Paracrine regulation of endometrial function: interaction between progesterone and corticotropin-releasing factor (CRF) and activin A

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Abstract

Under the influence of ovarian steroid hormones, endometrial cells are able to produce a wide variety of growth factors and peptide hormones that are believed to promote: (1) physiological growth and differentiation during the endometrial cycle; (2) decidualization, an essential preparative event for establishment of pregnancy; and (3) pathological growth and differentiation in endometriosis and cancer. Among the local factors produced by the human endometrium, corticotropin-releasing factor (CRF) and activin A have been evaluated in terms of localization and effects. CRF is a neuropeptide expressed by the epithelial and stromal cells of the human endometrium in increasing amounts from the endometrial proliferative to the secretory phase. CRF expression also increases in the pregnant endometrium, from early in the pregnancy until term. CRF-type 1 receptor mRNA is only expressed by stromal cells. Progesterone induces CRF gene expression and release from decidualized cells and CRF decidualizes cultured stromal endometrial cells. Urocortin, a CRF-related peptide, has been identified in endometrial epithelial and stromal cells, and its function is still under investigation. Activin A is a growth factor expressed in increasing amounts throughout endometrial phases by both epithelial and stromal cells. This growth factor is secreted into the uterine cavity with higher levels in the secretory phase. Maternal decidua expresses activin A mRNA in increasing amounts from early pregnancy until term. Human endometrium also expresses activin-A receptors and follistatin, its binding protein. Activin A decidualizes cultured human endometrial stromal cells (an effect reversed by follistatin) and modulates embryonic trophoblast differentiation and adhesion. Activin A is expressed in endometriosis and endometrial adenocarcinoma.

Keywords: Decidualization; Endometrium; Endometriosis; Endometrial cancer; Implantation; Placenta

1. Introduction

The human endometrium undergoes morphological and functional changes during the menstrual cycle, in order to prepare the local environment for blastocyst implantation, to establish pregnancy, and to regulate trophoblast invasion when pregnancy is achieved. In fact under the influence of ovarian steroid hormones, throughout the menstrual cycle endometrial cells differentiate to decidual cells, which are able to produce a wide variety of growth factors and peptide hormones. Thus, despite the changes that are achieved under the influence of ovarian steroid hormones, the growing belief is that steroid hormones also trigger local expression and synthesis of several proteins that are upregulated or induced throughout the menstrual cycle phases and in early pregnancy. These locally expressed peptides/proteins participate in paracrine signaling to other cell types, in order to locally modulate the endometrial functions; their dysfunction may initiate pathological conditions, such as endometriosis and endometrial adenocarcinoma.

In the present article, the endometrial expression, synthesis and putative roles of corticotropin-releasing factor (CRF) and activin A will be reviewed.

2. CRF in human endometrium

CRF is a 41-amino acid neuropeptide synthesized in the paraventricular hypothalamic nucleus and is involved in the regulation of the hypothalamus-pituitary-adrenal (HPA) axis response to stress via its induction of proopiomelanocortin (POMC) gene expression, as well as its
stimulation of ACTH [1]. CRF exerts its effects by bind-
ing to plasma membrane receptors that are coupled to Go protein and adenylate cyclase. To date, two distinct CRF receptor genes have been identified—CRF-R1 and three splice variants of the CRF-R2 (α, β, and γ), widely dis-
tributed throughout the body—thus suggesting that CRF and CRF-related ligands may play important roles in the physiology of several tissues.

CRF is also expressed in many tissues outside of the cen-
tral nervous system and is mainly present in the reproduc-
tive tissues, such as the human placenta and the endometrium. With respect to the endometrium, epithelial, and stromal cells both express CRF mRNA, and the concentrations of immunoreactive CRF in biopsies from human endometrium are significantly higher in the secretory than in the prolif-
erative phase [2]. CRF is detectable immunocytochemically in cultured decidual cells isolated from term decidua, but also in stromal cells decidualized in vitro by treatment with a mixture of medroxyprogesterone acetate (MPA), estradiol, and relaxin [3]. Furthermore, the CRF expression in human decidua is higher at term than in first and second trimesters of gestation [3–5], and the size of the CRF transcript in hu-
man endometrium appears to be identical to that found in the hypothalamus and the placenta.

In addition to the endometrium, the CRF transcript and its peptide product have been detected in the normal non-pregnant myometrium [6]. With respect to CRF re-
ceptors, both endometrial stromal cells [7] and the human myometrium are able to express the principal receptor for CRF ligand, the CRF receptor type 1 [8].

The CRF action is regulated by the CRF binding pro-
tein (CRF-BP), a 37-kDa protein of 322 amino acids, that blocks CRF-induced pituitary ACTH release [9]. CRF-BP is expressed in maternal decidua [10] but to date it is not clear whether the non-pregnant endometrium expresses and secretes CRF-BP throughout the menstrual cycle.

2.1. Putative role of CRF in normal human endometrium

Acting on different functions CRF may play some roles in endometrial physiology (Fig. 1).

In vitro, CRF induces decidualization of endometrial stro-
mal cells, as indicated by their morphological changes from elongated fibroblast-like cells into larger and round cells and by the release of prolactin in the medium [11]. These ef-
ffects are significantly enhanced when the cells are coincu-
bated with CRF and medroxy progesterone acetate (MPA);
in stromal cells CRF may mediate, via the CRF-R1 receptor, the cAMP-dependent pathway involved in the decidualizing effect of progesterone [12].

Furthermore, progesterins directly stimulate the expression of endometrial CRF in a cAMP-dependent manner [12]. It has been suggested that the expression of CRF by human en-
dometrium may have a role in modulating the inflammatory phenomena taking place in the endometrium, and in mod-
ulating the local immune function. In vivo, CRF has local inflammatory actions, and its immunoneutralization attenu-
ates the inflammatory response [13]. Cytokines play a very
important role in reproduction, i.e. embryo implantation, en-
dometrial development and trophoblast growth, and differ-
entiation by modulating the paracrine dialogue between the embryo and the endometrium [14].

The transcription of CRF in human endometrium is reg-
ulated by cytokines [15], as interleukin 1 (IL-1) and IL-6 both exert a strong stimulatory effect on its endometrial expression. With respect to the putative role on the local

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**Fig. 1.** Schematic summary of the putative roles played by CRF and activin A in human endometrium. Progesterone stimulates the expression and secretion of CRF and activin A in the human endometrium. In turn, CRF and activin A modulate several functions in the endometrium from cell differentiation until implantation and embryonic trophoblast growth and adhesion.
immune system at the feto-maternal interface, it is known that
CRF modulates endometrial expression of Fas Ligand (FasL) [16], a proapoptotic cytokine involved in the
immune privilege status of certain cell populations; for
example, when a FasL-expressing cell binds a Fas bearing
immunocyte, it triggers its death by apoptosis [17].

In fact, CRF directly improves rat blastocyst implanta-
tion and modulates early maternal tolerance, as it increases
the FasL expression and promotes the apoptosis of activ-
tated T lymphocytes. Female rats treated with antalarmin,
a CRF-R1 antagonist, showed a marked decrease in im-
plantation sites and live embryos and diminished endome-
trial FasL expression. These findings have suggested that
the blockade of CRF-R1 has an anti-mediation effect and
that the locally produced CRF promotes implantation and the
maintenances of early pregnancy by killing activated
T cells [16].

The administration of an anti-CRF antibody during the
early stages of rat pregnancy inhibits the attachment of the
embryo to the uterine implantation sites: intraperitoneal in-
jections of CRF antibodies at day 2 of pregnancy decrease
the number of fetuses within the uterus by 60%.

Another putative local action of CRF could be the regu-
lation of the endometrial vascular tone, as CRF may exert
vasodilatory effects [18], and it is known that the control
of the endometrial microvasculature tone is an important
key event in the mechanisms leading to implantation [19].
CRF can cause vasodilatation within the uterine cavity
indirectly through nitric oxide (NO) synthesis as the va-
sodilatory responses to CRF are attenuated by the NO
synthase inhibitor [20] and directly act on its receptors
[21].

2.2. CRF in endometrial cancer

Gene expression and biosynthesis of CRF and the ex-
pression of CRHR1 mRNA have also been demonstrated
in a tumor cell line derived from human endometrium,
namely adenocarcinoma Ishikawa cells [4]. CRF is able to
inhibit, in a dose- and time-dependent manner, the tumor
cell growth of human endometrial adenocarcinoma cells
via CRF-R1-mediated activation of cAMP-PKA (protein
kinase A) pathway [22]. The tumor produced by endome-
trial tumor cells may be secreted and acts locally to inhibit
cell growth via the activation of the R1 receptor subtype.
In fact CRF-R1 in physiological target tissues involves an
increase in intracellular cAMP production and the subse-
quent activation of PKA. The latter event did not cause
apoptosis in Ishikawa cells. In the absence of cytotoxic phe-
nomena, it may be postulated that CRF influences the cell
cycle by promoting differentiation in Ishikawa cells. The
activity exerted by CRF on differentiation of normal en-
dometrial cells seems to be translated into antiproliferative
action on endometrial-derived malignancies, and is medi-
ated through the expression of CRF receptors in neoplastic
cells [22].

3. Endometrial expression of urocortin

Urocortin is a 40-amino acid peptide belonging to the CRF
family, sharing 45% sequence homology with CRF [23],
binding CRF receptors with high affinity and increasing the
release of ACTH from dispersed rat anterior pituitary cells
[24]. It is expressed in different human reproductive tissues,
such as the ovary [25], the placenta, and the fetal membranes
[26], but also in pregnant [26,27] and non-pregnant human
endometrium [28]. In fact, urocortin mRNA is expressed by
stromal and epithelial endometrial cells [28] and by early
and term decidua [29]. Furthermore by immunohistochem-
istry urocortin has been localized in the endometrial luminal
and glandular epithelial cells, in stromal cells of both pro-
liferative and secretory phases, as well as in the myometrium
and the vascular smooth muscle cells.

The local expression of urocortin poses a new question on
the role played by the peptide in the human endometrium.
In fact, urocortin could be involved in the control of my-
ometrial tone [30], as well as in the modulation of endome-
trial inflammatory and vascular changes. In fact urocortin is
a highly potent vasodilator [31], and it may act locally in
the decidua to regulate uteroplacental vasoactivity.

Urocortin, such as CRF, may be involved in several en-
dometrial functions. Urocortin stimulates pituitary POMC
gene expression [23,24,32], and because the endometrium
also expresses and secretes POMC [33] and β-endorphin
[34], urocortin may stimulate endometrial POMC-derived
peptide secretion, as in placenta [30] and in the pituitary
[23,24,32]. Urocortin expression in vascular smooth mus-
cle cells [28] supports its participation in the process of en-
dometrial vascularization.

4. Activin A

Activin A was originally discovered as a dimeric glyco-
protein isolated from porcine ovarian fluid that enhanced
the release of follicle-stimulating hormone (FSH) from
the anterior pituitary [35]. Activin A is composed of two βA
linked to each other by disulfide bridges and belongs to the
transforming growth factor β (TGF-β) superfamily [36].
Subsequent studies have shown that it is produced by a wide
variety of tissues in the body, including brain, pituitary, pla-
centa, endometrium, gonads, and bone marrow. Such a wide
distribution suggests a host of different roles for activin
in modulating cell growth, differentiation, and apoptosis
[37].

The activity of activin A is tightly regulated by the local
extra-cellular levels of two proteins, follistatin (FS) and
follistatin-related gene (FLRG) that bind activin with high
affinity and neutralize its biological functions [38]. The
affinity of activin for these binding proteins is similar to that
for its receptors, and thus these proteins play a major role
in regulating activin bioavailability and function in target
tissues [39].
Activin receptors are divided into two subgroups, I and II, each of which has two forms, a and b (ActRIa, and ActRIIa, and ActRIBs). They are all transmembrane proteins containing a ligand-binding extracellular domain, a transmembrane domain, and a cytoplasmatic domain which is a predicted serine/threonine kinase. Type I receptors are essential for signal transduction, while type II receptors are responsible for binding ligand and inducing expression of type I receptors. Type I and II receptors form a stable complex upon ligand binding, resulting in phosphorylation of type I by type II receptors. Type II receptors appear to be constitutively active kinases [40].

4.1. Activin A in the healthy human endometrium

The study of activin has been hampered by difficulties in raising antisera specific to the βA subunit. In fact, the high sequence homology to TGF-β subunits, the similarity between βA and α subunits, the existence of precursors, alternative cleavage products and higher molecular weight forms all pose significant problems.

Activin A is expressed in human endometrium throughout the menstrual cycle, and its expression levels vary with the stage of the cycle. In fact, using polyclonal rabbit antibodies raised against the inhibin/activin βA subunit, Leung et al. observed significant differences in cytoplasmic βA subunit staining in glandular versus luminal epithelia, and between proliferative and secretory stages [41]. Weak and patchy staining was seen in proliferative tissues and a more uniform and intense staining in the secretory phase, which diminished after menses [41]. In addition to using βA antibodies, Otani et al. used a monoclonal antibody against human recombinant dimeric activin A, revealing similar staining patterns. Relatively low levels of endometrial activin A are detectable in the early proliferative phase, with a rise in the late proliferative phase, followed by decreased staining in early secretory phase, and culminating with intense staining throughout mid- and late-secretory phase and menstruation. Moreover, an intense staining was observed in human decidual tissues from 6 to 10 weeks of gestation, pointing to the decidua as an important source of activin A in early pregnancy [42].

With respect to the expression throughout pregnancy, βA mRNA levels are significantly increased in human decidua from early to term gestation, although the human placenta remains the main source of the large amounts of activin A that appear in maternal circulation during pregnancy [43].

Endometrial stroma and endothelial tissue produce little or no activin A, with the exception of decidualized stromal cells in the late secretory phase and resident migratory cells such as macrophages and neutrophils [41,42]. These findings were confirmed by in vitro studies that showed expression of activin A mRNA in both epithelia and stroma, albeit at a much higher level in epithelial cells [44].

βA subunit staining intensity during menstrual cycle increases from the mid- to late-secretory phase and continues at high levels in the cytoplasm of decidual cells during early pregnancy [42], when the levels of progesterone in the endometrium are raised. To confirm the relationship between the menstrual cycle and endometrial secretion, we have investigated the secretion of activin A and follistatin from human endometrium throughout the menstrual cycle. By using two-site enzyme immunoassay (ELISA), their levels were measured in uterine washing fluids retrieved by hydrodissection from healthy fertile women, according to the endometrial thickness and menstrual cycle days. Activin A, but not follistatin, levels were significantly higher in washing fluid collected during the secretory than in the proliferative phase of the menstrual cycle. In addition, a significant direct correlation was found between activin A, but not follistatin, and menstrual cycle day or endometrial thickness.

4.2. Activin A and endometrial adenocarcinoma

Activin A and its receptors are expressed in endometrial adenocarcinoma, as shown by RT-PCR on endometrial carcinoma cell lines (HEC-1A and HEC-1B) and tissue specimens [45]. It is also found at significantly higher levels relative to controls in culture media from the aforementioned cell lines and in uterine washings from patients with endometrial carcinoma, a finding that suggests that activin A is being secreted into the extracellular fluid by the tumor. Furthermore, serum levels of the protein are higher in patients than in controls and decrease shortly after removal of the tumor, suggesting tumoral activin A secretion into the circulation [45].

By immunohistochemistry the percentage of activin A expressing tumor cells was found to be higher in endometrial adenocarcinoma than in normal endometrial tissue, and poorly differentiated tumor tissue had a significantly higher percentage of cells stained than that of well or moderately differentiated tumors [44]. These results, taken together with the reported ability of activin A to enhance proliferation in certain cancer cell lines [46,47], suggest a role for activin A in endometrial tumorigenesis [44]. Such a role, however, might not be entirely straightforward. Activin A does indeed have a weak mitogenic effect on a cell line from a poorly differentiated estrogen-unresponsive adenocarcinoma (HEC 50), but also has a dose- and time-dependent inhibitory effect on the proliferation of a well-differentiated estrogen-responsive adenocarcinoma, an effect that is reversed by 17β estradiol [48]. In light of the important role of activin A in modulating cell growth and proliferation, and apoptosis [37], a growth inhibitory role for activin A, which malignant transformation effectively overcomes, may be hypothesized.

4.3. Activin A and endometriosis

Cultured endometriotic cells express mRNA for the inhibin/activin βA subunit and for activin type I and type IIb receptors [49]. Furthermore, endometriotic cysts contain
higher concentrations of activin A than found in peritoneal fluid, which in turn are higher than levels in serum [50]. However, since the peritoneal concentration of the protein does not differ between patients and controls, it is likely that activin A has at most a local modulating effect rather than a systemic role in the pathogenesis of endometriosis [49].

Endometriotic cells also express the βA subunit mRNA, and its relative abundance with respect to GADPH was found to be significantly decreased compared with cells from eutopic endometrium. It is conceivable that activin A may regulate proliferation and/or differentiation of endometriotic cells as occurs in other cell types belonging to steroid hormone responsive tissues, such as prostate, breast, and trophoblast [37]. At the same time, it is also noteworthy that low concentrations of TGF-β, a growth factor to which activin A has considerable homology, can stimulate mitosis in cultured endometrial stromal cells [51].

4.4. Activin A and steroids: in vitro evidences of a relationship

Steroid hormones activate local activin A synthesis and release. In fact, an approach using microarray technology revealed that activin βA expression was increased in endometrial stromal cells decidualized via exposure to cAMP-elevating agents (a well known ligand activating the endometrial PKA-dependent pathway [52]), and even more so in cells decidualized with progesterone, compared to non-decidualized cells [53]. Subsequent studies have confirmed the production of activin A by stromal cells decidualized in vitro through cAMP elevation. It was further shown that the addition of exogenous activin A with a decidualization stimulus (MPA + E2) had no effect on cell number, but resulted in a significantly elevated production of prolactin, a decidualization marker, compared to hormone treatment alone. The response was dose-dependent and the stimulatory effect was abolished by co-treatment with follistatin [54].

4.5. Activin A in human endometrium: putative effects and roles

The role of activin A and its binding proteins in the human endometrium remains poorly understood. What is clear is that human endometrium expresses activin A, its binding protein follistatin and its receptors, which are localized only in the stromal cells and show maximal expression in the secretory phase of the menstrual cycle. In light of the effect of activin A in promoting endometrial decidualization in vitro (an effect reversed by follistatin) and of variation in the pattern of expression and secretion of activin A (but not follistatin) across the menstrual cycle, we are hypothesizing that activin A has a physiological role in the endometrial function (Fig. 1).

In the secretory phase, the human endometrium undergoes decidualization in preparation for invasion by trophoblast if pregnancy occurs. In addition to its role in facilitating decidualization [54], endometrial activin A might have potent effects on embryonic and placental development during the early stages of human implantation, since both the human preimplantation embryo and the placenta, both expressing activin receptors [55,56], are targets of activin A. During the secretory phase of the menstrual cycle (at the time of blastocyst implantation), endometrial epithelial cell products are secreted predominantly into the uterine lumen and accordingly, activin A is present in the uterine fluid of cycling women [57] and in higher amounts during the secretory phase. Thus, the high activin A amounts produced by human endometrium might play a role in invasion and in support of placentation functions, because activin A is a well known regulator of the differentiation of proliferative cytotrophoblast into extravillous trophoblast cells of the anchoring villi [58]. With respect to human preimplantation embryos, activin A and follistatin were not detected in embryos from the four-cell to the morula stage. On the contrary, activin receptors were detectable in all cells of the embryos, and their transcripts were significantly enhanced with the presence of the endometrial stromal cells [55]. As follistatin counteracts the effect of activin A on cytotrophoblast [58] and its expression does not change throughout the menstrual cycle, the increased activin A expression and secretion from secretory endometrium may directly modulate the trophoblast growth, invasion, and differentiation, and thus the embryo implantation.

Overall, the human endometrium appears to be an important source and target of activin A. The derangement of activin A expression and secretion occurring in endometrial diseases such as endometriosis, endometrial hyperplasia, and adenocarcinoma [44–50] further support a possible role for activin A in endometrial pathophysiology.

References


