

**This review highlights key aspects of corticotropin releasing hormone (CRH) biology of potential relevance to the sexual dimorphism of the stress response and immune/inflammatory reaction, and introduces two important new concepts based on the regulatory potential of the human (h) CRH gene: (1) a proposed mechanism to account for the tissue-specific antithetical responses of hCRH gene expression to glucocorticoids, that may also explain the frequently observed antithetical effects of chronic glucocorticoid administration in clinical practice and (2) a heuristic diagram to illustrate the proposed modulation of the stress response and immune/inflammatory reaction by steroid hormones, from the perspective of the CRH system.**

**Key words:** Autoimmunity, Corticotropin releasing hormone, Estrogens, Glucocorticoids, Glucocorticoid receptor, Gonadal steroids, Heat shock proteins, Hormonal regulation of gene expression, Hypothalamic–pituitary–adrenal axis, Immune/inflammatory reaction, Sexual dimorphism, Stress response

## **Sexual dimorphism of stress response and immune/inflammatory reaction: the corticotropin releasing hormone perspective**

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### **Introduction**

Experimental support for the hypothesis that adrenocorticotropin (ACTH) secretion was controlled by hypothalamic factors,<sup>1</sup> was obtained in 1955.<sup>2,3</sup> In 1981, a 41 amino acid C terminal amidated peptide from ovine hypothalamus stimulating pituitary ACTH release *in vitro* was identified and characterized.<sup>4</sup> The biologically active form of this peptide, designated corticotropin releasing hormone (CRH), and also frequently referred to as corticotropin releasing factor (CRF), was synthesized and found to have potent ACTH-releasing actions *in vivo*.<sup>5</sup> CRH is the only permissive factor for the anterior pituitary release of ACTH known in man<sup>5,6</sup> and acts in synergy with arginine vasopressin (AVP) and, perhaps, other factors, to regulate pituitary ACTH secretion, and, therefore ultimately the activity of the pituitary–adrenal axis.<sup>7–9</sup>

Since its discovery, it has become evident that CRH has roles which are much wider than initially thought. Thus, coordination of the behavioral and physical components of the stress response and regulation of the immune/inflammatory reaction were unravelled as major overall roles of this neuropeptide.<sup>10,11</sup> In addition, this peptide was implicated in the pathophysiology of a large range of diseases associated with dysregulation of the stress system and autoimmunity.<sup>12</sup> Because of the central roles of CRH in homeostasis and pathogenesis of disease, knowledge of its gene

and its regulation would be essential for further progress. This brief review will outline the most critical aspects of CRH biology, will summarize the structure, function and regulation of the human (h) CRH gene by steroid hormones, and will introduce a tentative model to account for its tissue-specific antithetical hormonal responses. The implications of the regulatory potential of the hCRH gene for the sexual dimorphism of the stress response and immune/inflammatory reaction will also be discussed.

### **Overview of CRH biology**

CRH is synthesized as part of a prohormone, it is processed enzymatically, and in addition undergoes enzymatic modification to the amidated form. Mammalian CRH has homologies with non-mammalian vertebrate peptides  $\times$ CRH<sup>13</sup> and sauvagine<sup>14</sup> in amphibia (from frog brain/spleen and skin, respectively), urotensin-I in teleost fish<sup>15</sup> and the two diuretic peptides Mas-DPI and Mas-DPII from the tobacco hornworm *Manduca sexta*.<sup>16,17</sup> The vertebrate homologues have been tested and found to possess potent mammalian and fish pituitary ACTH-releasing activity. In addition, they decrease peripheral vascular resistance and cause hypotension when injected into mammals.<sup>15,18,19</sup>

The amino terminus of CRH is not essential for binding to the receptor, whereas absence of the carboxy terminal amide abolishes CRH binding to

its receptor. Oxidation of a methionine residue abolishes the biological activity of CRH, and this may be a mechanism for neutralization of the peptide *in vivo*.<sup>5</sup> CRH bioavailability is also regulated by binding to corticotropin releasing hormone binding protein (CRHBP),<sup>20</sup> with which it partially co-localizes in the rat central nervous system (CNS) and other tissues.<sup>21</sup> The human CRHBP gene has been assigned to 5q11.2–q13.3.<sup>22</sup> In the pituitary, CRH acts by binding to membrane receptors (CRHR) on corticotrophs, that couple to guanine nucleotide-binding proteins and stimulate the release of ACTH in the presence of  $Ca^{2+}$  by a cAMP-dependent mechanism.<sup>5,23,24</sup> CRH stimulation of cAMP production increases in parallel with the secretion of ACTH in rat pituitary corticotrophs<sup>25</sup> and human corticotroph cells.<sup>26</sup> In addition to enhancing the secretion of ACTH, CRH also stimulates the *de novo* biosynthesis of pro-opiomelanocortin (POMC).<sup>25,27</sup> CRH regulation of POMC gene expression in mouse tumorous corticotroph AtT 20 cells, involves the induction of c-fos expression by cAMP and  $Ca^{2+}$ -dependent mechanisms.<sup>28,29</sup>

Sequence analysis of hCRHR cDNAs isolated from cDNA libraries prepared from human corticotropinoma or total human brain mRNA, revealed homology to the G-protein coupled receptor superfamily.<sup>30,31</sup> The hCRHR cDNA sequences of the tumour and normal brain were aligned and found to be identical. The hCRHR gene has been assigned to 17q12–qter.<sup>32</sup> The sequences of mouse and rat CRHR cDNAs were also reported recently.<sup>31,33</sup> Human/rodent CRHR protein sequences differ primarily in their extracellular domains. In particular, positively charged arginine amino acid(s) are present in the third and fourth positions of the extracellular amino terminal domain of the rodent but not the human CRHR peptide. This might be responsible for the differential activity of the  $\alpha$ -helical 9-41 CRH antagonist<sup>34</sup> between rodents and primates (C. Kalogeras, personal communication).

Central sites of CRHR expression include the hypothalamus, the cerebral cortex, the limbic system, the cerebellum and the spinal cord.<sup>35</sup> This may explain the broad range of neural effects of intracerebroventricularly (i.c.v.) administered CRH, including arousal, increase of sympathetic system activity, elevations in systemic blood pressure, tachycardia, suppression of the hypothalamic component of gonadotropin regulation (GnRH), suppression of growth and inhibition of feeding and sexual behaviours characteristic of emotional stress.<sup>36–42</sup> Central injection of CRH in rats and monkeys thus induces complex physiological and behavioral responses, suggesting that central CRH pathways

coordinate the overall stress response.<sup>40</sup> High doses of CRH cause behaviors characteristic of anxiety, suggesting that the behavioral effects of CRH are dose-dependent, with low doses promoting adaptation and high doses being maladaptive.<sup>12,42</sup>

There is a broad peripheral expression of CRH and CRHR, including the peripheral nervous system, lung, liver, gastrointestinal tract, immune cells and organs, gonads and placenta.<sup>10,11,43</sup> The biological roles of extraneural CRH have not been fully elucidated as yet, although it is likely that it might participate in the auto/paracrine regulation of  $\beta$ -endorphin production and analgesia, and that it may modulate immune/inflammatory responses and gonadal function.<sup>12,41,44–46</sup> The current consensus is that CRH produced in high amounts in inflammatory sites of both animals and humans, designated immune CRH, is promoting inflammation by stimulating cytokine production by immune cells and/or by potentiating the proinflammatory activities of cytokines and other mediators of inflammation.<sup>46,47–53</sup>

Intravenous administration of CRH in humans causes a prompt increase in the release of ACTH into the blood, followed by the secretion of cortisol. The effect is specific for ACTH release and is inhibited by glucocorticoids. High cortisol levels reduce or abolish CRH action on the pituitary. CRH has been used as a diagnostic tool to differentiate causes of hypercortisolism and hypocortisolism, but does not have an established therapeutic role.<sup>24,54–56</sup> The clinical applications of CRH were recently reviewed extensively.<sup>11</sup>

The amidated active peptide form is stored within secretory granules. Stress stimulates a variety of endogenous substances, which excite the CRH neuron in the PVN<sup>12</sup> and cause the release of CRH into the portal system by the classical mechanism of membrane fusion.<sup>57</sup> Major intracellular signalling systems, such as the cyclic adenosine monophosphate (cAMP)-dependent protein kinase A (PKA) [cAMP/PKA] and the diacylglycerol (DAG)-dependent protein kinase-C (PKC) [DAG/PKC] pathways, appear to be involved in the regulation of CRH biosynthesis and release.<sup>58</sup> Theoretically, hormonal regulation of CRH biosynthesis and secretion, and CRHR-mediated signal transduction may occur in any of many steps.<sup>10</sup>

### hCRH gene structure and chromosomal localization

The hCRH gene consists of two exons separated by an intron in its 5' untranslated region (Fig. 1).<sup>59</sup> The rat and ovine CRH genes have a similar organization.<sup>60,61</sup> The hCRH gene has

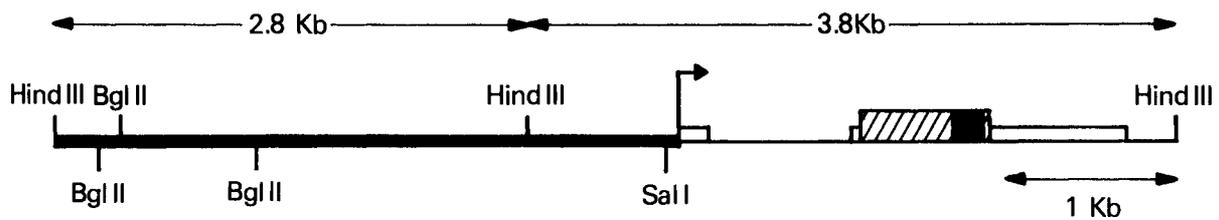


FIG. 1. Physical organization of a 6.6 kb DNA region containing the hCRH gene. The bent arrow marks the start and points towards the direction of transcription. The open boxes refer to the transcribed and untranslated mRNA sequences, the striped box to the translated prepro-hCRH mRNA and the small black box to the segment of the gene, coding the hCRH(1-41) peptide, which is derived from the precursor hCRH protein by post-translational proteolytic cleavage. The 5'-flanking region of the gene is indicated by a thick black line.

been mapped to the long arm of chromosome 8 (8q13).<sup>62</sup> The 3' untranslated region of the hCRH gene contains several polyadenylation sites, which may be utilized differentially in a potentially tissue-specific manner. CRH mRNA polyA tail length is regulated by phorbol esters in the human hepatoma CRH-expressing cell line NPLC, and this may have potential relevance for differential stability of CRH mRNA in various tissues *in vivo*.<sup>63</sup> Alignment of the human, rat and ovine CRH gene sequences has allowed the comparison of the relative degree of evolutionary conservation of their various segments. These comparisons revealed that the 330 bp long proximal segment of the 5' flanking region of the hCRH gene had the highest degree of homology (94%), suggesting that it may play a very important role in CRH gene regulation throughout phylogeny, which is crucial for survival. A conserved polypurine sequence feature of unknown biological significance is present at -829 of hCRH, -801 of the ovine CRH gene, as well as in the -400 bp 5' flanking region of POMC, rat growth hormone and other hormone genes.<sup>64</sup> The sequence of a 3.7 kb stretch into the 5' flanking region of the hCRH gene was also determined (Gene Bank accession no. x67661).<sup>65</sup> A gene bank search for homologous sequences identified a segment at position 2213-2580 with greater than 80% homology to members of the type O family of repetitive elements, and another at -2835 to -2972 with less homology to the 3' terminal half of the human AluI family of repetitive elements. The rest of the sequence was found to be novel and specific to the CRH gene. A computer program was used to identify consensus recognition sites of DNA binding proteins involved in transcriptional regulation in this region of the hCRH gene. The organization and spatial distribution of several putative responsive elements of potential relevance to CRH transcriptional regulation is shown in Fig. 2.<sup>65</sup>

### Regulatory elements of the hCRH gene

*CRE (cAMP-responsive enhancer, CREB enhancer sequence/PKA activation)*. Activation of adenylate cyclase by various effector systems elevates intracellular cAMP and stimulates protein phosphorylation by cAMP-dependent protein kinases and transactivation of genes with the consensus palindromic sequence 5'-TGACGTCA-3' in their 5' flanking regions.<sup>66</sup> cAMP-response element-binding protein (CREB), is a member of the bZIP or leucine zipper family of transcription factors, that is phosphorylated by several protein kinases, including the catalytic subunit of cAMP/PKA. CREB homo- or heterodimerizes through its leucine zippers, binds to DNA as a dimer, and modulates transcription of genes in response to hormonal stimulation of the cAMP pathways.<sup>67-70</sup> The gene encoding CREB contains at least 11 exons spanning over 40 kb and produces multiple CREB isoforms by alternative splicing.<sup>71,72</sup> This is in contrast to other bZIP members (e.g., c-jun<sup>72</sup> and C/EBP<sup>73</sup>), which are encoded by intronless genes.<sup>74</sup> The latter proteins can heterodimerize with some members of the CREB/ATF family of proteins.<sup>75,76</sup>

CRH regulation by the PKA pathway is well documented. Administration of cAMP increases CRH secretion from perfused rat hypothalami, and forskolin, an activator of adenylate cyclase, increases CRH secretion and CRH mRNA levels in primary cultures of rat hypothalamic cells.<sup>77</sup> Regulation of the hCRH gene by cAMP has also been demonstrated in the mouse tumorous anterior pituitary cell line AtT-20, stably or transiently transfected with the hCRH gene.<sup>78-80</sup> The hCRH 5' flanking sequence contains a perfect consensus CRE element that is conserved in the rat and sheep, and confers transcriptional activation to chloramphenicol acetyltransferase (CAT) reporters *in vivo*, as demonstrated with both human and rat CRH promoter-driven CAT constructs.<sup>81,82</sup>

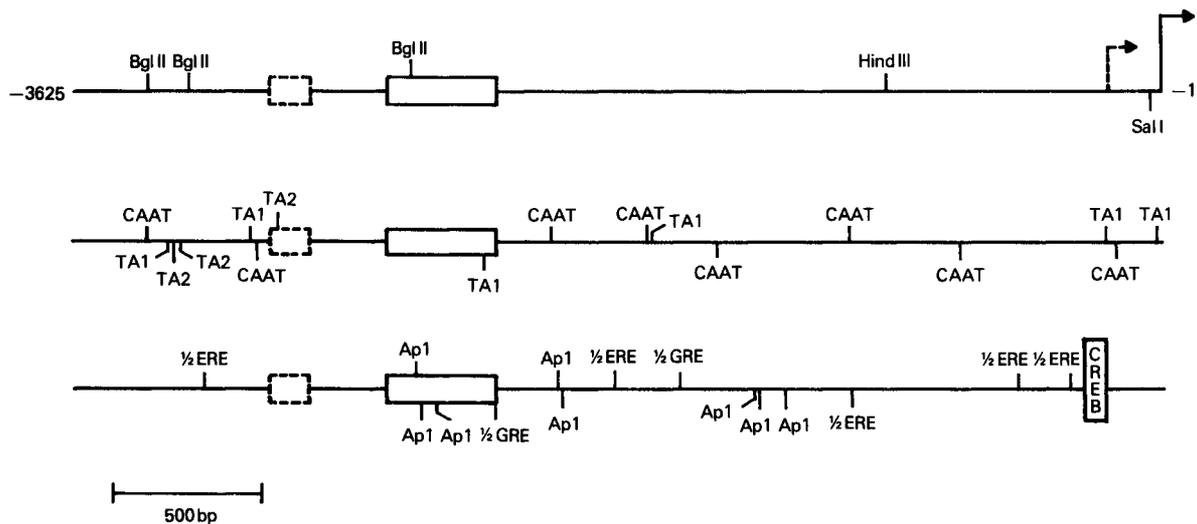


FIG. 2. Spatial distribution of putative regulatory features in the 3625 bp long hCRH 5' flanking region. *Top:* landmark restriction sites, the two mRNA initiation sites (solid and broken bent arrows point to the direction of transcription), the type O member of repetitive elements (solid box), and the partial Alu member of repetitive elements (broken box). *Middle:* locations of TATA box 1 [TA1: 5'TATAAA(+), 5'TTTATA(-)]; TATA box 2 [TA2: 5'TATAAT(+), 5'ATTATA(-)]; and CAAT box [5'CCAAT(+), 5'ATTGG (-)] consensus elements. *Bottom:* locations of CREB [5'ACGTCA(+), 5'TGATTCA(-), 5'TGACGTCA(+), 5'AGACGTCA(-)]; AP-1 [5'TGAAATCA(+), 5'TGATTCA(-), 5'TGAGACTT(+), 5'AAGTCTCA(-), 5'TGACTAA(+), 5'TTAGTCA(-), 5'TTAGTCAG(+), 5'CTGACTAA(-), 5'CTGACTAA(+), 5'TTAGTCAG(-)]; half palindromic ER sites (1/2 ERE) [5'GGTCA(+), 5'TGACC(-)]; and second half GR site (1/2 GRE) [5'TGTTCT(+), 5'AGAACA(-)] consensus elements. The (+) and (-) orientations of the elements are indicated by the arrow pointing above or below the midline, respectively.

*TRE (phorbol ester, 12-O-tetradecanoyl phorbol-13-acetate (TPA)- response element or AP-1 site/PKC activation).* Epidermal growth factor (EGF) and TPA elevate intracellular DAG and activate PKC and the transactivation of genes containing TPA response elements in their 5' flanking regions.<sup>85</sup> TRE or AP-1-binding proteins are the Jun and Fos families of the bZIP superfamily of transcription factors. These proteins interact through their carboxy-terminal leucine zippers and c-jun can form both homodimers with itself and heterodimers with c-fos, while c-fos can heterodimerize with c-jun but cannot form homodimers. TPA and the activation of PKC induce c-jun. This is due to positive autoregulation mediated by binding of c-jun homodimers or c-jun:c-fos heterodimers to an AP-1 site within the c-jun promoter.<sup>84,85</sup>

TPA, an activator of PKC, stimulates CRH mRNA levels and peptide secretion by 1.5- to 2-fold in primary cultures of rat hypothalamic cells.<sup>77</sup> TPA also increases CRH mRNA levels by almost 16-fold and CRH mRNA poly-A tail length by about 100 nucleotides in the human hepatoma cell line NPLC.<sup>63</sup> The proximal 0.9 kb 5' flanking the hCRH gene confers TPA inducibility to a CAT reporter in transient expression assays.<sup>86</sup> In the absence of a clearly discernible perfect TRE in this region, it has been suggested

that the CRE of the CRH promoter may, under certain conditions, elicit TRE-like responses thus conferring TPA responsivity to the CRE site.<sup>86</sup> Further upstream into the 5' flanking region of the hCRH gene, eight perfect consensus AP-1 binding sites have been detected.<sup>65</sup> Their ability to mediate TPA directed enhancement of hCRH gene expression has not been tested by conventional reporter gene assays as yet. EGF, however, has been shown to stimulate ACTH secretion in the primate and to directly stimulate CRH secretion by rat hypothalamic organ cultures *in vitro*.<sup>87</sup>

*GRE (glucocorticoid response element).* Glucocorticoids are the final effectors of the hypothalamic-pituitary-adrenal (HPA) axis and participate in the control of whole-body homeostasis and the organism's adaptive response to stress.<sup>10,11</sup> These hormones exert their effects through their cytoplasmic receptors. When in the ligand-unbound/inactive state, these receptors are in the form of a hetero-oligomer with a hsp90 dimer and other proteins.<sup>88</sup> The ligand-bound receptors dissociate from the hetero-oligomer, homodimerize and translocate into the nucleus, where they interact with DNA to transactivate appropriate hormone-responsive genes that contain the consensus sequence 5'-GGTA-

CANNNTGTTCT-3', GRE, in their 5' flanking regions.<sup>89,90</sup> The activated receptors also interact at the protein level with the c-jun component of the AP-1 transcription factor, preventing this factor from exerting its effects on TRE/AP1-responsive genes.<sup>91,92</sup> Consistent with the structural features of its promoter, the glucocorticoid receptor (GR) gene is constitutively expressed in all or most tissues of the organism.<sup>93-96</sup> Cellular hsp90 content appears to be an important determinant of a cell's sensitivity to glucocorticoids and the interaction of hsp90 with the unliganded form of GR appears to be a modulator of the glucocorticoid signal transduction cascade.<sup>97-102</sup>

Glucocorticoids play a key regulatory role in the biosynthesis and release of CRH.<sup>11,103</sup> Glucocorticoids down-regulate rat and ovine hypothalamic CRH peptide and mRNA levels.<sup>61,104</sup> Stably introduced hCRH gene in AtT-20 cells is also subject to negative glucocorticoid regulation.<sup>105</sup> Adrenalectomy and dexamethasone administration in the rat elicits differential CRH mRNA responses in the PVN and the cerebral cortex, stimulating and suppressing it respectively in the former, but not influencing it in the latter.<sup>106</sup> Glucocorticoids can also stimulate hCRH gene expression in other tissues such as the human placenta<sup>107</sup> and the central nucleus of the amygdala.<sup>108</sup> A construct containing the proximal 900 bp of the 5' flanking region of the hCRH gene coupled to a CAT reporter has been transiently expressed in COS cells and found to confer negative and positive glucocorticoid effects, depending on the cotransfection of a GRcDNA expression plasmid.<sup>86</sup> The molecular mechanism by which glucocorticoids regulate hCRH gene expression is somewhat obscure. Glucocorticoid suppression of hCRH gene expression might be mediated by the inhibitory interaction of the activated GR with the c-jun component of the AP-1 complex. On the other hand, glucocorticoid enhancement of hCRH gene expression might be mediated by the potentially active half perfect GREs present in the 5' flanking region of the gene.<sup>86</sup> Half GREs have been shown to confer delayed secondary glucocorticoid responses to another gene.<sup>109,110</sup> Direct experimental demonstration would be required to substantiate the functionality of the 1/2 GREs present in the 5' flanking region of the hCRH gene.

*ERE (estrogen response element):* Gonadal steroids are the final effectors of the hypothalamic-pituitary-gonadal (HPG) axis. They control reproduction and sexual dimorphic physiology and behavior.<sup>111</sup> Androgens and estrogens (E<sub>2</sub>) act by specific receptor-mediated processes to modulate the expression of genes with GRE or ERE (5'

GGTCANNNTGACC-3') consensus sequences in their 5' flanking regions, respectively.<sup>90</sup> The two ERE binding domains are exact palindromes, and half palindromic EREs can also mediate E<sub>2</sub> enhancement of gene expression.<sup>112</sup> Unlike the GR, androgen and estrogen receptors (ERs) are not constitutively expressed and have a narrower tissue distribution.<sup>111,113,114</sup> ER, although not studied as well as GR, appears to have similar hsp90-dependent activation characteristics.<sup>100,115</sup> It interacts with the c-jun and c-fos components of the AP1 binding proteins and thus also regulates gene expression from TRE sites in a negative or positive fashion.<sup>116,117</sup> It is not known whether 1/2 GREs may in some cases confer weak androgen receptor-mediated transactivation by analogy with the weak and delayed GR transactivation by glucocorticoids via the 1/2 GRE enhancer.<sup>109,110</sup> Some regions of the male brain possess aromatase activity to convert androgens to estrogens, which may then modulate CNS functions through the more commonly expressed ERs in brain areas of both sexes.<sup>111</sup>

Human female hypothalami have higher concentrations of CRH than the male ones.<sup>118</sup> Chronic estradiol treatment of ovariectomized rats stimulates PVN CRH peptide levels<sup>119</sup> and increases ACTH and corticosterone secretion basally and in response to stress.<sup>120</sup> In addition, rat PVN CRH mRNA levels increase in the afternoon of proestrous, at the approximate time of the E<sub>2</sub>-induced preovulatory surge of LH.<sup>121</sup> These findings indicate that gonadal steroids may have an effect on the CRH-secreting neuron and suggest bidirectional interactions between the HPA and HPG axes through their final effectors. A direct E<sub>2</sub> enhancement of the CAT reporter was found by using two overlapping hCRH 5' flanking region-driven CAT constructs in transient expression assays. Furthermore, the two perfect half palindromic EREs present in the common area of both CRH constructs, bound specifically to a synthetic peptide spanning the DNA binding domain of the human ER.<sup>122</sup> These findings demonstrate that hCRH gene expression is under direct E<sub>2</sub> regulation.

*TATA:* The TATA box is a highly conserved structural feature, present -20 to -30 from the transcription start site of many genes. This element appears to position the transcription start site by eukaryotic polymerase II, and participates in transcriptional control of gene expression.<sup>123</sup> Most genes that do not have a TATA box in their promoter region are constitutively expressed and have multiple transcription initiation sites.<sup>95</sup> The hCRH gene has two proximal TATA boxes located at -30 and -195 in its 5' flanking region

(Fig. 2). The promoter-like properties of the more distal TATA box were studied *in vitro* after deletion of the more proximal TATA box.<sup>86</sup> These studies demonstrated that the -195 TATA box was active in initiating transcription and was responsive to cAMP, TPA and glucocorticoids.

The majority of hCRH transcripts in most tissues and cell lines studied initiate at +1. However, transcripts starting at -163 and -130 have also been detected in some tissues and cell lines, suggesting that the -195 TATA box is transcriptionally active *in vivo* in a variety of sites of CRH expression, contributing up to 30–40% of the total pool of cytoplasmic hCRH mRNA.<sup>80,124</sup> Additional upstream, potentially active start sites are present in this gene, and the ratio of transcripts initiating at such sites might also be tissue-specific.<sup>60</sup> Regarding the physiological significance of the longer transcripts, it was proposed that these might have a higher degree of secondary structure and might be more stable and long-lived than the short ones.<sup>86</sup>

TATA boxes have also been implicated in transcriptional regulation by the p53 growth suppressor gene. More specifically, p53 appears to control cellular activity by suppressing the transcription of genes with a TATA box in their promoter region through direct interaction.<sup>125</sup> The region -1.7 to -3.3 kb flanking the hCRH gene has a total of eight additional TATA boxes, which might also interact with p53 and influence CRH gene expression.<sup>65</sup>

*Other elements:* Tissue-specific and other elements, potentially unique to the CRH gene, may be involved in the control of its expression. Their identification will require detailed analysis by both conventional and transgenic functional assays, and their characterization will provide a better understanding of the control of the HPA axis.

### Tissue-specific and stress-related expression of the hCRH gene

As discussed above, the CRH gene is widely expressed throughout the body, suggesting that its product may have autocrine or paracrine actions. Strong evidence for the presence of tissue-specific enhancers in the human and primate CRH gene is its expression in the placenta and decidua.<sup>126</sup> Such expression is absent in the placenta of rodents,<sup>127</sup> suggesting that these enhancers may be carried in a segment of the regulatory region of the gene potentially subject to rearrangement in the non-expressing species.<sup>65</sup> Alternatively, the presence of tissue-specific repressor sequences in rodents may

account for the observed differences in placental expression of the CRH gene between these species and primates.

Differential distribution of short and long hCRH mRNA transcripts has been detected in several tissues and under varying physiological conditions.<sup>80,124</sup> Tissue-specific and/or stress-dependent differential utilization of the two hCRH promoters, may explain these observations. Differential mRNA stability would then be a particularly important feature in CRH homeostasis, primarily in conditions of chronic stress, since in the latter case sustained production of CRH would be required and the long stable mRNAs produced by activation of the distal promoter would be beneficial to the organism.<sup>86</sup>

### Harmony in antithesis. Hormonal regulation of the hCRH gene

Apparently, depending on its site of expression, the hCRH gene responds antithetically to glucocorticoids.<sup>65,77,107,108</sup> The antithetical glucocorticoid effects on a hCRH promoter-drive CAT construct may be explained by the stimulation of the half GREs by high levels of ligand-bound GR, which presumably override the blockade of transcription exerted by lower levels of the activated GR through interaction with cjun-cfos, as discussed above. Is this mechanism physiologically relevant, particularly in view of the constitutive expression of the GR gene? Potentially yes, especially if one takes into account the heat shock proteins (hsp).

Although the GR content of various tissues is similar,<sup>101,113</sup> their sensitivity to glucocorticoids may vary substantially,<sup>125</sup> suggesting that some other cellular factor(s) is the principal modulator of glucocorticoid effect. There is a high tissue-specific fluctuation of hsp90 supporting a correlation between tissue hsp90 content and the sensitivity of this tissue to glucocorticoids.<sup>101</sup> For instance, tissues with high hsp90 content appear to be quite sensitive to glucocorticoids in contrast with tissues with a low hsp90 content,<sup>101,128</sup> and as hsp90 content increases one would expect a parallel increase of the effective concentration of tissue GR.<sup>97,101</sup> The relatively small fluctuation of hsp90 levels during immobilization stress,<sup>102</sup> on the other hand, suggests that the other proteins of the unbound GR hetero-oligomer<sup>99</sup> might also participate in the control of the sensitivity of tissues to glucocorticoids during stress.<sup>101</sup> Very little is known about the tissue-specific expression and the regulation of these proteins during stress, inflammation or debilitating disease. A proposed model summarizing these observations is shown in Fig. 3. This model

## Glucocorticoids Stimulate

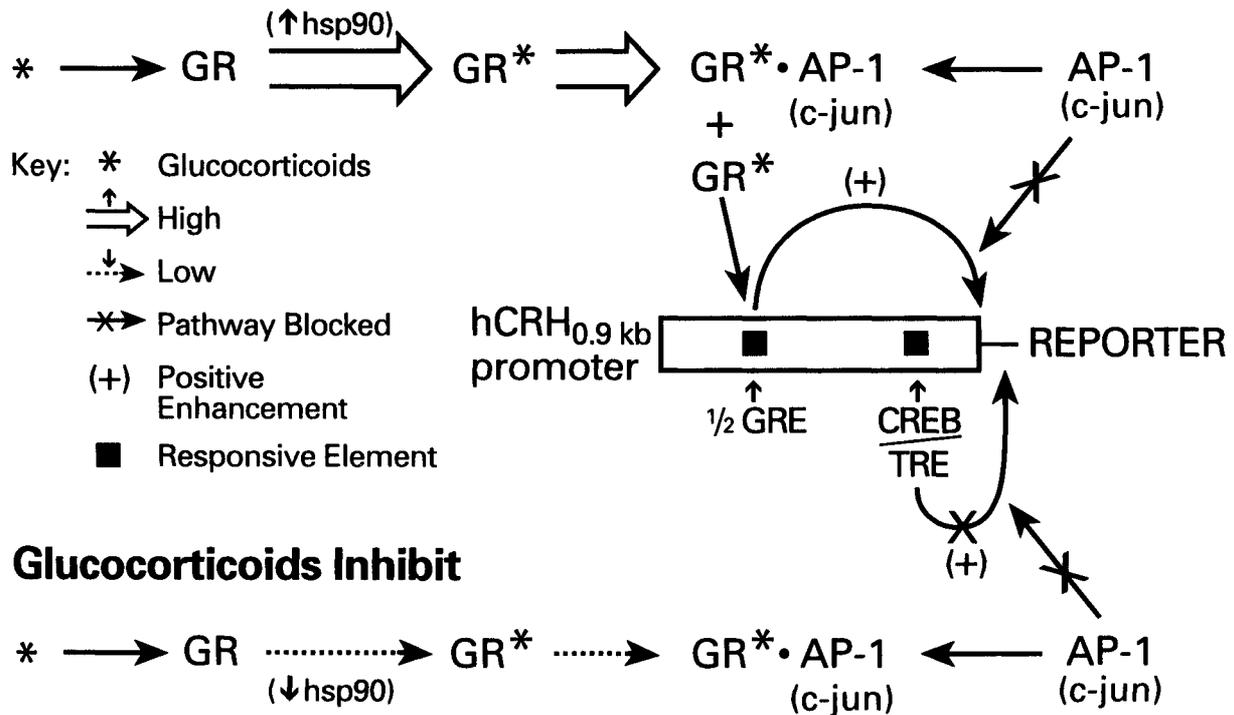


FIG. 3. A proposed model explaining the mechanism of tissue-specific antithetical effects of glucocorticoids on hCRH gene expression. In this model it is assumed that basal expression of the hCRH gene is under constant AP-1 enhancement. Elevation of ambient glucocorticoid levels may elicit activation of GR to different degrees, depending on the tissue hsp90 content.<sup>101</sup> Stimulation of hCRH gene expression would be predicted in tissues with high hsp90 content, such as testis, thymus or pineal, mediated by excess activation of GR that bypasses the AP-1 block and enhances transcription through the 1/2 GRE element. Inhibition would be expected in tissues with a low hsp90 content such as liver, spleen or pituitary, mediated by suppression of AP-1 enhancement through inactive complex formation with activated GR. Immunophilins, hsp70 and other factors interacting with the GR/hsp90 complex<sup>99</sup> may also modulate the activation of GR.

introduces a hypothetical general mechanism to account for the differential sensitivity and direction of effects of various tissues to glucocorticoids. This mechanism involves genes that are regulated by both the growth-promoting AP1 factors and by the differentiation-promoting, anti-growth glucocorticoid hormones. Since the hCRH gene contains both types of enhancers in its promoter region, it may potentially respond as outlined in Fig. 3. The mechanism proposed in Fig. 3 may also explain the frequently observed antithetical effects of chronic glucocorticoid administration in clinical practice.

### Potential implications of CRH gene regulation for the sexual dimorphism of the stress response and the immune/inflammatory reaction

Both the stress response and the immune/inflammatory reaction are associated with sexual dimorphism, both being more robust in the female or castrated male than in the intact male.

The basis of this dimorphism may be gonadal steroid regulation of the components of the stress response. The demonstration of direct E<sub>2</sub> effects on hCRH gene expression,<sup>122</sup> implicates the CRH gene and, therefore, the HPA axis, as a potentially important target of ovarian steroids and a potential mediator of gender-related differences in the stress response and HPA axis activity. These effects of E<sub>2</sub> on the CRH neuron suggest that the HPG axis, which is known to be inhibited by hormones of the HPA axis at the hypothalamic, pituitary, gonadal and sex steroid target tissue levels during stress,<sup>12,129</sup> also appears to influence the latter in a positive fashion, by slightly enhancing CRH gene transcription. Thus, these data support a mutual, bidirectional interaction between the HPG and HPA axes, as depicted heuristically in Fig. 4.

The slightly enhanced CRH neuron activation by estrogen may not only explain why normal women have a slightly higher ACTH response to oCRH than normal men, as well as a slightly decreased ability of the glucocorticoid negative

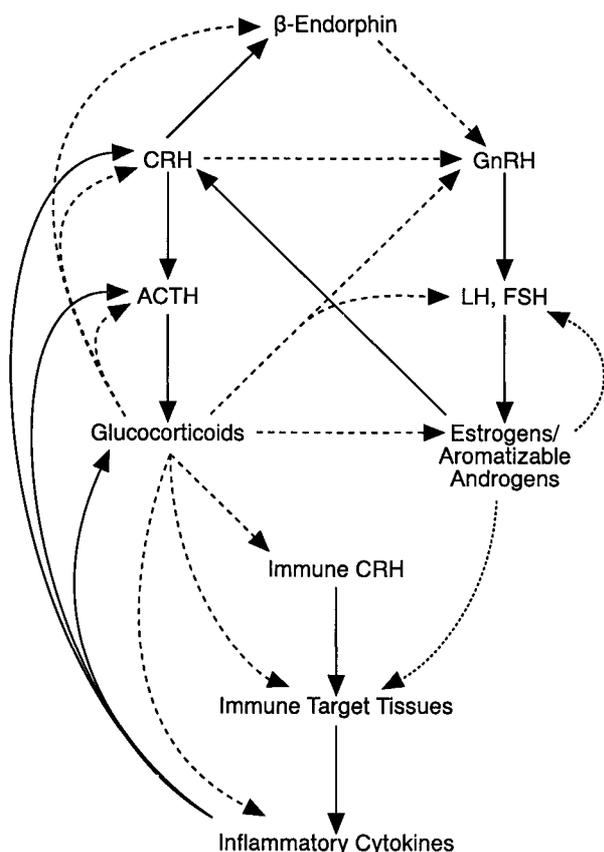


FIG. 4. A hypothetical model of the interactions between the HPG and HPA axes and the immune/inflammatory response. Solid lines indicate stimulation, broken lines inhibition, and the dotted lines conditional inhibition or stimulation. This model suggests that the interactions between these axes are not unidirectional, with the HPA axis inhibiting the HPG axis at multiple levels, as reviewed,<sup>12</sup> but bidirectional, with estrogen potentially stimulating both the CRH neuron, and therefore, the HPA axis, the peripheral production of immune CRH, and, hence, the immune/inflammatory response.<sup>122</sup> Immune CRH has been shown to exert proinflammatory effects *in vitro* and *in vivo*, including enhanced production of cytokines and other mediators of inflammation, which in turn stimulate hypothalamic production of CRH, pituitary production of ACTH and adrenal production of glucocorticoids. Although not included in the figure, gonadal steroid/cytokine interactions have been demonstrated for IL-6 actions.<sup>160</sup>

feedback to shut off the ACTH and cortisol responses,<sup>129</sup> but may also provide an explanation as to why various emotional disorders characterized by elevated CRH secretion,<sup>12</sup> such as depression and anxiety, have a higher incidence in women than in men.<sup>130</sup> Also, the same findings may explain why puberty/adolescence, the postpartum and the perimenopausal period, during all of which marked changes in estrogen production take place, are characterized by increased incidence of emotional disorders.

In addition to explaining the slightly increased, basal and stress stimulated HPA axis function in the female gender, the E<sub>2</sub>-induced enhancement

of the CRH neuron may also help explain the paradox of the negative E<sub>2</sub> feedback effect on the GnRH neuron,<sup>131</sup> which, unlike the PVN, lacks ERs.<sup>132</sup> The negative E<sub>2</sub> feedback might, thus, be exerted indirectly, via a subgroup of CRH neurons. CRH has been reported to suppress GnRH secretion through both a direct and an indirect, arcuate nucleus POMC/ $\beta$ -endorphin-mediated path.<sup>12,133</sup>

The immune/inflammatory reaction is greater in female than in male animals and humans, and in keeping with this, autoimmune inflammatory disease has a significantly higher prevalence in the female than the male sex of several species.<sup>134-136</sup> Estrogens, generally, have been shown to activate some components of the immune/inflammatory reaction, while androgens suppress it.<sup>136-138</sup> Markedly elevated secretion of immune CRH in various inflammatory sites has been demonstrated in the Lewis rat,<sup>44,47</sup> an animal model of increased susceptibility to autoimmune inflammatory disease, in which decreased hypothalamic CRH secretion and, hence, diminished glucocorticoid production and defective suppression of inflammation has been demonstrated.<sup>139,140</sup> Although the decreased production of CNS CRH and increased secretion of immune CRH are associated with the high susceptibility of this animal to autoimmune inflammatory disease in both sexes, both the susceptibility and the actual inflammatory responses, including expression of immune CRH in peripheral inflammatory sites, are greater in the female than the male.<sup>137,138</sup> E<sub>2</sub>-mediated enhancement of immune CRH secretion might be a partial explanation for this sexual dimorphism in the Lewis rat, as well as, albeit to a lesser extent, in other rat strains or animal species.<sup>141</sup>

Inflammatory sites, such as the arthritic joints of patients with rheumatoid arthritis, contain high levels of immunoreactive CRH in the synovial fluid and inside cultured synoviocytes.<sup>48</sup> Interestingly, patients with rheumatoid arthritis have been shown to have poor or deficient responses of their HPA axes to the stress of major surgery, when compared with patients with osteoarthritis having similar surgery.<sup>142</sup> Rheumatoid arthritis patients also have inappropriately normal or low normal basal diurnal concentrations of plasma cortisol<sup>142,143</sup> making them strictly analogous to the Lewis rat model of autoimmune/inflammatory disease.<sup>139,140</sup>

The above studies suggest that homeostatic regulation involves complex mutual interactions between the reproductive axis, HPA axis and the immune system, in which E<sub>2</sub> and CRH may play central roles (Fig. 4). Certainly, other molecules involved in the regulation of these axes,

such as several neurotransmitters, cytokines and lipid mediators, also participate in the above interactions and may contribute to their sexual dimorphism.<sup>144</sup> Thus, a neurotransmitter, serotonin, has been shown to stimulate both CRH and ACTH secretion.<sup>145,146</sup> The inflammatory cytokines tumour necrosis factor- $\alpha$ ,<sup>147,148</sup> interleukin-1<sup>148-150</sup> and interleukin-6,<sup>148,149,151</sup> have been shown to activate acutely hypothalamic CRH secretion and, more chronically, pituitary ACTH<sup>147,152,153</sup> and, perhaps, glucocorticoid secretion,<sup>151,152</sup> whereas several lipid mediators of inflammation cause CRH, ACTH and glucocorticoid secretion.<sup>150,154-157</sup> These findings explain the original pioneering studies, in which CRH-, ACTH- and glucocorticoid-releasing bioactivities were found in the serum or supernatants of stimulated immunocytes.<sup>155,158,159</sup> Thus, immune CRH, by participating in the regulation of the immune response at the level of the leukocyte, may be also viewed as a peripheral coordinator of immune-neuroendocrine interactions.

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