et al.* 1989). However, apart from any endocrine action, the peptide is known to exert paracrine influences within the placenta, since *in vitro* experiments have shown that human placental tissue can secrete CRH, which in turn can induce placental ACTH release (Petraglia *et al.* 1987; Saijonmaa *et al.* 1988). Moreover, several substances implicated in parturition including prostaglandins, interleukins, neurotransmitters, peptides and steroids have been shown to modulate CRH release from cultured human placental cells (Jones *et al.* 1989; Petraglia *et al.* 1989).

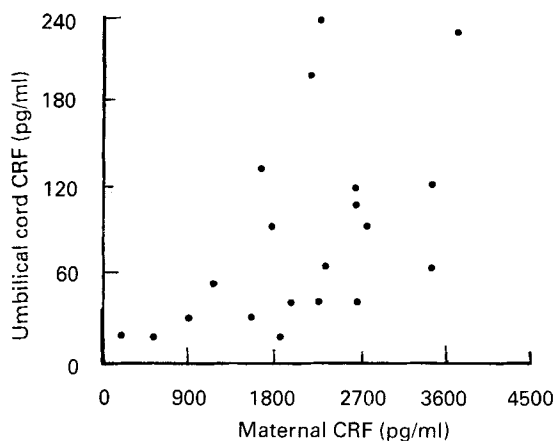


Fig. 2. Correlation between maternal corticotrophin-releasing hormone (CRH, pg/ml) and umbilical cord CRH (pg/ml) at delivery. Points represent one observation. Correlation r_s 0.54. $P < 0.01$.

Table 1. Range of maternal and fetal stress-axis hormones

| | Mother | Fetus |
|--|---------|--------|
| Corticotrophin-releasing hormone (pg/ml) | 31–1262 | 94–175 |
| Adrenocorticotrophic hormone (pg/ml) | 7–135 | 3–87 |
| Cortisol (nmol/l) | 135–545 | 74–324 |

THE FETAL STRESS AXIS

With such dramatic changes occurring in the maternal stress axis, how is the fetus affected? It is thought that the placenta also secretes CRH into the fetal compartment, since CRH has been demonstrated in umbilical cord plasma at delivery and a close correlation between maternal and fetal CRH levels has been noted by several groups (Goland *et al.* 1986, 1990; Campbell *et al.* 1987; Sasaki *et al.* 1987). Fetal plasma CRH levels are only 3–4% of those found in the mother, however (see Fig. 2). It is possible that any placental CRH secreted into the fetal circulation could be involved in the stimulation and maturation of the fetal pituitary–adrenal system. With the advent of cordocentesis as an accepted procedure, access to the profile of stress hormones in the fetus at earlier stages of gestation have now become possible.

Our recent work (Wolfe & Linton, 1990) has attempted to correlate CRH, ACTH and cortisol in both mother and fetus. Maternal and fetal umbilical blood samples, taken antenatally between 18 and 36 weeks gestation in twenty-four subjects, were examined along with fetal blood gases. The ranges of these stress hormones are displayed in Table 1. There is a significant rise in maternal plasma CRH, ACTH and cortisol with gestational age ($P < 0.05$), but no significant relationship exists between them. Surprisingly a significant negative correlation of fetal CRH with gestational age was observed, the opposite relationship to that found in the mother. This falling level of fetal CRH between

18–36 weeks gestation occurs at a time when maternal CRH is rising rapidly and may indicate maturation of the axis, diminishing amounts of CRF being required as pregnancy advances to stimulate the fetal pituitary corticotroph. Fetal ACTH and cortisol do not change significantly. As in the mother, there is no relationship between these hormones in the fetus and no correlation between maternal and fetal axes. In the fetus there is a significant downward trend in P_{O_2} , pH and glucose with gestational age and an increase in P_{CO_2} , with no significant trend in lactate, confirming previous findings (Nicolaidis *et al.* 1989). There is no significant correlation between any of these blood gas measurements and the maternal or fetal stress-axis hormones.

These findings are the first to report all the stress-axis hormones and blood gases in both mother and fetus in the third trimester. The numbers are small and some of the pregnancies ended in the delivery of a growth-retarded fetus, but the study indicates the range of concentrations of the various hormones in the maternal and fetal circulation and their changes, if any, with gestational age. Important information is lacking from the window of 35 weeks to term to show how and when fetal plasma CRH rises from the low levels measured at 35–36 weeks to the higher levels reported at delivery. A further crucial question is whether the CRH:BP exists in the fetal circulation in non-saturated quantities. It will be important to investigate this period of gestation for all the stress-axis hormones to confirm or refute the possibility of a fetal stress signal for the onset of parturition.

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