Placental corticotropin-releasing hormone may be a stimulator of maternal pituitary adrenocorticotropic hormone secretion in humans.

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Research Article

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Placental Corticotropin-releasing Hormone May Be a Stimulator of Maternal Pituitary Adrenocorticotropic Hormone Secretion in Humans

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Abstract

To clarify the physiological role of placental corticotropin-releasing hormone (CRH), we measured plasma CRH, ACTH, and cortisol throughout pregnancy. Cerebrospinal fluid (CSF) CRH levels and ACTH responsiveness to synthetic CRH were also quantified in pregnant and nonpregnant women. Maternal plasma CRH levels, which increased progressively during pregnancy, correlated well with both ACTH and cortisol in early labor, delivery, and postpartum samples, and also with cortisol levels in samples before labor. CSF CRH levels in term pregnant women did not differ from those of nonpregnant women. CRH infusion that attained similar plasma CRH levels to those found in late pregnancy elicited significant ACTH release in vivo and regular CRH test provoked normal ACTH response during early pregnancy but no response during late pregnancy. We concluded that: (*a*) maternal pituitary-adrenal axis correlates well with plasma CRH levels, which are high enough to provoke ACTH release from maternal pituitary; (*b*) hypothalamic CRH secretion in term pregnant women is not exaggerated; and (*c*) maternal pituitary is responsive to synthetic CRH in early but not late pregnancy, suggesting that maternal pituitary-adrenal axis is already activated by high circulating CRH. Placental CRH may be an important stimulator of the maternal pituitary-adrenal axis during pregnancy.

Introduction

Corticotropin-releasing hormone (CRH)[1] of hypothalamic origin is thought to be the major neuroregulator of anterior pituitary corticotrophic cell function (1). We recently reported the presence of immunoreactive CRH in human placental tissue and plasma of women during pregnancy, labor, and delivery (2–4). We also isolated and characterized placental CRH. Partial sequencing indicated that 32 amino acids of this peptide were identical to those of rat and human CRH, and comparative peptide mapping with rat CRH provided further evidence that the placental CRH-like peptide is very homologous if not identical to rat CRH (5). We postulated that placental CRH stimulated the maternal pituitary-adrenal axis during pregnancy because plasma CRH levels were high and such materials were biologically active (3). However, it is still controversial whether pituitary ACTH secretion could be regulated by extrahypothalamic organs such as placenta. The aims of the present study were to determine (*a*) whether plasma CRH correlates with the changes in ACTH or cortisol during pregnancy, labor, and delivery; (*b*) whether endogenous hypothalamic CRH secretion is elevated or suppressed during pregnancy; (*c*) whether plasma CRH levels obtained at delivery are high enough to stimulate maternal anterior pituitary corticotrophic cells; and (*d*) whether the maternal pituitary ACTH is responsive to exogenous synthetic CRH, thereby elucidating the physiological functions of placental CRH.

Methods

Subjects. All subjects gave us written informed consent for participation in this study. The study was approved by the Departmental Clinical Research Committee. Samples were obtained from 186 women aged 19–35 yr during pregnancy, labor, and delivery, and 15 nonpregnant women aged 19–25 yr. 33 women were in the first trimester of pregnancy (up to 14 wk), 50 were in the second trimester (15–27 wk), and 49 were in the third trimester (28–40 wk). 18 women were studied during early labor (cervical dilatation < 3 cm) and 36 at the time of uncomplicated vaginal delivery; maternal samples were also collected 1 and 2 h postpartum from 19 of them. The mean birth weight of infants was 3,360 g (range, 2,730–3,800 g). All were judged to be healthy term infants, and mean Apgar scores were 8.5 at 1 min. Cerebrospinal fluid (CSF) samples were obtained by lumbar puncture from 12 women without endocrine abnormalities and 10 pregnant women (37–40 wk of gestation) at lumbar anesthesia for cesarean section to save cephalopelvic dissociation. CSF samples were immediately acidified by adding 1 N HCl (final concentration 0.1 N) and stored at −80°C until extraction. Peripheral blood samples were also obtained from all of these subjects by venipuncture from the antecubital vein just before lumbar puncture. Blood was collected in ice-chilled glass tubes containing EDTA (disodium, 1 mg/ml) and immediately centrifuged at 4°C. Plasma was stored at −20°C until extraction or hormone assay.

Extraction. Plasma (1.5 ml) was extracted with Sep-Pak C-18 cartridges (Waters Associates, Milford, MA) for measurement of immunoreactive CRH as described previously (2, 3). The recovery of synthetic rat CRH added to hormone-free plasma (charcoal-treated plasma) was 72.4±2.5% (±SEM; $n = 4$). CSF (1 ml) was extracted with Sep-Pak C-18 cartridges for measurement of immunoreactive CRH. The recoveries of synthetic rat CRH added to charcoal-treated CSF were 86.3±3.8% ($n = 6$). The CSF and plasma values were corrected according to these recoveries.

CRH test in normal and pregnant women. Five nonpregnant women aged 19–23 yr with no evidence of endocrinological disease were given synthetic rat CRH infusion. Pure synthetic rat CRH (Peninsula Laboratories, Inc., Belmont, CA) was prepared as described previously (6) and found to be nonpyrogenic. After an overnight fast an antecubital vein was catheterized and 0.15 M saline with 0.25%

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1. *Abbreviations used in this paper:* CRH, corticotropin-releasing hormone; CSF, cerebrospinal fluid.

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human serum albumin was infused from a syringe ram pump for a basal period of 60 min. Pure rat CRH was then added to the infusion at a rate of 40 ng/kg per min for 10 min, then doses of CRH were decreased to 10 ng/kg per min for 140 min. Blood samples were drawn at 10-min intervals. These five women also were given 100 μg CRH as a bolus at 0900 h 1 wk after the CRH infusion test. Six pregnant women (three subjects of early second trimester and three of late third trimester) were also given 100 μg CRH as a bolus at 0900–1100 h. The pregnant women and the fetuses were monitored with a standard cardiotokograph throughout the whole test.

RIA. Details of the rat CRH RIA were described previously (2–4). Synthetic rat CRH was used for standard and iodination. Antiserum (MCR-0297) was against synthetic rat CRH. Other hypothalamic peptides did not react with this antiserum. All CSF and plasma samples were analyzed in duplicate. Plasma ACTH and cortisol were measured by RIA as described previously (4).

Statistics. The unpaired *t* test was used to compare CSF or plasma values obtained from pregnant and nonpregnant women. Correlations between the biochemical measurements were assessed using linear regression analysis.

Results

Immunoreactive CRH, ACTH, and cortisol concentrations throughout pregnancy are summarized in Table I. Immunoreactive CRH concentration in first trimester women was not significantly different from that in nonpregnant women. CRH concentrations increased progressively during pregnancy, and such increases were statistically significant. Plasma CRH was even higher in women in early labor, and the highest CRH levels were found at delivery. Plasma CRH declined promptly in the postpartum period. These changes of the plasma CRH during pregnancy were consistent with our previous report (3). Although there were not significant correlations between maternal plasma CRH and ACTH in samples during pregnancy (first, second, and third trimesters), there was a significant correlation between maternal plasma CRH and cortisol (r = 0.419, $P < 0.001$, n = 132; Fig. 1). In addition, maternal plasma CRH concentrations in samples of early labor, delivery, and postpartum periods correlated well with both plasma ACTH (r = 0.803, $P < 0.001$, n = 92) and cortisol (r = 0.251, $P < 0.05$) levels (Fig. 2).

Table I. Immunoreactive CRH, ACTH, and Cortisol Concentrations (Mean±SD) in Nonpregnant and Pregnant Women

	No. of subjects	CRH	ACTH	Cortisol
			pmol/liter	
Nonpregnant women	15	1.3±0.5	3.9±1.1	292±115
Pregnant women				
First trimester	33	1.2±0.8*	4.5±2*	432±101‡
Second trimester	50	7.9±8.1§	5.1±2.9*	649±124§
Third trimester	49	117±110§	5.2±2.8*	817±158§
Early labor	18	409±174§	7.6±7*	1,011±152§
Delivery	36	959±654§	44.3±29§	1,807±489§
1 h postpartum	19	153±88§	12.7±6.4§	1,701±317
2 h postpartum	19	68±60§	6.4±3.9§	1,468±282

* Not significant vs. nonpregnant women; ‡ $P < 0.01$ vs. nonpregnant women; § $P < 0.01$ vs. the level in the preceding group.

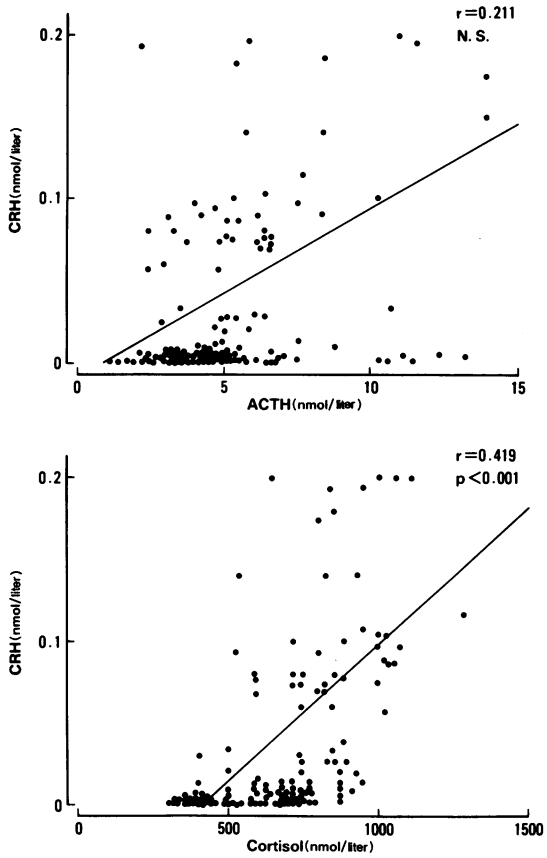

Figure 1. Correlations between CRH and ACTH (*top*) or cortisol (*bottom*) concentrations in samples obtained during pregnancy (first, second, and third trimesters).

Immunoreactive CRH in CSF. Dilution curves of CSF extracts were parallel to that of CRH standard (data not shown). Mean immunoreactive CRH levels in CSF of pregnant women (12.9±4.6 pmol/liter, ±SD, n = 10) were not different from those in nonpregnant women (13.5±5.6 pmol/liter, n = 12). There were no significant correlations between CSF CRH and plasma CRH, plasma ACTH, or plasma cortisol in either pregnant or nonpregnant women (data not shown). Gel filtration of CSF extracts of pregnant and nonpregnant women revealed one peak of CRH immunoreactivity in the position of synthetic rat CRH (data not shown).

CRH test in normal and pregnant women. To know whether immunoreactive CRH levels obtained at delivery are capable of stimulating maternal anterior pituitary corticotrophic cells, we gave rat CRH in graded doses to five normal subjects. In the protocol used, immunoreactive CRH levels were reached at 400 pmol/liter, which was comparable to or lower than maternal plasma CRH levels. These CRH concentrations elicited significant ACTH release in normal women (Fig. 3). These ACTH responses were significantly greater than that of a bolus injection of 100 μg CRH (plasma ACTH after CRH: 0 min, 2.2±0.4, mean±SD; 15 min, 4.9±1.8; 30 min, 5.6±1.7; 45 min, 5.2±1.6; 60 min, 4.5±1.2 nmol/liter). The CRH test in pregnant women was well tolerated and caused no subjective side effects, and cardiotokograph monitoring was also normal. A normal pattern of ACTH secretion in response to CRH could be observed in the early second trimester

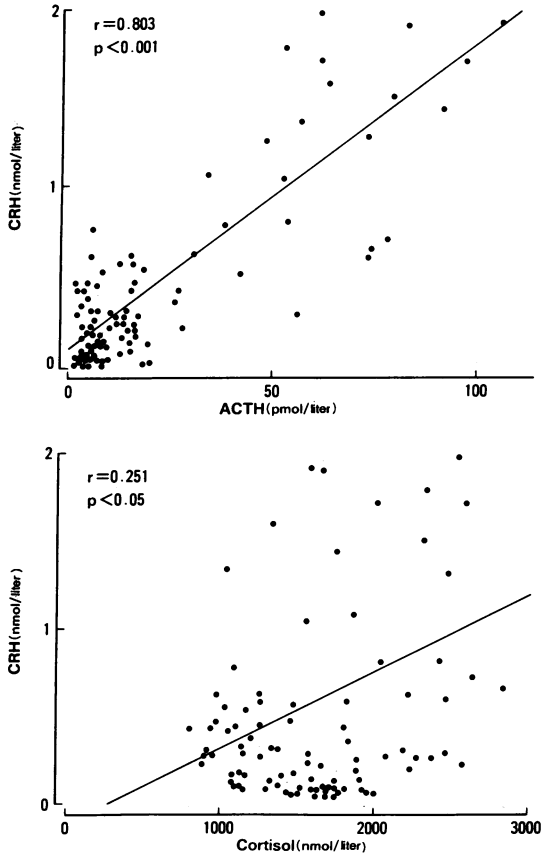

Figure 2. Correlations between CRH and ACTH (*top*) or cortisol (*bottom*) concentrations in samples obtained during early labor, delivery, and 1 and 2 h postpartum.

women. In contrast, CRH had no effects on ACTH secretion in the late third trimester women (Fig. 4).

Discussion

The functions of hypothalamic-pituitary-adrenal axis are altered during pregnancy. For example, both total plasma cortisol and plasma free cortisol are significantly elevated during pregnancy (7–9), but plasma ACTH levels are not suppressed; likewise, dexamethasone, a potent synthetic glucocorticoid, does not completely suppress maternal plasma or urinary cortisol or plasma ACTH in pregnant women (10–12). Increases in corticosteroid binding globulin, an extrapituitary source of plasma ACTH, a progesterone-modulated decrease in the sensitivity of pituitary corticotrophs or hypothalamus to glucocorticoid feedback inhibition (7), and an extrahypothalamic source of stimulatory input to the maternal corticotrophs (2, 3) have been suggested as possible explanations of this phenomenon.

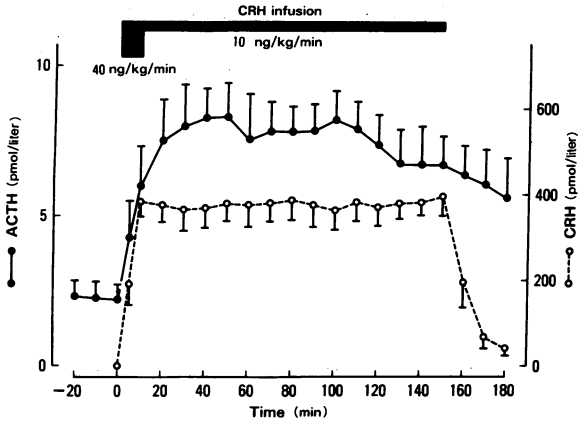

Figure 3. Responses (mean±SD, n = 5) of plasma ACTH (*closed circles*) and CRH (*open circles*) concentrations to synthetic rat CRH infusion at doses of 40 ng/kg per min for 10 min and then 10 ng/kg per min for 140 min.

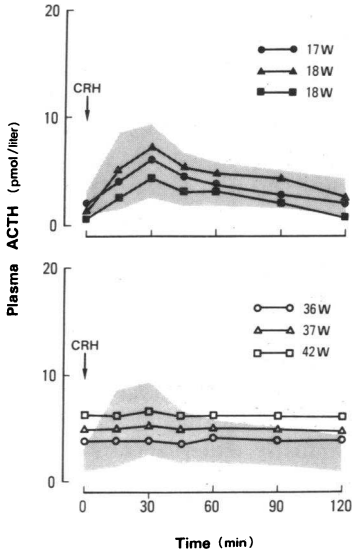

Figure 4. Plasma ACTH responses to synthetic CRH in pregnant women. *Top,* Early second trimester; *bottom,* late third trimester. Shaded areas indicate ACTH response to CRH (mean±2 SD area) in 10 normal women. Synthetic CRH was given at zero.

We and others have previously reported the presence of immunoreactive CRH in plasma of nonpregnant women as well as in the plasma of women at all stages of pregnancy (2–4, 13, 14). We found that plasma CRH increased progressively throughout pregnancy. The rise of plasma CRH during pregnancy correlated well with the weeks of pregnancy. This gradual rise suggests that such material is of extrahypothalamic origin. Our present and previous results are consistent with the view that the source of this plasma CRH during pregnancy is the placenta. However, the physiological significance of placental CRH was not yet completely defined. We previously reported that placenta contained significant amounts of immunoreactive CRH and such material was physicochemically indistinguishable from synthetic rat CRH (5). Umbilical cord CRH levels were much lower than those in corresponding maternal plasma, suggesting that placental CRH is preferentially secreted into maternal circulation (3). We also suggested in the present study that maternal plasma CRH levels are high enough to stimulate maternal pituitary ACTH secretion. From these observations, placental CRH seems to have its role in the maternal rather than fetal side. Recently, however, Orth and Mount (15) and Suda et al. (16) reported the presence of a specific, high affinity binding protein for CRH in human plasma, and concluded that CRH present in maternal plasma may be inactive in vivo due to this protein. However, we think such speculations are unlikely for several reasons. First, maternal plasma CRH, which was biological active and high enough to stimulate maternal pituitary ACTH secretion, correlated well with plasma cortisol levels during pregnancy and

with both plasma ACTH and cortisol during delivery. Absence of significant correlation between plasma CRH and ACTH before the start of labor may be explained by the gradual rise in plasma CRH during the progress of pregnancy. Elevation of plasma cortisol mediated by the hypersecretion of pituitary ACTH due to the placental CRH may inhibit (but not completely) its own ACTH secretion. Detection of plasma ACTH in spite of the significant increase of plasma-free cortisol during pregnancy may support this hypothesis. Second, the bound CRH to CRH binding protein still has bioactivity (16). Third, maternal plasma CRH levels did not show any significant variation during a 3-h observation when plasma samples were obtained at 20-min intervals in late pregnancy (Sasaki, A., unpublished observations); in contrast, plasma CRH after a bolus injection of synthetic rat CRH was rapidly eliminated from peripheral plasma (17). Similarly, maternal plasma CRH levels rapidly declined after the delivery of the placenta. CRH binding protein did not increase during pregnancy (16), suggesting that this protein is not of placental origin. Significant ACTH release during continuous CRH infusion, which attained similar plasma CRH levels with those of term pregnant women, was observed in nonpregnant women, suggesting that placenta is continuously secreting unbound CRH into maternal circulation and placental CRH, in such a situation, may have potential to stimulate maternal pituitary ACTH secretion. Fourth, the maternal corticotroph is normally responsive to synthetic CRH in early but not late pregnancy. For this explanation it is speculated that in late pregnancy the pituitary corticotroph is getting maximal stimulation by high circulating CRH and there is no more room to respond against further stimulation with synthetic CRH, or that an extrapituitary source of ACTH elicits hypersecretion of cortisol from the maternal adrenal cortex and inhibits maternal pituitary ACTH secretion. The former possibility was confirmed by Schulte et al. (18), and the latter speculation is unlikely because the placenta, which seems to be the extrapituitary ACTH source during pregnancy, contains only a small amount of ACTH (19) when compared with that of CRH. In addition, hypocortisolism should occur after delivery of the placenta as seen after tumor removal in patients with Cushing's syndrome due to an adrenal adenoma. However, hypocortisolism was not observed postpartum. Fifth, recently several cases of Cushing's syndrome that resolved on excision of ectopic CRH (but not ACTH)-producing tumor have been reported (20, 21). Such cases are rare, but they explain the possibility that ectopic CRH production in both extrahypothalamic tumors and placenta may elicit hypersecretion of cortisol despite the presence of CRH binding protein in peripheral plasma.

In the present study we showed that CSF CRH levels in pregnant women are not different from those of nonpregnant women in spite of significant differences of plasma CRH, ACTH, and cortisol levels. Although the origin and the physiological significance of CRH in CSF has not yet been elucidated several workers have suggested that CSF CRH might be a possible indicator of endogenous hypothalamic CRH secretion in humans (22–26). If this is true our results suggest that the pituitary-adrenal axis hyperactivity during late pregnancy is, at least, not due to the hypothalamic CRH hypersecretion.

The regulation of placental CRH secretion is not yet understood. It would, however, be doubtful that the secretory pattern of this placental hormone may be identical to that of hypothalamus. It also remains unclear whether placental CRH is secreted in response to stress. It has been reported that basal CRH secretion from fragments of human placental tissue were not affected by dexamethasone (27). More interestingly, glucocorticoids stimulate placental CRH synthesis and release in primary culture of human placenta (28). The latter findings suggest that plasma cortisol could stimulate, via placental CRH, a further rise in maternal plasma cortisol levels, completing a positive feedback loop that would be terminated at delivery.

Recently Campbell et al. (13) studied changes in plasma CRH levels throughout pregnancy and the relationship between plasma CRH and cortisol levels, and concluded that placental CRH has no effect on the maternal pituitary adrenal axis during pregnancy. The differences in experimental conditions (sample numbers and time of sampling in limited stages of pregnancy) may be responsible for these different results. In summary, based on our present results we conclude that a rise in plasma ACTH and cortisol during pregnancy, labor, and delivery is closely related to the rise of maternal plasma CRH. Since the maternal plasma CRH is biologically active and of placental origin (3) our results suggest that placental CRH may be a physiological stimulator of maternal anterior pituitary corticotrophic cells during pregnancy, labor, and delivery. Hypercortisolism during pregnancy may be partly due to the increase in corticosteroid binding globulin and partly to the placental CRH. Placental CRH may provoke the hypersecretion of cortisol and protect the mother from the stress of pregnancy and labor. We suppose that complete clinical signs of Cushing's syndrome will not occur during pregnancy because high circulating CRH is of short duration. However, we can see some similarity of clinical features such as obesity, hypertension, and striae in the skin (29) between patients with Cushing's syndrome and pregnant women. The mechanisms governing the regulation of CRH synthesis and release by the placenta, and whether other factors with ACTH-releasing activity are participating in the rise of ACTH during labor are important issues that remain to be elucidated.

While this paper was in preparation Schulte et al. (30) reported that CRH had no effect on ACTH and cortisol secretion in women in late pregnancy, and a normal pattern of ACTH and cortisol secretion in response to CRH could be observed in the same women postpartum. These findings are in agreement with our present report.

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