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Review

Does the CRH Binding Protein Shield the Anterior Pituitary from Placental CRH?

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Corticotropin releasing factor (CRH) is released from the hypothalamus and travels to the anterior pituitary where it stimulates the release of adrenocorticotropin (ACTH). In turn, ACTH travels through the blood and stimulates the release of cortisol from the adrenal. The placenta is also a source of CRH and is responsible for the dramatic rises in CRH plasma levels in the third trimester of pregnancy. A CRH binding protein may stop placental CRH from overstimulating the pituitary and may contribute to the reason that pregnant women show only mildly elevated levels of ACTH in the blood. There is evidence to suggest, however, that the CRH binding protein does not completely shield the corticotrope from placental CRH.

Key Words: CRH; placenta; ACTH; steroid; CRH-binding protein.

Introduction

The pattern of hypothalamic control of adrenocorticotropin (ACTH) and cortisol release is well known. Corticotropin-releasing factor (CRH) is released from the hypothalamus in response to stress. On reaching the corticotropes of the anterior pituitary, CRH stimulates the release of ACTH and β-endorphin. ACTH in turn is released into the bloodstream and directs its target tissue to the adrenal cortex to increase the synthesis and secretion of steroid hormones.

It is now well appreciated that the hypothalamus is not the only tissue capable of CRH synthesis, and ACTH can be produced at sites other than the pituitary. The placenta is a rich source of CRH and ACTH *(1)* and is believed to be responsible for the plasma levels of CRH, which rise throughout pregnancy to peak at parturition *(2)*. Indeed it has been noted that the placenta may be able to boost the levels of CRH in the mother to 1000 times that found in the nonpregnant individual *(3)*. Interestingly, the pregnant woman does not show dramatically elevated levels of blood ACTH. Indeed, levels of ACTH in the pregnant woman's circulation are in the high, but still normal range *(4)*. Furthermore, rises in corticosteroid binding globulin and unbound cortisol during pregnancy are gentle rather than dramatic (reviewed in *2*). Free cortisol only reaches an approximate two- to threefold increase in the latter stages of gestation, and it is rare for women in the third trimester to display cortisol levels higher than that seen in mild Cushing's syndrome *(5–7)*. Recently, Magiakou and colleagues *(7)* performed a study in which they collected plasma samples at 30-min intervals over 24 h from women in the third trimester of pregnancy. These samples were analyzed for hormones, including CRH, ACTH, and cortisol. No correlation between CRH and ACTH was observed, and a positive correlation between CRH and cortisol was weak and only apparent at night. These finding raised the following question: Why do the extraordinarily high levels of CRH not stimulate the pregnant woman's pituitary to release pathophysiological levels of ACTH?

An attractive answer to the conundrum was provided by the finding of a CRH binding protein in human plasma that was shown in in vitro studies to attenuate the action of CRH *(8–11)*. There is evidence, however, that there are additional factors that may account for the dampened bioactivity of placental CRH on the anterior pituitary, and some authors suggest that the CRH binding protein "partly explain(s)" the absence of anterior pituitary overstimulation *(10)*. Indeed, the extent to which the binding protein protects the corticotrope from placental CRH is often a topic of debate among scientists in the field (*see* Fig. 1). In future studies on CRH levels in pregnancy, it is likely that many investigators will include an analysis of bound vs free CRH.

The CRH Binding Protein in Pregnancy

Various forms of the CRH binding protein have been found, and these are thought to be differentially cleaved

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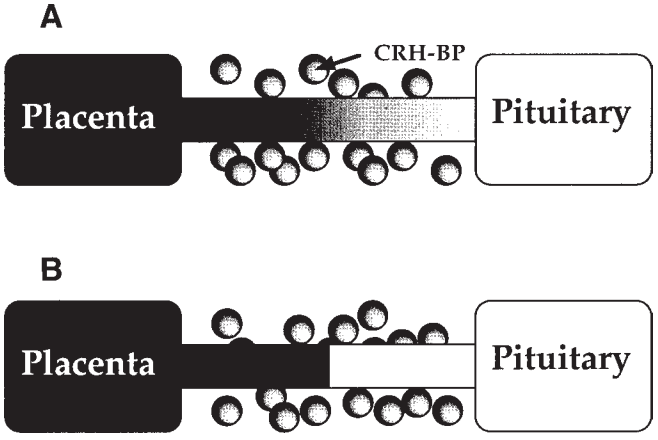

Fig. 1. Two hypotheses on the ability of the CRH binding protein to shield the anterior pituitary from placental CRH. CRH activity is indicated by the intensity of red color. **(A)** The CRH binding protein attenuates the activity of placental CRH, but leaves residual activity. **(B)** The CRH binding protein forms an impenetrable shield that completely prevents any interaction of placental CRH with the anterior pituitary.

products cleaved from a 322 amino acid protein precursor *(11,12)*. The mature protein's molecular weight has been reported in the range of 33–37 kDa and contains one possible glycosylation site *(11,12)*. The mature protein contains 10 cysteine residues, and these may function to stabilize its structure and activity *(11–13)*. All mammals tested so far have been shown to produce the binding protein in the brain *(12)*. In contrast, only humans have been found to produce CRH binding protein in the liver and placenta *(12,14)*. The CRH binding protein has also been found to be produced by the corticotropes of the anterior pituitary in rat *(15)*, and the expression of CRH binding protein DNA has been demonstrated in murine pituitary *(16)*. The synthesis of the CRH binding protein in the anterior pituitary is increased by glucocorticoids, and this regional and positive effect on the expression of CRH binding may help to reduce an anterior pituitary response to extrapituitary CRH *(12)*.

It has been proposed that the interaction between CRH and its binding protein results in dimerization of the protein and clearance of CRH from the circulation *(17–19)*. Decreased levels of CRH binding protein in late pregnancy (*20*; *see* Fig. 2) indicate that placental CRH may be saturating the binding protein pool and causing an increased clearance of the binding protein–ligand complex in the latter stages of gestation *(12)*. It is not known at present whether the rate of synthesis or degradation of the binding protein is affected by pregnancy, but this would provide an alternate reason for decreased amounts of CRH binding protein concentration in late gestation. Indeed it has been shown that a bolus injection of CRH into nonpregnant individuals results in a reduction in the amount of CRH binding protein complexed with CRH *(18)*. It has been proposed that this reduction is similar to that seen in pregnancy *(18)*. This phenomenon may produce higher levels of free CRH in the last 6 wk of pregnancy *(7)*.

The Effects of CRH Binding Protein on CRH Activity

In vitro experiments have shown that CRH binding protein can attenuate CRH activity on pituitary cells, but it is not yet certain whether the CRH binding protein can obliterate the CRH response. Linton et al. *(10)* have tested the effect of the binding protein on the ACTH-releasing activity of CRH on cultured pituitary cells. Even at the highest concentrations of CRH binding protein used (100 ng/mL), CRH (1.5 ng/mL) still retained approx 24% of its ability to activate the corticotrope *(10)*. Potter et al. *(11)* have transfected COS7 cells with vectors containing DNA sequences that code for rat and human CRH binding protein. Cultured media from these genetically engineered cells were shown to dampen the ACTH-releasing activity of CRH. CRH at a concentration of 0.5 n*M* retained approx 40% of its bioactivity. However, the concentration of CRH binding protein in these experiments was unknown. As expected, higher concentrations of CRH resulted in a dose-dependent reduction in the ability of the CRH binding protein to attenuate the bioactivity of CRH. Indeed, the CRH binding protein-conditioned media appeared to be close to ineffective in reducing the bioactivity of 5 n*M* CRH *(11)*. This result indicated that as concentrations of CRH rise as they do in pregnancy, a constant or falling level of CRH binding protein would progressively become a weaker obstacle to the effects of CRH on the corticotrope.

Furthermore, it has been suggested that it takes approx 200 s for the binding protein to bind maximally and inactivate CRH *(10,21)*. The brain is perfused with blood at the rate of approx 750 mL/min. Although the flow through the hypophysial portal system is several orders of magnitude lower, it is conceivable that some CRH that has been released from the placenta is able to reach the hypophysial portal system and the pituitary without being obstructed by CRH binding protein in the circulation. In addition, a portion of CRH in the blood of pregnant women is in the unbound form. It has been pointed out that unbound levels of CRH have been estimated at between 7 and 38% *(2)*, which indicates that the concentration of "full-strength" CRH is still 70–380 times that found in the blood of the nonpregnant individual. If in vitro results are accurate in predicting that the maximum inhibition the CRH binding protein can exert is approx 80%, then it is possible that the levels of CRH that can reach as high as 1 n*M* during pregnancy (reviewed in *22*) are still able to activate CRH receptors (CRH-Rs). Indeed, large-mol-wt placental CRH, which is believed to be a complex of CRH with the binding protein, has been shown to retain enough potency to stimulate the output of superfused ovine pituitary cells *(23)*. About

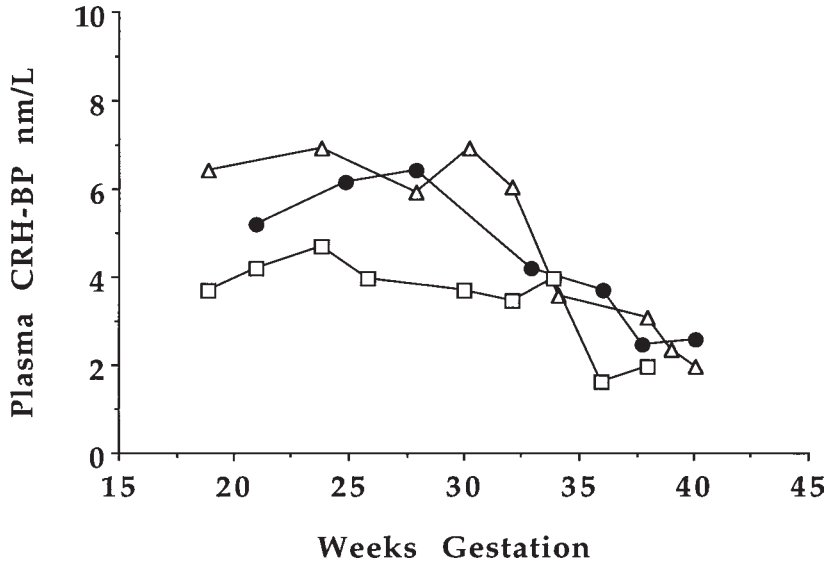

Fig. 2. Falling levels of CRH binding protein in three women during pregnancy. Redrawn with permission from ref. *(20)*. © The Endocrine Society.

30% of placental CRH disassociates, however, from the binding protein during its passage through a superfused pituitary cell column *(23)*. It is not known therefore whether this activity represents retained activity of the CRH/binding protein complex or the ability of placental CRH to disassociate from its binding protein in order to stimulate the corticotrope in vitro.

It would appear that there are several avenues that placental CRH can take to evade an obstruction in the path to the corticotrope. These are:

1. Incomplete ability of the CRH binding protein to counter CRH bioactivity.
2. The ability of a significant amount of placental CRH to escape the activity-reducing effect of the binding protein.
3. A time delay before CRH binding protein can attenuate CRH activity.
4. A decrease in the capacity of circulating CRH binding protein to counteract CRH activity as gestation progresses (*see* Fig. 1).

If placental CRH is performing important physiological functions, a system may have evolved whereby its actions are not completely blocked by the binding protein.

Heterogeneous CRH Receptors (CRH-Rs)

CRH-Rs belong to the G-protein-linked receptor family, which has seven regions of polypeptide that traverse the membrane *(24,25)*. In the human, the CRH-R type 1 (CRH-R1) and CRH-R1α and CRH-R1β subtypes have been identified in the anterior pituitary *(24,25)*. An additional 29 amino acid region differentiates the CRH-R1β subtype from the CRH-R1α. The extra 29 amino acid insert appears to reduce the ability of CRH-R1β to modulate the cAMP signaling system as compared with CRF-R1α *(24,25)*. Additionally, a variant version of the receptor that was found to be shorter than CRH-R1α was originally identified in central nervous tissue *(26)*. Interestingly, CRH-Rc was the only type of CRH receptor in the individual in which it was discovered *(26)*.

The CRH-R1 is highly expressed in the brain and pituitary. A second class of CRH-R, CRH-R2, has been identified and appears to predominate in heart and skeletal tissue *(27,28)*. In the rat, it has been discovered that there are two subtypes of CRH-R2, designated CRH-R2α and CRH-R2β *(29)*. A recent report indicates that there are a also a number of CRH-R2 isoforms present in the human *(30)*.

CRH-Rs have also been identified in the placenta *(31,32)*. Subgroups identified so far include the CRH-R1α and CRH-Rc, but no CRH-R2 has been found in the placenta *(33)*. Histochemical studies indicate that the syncytiotrophoblast cells are the major producers of CRH-Rs *(33)*. As CRH is also produced in the syncytiotrophoblast, it has been proposed that the placental CRH receptors are modulating autocrine and paracrine signals in the placenta *(33)*. Additionally or alternatively, the placental CRH-Rs may be binding placental CRH and directing it to a degradative pathway. This mechanism could prevent some placental CRH from reaching the anterior pituitary.

The existence of a heterogeneous collection of CRH-Rs presents an interesting and important question. Is there heterogeneity in the way that the CRH binding protein prevents interaction of CRH with a receptor type? Additionally, it should be remembered that most of the research into the CRH binding protein effects on the corticotrope has been performed in vitro. How much protection the CRH binding protein affords the corticotrope in vivo is as yet an unknown quantity. It will also be important to determine whether the CRH and binding protein complex is able to desensitize the corticotropes to subsequent stimulation with

CRH. If the CRH/CRH binding protein complex is able to desensitize the corticotropes to subsequent CRH exposure, then this would suggest that the hypothalamic–pituitary axis can be disturbed by the complex when levels rise during pregnancy.

Animal Models for the Effects of CRH Binding Protein in Pregnancy

Studies on the effects of the CRH binding protein on the release of CRH and vasopressin have yielded valuable information on how the CRH binding protein feeds back on homeostatic controls in the pituitary *(34)*. Transgenic mice have been produced that express high concentrations of CRH binding protein cDNA in the anterior pituitary. In these mice, CRH output from the anterior pituitary is elevated by 82%, and vasopressin secretion is increased by 35%. The authors concluded that CRH binding proteins are important regulators of the hypothalamic-pituitary-adrenal (HPA) axis *(34)*.

Several groups have begun prospecting for animal species that will provide models for studying the effects of CRH and its binding protein on the HPA axis during pregnancy. Domestic animals, such as the horse, may not encounter the same disturbances to the HPA axis as the human throughout gestation, and an equine CRH binding protein does not appear to exist *(35)*. Neither is there an apparent rise in plasma levels of CRH during pregnancy in the horse *(35)*.

Because domestic animals, such as the horse (as well as sheep and rats), do not display circulating placental CRH, two groups *(36,37)* began studying primate species to determine whether the HPA–placental axis of these animals displayed similarities to the human situation. These studies discovered that the baboon has elevated levels of CRH immunoreactivity during gestation *(36,37)*, and baboon CRH is similar to human CRH as judged by gel-filtration chromatography *(36,37)* and high-performance liquid chromatography (HPLC) *(37)*. The dynamics of the baboon's changes in plasma CRH during pregnancy do not, however, exactly mirror those of the human. Baboon CRH levels peak in the first half of pregnancy, but remain elevated until shortly after delivery. Furthermore, unlike the human situation, the baboon does not appear to have a CRH binding protein *(37)*. A landmark finding was that baboon free and total cortisol does not become significantly elevated or change with time of gestation *(37)*. This result showed that in the baboon at least, there was a mechanism or mechanisms other than the CRH binding protein that prevented placental CRH from inducing Cushing's syndrome during pregnancy. Recently, Smith and coworkers *(38)* extended their research to chimpanzees and gorillas, and found that these higher primates, like humans, display a rise in CRH throughout pregnancy and do possess a CRH binding protein. Ethical considerations in the use of these animals will restrict the type of experiments that can be conducted using them as a model. Nonetheless, it will be extremely interesting to see the results of future nondestructive and considerate research on the changes in the HPA–placental axis in higher primates. It will also be very interesting to see whether the baboon is completely free of any mechanism that reduces the ability of CRH to interact with CRH-Rs.

Is the HPA Axis Disturbed by Placental CRH During Pregnancy?

It has been noted that nonpregnant individuals have levels of CRH binding proteins similar to that found in the pregnant woman and yet can still respond to CRH injected into the bloodstream *(22)*. On the other hand, it has been pointed out that a bolus injection of CRH is not a complete model of the way the placenta more slowly infuses the bloodstream with CRH, and slow infusion perhaps gives the CRH binding protein more time to attenuate the CRH signal before it reaches the pituitary *(10)*. The placental output of CRH has been estimated at 690 ng/h for a 500-g placenta *(39)*. In the future, experiments that infuse CRH into the bloodstream (at a rate similar to the output of the placenta) of nonpregnant individuals would indicate whether release of CRH from the placenta acts as a secretagog for the anterior pituitary. Previous in vitro experiments have gauged the effects of a long-term exposure to free CRH and shown that superfused anterior pituitary cells respond to the start of CRH exposure with an explosive initial phase release of β-endorphin *(40)*. The pituitary cells soon become desensitized, however, and markedly reduce the rate of hormone release. The rate of β-endorphin output stabilizes at a level slightly higher than baseline, whereas the stimulus continues and then returns to normal after the stimulus is removed. If the corticotrope of the pregnant woman behaves in a similar fashion to prolonged exposure to CRH, this resetting of the corticotrope could participate in the explanation of the gently (not pathologically) elevated levels of ACTH in the latter stages of pregnancy. Perhaps it is time for experiments on superfused pituitary cells that recreate pregnancy by using sustained doses of CRH in the presence of appropriate amounts of CRH binding protein to study the dynamic phases of the corticotrope response in a pregnancy model.

In the model of corticotrope desensitization in pregnancy, ACTH is being produced at an elevated, but submaximal level. Studies on transgenic mice in which the CRH gene was overproduced show that ACTH and glucocorticoid levels are elevated in this situation *(41)*. These mice did, however, show pronounced Cushingoid syndromes, such as excess adipose tissue and muscle wasting. It would be interesting to find out whether the corticotropes are desensitized under these conditions and whether they are still able to respond to higher doses of CRH. Nonetheless, whether results gained in the transgenic murine model

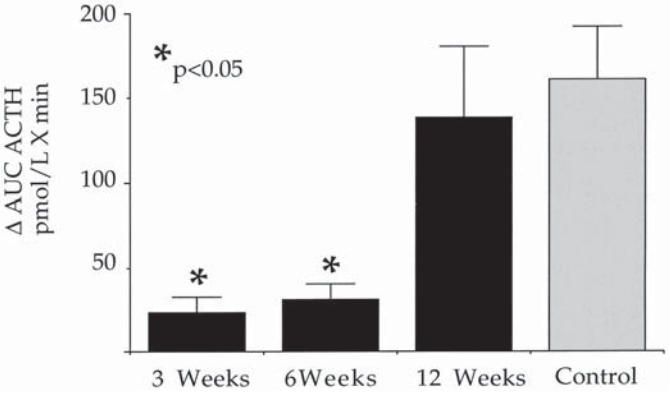

Fig. 3. Stimulated ACTH responses to ovine CRH (1 μg/kg) in women 3, 6, and 12 wk postpartum and a control response by nonpregnant women. Values = mean ± SE. Reproduced with permission from ref. *6*. © The Endocrine Society.

with overproduced hypothalamic CRH can be extrapolated to model the effects of placental CRH in the human is not yet known. A case study on a single patient with a prostate tumor that was a source of ectopic CRH is also difficult to correlate with the healthy HPA–placental axis. The subject in this study displayed elevated ACTH and cortisol levels, but did not appear to be displaying normal glucocorticoid negative feedback on the hypothalamic–adrenal axis *(42)*. Additional tumors on the median eminence and infundibulum in this case further complicate the analysis of the findings in relation to placental CRH effects.

In support of the theory that pregnant women have a pituitary that is desensitized to CRH, in vivo studies have shown that pregnant women display a blunted response to a bolus injection of 1 μg/kg CRH *(6,43)*. This depression of the corticotropes lasts for at least 6 wk following delivery, but returns to normal by 12 wk postpartum (*6*; *see* Fig. 3). An intriguing extension on the theory that pregnant women's pituitary is desensitized to CRH is that disturbances in the hypothalamic–pituitary axis may contribute to postpartum depression *(3,6)*. In support of this proposal, it has been shown that women who display depression postpartum have a lower response to CRH (measured at 3, 6, and 12 wk postpartum) as compared to nondepressed new mothers *(6)*. If the theory of pituitary desensitization is correct, then how does the hypothalamus retain control of the pituitary during pregnancy? (*See* Fig. 4.) Perhaps rapid pulsatile doses of hypothalamic CRH that are free of binding protein retain their power to influence the corticotrope *(10)*. Additionally or alternatively, perhaps other hypothalamic factors, such as vasopressin, are endowed with a greater responsibility in modulating ACTH release during the latter stages of gestation *(22,36)*.

Conclusion

As researchers around the globe probe deeper into the changes in the HPA–placental axis during pregnancy, the

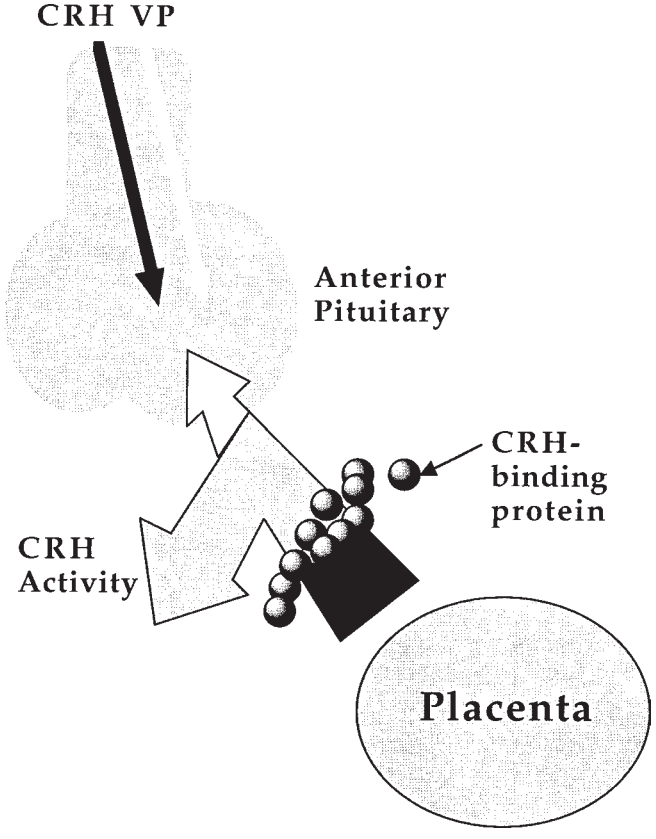

Fig. 4. A model that incorporates theories on why the anterior pituitary is not overstimulated by placental CRH. The intensity of placental CRH activity is reduced by the CRH binding protein. The CRH activity that is left after the CRH attenuation desensitizes the pituitary to CRH. This forms a second stage of reduction in the power of the placenta to influence the corticotropes. The pituitary may still be influenced by concentrated pulses of free CRH from the hypothalamus. It has been questioned, however, whether hypothalamic CRH can provide a "full-power" stimulus to the corticotrope. Hypothalamic vasopressin (VP) is still able to regulate the release of ACTH.

"road map" describing the endocrine and metabolic functions of the axis is becoming increasingly complex. Recent findings provide new factors that must be included into the formula. To illustrate, the discovery of new ligands for the CRH binding protein, such as urocortin *(44)*, has alerted investigators to the possibility that there are additional ligands for the CRH binding protein. Although the CRH binding protein is thought to prevent urocortin from interacting with the CRH receptor *(45)*, ligands, such as urocortin, may function to increase the amount of free CRH in the bloodstream. Indeed, advances in the field of CRH involvement in pregnancy have been progressing at an astounding rate in the last decade. This research provides a very concrete hope for the future in formulating strategies to counteract conditions, such as premature birth, which are perhaps in part caused by a nonability of the placenta to regulate a healthy level of CRH output *(46)*. Knowledge of

the effects of placental CRH may soon be put to practical use in combating conditions, such as postpartum depression.

Many investigators believe with good reason that the CRH binding protein does shield the corticotrope from CRH, but are also aware of the possibility that this shielding is not complete. There may be other mechanisms that prevent the pregnant woman from becoming pathologically Cushingoid.

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