CLINICAL STUDY

Maternal plasma corticotrophin-releasing factor and urocortin levels in post-term pregnancies

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Abstract

Objective: Corticotrophin-releasing factor (CRF) and urocortin are two placental neuropeptides that are involved in the mechanisms of labour by modulating myometrial activity. Maternal plasma levels of both CRF and urocortin are increased at term and preterm labour, whilst those of CRF are reduced in women who are destined to experience post-term delivery. The present study evaluated maternal plasma levels in term and post-term pregnancies out of labour.

Design: A group of healthy pregnant women was enrolled and subdivided as follows: (i) at term out of labour ($n = 19$; 276 ± 0.7 days of gestation; samples collected at the time of elective caesarean section due to previous uterine surgery); (ii) post-term ($n = 19$; 291 ± 1.4 days of gestation), from whom samples were collected before induction of labour.

Methods: Urocortin and CRF measurements by radioimmunoassay; digital palpatory cervical examination and Bishop score computation; cervical length and funnelling presence assessment by transvaginal ultrasonography.

Results: Maternal plasma CRF concentrations were significantly ($P < 0.05$) lower whilst those of urocortin were unchanged in post-term compared with term pregnancy. However, CRF and urocortin levels were both significantly ($P < 0.05$ and $P < 0.001$ respectively) higher in pregnancies delivered within 12 h of labour induction than in those that remained undelivered, and were significantly correlated with the induction-delivery interval (CRF: $r = -0.676$, $P = 0.0015$; urocortin: $r = -0.783$, $P < 0.0001$).

Conclusions: CRF and urocortin levels are decreased and unchanged, respectively, in post-term pregnancy when compared with term pregnancy. Both CRF and urocortin correlate with the time of labour onset after induction. Since CRF derives from the placenta, and urocortin from the fetus, the concerted expression of these neuropeptides appears to be relevant in determining the length of human gestation.

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Introduction

Prolonged gestation is largely an enigma but may be due to a defect in the hormonal milieu that leads to the onset of labour (1, 2). Indeed, the duration of pregnancy is determined by a shift in the equilibrium between inhibitors and activators of myometrial contractility, so that parturition results from a complex interplay between several placental players able to influence molecular pathways driving the onset of labour (2). Corticotrophin-releasing factor (CRF) and urocortin are local peptides that may be part of the hormonal milieu controlling uterine contractility (3).

CRF is a 41-amino acid neuropeptide expressed by gestational tissues throughout pregnancy and secreted

into the maternal circulation (2, 3). Urocortin is a 40-amino acid neuropeptide, sharing 45% sequence identity to CRF (4), which is also expressed by the human placenta, decidua and foetal membranes (5). Binding to diverse receptor subtypes expressed by the human placenta and foetal membranes (6), CRF and urocortin stimulate the local release of uterotonins, i.e. adrenocorticotrophin (ACTH), oxytocin and prostaglandins (7–10). Furthermore, the binding of CRF and urocortin to their myometrial receptors triggers different pathways that result in myometrial quiescence when activated by CRF, and myometrial contractility when stimulated by urocortin (11). Finally, both CRF (12) and urocortin (13) levels are increased in the maternal circulation at term and preterm labour. Although it is

possible that their increased levels at labour may be a consequence rather than a cause of human parturition, the *in vitro* evidence for a role of CRF in myometrial activity (11), together with the finding of reduced levels of plasma CRF in women who are destined to experience post-term delivery (14), suggest that CRF may act directly as a trigger for parturition in humans.

Since no data are as yet available on urocortin, in the present study we measured its maternal plasma levels in term and post-term pregnancies out of labour.

Materials and methods

A group of healthy pregnant women ($n = 38$) was enrolled in our Division of Obstetrics and Gynaecology, and subdivided as follows: (i) at term out of labour ($n = 19$; 276 ± 0.7 days of gestation; samples collected at the time of elective caesarean section due to previous uterine surgery); (ii) post-term ($n = 19$; 291 ± 1.4 days of gestation; samples collected before induction of labour).

Written informed consent was obtained from each pregnant woman and the permission of the Local Human Investigation Committee was granted for the study. The exclusion criteria were multiple pregnancies, diabetes, hypertension, foetal anomaly, maternal or foetal infection, foetal growth restriction, cardiotocographic evidence of foetal distress and an Apgar score at 1 min of <7 . All pregnancies were dated by ultrasound, with measurement of the biparietal diameter, head circumference, femur length, and abdominal circumference; their clinical characteristics are summarized in Table 1.

In all post-term pregnancies, a digital palpatory cervical examination was performed, the Bishop score was assigned (15), and all five parameters of the Bishop scoring system (dilatation, effacement, station, cervical consistency, and cervical position) were recorded separately. After the digital examination and before starting labour induction, patients were submitted to transvaginal ultrasonography with the use of Siemens Sonoline ELEGRA real-time ultrasound scan equipment (Erlangen, Germany) with a 4.5–7.0 MHz transvaginal probe, to measure the cervical length and to assess the presence of funnelling. Funnelling was defined as a V- or Y-shape triangle with a protrusion of the amniotic

membranes 3 mm or more into the internal cervical os as measured along the lateral border of the funnel (16). Labour was induced by administering an intravaginal prostaglandin pessary (dinoprostone; proposs 10 mg; Ferring AB, Limhamn, Sweden), that was removed after the 12-h observation period if there were no cervical changes or in any of the following events: initiation of labour, spontaneous rupture of membranes, amniorhexis, uterine hyperstimulation or hypertonus, foetal distress, or secondary systemic effects such as nausea, fever, vomiting, diarrhoea, tachycardia or hypertension. If an increase in the Bishop score of 3 or more points above the baseline was achieved, the pessary was removed, amniotomy was performed if possible and oxytocin was started as necessary. Oxytocin infusion was not begun until at least 30 min after removal of the pessary. Our protocol included intravenous oxytocin as recommended by the American College of Obstetricians and Gynecologists (17) and was to be continued for a maximum of 8 h unless contraindications arose (17). Amniotomy was performed at the discretion of the attending physician. The onset of active labour was considered as 4 cm dilatation with regular contractions every 2–3 min.

Blood samples were drawn by using a polypropylene syringe and a butterfly needle and then transferred to chilled tubes containing ethylenediaminetetra-acetic acid (10 mg/ml blood). The tubes were centrifuged immediately at 4°C (3000 *g* for 10 min). All plasma samples were kept at -80°C until assay.

Urocortin assay

Maternal plasma levels were measured using previously published methodology (13), except for the delayed addition of tracer to improve assay sensitivity. Briefly, duplicate 100 μl aliquots of plasma extract or human urocortin (1–40) standard were mixed with 100 μl assay buffer containing rat urocortin (1–40) anti-serum at 1:2100 dilution and incubated for 40 h at 4°C . Buffer (100 μl) containing approximately 2000 c.p.m. iodine¹²⁵-labelled human urocortin (1–40) was then added and the tubes incubated for a further 6 h prior to the addition of pre-precipitated sheep anti-rabbit second antibody, as previously described (13). The specificity of the urocortin anti-serum had been checked by measuring the cross-reactivity of peptides with sequence homology in the urocortin assay, i.e. human CRF (1–20) and human CRF (1–41) (Peninsula Laboratories, St Helens, Merseyside, UK), human urocortin II (Stresscopin-related peptide (6–43) NH_2) and human urocortin III (Stresscopin (3–40) NH_2) (Phoenix Pharmaceuticals Inc., Belmont CA, USA) as well as with ACTH, sauvagine and urotensin 1 (Sigma Chemicals Co, St Louis, MO, USA) and thyroglobulin; none of these molecules displayed significant cross-reactivity even at high concentration (1 $\mu\text{g}/\text{ml}$). Urocortin levels in the study

Table 1 Summary of clinical data.

	At term not in labour	Post-term not in labour
Number of subjects	19	19
Parity	1.05 ± 0.3	1.2 ± 0.5
Maternal age (years)	28.7 ± 1.2	28.6 ± 2.0
Gestation days at delivery	276 ± 0.7	291 ± 1.4
Birth weight (g)	3344 ± 66	3360 ± 56

samples were measured in a blinded fashion in a single assay; the assay had a sensitivity of approximately 50 pg/ml with intra-assay and interassay variations of 8% and 13% respectively.

CRF assay

CRF was measured with the CRF-specific immunoradiometric assay as described previously (18), using human CRF (Peninsula Laboratories) as standard. The assay had a sensitivity of approximately 40 pg/ml with intra-assay and interassay variations of 5% and 11% respectively.

Statistical analysis

After normality testing had confirmed that both CRF and urocortin levels were normally distributed, the data were expressed as means $\pm$ standard error (S.E.), and analysed for statistically significant differences by unpaired *t*-test. When two groups were compared, the paired *t*-test was used to compute statistical significance. Correlation between CRF and urocortin levels with the induction delivery interval (in min) was calculated by using the Pearson's correlation test. Statistical significance was assumed whenever $P < 0.05$.

Results

Table 2 shows the characteristics of post-term patients subjected to labour induction. The group of patients ($n = 9$) who achieved delivery within 12 h had the same parity, cervical length, Bishop score and presence of funnelling as the remaining patients ($n = 10$) who delivered after 12 h of prostaglandin exposure. Neither cervical length nor Bishop score were significantly correlated with plasma CRF or urocortin concentrations.

CRF and urocortin were measurable in all plasma samples evaluated. In detail, CRF concentrations were significantly ($P < 0.05$) lower (1.36 ± 0.3 ng/ml) whilst maternal plasma urocortin levels were unchanged (99.65 ± 4.1 pg/ml) in post-term compared

with term (CRF: 2.61 ± 0.3 ng/ml; urocortin: 91.1 ± 1.8 pg/ml) pregnancy (Fig. 1). However, maternal plasma CRF (1.52 ± 0.2 ng/ml) and urocortin (112.2 ± 4.8 pg/ml) levels were both significantly ($P < 0.05$ and $P < 0.001$ respectively) higher in post-term pregnancies delivered within 12 h of labour induction than in those that remained undelivered after 12 h of induced labour (CRF: 0.69 ± 0.2 ng/ml; urocortin: 88.33 ± 3.7 pg/ml) (Fig. 2), and were significantly correlated with the induction-delivery interval (CRF: $r = -0.676$, $P < 0.0015$; urocortin: $r = -0.783$, $P < 0.0001$) (Fig. 3).

Discussion

The onset of labour is timed by a complicated and not yet fully discovered mechanism, and is characterised by changes in many of the gestational tissues, which are prerequisites for the onset of labour. These include the completion of foetal organ maturation, ripening of the cervix and activation of the contractile apparatus of the myometrium (which is maintained in a quiescent state throughout pregnancy until this time) (2). In this regard, both CRF and urocortin have already been evaluated for their differing modulatory effects on uterine contractility. Whilst urocortin indirectly triggers a univocal myometrial response, i.e. activation of contractility (10, 11), CRF on the other hand promotes

Figure 1 Maternal plasma CRF (A) and urocortin (B) levels in patients at term and post-term pregnancy. * $P < 0.05$.

Table 2 Characteristics of the patients who succeeded or failed to deliver by the vaginal route within 12 hours of labour induction.

	Delivery within 12 h		<i>P</i> value
	Yes ($n = 9$)	No ($n = 10$)	
Age	28.7 ± 1.7	29.3 ± 1.9	NS
Parity	1.2 ± 0.5	1.2 ± 0.5	NS
Bishop score (total)	3 (2 – 5)	3 (2 – 5)	NS
Bishop score (dilatation + effacement)	2 (1 – 2)	2 (1 – 2)	NS
Cervical length (mm)	22.7 ± 1.0	23.2 ± 2.0	NS
Funnelling present	4 (47%)	3 (30%)	NS

NS, not significant.

Figure 2 Maternal plasma CRF (A) and urocortin (B) levels and induction-delivery interval: levels were significantly higher in patients who proceeded to delivery within 12 h from induction. * $P < 0.05$.

relaxation of the myometrium during most of pregnancy, but contraction in late pregnancy when the myometrial intracellular pathways have already been primed by uterotonic agents (11). Taken together, these data implicate CRF and urocortin as activators of multiple mechanisms that together contribute to the initiation of parturition and labour, and that also involve local events in the human placenta such as the induction of secretion of several uterotonic (3, 11, 12).

In the present study we found that levels of CRF were lower, whilst those of urocortin were unchanged in post-term compared with term pregnancies out of labour, suggesting a possible delay in the hormonal changes associated with the onset of labour, since concentrations of both neurohormones have been reported as significantly increased at term and preterm labour (2, 3, 12–14). This evidence suggests that in the presence of lower CRF secretion, the cascade of events that induces labour and signals the end of pregnancy is not activated, and therefore pregnancy will continue (14). Urocortin levels, however, were not reduced in post-term pregnancy; actually they were higher in the

Figure 3 Maternal plasma CRF (A) and urocortin (B) levels were significantly and inversely correlated to the induction-delivery interval (min).

subset of patients that responded rapidly to induction than in term gestation controls. This observation suggests that urocortin may not be as important in labour initiation as it is in sensitisation to labour inducers. In fact, we have previously shown that urocortin does not initiate contraction but greatly amplifies the contractile response of myometrial strips stimulated *in vitro* with prostaglandin (10).

The second result of the present study, relating to the higher levels of CRF and urocortin in pregnancies delivered within 12 h of labour induction than in those that remained undelivered, would support the idea that the combined effects of both neuropeptides are of relevance in predisposing a myometrial response to labour inducers (i.e. prostaglandins).

Whatever the role of circulating CRF and urocortin in post-term pregnancy, their sources warrant discussion. Previous work has shown that foetal urocortin levels are higher (13), whilst those of CRF are considerably lower (19, 20) than those in the mother, suggesting a placental source for CRF, and a foetal origin for urocortin levels in maternal blood. Further underlining this

difference is the evidence that placental CRF mRNA expression increases throughout gestation (21), whilst that of urocortin does not (22), and that maternal plasma CRF levels increase until term (3, 23), whilst urocortin concentrations are constant during gestation (24). Together, these findings led us to hypothesise the existence of a 'foetal' as well as a 'placental' clock (14) that, through the secretion of urocortin and CRF respectively, may determine the length of human gestation.

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