European Journal of Endocrinology (2006) **155** S71–S76 ISSN 0804-4643

Corticotropin-releasing hormone physiology

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Abstract

Corticotropin-releasing hormone (CRH), also known as corticotropin-releasing factor, is a highly conserved peptide hormone comprising 41 amino acid residues. Its name derives from its role in the anterior pituitary, where it mediates the release of corticotropin (ACTH) leading to the release of adrenocortical steroids. CRH is the major hypothalamic activator of the hypothalamic–pituitary–adrenal (HPA) axis. Major functions of the HPA include: (i) influencing fetal development of major organ systems including lung, liver, and gut, (ii) metabolic functions, including the maintenance of normal blood glucose levels during the fasting state via glycogenolysis and gluconeogenesis, (iii) modulation of immune function, and (iv) maintenance of cardiovascular tone. In addition, CRH, acting both directly and via the HPA, has a role in regulating several neuroendocrine functions including behavior, food intake, reproduction, growth, immune function, and autonomic function. CRH has been localized to the paraventricular nucleus (PVN) of the hypothalamus, which projects to the median eminence and other hypothalamic and midbrain targets. The *CRH* gene is composed of two exons. The *CRH* promoter contains a cAMP-response element, and the intron contains a restrictive element-1/neuron restrictive silencing element (RE-1/NRSE) sequence. Recently, a family of CRH-related peptides, termed the urocortins, has been identified. These peptides probably play a role in integrating multiple aspects of the stress-response, although their functions are largely unknown. Both CRH and the urocortins interact with two transmembrane G-protein-coupled cell surface receptors, CRH-R1, and CRH-R2, which differ in their patterns of tissue distribution. In addition, the binding affinities for CRH and the urocortins to the two receptors differ considerably, and may contribute to the different actions of these peptides.

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Introduction

The mammalian response to stress exists to maximize an individual's chances of surviving a life-threatening experience, to allow the organism the chance to reproduce and thereby increase its contribution to the gene pool at a later time. The stress-response is classically divided into three categories: behavioral, autonomic, and hormonal responses. Behavioral responses govern the conscious response to a stressor, and include fear, anxiety, heightened vigilance, clarity of thought, anorexia, and decreased libido. Autonomic responses, mediated by the sympathetic nervous system, mobilize certain organ systems to carry out fight or flight activities. These include increased heart rate, cardiac output, breathing rate, and bronchodilation, increased blood flow to brain and muscle, decreased blood flow to skin and gut, activation of innate immunity, maintenance of blood glucose, and increased free fatty acid generation. Hormonal responses largely provide fuel for these activities (during a time of induced anorexia) and include, maintenance of blood glucose, increased free fatty acid generation, maintenance of blood pressure, and restraint of the immune system.

Corticotropin-releasing hormone (CRH), a 41 amino acid neuropeptide, discovered in 1981 by Wiley Vale (1) is likely involved in all three types of stress-response. Behavioral responses may involve CRH present in the cerebral cortex and amygdala. Autonomic responses are controlled in part by brainstem fibers descending from the locus coeruleus, which receives CRH-containing fibers from the amygdala (2) and paraventricular nucleus (3). Hormonal responses center on activation of the hypothalamic–pituitary–adrenal (HPA) axis, which is initiated by CRH present in the paraventricular nucleus of the hypothalamus (PVH).

This paper was presented at the 4th Ferring Pharmaceuticals International Paediatric Endocrinology Symposium, Paris (2006). Ferring Pharmaceuticals has supported the publication of these proceedings.

Anatomic distribution

CRH is present in the nerve cell bodies in and near the dorsomedial parvicellular division of the

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DOI: 10.1530/eje.1.02247
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paraventricular nucleus (PVN) of the hypothalamus. These neurons send axons to the median eminence and other hypothalamic and midbrain targets. Although the exact inputs are not known, these neurons are largely controlled by serotoninergic input from the amygdala and hippocampus of the limbic system, and brainstem regions, involved in autonomic functions. *CRH* expression has also been localized in many other parts of the CNS including the cerebral cortex, limbic system, cerebellum, locus coeruleus of the brain stem, and dorsal root neurons of the spinal cord. It is also present in the adrenal medulla and sympathetic ganglia of the autonomic nervous system, as well as in the fetal lung, the T-lymphocytes of the immune system, and the gastrointestinal tract.

Gene and protein structure

The *CRH* gene is composed of two exons and an 800 bp intron, and is highly conserved among vertebrate species. The entire protein-coding region is contained in the second exon. Human CRH is processed from a preproCRH molecule, 196 amino acids in length, which contains a hydrophobic signal sequence, required for secretion, at its amino-terminal end. The middle of the molecule contains 124 amino acids of unknown function. The carboxyl-terminal end of preproCRH contains 41 amino acid sequence of the mature peptide hormone, separated from the amino-terminus by a protease-sensitive basic residue. Amidation of the carboxyl terminus of CRH is required for biological activity. Human, rat, and mouse CRH have amino acid sequence identity and ovine CRH differs by seven amino acid residues.

The promoter region of the *CRH* gene contains several putative *cis*-regulatory elements including, a cAMP-response element (CRE), AP-1 sequences, and glucocorticoid-response elements. The intron contains a restrictive element-1/neuron restrictive silencing element (RE-1/NRSE) sequence, which binds restrictive element silencing transcription factor/neuron restrictive silencing factor (REST/NRSF), and functions to restrict CRH expression to neuronal cells (4).

Regulation of gene expression

The stimulation and inhibition of *CRH* expression by cAMP-dependent protein kinase A and glucocorticoids respectively are mediated by changes in *CRH* gene transcription. Stimulation occurs following the cAMP-induced phosphorylation of cAMP-regulatory element-binding (CREB) protein, which binds to the CRE located in the CRH promoter and recruits the transcriptional activator, CREB-binding protein to the transcriptional apparatus (5). Experimental evidence suggests that glucocorticoid-mediated repression of CRH transcription results from a direct interaction between the activated cAMP-dependent transcription factor CREB and the activated glucocorticoid receptor (6). Transcription factor-mediated changes in chromatin structure, likely working through tissue-specific regulatory elements in the *CRH* gene, contribute to tissue-specific regulation. The brain serves as an example of tissue-specific glucocorticoid responsiveness. Glucocorticoids have a negative regulatory effect on *CRH* gene transcription in the PVN where negative feedback regulation on the HPA axis occurs (7). However, in the amygdala – a brain region involved in the behavioral stress-response – glucocorticoids increase *CRH* expression (8).

The RE-1/NRSE present within the *CRH* intron binds REST/NRSF, which inhibits transcription of the *CRH* gene by recruitment of histone deacetylase 4. *In vitro* studies suggest that, by this mechanism, *CRH* is restricted to expression within, and excluded from expression outside, the nervous system.

Regulation of CRH secretion

A number of neurotransmitters have been implicated in both the positive and negative regulation of CRH release. Acetylcholine, norepinephrine, histamine, and serotonin increase hypothalamic CRH release, while gamma-aminobutyric acid inhibits it. Additional factors that have been implicated in the regulation of CRH release include, angiotensin, vasopressin, neuropeptide-Y, substance P, atrial natriuretic peptide, activin, melanin-concentrating hormone, β-endorphin, and possibly CRH itself. In addition, cytokines such as interleukin-1β, tumor necrosis factor, eicosanoids, and platelet-activating factor have all been shown to activate the HPA axis by increasing hypothalamic CRH expression (see below).

With adrenalectomy, two groups noted that CRH-containing cells of the PVH, which project to the anterior pituitary via the median eminence, acquired expression of arginine vasopressin (AVP) (9, 10). This suggested that CRH and AVP might work together to stimulate the secretion of pituitary ACTH, which was subsequently shown to be correct (11).

Orthologs and paralogs

Invertebrates possess two CRH paralogs, termed diuretic hormones, which are structural orthologs of CRH (12). In mammals, the CRH family consists of four paralogous peptides, CRH, and urocortins I, II, and III, located on separate chromosomes. Fishes and amphibians possess CRH and a second paralog, urotensin I or sauvagine respectively. The invertebrate peptides appear to function in diuresis and feeding, and clearly evolved long before the Precambrian explosion. The vertebrate functions of CRH in the regulation of the HPA axis are conserved across the chordate lineage. The functions

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of urotensin I, sauvagine, and the urocortins are not yet resolved, so it is unclear whether their actions have been conserved across evolution (12).

When CRH was initially identified, it was felt to be the ortholog of fish urotensin and amphibian sauvagine. The later identification of factors more closely related to CRH in these species prompted the search for the true mammalian ortholog of urotensin. These efforts led to the discovery of urocortin I, a 122 amino acid propeptide, which undergoes a cleavage event to form the mature 40 amino acid peptide (13). Urocortin was first cloned in rats and shares 45% sequence identity with rat CRH. Rat and human urocortin are 95% identical within the mature peptide region.

The primary site of urocortin I expression is the Edinger–Westphal nucleus, although it is found in scatter sites throughout the brain, including the hypothalamus and pituitary. In addition, it is found in human placenta and fetal membranes. Like CRH, it binds to CRH-BP with high affinity, suggesting that the binding protein may modulate its function. Urocortin binds to all CRH receptors with higher affinity than does CRH, and to CRH-R2β-receptor with 40-fold higher affinity. Urocortin serves as a potent ACTH secretagog (although due to its restricted anatomic distribution is unlikely to be an important regulator of ACTH release) and appears to mediate some stress-induced behaviors, including increased anxiety and possibly anorexia. It may also be involved in sodium and water balance. The identification of urocortin I established that CRH was not the sole mediator of the stress-response. It further raised the possibility that urocortin may mediate some of the behaviors previously attributed to CRH. The search for additional CRH-related peptides that might be involved in regulating the stress-response has lead to the identification of two additional factors.

Based on the above observations, the existence of additional CRH-like molecules was proposed and subsequently identified based on sequence homology to CRH, urocortin I, and CRH-like factors identified in lower species. Searching the public DNA database, urocortin II (also termed stresscopin-related peptide) was recently identified based on its similarity to consensus amino acid sequences of known CRH-related peptides (14, 15). It encodes a 38 amino acid peptide that is a selective CRH-R2 agonist with no affinity for CRH-R1. It is expressed in the hypothalamic magnocellular neurons of the paraventricular and supraoptic nuclei. Based on experimental data, it may be involved in appetite suppression, without a role in more generalized behavioral responses.

Using a two part search strategy of the human DNA database, a third CRH-like peptide was identified and named stresscopin (also termed urocortin III) (15–17). Human urocortin III encodes a 161 amino acid prepropeptide with a mature peptide product of 40 amino acids. Receptor studies show selective CRH-R2 binding. Behavioral studies in mice demonstrate that urocortin III, as with urocortin II, results in suppression of appetite, decreased gastric emptying, and decreased heat-induced edema. Consistent with the lack of binding to CRH-R1, urocortin II and III have essentially no role in mediating ACTH release and elevation of glucocorticoid levels. A broad tissue survey demonstrates urocortin III expression in brain, gastrointestinal tract, adrenal, pancreas, thyroid, skeletal and cardiac muscles, and spleen.

Receptors

CRH binds to sites in the anterior lobe of the human pituitary that correlate with the distribution of corticotrophs. CRH receptors in the anterior pituitary gland are low capacity, high affinity receptors, with a K_d for CRH binding of about 1 nM. To date, two CRH receptor genes have been identified in humans and other mammals (18, 19), with a third gene described in the catfish (20). Both receptors consist of seven transmembrane regions coupled to adenylate cyclase via G_s. Both receptors have at least one additional isoform resulting from an alternate splicing event.

The tissue distribution of the type 1 and 2 receptors varies considerably (21, 22). CRH-R1 is expressed throughout the brain, being concentrated on anterior pituitary corticotrophs. The distribution of the CRH-R2 splice variants, in contrast, is quite distinct. CRH-R2α is expressed exclusively in the brain, being more widely distributed than CRH-R1. CRH-R2β is expressed primarily in the periphery, with the highest levels found in heart and skeletal muscle. The distinct localization of these receptor types suggests they may have functionally different roles.

CRH-R1 binds and is activated by both CRH and urocortin I (see below). This receptor mediates the actions of CRH at the corticotroph as well as some aspects of the behavior stress-response, including fear and anxiety. CRH-R2 binds urocortin with over 20-fold higher affinity, compared with CRH (see below). Experimental evidence suggests that this receptor may be involved in vasodilation and blood pressure control, consistent with its anatomic localization (22).

Mice deficient in CRH-R1 and CRH-R2 have provided important clues to help define their functional roles with CRH-R1 mediating and CRH-R2 attenuating behavioral stress-responses (23–25). Studies employing specific agonists and antagonists further suggest that CRH-R2 likely mediates pro-anxiety as well as anxiolytic behaviors in a brain region-specific manner. CRH-R2 has been shown to reduce feeding behavior and gastrointestinal motility (26).

CRH, acting via the type 1 CRH receptor, stimulates adenylate cyclase activity increasing cAMP levels in anterior pituitary corticotrophs. CRH stimulates ACTH release via the cAMP-protein kinase A pathway, which is

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responsible for both the increase in pro-opiomelanocortin (POMC) transcription and peptide synthesis, as well as for the rise in intracellular calcium, results in ACTH secretion. Forskolin, a direct stimulator of adenylate cyclase activity, and 8-bromo-cAMP, a cAMP analog, both markedly stimulate ACTH release, and increase CRH-stimulated ACTH release. CRH mediates its stimulation of POMC transcription via the POMC CRH responsive element.

Role in physiology

Much has been learned about the physiology of CRH by administering it and its antagonists to animals and humans, by measuring the concentration of CRH mRNA and peptide in various tissues, and genetically removing it from mice using gene-targeting methods. CRH-deficient mice have life-long deficiency, and therefore do not accurately model the consequences of acute CRH deficiency (27). Nevertheless, these mice have been useful to indicate the essential role of CRH and glucocorticoids for proper fetal lung development (28). CRH is also required for a robust adrenal response to a variety of stressors (29) including fasting (30).

Endocrine system

CRH is the key factor in the HPA axis leading to the release of ACTH, which acts on the adrenal cortex to release glucocorticoids and other steroid hormones including androgens and to a lesser extent aldosterone. CRH can also directly stimulate glucocorticoid release from the adrenal gland. Vasopressin, co-synthesized with CRH in hypothalamic PVN neurons, acts in concert with CRH to stimulate ACTH secretion, as discussed above.

As with other endocrine systems, this axis is organized and regulated through a series of negative feedback loops. The negative regulation of glucocorticoids on CRH and ACTH release represents long and short feedback loops respectively. The ability of ACTH from the pituitary to inhibit CRH release is another example of a short feedback loop. CRH is required for the stimulation of pituitary ACTH gene expression, which occurs with loss of negative feedback during adrenal insufficiency (31).

Behavioral effects

As an important integrator of the stress-response, CRH and CRH-related peptides (see below) have been implicated as mediators of behavioral responses to stress. CRH, as well as urocortin III, are present within the amygdala, an area of the brain, which mediates behaviors associated with fear and anxiety, such as decreased exploration and appetite. Consistent with this idea, CRH-R1-deficient mice exhibit decreased anxiety-related behaviors, and CRH-R2-deficient mice have increased anxiety-related behaviors (22). However, CRH-deficient mice have normal behavioral stress-responses, which are blocked by a CRH-R1-specific antagonist, suggesting that another CRH-related peptide, acting through the CRH-R1 receptor, is anxiogenic (32).

The circadian rhythm is generated in the suprachiasmatic nucleus. Signals are sent via efferent inputs to the PVN, which modulates CRH release. In response, ACTH is secreted in a pulsatile fashion, which gives rise to a corresponding rise in glucocorticoid levels. In humans, peak hormonal levels are seen in the early morning hours. In rodents and other nocturnal animals, the peak occurs in the evening. CRH is required for the presence of this rhythm, as CRH-deficient mice have a very low or absent rhythm in cortisol secretion. However, the rhythm is restored by infusion of constant amounts of CRH into these animals, indicating that variation in CRH is not required for rhythm generation (33).

Immune system

There is increasing evidence that the neuroendocrine system and the immune system work together in the regulation of both the stress and the immune response. CRH is thought to have both direct and indirect effects on the immune system. Indirectly, CRH may down-regulate inflammation through the release of glucocorticoid, whereas a direct pro-inflammatory action of CRH may occur in peripheral tissues. These direct actions are not completely understood but may include: mast cell degranulation, nitric oxide-dependent vasodilatation, enhanced vascular permeability, leukocyte proliferation, and cytokine release by activated leukocytes. Further support for a direct effect comes from experimental animal systems where s.c. inflammation gives rise to immunoreactive CRH within leukocytes, monocytes, macrophages, fibroblasts, endothelial, epidermal, and synovial lining cells. These studies have been extended to show that CRH antisera decrease the inflammatory response.

The integration of the neuroendocrine and immune response to stress relies on the balancing effects of multiple factors. Several such factors, including CRH and interleukin-1 (IL-1), have been shown to manifest both stimulatory and inhibitory potential. IL-1, produced by stimulated macrophages and monocytes, serves as a potent pro-inflammatory compound. In contrast, IL-1 has also been shown to act centrally, by directly stimulating the HPA axis, to increase secretion of CRH and ACTH leading to increased levels of anti-inflammatory glucocorticoid. These dual actions of IL-1 and CRH to mediate both pro- and anti-inflammatory responses may illustrate a fundamental regulatory mechanism integrating the immune system with the HPA axis.

Gastrointestinal system

The stimulation of colonic motility is a common response to a variety of stressors. This gastrointestinal

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response to stress, as measured by fecal defecation in rodent studies, can be mimicked by CRH infusion into either the brain or periphery and is blocked by CRH-R1 antagonists. Its administration induces bowel emptying by increasing colonic motility and inhibits gastric acid secretion and gastric emptying. Indirectly, as part of the neuroendocrine stress-response, CRH also inhibits feeding behavior even in food-deprived experimental animals. It is possible that one of the CRH-related peptides, such as urocortin I or III, rather than CRH, is the endogenous peptide that mediates these gastrointestinal actions.

Reproductive system

There is considerable evidence that stress has a major negative effect on reproduction. As part of the neuroendocrine response to stress, CRH has been implicated as a major inhibitor of reproductive function in both sexes. Intense or prolonged stress, for example, has been shown to inhibit gonadotropin secretion. Additional central effects include attenuation of sexual behavior. However, CRH-deficient mice have normal stressor-induced inhibition of reproductive function, indicating that CRH is not absolutely required for this function (34).

Placental CRH production is found only among primates, being derived from the synciotrophoblasts (35). It is secreted into both the maternal and fetal circulations. During the second half of gestation, both humans and chimpanzees show an exponential rise in maternal CRH blood levels. The fetal concentration of CRH is approximately one tenth that found in the maternal circulation. The role of placental CRH is not clear, but it may function in the initiation of parturition and/or in the regulation of fetal development (36). Unlike hypothalamic CRH, which is inhibited by cortisol (as part of a negative feedback loop), rising levels of cortisol secreted by the fetal adrenal glands appear to stimulate placental CRH (37). Placental CRH, via the umbilical vein, enters the fetal circulation, and may stimulate fetal ACTH release to cause a further increase in fetal cortisol release, thus completing a positive feedback loop. This may explain in part the marked, 10- to 100-fold rise in maternal CRH levels that occur in the last part of human pregnancy. The rise in fetal cortisol is also required for the proper maturation of lung, liver, bowel, and other tissues. The stimulation of fetal ACTH by placental CRH would also promote the synthesis and secretion of fetal DHEA-sulfate, which is the obligate precursor for estradiol produced by the placenta. Estradiol is necessary for the production of placental oxytocin and prostaglandin, which are the major effectors of uterine contraction during labor and delivery. The concomitant stimulation of both fetal cortisol and DHEA would thus couple the glucocorticoid effects on fetal organ maturation with the timing of parturition, of obvious benefit for postnatal survival (38).

CRH-binding protein

The CRH-binding protein (CRH-BP) was first inferred from studies in pregnant humans in whom a high level of immunoreactive CRH was detected in peripheral blood (39). Levels as high as 10 ng/ml have been documented during pregnancy, which is 10–100 times higher than CRH levels in the hypophysial portal system. The presence of CRH-BP is thought to block the effects of CRH in the pregnant mother, thus preventing or attenuating the activation of her HPA axis. The role of CRH-BP is not completely understood, though its intracellular distribution in corticotrophs and its association with neurons, both synthesizing CRH and regulating its release, suggests it may serve to modulate the effects of CRH. In addition to humans, it is expressed in great apes, which also have high levels of CRH during pregnancy, and in the rat. In humans, it is synthesized in liver, placenta, and brain, while in rats its expression is restricted to the brain.

Summary and conclusions

CRH was aptly named when it was discovered 25 years ago, since its major function has been found to be the major regulator of ACTH release. It has been postulated to have many other functions, from the mediator of stress-related behaviors, a trigger for parturition and an activation of the innate immune system. Although most of these putative functions remain to be conclusively proven, much evidence in many different areas is highly supportive. In addition to CRH, there exist three mammalian paralogs, urocortins I, II, and III. Again, the functions of these three peptides are not known with certainty, but several intriguing hypotheses exist regarding their regulation of various physiological systems. One can only hope that the next 25 years will be as exciting as these past 25 years, and lead to further answers regarding the mammalian stress-response and its regulation.

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Received 10 May 2006
Accepted 7 July 2006

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