ELSEVIER

Biochemical Pharmacology 65 (2003) 917–921

Commentary

Biochemical Pharmacology

www.elsevier.com/locate/biochempharm

Corticotropin-releasing hormone (CRH) and immunotolerance of the fetus

Antonis Makrigiannakis[a,*], Emmanuel Zoumakis[b], Sofia Kalantaridou[b], Nikos Mitsiades[b], Andreas Margioris[a], George P. Chrousos[b], Achilleas Gravanis[a]

[a]*Laboratory of Pharmacology, School of Medicine, University of Crete, Heraklion 71110, Greece*
[b]*Pediatric and Reproductive Endocrinology Branch, NICHD, NIH, Bethesda, MD 20892, USA*

Abstract

The hypothalamic neuropeptide corticotropin-releasing hormone (CRH) is produced by several tissues of the female reproductive system, including the endometrial glands and decidualized stroma, as well as the trophoblast, syncytiotrophoblast, and placental decidua. CRH is also secreted at inflammatory sites and possesses potent pro-inflammatory properties influencing both innate and acquired immune processes. Recent experimental findings show that uterine CRH participates in local immune phenomena associated with early pregnancy, such as differentiation of endometrial stroma to decidua and protection of the fetus from the maternal immune system. CRH induces the expression of apoptotic Fas ligand (FasL) on invasive extravillous trophoblast and maternal decidual cells at the fetal–maternal interface. Furthermore, CRH increases the apoptosis of activated T lymphocytes through FasL induction, participating in the processes of both implantation and early pregnancy tolerance.

Keywords: Endometrium; CRH; Implantation; FasL; Decidualization; Apoptosis

1. Introduction

The hypothalamic neuropeptide CRH is produced in several reproductive organs, including the endometrial glands and decidualized stroma, as well as the trophoblast, syncytiotrophoblast, and placental decidua [1–3]. Similarly, the *CRH-R1* gene is expressed in human endometrial stromal cells [4]. The biologic role of intrauterine CRH is not fully understood. However, it has been suggested that CRH might participate in local immune phenomena associated with embryo implantation [5]. This hypothesis is supported by recent findings showing that CRH is secreted at inflammatory sites and possesses potent pro-inflammatory properties influencing both innate and acquired immune processes. One of the early effects of ''immune'' CRH is the degranulation of mast cells and the release of histamine and several inflammatory cytokines, including TNF-α and IL-6 [6]. During blastocyst implantation, the maternal endometrial response to the invading semi-allograft has characteristics of an acute, aseptic inflammatory response; yet, once implanted, the embryo suppresses this response and prevents rejection. Simultaneously, the immune system of the mother prevents a graft versus host reaction deriving from the immune system of the fetus. The Fas receptor (Fas) and its ligand (FasL) play an important role in the regulation of immune tolerance. The major function of the Fas–FasL interaction is the induction of apoptosis in activated cells carrying Fas [7]. FasL is expressed on the surface of the fetal cytotrophoblast as well as maternal decidual cells of the placenta, i.e. in cells located in the interface between the fetal placenta and the maternal endometrium. Abnormalities of maternal immune tolerance to the fetal semi-allograft and vice versa have been implicated in several common disease processes of pregnancy, including recurrent early miscarriage, pre-eclampsia, and eclampsia.

We have reported [5] that locally produced embryonic and endometrial CRH might play a role in both the aseptic inflammatory process of implantation and the anti-rejection process that protects the fetus from the maternal immune system.

* Corresponding author. Tel.: +30-28-10-394-531; fax: +30-28-10-394-530.
E-mail address: makrigia@med.uoc.gr (A. Makrigiannakis).

Abbreviations: CRH, corticotropin-releasing hormone; CRH-R1, CRH receptor type 1; cAMP, cyclic AMP; EGF, epidermal growth factor; FasL, Fas ligand; IL, interleukin; PGE_2, prostaglandin E_2; TNF-α, tumor necrosis factor α.

0006-2952/02/$ – see front matter
PII: S0006-2952(02)01547-2

2. Expression and regulation of endometrial CRH

The CRH transcript and its peptide product are present in normal and tumoral human glandular endometrial cells [3]. Epithelial cells of the rat uterus also express the *CRH* gene [5]. The size of the endometrial CRH transcript is approximately 1.3 kb, i.e. similar to that isolated in the human hypothalamus and placenta. Ir-CRH is localized mainly in glandular cells [3]. Both epithelial and decidualized stromal cells from the uteri of rats in early pregnancy 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 express the *CRH* gene in both the human and the rodent uterus. The CRH transcript and peptide product are easily detectable in human decidua and in stromal cells that have been decidualized, *in vitro*, by a mixture of progesterone, relaxin, and estrogens. These data suggest that the cycling human endometrium possesses all the necessary enzymes for the post-translational processing of pre-proCRH, giving rise to a fully bioactive end product. The principal receptor for the CRH ligand, the CRH-R1, is also present in both epithelial and stromal cells of the human endometrium [4], as well as in the human myometrium [8]. Known inducers of hypothalamic CRH, 8-bromo-cAMP, forskolin, and EGF, stimulate the activity of the *CRH* promoter [9]. Estrogens suppress the activity of the *CRH* promoter in the endometrium. Glucocorticoids decrease the activity of the *CRH* promoter, via activation of cAMP- and EGF-dependent pathways. Indeed, the inhibitory effect of 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 the 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. Finally, the cytokines IL-1 and IL-6 have been shown to stimulate the activity of the *CRH* promoter, introduced in human endometrial cells [10]. This effect appears to be mediated via prostaglandins, in accordance with what has been described in the hypothalamus and placenta.

3. Involvement of uterine CRH in the differentiation of endometrial stroma to decidua

CRH is present at inflammation sites in both humans and rodents, and its immunoneutralization appears to attenuate the inflammatory response drastically [11]. Interestingly, in the human endometrium, a phenomenon with characteristics of an aseptic inflammatory reaction takes place during the differentiation of endometrial stroma to decidua. It has been shown that CRH induces the decidualization of endometrial stroma [12,13] and that it potentiates the decidualizing effect of progesterone. Furthermore, progestins stimulate the expression of endometrial CRH in a cAMP-dependent manner [14]. 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 group of modulators. It should also be stressed that these types of local factors usually exert their effect in a paracrine manner. Indeed, endometrial stroma produces several inflammatory factors, including PGE_2, IL-1, and IL-6. In humans, PGE_2 enhances, while IL-1 inhibits, the decidualizing effect of progesterone. Furthermore, PGE_2 and IL-1 and IL-6 are also major inducers of endometrial CRH. 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 [13]. Thus, it is 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 [13]. 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 [15]. 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 very interesting that progesterone *per se* induces the expression of the *CRH* gene in the stromal cells of the human endometrium [14]. Thus, it is possible that progesterone-driven CRH may exert an inhibitory effect on endometrial decidualization through induction of a local inhibitor, possibly IL-1, establishing a complex feedback system, fine tuning the response of stroma cells to these factors. It appears that a close interaction takes place within the human endometrium involving CRH, prostanoids, and cytokines. The following sequence of events may take place during decidualization: (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. inhibits the enhancer PGE_2, induces the inhibitor IL-1, and stimulates the inducer IL-6; and (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.

4. Role for CRH in supporting blastocyst implantation and early maternal tolerance

It is known that during implantation the blastocyst secretes several inflammatory mediators, including IL-1,

IL-6, and PGE_2. Blastocyst-deriving IL-1 plays an essential role in implantation. In fact, in mice, blockade of its effect by the specific antagonist IL-1ra inhibits implantation [16]. As mentioned earlier, IL-1 and PGE_2 are inducers of CRH expression in human endometrial cells. 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 inflammatory response at the site of implantation, rendering the endometrial surface "adhesive" for the attachment 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 [5].

In vivo experiments in the mouse have shown that intraperitoneal injections of CRH antibodies at day 2 of pregnancy decrease the number of fetuses within the uterus by 60% [17]. 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 1 of pregnancy) results in a 70% reduction in the number of implantation sites [18]. Thus, blocking CRH has an anti-nidation effect when it 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 abolish nidation completely, 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 [19,20]. Recent experimental findings show that CRH participates in the nidation of the fertilized egg by inhibiting local maternal immune response to the implanted embryo [18]. Indeed, CRH stimulates the expression of the pro-apoptotic FasL protein 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 (Fig. 1). Expression of FasL by fetal extravillous trophoblast cells can induce apoptosis of activated T lymphocytes expressing increasing numbers of the Fas membrane protein. 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. 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 (Fig. 1). 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. 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.

Our data clearly demonstrate that CRH stimulates the expression of FasL in EVT cells, thus potentiating their ability to induce apoptosis of the surrounding activated T lymphocytes (Fig. 1). This effect of CRH appears to be specifically mediated through its type 1 receptor. These findings are in agreement with previously published reports suggesting that expression of FasL by fetal extravillous trophoblast cells can induce apoptosis of activated T lymphocytes expressing increasing amounts of the Fas membrane protein [21,22]. It should be noted here that mice with missense or inactivating mutations of the FasL gene (*gld*) can reproduce, suggesting that trophoblast FasL expression is not obligatory for maternal immunotolerance. Thus, in the absence of a functional Fas–FasL system, other mechanisms supporting maternal immunotolerance are sufficient to prevent total pregnancy failure but at the cost of reproductive efficiency. The observed effect of CRH in implantation and in early pregnancy appears to be primarily mediated by the Fas–FasL system for the following reasons: (a) CRH had no effect on the expression of TRAIL protein in trophoblastic JEG3 cells; (b) neutralizing antibodies raised against FasL inhibited the CRH-induced apoptosis of activated T lymphocytes by 70%; (c) exposure of cells not expressing Fas (WS1 cells) to CRH and co-cultured with activated T lymphocytes showed very little evidence of T cell apoptosis (2–5%), and (d) non-activated T lymphocytes, which express neither the Fas protein nor the TRAIL system, when co-cultured with extravillous trophoblasts showed very little evidence of apoptosis (2–5%).

It has been suggested that FasL of maternal and fetal origin limits the migration of fetal cytotrophoblast cells into maternal tissue and vice versa. Our data strengthen this hypothesis, suggesting that CRH of fetal and maternal origin regulates FasL production, thus affecting the invasion process through a local auto-paracrine regulatory loop of cytotrophoblast cells, regulating their own apoptosis (Fig. 1).

If CRH-R1 blockade by antalarmin and other compounds prevents implantation by reducing the inflammatory-like reaction of the endometrium to the invading blastocyst, they might represent a new class of nonsteroidal inhibitors of pregnancy at its very early stages. Given the promising future of CRH antagonists in the therapy of depression and anxiety disorders, their ability to cause hypofertility or early miscarriages should be seriously taken into account. On the other hand, the lack of an abortifacient or fetotoxic effect in mid and late gestation suggests that CRH antagonists could be used to protect the fetus from maternal stress and/or to prevent premature

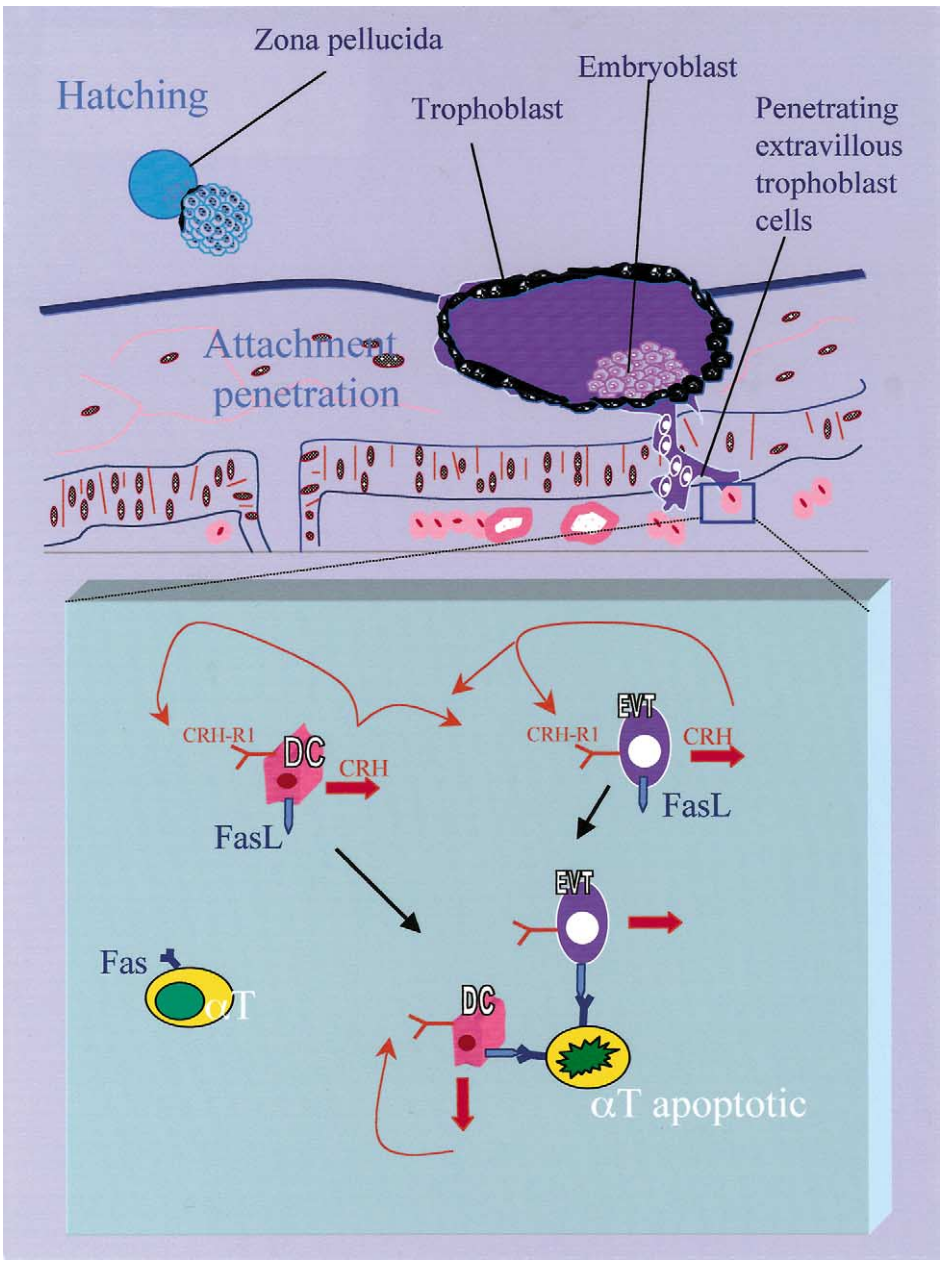

Fig. 1. Schematic presentation of the early phases of human implantation. CRH produced locally by EVT and decidual cells acts in an autocrine-paracrine fashion, through CRH-R1, to stimulate FasL expression and to potentiate the ability of these cells to cause apoptosis of activated maternal T lymphocytes (Fas receptor positive). CRH: corticotropin-releasing hormone; CRH-R1: CRH receptor type 1; DC: decidual cell; EVT: extravillous trophoblast; Fas: Fas receptor; FasL: Fas ligand; T: T lymphocyte.

labor and delivery, another potential use of this class of compounds.

References

[1] Usui T, Nakai Y, Tsukada T, Jingami H, Takahashi H, Fukata J, Imura H. Expression of adrenocorticotropin-releasing hormone precursor gene in placenta and other nonhypothalamic tissues in man. Mol Endocrinol 1988;2:871–5.

[2] Suda T, Tomori N, Tozawa F, Mouri T, Demoura H, Shizume K. Distribution and characterization of immunoreactive corticotropin-releasing factor in human tissues. J Clin Endocrinol Metab 1984;59:861–6.

[3] Makrigiannakis A, Zoumakis E, Margioris AN, Theodoropoulos P, Stournaras C, Gravanis A. The corticotropin-releasing hormone (CRH) in normal and tumoral epithelial cells of human endometrium. J Clin Endocrinol Metab 1995;80:185–9.

[4] Di Blasio AM, Peroci Giraldi F, Viganò P, Petraglia F, Vignali M, Cavagnini F. Expression of corticotropin-releasing hormone and its R1 receptor in human endometrial stromal cells. J Clin Endocrinol Metab 1997;82:1594–7.

[5] Makrigiannakis A, Margioris AN, Le Goascogne C, Zoumakis E, Nikas G, Stournaras C, Psychoyos A, Gravanis A. Corticotropin-releasing hormone (CRH) is expressed at the implantation sites of early pregnant rat uterus. Life Sci 1995;57:1869–75.

[6] Theoharides TC, Singh LK, Boucher W, Pang X, Letourneau R, Webster E, Chrousos G. Corticotropin-releasing hormone induces skin mast cell degranulation and increased vascular permeability, a

possible explanation for its proinflammatory effects. Endocrinology 1998;139:403–13.

[7] Runic R, Lockwood CJ, Ma Y, Dipasquale B, Guller S. Expression of Fas ligand by human cytotrophoblasts: implication in placentation and fetal survival. J Clin Endocrinol Metab 1996;81:3119–22.

[8] Hillhouse EW, Grammatopoulos D, Milton NG, Quartero HW. The identification of a human myometrial corticotropin-releasing hormone receptor that increases in affinity during pregnancy. J Clin Endocrinol Metab 1993;76:736–41.

[9] Makrigiannakis A, Zoumakis E, Margioris AN, Stournaras C, Chrousos GP, Gravanis A. Regulation of the promoter of the human corticotropin-releasing hormone gene in transfected human endometrial cells. Neuroendocrinology 1996;64:85–93.

[10] Makrigiannakis A, Margioris A, Zoumakis E, Stournaras C, Gravanis A. The transcription of corticotropin-releasing hormone in human endometrial cells is regulated by cytokines. Neuroendocrinology 1999;70: 451–9.

[11] Karalis K, Sano H, Redwine J, Listwark S, Wilder RL, Chrousos GP. Autocrine or paracrine inflammatory actions of corticotropin-releasing hormone in vivo. Science 1991;254:421–3.

[12] Ferrari A, Petraglia F, Gurpide E. Corticotropin releasing factor decidualizes human endometrial stromal cells in vitro. Interaction with progestin. J Steroid Biochem Mol Biol 1995;54:251–9.

[13] Zoumakis E, Margioris A, Stournaras C, Dermitzaki I, Angelakis E, Makrigiannakis A, Koumantakis E, Gravanis A. Corticotropin-releasing hormone (CRH) interacts with inflammatory prostaglandins and interleukins and affects decidualization of human endometrial stroma. Mol Hum Reprod 2000;6:344–51.

[14] Makrigiannakis A, Margioris A, Chatzaki E, Zoumakis E, Chrousos G, Gravanis A. The decidualizing effect of progesterone may involve direct transcriptional activation of corticotropin-releasing hormone (CRH) from human endometrial stroma cells. Mol Hum Reprod 1999;5:788–96.

[15] Frank GR, Brar AK, Jikihara H, Cedars MI, Handwerger S. Interleukin-1β and the endometrium: an inhibitor of stromal cell differentiation and possible autoregulator of decidualization in humans. Biol Reprod 1995;52:184–91.

[16] Sheth KV, Roca GL, Al-Sedairy ST, Parhar RS, Hamilton CJCM, Al-Abdul Jabbar F. Prediction of successful embryo implantation by measuring interleukin-1-alpha and immunosuppressive factor(s) in preimplantation embryo culture fluid. Fertil Steril 1991;55: 952–8.

[17] Athanassakis I, Farmakiotis V, Aifantis I, Gravanis A, Vassiliadis S. Expression of corticotropin-releasing hormone in the mouse uterus: participation in embryo implantation. J Endocrinol 1999;163:221–7.

[18] Makrigiannakis A, Zoumakis E, Kalantaridou S, Coutifaris C, Margioris A, Coukos G, Rice KC, Gravanis A, Chrousos GP. Corticotropin-releasing hormone promotes blastocyst implantation and early maternal tolerance. Nat Immunol 2001;2:1018–24.

[19] Muglia L, Jacobson L, Dikkes P, Majzoub JA. Corticotropin-releasing hormone deficiency reveals major fetal but not adult glucocorticoid need. Nature 1995;373:427–32.

[20] Turnbull AV, Smith GW, Lee S, Vale WW, Lee K-F, Rivier C. CRF type I receptor-deficient mice exhibit a pronounced pituitary-adrenal response to local inflammation. Endocrinology 1999;140:1013–7.

[21] Itoh N, Yonehara S, Ishii A, Yonehara M, Mizushima S, Sameshima M, Hase A, Seto Y, Nagata S. The polypeptide encoded by the cDNA for human cell surface antigen Fas can mediate apoptosis. Cell 1991; 66:233–43.

[22] Yamashita H, Otsuki Y, Matsumoto K, Ueki K, Ueki M. Fas ligand, Fas antigen and Bcl-2 expression in human endometrium during the menstrual cycle. Mol Hum Reprod 1999;5:358–64.