REVIEW

Corticotropin-releasing hormone signals adversity in both the placenta and the brain: regulation by glucocorticoids and allostatic overload

J Schulkin

Department of Research, American College of Obstetricians and Gynecologists, Department of Physiology and Biophysics, Georgetown University, Washington DC, USA

(Requests for offprints should be addressed to J Schulkin, Department of Research, American College of Obstetricians and Gynecologists, 409 12th Street SW, Washington DC, 20024–2188, USA)

Abstract

Glucocorticoids regulate corticotropin-releasing hormone (CRH) gene expression in the placenta and the brain. In both the placenta and two extrahypothalamic sites in the brain (the amygdala and the bed nucleus of the stria terminalis), glucocorticoids elevate CRH gene expression. One functional role of the elevation of CRH by glucocorticoids may be to signal adversity. When CRH is over-expressed in the placenta, it may indicate that the

pregnancy is in danger, and preterm labor may result. When CRH is over-expressed in the brains of animals, they may become more fearful. Both situations possibly reflect allostatic mechanisms and vulnerability to allostatic overload, a condition in which biological tissue may be compromised.

Journal of Endocrinology (1999) **161**, 349–356

Introduction

Preterm delivery accounts for up to 11% of all births and is a leading factor in neonatal morbidity; consequences of preterm birth include low birth weight and decreased respiratory function (Center for Disease Control 1997). Several physiological systems linked to the placenta may play a role in preterm labor (see e.g. Goland *et al.* 1992, MacGregor *et al.* 1995, Pepe & Albrecht 1995).

A large number of hormones are produced in the placenta. The list includes but is not limited to: corticotropin-releasing hormone (CRH), urocortin, adrenocorticotropin (ACTH), angiotensin, atrial natriuretic factor, prolactin, oxytocin, cytokines, gonadotropin releasing hormone, opioids, somatostatin, prostaglandin and parathyroid hormone (see e.g. Taniguchi *et al.* 1991, Ahmed *et al.* 1992, Lefebvre *et al.* 1992, Bramley *et al.* 1994, Lim & Gude 1995, Petraglia *et al.* 1990a, 1996). These placental peptides are homologous in structure to those in the fetal and in the mature brain (Challis *et al.* 1995).

The hypothesis in this review is that CRH is elevated during adverse events in pregnancy, that it may be a predictor of preterm labor when there are conditions of adversity (see e.g. Wolfe *et al.* 1988b, Goland *et al.* 1993),

and that it is altered in the brain when we experience fear and anxiety (Gold *et al.* 1984, Nemeroff *et al.* 1984, Nemeroff 1992), or a sense of potential harm (Kalin *et al.* 1989, Koob 1993, Schulkin *et al.* 1994). In the case of adverse events in pregnancy, CRH may be over-expressed in the placenta; in the case of fear and anxiety, CRH may be over-expressed in the brain.

Expression of corticotropin-releasing hormone in the placenta

The activation of the fetal hypothalamic–pituitary–adrenal axis promotes the maturation of the fetus and parturition (Challis *et al.* 1995). CRH is a peptide hormone which was originally discovered in the paraventricular nucleus of the hypothalamus and which is fundamental to the regulation of pituitary secretion of ACTH (Vale *et al.* 1981). CRH was subsequently isolated in the placenta, and found to be identical to CRH in the hypothalamus (Shibasaki *et al.* 1982), and in extrahypothalamic sites in the brain (Swanson *et al.* 1983). In placental tissue, CRH is largely localized in syncytiotrophoblast and intermediate trophoblast cell bodies (see e.g. Firn *et al.* 1988; F Petraglia & P Sawchenko, unpublished data) (Fig. 1).

Figure 1 Corticotropin-releasing hormone immunostaining in the human placenta (arrows) (courtesy of F Petraglia and P Sawchenko).

CRH in humans is not detectable in plasma except during pregnancy (Sasaki *et al.* 1984, Cambell *et al.* 1987, Goland *et al.* 1988, Wolfe *et al.* 1988a). In the second and third trimesters of normal pregnancy, CRH is elevated in maternal plasma that is derived from the placenta (Fig. 2). At the same time, both fetal and maternal ACTH and cortisol levels are elevated (Challis *et al.* 1995, Goland *et al.* 1995). Following parturition, CRH levels in the plasma rapidly decrease to nadir levels (Laatikainen *et al.* 1987). In other words, CRH gene expression in placental trophoblast cells rises towards the end of pregnancy in several species, including humans (Firn *et al.* 1988, Riley *et al.* 1991), gorillas (Robinson *et al.* 1989), and rhesus monkeys (Wu *et al.* 1995), although there appear to be species in which this is not the case (e.g. baboons; Goland *et al.* 1992, Smith *et al.* 1993).

Figure 2 Concentration of corticotropin-releasing hormone in placental tissue at various stages of human gestation (adapted from Firn *et al.* 1988).

Figure 3 Maternal plasma levels of corticotropin-releasing hormone in pregnant women at 30 to 35 weeks' gestation receiving betamethasone and in control patients (basal) (adapted from Marinoni *et al.* 1998).

Regulation of CRH by glucocorticoids

Classically, glucocorticoids are known to restrain CRH production by negative feedback (Munck *et al.* 1984) but, importantly, in one early study it was reported that dexamethasone treatment did not suppress levels of CRH in the plasma of pregnant women (Tropper *et al.* 1987). It was then demonstrated that glucocorticoids do not inhibit the production of CRH in the placenta as expected; rather, they increase CRH gene expression in the placenta (Robinson *et al.* 1988, Jones *et al.* 1989). Glucocorticoids increase CRH gene expression in primary cultures of human placental trophoblasts. These effects are dose related, and may be greater in response to dexamethasone than for cortisol, suggesting that these effects are dependent upon type II glucocorticoid receptor sites (Jones *et al.* 1989, Challis *et al.* 1995). Moreover, a recent study demonstrated that pregnant women treated with betamethasone after 30 weeks gestation had increased CRH levels in both plasma and placental tissue (Marinoni *et al.* 1998) (Fig. 3). An additional study also revealed that pregnant patients at 24 weeks also have increased levels of plasma CRH following betamethasone treatment (Korebrits *et al.* 1998). Thus, in marked contrast to glucocorticoids' well known inhibition of CRH via type II glucocorticoid receptor sites at the level of the paraventricular nucleus of the hypothalamus, and the negative restraint of the hypothalamic-pituitary-adrenal axis (Munck *et al.* 1984, Sawchenko 1987, Swanson & Simmons 1989), glucocorticoids increase CRH gene expression in the placenta.

Placental CRH expression under duress

The most consistent fact about CRH detected in plasma in pregnant women is its link to both potential maternal-fetal distress, or greater metabolic and physiological demands, in women who go on to experience preterm labor (Wolfe

Figure 4 Human placental corticotropin-releasing hormone peptide content in pregnancies complicated by pre-eclampsia and in uncomplicated pregnancies (adapted from Goland *et al.* 1995).

et al. 1988b, Warren *et al.* 1992, Berkowitz *et al.* 1996). For example, CRH and glucocorticoid levels are increased following bacterial infectious diseases (Petraglia *et al.* 1995), pre-eclampsia (Goland *et al.* 1995), diabetes (Wolfe *et al.* 1988b), growth retarded fetal development (Goland *et al.* 1993), multiple gestation (Wolfe *et al.* 1988b), and psychosocial stress (Hobel *et al.* 1999) (Fig. 4). In other words, for conditions of adversity, or great metabolic demand, CRH may play an important role in parturition, and in some instances may predict vulnerability to preterm delivery (c.f. McLean *et al.* 1995, Berokowitz *et al.* 1996, Bisits *et al.* 1998).

CRH and parturition

Oxytocin and prostaglandin, linked to parturition, enhance CRH gene expression in placental tissue (Petraglia *et al.* 1989). Bacterial microbial induced cytokine production may facilitate parturition via changes in CRH; there is a well established link between cytokine production, the release of CRH (Chrousos 1996) and a reduction in reproductive fitness (Ferin 1995).

Decreases in progesterone levels lead to the upregulation of CRH in the placenta, perhaps by enhanced binding of glucocorticoids (Karalis *et al.* 1996). In addition, decreases in CRH binding protein are linked to the upregulation of CRH (Behan *et al.* 1996). Finally, a recent study found that a CRH type I receptor antagonist can delay parturition in sheep (Chan *et al.* 1998).

Clearly, there is more than one mechanism to facilitate an event as important as birth—after all, the end-point of evolution is successful reproduction. For example, estrogen is known to increase oxytocin gene expression in the placenta, which facilitates the onset of labor. An important point is that the steroid is sustaining or increasing the peptide, which then contributes to the onset of parturition.

In general, steroids act to regulate peptides (see e.g. Pfaff 1980, Petraglia *et al.* 1990b, Herbert 1993, Schulkin 1998). In the placenta, glucocorticoids increase CRH expression. What seems clear about CRH is its over-expression by the placenta under adverse pregnancy conditions, which might reflect allostatic regulation and allostatic overload.

Allostasis and allostatic overload

The concept of allostasis is tied to systems in which there is no clear physiological set point. That is, the set point is fluid and changing (Sterling & Eyer 1988). Allostasis refers to the ability to achieve stability through change. Whereas homeostatic systems such as blood oxygen, blood pH, and body temperature must be maintained within a narrow range, allostatic systems are more labile, allowing them to adjust to external and internal circumstances (McEwen & Stellar 1993). Both homeostatic (Bernard 1865; Cannon 1932, Richter 1943) and allostatic regulation help maintain the internal milieu (Sterling & Eyer 1988, Schulkin *et al.* 1994, 1998, McEwen 1998). It is the latter concept that we hypothesize has relevance to understanding the endocrine mechanisms that may underlie preterm labor induced by maternal–fetal distress.

Allostatic responses, within limits, may prove adaptive and protective. Hormones such as CRH or cortisol can be destructive when they are activated for long periods of time, or when the body is unable to stop producing them (Sapolsky 1992, McEwen 1998). Glucocorticoids, such as cortisol, restrain hormone production by inhibiting CRH gene expression in the paraventricular nucleus of the hypothalamus (see e.g. Munck *et al.* 1984, Sawchenko 1987). In this context, the event is protective. If high cortisol levels continue to persist, it may be destructive (e.g. bone demineralization, immune defense degradation). Biological tissue is compromised when pushed beyond its limits. The CRH that is hypersecreted in pregnant women following situations of bacterial infectious diseases, pre-eclampsia or hypertension, multiple gestations, and psychosocial stress may be conditions of allostatic overload. Under conditions of allostatic overload, parturition may be adaptive to both mother and fetus, by both reducing stress on the pregnant woman and removing the fetus from an over-stressed environment.

Expression of CRH in the brain

Corticotropin-releasing hormone and binding protein are widely distributed in the central nervous system (see e.g. Swanson *et al.* 1983, Sawchenko 1993, Behan *et al.* 1996, Gray & Birnman 1996). In addition to the paraventricular nucleus and lateral region of the hypothalamus, CRH is found in the frontal cortex and regions of the thalamus, as well as brainstem regions near the locus coeruleus

Figure 5 Corticotropin-releasing hormone gene expression in the lateral bed nucleus of the stria terminalis in (B) rats treated with corticosterone and (A) those that were untreated (from Makino *et al.* 1994b).

(Valentino *et al.* 1992). At least two CRH receptors sites are found in the brain (Potter *et al.* 1994, Lovenberg *et al.* 1995).

Two regions in which CRH is particularly conspicuous are in the central nucleus of the amygdala and the lateral bed nucleus of the stria terminalis (Ju *et al.* 1989, Moga *et al.* 1989, Makino *et al.* 1994b). Both regions are known to influence the regulation of the hypothalamic–pituitary–adrenal axis (Herman & Cullian 1997). The lateral bed nucleus of the stria terminalis is particularly important in regulating CRH gene expression in the paraventricular nucleus of the hypothalamus under a number of conditions (Herman & Cullian 1997). The bed nucleus of the stria terminalis (e.g. extended amygdala) is ideally situated to regulate the paraventricular nucleus, because of the massive anatomical connectivity between these two regions (Alheid *et al.* 1996).

Regulation of CRH by glucocorticoids

Importantly, CRH gene expression is differentially regulated in the brain by glucocorticoid hormones (Swanson & Simmons 1989, Imaki *et al.* 1991, Tanimura & Watts 1998). In the parvocellular region of the paraventricular nucleus, glucocorticoid activation decreases CRH gene expression, while in the central nucleus of the amygdala and lateral bed nucleus of the stria terminalis, CRH gene expression is enhanced by glucocorticoids (Swanson & Simmons 1989, Makino *et al.* 1994a,b, Watts & Sanchez-Watts 1995, Watts 1996) (Fig. 5). These effects on

the upregulation of CRH gene expression may be mediated by both type I and type II glucocorticoid receptor sites (Watts & Sanchez-Watts 1995, Tanimura & Watts 1998).

Corticotropin-releasing hormone regulation by negative feedback is not axiomatic even at the level of the paraventricular nucleus (Swanson & Simmons 1989, Tanimura & Watts 1998). Interestingly, under various conditions, glucocorticoids in the paraventricular nucleus of the hypothalamus *stop* inhibiting CRH gene expression (Pich *et al.* 1993a, Kalin *et al.* 1994, Albeck *et al.* 1997, Tanimura & Watts 1998). These findings at the level of the paraventricular nucleus suggest a breakdown of normal regulatory function and, together with the elevated levels of CRH gene expression in the central nucleus of the amygdala and lateral bed nucleus of the stria terminalis, may reflect allostatic overload (Schulkin *et al.* 1998). Consistent with this, CRH is elevated in the cerebrospinal fluid, together with elevated systemic levels of cortisol, in people who are chronically fearful/anxious and depressed (Nemeroff *et al.* 1984, Gold *et al.* 1988, Kling *et al.* 1993, Nemeroff 1992). This condition is also one in which there is a vulnerability to bone loss (Michelson *et al.* 1996), or hippocampal deterioration (Sheline *et al.* 1996), which are perhaps also examples of allostatic overload (McEwen 1998).

Behavioral effects of elevated CRH

Elevated levels of CRH (Koob & Bloom 1985) in specific regions of the brain that include the central nucleus of the

amygdala and the lateral bed nucleus of the stria terminalis increase the likelihood that an event will be perceived as dangerous (see e.g. Koob 1993, Kalin *et al.* 1994, Lee & Davis 1997). These two neural sites underlie the behavioral response to fear and anxiety (LeDoux 1987, 1996, Rosen & Schulkin 1998).

Transgenic mice with overexpression of CRH in the brain demonstrate exaggerated fear responses (Stenzel-Poore *et al.* 1994). Fear responses are attenuated by central pharmacological blockade of CRH expression (Swerdlow *et al.* 1989, Koob 1993), but not peripheral blockade of CRH expression (Pich *et al.* 1993b). In particular, antagonists of CRH directed at the central nucleus of the amygdala reduce a number of fear-induced behavioral responses (Swerdlow *et al.* 1989, Koob 1993). Infusion of CRH directly into the lateral or third ventricle (e.g. Koob 1993) or the bed nucleus of the stria terminalis (extended amygdala) facilitates fear responses (Lee & Davis 1997).

Interestingly, it has been suggested that pharmacological blockade of type I CRH receptor sites reduces fear-related behavioral responses (Deak *et al.* 1999) and, perhaps together with decreased levels of central CRH binding protein, facilitates the binding of CRH and the expression of fear. It should also be remembered that the type I CRH receptor antagonist has been reported to delay parturition (Chan *et al.* 1998).

Animal studies suggest that excessive prenatal stress and fear can have long term impact on the offspring (Weinstock 1996). One study, for example, has shown that aversive stress results in elevated cortisol levels in both the mother and the fetus(es). When sacrificed as young adults, the offspring demonstrate increased levels of CRH (49%) in the central nucleus of the amygdala compared with control rats. Prenatal stress experiences can increase fear responses when these animals are provoked as adults (Cratty *et al.* 1995). Consistent with this picture are the experiments demonstrating that CRH injections during pregnancy in rats increase the vocalization of the offspring when tested in isolation chambers during ontogeny (Williams *et al.* 1995).

Glucocorticoid and CRH elevation decrease reproductive fitness and decrease sexual behavior (e.g. Nappi & Rivier 1995). When glucocorticoids are elevated under duress, testosterone or estrogen is reduced in most species that have been studied (Sapolsky 1992). The state of combating disease or experiencing fear (or psychological stress) are metabolically expensive events, and thereby reduce the hormones of reproduction and the likelihood of successful reproduction (Wasser 1996). Interestingly, in experiments in which there is elevated estrogen there is decreased CRH expression in the brain (Patchev & Almeida 1996).

Importantly, rather than restraining behavioral effects, glucocorticoids, via central activation of CRH, enhance these effects (Schulkin *et al.* 1998). For example, glucocorticoids facilitate CRH-induced fear responses (Lee *et al.*

Figure 6 Potentiation of glucocorticoids on corticotropin-releasing hormone-related startle reactions and seizures (adapted from Lee *et al.* 1994, Rosen *et al.* 1994).

1994). Rats treated with glucocorticoids systemically have increased fear-related startle responses to central CRH infusions.

When given centrally high doses of CRH also facilitate the vulnerability to seizures (Weiss *et al.* 1986). This event is linked to the activation of the amygdala (Weiss *et al.* 1986, Helfer *et al.* 1996). While it was hypothesized that glucocorticoids should restrain CRH-related seizures, we found that glucocorticoids facilitated the onset of CRH-induced seizures (Rosen *et al.* 1994) (Fig. 6). Moreover, we also found that, rather than restraining cocaine-induced seizures, which are linked to CRH expression in the amygdala, glucocorticoids actually increase them (Kling *et al.* 1993).

Conclusion

The underlying hypothesis is that CRH is a signal of danger in both the placenta and the brain. In one context, the impact of elevated glucocorticoids on CRH gene expression may render women more vulnerable to pre-term labor. In the other context, glucocorticoids facilitate the perception of danger. Glucocorticoids act to magnify and sustain the CRH signal.

Glucocorticoid regulation of CRH gene expression in the placenta and in the amygdala and bed nucleus of the stria terminalis look remarkably similar. While glucocorticoids restrain CRH gene expression in the parvocellular region of the paraventricular nucleus of the hypothalamus, they magnify the effects of CRH in these other areas. This induction of CRH gene expression under certain conditions may contribute to regulatory or allostatic overload.

It should be noted that we do not know whether the mechanisms that underlie the placental increase of CRH by glucocorticoids are the same for both the brain and the placenta. Nor do we know whether the induction of CRH gene expression in the placenta originates with the mother, the fetus, or even the placenta itself. What is clear, however, is that, contrary to the belief that glucocorticoids

always react to restrain the expression of CRH, glucocorticoids facilitate CRH expression in the placenta and in regions of the brain that underlie the experience of adversity, and it is this CRH expression that may be significant in preterm labor (e.g. allostatic overload) at the level of the placenta, and in the brain to sustain the experience of adversity.

References

- Ahmed MS, Cemerik B & Aghas A 1992 Properties and functions of human placental opioid system. *Life Sciences* **50** 83–97.
- Albeck DS, McKittrick CR, Blanchard DC, Blanchard RJ, Nikulina J, McEwen BS & Sakai RR 1997 Chronic social stress alters levels of corticotropin-releasing factor and arginine vasopressin mRNA in rat brain. *Journal of Neuroscience* **17** 4895–4903.
- Alheid GF, deOlmos J & Beltramino CA 1996 Amygdala and extended amygdala. In *The Rat Nervous System*, edn 2. Ed G Paxinos. San Diego: Academic Press.
- Behan DP, Grigoridis DE, Lovenberg T, Chalmers D, Heinrichs S, Liaw C & DeSouza EB 1996 Neurobiology of corticotropin releasing factor (CRF) receptors and CRF-binding protein: implications for the treatment for CNS disorders. *Molecular Psychiatry* **1** 265–277.
- Berkowitz GS, Lapinski RH, Lockwood CJ, Florio P, Blackmore-Prince C & Petraglia F 1996 Corticotropin-releasing factor and its binding protein: maternal serum levels in term and preterm deliveries. *American Journal of Obstetrics and Gynecology* **174** 1477–1483.
- Bernard C 1865 *An Introduction to the Study of Experimental Medicine*. 1957 edn. New York: Dover Press.
- Bisits A, Madsen G, McLean M, O'Callaghan S, Smith R & Giles W 1998 Corticotropin-releasing hormone: a biochemical predictor of preterm delivery in a pilot randomized trial of the treatment of preterm labor. *American Journal of Obstetrics and Gynecology* **178** 862–866.
- Bramley TA, McPhie CA & Menzies GS 1994 Human placental gonadotrophin-releasing hormone (GnRH) binding sites. III Changes in GnRH binding levels with stage of gestation. *Placenta* **15** 733–745.
- Cambell EA, Linton EA, Wolfe CDA, Scraggs PR, Jones MT & Lowry PJ 1987 Plasma corticotropin releasing hormone concentrations during pregnancy and parturition. *Journal of Clinical Endocrinology and Metabolism* **64** 1054–1059.
- Cannon WB 1932 *The Wisdom of the Body*. New York: Norton Press.
- Center for Disease Control 1997 Report of final statistics **46** 80 (Table 44).
- Challis JRG, Matthews SG, VanMeir C & Ramirez MM 1995 Current topic: the placental corticotropin-releasing hormone–adrenocorticotropin axis. *Placenta* **16** 481–502.
- Chan EC, Falconer J, Madsen G, Rice K, Webster E, Chrousos GP & Smith R 1998 Corticotropin-releasing hormone type I receptor antagonist delays parturition in sheep. *Endocrinology* **139** 3357–3360.
- Chrousos GP 1996 The hypothalamic–pituitary–adrenal axis and immunomediated inflammation. *New England Journal of Medicine* **332** 1351–1362.
- Cratty MS, Ward HE, Johnson EA, Azzaro AJ & Birkle DL 1995 Prenatal stress increases CRF content and release in rat amygdala minces. *Brain Research* **675** 297–302.
- Deak T, Nguyen KT, Ehrlich AL, Watkins LR, Spencer RL, Maier SF, Licino J, Wong ML, Chrousos GP, Webster E & Gold PW 1999 The impact of a nonpeptide corticotropin-releasing hormone antagonist antalarmin on behavioral and endocrine responses to stress. *Endocrinology* **140** 79–86.
- Ferin M 1995 The antireproductive role of corticotropin releasing hormone and interleukin-1 in the female rhesus monkey. *Annales d'Endocrinologie* **56** 181–186.
- Firm DM, Emanuel RL, Robinson BG, Smas CM, Alder GK & Majzoub JA 1988 Characterization and gestational regulation of corticotropin releasing hormone messenger RNA in human placenta. *Journal of Clinical Investigation* **82** 287–292.
- Goland RS, Wardlaw SL, Blum M, Tropper PJ & Stark RL 1988 Biologically active corticotropin-releasing hormone in maternal and fetal plasma during pregnancy. *Journal of Obstetrics and Gynecology* **159** 884–890.
- Goland RS, Sharon SL, Fortman JD & Stark RI 1992 Plasma corticotropin releasing hormone factor concentrations in the baboon during pregnancy. *Endocrinology* **131** 1782–1786.
- Goland RS, Jozak S, Warren WB, Conwell IM, Stark RI & Tropper PJ 1993 Elevated levels of umbilical cord plasma corticotropin-releasing hormone in growth-retarded fetuses. *Journal of Clinical Endocrinology and Metabolism* **77** 1174–1179.
- Goland RS, Tropper PJ, Warren WB, Stark RI, Jozak SM & Conwell IM 1995 Concentrations of corticotropin-releasing hormone in the umbilical cord blood of pregnancies complicated by pre-eclampsia. *Reproduction, Fertility and Development* **7** 1227–1230.
- Gold PW, Chrousos G, Kellner C, Post R, Roy A, Augerinos P, Schulte H, Oldfield E & Loriaux DL 1984 Psychiatric implications of basic and clinical studies with corticotropin-releasing hormone. *American Journal of Psychiatry* **141** 619–627.
- Gold PW, Goodwin FK & Chrousos GP 1988 Clinical and biochemical manifestation of depression: relation to the neurobiology of stress. *New England Journal of Medicine* **319** 348–353.
- Gray TS & Birmaman EW 1996 The amygdala: corticotropin releasing hormone, steroids and stress. *Critical Reviews in Neurobiology* **10** 155–168.
- Helfer V, Deransart C, Maresc C & Depaulis A 1996 Amygdala kindling in the rat: anxiogenic-like consequences. *Neuroscience* **73** 971–978.
- Herbert J 1993 Peptides in the limbic system: neurochemical codes for co-ordinated adaptive responses to behavioural and physiological demand. *Progress in Neurobiology* **41** 723–791.
- Herman JP & Cullian WE 1997 Neurocircuitry of stress: central control of the hypothalamic–pituitary–adrenocortical axis. *Trends in Neurosciences* **20** 78–84.
- Hobel CJ, Dunkel-Schetter C, Roesch SC, Castro LC & Arora CP 1999 Maternal plasma corticotropin-releasing hormone associated with stress at 20 weeks' gestation in pregnancies ending in preterm delivery. *American Journal of Obstetrics and Gynecology* **180** S257–263.
- Imaki T, Nahan JL, Rivierck C & Vale W 1991 Differential regulation of corticotropin-releasing hormone mRNA in rat brain regions by glucocorticoids and stress. *Journal of Neuroscience* **11** 585–599.
- Jones SA, Brooks AN & Challis JRG 1989 Steroids modulate corticotropin-releasing hormone production in human fetal membranes and placenta. *Journal of Clinical Endocrinology and Metabolism* **125** 2751–2757.
- Ju G, Swanson LW & Simerly RB 1989 Studies on the cellular architecture of the bed nuclei of the stria terminalis in the rat. II Chemoarchitecture. *Journal of Comparative Neurology* **280** 603–621.
- Kalin NH, Shelton SE & Barksdale CM 1989 Behavioral and physiological effects of corticotropin releasing hormone administered to infant primates undergoing maternal separation. *Neuropsychopharmacology* **2** 97–104.
- Kalin NH, Takahashi LK & Chen FL 1994 Restraint stress increases corticotropin releasing hormone mRNA content in the amygdala and the paraventricular nucleus. *Brain Research* **656** 182–186.
- Karalis L, Goodwin G & Majzoub JA 1996 Cortisol blockade of progesterone: a possible molecular mechanism involved in the initiation of labor. *Nature Medicine* **2** 556–560.

- Kling MA, Rubinow DR, Doram AR, Roy A, Davis CL, Calabrese JR, Nieman LK, Post RM, Chrousos GP & Gold PW 1993 Cerebrospinal fluid immunoreactive somatostatin concentrations in patients with Cushing's disease and major depression: relationship to indices of corticotropin-releasing hormone and cortisol secretion. *Neuroendocrinology* **57** 79–88.
- Kling MA, Glowa JF, Pluznik D, Demas J, DeBellis MD, Gold PW & Schulkin J 1993 Facilitation of cocaine-kindling by glucocorticoids in rats. *Brain Research* **372** 345–351.
- Koob GF 1993 The role of corticotropin-releasing hormone in behavioral responses to stress. In *Corticotropin-Releasing Factor*. Eds K Chadwick, J Marsh & K Ackrill. New York: John Wiley and Sons.
- Koob GF & Bloom FE 1985 Corticotropin-releasing factor and behavior. *Federation Proceedings* **44** 259–263.
- Korebrits C, Yu DHT, Ramirez MM, Marinoni E, Bocking AD & Challis JRG 1998 Antenatal glucocorticoid administration increases corticotrophin-releasing hormone (CRH) in maternal plasma. *British Journal of Obstetrics and Gynaecology* **105** 556–561.
- Laatikainen T, Virtanen T, Raisanen I & Salminen K 1987 Immunoreactive corticotropin releasing factor and corticotropin during pregnancy, labor and puerperium. *Neuropeptides* **10** 343–353.
- LeDoux JE 1987 Emotion. In *Handbook of Physiology. The Nervous System*, pp 419–459. Ed ER Kandel. Bethesda, MD: American Physiological Society.
- LeDoux JE 1996 *The Emotional Brain*. New York: Simon and Schuster.
- Lee Y & Davis M 1997 Role of the hippocampus, the bed nucleus of the stria terminalis and the amygdala in the excitatory effect of CRH on the acoustic startle reflex. *Journal of Neuroscience* **17** 6434–6446.
- Lee Y, Schulkin J & Davis M 1994 Effect of corticosterone on the enhanced acoustic startle reflex by corticotropin-releasing factor. *Brain Research* **666** 93–99.
- Lefebvre DL, Gaid A & Zingg HH 1992 Expression of the oxytocin gene in rat placenta. *Endocrinology* **130** 1185–1192.
- Lim AT & Gude NM 1995 Atrial natriuretic factor production by the human placenta. *Journal of Clinical Endocrinology and Metabolism* **80** 3091–3093.
- Lovenberg TW, Chalmers DT, Liu C & DeSouza EB 1995 CRF2 beta receptor mRNAs are differentially distributed between the rat central nervous system and peripheral tissues. *Endocrinology* **136** 4139–4142.
- McEwen BS 1998 Protective and damaging effects of stress mediators. *New England Journal of Medicine* **338** 171–179.
- McEwen BS & Stellar E 1993 Stress and the individual. *Archives of Internal Medicine* **153** 2093–2101.
- MacGregor JA, Jackson GM, Lachelin GC, Goodwin TM, Artal R, Hastings C & Dullien V 1995 Salivary estriol as risk assessment for preterm labor: a prospective trial. *American Journal of Obstetrics and Gynecology* **173** 1337–1342.
- McLean M, Bisits A, Davies J, Woods R, Lowry P & Smith R 1995 A placental clock controlling the length of human pregnancy. *Nature Medicine* **1** 460–463.
- Makino S, Gold PW & Schulkin J 1994a Effects of corticosterone on CRH mRNA and content in the bed nucleus of the stria terminalis: comparison with the effects in the central nucleus of the amygdala and the paraventricular nucleus of the hypothalamus. *Brain Research* **657** 141–149.
- Makino S, Gold PW & Schulkin J 1994b Corticosterone effects on corticotropin releasing hormone mRNA in the central nucleus and the parvocellular region of the paraventricular nucleus of the hypothalamus. *Brain Research* **640** 105–112.
- Marinoni E, Korebrits C, DiIorio R, Cosmi EV & Challis JRG 1998 Effect of betamethasone *in vivo* on placental corticotropin-releasing hormone in human pregnancy. *American Journal of Obstetrics and Gynecology* **178** 770–778.
- Michelson D, Stratakis C, Hill L, Reynolds J, Galliven E, Chrousos G & Gold P 1996 Bone mineral density in women with depression. *New England Journal of Medicine* **335** 1176–1181.
- Moga MM, Saper CB & Gray TS 1989 Bed nucleus of the stria terminalis: cytoarchitecture, immunohistochemistry, and projection to the parabrachial nucleus in the rat. *Journal of Comparative Neurology* **283** 315–332.
- Munck A, Guyre PM & Holbrook NJ 1984 Physiological regulation of glucocorticoids in stress and their regulation to pharmacological actions. *Endocrine Reviews* **5** 25–44.
- Nappi RE & Rivier S 1995 Corticotropin-releasing factor (CRF) and stress-related reproductive failure: the brain as a state of the art or the ovary as a novel clue? *Journal of Endocrinological Investigation* **18** 872–880.
- Nemeroff CB 1992 New vistas in neuropeptide research in neuropsychiatry: focus on corticotropin-releasing factor. *Neuropsychopharmacology* **6** 69–75.
- Nemeroff CB, Widerlov E, Bissette G, Walleus H, Karlsson I, Eklund K, Kilts CD, Loosen PT & Vale W 1984 Elevated concentrations of corticotropin-releasing factor-like immunoreactivity in depressed patients. *Science* **26** 1342–1343.
- Patchev RK & Almeida OF 1996 Gonadal steroids exert facilitating and 'buffering' effects on glucocorticoid-mediated transcriptional regulation of corticotropin-releasing hormone and corticosteroid receptor genes in rat brain. *Journal of Neuroscience* **16** 7077–7084.
- Pepe GJ & Albrecht ED 1995 Actions of placental and fetal adrenal steroid hormones in primate pregnancy. *Endocrine Reviews* **16** 608–648.
- Petraglia F, Sutton S & Vale W 1989 Neurotransmitters and peptides modulate the release of immunoreactive corticotropin-releasing factor from cultured human placental cells. *American Journal of Obstetrics and Gynecology* **160** 247–251.
- Petraglia F, Volpe A, Genazzani AR, Rivier J, Sawchenko PE & Vale W 1990a Neuroendocrinology of the human placenta. *Frontiers in Neuroendocrinology* **11** 6–37.
- Petraglia F, Vaughan J & Vale W 1990b Steroid hormones modulate the release of immunoreactive gonadotropin-releasing hormone from cultured human placental cells. *Journal of Clinical Endocrinology and Metabolism* **70** 1173–1178.
- Petraglia F, Aguzzoli P, Florio P, Baumann AD, Genazzani C, DiCarlo C & Romero R 1995 Maternal plasma and placental immunoreactive corticotrophin-releasing factor concentrations in infection-associated term and pre-term delivery. *Placenta* **16** 157–164.
- Petraglia F, Florio P, Gallo R, Simoncini T, Saviozzi M, DiBlasio AM, Vaughan J & Vale W 1996 Human placenta and fetal membranes express human urocortin mRNA and peptide. *Journal of Clinical Endocrinology and Metabolism* **3806**–3810.
- Pfaff DW 1980 *Estrogens and Brain Function*. Berlin: Springer-Verlag.
- Pich EM, Koob GF, Heilig M, Menzaghi F, Vale W & Weiss F 1993a Corticotropin-releasing factor release from the mediobasal hypothalamus of the rat as measured by microdialysis. *Neuroscience* **55** 695–707.
- Pich EM, Heinrichs SC, Rivier C, Miczek KA, Fisher DA & Koob GF 1993b Blockade of pituitary-adrenal axis activation induced by peripheral immunoneutralization of corticotropin-releasing factor does not affect the behavioral response to social defeat stress in rats. *Psychoneuroendocrinology* **18** 495–507.
- Potter E, Sutton S, Donaldson C, Chen R, Perrin M, Lewis K, Sawchenko PE & Vale W 1994 Distribution of corticotropin-releasing factor receptor mRNA expression in the rat brain and pituitary. *Proceedings of the National Academy of Sciences of the USA* **91** 8777–8781.
- Richter CP 1943 Total self-regulatory functions in animals and human beings. *Harvey Lecture Series* **38** 63–103.
- Riley SC, Walton JC, Herlick JM & Challis JRG 1991 The localization and distribution of corticotropin-releasing hormone in the human placenta and fetal membranes throughout gestation. *Journal of Clinical Endocrinology and Metabolism* **72** 1001–1007.

- Robinson BG, Emanuel RI, Friom DM & Majzoub JA 1988 Glucocorticoid stimulates expression of corticotropin-releasing hormone gene in human placenta. *Proceedings of the National Academy of Sciences of the USA* **85** 5244–5248.
- Robinson BG, Arbisser JL, Emanuel RL & Majzoub JA 1989 Species specific placental corticotropin releasing hormone messenger RNA and peptide expression. *Molecular and Cellular Endocrinology* **62** 337–341.
- Rosen JB & Schulkin J 1998 From normal fear to pathological anxiety. *Psychological Review* **105** 325–350.
- Rosen JB, Pishevar SK, Weiss SRB, Smith MA, Gold PW & Schulkin J 1994 Glucocorticoid potentiation of corticotropin releasing hormone-induced seizures. *Neuroscience Letters* **174** 113–116.
- Sapolsky RM 1992 *Stress, The Aging Brain, and the Mechanisms of Neuron Death*. Cambridge, MA: MIT Press.
- Sasaki A, Liotta AS, Luckey MM, Margioris AN, Suda T & Krieger D 1984 Immunoreactive corticotropin-releasing factor is present in human maternal plasma during the third trimester of pregnancy. *Journal of Clinical Endocrinology and Metabolism* **59** 812–814.
- Sawchenko PE 1987 Evidence for a local site of action for glucocorticoids in inhibiting CRF and vasopressin expression in the paraventricular nucleus. *Brain Research* **403** 213–223.
- Sawchenko PE 1993 The functional neuroanatomy of CRF. In *Corticotropin Releasing Factor*. Eds DJ Chadwick, J Marsh & K Ackrill. New York: John Wiley and Sons.
- Schulkin J 1998 *The Neuroendocrine Regulation of Behavior*. Cambridge: Cambridge University Press.
- Schulkin J, McEwen BS & Gold P 1994 Allostasis, amygdala, and anticipatory angst. *Neuroscience and Biobehavioral Reviews* **18** 385–396.
- Schulkin J, Gold P & McEwen BS 1998 Induction of corticotropin releasing hormone gene expression by glucocorticoids. *Psychoneuroendocrinology* **23** 219–243.
- Sheline YI, Wang PW, Gado MH, Csernansky JG & Vannier MW 1996 Hippocampal atrophy in recurrent major depression. *Proceedings of the National Academy of Sciences of the USA* **93** 3908–3913.
- Shibasaki T, Odagiri E, Shizume K & Ling N 1982 Corticotropin-releasing factor-like activity in human placental extracts. *Journal of Clinical Endocrinology and Metabolism* **55** 384–386.
- Smith R, Chan EC, Bowman ME, Harewood WJ & Phippard AF 1993 Corticotropin releasing hormone in baboon pregnancy. *Journal of Clinical Endocrinology and Metabolism* **76** 1063–1068.
- Stenzel-Poore MP, Heinrichs SC, Riverst S, Koob GF & Vale WW 1994 Overproduction of corticotropin-releasing hormone in transgenic mice: a genetic model of anxiogenic behavior. *Journal of Neuroscience* **14** 2579–2584.
- Sterling P & Eyer J 1988 Allostasis: a new paradigm to explain arousal pathology. In *Handbook of Stress, Cognition and Health*. Eds S Fisher & J Reason. New York: John Wiley and Sons.
- Swanson LW & Simmons DM 1989 Differential steroid hormone and neural influences on peptide mRNA levels in corticotropin-releasing hormone cells of the paraventricular nucleus: a hybridization histochemical study in the rat. *Journal of Comparative Neurology* **285** 413–435.
- Swanson LW, Sawchenko PE, Rivier J & Vale WW 1983 Organization of ovine corticotropin releasing hormone immunoreactive cells and fibers in the rat brain: an immunohistochemical study. *Neuroendocrinology* **36** 165–186.
- Swerdlow NR, Britton KT & Koob GF 1989 Potentiation of acoustic startle by corticotropin-releasing factor (CRF) and by fear are both reversed by alpha-helical CRF(9–41). *Neuropsychopharmacology* **2** 285–292.
- Taniguchi T, Matsuzaki N, Kameda T, Shimoya K, Jo T, Saji F & Tanizawa O 1991 The enhanced production of placental interleukin-1 during labor and intrauterine infection. *American Journal of Obstetrics and Gynecology* **165** 131–137.
- Tanimura SM & Watts AG 1998 Corticosterone can facilitate as well as inhibit corticotropin releasing hormone gene expression in the rat hypothalamic paraventricular nucleus. *Endocrinology* **139** 3830–3836.
- Tropper PJ, Goland RS, Wardlaw SL, Fox HE & Frants AG 1987 Effects of betamethasone on maternal plasma corticotropin releasing factor, ACTH and cortisol during pregnancy. *Journal of Perinatal Medicine* **15** 221–224.
- Vale W, Spiess J, Rivier C & Rivier J 1981 Characterization of a 41-residue ovine hypothalamic peptide that stimulates secretion of corticotropin and beta-endorphin. *Science* **213** 1394–1397.
- Valentino RJ, Page ME, Van Bockstaele E & Aston-Jones G 1992 Corticotropin releasing factor immunoreactive cells and fibers in the locus coeruleus region: distribution and sources of input. *Neuroscience* **48** 689–705.
- Warren WB, Patrick SL & Goland RS 1992 Elevated maternal plasma corticotropin-releasing hormone levels in pregnancies complicated by preterm labor. *American Journal of Obstetrics and Gynecology* **166** 1198–1204.
- Wasser SK 1996 Reproductive control in wild baboons measured by fecal steroid. *Biology of Reproduction* **55** 393–399.
- Watts AG 1996 The impact of physiological stimuli on the expression of corticotropin releasing hormone and other neuropeptide genes. *Frontiers in Neuroendocrinology* **17** 281–326.
- Watts AG & Sanchez-Watts G 1995 Region specific regulation of neuropeptide mRNAs in rat limbic forebrain neurones by aldosterone and corticosterone. *Journal of Physiology* **484** 721–736.
- Weinstock M 1996 Does prenatal stress impair coping and regulation of hypothalamic–pituitary–adrenal axis? *Neuroscience and Biobehavioral Reviews* **21** 1–10.
- Weiss SR, Post RM, Gold PW, Chrousos G, Sullivan TL, Walker D & Pert A 1986 Corticotropin releasing hormone-induced seizures and behavior: interaction with amygdala kindling. *Brain Research* **372** 345–351.
- Williams MT, Hennessy MB & Davis HN 1995 Corticotropin releasing hormone administered to pregnant rats alters offspring behavior and morphology. *Pharmacology, Biochemistry and Behaviour* **52** 161–167.
- Wolfé CDA, Patel SP, Campbell EA, Linton EA, Anderson J, Lowry PJ & Jones MT 1988a Plasma corticotrophin-releasing factor (CRF) in normal pregnancy. *British Journal of Obstetrics and Gynaecology* **95** 997–1002.
- Wolfé CDA, Patel SP, Linton EA, Campbell EA, Anderson J, Dornhorst A, Lowry PJ & Jones MT 1988b Plasma corticotrophin-releasing factor (CRF) in abnormal pregnancy. *British Journal of Obstetrics and Gynaecology* **95** 1003–1006.
- Wu WX, Unno S, Giussani DA, Mecnas CA, McDonald TJ & Nathanielsz PW 1995 Corticotropin releasing hormone and its receptor distribution in fetal membranes and placenta of the rhesus monkey in late gestation and labor. *Endocrinology* **136** 4621–4628.

Received 7 July 1998

Revised manuscript received 14 September 1998

Accepted 3 November 1998