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Review

Stress neuropeptides in the human endometrium: Paracrine effects on cell differentiation and apoptosis

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ABSTRACT

Human endometrium exhibits characteristics of a neuroendocrine-like stress organ in addition to its classical role as the main target of ovarian steroid hormones. Indeed, the epithelial cells of human endometrium express the stress-associated neuropeptide genes corticotropin-releasing hormone (CRH), proopiomelanocortin, proenkephalin and prodynorphin. Furthermore, endometrium stroma cells also express CRH when they differentiate into decidual cells. Multiple lines of evidence suggest that the stress-associated neuropeptides of human endometrium are under the endocrine control of gonadal steroids as well as under an autocrine/paracrine regulation by prostanoids and interleukins. Endometrial stress-associated neuropeptides appear to exert their biological effect locally, i.e. within the uterus since human endometrium and myometrium also express the relevant receptors. More specifically, recent data suggest that endometrial CRH participates in the regulation of intrauterine inflammatory processes taking place in early pregnancy including stroma decidualization, blastocyst implantation and early maternal tolerance. Similarly, endometrial opioids participate in the regulation of uterine tissue remodeling via their effect on endometrial cell apoptosis. Thus, endometrial stress neuropeptides act as paracrine regulators of uterine cell differentiation and tissue remodeling as well as modulators of local immune responses.

Key words: endometrium, human, opioids, CRH, implantation, decidualization, apoptosis, differentiation

INTRODUCTION

Corticotropin-releasing hormone (CRH), a 41-amino acid neuropeptide, is the major endogenous regulator of endocrine, autonomic, immunological and behavioural adaptation to stress. In the central nervous system (CNS), CRH is synthesized principally within the paraventricular nucleus (PVN) of the hypothalamus. Its role there is the regulation of the hypothalamic-pituitary-adrenal (HPA) axis response to stress via its induction of the proopiomelanocortin (POMC) gene expression in the corticotroph cells of the anterior pituitary as well as its stimulation of

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ACTH[1-4]. The human CRH gene maps into the long arm of chromosome 8 and the size of its transcript is approximately 1.3 Kb. Outside the CNS, the CRH gene is expressed in a growing list of peripheral tissues including the adrenal cortex and medulla, lymphocytes, pancreas, lung, liver, stomach, duodenum, intestines, skin, etc.[5-10]. However, it should be emphasized that CRH is mainly present in the peripheral part of the reproductive tract including human placenta, the uterus and the gonads of both sexes[11-14].

CRH exerts its many biological effects by binding to plasma membrane receptors that are coupled to G_s protein and adenylate cyclase. So far, two distinct CRH receptor genes have been identified[15,16]. The first, the CRH-R1, binds CRH with high affinity, i.e. with a Kd of 0.95 to 2 nM. CRH-R1 was cloned from human and mouse pituitary and rat brain. Two splice variants of the second CRH receptor type (CRH-R2) have been isolated, differing only in their N-terminal domain. The CRH-R2a (kd: 7.2-22 nM) which was cloned from rat brain and the CRH-R2b (Kd: 10-29 nM) which was isolated from mouse heart. The CRH receptors are widely distributed in several tissues including the central and peripheral nervous system, the adrenals, retina, spleen, immune cells, heart, skeletal muscles, skin, gonads, placenta, endometrium and myometrium indicating the diverse and multiple roles that CRH appears to exert[17].

The endogenous opioid peptides (EOP) derive from three precursors of similar molecular size and sequence homology[18-20]. POMC is the precursor of ACTH, a-melanocyte-stimulating hormone (a-MSH), b-endorphin and lipotropin. Proenkephalin (PENK) is the precursor of enkephalins while prodynorphin (PDYN) of dynorphins, rimorphin, leumorphin and neo-endorphins. A variety of opioid receptor types and subtypes are present throughout the body[21-27]. Mu, kappa, delta, epsilon and sigma are the main opioid receptor types. Each EOP exhibits distinct binding activity towards each type of opioid receptor. Opioids are mainly localized within CNS including the hypothalamus and spinal cord as well as in both lobes of the pituitary. Opioid receptors are also widely distributed in peripheral tissues including the peripheral part of the sympathetic system and the adrenal cortex and medulla, as well as throughout the gastrointestinal tract, immune cells, heart and vasculature. However, as is the case with the CRH receptors, opioid receptors are heavily present in all reproductive tissues including human placenta, the endometrium and the gonads[28-39].

ENDOMETRIAL CRH

Expression: The CRH transcript and its peptide product are present in normal and tumoral human glandular endometrial cells[40,41]. In contrast to rat placenta where CRH is absent, the epithelial cells of rat uterus express the CRH gene[42]. The size of the endometrial CRH transcript is approximately 1.3 kb, i.e. similar to that isolated from the human hypothalamus and placenta. Immunofluoresence experiments in a normal human glandular endometrium reveal a cytoplasm rich in granules positive for immunoreactive (ir)-CRH. However, it should be noted that ir-CRH is localized only in glandular cells while endometrial stroma is negative for it[40]. Immunohistochemical data show that both epithelial and decidualized stromal cells of the early pregnant rat uterus contain ir-CRH, suggesting that epithelial cells in the endometrium are the main source of intrauterine CRH in the non-pregnant uterus, whereas decidualization of normal stromal cells which up to that moment did not express CRH express the CRH gene, a phenomenon taking place in both the human and rodent uterus[40-42]. The CRH transcript and peptide product are easily detectable in the human decidua and in stroma which has been decidualized, in vitro, by a mixture of progesterone, relaxin and estrogens[43]. The physico-chemical characteristics of ir-CRH in normal and tumoral human endometrium and rat uterus extracts are identical to synthetic 41-amino acid CRH peptide further supporting the immunoassay data[40]. These data suggest that the cycling human endometrium possesses all the necessary enzymes for the post-translational processing of preproCRH, giving rise to a fully bioactive end product. The principal receptor for the CRH ligand, the CRH-R1 receptor, is also present in both epithelial and stroma cells of the human endometrium[44,45], as well as in the human myometrium[29]. Interestingly, the affinity of the CRH receptors in the the human myometrium increases during the later stages of pregnancy resulting in an linear acceleration of cAMP production as pregnancy progresses which acts as a potent myometrial relaxant[46]. Appropriately enough, at term, a marked reduction in the ability of CRH to stimulate cAMP takes place most probably

due to a reduction of the number of G_sa subunits caused by the rising biological activity of oxytocin. It should be noted that oxytocin activates protein kinase C which, by phosphorylating the CRH receptor, leads to its severe desensitization towards CRH[47,48].

Regulation: Three known inducers of hypothalamic CRH, 8-bromo-cAMP, forskolin and epidermal growth factor (EGF) also stimulate the activity of a CRH promoter introduced into endometrial cells[49]. Indeed, it is widely recognized that cAMP is a major inducer of CRH expression in both hypothalamus and placenta and a stimulator of its secretion. It should be noted that all data available indicate that cAMP affects CRH gene expression rapidly without the mediation of de novo synthesis of regulatory proteins, supporting the hypothesis that the effect of cAMP is associated to factors within the nuclear compartment consistent with a direct effect on CRH gene transcription mediated via an effect on proximal promoter[49]. It has been shown that estrogens suppress the activity of CRH promoter in the endometrium in the same way they affect the transcription of hypothalamic CRH. Furthermore, estradiol is not capable of inhibiting forskolin- or EGF-induced CRH promoter, acting directly on pairs of functional half palindromic estrogen responsive elements (EREs) present in the promoter region of the CRH gene. Although glucocorticoids decrease the activity of the CRH promoter, the absence of the concensus glucocorticoid response element in CRH promoter and the inhibition of forskolin- or EGF-induced CRH promoter point to an effect via activation of cAMP- and EGF-dependent pathways. Indeed, the inhibitory effect of the glucocorticoids in the endometrium is in agreement with that described for the hypothalamus and exactly opposite to what has been found to take place in human placenta, suggesting that the regulation of the transcription of the CRH gene is cell-specific depending on the presence or absence of certain specific transcription factors[49]. Finally, the cytokines IL-1 and IL-6 have been shown to stimulate the activity of the CRH promoter introduced in human endometrial cells[50]. This effect appears to be mediated via prostaglandins, in accordance to what has been described in the hypothalamus and placenta.

ENDOMETRIAL OPIOID PEPTIDES

Expression: It is intriguing that all three opioid peptide precursors are synthesized by human endometrial cells[51,52]. The size of the POMC transcript present in the epithelial cells of human endometrium is approximately 1.2 kb long, i.e. similar to that present in the pituitary gland, while the size of the prodynorphin gene transcript is approximately 2.3 kb long, i.e. similar to that present in CNS[52]. Supporting these molecular biology data are the physico-chemical characteristics of ir-b-endorphin and ir-dynorphin present in the human endometrium. Indeed, both ir-peptides have been shown to have similar or identical characteristics to those found for authentic b-endorphin and the 8 kD dynorphin molecules. Furthermore, endometrial cells express multiple types of opioid receptors including the k1, k2 and k3 variants[53].

Regulation: It has been found that estrogens and the glucocorticoids suppress the secretion of endometrial b-endorphin while progesterone, dihydrotestosterone and the gonadal regulator GnRH do not significantly affect it[50]. Interestingly, the antiglucocorticoid-antiprogestin RU486 exhibits agonist effects, possibly acting via glucocorticoid receptors. Contrary to b-endorphin, the secretion of endometrial dynorphin is induced by GnRH and is not affected by all steroid hormones. As a consequence, the regulation of endometrial opioids appears to have similarities to that reported for opioids in the arcuate nucleus in the hypothalamus and the pituitary gland. Indeed, estrogens suppress the secretion of b-endorphin and the transcription of the POMC gene in rat hypothalamus, while GnRH induces the production of hypothalamic/pituitary dynorphins. The type-specific regulation of endometrial opioids suggests that each family of opioids possesses a quite distinct physiological role within the uterine cavity. The presence of high affinity opioid-binding sites on its organ implies that local opioids exert mainly autocrine and/or paracrine effects without excluding the possibility of systemic actions. It is hypothesized that while the endometrial dynorphins may exert their biological effects via the k1-opioid receptors, b-endorphin may affect principally the k2 type of opioid receptors.

PHYSIOLOGICAL ROLES OF ENDOMETRIAL STRESS-ASSOCIATED NEUROPEPTIDES

Uterine CRH modulates the differentiation of endometrial stroma to decidua: A growing number of published reports suggest that CRH exerts pro-in-

flammatory / procytokine effects. Indeed, it now appears certain that the CRH neuropeptide is an integral part of the immune phenomena taking place during the initiation of the inflammatory response. Supportive of this hypo-thesis are the ubiquitous presence of CRH at the sites of inflammation in both humans and rodents and the fact that its immunoneutralization appears to drastically attenuate the inflammatory response[54]. Interestingly, in the human endometrium, a phenomenon with characteristics of an aseptic inflammatory reaction takes place during the differentiation of endometrial stroma to decidua. More specifically, during decidualization the endometrial stroma is subjected to numerous functional changes including an increase in its vascular permeability, remarkable cell growth and remodeling of its extracellular matrix. It has been shown that CRH induces the decidualization of the endometrial stroma[55] and that it potentiates the decidualizing effect of progesterone. Furthermore, progestins stimulate the expression of endometrial CRH in a cAMP-dependent manner[56]. Indeed, in stromal cells, CRH may mediate, via the CRH-R1 receptor, the cAMP-dependent part of the decidualizing effect of progesterone, an effect blocked by the cAMP inhibitor Rp-Br-cAMP.

In addition to progesterone, several locally produced pro-inflammatory immune factors also exert a decidualizing effect. Thus, prostaglandins and interleukins are prominent members of this category of modulators. It should also be stressed here that these types of local factors usually exert their effect in a paracrine manner. Indeed, endometrial stroma produces several inflammatory factors, including prostaglandin E_2 (PGE_2), interleukin 1 (IL-1) and IL-6[57]. In humans, prostaglandin E_2 enhances, while interleukin-1 inhibits, the decidualizing effect of progesterone[58-60]. Furthermore, PGE_2 and interleukins IL-1 and IL-6 are also major inducers of endometrial CRH[50]. It is postulated that during the decidualizing process CRH interacts with these local factors. For instance, it has been shown that CRH inhibits the production of PGE_2 by human endometrial stromal cells[61]. It is thus possible that endometrial CRH, in addition to its direct decidualizing effect, may also alter the decidualizing action of progesterone via its influence on the locally produced modulators including PGE_2. In addition, CRH stimulates the production of both IL-1 and IL-6 in human endometrial stromal cells[61]. It should be remembered that IL-1 is a principal modulator of the decidualization process, blocking the differentiation of human endometrial stromal cells induced by ovarian steroids or cAMP[59]. The stimulatory effect of CRH on stromal IL-1 suggests that the former may exert its decidualizing effect either as a principal regulator or as a modulator of progesterone, the classical decidualizing effector**.** It is extremely interesting that progesterone per se induces the expression of the CRH gene in the stromal cells of human endometrium[56]. Thus, it is possible that progesterone-driven CRH may exert an inhibitory effect on endometrial decidualization through induction of a local inhibitor, possibly interleukin-1**,** establishing a complex close loop feedback system fine tuning the response of stroma cells to these factors (Figure 1). In conclusion, it now appears that a close interaction takes place within the human endometrium involving CRH, prostanoids and cytokines. Indeed, the following sequence of events may take place during decidualization (Figure 1): a) progesterone, in addition to its strong decidualizing effect, also induces the production of endometrial CRH; b) CRH participates in stromal decidualization, regulating local modulators of this process, i.e., it inhibits the enhancer PGE_2, induces the inhibitor IL-1 and stimulates the inducer IL-6; c) subsequently, endometrial PGE_2, IL-1 and IL-6 exert a positive effect on the expression of endometrial CRH completing this endometrial paracrine network.

Uterine CRH supports blastocyst implantation and early maternal tolerance: The Human endometrium is one of the principal examples where local modulators of the inflammatory response serve a key physiological function. Indeed, the classic modulators of inflammation appear to be part of the mechanism regulating the implantation of conceptus. It is now well established that the implanting blastocyst secretes several inflammatory mediators, including IL-1 and prostaglandin PGE_2[54]. Blastocyst-deriving IL-1 plays an essential role on implantation. In fact, in mice, blockade of its effect by the specific antagonist IL-1ra inhibits implantation[57]. In addition, measurement of the IL-1 levels in the peri-implantation area appears to be a good predictor of the outcome of pregnancy[62]. As mentioned above, IL-1 and PGE_2 are inducers of CRH expression in human endometrial cells[50]. Based on these data, it has been hypothesized that the blastocyst may modulate the expression of endometrial CRH through IL-1 and/or PGE_2 produced by it at the very site of nidation. Subsequently, endometrial CRH,

in association with other local factors, may then participate in a local inflammation-regulating response at the site of implantation, rendering endometrial surface "adhesive" for the attachement of the fertilized egg, culminating in the formation of the egg nidus. This hypothesis is supported by several lines of data showing a significantly higher concentration of the CRH transcript and its peptide product at the early implantation sites of pregnant rats compared to the inter-implantation uterine areas[42].

In vivo experiments in the mouse have shown that intra-peritoneal injections of CRH antibodies at day 2 of pregnancy decrease the number of fetuses within the uterus by 60%[63]. This observation is further supported by experiments in rats using antalarmin, a CRH-R1 specific antagonist. Indeed, administration of antalarmin to early pregnant rats (day one of pregnancy) results in a 70% reduction in the number of implantation sites[64]. Thus, blocking of CRH has an anti-nidation effect when this happens at a very early stage of pregnancy. It is evident that both methods of blocking the effects of uterine CRH (antibodies or antalarmin) do not completely abolish nidation, suggesting the presence of other, redundant mechanisms supporting the implanted embryo. This is also compatible with the fact that CRH and CRH-R1-knockout mice are not entirely sterile[65,66]. Recent experimental findings show that CRH participates in the nidation of the fertilized egg by inhibiting local maternal immune response to the implanted embryo. Indeed, CRH stimulates the expression of the pro-apoptotic FasL protein[67] in the decidual and trophoblastic cells, thus potentiating their ability to induce apoptosis of the surrounding maternal T lymphocytes activated by the presence of the embryo[64]. Expression of Fas ligand by fetal extravillous trophoblast cells can induce apoptosis of activated T lymphocytes expressing increasing numbers of the Fas membrane protein (Figure 2). Another role that is attributed to maternal and fetal FasL is that it limits the migration of fetal cytotrophoblast cells into maternal tissue and *vice versa*[68]. The intra-uterine presence of CRH, both in the maternal (decidua) and fetal (trophoblast) sites suggests that locally produced CRH regulates FasL production, thus affecting the invasion process through a local auto/paracrine regulatory loop of cytotrophoblast cells, regulating their own apoptosis. Therefore, inadequate CRH-mediated self-induction of FasL in extravillous trophoblasts might be involved in the pathophysiology of infertility and recurrent fetal resorption or miscarriage[69]. Abrogation of immune privilege at the placental-uterine interface or unbridled invasion of the trophoblast may have deleterious consequences for the developing fetus, as evidenced by the high rates of fetal morbidity and mortality, and for the mother, observed in pregnancies complicated by inflammation at maternal-fetal interfaces and preeclampsia/eclampsia.

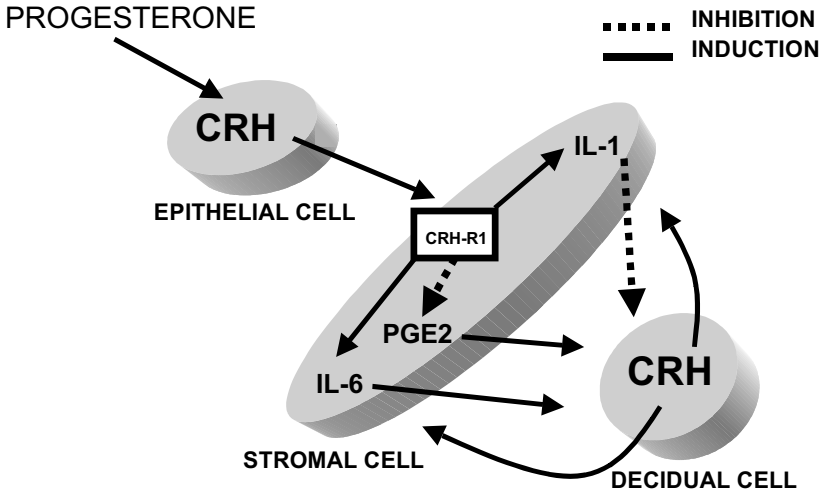

Figure 1. Endometrial CRH modulates the decidualization process. Endometrial CRH, under the influence of progesterone, may participate in a positive local feed-back loop between epithelial and stromal cells, facilitating decidualization of the latter via the production of PGE_2 and IL-6 from the stroma. On the other hand, the inhibitory effect of CRH on the release of PGE_2 from stroma cells could represent a negative local feed-back mechanism to control the process of decidualization.

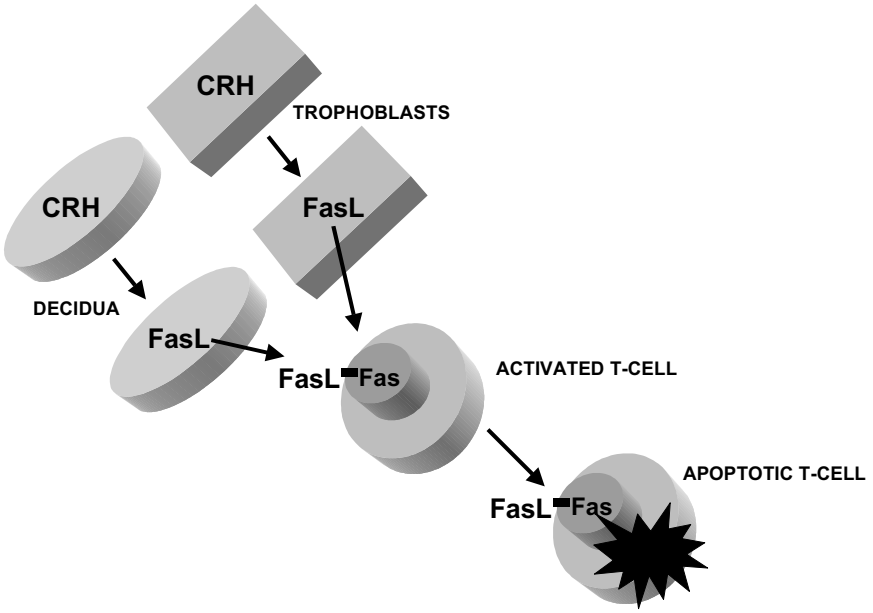

Figure 2. Uterine CRH affects the maternal immune tolerance. CRH produced locally by decidual cells and extravillous trophoblasts acts in an autocrine/paracrine fashion, through CRH-R1, to stimulate FasL expression and to potentiate the ability of these cells to cause apoptosis of activated maternal T lymphocytes (Fas receptor positive).

Uterine opioids modulate endometrial remodelling via their effect on apoptosis: The human endometrium expresses specific k-opioid binding sites and their endogenous ligands, the dynorphins[52,53]. The physiological role of endometrial opioids, including the dynorphins, remains largely speculative. However, recent findings suggest that locally secreted k-opioid peptides may play a role as paracrine modulators of endometrial stroma apoptosis. It should be noted that apoptosis plays a central role in endometrial physiology including the decidualization process, the implantation of the fertilized egg and menstruation. More specifically, decidualization in the immediate vicinity of the implanting blastocyst may in fact involve the entire endometrial stroma. Following a period of intense growth and differentiation, the anti-mesometrial decidua, in the area adjacent to trophoblast, begins to degenerate by apoptosis. Progression of apoptosis through the anti-mesometrial decidua enables trophoblast giant cells to gain access to maternal vessels. It should be noted here that programmed cell death of decidual cells in the anti-mesometrial decidua allows remodeling of the implantation chamber without disrupting the growth and development of the embryo or the integrity of the tissue.

Expression of several factors involved in the regulation of apoptosis are detectable in human endometrial stroma. Thus the Fas antigen, a member of tumour necrosis factor receptor (TNFR) family and a type-I membrane protein[67] induces apoptosis via cross-linking to Fas ligand (FasL)[70,71]. Members of the Bcl-2 family, such as the apoptosis inhibiting proteins Bcl-2 and Bcl-x_L and their apoptosis promoting homologues Bax and Bak have also been found in the human endometrium[72-74]. K-opioids agonists stimulate apoptosis of endometrial stroma cells[75]. This effect is dose-dependent and reversible by the general opioid antagonist naloxone, an observation indicating that this phenomenon is mediated via specific opioid-receptors. The pro-apoptotic effects of k-opioids is exerted through regulation of the pro- and anti-apoptotic proteins including the Fas and Bcl-2 families (Figure 3). Indeed, k-opioids cause a rapid but transient up-regulation of the apoptotic protein Fas, suggesting that their effect on accelerating apoptosis of endometrial stroma cells is mediated by activation of the Fas/FasL pro-apoptosis pathway. At the same time, recent data suggest that k-opioids stimulate the production of the anti-apoptotic members of the Bcl-2 family of proteins, the Bcl-2 and Bcl-x_L whereas it has no significant effect on the apoptosis-promoting homologues

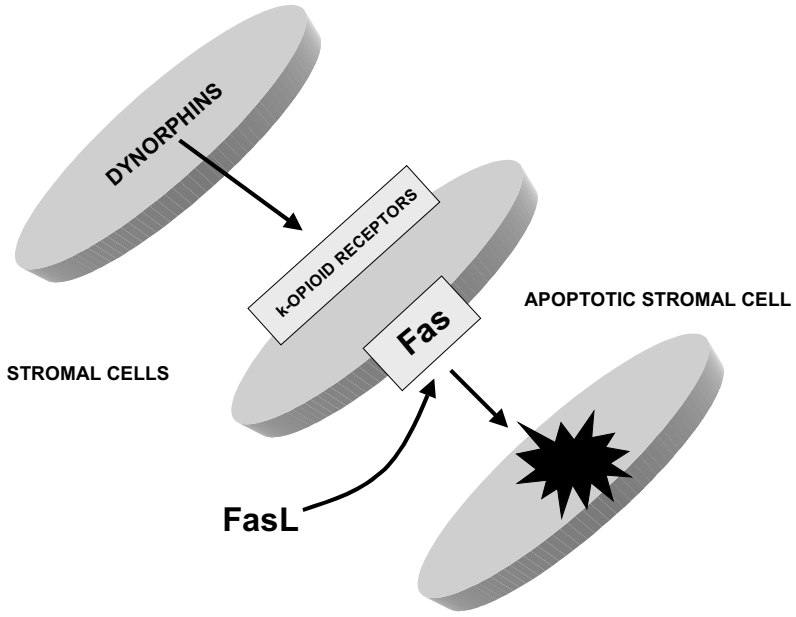

Figure 3. Uterine dynorphins regulate endometrial cell apoptosis and tissue remodelling. Dynorphins, produced by endometrial cells induce the expression of the pro-apoptotic Fas receptor on stromal cells, propagating their apoptosis and facilitating tissue remodeling during early pregnancy.

Table 1. Regulators of endometrial stress neuropeptides

Regulator	b-Endorphin	Dynorphin	CRH
Estradiol	inhibition	no effect	inhibition
Dexamethasone	inhibition	no effect	inhibition
RU486	inhibition	no effect	inhibition
Progesterone	no effect	no effect	induction
GnRH	no effect	induction	NO*
cAMP	NO	NO	induction
EGF	NO	NO	induction
Forskolin	NO	NO	induction
PGE_2	NO	NO	induction
IL-1	NO	NO	induction
IL-6	NO	NO	induction

*NO: No information

Bax, Bcl-x_s and Bak, implying a transient survival mechanism activated in stroma cells as a parallel rescue-response to apoptosis-inducing factors.

Conclusions: Endometrial stress neuropeptides act as paracrine regulators of uterine cell differentiation and tissue remodeling as well as modulators of local immune responses. Recent experimental findings suggest that endometrial CRH participates in intrauterine inflammatory processes, such as stroma decidualization, blastocyst implantation and early maternal tolerance. On the other hand, uterine opioids contribute in the regulation of endometrial tissue remodeling via their effect on cell apoptosis. The significance of uterine stress neuropeptides in endometrial pathophysiology merits further investigation.

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