

Corticotropin Releasing Hormone: a Diagnostic Marker for Behavioral and Reproductive Disorders?

Pasquale Florio¹, Maria Chiara Zatelli², Fernando M. Reis³, Ettore C. degli Uberti², and Felice Petraglia¹

¹ Chair of Obstetrics & Gynecology, Department of Pediatrics, Obstetrics & Reproductive Medicine, University of Siena, Siena, Italy, ² Section of Endocrinology, University of Ferrara, Ferrara, Italy, and ³ Department of Obstetrics and Gynecology, UFMG, Belo Horizonte, Brazil

TABLE OF CONTENTS

1. Abstract
2. Introduction
3. Regulation of CRH secretion from the hypothalamus
4. CRH test
5. Neuropsychiatric disorders and neurodegenerative diseases
6. CRH in human gestation
7. Pre-eclampsia
8. Preterm Labor
9. Summary and perspective
10. References

1. ABSTRACT

Corticotropin-releasing hormone (CRH) is a key mediator of endocrine, autonomic, behavioral, and immune responses to stress. The ability of CRH to induce hormonal stress responses has been used to investigate the functionality of the hypothalamus-pituitary-adrenal axis, and consequently the activity of hypothalamic CRH neuronal systems. Indeed, CRH administration to humans causes prompt release of ACTH, followed by secretion of cortisol, aldosterone and other adrenal steroids. CRH hypersecretion / hyperactivity has been associated to major depression, anxiety-related disorders, anorexia nervosa, Alzheimer's and Parkinson's diseases and progressive supranuclear palsy. During pregnancy the human placenta and its accessory membranes are the major sites of CRH synthesis and secretion. Placental CRH secretion is autonomous, but increasing evidence indicates that maternal or fetal conditions may influence such secretion. Therefore, the emerging concept is that in the event of acute or chronic metabolic, physical or infectious stress, the placenta takes part in a stress syndrome by releasing CRH, which may contribute to restore local blood flow and to influence the timing of delivery. The CRH released by the placenta is measurable in maternal plasma and other biological fluids and may be used to diagnose subclinical processes anteceding pregnancy complications such as pre-eclampsia and preterm delivery.

2. INTRODUCTION

Corticotropin-releasing hormone (CRH) is a 41-amino acid single chain polypeptide, and is the major physiologic ACTH secretagogue (1). This peptide is produced in parvocellular neurons of the hypothalamic paraventricular nucleus (PVN) (2) and is secreted into the pituitary portal circulation from axonal terminals in the external zone of the median eminence along with other ACTH secretagogues, such as arginine vasopressin (AVP), cholecystikinin, met-enkephalin and dynorphin (3). CRH is also synthesized by anterior pituitary corticotroph cells and stimulates ACTH secretion in an autocrine or paracrine fashion (4).

CRH is localized in a variety of peripheral tissues (5). CRH fibers originating in the hypothalamus are present in the intermediate lobe of the pituitary, and CRH has been detected in the adrenal medulla, as well as in lymphocytes, mast cells, testis (6,7), ovaries (8,9), pancreas, stomach, small intestine, myometrium, endometrium, placenta (10-12) and in inflammatory sites of a variety of species (13-15). CRH is not detectable in the circulation under normal circumstances. Very high levels have been measured in the plasma of pregnant women during the third trimester (16). Despite this, pregnant women have normal ACTH levels because the bioavailability of CRH is limited by a specific CRH-binding protein (CRH-BP), a 37-kilodalton protein of

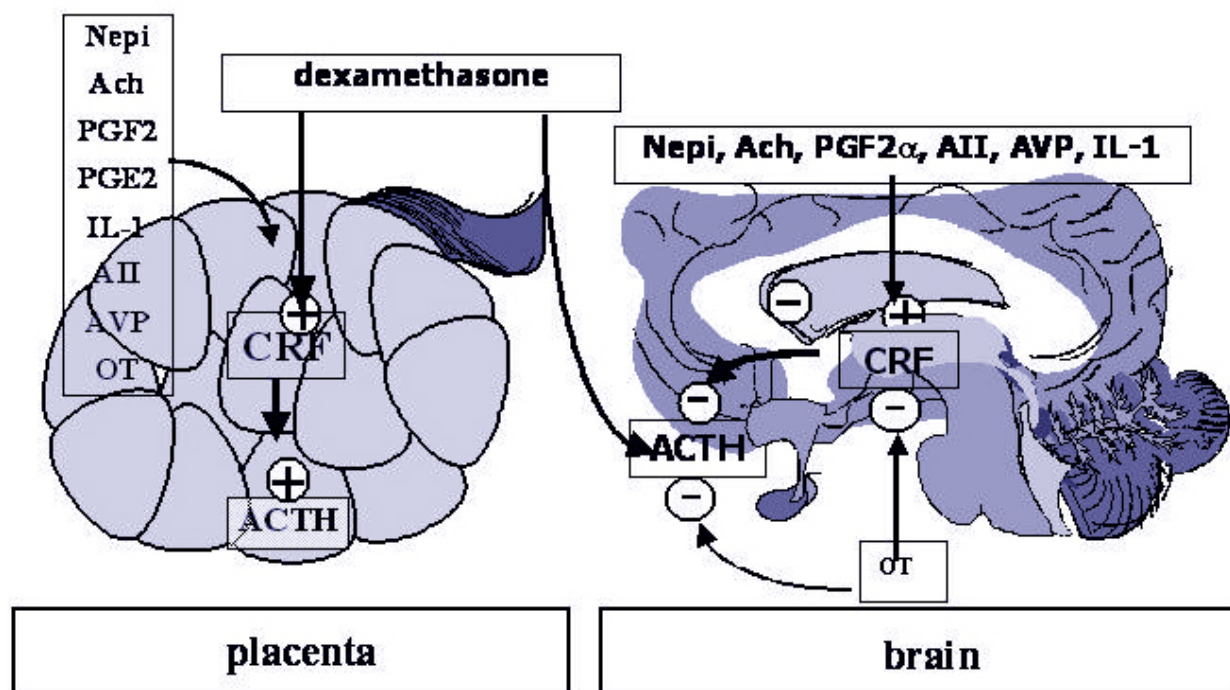


Figure 1. The mechanisms stimulating CRH release from medial basal hypothalamus are in part chemically identical to those operating in the human placenta. Prostaglandin F₂ (PGF₂) and E₂ (PGE₂), norepinephrine (Nepi), acetylcholine (Ach), angiotensin II (AII), arginine vasopressin (AVP), stimulate CRH in hypothalamus, as well as in placental cells. On the contrary, the effect of oxytocin (OT) on CRH and HPA hormones in human placenta, is different being stimulatory. In turns, placental CRH stimulates ACTH secretion from cultured human placental cells.

322 amino acids (17), mainly produced by the liver and placenta. Further sources of CRH-BP during pregnancy are decidua and fetal membranes (18).

3. REGULATION OF CRH SECRETION FROM THE HYPOTHALAMUS

CRH physiologically regulates basal and stress-induced release of ACTH, beta-endorphin and other POMC-derived peptides from the anterior pituitary (19) (Figure 1). ACTH released by CRH leads to secretion of cortisol and other adrenal steroids, such as dehydroepiandrosterone (DHEA) and, transiently, aldosterone (20). The plasma ACTH response to CRH is inversely correlated with the basal plasma cortisol concentration and is not influenced by the hour of day, however the corresponding cortisol response is maximized in late afternoon (21). Plasma ACTH and cortisol response to CRH are comparable in males and females (22), while plasma DHEA response is reduced in elderly men, and plasma cortisol response is blunted in obese subjects (21).

Hypothalamic CRH release is controlled by a variety of stimulatory and inhibitory inputs, as summarized in Figure 1. Norepinephrine has both stimulatory and inhibitory effects on CRH release, depending on the dose administered and the receptor subtype involved (23). Similarly, opioids can both inhibit and stimulate CRH release, depending on the nature of the opioid, the dose and

the receptor involved (24). Drugs acting at the GABA/benzodiazepine/chloride ionophore complex are potent inhibitors of CRH secretion. Glucocorticoids are potent inhibitors of CRH release, and therefore adrenalectomy results in marked elevations in the synthesis and release of CRH, and a consequent down-regulation of CRH binding and CRH-stimulated adenylate cyclase activity in the anterior pituitary, which can be prevented by glucocorticoid administration (25).

4. CRH TEST

CRH stimulation test represents a standard HPA axis challenge test, which is used to investigate the activity of hypothalamic CRH neuronal systems (26). CRH administration to humans causes prompt release of ACTH into the blood, followed by secretion of cortisol and other adrenal steroids including aldosterone (27). Since human and ovine CRH appear to have equal diagnostic value, most studies have used ovine CRH, which is more potent and longer acting than human CRH (27). The test is performed by injecting 100 microgram or 1 microgram/kg CRH intravenously, and measuring cortisol and ACTH at -5, -1, 0, 15, 30, 60, 90, and 120 minutes. Maximal ACTH response (2- to 4-fold above baseline) is evoked at 30 minutes and cortisol levels peak (over 20 microgram/dL) after 60 minutes or increase more than 10 microgram/dL above baseline (28). Despite great variations in ACTH response, the CRH test can be useful to demonstrate ACTH

deficiency or Cushing's disease. Dexamethasone pretreatment in the context of petrosal venous sampling can be useful for the diagnosis of ACTH-secreting pituitary adenoma using the CRH test, as it allows a sensitive and specific ACTH gradient, effectively distinguishing peripheral from pituitary excessive ACTH secretion (29). Pituitary responsiveness to CRH can be suppressed by circulating glucocorticoids, and therefore it may be difficult to distinguish a corticotroph adenoma from pseudo-Cushing's disease. In these circumstances, combining CRH administration with dexamethasone suppression test may be a useful diagnostic tool (30). In normal subjects and in pseudo-Cushing's disorder, cortisol levels are ≤ 1.4 microgram/dL, while an ACTH-secreting pituitary tumor is invariably present with 100% sensitivity and specificity when cortisol levels are > 4 microgram/dL after 15 minutes from CRH injection (30). CRH responsiveness (at least a 35% cortisol rise) is usually retained in ACTH-secreting adenomas but is not apparent in more than 90% of ectopic ACTH-producing tumors. Although this approach has 100% specificity, only 90% sensitivity is achieved, and the test does not distinguish ACTH-secreting adenomas from pseudo-Cushing's disorder (31).

5. NEUROPSYCHIATRIC DISORDERS AND NEURODEGENERATIVE DISEASES

Major depression is frequently characterized by hypercortisolemia and an abnormal dexamethasone suppression test. It has been suggested that these alterations might be due to CRH hypersecretion/hyperactivity, since in major depression CRH levels are significantly increased in the cerebrospinal fluid (CSF) (32), and significantly and positively correlate with the degree of post-dexamethasone suppression plasma cortisol levels (33). Furthermore, CRH binding sites in the frontal cerebral cortex of suicide victims are reduced, compared with controls, supporting the hypothesis that CRH is hypersecreted in major depression (34) and down-regulates CRH receptors. In addition, depressed patients show a blunted ACTH response to intravenously administered ovine or human CRH, compared with normal controls (35).

CRH may also be involved in **anxiety-related disorders**, since in panic disorder patients CRH test evokes a blunted ACTH response (36), most likely due to excess secretion of endogenous CRH. **Anorexia nervosa** is characterized by hypothalamic hypogonadism and hypercortisolism, with a markedly attenuated ACTH response to CRH test. These alterations return to normal after normal body weight is restored (19, 37).

In **Alzheimer's disease** cerebral cortical CRH content is decreased, while CRH receptors are increased. Moreover, it has been shown that CRH is reduced in the caudate (38), and in the CSF (39), with a significant correlation between CRH levels in CSF and the global neuropsychological impairment ratings, suggesting that a greater cognitive impairment is associated with a lower CSF concentrations of CRH (40). On the other hand, CRH immunostaining of PVN is increased in Alzheimer's disease as compared to controls (41). The increased expression

and/or release of CRH from the PVN would provide a reasonable explanation for the hypercortisolemia often seen in Alzheimer's patients. In **Parkinson's disease** with dementia CRH content is decreased in the cerebral cortex (42), but not in the hypothalamus (43). CRH was found to be reduced also in the frontal, temporal and occipital lobes of patients with **progressive supranuclear palsy**, but cerebral cortical reduction in CRH content might be a nonspecific consequence of the disease (42).

6. CRH IN HUMAN GESTATION

Human placenta, decidua, chorion and amnion produce CRH (44, 45). The structure of placental CRH mRNA is similar to that predicted for hypothalamic CRH mRNA (12), and intrauterine tissues are able to synthesize its mRNA and secrete the biological active peptide in response to different stress stimuli during pregnancy (46).

Some mechanisms stimulating CRH release from medial hypothalamic eminence in the brain are in part identical to those operating in the human placenta, since prostaglandins, neurotransmitters (norepinephrine, acetylcholine) and neuropeptides (angiotensin II, arginine vasopressin) stimulate the release of CRH from cultured placental cells, suggesting a close correlation between hypothalamic and placental regulation of CRH release (47) (Figure 1). Furthermore, in agreement with the regulation of the hypothalamic CRH, interleukin-1 stimulates the release of CRH from cultured placental cells, however interleukin-2 has no effect on it (47, 48).

On the contrary to the effect played on the hypothalamus, oxytocin has different effects being inhibitory to CRH/hypothalamus-pituitary-adrenal (HPA) axis (49), while stimulatory on CRH and ACTH secretion from cultured placental cells (50). Finally, glucocorticoids stimulate placental CRH synthesis and secretion in primary cultures of human placenta (51), and this stimulation is in contrast to the glucocorticoid suppression of CRH expression in hypothalamus.

Plasma CRH levels are low in nonpregnant women (less than 10 pg/mL) and become higher during the first and second trimesters of pregnancy. A clear increase is evident after the second trimester of gestation, rising steadily until term (52). There is no further change during the progression of spontaneous labor (53). Maternal CRH is secreted with no circadian rhythm, but with a pulsatile variation (54). Pulse frequency and duration of plasma CRH do not change according to gestational age, whereas pulse amplitude progressively increases through pregnancy. After spontaneous labor and delivery or elective cesarean section, maternal plasma CRH concentrations decrease to 50% of predelivery values by 20–30 minutes and become similar to those of nonpregnant women within 1–5 days postpartum (52, 55).

The diurnal rhythm for plasma ACTH, cortisol, and beta-endorphin is maintained in pregnant women; however, CRH does not have a circadian rhythm (54, 56). These observations suggest that during pregnancy pituitary

CRH as a diagnostic marker

ACTH release is regulated centrally whereas placental CRH is produced independently from the maternal hypothalamus-pituitary-adrenal axis. Indeed, maternal plasma CRH correlates significantly with beta-endorphin during pregnancy but not at labor, suggesting that placental CRH has a minor role in the regulation of pituitary-corticotroph function at this time. In addition, there is no substantial change in maternal CRH levels in women exposed to either chronic psychosocial stress (57) or acute physical stress (58), reinforcing the concept that most of the CRH measurable in maternal circulation comes from the placenta and does not reflect the stress-induced hypothalamic CRH release.

CRH is measurable in spot urine, and the concentrations of urinary CRH are elevated during pregnancy (approximately 55 pg per micromol of creatinine) and correlate with gestational age. The amount of CRH excreted is very low: only 0.03% is detectable in urine, whereas 99.97% of filtered CRH is reabsorbed or metabolized by the kidney (59). CRH is also measurable in fetal circulation, and a linear correlation exists between maternal and fetal plasma CRH levels. The CRH levels in umbilical cord blood are 20–30-fold lower than in maternal circulation (55, 60), with higher concentrations in the venous than in the arterial cord blood, which suggests placenta as a major source (61).

Amniotic fluid CRH levels measured at 16–18 weeks are similar to those of maternal plasma. Higher levels are found at term, with CRH concentrations similar to those of cord plasma but 20–30-fold less than in maternal plasma (62). A significant correlation between the amniotic fluid and maternal plasma CRH levels obtained simultaneously suggests a placental origin for amniotic CRH (62, 63), but it is unclear how CRH is transported to the amniotic fluid.

CRH-BP is measurable in maternal plasma, and levels remain stable in nonpregnant women and until the third trimester of pregnancy. Maternal plasma CRH-BP concentrations slightly decrease between 26 and 30 weeks, then significantly and rapidly decrease in the last 4–6 weeks before labor (64–66). During the first 24 hours postpartum, this protein returns to approximately nonpregnant levels. Thus, opposite changes in concentration of CRH and CRH-BP in maternal plasma occur at term.

7. PRE-ECLAMPSIA

Preeclampsia is a multisystemic syndrome specific to pregnancy and is a leading cause of maternal and neonatal morbidity and mortality. It is characterized by hypertension arising *de novo* during the second half of gestation associated with diffuse endothelial damage and renal dysfunction, which is manifested as massive proteinuria. The current requirements for a clinical diagnosis of preeclampsia are 300 mg or more of urinary protein excretion per 24 h, and hypertension, defined as blood pressure of 140/90 mm Hg or higher, first diagnosed after 20 weeks of gestation.

Increased maternal serum CRH levels are a frequent feature of pregnancies complicated by preeclampsia. Increased placental synthesis of CRH is evident at both the mRNA and protein levels (67,68). Umbilical cord plasma concentrations of CRH are higher in preeclampsia than in normotensive pregnancies, and concentrations are higher in venous than in arterial cord blood, indicating the secretion of CRH from the placenta into the fetal circulation (69). The intimate mechanisms leading to excessive placental production of CRH in preeclampsia are not known.

Relatively high CRH concentrations may be detectable as early as the second trimester in women who subsequently develop preeclampsia (70). However, in a selected sample of women at high risk of developing gestational hypertension, we have observed that a consistent elevation of maternal CRH levels did not occur before the onset of hypertension (66). CRH levels are actually lower in women with an increased uterine artery resistance at mid-gestation, which is a high risk group for subsequent gestational hypertension and preeclampsia (71). However, at 28–29 weeks gestation it is possible to use maternal plasma CRH (increased) and CRH-BP (decreased) levels in hypertensive women to predict who will proceed to develop preeclampsia (72) (Figure 2).

8. PRETERM LABOR

Preterm delivery is defined as delivery occurring before 37 completed gestational weeks. It complicates nearly 10% of all births and accounts for approximately 70% of all neonatal deaths throughout the world. A laboratory test that could recognize at early gestation the women with higher probability to have spontaneous preterm labor and delivery would be extremely useful to improve perinatal outcomes.

In this context, the assessment of maternal CRH has been a promising tool since elevated CRH levels were observed in pregnancies complicated by preterm labor (73). The evolution of maternal plasma CRH concentrations parallels the CRH curve of normal pregnancy but the level is displaced upward (74). This discrimination is detectable before any clinical manifestation of uterine contractility, a fact that prompted the design of controlled studies focusing on the value of CRH measurement for prediction of preterm labor or impending preterm birth. These studies showed that the predictive power of CRH increases with gestational age and probably achieves clinical significance only at third trimester (Figure 3).

This view is supported by the following evidence. An extensive cross-sectional study of asymptomatic women revealed a poor discrimination between pregnancies ending at term and preterm on the basis of second-trimester maternal serum CRH concentrations (75). A large cohort study of low-risk women starting at 15–20 wk of gestation (76) confirmed that the measurement of maternal serum CRH would not satisfy the requisites of a screening test for preterm delivery in a low-risk population. Recent data from a prospective study showed that a single CRH measurement

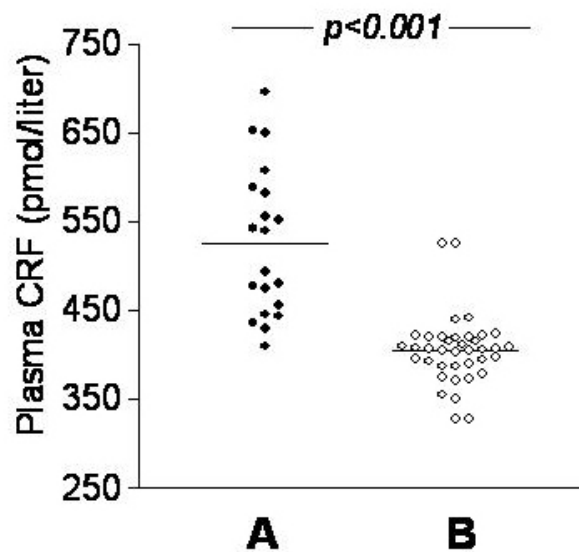


Figure 2. Maternal plasma CRH concentrations in women with gestational hypertension who later progressed to preeclampsia (A) and in those who did not have further complications and were eventually diagnosed as transient hypertension (B). The horizontal lines indicate the group means. Modified from Florio *et al.* 2004 (72).

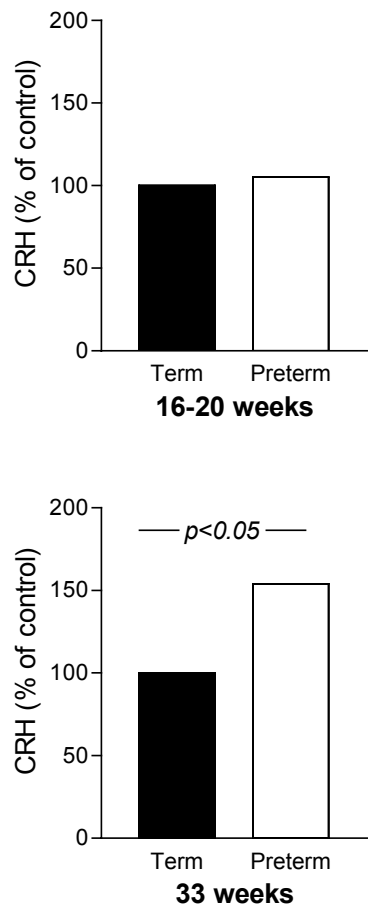


Figure 3. The effect of gestational age on preterm labor prediction by a single CRH measurement in asymptomatic women. CRH fails to discriminate between the two outcome groups when assessed at 16-20 weeks (77) but achieves a much better risk prediction at 33 weeks (9).

between 16 and 20 weeks does not predict the occurrence of preterm birth even in a high risk population, with an overall incidence of 36% of birth at less than 37 weeks (77).

At third trimester, however, there seems to be a better correlation between maternal CRH and the risk of preterm birth. A single CRH measurement at 33 weeks in asymptomatic women has detected a threefold increased risk of preterm birth among those with "high" CRH levels (78). Finally, CRH is useful to predict impending birth among women presenting with preterm labor in the third trimester (79).

There is no doubt that placental CRH plays a role in the control of human parturition, but its precise role is still a matter of debate. The finding of elevated midtrimester CRH levels in women who will have preterm delivery does not necessarily represent an early release of the cascade of parturition, since many women with relatively high midtrimester CRH levels still proceed to term. The precocious elevation of plasma CRH levels could be an epiphenomenon rather than a trigger for the mechanisms leading to preterm labor (80). An unsolved paradox emerges from biochemical evidence for a myometrial relaxing effect of CRH before term, favoring uterine quiescence, in contrast to its indirect uterotonic effect *in vitro* and its increased bioavailability at term (81). This apparently dual role of CRH might explain its limited predictive power for preterm labor. Future research should contemplate this complexity of CRH regulation and try to improve its predictive performance by combining it with clinical, biophysical and biochemical data, such as CRH-BP (80).

9. SUMMARY AND PERSPECTIVE

More than 20 years after CRH biochemical characterization and the subsequent development of a specific radioimmunoassay, this hormone remains a promising diagnostic tool in behavioral and reproductive conditions. It has not yet been introduced in clinical practice as a routine diagnostic marker, but only as a pharmacological stimulus to assess pituitary ACTH release. This is because CRH, like any hypothalamic hormone, circulates in very low concentrations in peripheral blood. However, placental CRH is released abundantly into maternal circulation and becomes a measurable marker that increases in maternal plasma before clinical signs of preterm labor or pre-eclampsia and also in the rests of spontaneous abortion (82). The jump from this evidence to the clinical use of CRH measurement will require further progress in understanding CRH's functional regulation and improving its reliability through combination with other diagnostic markers.

10. REFERENCES

1. Vale W, C. Rivier, M.R. Brown, J. Spiess, G. Koob, L. Swanson, L. Bilezikjian, F. Bloom & J. Rivier: Chemical and biological characterization of corticotropin releasing factor. *Recent Prog Horm Res* 39, 245-70 (1983)

2. Swanson LW, P.E. Sawchenko, J. Rivier & W.W. Vale: Organization of ovine corticotropin-releasing factor immunoreactive cells and fibers in the rat brain: An immunohistochemical study. *Neuroendocrinology* 36, 165-86 (1983)

3. Antoni FA: Hypothalamic control of adrenocorticotropin secretion: advances since the discovery of 41-residue corticotropin-releasing factor. *Endocr Rev* 7, 351-78 (1986)

4. Pecori-Giraldi F & F. Cavagnini: Corticotropin-releasing hormone is produced by rat corticotropes and modulates ACTH secretion in a paracrine/autocrine fashion. *J Clin Invest* 101, 2478-84 (1998)

5. MJ Owens & CB Nemeroff: Neurotransmitter regulation of the CRH secretion in vitro. In: Corticotropin-releasing factor: Basic and clinical studies of a neuropeptide. Eds: De Souza EB, Nemeroff CB, CRC Press, Boca Raton (1990)

6. Fabbri A, J.C. Tinajero & M.L. Dufau: Corticotropin-releasing factor is produced by rat Leydig cells and has a major local antireproductive role in the testis. *Endocrinology* 127, 1541-43 (1990)

7. Ullisse S, A. Fabbri & M.L. Dufau: Corticotropin-releasing factor receptors and actions in rat Leydig cells. *J Biol Chem* 264, 2156-63 (1989)

8. Mastorakos G, E.L. Webster, T.C. Friedman & G.P. Chrousos: Immunoreactive corticotropin releasing hormone (CRH) and its binding sites in the rat ovary. *J Clin Invest* 92, 961-8 (1993)

9. Mastorakos G, C.D. Scopa, A. Vryonidou, T.C. Friedman, D. Kattis, C. Phenekos, M.J. Merino & G.P. Chrousos: Presence of immunoreactive corticotropin releasing hormone (CRH) in normal and polycystic human ovaries. *J Clin Endocrinol Metab* 79, 1191-7 (1994)

10. Petraglia F, S. Tabanelli, M.C. Galassi, G.C. Garuti, A.C. Mancini, A.R. Genazzani & E. Gurpide: Human decidua and in vitro decidualized endometrial stromal cells at term contain immunoreactive corticotropin-releasing factor (CRH) and CRH messenger ribonucleic acid. *J Clin Endocrinol Metab* 74, 1427-31 (1992)

11. Mastorakos G, C.D. Scopa, L.C. Kao, A. Vryonidou, T.C. Friedman, D. Kattis, C. Phenekos, D. Rabin & G.P. Chrousos: Presence of immunoreactive corticotropin-releasing hormone in human endometrium. *J Clin Endocrinol Metab* 81, 1046-50 (1996)

12. Grino M, G.P. Chrousos & A.N. Margioris: The corticotropin releasing hormone gene is expressed in human placenta. *Biochem Biophys Res Commun* 148, 1208-14 (1987)

13. Chrousos GP: The Hypothalamic-Pituitary-Adrenal axis and the immune/inflammatory reaction. *N Engl J Med* 332, 1351-62 (1995)

14. Scopa CD, G. Mastorakos, T.C. Friedman, M. Melachrinou, M.J. Merino MJ & G.P. Chrousos: Presence of immunoreactive corticotropin releasing hormone (CRH) in thyroid lesions. *Am J Pathol* 145, 1159-67 (1994)
15. Mastorakos G, E.A. Bouzas, P.B. Silver, G. Sartani, T.C. Friedman, C.C. Chan, R.R. Caspi & G.P. Chrousos: Immune corticotropin-releasing hormone is present in the eyes of and promotes experimental autoimmune uveoretinitis in rodents. *Endocrinology* 136, 4650-8 (1995)
16. Sasaki A, O. Shinkawa, A.N. Margioris, A.S. Liotta, S. Sato, O. Murakami, M. Go, Y. Shimizu, K. Hanew & K. Yoshinaga: Immunoreactive corticotropin-releasing hormone in human plasma during pregnancy, labor, and delivery. *J Clin Endocrinol Metab* 64, 224-9 (1987)
17. Potter E, D.P. Behan, W.H. Fischer, E.A. Linton, P.J. Lowry & W.W. Vale: Cloning and characterization of the cDNAs for human and rat corticotropin releasing factor-binding proteins. *Nature* 349, 423-6 (1991)
18. Petraglia F, E. Potter, V.A. Cameron, S. Sutton, D.P. Behan, R.J. Woods, P.E. Sawchenko, P.J. Lowry & W. Vale: Corticotropin-releasing factor-binding protein is produced by human placenta and intrauterine tissues. *J Clin Endocrinol Metab* 77, 919-24 (1993)
19. Owens MJ & C.B. Nemeroff: Physiology and pharmacology of corticotropin-releasing factor. *Pharmacol Rev* 43, 425-73 (1991)
20. Conaglen JV, R.A. Donald, E.A. Espiner, J.H. Livesey & M.G. Nicholls: The effect of ovine corticotropin-releasing factor on catecholamine, vasopressin, and aldosterone secretion in normal man. *J Clin Endocrinol Metab* 58, 463-6 (1984)
21. DeCherney GS, C.R. DeBold, R.V. Jackson, W.R. Sheldon, D.P. Island & D.N. Orth: Diurnal variation in the response of plasma adrenocorticotropin and cortisol to intravenous ovine corticotropin-releasing hormone. *J Clin Endocrinol Metab* 61, 273-9 (1985)
22. Pavlov EP, S.M. Harman, G.P. Chrousos, D.L. Loriaux & M.R. Blackman: Responses of plasma adrenocorticotropin, cortisol, and dehydroepiandrosterone to ovine corticotropin-releasing hormone in healthy aging men. *J Clin Endocrinol Metab* 62, 767-72 (1986)
23. Plotsky PM, E.T.J. Cunningham & E.P. Widmaier: Catecholaminergic modulation of corticotropin-releasing factor and adrenocorticotropin secretion. *Endocrinol Rev* 10, 437-58 (1989)
24. degli Uberti EC, F. Petraglia, M. Bondanelli, A.L. Guo, A. Valentini, S. Salvadori, M. Criscuolo, R.E. Nappi & A.R. Genazzani: Involvement of mu-opioid receptors in the modulation of pituitary-adrenal axis in normal and stressed rats. *J Endocrinol Invest* 18, 1-7 (1995)
25. Wynn PC, J.P. Harwood, K.J. Catt & G. Aguilera: Regulation of corticotropin-releasing factor (CRH) receptors in the rat pituitary gland. Effects of adrenalectomy on CRH receptor and corticotroph responses. *Endocrinology* 116, 1653-9 (1985)
26. Plotsky P, M.J. Owens & C.B. Nemeroff: Psychoneuroendocrinology of depression: hypothalamic-pituitary-adrenal axis. *Psychiatr Clin North Am* 21, 293-307 (1998)
27. Trainer PJ, M. Faria, J. Newell-Price, P. Browne, P. Kopelman, D.H. Coy, G.M. Besser & A.B. Grossman: A comparison of the effects of human and ovine corticotropin-releasing hormone on the pituitary-adrenal axis. *J Clin Endocrinol Metab* 80, 412-7 (1995)
28. l'Allemand D, A. Penhoat, M.C. Lebrethon, R. Ardevol, V. Baehr, W. Oelkers & J.M. Saez: Insulin-like growth factors enhance steroidogenic enzyme and corticotropin receptor messenger ribonucleic acid levels and corticotropin steroidogenic responsiveness in cultured human adrenocortical cells. *J Clin Endocrinol Metab* 81, 3892-7 (1996)
29. Oldfield EH, J.L. Doppman, L.K. Nieman, G.P. Chrousos, D.L. Miller, D.A. Katz, G.B. Cutler & D.L. Loriaux: Petrosal sinus sampling with and without corticotropin-releasing hormone for the differential diagnosis of Cushing's syndrome. *N Engl J Med* 325, 897-905 (1991)
30. Yanovski JA, G.B. Cutler, G.P. Chrousos & L.K. Nieman: Corticotropin-releasing hormone stimulation following low-dose dexamethasone administration: a new test to distinguish Cushing's syndrome from pseudo-Cushing's states. *JAMA* 269, 2232-8 (1993)
31. Nieman LK, E.H. Oldfield, R. Wesley, G.P. Chrousos, D.L. Loriaux & G.B. Cutler: A simplified morning ovine corticotropin-releasing hormone stimulation test for the differential diagnosis of adrenocorticotropin-dependent Cushing's syndrome. *J Clin Endocrinol Metab* 77, 1308-12 (1993)
32. Nemeroff CB, E. Widerlov, G. Bissette, H. Walleus, I. Karlsson, K. Eklund, C.D. Kilts, P.T. Loosen & W. Vale: Elevated concentration of CSF corticotropin-releasing factor-like immunoreactivity in depressed patients. *Science* 226, 1342-4 (1984)
33. Roy A, D. Pickar, S. Paul, A. Doran, G.P. Chrousos & P.W. Gold: CSF corticotropin-releasing hormone in depressed patients and normal control subjects. *Am J Psychiatry* 143, 896-9 (1987)
34. Nemeroff CB, M.J. Owens, G. Bissett, A.C. Andorn & M. Stanley: Reduced corticotropin-releasing factor receptor binding sites in the frontal cortex of suicide victims. *Arch Gen Psychiatry* 45, 577-9 (1988)

35. Gold PW, D.L. Loriaux, A. Roy, M.A. Kling, J.R. Calabrese, C.H. Kellner, L.K. Nieman, R.M. Post, D. Pickar & W. Gallucci: Responses to corticotropin-releasing hormone in the hypercortisolism of depression and Cushing's disease. *N Engl J Med* 314, 1329-34 (1986)
36. Roy-Byrne PP, T. Uhde, R. Post, W. Gallucci, G.P. Chrousos & P.W. Gold: The corticotropin-releasing hormone stimulation test in patients with panic disorder. *Am J Psychiatry* 143, 896-9 (1986)
37. EB De Souza: Role of corticotropin-releasing factor in neuropsychiatric disorders and neurodegenerative diseases. In: Annual reports in medicinal chemistry. Ed: Seamon KB, Academic Press, San Diego (1990)
38. Bissette G, G.P. Reynolds, C.D. Kilts, W. Widerlov & C.B. Nemeroff: Corticotropin-releasing factor-like immunoreactivity in senile dementia of the Alzheimer type. *JAMA* 254, 3067-9 (1985)
39. May C, S.I. Rapoport, T.P. Tomai, G.P. Chrousos & P.W. Gold: Cerebral spinal fluid concentrations of corticotropin-releasing hormone (CRH) and corticotropin (ACTH) are reduced in Alzheimer's disease. *Neurology* 37, 535-8 (1987)
40. Pomara N, R.R. Singh, D. Deptula, P.A. LeWitt, G. Bissette, M. Stanley & C.B. Nemeroff: CSF corticotropin-releasing factor (CRH) in Alzheimer's disease: Its relationship to severity of dementia and monoamine metabolites. *Biol Psychiatry* 26, 500-4 (1989)
41. Powers RE, L.C. Walker, E.B. DeSouza, W.W. Vale, R.G. Struble, P.J. Whitehouse & D.L. Price: Immunohistochemical study of neurons containing corticotropin-releasing factor in Alzheimer's disease. *Synapse* 1, 405-10 (1987)
42. Whitehouse PJ, W.W. Vale, R.M. Zweig, H.S. Singer, R. Mayeux, M.J. Kuhar, D.L. Price & E.B. De Souza: Reduction in corticotropin-releasing factor-like immunoreactivity in cerebral cortex in Alzheimer's disease, Parkinson's disease, and progressive supranuclear palsy. *Neurology* 37, 905-9 (1987)
43. Conte-Devolx B, M. Grino, A. Nieoullon, F. Javoy-Agid, E. Castanas, V. Guillaume, M.C. Tonon, H. Vaudry & C. Oliver: Corticoliberin, somatocinin and amine contents in normal and parkinsonian human hypothalamus. *Neurosci Lett* 56, 217-22 (1985)
44. Challis JR, S.G. Matthews, C. Van Meir & M.M. Ramirez: Current topic: the placental corticotrophin-releasing hormone-adrenocorticotrophin axis. *Placenta* 16, 481-502 (1995)
45. Petraglia F, P. Florio, C. Nappi & A.R. Genazzani: Peptide signaling in human placenta and membranes: autocrine, paracrine, and endocrine mechanisms. *Endocr Rev* 17, 156-86 (1996)
46. Florio P, F.M. Severi, P. Ciarmela, G. Fiore, G. Calonaci, A. Merola, C. De Felice, M. Palumbo & F. Petraglia: Placental stress factors and maternal-fetal adaptive response: the corticotropin-releasing factor family. *Endocrine* 19, 91-102 (2002)
47. Reis FM & F. Petraglia: The placenta as a neuroendocrine organ. *Front Horm Res* 27, 216-28 (2001)
48. Petraglia F, S. Sutton & W. Vale: Neurotransmitters and peptides modulate the release of immunoreactive corticotropin-releasing factor from cultured human placental cells. *Am J Obstet Gynecol* 160, 247-51 (1989)
49. Plotsky PM, K.V. Thirivikraman & M.J. Meaney: Central and feedback regulation of hypothalamic corticotropin-releasing factor secretion. *Ciba Found Symp* 172, 59-75 (1996)
50. Petraglia F, P.E. Sawchenko, J. Rivier & W. Vale: Evidence for local stimulation of ACTH secretion by corticotropin-releasing factor in human placenta. *Nature* 328, 717-9 (1987)
51. Robinson BG, R.L. Emanuel, D.M. Frim & J.A. Majzoub: Glucocorticoid stimulates expression of corticotropin-releasing hormone gene in human placenta. *Proc Natl Acad Sci USA* 85, 5244-8 (1988)
52. Campbell EA, E.A. Linton, C.D.A. Wolfe, P.R. Scraggs, M.T. Jones & P.J. Lowry: Plasma corticotropin releasing hormone concentrations during pregnancy and parturition. *J Clin Endocrinol Metab* 63, 1054-9 (1987)
53. Florio P, L. Cobellis, J. Woodman, F.M. Severi, E.A. Linton & F. Petraglia: Levels of maternal plasma corticotropin-releasing factor and urocortin during labor. *J Soc Gynecol Investig* 9, 233-7 (2002)
54. Petraglia F, A.D. Genazzani, L. Aguzzoli, A. Gallinelli, D. de Vita, A. Caruso & A.R. Genazzani: Pulsatile fluctuations of plasma-gonadotropin-releasing hormone and corticotropin-releasing factor levels in healthy pregnant women. *Acta Obstet Gynecol Scand* 73, 284-9 (1994)
55. Stalla GK, H. Bost, J. Stalla, T. Kaliebe, H.G. Dorr, D. Pfeiffer, K. von Werder & O.A. Muller: Human corticotropin-releasing hormone during pregnancy. *Gynecol Endocrinol* 3, 1-10 (1989)
56. Chan EC, R. Smith, T. Lewin, M.W. Brinsmead, H.P. Zhang, J. Cubis, K. Thornton & D. Hurt: Plasma corticotropin releasing hormone, beta endorphin and cortisol inter relationship during human pregnancy. *Acta Endocrinol* 128, 339-44 (1993)
57. Petraglia F, M.C. Hatch, R. Lapinski, M. Stomati, F.M. Reis, L. Cobellis & G.S. Berkowitz: Lack of effect of psychosocial stress on maternal corticotropin-releasing factor and catecholamine levels at 28 weeks' gestation. *J Soc Gynecol Investig* 8, 83-8 (2001)

58. Gitau R, N.M. Fisk & V. Glover: Human fetal and maternal corticotrophin releasing hormone responses to acute stress. *Arch Dis Child Fetal Neonatal Ed* 89, F29-32 (2004)
59. Chan EC, M.W. Brinsmead, S.E. Chen, R. Nanra, B. Simm & M. McLean: Urinary corticotropin releasing hormone immunoreactivity is elevated during human pregnancy. *Gynecol Endocrinol* 4, 233-44 (1990)
60. Economides D, F. Linton, K. Nikolaides, C.H. Rodeck, P.J. Lowry & T. Chard: Relationship between maternal and fetal corticotropin releasing hormone-41 and ACTH levels in human mid-trimester pregnancy. *J Endocrinol* 114, 497-501 (1987)
61. Goland RS, S.L. Wardlaw, M. Blum, P.J. Tropper & R.I. Stark: Biologically active corticotropin-releasing hormone in maternal and fetal plasma during pregnancy. *Am J Obstet Gynecol* 159, 884-90 (1988)
62. Laatikainen TJ, I.J. Raisanen & K.R. Salminen: Corticotropin-releasing hormone in amniotic fluid during gestation and labor and in relation to fetal lung maturation. *Am J Obstet Gynecol* 159, 891-5 (1988)
63. Maser-Gluth C, U. Lorenz & P. Vecsei: In pregnancy, corticotropin-releasing-factor in maternal blood and amniotic fluid correlates with the gestational age. *Horm Metab Res* 16, 42-4 (1987)
64. Linton EA, A.V. Perkins, R.J. Woods, F. Eben, C.D.A. Wolfe & D.P. Behan: Corticotropin releasing hormone-binding protein: plasma levels decrease during the third trimester of normal human pregnancy. *J Clin Endocrinol Metab* 76, 260-2 (1993)
65. Perkins AV, F. Eben, C.D.A. Wolfe, H.M. Schulte & E.A. Linton: Plasma measurements of corticotropin-releasing hormone-binding protein in normal and abnormal human pregnancy. *J Endocrinol* 138, 149-57 (1993)
66. Petraglia F, P. Florio, C. Benedetto, C. Gallo, R.J. Woods, A.R. Genazzani & P.J. Lowry: High levels of corticotropin-releasing factor (CRH) are inversely correlated with low levels of maternal CRH-binding protein in pregnant women with pregnancy-induced hypertension. *J Clin Endocrinol Metab* 81, 852-6 (1996)
67. Ahmed I, B.P. Glynn, A.V. Perkins, M.G. Castro, J. Rowe, E. Morrison & E.A. Linton: Processing of procorticotropin-releasing hormone (pro-CRH): molecular forms of CRH in normal and preeclamptic pregnancy. *J Clin Endocrinol Metab* 85, 755-64 (2000)
68. Goland RS, I.M. Conwell & S. Jozak: The effect of pre-eclampsia on human placental corticotrophin-releasing hormone content and processing. *Placenta* 16, 375-82 (1995)
69. Laatikainen T, T. Virtanen, R. Kaaja & K. Salminen-Lappalainen: Corticotropin-releasing hormone in maternal and cord plasma in pre-eclampsia. *Eur J Obstet Gynecol Reprod Biol* 39, 19-24 (1991)
70. Leung TN, T.K. Chung, G. Madsen, C.W.K. Lam, P.K.W. Lam, W.A.W. Walters & R. Smith: Analysis of mid-trimester corticotrophin-releasing hormone and -fetoprotein concentrations for predicting pre-eclampsia. *Hum Reprod* 15, 1813-18 (2000)
71. Florio P, F.M. Severi, G. Fiore, L. Micheli, C. Bocchi, C. Nencini, I. Pezzani, G. Giorgi & F. Petraglia: Impaired uterine artery blood flow at mid gestation and low levels of maternal plasma corticotropin-releasing factor. *J Soc Gynecol Investig* 10, 294-7 (2003)
72. Florio P, A. Imperatore, F. Sanseverino, M. Torricelli, F.M. Reis, P.J. Lowry & F. Petraglia: The measurement of maternal plasma corticotropin-releasing factor (CRH) and CRH-binding protein improves the early prediction of preeclampsia. *J Clin Endocrinol Metab* 89, 4673-7 (2004)
73. Warren WB, S.L. Patrick & R.S. Goland: Elevated maternal plasma corticotropin-releasing hormone levels in pregnancies complicated by preterm labor. *Am J Obstet Gynecol* 166, 1198-1204 (1992)
74. McLean M, A. Bisits, J. Davies, R. Woods, P. Lowry & R. Smith: A placental clock controlling the length of human pregnancy. *Nat Med* 1, 460-3 (1995).
75. Berkowitz GS, R.H. Lapinski, C.J. Lockwood, P. Florio, C. Blackmore-Prince & F. Petraglia: Corticotropin-releasing factor and its binding protein: maternal serum levels in term and preterm deliveries. *Am J Obstet Gynecol* 174, 1477-83 (1996)
76. Leung TN, T.K. Chung, G. Madsen, M. McLean, A.M. Chang & R. Smith: Elevated mid-trimester maternal corticotrophin-releasing hormone levels in pregnancies that delivered before 34 weeks. *Br J Obstet Gynaecol* 106, 1041-6 (1999).
77. Sibai B, P.J. Meis, M. Klebanoff, M.P. Dombrowski, S.J. Weiner, A.H. Moawad, A. Northen, J.D. Iams, M.W. Varner, S.N. Caritis, M.J. O'Sullivan, M. Miodovnik, K.J. Leveno, D. Conway, R.J. Wapner, M. Carpenter, B. Mercer, S.M. Ramin, J.M. Thorp, A.M. Peaceman, S. Gabbe & Maternal Fetal Medicine Units Network of the National Institute of Child Health and Human Development: Plasma CRH measurement at 16 to 20 weeks' gestation does not predict preterm delivery in women at high-risk for preterm delivery. *Am J Obstet Gynecol* 193, 1181-6 (2005)
78. Wadhwa PD, T.J. Garite, M. Porto, L. Glynn, A. Chiczy-DeMet, C. Dunkel-Schetter & C.A. Sandman: Placental corticotropin-releasing hormone (CRH), spontaneous preterm birth, and fetal growth restriction: a prospective investigation. *Am J Obstet Gynecol* 191, 1063-9 (2004)
79. Korebrits C, M.M. Ramirez, L. Watson, E. Brinkman, A.D. Bocking & J.R.G. Challis: Maternal CRH is increased

with impending preterm birth. *J Clin Endocrinol Metab* 83, 1585-91 (1998)

80. Challis JRG: Maternal corticotropin-releasing hormone, fetal growth, and preterm birth. *Am J Obstet Gynecol* 191, 1059-60 (2004)

81. Challis JRG, S.G. Matthews, W. Gibb & S.J. Lye: Endocrine and paracrine regulation of birth at term and preterm. *Endocr Rev* 21, 514-50 (2000)

82. Madhappan B, D. Kempuraj, S. Christodoulou, S. Tsapikidis, W. Boucher, V. Karagiannis, A. Athanassiou & T.C. Theoharides: High levels of intrauterine corticotropin-releasing hormone, urocortin, tryptase, and interleukin-8 in spontaneous abortions. *Endocrinology* 144, 2285-90 (2003)

Key Words: Corticotropin-Releasing Hormone, CRH, Diagnostic Use, Pregnancy, Psychiatric Disorders, Review

Send correspondence to: Dr Felice Petraglia, M. D., Chair of Obstetrics and Gynecology, Departement of Pediatrics, Obstetrics and Reproductive Medicine, University of Siena, Policlinico "Le Scotte", Viale Bracci, 53100, Siena, Italy, Tel: 39-0577-233-453, Fax: 39-0577-233-454, E-mail: petraglia@unisi.it

<http://www.bioscience.org/current/vol12.htm>