MINIREVIEWS

Role of urocortin in pregnancy: An update and future perspectives

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Abstract

The activities of corticotropin-releasing factor (CRF) and related peptides are mediated a number of receptors with seven transmembrane domains that are coupled to the Gs and Gq proteins. These receptors are known as CRF-Rs. *In vitro* studies have evidenced that urocortin (UCN) and CRF provoke an increase in the contractility of the uterus which is induced by endometrial prostaglandin F2a. Furthermore, through trophoblasts, it stimulates the secretion of adrenocorticotropic hormone (ACTH) and prostaglandin PGE2 and has a vasodilatory effect on the placenta. While it is well known that the placenta produces considerable quantities of CRF, several studies have, however, excluded that the placenta can generate significant quantities of UCN. In the short term, the human fetal adrenal gland produces more cortisol and dehydroepiandrosterone sulfate. The gestational tissues express UCN3 and UCN2 mRNA in cytotrophoblast and syncytiotrophoblast cells, while UCN2 is only to be found in the maternal and fetal vessels and amniotic cells. Nevertheless, gestational tissues express UCN2 and UCN3 differentially and do not stimulate placental ACTH secretion. In term pregnancies, maternal plasma levels of CRF and UCN are lower than at the beginning of pregnancy and are correlated to labor onset. Conversely,

they do not decrease in post-term pregnancies. This evidence would seem to indicate that the fine-regulated expression of these neuropeptides is important in determining the duration of human gestation. In this scenario, low concentrations of UCN in the amniotic fluid at mid-term may be considered a sign of predisposition to preterm birth.

Key words: Urocortin; Corticotropin-releasing factor; Obstetrics; Gynecology; Inflammation

Core tip: Low concentrations of urocortin (UCN) in the amniotic fluid may be a sign of predisposition to preterm birth, since it correlates with a pro-inflammatory state. UCN is present at higher serum concentrations in women with endometriomas. Therefore, the measurement of UCN could be useful in distinguishing between endometriosis and other benign ovarian cysts. The therapeutic treatment with UCN decreases serum levels of proinflammatory cytokines in several experimental situations, so it is plausible that the same effects may occur in different obstetric and gynecological diseases in which inflammation plays a key role in the onset and progression of the disease.

INTRODUCTION

The urocortin (UCN) is a molecule composed of 40 amino acid residues belonging to the family of peptides related to corticotropin-releasing factor (CRF). Accumulating evidence suggests a key role for UCN in several physiological as well as pathological obstetric conditions. Nevertheless, so far no robust data are available in current literature which can be helpful to elucidate its possible therapeutic application(s). Considering these assumptions, the aim of this paper was to collect data about this topic in order to review the role of UCN during pregnancy. The MEDLINE data base between 1970 ± 2016 was searched by using the terms UCN combined with each of the following terms: Gynaecology, obstetrics, delivery, birth, placenta, and uterus. The relevance of articles was screened on the basis of information in the title and abstract.

BIOCHEMICAL AND GENETIC ASPECTS

The activation of CRH receptors type 1 (CRH-R1) by CRH or UCN stimulates multiple G proteins which influences various signaling cascades in a tissue-specific manner. In human myometrium and human embryonic kidney, the binding of UCN to CRH-R1α receptors activates both the Gs and Gq. These activate the adenylyl cyclase/protein kinase A and the phospholipase C/protein kinase C and ERK1/2 signaling pathways, respectively[1].

In this family, UCN1, UCN2 and UCN3 have been identified. UCN1 has a high affinity for both CRHR1 and CRHR2receptors. Human UCN1 mRNA is present in different central nervous system cells and various peripheral tissues including placenta, decidua, fetal membranes, endometrium, heart and gastrointestinal tract. UCN2 is mainly expressed in the heart. UCN2 has a number of various cardiovascular effects and due to fact it protects the myocardium against ischemic injury, it has provoked considerable interest recently. UCN2 also activates mitochondrial ATP-sensitive potassium channels and also blocks the opening of the mitochondrial permeability transition pore.

UCN3 was expressed in the pancreatic beta cells during mice embryonic development. UCN3 can be secreted from pancreatic alpha and beta cells and may have possible paracrine and/or autocrine effects in the islet. Its potent biological action is mediated by CRF receptor type 1 (CRF-R1) and receptor type 2 (CRF-R2), causing adrenocorticotropic hormone (ACTH) release, vasodilation[2], increased cardiac inotropism, reduction of vascular permeability[3], appetite suppression[4] and increased secretion of atrial natriuretic peptide and brain natriuretic peptide[5]. The UCN sequence is 45% homologous to that of CRF[6].

The human UCN consists of 124 amino acids, 80 of which form the "precursor peptide". The amino acids Arg-Arg in position 81-82 and Lys-Gly in position 123-124 undergo a process of proteolysis, constituting respectively the site of detachment from the precursor and the C-terminal sequence. The UCN binds to the same protein in plasma, the CRF-BP (CRF-binding protein) that carries the CRF. The actions and the tissue distribution of CRF-BP have led to the hypothesis that this protein may modulate the action of UCN in different time frames and in different parts of the brain. UCN3 mRNA expression is found in areas of the brain including the hypothalamus, amygdala, and brainstem, but is not evident in the cerebellum, pituitary, or cerebral cortex. The inactivation of CRF-BP induces an increase of CRF and free UCN[7]. The UCN gene is located on the short arm of chromosome 2 (2p23-p21). The gene structure is similar to that of CRF, articulated on two exons and an intron. The coding region is located entirely on the second exon. Moreover, the UCN and CRF have common sites of transcription initiation, such as the TATA-box, the CRE (cAMP response element), GATA binding sites and C/EBP[8]. Conversely, the regional distribution of immunoreactive UCN has been studied with radioimmunoassay techniques in the human brain and is partly different from that of the CRF, although

it has been demonstrated that the UCN mRNA is expressed in every brain region[9]. Indeed, UCN has been found in several brain regions, including the hypothalamus, bridge, cerebral cortex and cerebellum, whereas CRF is present in higher concentrations in the frontal and parietal cortex and the hypothalamus, while its concentration at the pontine level is minimal. CRF activity and their related peptides are mediated by receptors with seven transmembrane domains that are coupled to the G protein[10,11]. Compared to the CRF, the UCN binds with six times greater affinity to CRF-R1 and with forty times greater affinity to CRF-R2[12]. Another mechanism of UCN action is its effect on calcium channels. In mouse spermatogenic cells, UCN has been shown to act by reducing L-type calcium currents of acute isolated cardiac myocytes and T-type calcium currents through the inhibition of the calcium channel instead of binding to its CRF-R2. Secondly, UCN may also reduce intracellular calcium through the inhibition of calcium channels in vascular smooth muscle cells. In this way, UCN could increase the gene expression of ATP-sensitive potassium channels[13]. As already mentioned, elevated levels of UCN are correlated with the reduction of appetite and blood pressure through an increase in cardiac contractility, activity and anxiety[14-16].

The human cardiovascular system also expresses high numbers of CRF receptors. Therefore, both UCN2 and UCN3 have been found to produce potent, sustained, direct and endothelium-independent vasodilating effects in an *in vitro* human internal mammary artery model of endothelin-1 induced constrictions. UCN1 also produced endothelium-dependent vasodilating effects in this model, which were putatively mediated (unlike *in vivo* rodent studies) by nitric oxide and, downstream, by the cyclic guanine 3',5' monophosphate-dependent stimulation of calcium-activated K^+ channels in vascular smooth muscle.

The NO/cGMP signaling pathway seems to be important in vascular adaptation and placental physiology during pregnancy and labor because it mediates the vasodilatory effects of agonists in resistance vessels, especially the stem villous arterioles, and this helps maintain the low vascular resistance of the fetoplacental circulation. There is considerable evidence which indicates that this NO/cGMP pathway mediates the vasodilatory actions of CRH and UCN in human fetoplacental circulation[14,17]. UCN also correlates with the onset of labor, spontaneous abortion and pre-eclampsia[14]. Endometrium, myometrium, ovaries and/or placenta are also known to express CRF and UCN[16,18].

IMPLICATIONS IN OBSTETRICS DURING PREGNANCY

Reproductive UCN
Evidence that the concentration of free CRF increases during pregnancy and that CRF increases placental vasodilation and myometrial contractility has led several researchers to investigate whether UCN has a similar role during gestation. *In vitro* studies have demonstrated that both UCN and CRF increase uterine contractility induced by the endometrial prostaglandin F2a. This mechanism is probably due to the accelerated procontractile effect of PGE2 by up-regulating the expression of PGE2 receptor and by increasing the prostaglandin alfa receptor. Furthermore, it stimulates ACTH and prostaglandin PGE2 secretion by the trophoblast and has a vasodilatory effect on the placenta. In addition to stimulatory effects on prostaglandin production, UCN also up-regulated myometrial expression of proinflammatory cytokines *via* CRH-R2. A positive feedback loop between UCN and inflammatory cytokines therefore probably exists because UCN expression was increased by the inflammatory stimulus tumor necrosis factor alpha, probably through NF-κB signaling[18]. Moreover, UCN decreases placental gene and protein expression of 15-hydroxyprostaglandin dehydrogenase, a PGE metabolizing enzyme, an effect that is reversed by selective CRH-R2 antagonist astressin. These effects echo the stimulatory effect of UCN on prostaglandin production. All of these effects are inhibited by astressin, a CRF receptor antagonist. While there is a substantial similarity in action of CRF and UCN regarding myometrial contractility and placental functions, similar relationships were not observed when investigating the ability of the placenta to produce UCN[19]. It has already been demonstrated that the placenta synthesizes considerable quantities of CRF, but studies have shown that the placenta does not produce significant quantities of UCN. This excludes the possibility that greater amount of free CRF present in circulation during pregnancy may be a result of a dislocation of the CRF from CRF-BP by UCN[20]. On the other hand, the possible role of UCN in ovarian steroidogenesis is still far from being completely elucidated.

Ovarian cycle
Immunohistochemical methods were adopted to detect UCN1 in both granulosa and theca cells of dominant and non-dominant follicles, as well as in atretic follicles[21]. UCN1 was also found in luteinized granulosa and thecal cells in the mid- and late-phase corpus luteum[21]. Synthesized locally, it acts on steroidogenic luteal cells, in particular, during luteal regression, through the CRF-R[21]. Immunohistochemistry methods also revealed that mRNA levels of CRF and CRF-R1 were significantly higher in the regressing corpus luteum compared to either the mid-luteal phase or pregnant corpus luteum[21]. However, no significant difference was revealed between the expression of these genes in the mid-luteal phase of the menstrual cycle and early pregnancy[21,22]. Nevertheless, no evidence was found concerning the potential role of UCN 2 and 3 in the ovary in those studies[21,22].

Menstrual cycle
During the menstrual cycle, UCN mRNA has been shown

to be expressed in endometrial epithelial and stromal cells during both endometrial phases (proliferative and secretory phase)[23]. The highest concentrations of UCN1 and mRNA were found in the secretory phase, while they were higher in the late phase compared to the early secretory phase[24].

Placental and myometrial UCN

Placental and myometrial UCN2 may play a role in the endocrine-inflammatory processes of parturition, thus representing a potential target for treating inflammation-induced obstetric complications[25]. In the short term, the human fetal adrenal gland increases the production of cortisol and dehydroepiandrosterone sulfate (DHEA-S). DHEA-S, which acts as a substrate for the production of placental estrogen, induces important changes involved in childbirth. CRF, UNC, ACTH stimulate all elements of the pathway of DHEA-S and also activated synthetic CRF-R1. The consequential increase in levels of DHEA-S may be used for the synthesis of estrogens in the placenta and contribute to the process that leads to birth[26]. The estradiol E2 increases the activity of the promoter UCN *via* ER-, and decreases the activity of the human UCN promoter through ER-α. There is evidence that estrogens exert a direct transcriptional regulation and differential gene UCN[27]. The placenta, decidua and fetal membranes express mRNA UCN2-UCN3, localized in cytotrophoblast and syncytiotrophoblast cells, while only in the maternal and fetal vessels and amniotic cells can UCN2 be found. Gestational tissues differentially express UCN2 and UCN3 and do not stimulate the secretion of placental ACTH[28].

UCN and delivery

In term pregnancies, maternal plasma levels of CRF and UCN are lower and correlated to labor onset. Conversely, they do not decrease in post-term pregnancies (when the labor did not physiologically occur). The fine-regulated expression of these neuropeptides would, therefore, seem to be important in determining the length of human gestation[29]. In this view, low concentrations of UCN in the amniotic fluid at mid-term may be considered a sign of predisposition to preterm birth[30].

Furthermore, placental CRF ant its receptors are highly expressed during the premature rupture of membranes (pPROM) with chorioamnionitis, suggesting that placental expression of stress-related pathways is activated in infective processes[31]. Interestingly, a report in a recent paper[32] illustrates the results of a cohort study which was carried on pregnant women at a gestational age of 28-36 wk. The subjects were admitted to a labor ward with spontaneous preterm labor. A blood sample was obtained from all participants and serum UCN was measured. The women were monitored up to delivery in order to compare serum UCN in women with preterm delivery with those delivering at term (37 wk or more). This study demonstrates that serum UCN cannot be used to differentiate between women who delivered preterm and women with signs of preterm labor. Furthermore, UCN1 concentrations in midtrimester amniotic fluid were analysed in 22 pregnant women with preterm deliveries and 45 women who delivered at term using an enzyme-linked immunosorbent assay. This study[33] indicates the possibility that low UCN1 concentrations in midtrimester amniotic fluid may be used as an indicative marker of preterm birth.

UCN and preeclampsia

An investigation was also carried out to study the possible role of the molecule in the prediction of preeclampsia. Syncytiotrophoblast cells express UCN1 during pregnancy and this has been found to provoke the vasodilation of placental vascular tissue through paracrine or endocrine mechanisms[34,35]. Vascular endometrial cells express CRF-R2 and this makes UCN a strong vasodilator[36]. Pregnancies characterized by abnormal placental function (*e.g.*, preeclampsia) have high maternal plasma CRF levels and reduced CRF-R1 expression[37]. This is why changes of this kind may lead to abnormal vascular resistance and preeclampsia[38,39]. In two interesting papers[40,41] it was demonstrated that CRF, UCN1 and UCN2 may positively regulate the placental pathway of nitric oxygen (NO)/cGMP, thus provoking a poorly perfused feto-placental unit, dysregulation of the vascular resistance balance and, finally, preeclampsia.

One interesting paper reported that placental UCN2 and UCN3 expression are sensitive to O_2 tensions and mediated by HIF-1α. During early pregnancy, UCN2/UCN3 could influence the proliferation of trophoblast and the establishment of pregnancy. In preeclamsia placentae, the increased expression of both peptides possibly indicate a response to oxidative stress[42]. Finally, human endometrium expresses both UCN and CRF, CRF-R1 and CRF-R2. The activation of CRF-R1 inhibits cell development and the proliferation of a line of tumor cells that derive from the human endometrium. Furthermore, it has been suggested that the signaling pathway of UCN is involved in the tumorigenesis of different tissues[43]. In fact, increased numbers of highly activated mast cells were observed in peritoneal endometriosis tissue. Affected tissue was also found to stain strongly for CRH or UCN, which indicates they might be associated with activated mast cells. These processes could play a role in fibrosis, inflammation, low fertility or spontaneous abortions associated with endometriosis. UCN increases in women with endometriomas, and measuring it might be of use to differentiate between endometriosis and other benign ovarian cysts[44].

Data from animal studies which indicate possible therapeutic applications

Gonzalez-Rey *et al*[22] refers, in a study conducted in 2006, to the therapeutic effects of UCN and adrenomedullin (AM) on the colonic mucosa of mice. The use of

Table 1 Corticotropin-releasing factor, urocortin, corticotropin-releasing factor receptor 1 and 2 actions during pregnancy and labor

Peptide	Actions during pregnancy and labor
CRF	It is present in the syncytiotrophoblasts and intermediate trophoblasts during early implantation, and allows the necessary dilation of uterine and placental vessels
	The circulating concentration of CRF increases progressively during pregnancy
	At the end of pregnancy, the levels of CRF-binding protein fall dramatically, so the concentration of free CRF increases: this causes the stimulation of uterine smooth muscle contractions and the triggering of labor
UCN	UCN stimulates the secretion of ACTH and prostaglandin PGE2 by the trophoblast and has a vasodilatory effect on the placenta
	It induces uterine contractions by increasing myometrial contractile response to prostaglandins
	The levels of circulating UCN are higher during preterm labor, suggesting a key role of this peptide in triggering this condition
CRF-R1	During early implantation, the binding of CRF to CRF-R1 induces the expression of apoptotic FasL on invasive extravillous trophoblast and maternal decidual cells, allowing the physiological immune tolerance at the fetal-maternal interface
	It is strongly upregulated in the myometrium and fetal membranes during labor
CRF-R2	It is expressed in cultured extravillous trophoblasts at both mRNA and protein levels
	Its interaction with CRF and UCN may inhibit angiogenesis during early placentation

ACTH: Adrenocorticotropic hormone; CRF: Corticotropin-releasing factor; UCN: Urocortin; CRF-R1: Corticotropin-releasing factor receptor 1; CRF-R2: Corticotropin-releasing factor receptor 2.

UCN or AM considerably reduced the mRNA expression of inflammatory cytokines (TNF-α, IFN-γ, IL-6, IL-1α, IL-1β, IL-12, IL-18, IL-17, IL-15), macrophage migration inhibitory factor, chemokines (RANTES), macrophage inflammatory protein (MIP-1α, MIP-1β, MIP-3β), monocyte protein (MCP-1, MCP-3), inducible protein (IP-10 and MIP-2), and chemokine receptors (CCR-1, CCR-2, CCR-3, CCR-5 and CCR-7) in the colonic mucosa of pathological rats. Furthermore, after the two points had been treated with AM/UCN there was a rise in the levels of the anti-inflammatory cytokine IL-10 and receptors CCR-4 and CCR-8. *In vitro*, lymphoid peripheral mononuclear cells, that had been isolated from mice treated with AM or UCN, reduced their levels of pro-inflammatory factors (TNF-α, IL-6 and MIP-2). This suggests that UCN/AM administers the deactivation of the inflammatory response of the colonic mucosa. Treatment with UCN/AM provoked a drop in the serum levels of proinflammatory cytokines TNF-α, IL-1β, IL-6, and MIP-2 and ASA, which is a hepatic acute phase protein involved in inflammatory tissue damage. The therapeutic effect of UCN and AM works by reducing the local and systemic levels of a wide range of inflammatory mediators, including cytokines, chemokines and the acute phase serum amyloid protein A. UCN and AM could be utilized for therapeutic septic shock, also in combination with other immunomodulatory agents. Alternatively, they could be used together with other anti-inflammatory factors in other therapies[45]. UCN is not currently commercially available for administration despite its promising role in reducing the inflammatory phenomena typical of several obstetrics and gynecological diseases. These interesting considerations could lead to its use in clinical practice if these data are confirmed by further studies.

CONCLUSION

UCN plays a significant role in human reproduction influencing the mechanisms of steroidogenesis in the ovary, the maintenance of placental function and labor (summarized in Table 1). Low concentrations of UCN in the amniotic fluid may be a sign of predisposition to preterm birth, since it correlates with a pro-inflammatory state. UCN can be found at higher concentrations in women with endometriomas, and measuring it could be important in differentiating between endometriosis and other benign ovarian cysts. The therapeutic treatment with UCN and AM decreases serum levels of proinflammatory cytokines in several experimental situations, so it is plausible that the same effects may occur in different obstetrics and gynecological diseases in which inflammation plays a key role in the onset and progression of the disease. Nevertheless, more pre-clinical studies are needed which may clarify the possible therapeutic effect(s) of UCN and its side effect(s), before hypothesizing its possible role in clinical practice.

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