Maternal plasma corticotropin-releasing factor (CRF) and CRF-binding protein (CRF-BP) levels in post-term pregnancy: effect of prostaglandin administration

P Florio, P J Lowry, C Benedetto, L Galleri, M Torricelli, A Giovannelli, R Battista, F M Reis and F Petraglia

Department of Pediatrics, Section of Obstetrics and Gynecology, Obstetrics and Reproductive Medicine, University of Siena, Policlinico ‘Le Scotte’ Viale Bracci, 53100 Siena, Italy 1Department of Biochemistry and Physiology, University of Reading, Whiteknights, Reading, UK and 2Department of Obstetrics and Gynecology, University of Torino, Torino, Italy

(Correspondence should be addressed to P Florio; Email: florio@unisi.it)

Abstract

Objective: Placental corticotropin-releasing factor (CRF) affects myometrial contractility and the secretion of several uterotonins such as prostaglandins (PGs); however, the activity of CRF is counteracted by CRF-binding protein (CRF-BP). At term and pre-term labor, CRF levels in maternal plasma are highest whereas those of CRF-BP are falling, and the cause of this fall is unknown. Thus, in this study, we investigated the effect of PG administration for labor induction on maternal plasma CRF and CRF-BP concentrations.

Design: Maternal plasma CRF and CRF-BP levels were assayed before and after (2 h later) induction of labor by intracervical administration of prostaglandin E2 (PGE2), and at delivery in a group of healthy post-term pregnancies (n=18). Controls were women at term out of labor (n=22), who subsequently progressed to deliver a healthy singleton baby.

Methods: CRF was measured by two-site immunoradiometric assay, and CRF-BP was assayed by radioimmunoassay.

Results: Maternal plasma CRF levels were significantly (P<0.0001) lower and CRF-BP significantly (P<0.0005) higher in post-term than in term pregnancies. With respect to induction of labor, 2 mg PGE2 were sufficient to increase maternal plasma CRF levels at delivery (P<0.005). While 0.5 mg PGE2 significantly decreased maternal plasma CRF-BP levels at delivery (P<0.001), 2.0 mg PGE2 significantly reduced CRF-BP concentrations both after 2 h (P<0.05) and at delivery (P<0.0001).

Conclusions: In the light of the well-known stimulation of prostaglandin release by CRF, these data suggest a positive feedback effect of PGE2 on maternal CRF release during induced labor.

Introduction

The mechanisms underlying the onset of parturition emphasize the role played by the paracrine intrauterine interactions (1, 2). Several locally produced factors, corticotropin-releasing factor (CRF), adrenocorticotropin (ACTH), prostaglandins (PGs), and oxytocin (OT) modulate myometrial contractility (2–4).

In addition to CRF, the human placenta also expresses specific CRF receptors (5), by which CRF stimulates secretion of prostaglandin (6–8), ACTH (8, 9), and OT (10), all specific uterotonins. Moreover, as term approaches, the human myometrium expresses specific CRF receptors having increased affinity (11), by which CRF may potentiate PG-induced myometrial contractility, an effect reversed by the addition of a synthetic CRF antagonist (12).

Intrauterine tissues also express CRF-binding protein (CRF-BP) (13), a 37 kDa protein of 322 amino acids able to bind CRF with high affinity (14) and blocking the CRF actions on intrauterine target organs (14, 15). It counteracts the local CRF-induced secretion of ACTH (13, 16) and PG (7) as well as the effect of CRF on PG-induced myometrial contractility (7). CRF is released into maternal circulation from early in gestation and its peripheral plasma concentration increases until the third trimester; when a more incisive rise associated with the onset of parturition occurs (17, 18). On the contrary, CRF-BP levels do not change until late gestation (19–22), when they fall rapidly in the last 3 weeks before delivery (19, 23, 24). Thus, reciprocal changes of CRF and CRF-BP levels occur in the biological fluids of pregnancy, with higher CRF levels in the presence of lower CRF-BP concentrations at term as well as at pre-term labor, suggesting an involvement of both CRF and CRF-BP in the events controlling the length of human pregnancy (1, 4).

The fact that both CRF and PGs interact with the myometrium, and that PGs have been reported to increase placental CRF secretion from cultured human...
placental cells prompted us to examine the effect of PGE₂ on maternal plasma CRF and CRF-BP levels.

Materials and methods

Patients

A group of 40 healthy pregnant women were consecutively enrolled. Informed written consent was obtained from all patients prior to inclusion in the study for which local Human Investigation Committee approval was obtained.

In a longitudinal study, we included a group of healthy post-term pregnancies (n = 18; age range from 27 to 39 years; gestational age: 41.5 ± 0.14 weeks (mean ± S.E.M.)); Table 1, who underwent induction of labor by intracervical administration of PGE₂ (Prepidil Gel, Pharmacia & Upjohn, Sweden) 0.5 (n = 10) and 2.0 mg (n = 8). Blood samples were collected before and after (2 h later) PGE₂ administration, and at delivery. None of these patients received OT during the course of labor induction.

As a control group, a blood sample was collected from women at term in absence of labor (n = 22; age range from 25 to 38 years, between 40 and 41 gestational weeks; Table 1). They subsequently (4 ± 2 days interval (mean ± S.E.M.) from blood collection) progressed to deliver a healthy singleton baby, without any obstetric complications at delivery.

Gestational age was calculated on the basis of the last menstrual period and confirmed by the use of ultrasound evaluation (Real Time Ultrasound Scan Equipment, Siemens Sonoline Elegra, Germany Millenium edition, with a probe at 2.5–5.0 MHz). The exclusion criteria were multiple pregnancies, diabetes, hypertension, fetal anomaly, maternal or fetal infection, fetal growth restriction, cardiocographic evidence of fetal distress, and an Apgar score at 1 min of < 7.

Blood samples were drawn from the antecubital vein with a polypropylene syringe and a butterfly needle, and then transferred to chilled tubes containing ethylenediaminetetraacetic acid (10 mg/ml blood) and aprotinin (50 µl/tube from a solution of 20 000 UI/ml; Trasylool 100 000 UIC; Bayropharm, Milan, Italy). The tubes were immediately centrifuged at 4 °C (3000 g for 10 min). All plasma samples were kept at −80 °C until assay.

CRF-BP assay

CRF-BP levels were measured by RIA. Purified recombinant CRF-BP was radioiodinated by the glucose oxidase/lactoperoxidase method and was separated on a 90x1 cm bed of Sephadryl S200 developed with 0.05 ml/l phosphate buffer (pH 7.4), containing 0.5% BSA and 0.1% sodium azide at a flow rate of 3 ml/h, collecting fractions every 20 min. Only a radiolabel constituting the peak eluting with a Kav of 0.46 was used as tracer for the CRF-BP RIA. Seventy-nine percent of the radioactivity from these peak fractions was precipitated by the addition of an excess of the rabbit antibody raised against recombinant CRF-BP as used in the RIA. The immunoassay was performed essentially as described previously (21). Briefly, CRF-BP stocks (3.28 mg/l) were prepared in aliquots of 0.5 ml in sheep serum and stored frozen at −20 °C. Assay standards were prepared by dilution of stock aliquots in 0.05 M phosphate buffer (pH 7.4), containing 0.5% wt/vol BSA and 0.1% wt/vol sodium azide to obtain a range of concentrations from 0.9 to 464 mg/l. To 50 ml of the above buffer were added 50 ml standard or a column fraction, 100 ml tracer containing 20 000 c.p.m. 125I-CRF-BP, and 100 ml rabbit anti-CRF-BP antibody diluted 4000-fold in the same buffer. Standard and samples were prepared in duplicate, and the assay was incubated for 16 h at 4 °C before separation. Separation was achieved by a precipitating antibody consisting of 10% sheep anti-rabbit antiserum directed against the Fc fragment containing 0.5% vol/vol normal rabbit serum and 4% polyethylene glycol 6000. Inclusion of hCRF in standards or human plasma samples in concentrations ranging from 1.6 to 25 mg/l had no effect on CRF-BP measurements (21). Assay sensitivity was 3.125 ng/ml. Intra-assay coefficient of variation was 7%.

CRF assay

Maternal plasma samples were submitted to an extraction procedure as previously described (21). The acid extraction method we used to strip CRF from the

Table 1 Clinical characteristics of study groups. Values are expressed as means ± S.E.M.

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>PGE₂ 0.5 mg</th>
<th>PGE₂ 2.0 mg</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>22</td>
<td>10</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Parity</td>
<td>1.18 ± 0.18</td>
<td>1.28 ± 0.19</td>
<td>1.16 ± 0.16</td>
<td>NS</td>
</tr>
<tr>
<td>Maternal age (years)</td>
<td>30.18 ± 1.55</td>
<td>30.4 ± 1.5</td>
<td>32.5 ± 1.2</td>
<td>NS</td>
</tr>
<tr>
<td>Weeks at delivery</td>
<td>40.4 ± 0.5</td>
<td>41.3 ± 0.23</td>
<td>41.6 ± 0.14</td>
<td>NS</td>
</tr>
<tr>
<td>Birthweight (g)</td>
<td>3344 ± 66.31</td>
<td>3285 ± 73.5</td>
<td>3421 ± 52.44</td>
<td>NS</td>
</tr>
<tr>
<td>Apgar &lt; 7 at 1 min</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>NS</td>
</tr>
<tr>
<td>Placental weight (g)</td>
<td>556.6 ± 13.65</td>
<td>546.0 ± 28.53</td>
<td>550.3 ± 31.83</td>
<td>NS</td>
</tr>
<tr>
<td>CRF (pmol/l)</td>
<td>3708.31 ± 176.9</td>
<td>1740.51 ± 386.22</td>
<td>1163.32 ± 378.81</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>CRF-BP (nmol/l)</td>
<td>42.66 ± 3.78</td>
<td>74.25 ± 12.04</td>
<td>83.09 ± 10.78</td>
<td>&lt;0.0005</td>
</tr>
</tbody>
</table>

NS, not significant.
CRF-BP allowed us to measure total CRF (25). Briefly, cyclohexyl columns (500 mg; Bondelut, AnalyLichem International, Arbor City, CA USA) were washed with methanol (0.5 ml) and a 2 ml mixture of formic acid plus triethylamine and 0.2% β-mercaptoethanol (pH 3) and loaded slowly into the column. The peptide was finally eluted with a mixture of 75% acetonitrile, 25% triethylamine, and 0.2% mercaptoethanol (2 ml). The final recovery of the peptide evaluated with cold (100 ng) or labeled (125I) CRF was 85%. All extracted samples were then dried in a speed vacuum concentrator (Savant, Hicksville, NJ, USA). All reagents were purchased from Sigma–Aldrich Corp.

Each dried sample was redissolved in buffer (0.1% BSA, 0.05% Triton X-100, and PBS (pH 7.3)), and CRF concentrations were measured by two-site immunoradiometric assay (IRMA) as previously described (21), except that the immunoglobulin G for radiiodination was purified from a sheep antibody raised against the hCRF (sequence 36–41) conjugate, and the second epitope antibody was from a rabbit that had been immunized with the hCRF (sequence 1–20) conjugate. The assay sensitivity was 10 pg/ml. Inter-assay coefficient of variation was 6%.

**Statistical analysis**

Results are expressed as mean and s.e.m. Statistical significance of hormone concentrations was assessed using Mann–Whitney U test for comparison between term and post-term pregnancies. Friedman test followed by post hoc Dunn’s test was applied to evaluate the statistical significance of hormone changes throughout induction of labor.

Results were considered statistically significant when \( P < 0.05 \).

**Results**

**Clinical findings**

Table 1 shows the clinical characteristics of all patients. There were no significant differences between maternal age, parity, and gestational age. In addition, all patients had a vaginal delivery, without difference in the Apgar score, birthweight, and placental weight.

**Hormone levels**

CRF and CRF-BP were measurable in all plasma samples and correlated with the duration of labor \( (r = -0.58 \) and \( r = 0.57 \) for CRF and CRF-BP respectively; \( P < 0.05 \)).

Maternal plasma CRF levels were significantly lower \( (P < 0.0001) \) in post-term pregnancies than in control women (Table 1; Fig. 1A). With respect to induction of labor, whilst the administration of 0.5 mg PGE2 failed to affect CRF levels at the prescribed time points (Fig. 2A; Table 2), the intracervical administration of 2 mg PGE2 was associated with significantly increased \( (P < 0.005) \) maternal plasma CRF levels at delivery (Fig. 3A; Table 2).

Maternal plasma CRF-BP levels were significantly lower \( (P < 0.0005) \) in control subjects than in post-term pregnancies (Fig. 1B; Table 1). In addition, while the administration of 0.5 mg PGE2 significantly decreased \( (P < 0.001) \) maternal plasma CRF-BP levels only at delivery, PGE2 at 2.0 mg significantly reduced CRF-BP concentrations both after 2 h \( (P < 0.05) \) and at delivery \( (P < 0.0001) \; (Fig. 3B; Table 2).\)

**Discussion**

The present study shows that post-term pregnancies had significantly lower CRF and higher CRF-BP levels, and primarily that the therapeutic use of PGs affects maternal CRF and CRF-BP levels.

CRF and CRF-BP are expressed by human placenta and secreted differentially during pregnancy. In fact, while CRF concentrations increase progressively until the third
trimester (1, 3, 4, 18), the concentrations of CRF-BP do not change significantly during most of gestation (19–22), but they fall during the final weeks of normal pregnancy (19, 23, 24) and still further with the onset of labor (19). In pre-term labor, the highest CRF and lowest CRF-BP concentrations are measurable in maternal and fetal circulation, as well as in amniotic fluid (19, 20, 22–24, 26). During the course of spontaneous labor at term, there is no further change in maternal plasma CRF (27), and both CRF and CRF-BP return to normal non-pregnancy levels within 48 h post partum (28, 29). The dynamics of these proteins in maternal circulation through the various stages of spontaneous labor either pre- or post-term remains unknown.

In the present study, we found significantly lower CRF and higher CRF-BP levels in post-term pregnancies, suggesting that the lack of the reciprocal changes in CRF and CRF-BP levels may delay the onset of labor. As already mentioned, CRF-BP, because it binds CRF, has been postulated to affect levels of ‘free’ CRF and, thus, its bioactivity (14–16), counteracting placental CRF-induced secretion of ACTH (13, 30) and PG (7) as well as the effect of CRF on PG-induced myometrial contractility (7). Consequently, the failure of CRF levels to increase and CRF-BP levels to decrease in the last weeks of gestation may postpone the spontaneous onset of labor. Our data describing the impairment of CRF and CRF-BP secretion in post-term deliveries agree with that reported by McLean et al. (20), who first suggested that CRF and CRF-BP levels trigger parturition after a predetermined length of gestation.

In the present in vivo study, we have also found that the intracervical administration of PGE2 increased maternal plasma CRF levels at delivery and decreased maternal plasma CRF-BP levels, extending the observation in vitro that PGE2 stimulates both the placental secretion (9) and expression (31) of CRF in a dose-dependent manner. We found that PGE2 increased CRF levels only at the highest dose (2.0 mg), while the effect on maternal plasma CRF-BP concentrations was obtained using a lower dose (0.5 mg), with an early effect (after 2 h) when the highest dosage was used. Because the average concentrations of CRF in the two treatment groups differed at baseline, it can be argued that the group treated with 0.5 mg PGE2 might have responded similarly to the group receiving 2.0 mg PGE2, if they had the same CRF concentrations pre-treatment. However, the increase of plasma CRF concentrations following 2.0 mg PGE2 administration was consistently seen in all patients, covering a wide range of pre-treatment CRF concentrations, contrasting with no effect of 0.5 mg PGE2 administration even in the women with the lowest baseline CRF levels.

In summary, CRF concentrations increased and CRF-BP concentrations decreased in maternal plasma after intracervical administration of PGE2 for labor induction in post-term gestation. Taken together with the observation that CRF stimulates prostaglandin release, these data suggest a positive feedback effect of PGE2 on maternal CRF release during induced labor. Thus, in addition to the...
direct PG effects on cervical dilatation and myometrial contraction, the mechanism of labor induction with PGE₂ may also benefit from decreased CRF-BP and increased CRF levels in maternal circulation.

**References**


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