0021-972X/05/$15.00/0
Printed in U.S.A.

The Journal of Clinical Endocrinology & Metabolism 90(9):5361–5365

doi: 10.1210/jc.2005-0109

High Fetal Urocortin Levels at Term and Preterm Labor

P. Florio, M. Torricelli, L. Galleri, G. De Falco, E. Leucci, G. Calonaci, E. Picciolini, G. Ambrosini, E. A. Linton, and F. Petraglia

Departments of Pediatrics, Obstetrics, and Reproductive Medicine (P.F., M.T., L.G., G.C., E.P., F.P.) and Human Pathology and Oncology (G.D.F., E.L.), University of Siena, 53100 Siena, Italy; Department of Gynecological Science and Reproductive Medicine, University of Padua School of Medicine (G.A.), 35100 Padua, Italy; and Nuffield Department of Obstetrics and Gynecology, John Radcliffe Hospital, University of Oxford (E.A.L.), Oxford OX3 9DU, United Kingdom

Context: Placental urocortin has a role in the cascade of events leading to parturition by stimulating myometrial contractility and placental uterotonins secretion.

Objective: The objective of this study was to evaluate urocortin levels in maternal and fetal [umbilical cord artery (UCA) and vein (UCV)] plasma at term and preterm labor.

Design: The study design was a controlled cross-sectional study performed from November 2003 to June 2004.

Setting: This study was performed at the Division of Obstetrics and Gynecology, University of Siena (Siena, Italy).

Patients: Plasma samples were collected at term in the absence of labor (TNL; n = 27; 39.3 ± 0.1 gestational weeks), at term spontaneous vaginal delivery (TL; n = 24; 40.1 ± 0.2 gestational weeks), and at preterm labor (PTL; n = 19; 32.4 ± 0.4 gestational weeks). Changes in urocortin mRNA expression were also evaluated in placentas collected from TNL (n = 11), TL (n = 11), and PTL (n = 10).

Intervention: Urocortin levels were measured by specific RIA. Changes in placental mRNA expression were determined by real-time quantitative RT-PCR analysis.

Results: Maternal and UCA plasma urocortin levels were significantly ($P < 0.0001$ for all) higher in TL and PTL than in TNL. Furthermore, UCA concentrations were significantly ($P < 0.0001$ for all) higher than and correlated with maternal concentrations (TNL: r = 0.45; $P < 0.05$; TL: r = 0.959; $P < 0.0001$; PTL: r = 0.7719; $P < 0.0001$). UCV levels were significantly ($P < 0.001$) higher in TL and PTL than in TNL and were significantly ($P < 0.0001$ for all) higher than and significantly ($P < 0.0001$ for all) correlated with maternal values, but were significantly ($P < 0.0001$ for all) lower than and correlated with UCA values (TNL: r = 0.9548; $P < 0.0001$; TL: r = 0.927; $P < 0.0001$; PTL: r = 0.838; $P < 0.0001$). Placental urocortin mRNA expression did not differ among TNL, TL, and PTL samples.

Conclusions: Fetal urocortin secretion is increased in term and preterm labor. Whether these changes are a consequence rather than a cause of human parturition remains to be addressed. **(*J Clin Endocrinol Metab* 90: 5361–5365, 2005)**

PARTURITION RESULTS FROM a complex interplay of a variety of different maternal and fetal factors that act upon the myometrium to trigger molecular pathways involved in the development of coordinated uterine contractility (1). However, the molecular mechanisms driving the onset of human labor remain uncertain, although several key players have been identified. Among these players are the activated maternal and fetal hypothalamo-pituitary-adrenal axes, the primary function of which is to control the response of the body to stress (2). A key component of the stress axis is a larger family of stress-related peptides that includes corticotropin-releasing factor (CRF) and urocortin (3). Indeed, recent data have indicated a role for both CRF and urocortin in the regulation of smooth muscle contractility through binding to diverse CRF receptor subtypes (4). The evidence that CRF and urocortin stimulate the placental release of ACTH (5, 6) and the uterotonins, oxytocin (7) and prostaglandins (6, 8), suggests that both neuropeptides are involved in the cascade of events leading to parturition through the activation of more than one pathway.

CRF and urocortin are expressed in intrauterine tissues (human placenta, decidua, and fetal membranes) throughout pregnancy (9), but they are secreted with a different pattern, because maternal plasma CRF levels increase until term (10), whereas urocortin concentrations are constant during gestation (11), paralleling similar time courses of placental CRF (12) and urocortin (13) mRNA expression. With respect to parturition, it is well known that maternal levels of both CRF (10) and urocortin (14) are increased at term labor, and that women with preterm labor (PTL) have maternal plasma CRF levels significantly higher than those detected in normal pregnancy (15). However, no data are as yet available showing whether circulating urocortin levels differ according to the presence of spontaneous term and PTL.

Consequently, in the present study we evaluated the levels of urocortin in the maternal and fetal circulation as well as urocortin mRNA expression in placental tissues collected from women delivered by elective caesarean section at term and preterm.

Subjects and Methods

In this controlled cross-sectional study, we evaluated 70 women with singleton pregnancies who received perinatal care from November 2003

First Published Online June 14, 2005

Abbreviations: CRF, Corticotropin-releasing factor; PTL, preterm labor; TL, term spontaneous vaginal delivery; TNL, term in the absence of labor; UCA, umbilical cord artery; UCV, umbilical cord vein.

to June 2004 at the Division of Obstetrics and Gynecology, University of Siena (Siena, Italy), a tertiary clinical care center. Written informed consent was obtained from each pregnant woman, and the permission of the local human investigation committee was granted for the study. All pregnancies were dated by ultrasound, with measurement of the biparietal diameter, head circumference, femur length, and abdominal circumference, and their clinical characteristics are summarized in Table 1.

Urocortin levels were evaluated in maternal and fetal plasma collected at term (39.3 ± 0.1 gestational weeks) in the absence of labor (TNL; n = 27; after elective caesarean section due to previous uterine surgery), at term (40.1 ± 0.2 gestational weeks) spontaneous vaginal delivery (TL; n = 24), and at PTL (32.4 ± 0.4 gestational weeks) labor due to premature rupture of membranes (n = 19).

The exclusion criteria were multiple pregnancies, diabetes, hypertension, fetal anomaly, maternal or fetal infection, fetal growth restriction, cardiotocographic evidence of fetal distress, and an Apgar score at 1 min of less than 7.

Umbilical cord blood samples were collected [separately from umbilical cord artery (UCA) and vein (UCV)] immediately after fetus delivery and cord clamping and before placental detachment. Blood samples were drawn using a polypropylene syringe and a butterfly needle and then transferred to chilled tubes containing EDTA (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 and fetal plasma urocortin levels were measured using previously published methodology (14, 16), 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) antiserum at a 1:2,100 dilution and incubated for 40 h at 4 C. One hundred microliters of buffer containing approximately 25,000 cpm ^{125}I-labeled human urocortin-(1–40) were then added, and the tubes were incubated for an additional 6 h before the addition of preprecipitated sheep antirabbit second antibody, as previously described (14, 16). The specificity of the urocortin antiserum 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. Helen's 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] as well as with ACTH, sauvagine, and urotensin 1 (Sigma-Aldrich Corp., St. Louis, MO) and thyroglobulin. None of these molecules displayed significant cross-reactivity even at a high concentration (1 mg/ml).

Urocortin levels were measured in a blinded fashion in a single assay. The assay had a sensitivity of approximately 50 pg/ml, with intra- and interassay variations of 8% and 13%, respectively.

Placental tissues collection, RNA extraction, and cDNA preparation

Specimens of human placenta were collected from pregnant women delivered at term (n = 11 in the absence of labor due to elective caesarean section; n = 11 due to spontaneous vaginal delivery) and preterm (n = 10). Total RNA was extracted from frozen tissue samples using a commercially available kit (TRIzol, Invitrogen Life Technologies, Inc., Milan, Italy). Approximately 5 μg total RNA were subsequently treated with deoxyribonuclease (DNase I Set, Promega Corp., Milan, Italy). Quantification of total RNA was performed by measuring absorbance at OD_{260}.

TABLE 1. Summary of clinical data

	TNL	TL	PTL
No.	27	24	19
Parity	1.05 ± 0.3	1.2 ± 0.1	1.2 ± 0.5
Maternal age (yr)	28.7 ± 1.2	27.9 ± 1.1	28.6 ± 2.0
Wk at delivery	39.3 ± 0.1	40.1 ± 0.2	32.4 ± 0.4[a]
Birth weight (g)	3344 ± 66	4311 ± 56[a]	1902 ± 116[a]

[a] $P < 0.01$ *vs.* control group (by Newman-Keuls test).

The quality of total RNA was controlled by running 1.5% agarose gels buffered in 89 mM Tris, 89 mM boric acid, and 2 mM EDTA (pH 8.3) and was assessed as acceptable if strong and intact 28S rRNA and 18S rRNA bands were visible under UV light after staining with ethidium bromide. No bands of genomic DNA were observed in agarose gels after deoxyribonuclease treatment. cDNA synthesis from total RNA (1 μg) was carried out in a reaction volume of 20 μl containing 50 mM Tris-HCl (pH 8.3), 75 mM KCl, 3 mM $MgCl_2$, 10 mM dithiothreitol, 5 μM random hexamer primer, 2.7 mM deoxynucleoside triphosphate, and 10 U/μl SuperScript II reverse transcriptase (all reagents obtained from Invitrogen Life Technologies, Inc.). RNA was initially denatured at 85 C for 5 min. The reaction mixture was then added, and RT was performed at 42 C for 90 min. The reaction was stopped by denaturing the enzyme at 85 C for 15 min. The cDNA was immediately subjected to real-time quantitative RT-PCR. For each RNA sample, a parallel reaction tube was prepared as described above, but without reverse transcriptase (RT-negative control).

Real-time quantitative RT-PCR analysis

To quantify mRNA expression of urocortin, real-time quantitative RT-PCR (TaqMan PCR, Applied Biosystems, Weiterstadt, Germany) using an Opticon 2 (MJ Research, Bio-Rad Laboratories, Waltham, MA) was performed. All samples were run in duplicate on 96-well optical PCR plates (Applied Biosystems) with a TaqMan Universal PCR Master Mix (Applied Biosystems). Standard RNA preparations were included in every RT-PCR run. TaqMan probes for hypoxanthine phosphoribosyltransferase (assay identification no. Hs99999909_m1; GenBank mRNA no. NM_000194) and urocortin (assay identification no. Hs00175020_m1; GenBank mRNA no. NM_003353) were taken from the commercially available Assays on Demand (Applied Biosystems). All assays for the target sequences investigated were optimized to the universal PCR protocol of the manufacturer to investigate different target mRNAs on one plate. After initial denaturation for 10 min at 95 C, denaturation at the subsequent 40–50 cycles was performed for 15 sec at 95 C, followed by primer annealing and elongation at 60 C for 1 min. The $\Delta\Delta C_T$ method (17) was applied as a comparative method of quantification.

Statistical analysis

After normality testing had confirmed that urocortin levels were normally distributed, the data were expressed as the mean ± SE and analyzed for statistically significant differences by one-way ANOVA, followed by the *post hoc* Newman-Keuls multiple comparison test for multiple comparison. When only two groups were compared, the paired *t* test was used to compute statistical significance. Correlation between maternal and fetal as well as between UCA and UCV plasma urocortin levels was calculated using Pearson's correlation coefficient test. Statistical significance was assumed whenever $P < 0.05$.

Results

Maternal and fetal urocortin levels

Urocortin was measurable in all samples evaluated. In detail, maternal plasma urocortin levels were highest at term (133.0 ± 7.7 pg/ml) and preterm (132.2 ± 3.6 pg/ml) labor and were significantly ($P < 0.0001$) higher than those in patients delivered by elective cesarean section (86.8 ± 3.5 pg/ml; Fig. 1A). With respect to the fetal circulation, urocortin levels in the UCA were highest at term (196.5 ± 9.1 pg/ml) and preterm (192.9 ± 14.7 pg/ml) labor and were significantly ($P < 0.0001$) higher than in samples collected at TNL, *i.e.* after elective cesarean section (147.7 ± 4.5 pg/ml), significantly ($P < 0.0001$ for all) higher than those in the maternal circulation (Fig. 1A), and significantly correlated to maternal concentrations at TNL (r = 0.45; $P < 0.05$), at term (r = 0.959; $P < 0.0001$), and PTL (r = 0.7719; $P < 0.0001$; Fig. 1B).

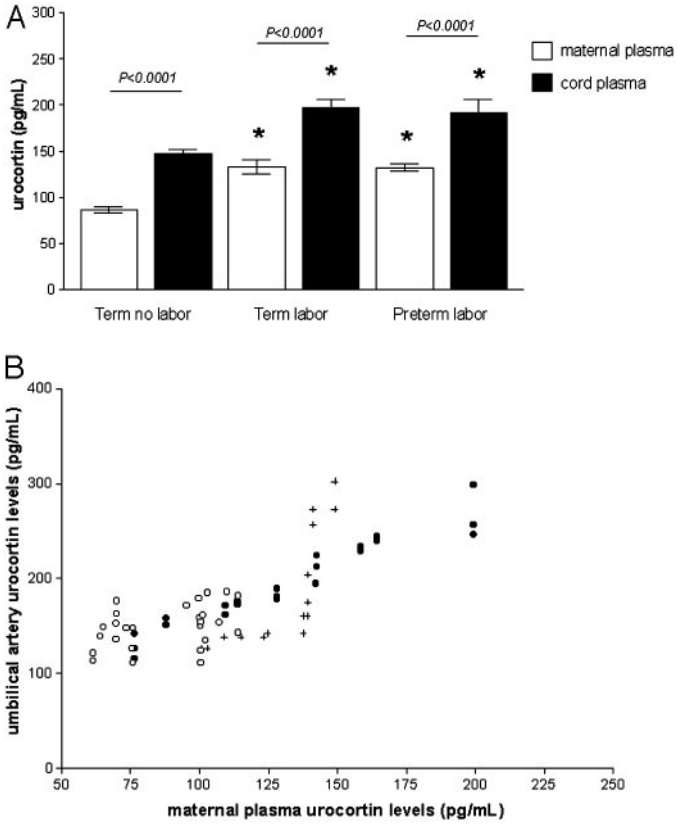

FIG. 1. A, Mean ± SE maternal (□) and fetal (■) urocortin levels at TNL, TL, and PTL. *, $P < 0.0001$ *vs.* TNL. B, Correlation between umbilical artery and maternal plasma urocortin levels at TNL (○; r = 0.45; $P < 0.05$), TL (●; r: 0.959; $P < 0.0001$), and PTL (+; r = 0.7719; $P < 0.0001$).

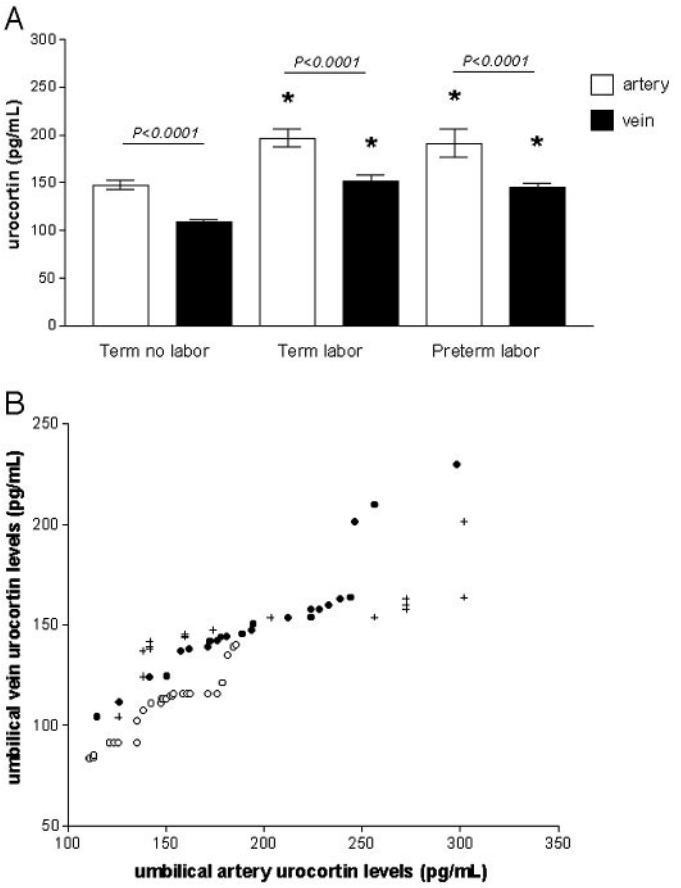

FIG. 2. A, Mean ± SE urocortin levels in UCA (□) and UCV (■). *, $P < 0.0001$ *vs.* TNL. B, Correlation between UCA and UCV urocortin levels at TNL (○; r = 0.9548; $P < 0.0001$), TL (●; r = 0.927; $P < 0.0001$), and PTL (+; r = 0.838; $P < 0.0001$)r.

In the UCV, urocortin levels were highest at term (151.75 ± 5.81 pg/ml) and preterm (144.9 ± 4.9 pg/ml) labor, significantly ($P < 0.0001$) higher than in samples collected after elective cesarean section (108.4 ± 3.1 pg/ml; Fig. 2A). In addition, in each condition evaluated, umbilical venous urocortin concentrations were significantly ($P < 0.0001$ for all) lower than (Fig. 2A) and correlated to umbilical arterial levels at TNL (r = 0.9548; $P < 0.0001$) and at term (r = 0.927; $P < 0.0001$) and PTL (r = 0.838; $P < 0.0001$; Fig. 2B). Finally, umbilical venous levels were significantly ($P < 0.0001$ for all) higher than and significantly ($P < 0.0001$ for all) correlated to maternal concentrations (TNL: r = 0.9114; TL: r = 0.9330; PTL: r = 0.8853; Fig. 3A).

Placental urocortin mRNA levels

As depicted graphically in Fig. 3B, no significant differences in urocortin mRNA expression were observed between samples collected after term labor, PTL, and elective caesarean section. The expression of urocortin mRNA was unequivocally detectable in the patient samples compared with RT-negative controls.

Discussion

The present study is the first to show that maternal and fetal plasma urocortin levels were increased at term and PTL compared with those after elective caesarean section. Because urocortin is a neuropeptide belonging to the CRF family (18) and is expressed by human syncytiotrophoblast throughout pregnancy (13, 19), we expected the highest levels to occur in the presence of PTL, higher than at term delivery, as previously reported for CRF (10). This was not the case, however. The present findings and the evidence that maternal plasma CRF levels increases until term (10), whereas urocortin concentrations are constant during gestation (11), and that placental CRF mRNA expression increases throughout gestation (12), whereas that of urocortin does not (13), lead us to suggest that the secretory pattern of urocortin differs from that of CRF. This hypothesis is also supported by the fact that fetal urocortin levels were higher, whereas those of CRF were considerably lower (20, 21), than those in the mother. Furthermore, we found that urocortin concentrations in the umbilical artery were higher than those in the corresponding vein, and that placental urocortin mRNA expression did not change with labor, whether term or preterm, thus indicating a release of urocortin from the feto-placental compartment into the maternal circulation.

The finding of significant veno-arterial and feto-maternal differences raises new questions about the source of urocortin in the fetal circulation. In the rat, urocortin mRNA and protein have been identified in the hypothalamus (22–25),

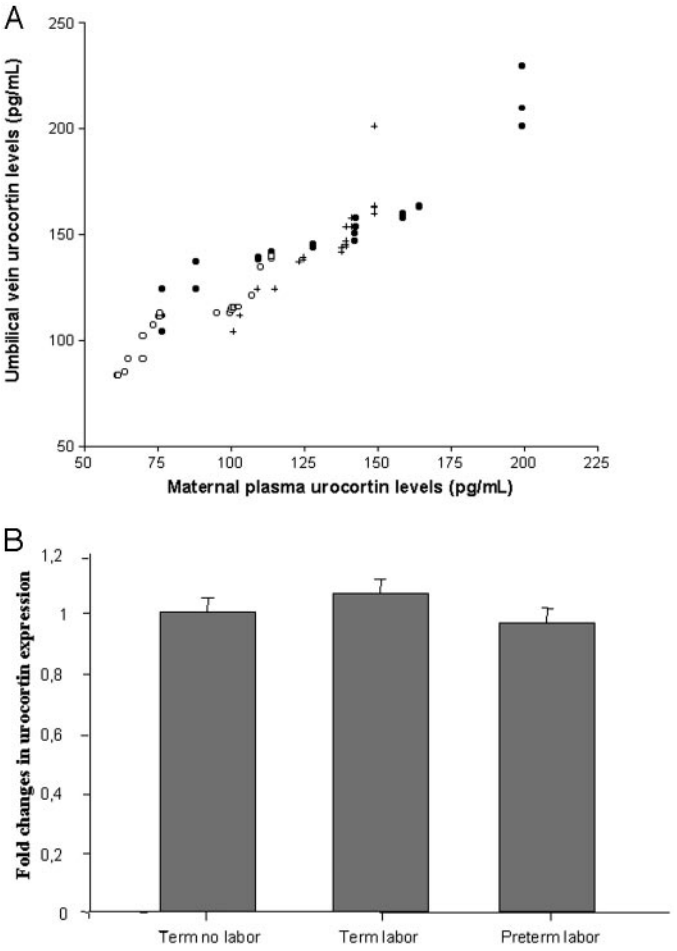

FIG. 3. A, Correlation between UCV and maternal plasma urocortin levels at TNL (○; r = 0.9114; $P < 0.0001$), TL (●; r = 0.9330; $P < 0.0001$), and PTL (+; r = 0.8853; $P < 0.0001$). B, Expression of urocortin mRNA in placental samples collected after TL, PTL, and elective cesarean section. Results of real-time quantitative RT-PCR demonstrate that urocortin mRNA expression does not differ in TL and PTL, and elective cesarean section tissues. TaqMan runs were performed with cDNA preparations reversibly transcribed in the same RT reaction from 1 μg total RNA in each case. The $\Delta\Delta C_T$ method was applied as a comparative method of quantification.

and despite the lack of evidence for urocortin mRNA or protein expression in either the human paraventricular nucleus of the hypothalamus or in the pituitary stalk (26), as occurs in the hypothalamus of the adult sheep (27), high levels of urocortin mRNA expression have been identified in the pituitary glands of humans (28), rats (29), and fetal sheep (30). Other putative relevant fetal sources may be the heart, kidney, and digestive system, because these organs, at least in adult animals, are able to express urocortin (31).

Whatever the source of urocortin in the fetal circulation, the role of the increased levels of the peptide at term and PTL warrants consideration. Indeed, the evidence that urocortin is an important regulator of pituitary ACTH output in several species (18, 32, 33) suggests that urocortin may not act solely as a traditional hypothalamic releasing peptide, but, rather, that urocortin produced locally in the pituitary might regulate ACTH output in a paracrine/autocrine manner. Fetal urocortin could be an additional contributor to the prepartum activation of the fetal HPA axis (1, 34), ensuring concurrent increases in circulating fetal concentrations of ACTH and cortisol and resulting in birth (1, 34).

Human labor is a physiological event involving an integrated series of uterine changes and may be regarded as both a release from the inhibitory effects of pregnancy on the myometrium and an active process mediated by uterine stimulants, so that at labor the uterus switches from quiescence to a state of coordinated contractility. Urocortin may have a role in regulating the molecular mechanisms underlying these complex physiological events, because it directly enhances myometrial contractility by augmenting the myometrial contractile response to prostaglandin $F_{2\alpha}$ and by activating the MAPK signaling pathways, which have been proposed to be involved in the regulation of myometrial contractility by uterotonins (35, 36), through the activation of the CRF-1α and -2β receptor subtypes (37). However, the possibility that the higher levels of urocortin measured as apparently coming from the fetus are the result of a degradation of some of the urocortin as it passes through the placenta could also be considered.

In conclusion, we found that urocortin levels were significantly higher in the fetal than in the maternal circulation, suggesting that in addition to the placenta (13, 19), a fetal source of the peptide exists. Because urocortin triggers myometrial contractility, stimulates ACTH release, and, in turn, is regulated by cortisol, its increased secretion at labor in the fetal circulation would support the role of the human fetus in sustaining hypothalamic-pituitary-adrenal axis activation, leading to the mechanisms of adaptations to the postnatal life (34). However, the possibility that increased urocortin levels at labor may be a consequence rather than a cause of human parturition should also be considered.

Acknowledgments

Received January 20, 2005. Accepted June 8, 2005.

Address all correspondence and requests for reprints to: Dr. Felice Petraglia, Department of Pediatrics, Obstetrics, and Reproductive Medicine, University of Siena, Policlinico Le Scotte, viale Bracci, 53100 Siena, Italy. E-mail: petraglia@unisi.it.

References

1. **Challis JRG, Matthews SG, Gibb W, Lye SJ** 2000 Endocrine and paracrine regulation of birth at term and preterm. Endocr Rev 21:514–550
2. **Orth DN** 1992 Corticotropin-releasing hormone in humans. Endocr Rev 13: 164–191
3. **Perrin MH, Sutton SW, Cervini LA, Rivier JE, Vale WW** 1999 Comparison of an agonist, urocortin, and an antagonist, astressin, as radioligands for characterization of corticotropin-releasing factor receptors. J Pharmacol Exp Ther 288:729–734
4. **Papadopoulou N, Chen J, Randeva HS, Levine MA, Hillhouse EW, Grammatopoulos DK** 2004 Protein kinase A-induced negative regulation of the corticotropin-releasing hormone R1α receptor-extracellularly regulated kinase signal transduction pathway: the critical role of Ser^{301} for signaling switch and selectivity. Mol Endocrinol 18:624–639
5. **Petraglia F, Sawchenko PE, Rivier J, Vale W** 1987 Evidence for local stimulation of ACTH secretion by corticotropin-releasing factor in human placenta. Nature 328:717–719
6. **Petraglia F, Florio P, Benedetto C, Marozio L, Di Blasio AM, Ticconi C, Piccione E, Luisi S, Genazzani AR, Vale W** 1999 Urocortin stimulates placental adrenocorticotropin and prostaglandin release and myometrial contractility *in vitro*. J Clin Endocrinol Metab 84:1420–1423
7. **Florio P, Lombardo M, Gallo R, Di Carlo C, Sutton S, Genazzani AR, Petraglia F** 1996 Activin A, corticotropin-releasing factor and prostaglandin $F_{2\alpha}$ increase immunoreactive oxytocin release from cultured human placental cells. Placenta 17:307–311

8. **Jones SA, Challis JR** 1990 Effects of corticotropin-releasing hormone and adrenocorticotropin on prostaglandin output by human placenta and fetal membranes. Gynecol Obstet Invest 29:165–168
9. **Florio P, Vale W, Petraglia F** 2004 Urocortins in human reproduction Peptides 25:1751–1757
10. **Florio P, Severi FM, Ciarmela P, Fiore G, Calonaci G, Merola A, De Felice C, Palumbo M, Petraglia F** 2002 Placental stress factors and maternal-fetal adaptive response: the corticotropin-releasing factor family. Endocrine 19:91–102
11. **Glynn BP, Wolton A, Rodriguez-Linares B, Phaneuf S, Linton EA** 1998 Urocortin in pregnancy. Am J Obstet Gynecol 179:533–539
12. **Frim DM, Emanuel RL, Robinson BG, Smas CM, Adler GK, Majzoub JA** 1988 Characterization and gestational regulation of corticotropin-releasing hormone messenger RNA in human placenta. J Clin Invest 82:287–292
13. **Florio P, Rivest S, Reis FM, Simoncini T, Martinelli P, Genazzani AR, Petraglia F** 1999 Lack of gestational-related changes of urocortin gene expression in human placenta. Prenat Neonat Med 4:296–300
14. **Florio P, Cobellis L, Woodman J, Severi FM, Linton EA, Petraglia F** 2002 Levels of maternal plasma corticotropin-releasing factor and urocortin during labor. J Soc Gynecol Invest 9:233–237
15. **Berkowitz GS, Lapinski RH, Lockwood CJ, Florio P, Blackmore Prince C, Petraglia F** 1996 Corticotropin-releasing factor and its binding protein: maternal serum levels in term and preterm deliveries. Am J Obstet Gynecol 174:1477–1483
16. **Florio P, Calonaci G, Severi FM, Torricelli M, Bocchi C, Fiore G, Linton EA, Petraglia F** 2004 Reduced maternal plasma urocortin concentrations and impaired uterine artery blood flow at human mid pregnancy. J Soc Gynecol Invest 12:191–194
17. **Livak KJ, Schmittgen TD** 2001 Analysis of relative gene expression data using real-time quantitative PCR and the 2(-delta delta C(T)) method. Methods 25:402–408
18. **Donaldson, CJ, Sutton SW, Perrin MH, Corrigan AZ, Lewis KA, Rivier JE, Vaughan JM, Vale WW** 1996 Cloning and characterization of human urocortin. Endocrinology 137:2167–2170
19. **Petraglia F, Florio P, Gallo R, Simoncini T, Saviozzi M, Di Blasio AM, Vaughan J, Vale W** 1996 Human placenta and fetal membranes express human urocortin mRNA and peptide. J Clin Endocrinol Metab 81:3807–3810
20. **Goland RS, Wardlaw SL, Stark RI, Brown Jr LS, Frantz AG** 1986 High levels of corticotropin-releasing hormone immunoactivity in maternal and fetal plasma during pregnancy. J Clin Endocrinol Metab 63:1199–1203
21. **Nodwell A, Carmichael L, Fraser M, Challis J, Richardson B** 1999 Placental release of corticotrophin-releasing hormone across the umbilical circulation of the human newborn. Placenta 20:197–202
22. **Bittencourt, JC, Vaughan J, Arias C, Rissman RA, Vale WW, Sawchenko PE** 1999 Urocortin expression in rat brain: evidence against a pervasive relationship of urocortin-containing projections with targets bearing type 2 CRF receptors. J Comp Neurol 415:285–312
23. **Kozicz, T, Yanaihara H, Arimura A** 1998 Distribution of urocortin-like immunoreactivity in the central nervous system of the rat. J Comp Neurol 391: 1–10
24. **Morin, SM, Ling N, Liu XJ, Kahl SD, Gehlert DR** 1999 Differential distribution of urocortin- and corticotropin-releasing factor-like immunoreactivities in the rat brain. Neuroscience 92:281–291
25. **Oki, Y, Iwabuchi M, Masuzawa M, Watanabe F, Ozawa M, Iino K, Tominag T, Yoshimi T** 1998 Distribution and concentration of urocortin, and effect of adrenalectomy on its content in rat hypothalamus. Life Sci 62:807–812
26. **Iino, D, Sasano H, Oki Y, Andoh N, Shin RW, Kitamoto T, Takahashi K, Suzuki H, Tezuka F, Yoshimi T, Nagura H** 1999 Urocortin expression in the human central nervous system. Clin Endocrinol (Oxf) 50:107–114
27. **Cepoi, D, Sutton S, Arias C, Sawchenko P, Vale WW** 1999 Ovine genomic urocortin: cloning, pharmacologic characterization, and distribution of central mRNA. Mol Brain Res 68:109–118
28. **Iino, K, Sasano H, Oki Y, Andoh N, Shin RW, Kitamoto T, Totsune K, Takahashi K, Suzuki H, Nagura H, Yoshimi T** 1997 Urocortin expression in human pituitary gland and pituitary adenoma. J Clin Endocrinol Metab 82: 3842–3850
29. **Wong ML, al-Shekhlee A, Bongiorno PB, Esposito A, Khatri P, Sternberg EM, Gold PW, Licinio J** 1996 Localization of urocortin messenger RNA in rat brain and pituitary. Mol Psychiatry 1:307–312
30. **Holloway AC, Howe DC, Chan G, Clifton VL, Smith R, Challis JR** 2002 Urocortin: a mechanism for the sustained activation of the HPA axis in the late-gestation ovine fetus? Am J Physiol 283:E165–E171
31. **Baigent SM, Lowry PJ** 2000 mRNA expression profiles for corticotrophin releasing factor (CRF), urocortin, CRF receptors and CRF-binding protein in peripheral rat tissues. J Mol Endocrinol 25:43–52
32. **Asaba, K, Makino S, Hashimoto K** 1998 Effect of urocortin on ACTH secretion from rat anterior pituitary in vitro and in vivo: comparison with corticotropin-releasing hormone. Brain Res 806:95–103
33. **Ozawa, M, Oki Y, Watanabe F, Iino K, Masuzawa M, Iwabuchi M, Yoshimi T** 1998 Effect of urocortin and its interaction with adrenocorticotropin (ACTH) secretagogues on ACTH release. Peptides 19:513–518
34. **Challis JR, Sloboda D, Matthews SG, Holloway A, Alfaidy N, Patel FA, Whittle W, Fraser M, Moss TJ, Newnham J** 2001 The fetal placental hypothalamic-pituitary-adrenal (HPA) axis, parturition and post natal health. Mol Cell Endocrinol 185:135–144
35. **Nohara A, Ohmichi M, Koike K, Masumoto N, Kobayashi M, Akahane M, Igekami H, Hirota K, Miyake A, Murata Y** 1996 The role of mitogen-activated protein kinase in oxytocin-induced contraction of uterine smooth muscle in pregnant rat. Biochem Biophys Res Commun 229:938–944
36. **Ohmichi M, Koike K, Kimura A, Masuhara K, Ikegami H, Ikebuchi Y, Kanzaki T, Touhara K, Sakaue M, Kobayashi Y, Akabane M, Miyake A, Murata Y** 1997 Role of mitogen-activated protein kinase pathway in prostaglandin $F_{2\alpha}$-induced rat puerperal uterine contraction. Endocrinology 138: 3103–3111
37. **Grammatopoulos D, Randeva H, Levine MA, Katsanou E and Hillhouse EW** 2000 Urocortin, but not corticotropin-releasing hormone (CRH), activates the mitogen-activated protein kinase signal transduction pathway in human pregnant myometrium: an effect mediated via R1α and R2β CRH receptor subtypes and stimulation of G_q proteins. Mol Endocrinol 14:2076–2091

JCEM is published monthly by The Endocrine Society (http://www.endo-society.org), the foremost professional society serving the endocrine community.