Maternal plasma corticotropin-releasing hormone associated with stress at 20 weeks' gestation in pregnancies ending in preterm delivery

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OBJECTIVE: This study tested the hypothesis that maternal stress is associated with elevated maternal levels of corticotropin releasing hormone and activation of the placental-adrenal axis before preterm birth.
STUDY DESIGN: In a behavior in pregnancy study, 524 ethnically and socioeconomically diverse women were followed up prospectively and evaluated at 3 gestational ages: 18 to 20 weeks, 28 to 30 weeks, and 35 to 36 weeks. Maternal variables included demographic data, medical conditions, perceived stress level, and state anxiety. Maternal plasma samples were collected at each gestational age. Eighteen case patients with spontaneous onset of preterm labor were matched against 18 control subjects who were delivered at term, and their samples were assayed for corticotropin-releasing hormone, adrenocorticotropic hormone, and cortisol by means of radioimmunoassay. Statistical tests were used to examine mean differences in these hormones. In addition, the relationship between stress level and each hormone was tested with a Pearson correlation coefficient and hierarchic multiple regressions in each group.
RESULTS: Patients who had preterm delivery had significantly higher plasma corticotropin-releasing hormone levels than did control subjects at all 3 gestational ages ($P < .0001$). Analyses did not find any differences in reported levels of stress between 18 to 20 weeks' gestation and 28 to 30 weeks' gestation. A hierarchic multiple regression indicated that maternal stress level at 18 to 20 weeks' gestation and maternal age accounted for a significant amount of variance in corticotropin-releasing hormone at 28 to 30 weeks' gestation, after controlling for corticotropin-releasing hormone at 18 to 20 weeks' gestation ($P < .001$). In addition, patients who were delivered preterm had significantly elevated plasma levels of adrenocorticotropic hormone at all 3 gestational ages ($P < .001$) and significantly elevated cortisol levels at 18 to 20 weeks' gestation and 28 to 30 weeks' gestation ($P < .001$).
CONCLUSION: Maternal plasma levels of corticotropin-releasing hormone are significantly elevated at as early as 18 to 20 weeks' gestation in women who are subsequently delivered preterm. Changes in corticotropin-releasing hormone between 18 to 20 weeks' gestation and 28 to 30 weeks' gestation are associated with maternal age and stress level at 18 to 20 weeks' gestation. Maternal stress and corticotropin-releasing hormone levels may be potential markers for the patient at risk for preterm birth. Activation of the placental maternal pituitary-adrenal axis is consistent with the classic endocrine response to stress. (Am J Obstet Gynecol 1999;180:S257-63.)

Key words: Corticotropin-releasing hormone, maternal stress, preterm birth, placental-maternal pituitary-adrenal axis

Corticotropin-releasing hormone (CRH) levels rise throughout the second and early third trimesters of pregnancy and then increase exponentially in the final 6 weeks before the onset of parturition at term.[1-4] Significantly higher levels of CRH are found in the umbilical vein than in the umbilical artery,[4] and the placenta has been noted to be the source of CRH.[5] There is evidence to suggest that placental CRH is biologically active and could stimulate the pituitary-adrenal axes of both the mother and fetus. The secretion of CRH by trophoblast cells is stimulated by glucocorticoids, suggesting a positive feedback loop exists between both the fetal and maternal pituitary-adrenal axes and placental CRH.[6, 7] This progressive stimulation of the placental-adrenal axes of both the fetus and mother could play a role in the initiation of parturition.[3]

Several studies suggest early activation of placental se-

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cretion of CRH in preterm labor.[1, 8, 9] None of these investigators, however, has proposed a clinically relevant mechanism for activating placental production of this peptide. Petraglia et al[10] identified several neurotransmitters and peptides that modulate the release of immunoreactive CRH from cultured human placental cells. These include norepinephrine, epinephrine, acetylcholine, angiotensin II, interleukin 1, arginine vasopressin, and oxytocin.[10] Because CRH has been clearly implicated in the mediation of the response to stress and because stress hormones such as epinephrine, norepinephrine, and cortisol are known to modulate CRH production in placental tissue in vitro, we proposed to study the role of maternal stress in activating the rise in CRH level before preterm birth.[6, 11]

Methods

Subjects. We prospectively followed up 524 ethnically and socioeconomically diverse women to assess the effects of maternal behaviors and biologic markers during pregnancy on outcome. We assessed fetal and maternal conditions at 3 gestational ages: time 1 (18-20 weeks' gestation), time 2 (28-30 weeks' gestation), and time 3 (35-36 weeks' gestation). We then assessed outcome at delivery. Forty-seven women were delivered preterm: 22 idiopathic, 16 preterm premature rupture of membranes, and 9 indicated. Blood samples were available from 18 case patients who had spontaneous onset of preterm labor (12 idiopathic, 2 mild preeclampsia, 2 transient hypertension, and 2 preterm premature rupture of membranes). Eighteen patients who were delivered at term were matched against the preterm group for age, previous birth outcome, smoking status, and race to constitute a control group. All patients were assessed between 9 o'clock AM and 1 o'clock PM.

Measures of response to stress. An 8-item abbreviated version of the Perceived Stress Scale, developed by Cohen et al,[12] was administered to measure general feelings of stress during pregnancy. This scale quantifies the perception of situations in one's life as unpredictable, uncontrollable, and taxing. It has been used as a measure of chronic stress level in studies of stress effects on health and in several studies with pregnant women.[13-15] Items ask participants how often in the last month they felt unable to control important things in life, deal with daily hassles, cope with life changes, handle personal problems, control irritations, and overcome difficulties, and also how often they felt that things were going well and they were "on top of things." Responses are provided on a 5-point scale anchored by *1* (never) and *5* (almost always). This scale was administered by interview at each gestational age. A shortened 10-item version of Spielberger's State Anxiety Inventory was used to measure subjective feelings of anxiety during pregnancy.[16] This scale is psychometrically sound, can be used on repeated occasions, and has been widely used in previous pregnancy studies.[17] It presents participants with a list of adjectives, including *calm, tense, at ease, nervous, jittery, relaxed, worried, steady,* and *frightened,* to describe their feelings during the last few days. Responses are rated on a 4-point scale anchored by *1* (not at all) and *4* (very much). Positive adjectives are recoded to reflect more anxiety. This scale was administered at each gestational age.

Estimates of reliability for the stress variables were high (Perceived Stress Scale at time 1, Cronbach α = .84; Perceived Stress Scale at time 2, α = .90; State Anxiety Inventory at time 1, α = .86; State Anxiety Inventory at time 2, α = .91). Perceived Stress Scale and State Anxiety Inventory scores were highly intercorrelated (time 1 $r[34] = .61$, $P < .01$; time 2 $r[34] = .69$, $P < .01$). On the basis of these correlations, Perceived Stress Scale and State Anxiety Inventory scores at each gestational age were standardized and summed to create stress level composites (stress level composite at time 1 and stress level composite at time 2).

Hormonal assays. At each gestational age blood was collected in chilled glass tubes containing ethylenediaminetetraacetic acid (1 mg/mL blood) and aprotinin (500 kIU/mL blood) and centrifuged at 4°C. Plasma was stored at –70°C until extraction. Plasma was extracted with Sep-Pak C-18 cartridges (Waters Associates, Milford, Mass) for measurement of immunoreactive CRH and adrenocorticotropic hormone (ACTH) levels. Acidified plasma was loaded onto the columns previously activated with 60% acetonitrile in 1% trifluoroacetic acid. The columns were washed twice with 3 mL 1% trifluoroacetic acid. The absorbed peptide was eluted with 3 mL acetonitrile, trifluoroacetic acid buffer and eluant dried in a speed vacuum concentrator (Savant Instruments, Micksville, NY). The dried extracts were stored at –80°C and resuspended in radioimmunoassay buffer at the time of assay. Plasma CRH and ACTH levels were each measured by specific double-antibody radioimmunoassays. Specific polyclonal rabbit antisera and the iodinated peptides were obtained from Peninsula Laboratories (Belmont, Calif). Cross-reactivities of CRH antiserum were 100% for human and rat CRH and none for the precusor of CRH (0%) and human ACTH (0%). ACTH antiserum had cross-reactivities of 100% for human, rat, and 1-24 (human) ACTH fragment; 1% for β-endorphin; and none (0%) for human CRH. The detailed radioimmunoassay procedure has been described previously.[18] Cortisol levels in plasma were measured with the double-antibody cortisol kit (Diagnostic products, Los Angeles, Calif).

Results

Bivariate and multivariate analyses were conducted to test the study hypotheses. First, mean differences in CRH, ACTH, cortisol, and stress levels between the

preterm and matched control groups were tested with analyses of variance and planned comparisons. The within-subject residual from each overall repeated measure analysis of variance was used as the error term in all comparisons. For tests of group differences at time 2, time 1 levels were used as covariates to reflect changes. Second, bivariate correlations among CRH level, ACTH level, cortisol level, stress level, demographic variables, and medical variables were examined separately for each group, primarily as a guide to planning simple regression analyses. Third, multiple regression equations were specified to examine the joint contributions of stress level, demographic variables, and medical variables in predicting hormone levels.

Before these analyses were conducted, t tests and χ^2 tests were performed to test for differences in demographic variables between the 2 groups. There were no significant differences ($P < .05$) between the preterm and matched control groups, in terms of mean (± SD) maternal age (27.7 ± 5.9 years and 28.1 ± 5.7 years, respectively), mean (± SD) number of medical risk factors (3.2 ± 1 and 2.7 ± 1.2, respectively), parity (22.2% and 38.9% primiparous, respectively; 77.8% and 61.1% multiparous, respectively), education (86.7% and 69.2% high school graduates, respectively; 0.0% and 23.1% college graduates, respectively; 13.3% and 7.7% other, respectively), marital status (33.3% and 61.1% married, respectively; 66.7% and 38.9% single, respectively), ethnicity (11.1% and 11.1% white non-Hispanic, respectively; 27.8% and 27.8% Hispanic, respectively; 55.6% and 55.6% African American, respectively; 5.6% and 5.6% Asian, respectively), and annual family income (50.0% and 41.2% <$20,000, respectively; 27.8% and 23.5% $20,000-$39,999, respectively; 16.7% and 5.9% $40,000-$49,999, respectively; 5.6% and 29.4% ≥$50,000, respectively).

Tests revealed that the preterm group had significantly greater plasma CRH levels ($t[34] = 27.47$, $P < .001$), ACTH levels ($t[34] = 9.93$, $P < .001$), and cortisol levels ($t[34] = 14.33$, $P < .001$) than did matched control subjects at time 1 (Table I; Figs 1 through 3). At time 2 the preterm group had significantly greater changes in ACTH levels ($t[33] = 2.28$, $P < .03$) and cortisol levels ($t[33] = 3.14$, $P < .005$) than did the control group, but changes in CRH levels from time 1 to time 2 were not significantly different from those in the control group ($P > .05$). However, Fig 1 illustrates that absolute levels of CRH at time 2 remained significantly greater in the preterm group than in the control group. Stress levels did not differ significantly between groups at time 1 or time 2. The size of the preterm group at time 3 was reduced by early delivery, but Figs 1 and 2 indicate that levels of CRH and ACTH at 35 to 36 weeks' gestation in the few women left who were delivered before 37 weeks' gestation were greater than those in the control subjects.

Bivariate correlational analyses (Table II) indicated significant associations in the preterm group between stress level at time 1 and CRH level at time 2 ($r = +.48$, $P < .05$). Maternal age was the only other variable correlated with CRH level in the preterm group ($r = -.53$, $P < .05$). Separate hierarchic regression equations predicting CRH level at time 2 in each group were conducted with variables entered in 3 steps. CRH level at time 1 and maternal age were entered at step 1, stress level at time 1 was entered at step 2, and stress level at time 2 was entered at step 3. The equation was significant for the preterm group ($F[4.13] = 16.55$, $P < .001$) and was not significant for the matched control group. In the preterm group, CRH level at time 1 ($\beta = .60$, $P < .01$) and maternal age ($\beta = .40$, $P < .05$) were significant predictors of CRH level at time 2. Moreover, in step 2, stress level at time 1 was a significant predictor of CRH level changes from time 1 to time 2 ($\beta = .35$, $P < .05$). The time 2 stress level entered in step 3 did not add to the prediction of changes in CRH level ($\beta = .11$, $P > .05$). That is, change in stress level from time 1 to time 2 did not add to prediction of CRH level change from time 1 to time 2.

Table I. Mean (± SD) stress and hormone levels

Variable	*Preterm group**	*Control group*
Stress level composite		
Time 1	0.28 ± 1.56	–0.28 ± 2.01
Time 2	0.10 ± 1.68	–0.10 ± 2.03
CRH level (pg/mL)		
Time 1	22.90 ± 2.41	6.90 ± 0.54
Time 2	334.23 ± 15.57	238.87 ± 25.52
Time 3	927.10 ± 22.91	818.52 ± 33.44
ACTH level (pg/mL)		
Time 1	22.62 ± 1.02	19.10 ± 1.10
Time 2	24.96 ± 0.81	21.69 ± 1.09
Time 3	26.31 ± 0.64	23.10 ± 0.72
Cortisol level (μg/mL)		
Time 1	20.86 ± 0.63	17.52 ± 0.76
Time 2	26.92 ± 0.67	24.74 ± 0.58
Time 3	31.60 ± 0.73	31.52 ± 0.70

*The number of women in the preterm group at time 2 was 17 and that at time 3 was 7.

Comments

In this study we observed, at as early as 18 to 20 weeks' gestation, significantly greater maternal CRH levels in a group of women who subsequently had spontaneous delivery of preterm infants than in a matched control group. Levels of CRH remained significantly greater in the preterm group at 28 to 30 weeks' gestation, and at 35 to 36 weeks' gestation among those who were not yet delivered. Elevated levels of CRH in patients destined to be delivered preterm have been reported by several investigators, but no clinically relevant mechanism has been proposed.[1, 8, 9] In 1984 we proposed that maternal stress could play a central role in initiating a cascade of events leading to preterm labor.[19] In the past 15 years we have

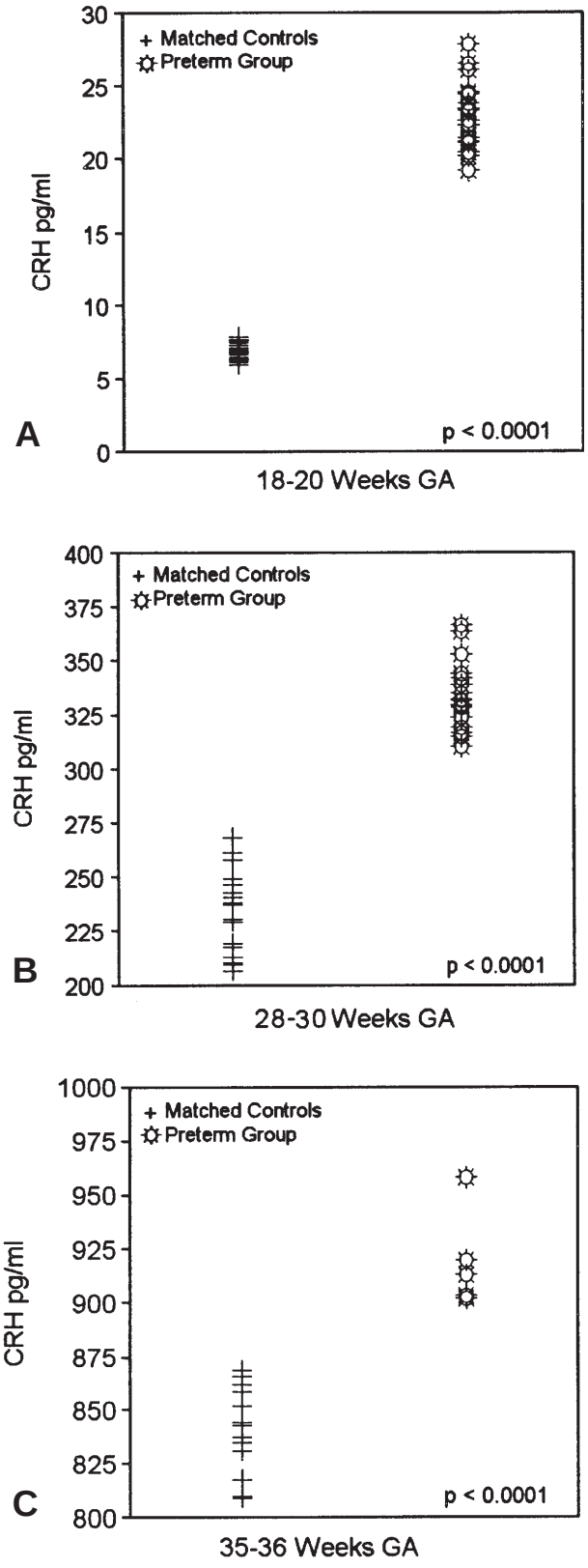

Fig 1. Individual data points for maternal plasma CRH level for the preterm group and matched controls at time 1 (18-20 weeks' gestation, **A**), time 2 (28-30 weeks' gestation, **B**) and time 3 (35-36 weeks' gestation, **C**).

seen an improved understanding of the biobehavioral mechanisms that make up the stress response.[11] As noted earlier, CRH gene expression by the placenta can be initiated by several neurotransmitters and peptides common in the classic neuroendocrine response to stress.[10]

In this study we also observed a significant association between psychosocial stress level as early as 18 to 20 weeks' gestation and significant elevations of CRH level at 28 to 30 weeks' gestation. Stress level also predicted significant changes in CRH level between 18 to 20 weeks' gestation and 28 to 30 weeks' gestation. We observed no overlap between CRH levels in patients who were delivered preterm and CRH levels in those who were delivered at term, suggesting that gene expression is turned on earlier than 18 to 20 weeks' gestation. This is consistent with the observations of McLean et al,[9] who suggest that these changes are consistent with the setting of a biologic clock for early delivery. We are the first to suggest that maternal stress could be an early trigger to initiate CRH gene expression in the placenta and program this biologic clock for preterm delivery. We have preliminary data showing that our stress level measures correlated significantly with urinary norepinephrine levels in the patients followed up in this study.[20] Norepinephrine is among the neurotransmitters known to turn on CRH gene expression in the placenta. It should be noted that some patients in this study were delivered preterm and had elevated CRH levels but did not have stress, as determined by the questionnaire, which suggests that there may be other causes for CRH gene expression, such as inflammation.[11]

The elevations in maternal CRH level observed in this study most likely represent biologically active hormone because of the observed significant elevations in maternal ACTH and cortisol levels when compared to controls at 18-20 and 28-30 weeks. An alternative explanation could be the role of vasopressin release during stress and its effect on ACTH release. It was shown by Goland et al[21] that chronic placental CRH stimulation during pregnancy leads to an enhanced ACTH release as a result of vasopressin release during stress. In addition, vasopressin is among the peptides known to stimulate CRH gene expression in the placenta.[10] Our data suggest that there is an accelerated activation of the placental maternal adrenal axis in patients who are at risk for preterm delivery. More than a decade ago, Carr et al[22] showed progressive increases in both ACTH and cortisol levels throughout pregnancy. Elevations of ACTH level may also be of placental origin because, as Rees et al[23] and Petraglia et al[24] showed, the placenta can secrete ACTH in response to CRH. Whatever its source, its downstream effect results in a significant elevation of maternal cortisol levels, and cortisol acts as a positive feedback loop for CRH gene expression at the level of the placenta but not the hypothalamus.[6]

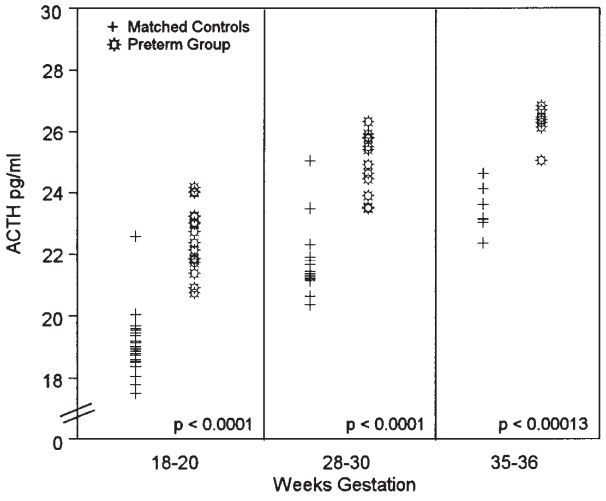

Fig 2. Individual data points for maternal plasma ACTH level for the preterm group and matched control subjects for time 1 (18-20 weeks' gestation), time 2 (28-30 weeks' gestation), and time 3 (35-36 weeks' gestation).

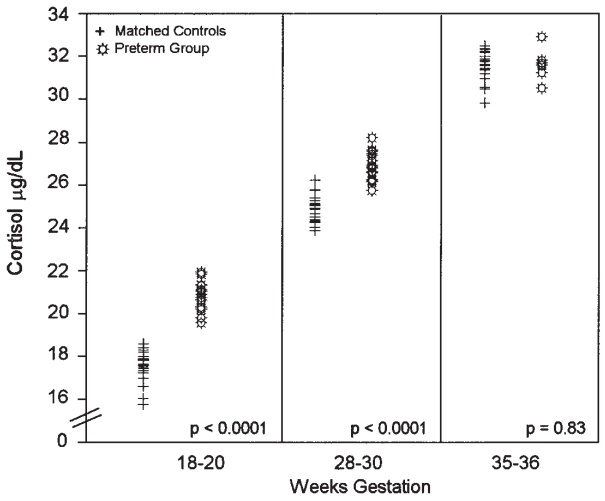

Fig 3. Individual data points for maternal plasma cortisol level for the preterm group and matched control subjects at time 1 (18-20 weeks' gestation), time 2 (28-30 weeks' gestation), and time 3 (35-36 weeks' gestation).

Table II. Correlations among stress level, CRH level, and maternal age for preterm and control groups

	CRH level, time 1	*CRH level, time 2*	*Stress, time 1*	*Stress, time 2*	*Maternal age*
CRH level, time 1	1.00	0.78*	0.23	0.11	–0.25
CRH level, time 2	0.66*	1.00	0.46†	0.34	–0.53†
Stress level, time 1	–0.38	–0.48†	1.00	0.83*	0.07
Stress level, time 2	–0.33	–0.47†	0.84*	1.00	–0.05
Maternal age	–0.17	0.17	–0.41	–0.18	1.00

Correlations above the diagonal of 1.00 values are for the preterm group; correlations below that diagonal are for the control group.
*$P < .01$.
†$P < .05$

The plasma of pregnant women contains a circulating binding protein specific for CRH, and it has been suggested that this protein may protect the maternal pituitary gland against the progressive increase in CRH levels during pregnancy.[25, 26] In pregnancies complicated by preterm labor and preeclampsia, in which significant elevations in CRH levels have been reported, there is concern that the excess CRH may not be biologically active. We did not measure CRH binding protein in this study; however Perkins et al[27] reported that plasma levels of CRH binding protein were significantly reduced in patients with preterm labor or preeclampsia.

The observed changes in neuropeptide concentrations that we believe to be a response to maternal stress are consistent with a mechanism to prepare the fetus for an untimely delivery. Set into action are a series of endocrine events both autocrine and paracrine to prepare the fetus and uterus for parturition. First, elevated levels of CRH appear in the fetal circulation, as shown by Goland et al,[4, 28] suggesting that this peptide is available to activate the fetal pituitary-adrenal axis, which is considered to be mature during the second trimester.[29] We previously showed by time trend analysis of unconjugated estriol levels that the fetal pituitary-adrenal axis is sensitive to maturational events near term, showing a peak between 32 and 34 weeks' gestation that is followed by a nadir at 35 weeks' gestation and then a second steep rise between 35 and 37 weeks' gestation.[30] These data suggest that the fetal pituitary-adrenal axis is undergoing maturation consistent with a clock that has a set time for parturition.[9] The acute rise in maternal CRH level near term may account for these maturational observations, providing further stimulation of fetal ACTH and adrenal synthesis and secretion of cortisol and dehydroepiandrosterone.[31] Moreover, there is direct evidence of a sudden rise in fetal corticoid levels late in human gestation consistent with the second steep rise in estriol levels.[32] The positive feedback loop between the fetal adrenal gland (increased corticoid levels) and placental production of CRH provides a mechanism for an expo-

nential rise in levels of CRH and estrogens, 2 important mediators of labor.[33] Data from various investigators showing an early activation of CRH gene expression in the placenta provide a mechanism through which these maturational events could occur early in the case of preterm labor. Recently, McGregor et al[34] found an early estriol surge occurring in a group of women approximately 3 weeks before preterm delivery, which matches our earlier observations of an estriol increase occurring near term. This relationship between placental CRH level and the fetal pituitary-adrenal axis could be an attempt by the maternal-fetal-placental unit to bring about early fetal maturation to increase the chances for survival in case the fetus is delivered early.

So far we have centered our discussion on the physiologic significance of placental CRH on maternal and fetal pituitary-adrenal axis activation. Jones et al[7] proposed a paracrine interaction of tissues other than the placenta that secrete CRH in the presence of cortisol, such as the amnion, chorion, and decidua. Thus the hypercorticoid state brought about by the elevations of ACTH and cortisol levels could add a third dimension to this complex interaction. These same investigators showed that both CRH and ACTH are capable of stimulating prostaglandin production (prostaglandin E_2 and $F_{2\alpha}$) by amnion cells in early and late pregnancy.[35, 36] The myometrium is the final target tissue at which these hormones play their primary role in initiating parturition. Apparently CRH itself has no intrinsic action on the myometrium[37]; however, CRH can potentiate the action of oxytocin on myometrial contractility, which is prostaglandin dependent.[38] A myometrial CRH receptor that increases in affinity during pregnancy has been found.[39] The precise role that this receptor plays in the mechanisms influencing parturition is at present unknown.

It thus appears that during pregnancy the placenta is a source of a regulatory factor (CRH) that can interact with both the maternal and fetal pituitary-adrenal axes to initiate a cascade of endocrine events, both paracrine and autocrine, that determine the timing of delivery. We observed a significant relationship between maternal stress level and elevations in maternal plasma CRH levels during the second trimester in women who were subsequently delivered preterm. Maternal stress may be among the clinical conditions contributing to the cascade of events leading to preterm birth, and CRH level may be a biologic marker that will help to discriminate patients at risk.

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