[Frontiers in Bioscience 1, e1-8 January 1, 1996]

CORTICOTROPIN RELEASING HORMONE (CRH) IN NORMAL AND PREGNANT UTERUS: PHYSIOLOGICAL IMPLICATIONS

Zoumakis E, Makrigiannakis A, Margioris A, Stournaras C, Gravanis A[1]

Departments of Pharmacology, Clinical Chemistry, Biochemistry, Medical School, University of Crete, Iraklion 71110, Greece

TABLE OF CONTENTS

1. ABSTRACT

Corticotropin-releasing hormone, is a hypothalamic neuropeptide, responsible not only for the endocrine but also the autonomic, immunological and behavioural responses of mammalian organisms to stress. CRH is also expressed in female reproductive tissues, such as placenta and uterus. Multiple sites within the pregnant uterine cavity express the CRH gene, including the trophoblasts, fetal membranes (chorion, amnion) and decidua. The trophoblastic syncytium appears to be the major source of placental CRH. It is postulated that placental CRH influences the HPA axis of either mother or fetus and participates at the initiation of labour. Recent findings show that human and rat uterus express the CRH gene. Epithelial cells of both species are the main source of endometrial CRH, while stroma does not seem to express it, unless it differentiates to decidua. Estrogens and glucocorticoids inhibit and prostaglandin E2 stimulates the promoter of human CRH gene in transfected human endometrial cells, suggesting that endometrial CRH gene expression is under the control of these agents. Moreover, in rats, endometrial CRH expression is significantly higher at the implantation sites, compared to at the inter-implantation uterine regions. Given the proinflammatory/vasoregulatory properties of CRH, we hypothesize that endometrial CRH may participate in the regulation of intrauterine phenomena, such as blastocyst implantation, endometrial vascularization and myometrial contractility.

Received 11/2/95; Accepted 12/14/95

[1] Correspondence should be addressed to : Dr A. Gravanis, Department of Pharmacology, Medical School, University of Crete, Iraklion 71110 Greece.
E-mail : gravanis@iesl.forth.gr

2. INTRODUCTION

Corticotropin-releasing hormone (CRH), is a 41-amino acid peptide, responsible not only for the endocrine but also the autonomic, immunological and behavioural responses of mammalian organisms to stress (1).

This neuropeptide is mainly synthesized in the hypothalamus and is secreted into the hypophysial-portal circulation in response to stress (1-3). Its major role is the regulation of the hypothalamus-pituitary-adrenal (HPA) axis through induction of the proopiomelanocortin (POMC) gene and the secretion of corticotropin (ACTH) from anterior pituitary (4). The CRH gene is also expressed in other intracranial sites and in an increasing number of peripheral tissues, many of which are unrelated to the activity of HPA axis. Many investigators have shown that immuno-reactive CRH (ir-CRH) and CRH mRNA are present in the adrenal medulla, lymphocytes, pancreas, lung, liver, stomach and duodenum (5-10). Furthermore, they are present in human placenta and in a number of reproductive tissues (table 1), including ovaries and testes. CRH is detectable in rat and human inflammatory sites, acting as an autocrine or paracrine proinflammatory regulator (11). It has been shown that

Table 1: Reproductive Tissues Expressing The Crh Gene

Male	Testes	Leydig cells, germ cells
Female	Ovaries	Follicles, corpora lutea
	Pregnant uterus	amnion chorion syncytiotrophoblast decidua
	non-pregnant uterus	glandular endometrium decidualized stroma

immunoneutralization of local CRH attenuates the inflammatory response.

3. CRH in plasma

Low concentrations of ir-CRH are detected in blood plasma obtained from the peripheral circulation and in the plasma of portal vessels supplying the anterior pituitary (12). It is also detectable in blood plasma of pregnant women, originating from placenta, undergoing a sharp increase during the third trimester of pregnancy and at parturition (13).

4. CRH binding protein

The amount of CRH that is secreted into the blood is probably modulated by a specific circulating CRH-binding protein (CRHBP) (14-16), which is mainly synthesized in the liver and is also expressed inthe human brain, playing an important physiological role in CRH availability.

5. CRH receptors

Two types of the human CRH receptor have been identified so far, probably resulting from alternative splicing of a single gene (17-19). These variants are identical except for a 29-amino acid insert present in the first intracellular loop of the type II receptor. CRH exerts its effects by binding to these plasma membrane receptors, coupled to G_s protein and adenylate cyclase. hCRH receptor type I binds to CRH with high affinity (k_d:1-6.6 nM), while the signalling defect of the type II receptor (k_d:18-25 nM) is due to deficient coupling to the G protein(s). The CRH receptors are widely distributed in many tissues (central and peripheral nervous system, adrenals, spleen, heart and skeletal muscle, ovary, testes and myometrium (20-26)), suggesting that hCRH may play an important role in the physiology of these organs.

6. CRH in reproductive tissues

6.1 Testes

Many investigators have shown that ir-CRH is present in the testis of several animal species, including humans (27,28) and is localized in Leydig and germ cells and in spermatozoa (29). Testicular CRH acts via specific receptors to exert autocrine inhibitory actions on Leydig cell steroidogenesis (29,30). In the rat testis, CRH acts as an antireproductive hormone and as a major local inhibitory regulator of Leydig cell function. Meanwhile, recently published data demonstrate that in mouse Leydig cells CRH exerts stimulatory effects on steroidogenesis (31).

6.2 Ovaries

The human ovary is another tissue where CRH and its receptors are also present (32,33). Ir-CRH is localized in thecal cells surrounding the ovarian follicles, in luteinized cells of the stroma and in a number of cells within the corpora lutea. It is also detected in the follicular fluid. Additionally, CRH receptors are found in the theca and stroma cells surrounding the follicles and on cumulus oophorus. The physiological significance of ovarian CRH is still unknown. However, it is postulated that it might play an important role in inflammatory-like phenomena (ovulation, luteolysis) and/or steroidogenesis taking place in the female gonad.

7. CRH in the pregnant uterus

7.1 Placenta

Multiple sites within the pregnant uterine cavity express the CRH gene, including the trophoblasts, fetal membranes (chorion, amnion) and decidua. The trophoblastic syncytium appears to be the major source of uterine CRH. Plasma CRH in pregnant women, the source of which is placenta, undergoes a sharp increase during the third trimester of pregnancy. Thus, placental CRH increases progressively during pregnancy with a time course similar to that of ir-CRH in maternal plasma. The CRHBP is also produced by placenta and intrauterine tissues and may represent one of the major mechanisms to control CRH activity during pregnancy (34-42).

Glucocorticoids positively regulate CRH gene in human placenta (41) in contrast to their suppressive effects in hypothalamus. Cytokines have a stimulatory effect on placental CRH. Indeed, IL-1 is

present in human placenta, stimulating placental CRH secretion. Cytokines also stimulate the release of placental PGE_2 and PGE_{2a} which in turn stimulate CRH and ACTH secretion. On the other hand, glucocorticoids inhibit the production of cytokines, possibly counterbalancing their direct stimulatory effect on placental CRH production (34,47).

The role of placental CRH in maternal-fetal physiology is unknown. Since it is secreted into both maternal and fetal circulation, it could function to influence the HPA axis of either mother or fetus, providing an additional adaptation mechanism of the maternal HPA axis to the demands of pregnancy and regulating the growth of fetal adrenals. It is also postulated that CRH participates at the initiation of labour regulating myometrial contractility by increasing the release of intrauterine prostaglandins and by sensitising myometrium to oxytocin (35,46).

7.2 Decidua

Ir-CRH and CRH mRNA are present in human decidua and in the "*in vitro*" decidualized stromal endometrial cells (48). There is a gestational age-related increase in decidual mRNA levels.

8. CRH in the non-pregnant uterus

8.1 Expression and localization of endometrial CRH

The CRH mRNA and its peptide product are both present in normal human glandular endometrial cells as well as in neoplastically transformed human endometrial cells (Ishikawa cell line) (49). The size of the CRH transcript is about 1.3 kilobases (kb), similar or identical to that of its human placenta and rat hypothalamus counterparts. The epithelial cells of rat uterus also express the CRH gene, in common with epithelial cells of human endometrium (50). A CRH transcript is identified in RNA extracts of rat uteri having a size of about 1.3 kb, i.e. similar or identical to that present in human placenta. immunofluorescence for CRH in normal human glandular endometrium and in Ishikawa cells reveals a cytoplasm rich in granules positive staining for ir-CRH. The bulk of ir-CRH present in extracts from normal human endometrium and in Ishikawa cell extracts and culture media, has the same chromatographic profile as synthetic 41-amino acid CRH, suggesting that endometrial ir-CRH is authentic CRH. However, in gel filtration chromatography from normal epithelial cells as well as from Ishikawa cells, a substantial amount of ir-CRH is detected, with an apparent molecular size of approximately 10 kd. This may correspond to the precursor preproCRH. The finding that most ir-CRH in the normal human endometrial cells corresponds to the 41-amino acid sized hypothalamic CRH suggests that the cycling human endometrium possesses all the necessary enzymes for the posttranslational processing of preproCRH, thus giving rise to bioactive end product. Ishikawa cells also retain this characteristic. Additionally the bulk of ir-CRH present in extracts from rat uteri has the same chromatographic profile as synthetic 41-amino acid CRH suggesting that the rat uterine ir-CRH is also the authentic CRH. Ir-CRH is localised only in the glandular cells while the stroma is negative in ir-CRH staining. In addition, immunohistochemical data show that both epithelial and decidualized stromal cells of the early pregnant rat uterus contain ir-CRH, suggesting that epithelial endometrial cells are the main source of intrauterine CRH in the non-pregnant uterus, whereas decidualization of normal stromal cells, which do not express CRH, results in the induction of the expression of this gene in both humans and rodents (48,50).

8.2 Potential physiological implications of endometrial CRH

The role of endometrial CRH within the uterine cavity is still largely unknown. Clues regarding its physiological function derive from the study of the regulation of endometrial CRH gene promoter. In homologous transfection experiments with the 0.9 kb flanking region of hCRH gene in Ishikawa human endometrial cells, we have demonstrated that estradiol, dexamethasone and indomethacin inhibit while prostaglandin PGE_2 induces in a dose dependent fashion the activity of the hCRH promoter (table 2). These findings provide evidence for direct transcriptional regulation of endometrial CRH gene by these agents and suggest that endometrial CRH is under the negative control of estrogens and glucocorticoids and under the positive control of PGE_2 (51). In addition, Br-cAMP and forskolin (FSK), two inducers of protein kinase A (PKA), increase the activity of the CRH promoter. Furthermore, epidermal growth factor (EGF), a known activator of protein kinase C (PKC), stimulates it.

The expression of the CRH gene is induced in the implantation sites of the early pregnant rat uterus, since the CRH mRNA and its peptide product present in the implantation sites are in significantly higher concentrations compared to the inter-implantation regions (50).

Human endometrial cells express the proopiomelanocortin (POMC) gene and synthesize its peptide end products (52,53) the secretion of which is inhibited by estrogens and glucocorticoids. The co-expression in endometrial epithelial cells of both CRH and POMC suggests that uterine CRH may have an autocrine/paracrine effect on locally produced POMC-derived peptides, as in the case for other peripheral tissues, such as placenta and testes (29,40). Thus, the inhibitory effects of estrogens or glucocorticoids on endometrial POMC could be the result of a preceding inhibition of endometrial CRH.

Table 2: Factors Regulating Endometrial Crh Transcription

Estrogens	inhibit
Glucocorticoids	inhibit
Progesterone	no effect
PGE_2	induces
Implantation	induces

It is postulated that CRH participates in the inflammatory phenomena, possessing procytokine-like properties. Actually, CRH is detectable in rat and human inflammatory sites. Immunoneutralization of this CRH attenuates the inflammatory response (11). The reaction of uterus to the invading blastocyst has many characteristics of inflammation (54). Indeed, several immune mediators of the inflammatory response, such as Interleukin-1 (IL-1) and Interleukin-6 (IL-6), are produced in the endometrium and IL-1 and Tumor Necrosis Factor-α (TNF-α) receptors are expressed in endometrial cells (55,56). The implanting blastocyst secretes inflammatory mediators, including IL-1 and PGE_2 and has been suggested that blastocyst-deriving IL-1 plays an essential role in implantation, since in mice blockage of its action by the antagonist IL-1ra inhibits it (57). Furthermore, measurement of IL-1 levels in the periimplantation embryo culture fluid has predictive value for the successful outcome of implantation (58). It is now known that IL-1 and PGE_2 are major inducers of CRH expression in human placenta and rat hypothalamus (59,60). Moreover, the implantation sites of the early pregnant rat uterus, contain significantly higher concentrations of ir-CRH and CRH mRNA compared to the uterine tissue in-between the implantation sites, suggesting that uterine CRH may play a role in endometrial decidualization and egg implantation. Thus, the following sequence of events may take place during implantation: the blastocyst secretes PGE_2 at the site of nidation which, among other effects, induce through the uterine epithelium the local production of pharmacodynamicaly potent concentrations of CRH, facilitating the subsequent endometrial inflammatory reaction associated with egg implantation and the formation of egg nidus. At the same time, endometrial CRH may also regulate the induced inflammatory reaction by augmenting the production of local (uterine) β-endorphin which may facilitate immunosupression at the site of nidation inhibiting the rejection of the semi-xenograft. At the same time, blastocyst-deriving estrogens could exert a local inhibitory effect on endometrial CRH establishing a local regulatory system of endometrial reaction to the implanting blastocyst.

Additionally, the repressive effects of dexamethasone and RU486 on the activity of the CRH promoter suggest that these molecules could regulate the CRH related intrauterine immune phenomena. Recently the presence of a rich network of uterine lymphokines, has been described (55). These lymphokines are mainly synthesized from endometrial stromal or epithelial cells, they affect blastocyst attachment and implantation, trophoblast outgrowth or menstruation and they are inhibited by glucocorticoids and antiglucocorticoids e.g RU486 (61,62).

Another action of endometrial CRH could be its participation in endometrial vascularization. Indeed, peripheral CRH has been found to exert vasodilatory effects (63). On the other hand, during implantation, there is a major increase in vascular permeability surrounding the implanting embryo. Endometrial capillaries adjacent to the primary decidual zone become dilated. It has been suggested that local inflammatory mediators regulate the vascular changes in the endometrium during the implantation process. Thus, endometrial CRH may be part of these sequences of events that may be under the control of factor(s) originating from the blastocyst (fig.1).

Uterine CRH, apart from its effects on the endometrium, may play a local role in the regulation of myometrial tone. Specifically, multiple isoforms of the CRH receptor have been detected in human myometrium (17). Additionally, CRH induces the release of prostaglandins PGE_2 and PGF_{2a}, strong inducers of myometrial tone. Furthermore, CRH potentiates the stimulatory effect of oxytocin on myometrial tone (64). CRH may also play a role in the regulation of myometrial tone through its effect on endometrial β-endorphin since β-endorphin has a relaxant effect on smooth muscles (fig.1) (65). During the peri-implantation period, endometrial CRH and β-endorphin may insure the necessary uterine relaxation for the efficient implantation of blastocyst. Thus, the blastocyst may regulate the myometrial tone, the increase of which at the moment of implantation could lead to its expulsion. The prevention of this phenomenon may be controlled by the blastocyst itself, secreting IL-1 and PGE_2 and inducing the synthesis and secretion of endometrial CRH.

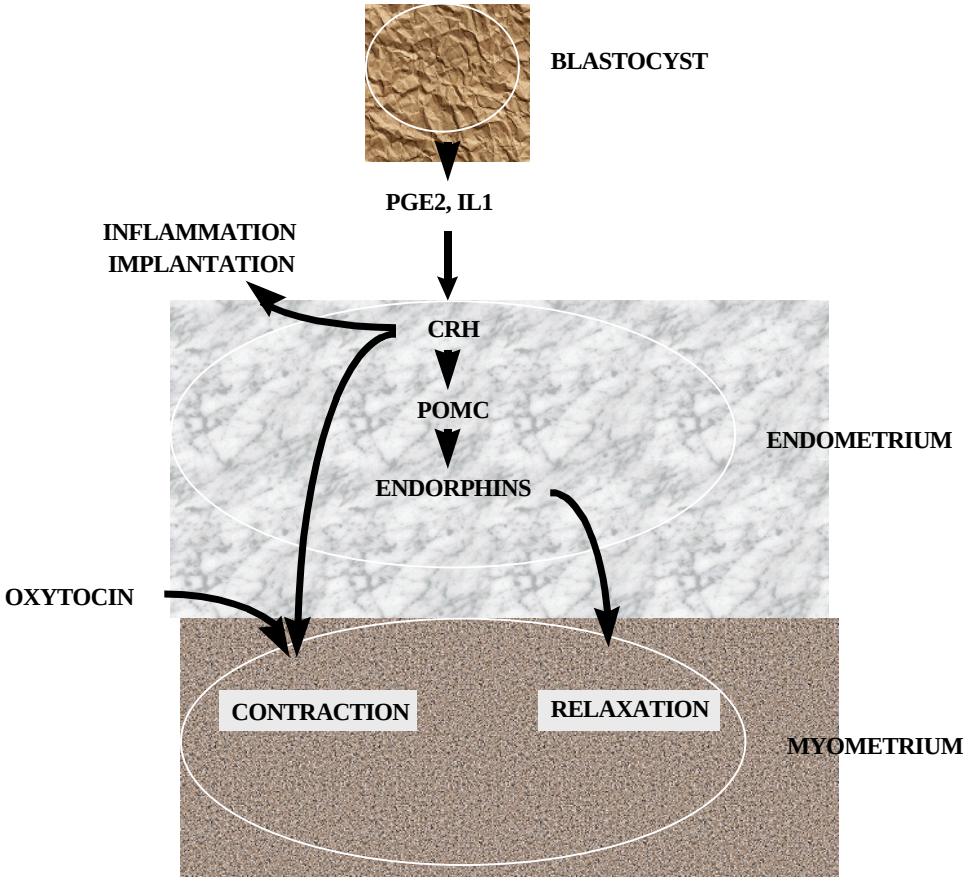

Figure 1 : Potential implications of endometrial CRH in physiological events of early pregnancy.

9. ACKNOWLEDGEMENTS

This work was supported by grants to A.G from the General Secretariat of Research and Technology, the Committee of Biomedical Research, Ministry of Health and Medicon Hellas.

10. REFERENCES

1. W. Vale, J. Spiess, C. Rivier, J. Rivier: Characterization of a 41-residue ovine hypothalamic peptide that stimulates secretion of corticotropin and β-endorphin. *Science* 213:1394-7 (1981)

2. W. Vale, C. Rivier, M.R. Brown, J. Spiess, G. Koob, A. Swanson: Chemical and biological characterization of corticotropin-releasing factor. *Recent Prog Horm Res* 39:245-67 (1983)

3. F.A. Antoni: Hypothalamic control of adrenocorticotropin secretion: advances since the discovery of 41-residue Corticotropin-releasing factor. *Endocr Rev* 7:351-362 (1986)

4. D. Orth: Corticotropin-releasing hormone in humans. *Endocr Rev* 13:164-191 (1992)

5. Usui T, Nakai Y, Tsukada T, Jingami H, Takahashi H, Fucada J. Expression of Adrenocorticotropin-releasing hormone precursor gene in placenta and other nonhypothalamic tissues in man. *Mol Endocrinol* 1988;2:871-875

6. 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;59:861-868

7. Petrusz P, Merghenthaler I, Maderdrut L, Vigh S, Schally A. Corticotropin-releasing factor-like immunoreactivity in the vertebrate endocrine pancreas. *Proc Natl Acad Sci U S A* 1983; 80:1721-1725

8. Petrusz P, Merghenthaler I, Ordronneau P, Maderdrut L, Vigh S, Schally A. Corticotropin-releasing factor-like immunoreactivity in the gastro-

entero-pancreatic endocrine system. *Peptides* 1984;5:71-72

9. Dammrich J, Ormanns W, Kahaly G, Schrezenmeir J. Multiple peptide hormone producing adenocarcinoma of lung with neurotensinand CRF-like immunoreactivity. *Pathol Res Pract* 1988;183:670-4

10. Owens MJ, Nemeroff CB. Physiology and pharmacology of Corticotropin- releasing factor. *Pharmacol Rev* 1991;43:425-73

11. Karalis K, Sano H, Redwine J, Listwark S, Wilder R, Chrousos G. Autocrine or paracrine inflammatory actions of Corticotropin-Releasing Hormone *in vivo*. *Science* 1991;254:421-423

12. Suda T, Tomori N, Yajima F, Sumitomo T, Nakagami Y, Ushiyama T, Demura H, Shizume K. Immunoreactive corticotropin-releasing factor in human plasma. *J Clin Invest* 1985;76:2026-9

13. Jeske W, Soszynski P, Rogozinski W, Lukaszewicz E, Latoszewska W, Snochowska H. Plasma GHRH, CRH, ACTH, beta-endorphin, human placental lactogen, GH andcortisol concentrations at the third trimester of pregnancy. *Acta Endocrinol* (Copenh) 1989;120:785-9

14. Nishimura E, Billestrup N, Perrin M, Vale W. Identification and characterization of a pituitary corticotropin-releasing factor binding protein by chemical cross-linking. *J Biol Chem* 1987;262:12893-6

15. Orth DN, Mount CD. Specific high-affinity binding protein for human corticotropin-releasing hormone in normal human plasma. *Biochem Biophys Res Commun* 1987;143:411-7

16. Potter E, Behan DP, Fischer WH, Linton EA, Lowry PJ, Vale WW. Cloning and characterization of the cDNAs for human and rat corticotropin releasing factor-binding proteins. *Nature* 1991;349:423-6

17. 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

18. Smets G, Van-Gompel J, Meyer C, Abs R, de-Menten Y, Kloppel G. Corticotropin-releasing hormone receptors on human pituitary cells: autoradiography at the light microscopical level, quantification and immunocytochemistry. *Prog Histochem Cytochem* 1992;26:107-9

19. Polymeropoulos MH, Torres R, Yanovski JA, Chandrasekharappa SC, Ledbetter DH. The human Corticotropin-releasing factor receptor (CRHR) gene maps to chromosome 17q12-q22. *Genomics* 1995;28:123-4

20. Xiong Y, Xie LY, Abou-Samra AB. Signaling properties of mouse and human corticotropin-releasing factor (CRF) receptors: decreased coupling efficiency of human type II CRF receptor. *Endocrinology* 1995 ;136:1828-34

21. Perrin M, Donaldson C, Chen R, Blount A, Berggren T, Bilezikjian L, Sawchenko P, Vale W. Identification of a second corticotropin-releasing factor receptor gene and characterization of a cDNA expressed in heart. *Proc Natl Acad Sci U S A* 1995; 92:2969-73

22. Kishimoto T, Pearse RV 2nd, Lin CR, Rosenfeld MG. A sauvagine/corticotropin-releasing factor receptor expressed in heart and skeletal muscle. *Proc Natl Acad Sci U S A* 1995;92:1108-12

23. Lovenberg TW, Liaw CW, Grigoriadis DE, Clevenger W, Chalmers DT, De Souza EB, Oltersdorf T. Cloning and characterization of a functionally distinct corticotropin-releasing factor receptor subtype from rat brain. *Proc Natl Acad Sci U S A* 1995; 92: 836-40

24. Grigoriadis DE, Heroux JA, De Souza EB. Characterization and regulation of corticotropin-releasing factor receptors in the central nervous, endocrine and immune systems. *Ciba Found Symp* 1993;172:85-101

25. Chang CP, Pearse RV 2d, O'Connell S, Rosenfeld MG. Identification of a seven transmembrane helix receptor for corticotropin-releasing factor and sauvagine in mammalian brain. *Neuron* 1993;11:1187-95

26. Chen R, Lewis KA, Perrin MH, Vale WW. Expression cloning of a human corticotropin-releasing-factor receptor. *Proc Natl Acad Sci U S A* 1993; 90: 8967-71

27. Yoon DJ, Sklar C, David R. Presence of immunoreactive corticotropin-releasing factor in rat testis. *Endocrinology* 1988;122:759-61

28. Audhya T, Hollander CS, Schlesinger DH, Hutchinson B. Structural characterization and localization of corticotropin-releasing factor in testis. *Biochim Biophys Acta* 1989;995:10-6

29. Fabbri A, Tinajero JC, Dufau ML. Corticotropin-releasing factor is produced by rat Leydig cells and has a major local antireproductive role in the testis. *Endocrinology* 1990;127:1541-3

30. Dufau ML, Tinajero JC, Fabbri A. Corticotropin-releasing factor: an antireproductive hormone of the testis. *FASEB J* 1993;7:299-307

31. Huang BM, Stocco DM, Hutson JC, Norman RL. Corticotropin-releasing hormone stimulates steroidogenesis in mouse Leydig cells. *Biol Reprod* 1995; 53: 620-626

32. Mastorakos G, Webster E, Friedman T, Chrousos G. Immunoreactive Corticotropin-releasing hormone and its binding in the rat ovaries. *J Clin Invest* 1993;92:961-968

33. Mastorakos G, Scopa C, Vryonidou A, Friedman T, Kattis D, Phenekos C, Merino M, Chrousos G. Presence of immunoreactive corticotropin-releasing hormone in normal and polycystic human ovaries. *J Clin Endocrinol Metab* 1994;79:1191-1197

34. Grino M, Chrousos G, Margioris A. The Corticotropin-releasing hormone gene is expressed in human placenta. *Biochem Biophys Res Com* 1987; 148: 1208-1214

35. Margioris AN. Corticotropin releasing hormone and the placenta and fetal membranes. In: *Molecular aspects of placental and fetal membrane autacoids*, Brennecke S and Rice G (eds), CRC Press (publs) 1993;12:277-301

36. Linton EA, Perkins AV, Woods RJ, Eben F, Wolfe CD, Behan DP, Potter E, Vale WW, Lowry-PJ. Corticotropin releasing hormone-binding protein (CRH-BP): plasma levels decrease during the third trimester of normal human pregnancy. *J Clin Endocrinol Metab* 1993;76:260-2

37. Campbell EA, Linton EA, Wolfe CD, Scraggs PR, Jones MT, Lowry PJ. Plasma corticotropin-releasing hormone concentrations during pregnancy and parturition. *J Clin Endocrinol Metab*. 1987;64:1054-9

38. Frim D, Emanuel R, Robinson B, Smas C, Adler G, Majzoub J. Characterization and gestational regulation of Corticotropin-releasing hormone mRNA in human placenta. *J Clin Invest* 1988;82:287-292

39. Petraglia F, Sawchenko PE, Rivier J, Vale W. Evidence for local stimulation of ACTH secretion by corticotropin-releasing factor in human placenta. *Nature* 1987;328:717-719

40. Margioris AN, Grino M, Protos P, Gold P, Chrousos G. Corticotropin-releasing hormone and Oxytocin stimulate the release of placental proopiomelanocortin peptides. *J Clin Endocrinol Metab*. 1988;66:922-926

41. Robinson B, Emanuel R, Frim D, Majzoub J. Glucocorticoid stimulates expression of corticotropin-releasing hormone gene in human placenta. *Proc Natl Acad Sci U S A* 1988;85:5244-5248

42. Petraglia F, Potter E, Cameron VA, Sutton S, Behan DP, Woods RJ, Sawchenko PE, Lowry PJ, Vale W. Corticotropin-releasing factor-binding protein is produced by human placenta and intrauterine tissues. *J Clin Endocrinol Metab* 1993;77:919-24

43. Coukos G, Monzani A, Saletti C, Petraglia F. [Noradrenaline and interleukin-1 stimulate CRF secretion from human placental cells in culture]. *Medicina* (Firenze) 1988;8:441-2

44. Margioris AN, Grino M, Rabin D, Chrousos GP. Human placenta and the hypothalamic-pituitary-adrenal axis. *Adv Exp Med Biol* 1988;245:389-98

45. Riley SC, Challis JR. Corticotrophin-releasing hormone production by the placenta and fetal membranes. *Placenta* 1991;12:105-19

46. Quartero HW, Fry CH. Placental corticotrophin releasing factor may modulate human parturition. *Placenta* 1989;10:439-43

47. Sasaki A, Tempst P, Liotta AS, Margioris AN, Hood LE, Kent SB, Sato S, Shinkawa O, Yoshinaga K, Krieger DT. Isolation and characterization of a corticotropin-releasing hormone-like peptide from human placenta. *J Clin Endocrinol Metab* 1988; 67: 768-73

48. Petraglia F, Tabanelli S, Galassi C, Garuti GC, Mancini A, Genazzani A, Gurpide E. Human decidua and *in vitro* decidualized endometrial stromal cells at term contain immunoreactive CRF and CRF mRNA. *J Clin Endocrinol Metab* 1992;74:1427-1431

49. Makrigiannakis A, Zoumakis E, Margioris AN, Theodoropoulos P, Stournaras C, Gravanis A. The corticotropin-Releasing Hormone in normal and tumoral epithelial cells of human endometrium. *J Clin Endocrinol Metab* 1995;80:185-189

50. Makrigiannakis A, Margioris AN, LeGoascogne 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

51. Makrigiannakis A, Zoumakis E, Margioris AN, Stournaras C, Chrousos GP, Gravanis A. Estrogen and glucocorticoids repress the activity of the 5'-flanking region of human corticotropin-releasing hormone (CRH) gene in transiently transfected Ishikawa human endometrial cells. 1995 Abstract in *76th Endocrine Meeting*, Washington, MD

52. Makrigiannakis A, Margioris A, Markogiannakis E, Stournaras C, Gravanis A. Steroid hormones regulate the release of immunoreactive β-endorphin from the Ishikawa human endometrial cell line. *J Clin Endocrinol Metab* 1992;75:584-589.

53. Gravanis A, Makrigiannakis A, Stournaras C, Margioris AN. Interaction between steroid hormones and endometrial opioids. *Ann NY Acad Sci* 1994;734:245-256

54. Weitlauf HM. The physiology of reproduction. E. Knobil, J. Neil, eds. New York:Raven Press. 1988:231- 262

55. Tabibzadeh S. Cytokine regulation of human endometrial function. *Ann NY Acad Sci* 1991;622:89-98

56. Ininns E, Gatanaga M, Cappuccini F, Dett C, Yamamoto R, Granger G, Gatanaga T. Growth of the endometrial adenocarcinoma cell line AN3 CA is modulated by tumor necrosis factor and its receptor is up-regulated by estrogen *in vitro*. *Endocrinology* 1992;130:1852-1856

57. Simon C, Frances A, Piquette G, Danasouri I, Zurawski G, Dang W, Polan M. Embryonic implantation in mice is blocked by interleukin-1 receptor antagonist. *Endocrinology* 1992;134:521-529

58. Sheth KV, Roca GL, Al-Sedairy S, Parhar R, Hamilton G, Al-Abdul Jabbar F. Prediction of successful embryo implantation by measuring interleukin-1-alpha and immunosuppressive factor(s) in preimplantation. *Fertil Steril* 1991;55:952-957

59. Sapolsky R, Rivier C, Yamamoto G, Plotsky P, Vale W. Interleukin-1 stimulates the secretion of hypothalamic Corticotropin-releasing factor. *Science* 1987;238:522-524

60. Petraglia F, Garutti GC, Deramundo B, Angioni S, Genazzani A, Bilezikjian M. Mechanisms of action of interleukin-1 in increasing CRF and ACTH from cultured human placental cells. *Am J Obstet Gynecol* 1990;163:1307-12

61. Hill JA. Cytokines considered critical in pregnancy. *Am J Reprod Immunol* 1982;28:123-126

62. Antonakis N, Georgoulias V, Margioris A, Stournaras, Gravanis A. *In vitro* differential effects of the antiglucocorticoid RU 486 on the release of lymphokines from normal human lymphocytes. *J Steroid Biochem Mol Biol* 1994;51:67-72

63. Rogers PA. Early endometrial microvascular response during implantation in the rat. *Reprod Fertil Dev* 1992;4: 261-264

64. Quartero HW, Srivatsa G, Gillham B. Role of cyclic adenosine monophosphate in the synergistic interaction between oxytocin and CRH in isolated human myometrium. *Clin Endocrinol* 1992;36:141-145

65. G. Olson, R. Olson, A. Kastin: Endogenous opiates. *Peptides*1989;10:1253-1280