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Placental Stress Factors and Maternal-Fetal Adaptive Response

The Corticotropin-Releasing Factor Family

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The placenta and its accessory membranes amnion and chorion undertake the role of intermediary barriers and active messengers in the maternal-fetal dialog. They synthesize, metabolize, and serve as target to numerous hormones that regulate maternal and fetal physiology during pregnancy. Among these factors, corticotropin-releasing factor (CRF) has been one of the more investigated in the last decade. Increasing evidence indicates that in the event of acute or chronic metabolic, physical, or infectious stress, maternal or fetal physiologic and pathologic conditions may influence placental secretion of CRF. The current opinion is that the placenta actually takes part in a stress syndrome by releasing CRF, which may help to influence uterine perfusion, maternal metabolism, fluid balance, and possibly uterine contractility, thereby protecting the fetus from a hostile environment.

Key Words: Corticotropin-releasing factor ; urocortin; syncytrotrophoblast; adrenocorticotropic hormone; parturition; preeclampsia.

Introduction

Initiation, maintenance, and termination of pregnancy are related to placental functions. In fact, human placenta helps to maintain an equilibrium between the fetus and the mother, regulating the body functions of both organisms in a complementary way, providing a favorable uterine environment at implantation, regulating fetal growth during pregnancy, and directing the appropriate signals for the timing of parturition.

Several studies point to the ability of the placenta to produce brain, pituitary, gonadal, and adrenocortical hormones *(1–4)*. These placental hormones are chemically identical and as biologically active as their hypothalamic/gonadal counterparts, and when added to placental cell cultures, they modulate the release of both pituitary-like peptide hormones and gonadal/adrenal cortex-like steroid hormones. Moreover, the local intraplacental mechanism of control is in many aspects comparable to the organization of the hypothalamic-pituitary-target organ axes.

Human placenta, decidua, chorion, and amnion produce corticotropin-releasing factor (CRF) *(5,6)*, the well-known hypothalamic peptide involved in the endocrine adaptations of the hypothalamic-pituitary-adrenal (HPA) axis in response to stress stimuli *(7,8)*. Like the secretion of various hormones, placenta releases CRF both with autonomous mechanisms and under maternal or fetal influence. The event of acute or chronic physical or infectious stress may cause a placental response by releasing CRF into biologic fluids of pregnancy (maternal and fetal plasma, amniotic fluid).

This review illustrates the experimental and clinical studies showing the role of CRF in physiologic (parturition, life and work stress events) and pathologic stress conditions (preterm labor, intrauterine infection, hypertensive disorders of pregnancy) throughout gestation.

Expression and Localization of CRF and CRF-Related Peptides

CRF is a 41 amino acid peptide released from the medial eminence of the hypothalamus, acting at the corticotroph cells in the anterior pituitary to stimulate the release of adrenocorticotropic hormone (ACTH) and related peptides in response to stress events, and modulating behavioral, vascular, and immune response to stress *(7,8)*.

Immunoreactive CRF was first detected in the extracts of human placenta obtained after spontaneous delivery *(9)* and resulted as bioactive as rat hypothalamic CRF or synthetic ovine CRF on the release of immunoreactive ACTH and β-endorphin from cultures of rat anterior pituitary cells *(10)*. The content of immunoreactive CRF is higher in extracts of placenta obtained at term than in tissue obtained at 10 wk of gestation *(11,12)*. In addition, a progressive increase

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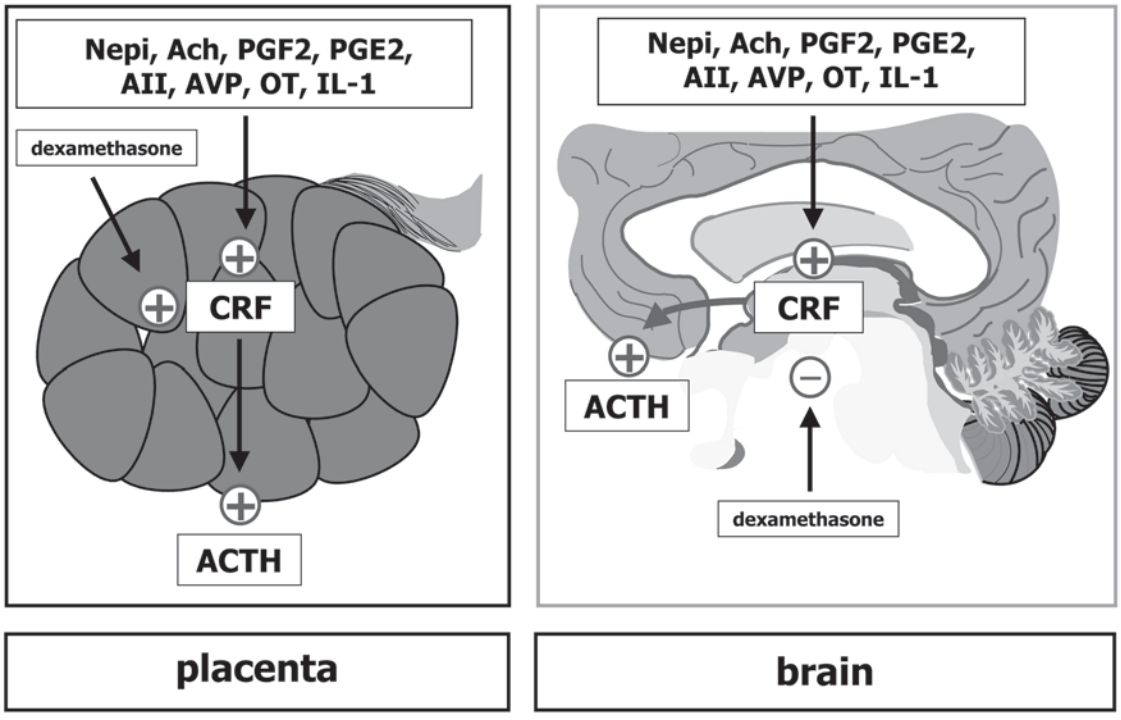

Fig. 1. The mechanisms stimulating CRF release from medial basal hypothalamus are in part chemically identical to those operating in the human placenta. PGF_2 (PGF2) and PGE_2 (PGE2), norepinephrine (Nepi), acetylcholine (Ach), angiotensin II (AII), and arginine vasopressin (AVP) stimulate CRF in the hypothalamus, as well as in placental cells. By contrast, the effect of oxytocin (OT) on CRF and HPA hormones in human placenta is different, being stimulatory. In turn, placental CRF stimulates ACTH secretion from cultured human placental cells.

in placental CRF content has been described during normal pregnancy, paralleling a similar time course of placental CRF mRNA expression, which starts from early gestation (7 to 8 wk) *(12,13)* (Fig. 1). The structure of this placental CRF mRNA is similar to that predicted for the hypothalamic CRF mRNA *(12)*.

Regarding the site of CRF production, some immunohistochemical studies demonstrated that CRF is located in cytotrophoblast cells, with a more intense CRF signal in syncytiotrophoblast *(5,14,15)*. Cytotrophoblast cells are transformed to syncytial cells, which release CRF factor when maintained in culture *(5,12,16,17)*.

CRF is also released from cultured amnion, chorion, and decidual cells at term *(16–18)* with an output similar to that by the placental cells *(16)*. Immunohistochemical localization of CRF in fetal membranes showed that CRF is distributed in the epithelial cells, in some cells of the subepithelial layer of amnion, and in cells of the reticular layer of chorion *(14,19)*. Immunoreactive CRF is present in decidual cells *(6,19)* and is also produced and secreted by endometrial stromal cells of healthy women *(20)*. Interestingly, the addition of CRF to endometrial cell culture induces the process of decidualization, indicated by prolactin release, which suggests a possible role of CRF in the differentiation of human endometrium *(21)*. CRF mRNA is expressed in decidual cells, and decidual CRF mRNA levels increase throughout gestation *(6)*.

CRF is bound to CRF-binding protein (CRF-BP), a 37-kDa protein of 322 amino acids, mainly produced by the human brain and liver *(22)*. It has been demonstrated that CRF-BP is able to bind circulating CRF and urocortin (discussed later), thus modulating their actions on pituitary gland *(23,24)*. Further sources of CRF-BP during pregnancy are placental trophoblast, deciduas, and fetal membranes *(22,25)*.

In situ hybridization demonstrated that CRF-BP mRNA in placenta is intensely expressed by syncytial cells, whereas rare, positively hybridized cells are observed within cytotrophoblast and mesenchymal cells *(25,26)*. A CRF/CRF-BP complex may be detected by immunohistochemistry in syncytiotrophoblast. Additionally, large decidual cells and chorionic cytotrophoblasts express CRF-BP mRNA and protein *(25)*. The existence of a binding protein for CRF led to the explanation of how despite high levels of CRF during the third trimester of pregnancy, there is not a dramatic increase in ACTH *(27,28)*. In fact, it was confirmed that most of the endogenous CRF in maternal plasma *(27)* as well as in amniotic fluid *(29)* is carrier bound and, therefore, has reduced bioactivity.

Urocortin is another component of the CRF family, and its sequence is similar to fish urotensin (63%) and human CRF (45%) *(30)*. Placental and decidual cells collected at 8–11 wk or 38–40 wk of gestation express urocortin mRNA and immunohistochemistry-localized urocortin staining in syncytial cells of trophoblast as well as in amnion, chorion,

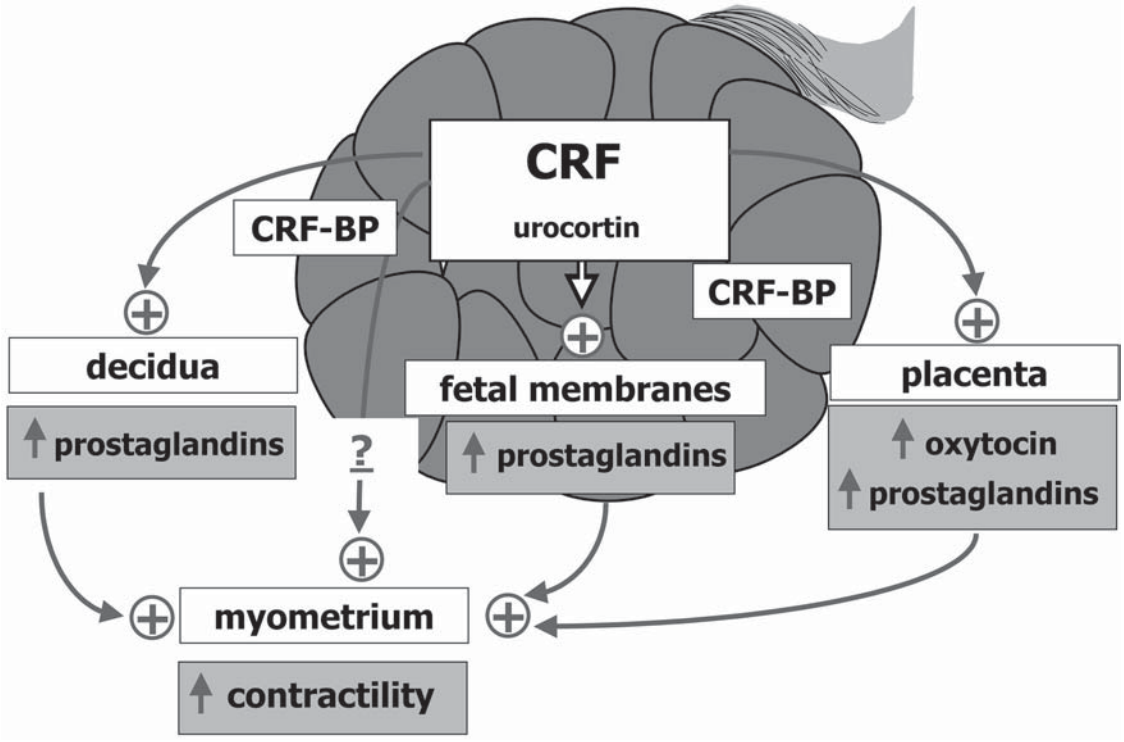

Fig. 2. Paracrine interactions between placental CRF and CRF-BP on myometrial contractility modulation. CRF stimulates, while CRF-BP inhibits CRF-induced release of prostaglandins from placenta, fetal membranes, and decidua, and the secretion of placental oxytocin. Prostaglandins and oxytocin in turn stimulate myometrial contraction.

and decidua of fetal membranes *(31,32)*. Urocortin mRNA expression in human placenta does not change throughout gestation *(32)*.

CRF and urocortin interact with two distinct receptors *(33)*: R1 (classified in R1a, R1b, R1c, and R1d subtypes) and R2 (R2a, R2b, and R2g subtypes) *(34,35)*. Fluorescent *in situ* hybridization and immunofluorescence demonstrated that syncytiotrophoblast cells and amniotic epithelium are the cell types expressing CRF-R1a, -Rc *(36)*, and -R2β mRNA *(37)*.

CRF receptors (mRNA and protein) have also been described in human myometrium *(38,39)* and endometrium *(20)*. In particular, recent findings show the presence in pregnant myometrium of subtypes 1a, 1b, 2a, and 2b and the variant -Rc, whereas only the 1a, 1b, and 2b receptors are detectable in nonpregnant myometrium *(39)* and endometrium *(20)*. Urocortin binds to CRF receptor types 1 and 2, with a particularly high affinity for type 2 receptor *(30,33)*.

Paracrine and Endocrine Actions of CRF and CRF-Related Peptides

Human Placenta and Fetal Membranes

The addition of CRF to primary trophoblast cell cultures stimulates ACTH secretion in a dose-dependent manner *(5, 40,41)*, and the concentration of CRF required for 50% of maximal stimulation of ACTH secretion is higher than the concentration necessary to release ACTH from cultured anterior pituitary cells *(5)*. Moreover, the addition of a CRF antagonist is able to block the CRF-induced ACTH release from placental cells *(5,40)*. CRF-induced ACTH secretion is mediated by cyclic adenosine monophosphate (cAMP) as second messenger, and evidence that this intracellular mechanism operates in placenta comes from the observation that dibutyryl cAMP and forskolin, a diterpene that stimulates adenylate cyclase activity, stimulate ACTH release from cultured trophoblast cells with the same intensity of CRF without potentiating the effect of CRF *(5)*.

CRF-BP reverses the CRF-induced ACTH release from placental cells *(22)* (Fig. 2), as in the pituitary *(23)*. This finding indicates a similarity between pituitary and placental CRF-induced ACTH release. However, in contrast to the corticosteroid negative feedback on pituitary ACTH secretion, glucocorticoids stimulate placental CRF secretion and mRNA expression *(5,18)*, and dexamethasone does not inhibit the effect of CRF on placental ACTH release *(5,18)* (Fig. 1). The addition of urocortin to cultured placental cells has a stimulatory effect on ACTH release equimolar to the CRF effect *(40)* (Fig. 2).

In vitro data support a role for CRF at labor (Fig. 2). In fact, CRF and ACTH stimulate the release of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and PGE_2 from cultured amnion, chorion, decidual, and placental tissues *(40,42–44)*. These effects are inhibited in the presence of antisera to CRF and to ACTH. Moreover, in placenta, but not in amnion or decidua, the

stimulatory effect of CRF on $PGF_{2\alpha}$ and PGE_2 output is attenuated in the presence of an antibody to ACTH, thus supporting the possibility of paracrine stimulation by CRF and ACTH of prostaglandin production in intrauterine tissues *(45)*.

CRF markedly stimulates the release of immunoreactive oxytocin from cultured placental cells in a dose-dependent fashion *(46)*. Moreover, the addition of CRF, but not of arginine vasopressin or neuropeptide Y, increases the release of immunoreactive oxytocin three- to fourfold from placental cells.

Myometrium and Uterine Contractility

Recent data point to the direct role played by CRF on myometrial contractility, owing to the fact that CRF mediates its actions in the human myometrium via activation of two distinct classes of CRH receptors, R1 and R2 *(47)*. By contrast, data exist on the net role played by CRF, some suggesting CRF as an important uterotonic *(43,48,49)*, and some as the main uterine quiescence factor *(47,50)*. It seems that different myometrial CRF receptors are recruited at labor, and that this recruitment may be dynamically and differentially modulated by the great hormonal changes occurring at term pregnancy, so that CRF actions in vivo may differ from actions reported in vitro, according to different myometrial CRF receptor expression and the induced affinity state *(51)*. A recent interpretation has proposed that, during pregnancy, CRF has a protective role in the myometrium through the prevention of uterine contractions. As a result, CRF actions in vitro *(40,43,44,48,49)* may depend on the different isoforms of myometrial CRF receptors expressed and on their induced affinity state in the tissues studied *(47,52)*. In addition, urocortin has been reported to modulate myometrial contractility directly *(40)*, and recent work has shown that urocortin, but not CRF, was able to induce different intracellular signals in the regulation of myometrial contractility by uterotonins *(52)*.

Vascular Effects

Several in vitro studies demonstrated that CRF has vasodilatory effects in a number of species *(53–57)*. In fact, CRF caused dilatation of the mesenteric arteries in vivo *(53)*, and in both rat and humans iv administration of CRF lowers arterial pressure owing to peripheral vasodilatation caused by a direct action on vascular smooth muscle *(54–57)*. However, in most animals and in nonpregnant humans, peripheral concentrations of CRF are low *(8)*, which suggests that CRF may play a minimal role in the control of vascular tone. By contrast, in the pregnant human, plasma CRF concentrations rise exponentially, peaking at term *(2,58)*. Recent investigations have shown that CRF is a potent vasodilator of the human fetoplacental circulation *(59)*, acting at concentrations comparable with plasma CRF levels in maternal and fetal circulation. Placental CRF may, therefore, have a significant physiologic role as a regulator of fetoplacental vascular tone. This effect is owing to endothelial independent pathways *(55–57)*, but in some species CRF may also operate via an endothelium-dependent mechanism, acting on specific receptors expressed by endothelium, as in the case of human umbilical vein endothelial cells *(60)*.

In the human fetoplacental circulation, CRF causes vasodilatation via a nitric oxide (NO)– and a cyclic guanosine 5'-monophosphate (cGMP)–mediated pathway, because the addition of a blocker of NO formation and inhibitors of cGMP formation respectively cause marked attenuation of CRF-stimulated vasodilatation. The addition of CRF to preconstricted placental vessels is able to attenuate all constrictor mechanisms without variation in CRF potency as a vasodilator agent. CRF-induced vasodilatation appears to be mediated by a CRF receptor, since the vasodilatory response to CRF is antagonized in the presence of a CRF receptor antagonist *(59,61)*. CRF-induced vasodilatation occurred at concentrations comparable to plasma CRF levels found in the maternal and fetal circulations *(2)*, and CRF is approx 50 times more potent than prostacyclin *(59,61)*.

Urocortin has the same effects as CRF. Administered intravenously in rats it is more potent than CRF in causing hypotension *(30,62)* and, with respect to placental circulation, it causes vasodilatation, reducing fetoplacental vascular resistance via CRF type 2 receptors, and is more potent than CRF *(63)*.

Because the fetal vessels of the human placenta are not innerved, control of blood flow in this vascular bed is partly dependent on locally produced and circulating vasoactive factors *(64)*. Syncytyiotrophoblast is the main source of CRF during pregnancy *(2,26,58,65)*, and, therefore, placental CRF may access the fetoplacental circulation to cause dilatation by paracrine or endocrine mechanisms. It may be released locally to affect the vascular smooth muscle and endothelium, or it may be secreted into the fetoplacental circulation and travel to its site of action through the placental vascular system. Finally, CRF may maximize the release of products such as proopiomelanocortin (POMC) peptides *(5,40)* or PGs *(58)* in vivo, by causing vasodilatation of placental vascular tissue *(66)* (Fig. 3).

Pregnancy is associated with various cardiovascular changes such as increased blood volume and cardiac output, and decreased blood pressure and peripheral vascular resistance. The decrease in peripheral vascular resistance occurring in pregnancy has been attributed to increased production of vasorelaxant, which acts on the vascular endothelium to cause the release of several relaxant factors *(67)*, as well as directly on vascular smooth muscle, causing relaxation *(68)*. It has been demonstrated that CRF, when administered chronically in pregnant rats, decreases blood pressure *(69)*, and it is also a potent relaxant of the uterine artery of pregnant rats, acting on both the endothelium (mediated by the NO-cGMP system) and the vascular smooth muscle *(70)*.

Animal studies revealed that reduced uterine blood flow and consequently hypoxia induce an increased expression and secretion of CRF, and thus ACTH and cortisol *(71)*.

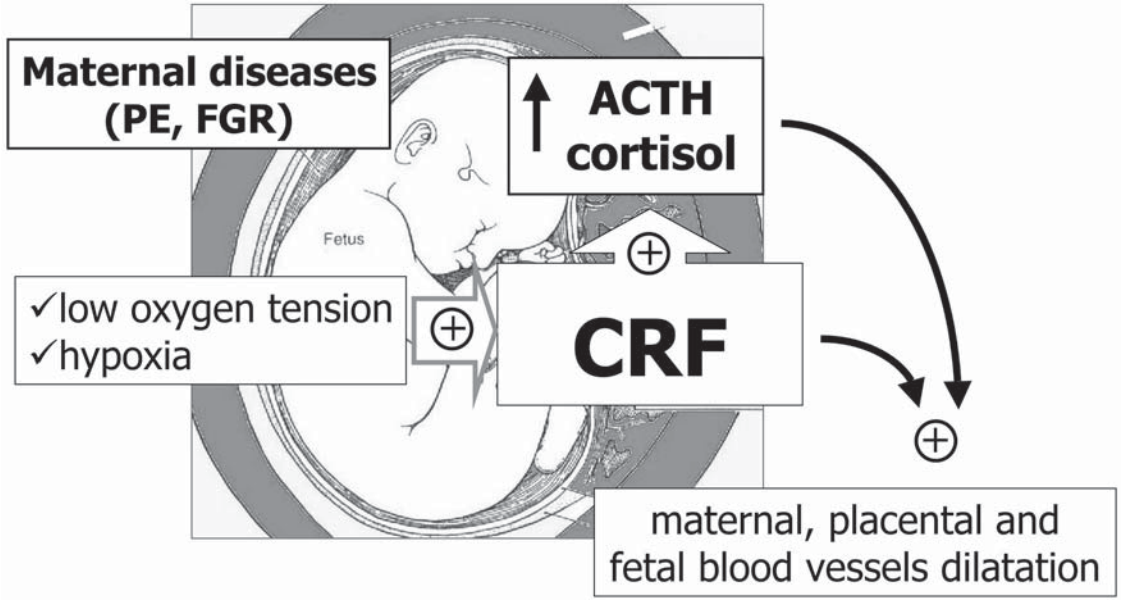

Fig. 3. Hypoxia regulates placental CRF production, which in turn stimulates placental ACTH and cortisol secretion. The final net effect may be the vasodilation of uterine and umbilical arteries, in order to promote uteroplacental and fetal blood flow. PE, preeclampsia; FGR, fetal growth restriction.

Because CRF acts as a vasodilator in placental circulation, increased CRF could act systemically or be released locally in the placenta as a compensatory mechanism to reduce uterine resistance to blood flow *(72)* (Fig. 3).

Effects on Maternal HPA Axis

The activity of the maternal HPA axis is increased in pregnant women, and high levels of free and bound cortisol circulate in pregnant women *(58)*. In fact, hypercortisolemia is characteristic of pregnancy, and the correlation between plasma CRF and salivary or urinary-free cortisol levels would suggest that placental CRF is responsible for these alterations, even though other factors may act in modulating maternal HPA axis function in pregnancy *(73–75)*. However, some discrepancies occur between CRF and ACTH. Plasma ACTH levels increase throughout pregnancy, remaining within the normal range of nonpregnant women *(76)*. This is probably because CRF-BP counteracts the secretory action of CRF on both maternal pituitary and placental ACTH *(23,25,58)*. Furthermore, evidence that the injection of exogenous CRF in pregnant women does not induce an increase in circulating ACTH suggests that high cortisol levels may desensitize maternal pituitary corticotrophs *(11,77,78)*.

Some discrepancies do exist in the HPA axis regulation between pregnant and nonpregnant women. The administration of exogenous glucocorticoid to pregnant women may increase maternal plasma and placental levels of immunoreactive CRF *(79)*, decreasing cortisol *(79–81)* and ACTH levels *(79)*. To date, it is unclear whether maternal plasma ACTH originates from the maternal pituitary, placenta, or both. The diurnal rhythm for plasma ACTH, cortisol, and β-endorphin is maintained in pregnant women; however, CRF does not have a circadian rhythm *(82,83)*. These findings and the fact that the changes in plasma CRF do not correlate with those of ACTH or cortisol throughout normal pregnancy or during labor *(82,84)* underlie the differences in HPA regulation in pregnancy. In addition, they help to verify that pituitary ACTH release is regulated centrally, and that placental CRF is not the only regulator of maternal ACTH and cortisol levels *(75,83)*.

Effects on Fetal HPA Axis

Placental CRF secreted into the fetal circulation may stimulate the production of pituitary ACTH as well as of adrenal hormones (Fig. 4). The effect of CRF on fetal pituitary ACTH release is potentiated by arginine vasopressin and possibly mediated by cAMP, and may be antagonized by dexamethasone *(85)*. Recent studies revealed a direct effect of CRF on dehydroepiandrosterone sulfate (DHEA-S) release from cultured fetal adrenal cells *(86)*. Expression of mRNA encoding type 1 CRF receptor was identified in midgestation human fetal adrenals *(86)*, suggesting that the fetal adrenal cortex may be directly responsive to CRF. Placenta of humans and higher primates uses DHEA-S supplied by the fetal adrenals as the main substrate for estrogen synthesis, and estrogens produced by the placenta play a pivotal role in the endocrine control of pregnancy and induce many of the key changes involved at parturition *(58)*.

Human CRF increased DHEA-S production by cultured human fetal adrenal cortical cells in a dose-dependent fashion and was as effective as ACTH at stimulating DHEA-S production, although it was considerably less potent than

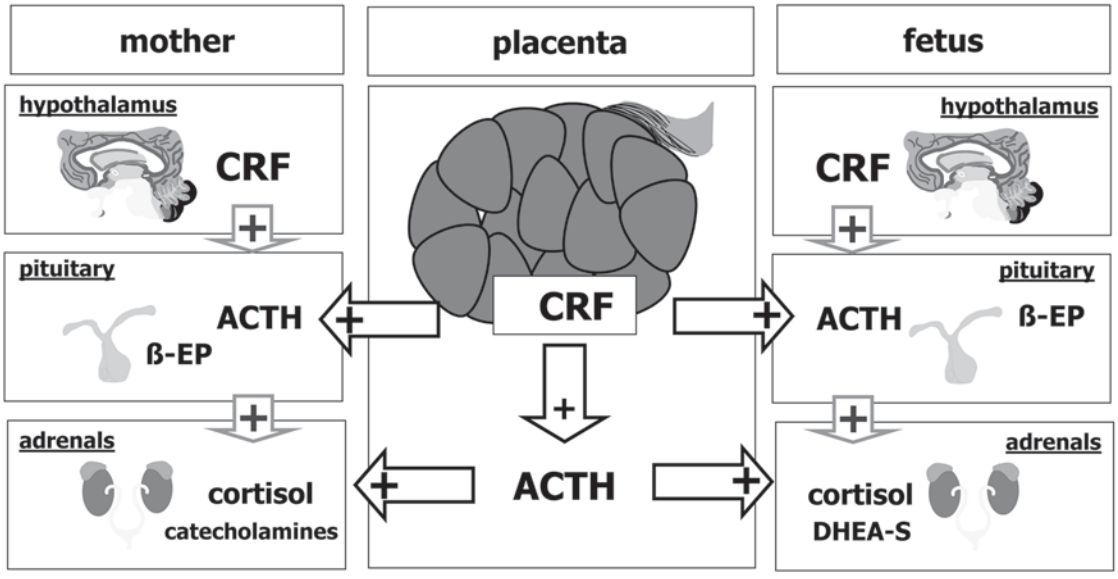

Fig. 4. Effects of placental CRF on maternal and fetal HPA.

ACTH in stimulating cortisol synthesis *(86)* (Fig. 4). CRF did not alter cell number, indicating that it is not mitogenic for fetal adrenal cortical cells. Therefore, placental CRF production, which rises exponentially during human pregnancy, may play a key role in promoting DHEA-S production by the fetal adrenals, which could lead to an increase in synthesis of placental estrogen *(58,86)*.

Regulation of Placental CRF Secretion

Some mechanisms stimulating CRF release from medial hypothalamic eminence in the brain *(7,8)* are identical to those operating in the human placenta (Fig. 1). In fact, prostaglandins, neurotransmitters, and peptides stimulate the release of CRF from cultured placental cells. Both PGF_2 and PGE_2 increase CRF concentration in culture medium with a dose-dependent effect *(5,87)*. Norepinephrine and acetylcholine are the most active neurotransmitters in increasing CRF release. The norepinephrine effect is reversed by prazosin, an α1-adrenergic antagonist, or yohimbine, an α2-adrenergic receptor antagonist. The involvement of both adrenergic receptor subtypes is further supported by the evidence that methoxamine and clonidine, α1- and α2-adrenergic receptor agonists, respectively, stimulate CRF release from placental cells *(88)*. Acetylcholine acts via a muscarinic receptor: atropine or hexamethonium, specific muscarinic receptor antagonists, reverses the effect of acetylcholine on CRF release. In addition, human placenta synthesizes acetylcholine and contains acetylcholine concentrations higher than in mammalian brain tissue *(89)*. Interestingly, the positive effect of norepinephrine and acetylcholine on placental immunoreactive CRF release agrees with the observation that these neurotransmitters stimulate CRF release from rat hypothalamic tissue in vitro and increase CRF levels in the hypophysial portal circulation *(90,91)*, suggesting a close correlation between hypothalamic and placental regulation of CRF release.

In agreement with the hypothalamic mechanisms of secretion, some neuropeptides also modulate placental CRF release. Angiotensin II and arginine vasopressin increase the release of placental CRF from cultured trophoblasts *(88)*. By contrast, oxytocin has different effects, being inhibitory to the CRF/HPA axis *(8,92,93)*, but stimulatory on CRF and ACTH secretion from cultured placental cells *(5,41)*. CRF and both groups of neurotransmitters (norepinephrine and acetylcholine) and neuropeptides (angiotensin II, arginine vasopressin, and oxytocin) are involved in the stress-induced responses of the neuroendocrine system *(91,94)*. The release of CRF from cultured placental cells during incubation with norepinephrine, acetylcholine, angiotensin II and arginine vasopressin, or oxytocin suggests a possible in vivo interaction among these substances. In agreement with regulation of hypothalamic CRF, whereas interleukin-1 (IL-1) stimulates the release of CRF from cultured placental cells, IL-2 has no effect *(88)*. Since indomethacin prevents CRF release induced by IL-1, it has been suggested that the action of IL-1 is mediated by prostaglandins *(87)*.

CRF and CRF-Related Peptides in Biologic Fluids: Changes Throughout Physiological Pregnancy

Normal Pregnancy

From intrauterine tissues, CRF is reversed into the maternal and umbilical cord plasma, as well as the amniotic fluid.

Plasma CRF levels are low in nonpregnant women (<10 pg/mL) and become higher during the first trimester of pregnancy, rising steadily until term *(84,95–99)*. CRF is also measurable in fetal circulation, and a linear correlation exists between maternal and fetal plasma CRF levels, despite the fact that umbilical cord plasma CRF levels are 20 to 30-fold lower than in maternal circulation *(84,100)*. In addition, CRF concentrations in umbilical venous plasma are higher than in the umbilical artery, supporting placenta as a major source of fetal plasma CRF *(101)*. A significant correlation between the amniotic fluid and maternal plasma CRF levels obtained simultaneously *(102)* suggests a placental source for amniotic CRF: amniotic fluid levels are similar to those circulating in cord plasma *(103)*.

CRF-BP is measurable in maternal plasma, and levels remain stable in nonpregnant women and during gestation until the third trimester of pregnancy *(22,99)*. At that time, maternal plasma CRF-BP concentrations significantly and rapidly decrease in the last 4–6 wk before labor *(22,99,104, 105)*, returning to approximately nonpregnant levels during the first 24 h postpartum. Thus, opposite changes in concentrations of CRF (higher) and CRF-BP (lower) in maternal plasma occur at term, so that the availability of bioactive CRF increases during the activation of labor. Cord blood CRF-BP levels are higher *(106)*, whereas amniotic fluid levels are lower than in maternal plasma and have a similar trend, decreasing until term pregnancy *(107)*.

Urocortin levels are undetectable during pregnancy, with no rise with increasing gestational age, as seen for CRF *(108)*. This lack of a rise in urocortin throughout pregnancy is also confirmed by other groups *(109,110)* and is further supported by the absence of gestational age–related changes in placental urocortin mRNA expression *(32)*.

Labor and Delivery

Several findings underlie the link between placental CRF and stress of parturition in humans. During spontaneous labor, maternal plasma CRF levels progressively rise, reaching the maximum values at the most advanced stages of cervical dilatation *(74,95,111,112)*. In addition, subjects who underwent elective cesarean delivery had plasma and amniotic fluid CRF levels significantly lower than patients after spontaneous vaginal delivery *(112)*. Moreover, the amount of CRF in placental extracts obtained at term after spontaneous vaginal delivery was significantly greater than the amount extracted from placentas obtained after cesarean delivery *(112)*. By contrast, during spontaneous physiologic labor, a significant decrease in CRF-BP levels in maternal plasma *(96,104)*, cord blood *(106)*, and amniotic fluid *(107)* has been observed.

With respect to urocortin, levels were higher at labor than those previously reported during pregnancy, but they did not change significantly at the different stages of labor when evaluated longitudinally. Some patients displayed a trend toward increasing levels, whereas others had variable concentrations *(113)*.

Role of Human Fetal Adrenal Glands in Maternal-Fetal Interaction and Labor

Findings reviewed in the present and other articles support the concept that placental CRF and CRF-related peptides drive the mechanisms leading to labor. However, for many years, investigators questioned whether there are fundamental differences between ovine pregnancy, in which the fetal adrenal gland plays a pivotal role in the process of parturition, and human pregnancy, in which the role of the fetal adrenal gland in this process is less clear *(58)*.

Primary regulation of growth and differentiation (steroidogenic) of the fetal adrenal gland is under the control of ACTH from the fetal pituitary gland, likely acting through several peptide growth factors *(58)*. More recent data indicate that CRF, produced by the placenta, also has the capacity to directly and preferentially stimulate the fetal adrenocortical production of DHE-S to as great an extent as ACTH, while stimulation of cortisol by CRF occurs to a much lesser degree than stimulation by ACTH *(86)* (Fig. 4).

Furthermore, placental CRF can stimulate production of POMC and some of its derivatives in the placenta, including ACTH, α-melanocyte-stimulating hormone, and β-endorphin in syncytiotrophoblast cells in vitro *(2,114)*. Although this placental ACTH may contribute to fetal adrenal regulation, it does not appear to be sufficient to sustain normal growth and function in anencephalic fetuses, suggesting that, at best, it plays a limited role in fetal adrenal development *(115)*. Placental CRF could, however, like fetal CRF, also stimulate fetal pituitary ACTH. Robinson et al. *(18)* suggested that placental CRF stimulates fetal pituitary ACTH, which then stimulates fetal adrenal DHEA-S, which is used by the placenta for conversion to estrogen by the process of aromatization *(58)*. This increase in estrogen then could serve as a trigger for the cascade of events leading to labor and parturition. In fact, estrogens increase uterine contractility by increasing myometrial excitability, increase myometrial responsivity to oxytocin and other uterotonic agents, as well as stimulate the synthesis and release of prostaglandins by fetal membranes *(58)*. Furthermore, estrogens stimulate proteolytic enzymes in the cervix, such as collagenase, which break down the extracellular matrix, permitting the cervix to dilate.

Consistent with the observation that CRF preferentially stimulates fetal adrenal DHEA-S directly was the observation that CRF increased the abundance of mRNAs encoding the enzymes for the conversion of androgen to estrogen *(86)*. It was therefore hypothesized that the rapid rise in placental CRF that occurs at the end of gestation at the time when CRF-BP decreases serves as the inciting event leading to placental aromatization *(115)*. The increasing estrogen, then, would initiate the chain of events terminating in labor

and delivery *(58)*. Thus, there may be a fetoplacental unit that involves fetal glucocorticoids and placental CRF as well as that involving fetal DHEA-S and placental estrogen.

Among the possible processes governing the initiation of human parturition are the following: First, the rise in placental CRF at the end of pregnancy stimulates fetal pituitary ACTH, which in turn stimulates increased fetal adrenal cortisol and DHEA-S production. The increasing concentrations of cortisol, in addition to maturating enzymes in organs critical for postnatal existence, further stimulate production of placental CRF by a feed-forward mechanism. The increasing production of DHEA-S provides additional substrate for placental aromatization to estrogen, which triggers the cascade leading to labor and delivery. Second, the increasing production of placental CRF directly and preferentially stimulates fetal adrenal DHEA-S, which is then converted by placental aromatization to estrogens that trigger the cascade leading to parturition. Third, CRF exerts a direct effect on the myometrium and fetal membranes to increase myometrial contractility.

CRF and CRF-Related Peptides in Biologic Fluids: Changes in Pathologic Pregnancy

Preterm Labor

Women with preterm labor have maternal plasma CRF levels significantly higher than those measured in the course of normal pregnancy *(116)*. Maternal plasma CRF levels are elevated before the diagnosis of labor in women who later develop preterm labor *(96)*. This finding suggests that the increase in CRF levels in patients with preterm labor is not owing to the process of labor itself, but indeed may be part of the mechanism controlling the onset of labor. The continued elevation of CRF preceding clinical evidence of uterine contraction suggests that CRF secretion is not sufficient to induce initiation of labor, and other factors are required in this event *(96)*. Maternal plasma CRF is higher in women with threatened preterm labor who give birth within 24 h from admission compared with those delivered after 24 h or with normal women at the same gestational age. This difference was observed at 28–32 wk as well as at 32–36 wk of gestation, but not before 28 wk *(99)*. Tocolytic treatment does not influence the CRF levels in maternal plasma with either indomethacin (a prostaglandin synthesis inhibitor) or nylidrin (a β-sympathomimetic agent) *(117)*. With indomethacin there is a fall of about 10% in CRF level, and with nylidrin about 10–20%, but these changes are not significant. On the other hand, cortisol levels fall significantly after the start of therapy. Furthermore, women who delivered preterm after cessation of contractions during tocolysis had higher CRF levels than women who proceeded to delivery at term.

McLean et al. *(96)* suggested that placental CRF production plays a role in determining the length of pregnancy and the timing of parturition. Their data indicate that circulating maternal concentrations of CRF, likely primarily of placental origin, were predictive of those women who proceeded to have normal-term, preterm, or postterm delivery. This suggests that the increase in CRF levels in patients with preterm labor is not owing to the process of labor itself, but indeed may be part of the mechanisms controlling the onset of labor. However, the continued elevation of CRF preceding clinical evidence of uterine contraction suggests that CRF secretion is not sufficient to induce initiation of labor, and other factors are required in this event *(95,96)*.

The evolution of maternal serum CRF concentrations in pregnancies destined to end preterm parallels the CRF curve of normal pregnancy, but the level is displaced upward *(118)*. This discrimination is detectable in advance of any clinical manifestation of uterine contractility, which prompted the design of controlled studies focusing on the value of second-trimester CRF level in predicting preterm birth.

Maternal and fetal plasma CRF-BP levels are low in preterm labor *(98,105,106)*, resembling the physiologic pattern observed at term. Because CRF-BP modulates the CRF-induced ACTH and prostaglandin release from decidual cells as well as the myometrial contractility activated by CRF *(44)*, the precocious fall in CRF-BP levels may be involved in the pathophysiology of preterm labor. Finally, recent evidence further suggests that not only are elevated levels of CRF linked to preterm labor and delivery, but also that CRF is one of several systems involved in this process *(119,120)*.

Life and Work Stress Events and Maternal-Fetal Adaptive Response

Throughout gestation, pregnant women shown an exponential increase in plasma CRF concentrations and an increased sensitivity to catecholamines *(2)*, associated with cardiovascular, metabolic, and endocrine changes. In nonpregnant women and in men, a close relationship among catecholamines, HPA axis, and stress events represents a classic finding of neuroendocrinology *(121)*; increased production of catecholamines and CRF characterizes the adaptative responses to stressful events, and a close relationship between catecholamines and the function of the HPA axis has been described *(122)*. Some reports describe a relationship among stressful life events or poor social circumstances, psychologic environment, and preterm delivery, suggesting that external environmental events could be one of the factors responsible for preterm labor *(123)*.

With respect to psychosocial stress events, Hobel et al. *(124)* showed a significant relationship between maternal psychosocial stress and high CRF levels at 18–20 wk of gestation, but no correlation was found at 28 wk gestation *(125)*. It has thus been suggested that placental CRF secretion may be independent of a condition of psychosocial stress, at least at this gestational age *(125)*. However, it is also possible that psychosocial stress may initiate a preterm labor by modulating other placental products, such as prostaglandins *(58, 119)*, whereas the CRF pathway is activated by other factors *(2)*. The absence of correlation between psychogenic

stressful stimuli and placental CRF may be evidence against the involvement of external stressors in the modulation of this neuroendocrine event of labor and/or on the existence of a protective mechanism against this activity. In fact, studies in rats have shown an attenuation of stress-induced catecholamine, CRF, and β-endorphin changes during pregnancy *(126)*, so it may be suggested to be a protective effect of pregnancy against the neuroendocrine and immune system responses to stress.

Chorioamnionitis

Several findings support a role for proinflammatory cytokines in the mechanisms responsible for preterm labor occurring in the setting of microbial invasion of the amniotic cavity *(127)*. The cytokine IL-1 stimulates production of CRF, and CRF in turn regulates cytokine production by immune effector cells *(128,129)*. It is well known that chorioamnionitis is the leading cause of preterm birth and neonatal complications, and it is the main cause of early neonatal brain damage, independently from gestational age *(130)*.

Nevertheless, a significant elevation of CRF levels in maternal plasma and placental extracts has been observed in the presence of microbial invasion of the amniotic cavity *(131)*, and because the immunologic and endocrinologic systems regulate each other extensively, there is potential for CRF to regulate inflammatory responses and vice versa. From this viewpoint, increased secretion of CRF in pregnancies complicated by intrauterine infection may help trigger uterine activity and preterm delivery, thereby helping the fetus to escape from a hostile environment.

Preeclampsia and Fetal Growth Restriction

Preeclampsia, defined as hypertension associated with proteinuria, complicates 2–8% of pregnancies and is an important cause of maternal and neonatal mortality *(132)*. It is associated with abnormal placentation, owing to the altered cytotrophoblast proliferation and invasion of endometrium, causing a reduced placental perfusion, the impairment of placental angiogenesis with insufficiency and failure of spiral artery remodeling *(132)*. The reduced and/or low perfusion of placenta and the fetus is consequently the main cause of fetal growth restriction, a complication of preeclampsia.

Maternal concentrations of CRF are greatly increased in preeclampsia *(99,133)*, in the presence of plasma CRF-BP levels significantly lower than in healthy control subjects *(99, 134)*. Additionally, cord venous plasma CRF concentrations are significantly higher in patients with preeclampsia and higher than in cord arterial plasma, indicating the secretion of CRF from the placenta into the fetal circulation *(133)*. In addition to CRF, the remaining hormones with vasodilatory actions involved in the stress response, such as ACTH and cortisol, are increased in the fetuses from preeclampsia pregnancies *(135)* as well as in fetal growth restriction fetuses *(136)*.

Concentrations of CRF in the fetal circulation are significantly increased in pregnancies complicated by abnormal umbilical artery flow-velocity waveforms, thus representing a stress-responsive compensatory mechanism in the human placenta *(137)*. It is not known whether this deranged secretion is part of the primary pathophysiology of these conditions or occurs as a secondary response to the increased vascular resistance in abnormal pregnancies. Recent findings suggest a role for CRF in vasodilatation of fetoplacental circulation, leading us to suggest that the increase in CRF secretion from human placental tissues in hypertensive pregnant women may be a compensatory response. This suggestion is also reinforced by the fact that the concentration of CRF in the fetal circulation is significantly increased in pregnancies complicated by abnormal umbilical artery flow-velocity waveforms—an important tool to assess the degree of uteroplacental insufficiency and, consequently, of chronic fetal hypoxia *(138)*—thus representing a stress-responsive compensatory mechanism in the human placenta *(137)*. Moreover, it is well known that corticosteroids can increase placental CRF secretion, both in vitro *(5,40)* and in vivo *(79)*. Evaluation of umbilical artery flow-velocity waveforms before and after administration of betamethasone in pregnancies with increased placental vascular resistance, as shown by umbilical artery absent end-diastolic flow, revealed that umbilical artery diastolic flow returned within 24 h after administration of betamethasone, consistent with decreased resistance. Thus, in pregnancies with umbilical artery absent end-diastolic flow, betamethasone treatment is associated with decreased placental vascular resistance, possibly induced via increased placental CRH secretion *(139)*.

Conclusion

In the past two decades, accelerated progress has been made in the understanding of the physiologic and pathologic functions of the placenta, mostly related to the discovery of several placental signaling molecules. The growing concept is that the placenta undertakes the role of intermediary barriers and active messengers in the maternal-fetal dialog, producing and releasing substances acting as endocrine, paracrine, and autocrine factors to control the secretion of other regulatory molecules. Placental CRF takes part in the regulation of maternal and fetal physiology during pregnancy, ranging from the control of placental anchoring to fetal growth and maturation, the fine regulation of uterine blood flow, and/or initiation of labor. Maternal or fetal physiologic and pathologic stress conditions influence such function, so that endogenous or exogenous stress stimuli stimulate placental CRF secretion, as part of an adaptive response of placenta to escape these adverse conditions.

In a scenario of maternal and/or fetal stress elicited by a number of pathologic conditions, placental CRF appears to play a role in coordinating the adaptive changes in uterine perfusion, maternal metabolism, fluid balance, and possibly uterine contractility.

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