Corticotropin releasing hormone and the immune/inflammatory response

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Abstract

Hypothalamic corticotropin-releasing hormone (CRH) acts as the major physiologic ACTH secretagogue. Moreover, CRH is distributed in the brain and spinal cord, adrenal medulla, testes, ovaries, gastrointestinal tract, pancreas, myometrium, endometrium, placenta, and diverse inflammatory sites. Immunoreactive CRH has been found in the cytoplasm of immune accessory cells (macrophages, endothelial cells, and tissue fibroblasts), and in inflammatory sites of both acute and chronic inflammation (synovial lining cell layers and blood vessels from the joints of patients with rheumatoid arthritis and osteoarthritis). Additionally, the local presence of CRH in the uveitic eyes, cytoplasm of inflammatory cells (macrophages, lymphocytes, and polymorphonuclear cells) infiltrating the iris, ciliary body, vitreous, retina, and choroid appears to be of pivotal importance in the process of experimental autoimmune uveoretinitis. Traditionally, hypothalamic CRH has been considered to act indirectly in an anti-inflammatory fashion, since the end product of the hypothalamic–pituitary–adrenal axis is cortisol, a well-known anti-inflammatory compound. However, CRH produced at peripheral inflammatory sites has been shown to participate in an autocrine/paracrine stimulation of inflammation. Thus, CRH may have a peripheral, primarily activating role on the immune system. The mechanisms of the CRH-mediated component of the immune/inflammatory response are still unclear. CRH in inflammatory sites seems to be involved in the activation of the Fas/Fas ligand system. Furthermore, locally produced embryonic and endometrial CRH plays a role in both the aseptic inflammatory process of implantation and the anti-rejection process that protects the fetus from the maternal immune system. There are two types of G-protein-coupled CRH receptors (CRH-R1 and CRH-R2). Pyrrolopyrimidine compounds, such as antalarmin, have been developed as CRH-R1 receptor antagonists. Confirming the peripheral pro-inflammatory actions of CRH, antalarmin has been shown to suppress experimental aseptic inflammation. Thus, antalarmin may represent the first in a new class of anti-inflammatory agents operating through CRH-R1. Studies of CRH genetics have provided new insights on the pathogenesis of rheumatoid arthritis in humans. DNA variation across the CRH gene-containing region has been examined in families with multiple cases of rheumatoid arthritis. Transmission Disequilibrium Test analysis showed significant association at the CRH locus.

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Introduction

The immune/inflammatory (I/I) response is influenced by the brain in a major way. This is achieved via regulation of peripheral nervous system functions and endocrine responses (1). Among other pathways, the brain regulates this response through the hypothalamic–pituitary–adrenal (HPA) axis, which is activated during stress (1). On the other hand, receptors for a number of hormones, neurotransmitters, and neuropeptides are carried by cells of the immune system,

leading to modulation of their responses by changes in neuroendocrine and/or autonomic activity (2). Products of the I/I response, such as eicosanoids and inflammatory cytokines influence brain function. Additionally, immune cells produce a number of hormones and neuropeptides, such as corticotropin-releasing hormone (CRH) and corticotropin (ACTH), which act locally as autacoids during both the early and the late stages of the I/I process (3). This locally produced CRH is subsequently called ‘peripheral CRH’.

Central and peripheral CRH. CRH receptors

CRH is a 41-amino acid peptide that plays a central role in organizing the HPA axis and the systemic response to

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stress (4). It acts as the main physiological ACTH stimulator (5, 6). CRH is synthesized by parvocellular neurons of the paraventricular nucleus of the hypothalamus and is secreted in the hypophyseal portal blood via projecting axons to the median eminence (7). In addition, CRH is distributed in the brain and the spinal cord. The plasma half-life of CRH in humans is 4 min (8). ACTH released by CRH leads to secretion of cortisol (F) and other adrenal steroids, such as DHEA and, transiently, aldosterone (9). There have not been noted any sex or age differences in plasma ACTH or F responses to CRH (10). Moreover, although ACTH response to CRH is not influenced by the hour of the day, the corresponding F response is maximized late in the afternoon (11).

Peripheral CRH has been found in the adrenal medulla, testes, ovaries, cardiovascular system, gastrointestinal tract, pancreas, lung, spinal cord, endometrium, and placenta (11–16), as well as in diverse inflammatory sites (1, 17, 18). Peripheral sensory afferent type C fibers and postganglionic sympathetic nerves also express CRH (19) and have been suggested as one more source of immune CRH. Another peripheral organ where CRH is locally produced is the skin, where CRH receptor type-1 (CRH-R1) isoforms are expressed in keratinocytes (20). Peripheral CRH has the same electrophoretic profile as hypophyseal CRH and the same expression pattern during acute inflammation as the acute phase reactants, substance P and tumor necrosis factor α (TNF- α), including their downregulation by glucocorticoids (GCs) (21). The majority of the plasma CRH is of non-hypothalamic origin, as CRH is rapidly decomposed enzymatically at the pituitary level. However, under certain circumstances, such as insulin-induced hypoglycemia and pregnancy, hypothalamic CRH release leads to increased plasma CRH concentration. Uniquely to humans, CRH in plasma is bound to a high-affinity binding protein (37 kDa; CRH-BP) (22), which plays a major role in limiting the CRH distribution or duration of activity (23).

The biological effects of CRH are mediated by at least two different receptors; CRH-R1 and CRH-R2, which belong to the G-protein-coupled receptor superfamily and typically are positively coupled to adenylate cyclase (24, 25). The CRH-R shares a 70% homology of their amino acid sequence. They are differentially expressed and appear to mediate selective actions of CRH at different tissues. CRH-R1 expression is highest in the cerebral cortex, striatum, amygdala, and cerebellum. CRH-R1s are also present on both epithelial and stromal cells of the human endometrium (26). CRH-R2s are mostly present in subcortical structures, such as the lateral septal nucleus, several nuclei of the hypothalamus, and choroid plexus (27). At the periphery, CRH-R2s are widely expressed in the gastrointestinal tract, lung, skeletal muscle, arteries, and heart muscle. Indeed, peripherally injected CRH augments gastrointestinal motility, inhibits gastric secretion, lowers blood pressure, affects heart output, and decreases

inflammatory reactions. The two receptors are probably involved in some coordinated ways to express the totality of the physiological responses to stress, including behavioral responses (27). The coupling of CRH receptor and G-protein stimulates intracellular cAMP pathway events in a variety of brain-derived and peripheral cell lines. These events include the crosstalk between CRH receptor-initiated signal transduction and activation of nuclear factor- κ B in T cells (28).

The immune/inflammatory response

The I/I response is the reaction to an injury of the vascularized connective tissue, characterized by the accumulation of fluid and leukocytes in extravascular tissues. It is considered to be a protective mechanism aiming to rid the organism of both the initial cause of cell injury (such as microorganisms, toxins, and antigens) and the consequences of such injury (e.g. necrotic cells and tissues). In this process, cellular and extracellular elements participate in a complex cooperative network. The cellular components of the I/I response include leukocytes, such as monocytes–macrophages, polymorphonuclear neutrophils, eosinophils and basophils, platelets, dendritic cells, mast cells, epithelial and endothelial cells, and fibroblasts (innate immune system), as well as lymphocytes T, B and natural killer (NK) (adaptive immune system). These cells cooperate using molecular signals, including cytokines (interleukines (ILs), colony-stimulating factors, interferons, TNFs, transforming growth factor, and chemokines), vasoactive amines (histamine and serotonin), plasma proteases (kinins and complement systems), arachidonic acid metabolites (prostaglandins, leukotrienes, and lipoxins), platelet-activating factor, nitric oxide, and neuropeptides.

The first step in the initiation of the I/I response is the activation of the innate immune system. Subsequently, the adaptive immune system is activated by the innate immune responses. As lymphocytes arrive at the inflammatory area, antigen-presenting cells (APCs), such as plasmacytoid dendritic cells, interstitial and Langerhans dendritic cells, and astrocytes present infectious agent antigens of macrophages to T cells. This is the ignition signal for the activation of the adaptive immunity consisting of cellular (T4, T8, and NK lymphocytes) and humoral (B lymphocytes, plasma cells, and antibodies) immunities. CD4 helper T cells are the regulators of this antigen-specific response. These cells can be subdivided on the basis of cytokines produced in Th1 T cells, which promote primarily the cellular/inflammatory immunity and Th2 cells, which have a primary role in the regulation of humoral immunity. The balance between Th1 and Th2 is important for the homeostasis within the immune system. GCs and catecholamines, the hormones released during stress, have a significant effect on this balance. GCs suppress the

production of IL-12, the main inducer of Th-1 responses (29, 30) and thus, they affect the Th-1/Th-2 balance leading to a Th-2 shift. In contrast to catecholamines, GCs also have a direct effect on Th-2 cells by upregulating their IL-4, IL-10, and IL-13 production (30, 31). On the other hand, the two major catecholamines, nor-epinephrine and epinephrine, potently inhibit the production of IL-12 by APCs thus suppressing the development of Th1 type cells (29, 32, 33).

CRH and the I/I response

Central CRH

The immune response balances between pro- and anti-inflammatory actions (34). Hypothalamic CRH has been considered to act indirectly in an anti-inflammatory fashion, since the final product of the HPA-axis stimulation is F, known for its anti-inflammatory actions. On the other hand, an excessive HPA response (for example, a state of stress or relative hypercortisol-emia), can increase susceptibility to infectious agents and tumorigenesis, but enhances resistance to auto-immune or aseptic inflammatory diseases. In contrast, a defective HPA-axis response (for example, relative GC-deficient state) causes resistance to infections and tumorigenesis, but increased susceptibility to auto-immune or aseptic inflammatory diseases. The effects of either a defective or an excessive HPA response have been ascertained in Fischer and Lewis rats, two highly inbred strains selected for their resistance (Fischer rats) or susceptibility (Lewis rats) to inflammatory disease. In Lewis rats, hypothalamic CRH neurons respond poorly to all neurotransmitters and the overall HPA-axis response to stress is decreased (1). Moreover, CRH-deficiency disrupts endogenous GC production and enhances allergen-induced airway inflammation and lung mechanical dysfunction in CRH knockout mice. Thus, inherited or acquired CRH deficiency could increase asthma severity in human subjects (35). Hypofunction of the HPA axis was also found in patients with Sjogren's syndrome (36) and sarcoidosis (37).

Peripheral CRH

The presence of CRH-specific binding sites in human lymphocytes secreting pro-opiomelanocortin-derived peptides (ACTH and β -endorphin) supports the direct involvement of CRH in the I/I response (38). By employing the rat air pouch model of carrageenin-induced acute aseptic chemical inflammation, immuno-reactive CRH (IrCRH) was detected in the inflamed area but not in the systemic circulation. CRH produced in peripheral inflammatory sites, in contrast to its systemic indirect immunosuppressive effects, acts as an autocrine or a paracrine inflammatory cytokine (21). IrCRH was found in the synovial lining cell layers and blood vessels

from the joints of patients with rheumatoid arthritis and osteoarthritis (39), whereas high CRH levels were found in the synovial fluids of the former patients (40). In addition, IrCRH was found in immune accessory cells from uveitic retinas and corpora vitrea from Lewis rats with experimentally induced autoimmune uveitis (18, 41). The local presence of CRH appears to be of pivotal importance in the process of experimental autoimmune uveoretinitis in rodents. Retinas from immunized B10.A mice treated with anti-CRH antibody showed significantly lower apoptosis and Fas and Fas ligand (FasL) expression than placebo-treated animals (42). Thus, CRH in inflammatory sites seems to be involved in the activation of the Fas/FasL system. Studies of immuno-neutralization *in vivo* with a highly specific anti-CRH polyclonal antiserum resulted in a major suppression of the inflammatory response (21). On the other hand, somatostatin analogs have significant anti-inflammatory effects *in vivo*, associated with suppression of pro-inflammatory cytokines and neuropeptides. CRH levels at experimentally induced inflammatory sites are lowered in the presence of somatostatin analogs (43). Furthermore, locally produced somatostatin mediates the anti-inflammatory actions of GCs (44).

Interestingly, CRH-deficient mice are resistant to experimental autoimmune encephalomyelitis (45). This effect of CRH is independent of its ability to increase corticosterone production, because adrenalectomized wild-type mice had similar disease course and severity to control mice. Thus, it seems that peripheral CRH exerts a pro-inflammatory effect in experimental autoimmune encephalomyelitis with a selective increase in Th1-type responses, indicating a novel contribution of peripheral CRH to the regulation of Th1-mediated inflammation. These findings might have implications for the treatment of Th1-mediated diseases such as multiple sclerosis.

Peripheral CRH exerts pro-inflammatory effects, possibly through mast cell activation. Mast cells are necessary for allergic reactions, but are increasingly implicated in acquired immunity and inflammatory diseases worsened by stress. Acute psychological stress induces CRH-dependent mast cell degranulation. In a similar way, CRH causes mast cell degranulation in human skin, releasing great amounts of histamine, which appears to be the principal mediator of the vasodilatory effects of CRH in human skin (46). In addition, CRH is synthesized and secreted by human mast cells acting in autocrine and paracrine fashion, especially in allergic inflammatory disorders exacerbated by stress (47).

CRH-deficient mice have reduced ileal secretion, histological damage, and inflammation in response to clostridium difficile (toxin A). In addition, the content of substance P (a sensory neuropeptide with a pivotal role in the mediation and amplification of the inflammatory signal in response to toxin A) at the inflammatory sites, is CRH-dependent. These results show the major

pro-inflammatory role of CRH in the pathophysiology of toxin A-mediated inflammatory diarrhea and indicate a substance P-linked pathway (48). Furthermore, IrCRH and CRH mRNA as well as CRH-binding sites are present in the inflammatory cells of rat joint tissue with streptococcal cell wall- and adjuvant-induced arthritis (39, 49).

CRH in the female reproductive system

Peripheral CRH and its receptors have been identified in most female reproductive tissues, including ovary, uterus, and placenta (11, 27, 50–53). Ovarian CRH is primarily found in the theca and stroma, and also in the cytoplasm of the oocyte (52, 53). CRH type-1 receptors are also detected in the ovarian stroma and the theca and in the cumulus oophorus of the graafian follicle (52, 54). Granulosa cells are devoid of the expression of CRH and CRH-R1 genes and peptides (54). Epithelial cells of the human endometrium and differentiated stromal cells also express the CRH gene (27, 55, 56). In addition, CRH-R1 are present in both epithelial and stroma cells of human endometrium and myometrium (57–59). Although it is known that CRH is expressed in myometrial smooth muscle cells with CRH transcript and immunoreactive peptide increasing significantly with pregnancy, it is not clear whether the peptide responsible for the direct activation of myometrial CRH receptors is circulating placental CRH acting by paracrine diffusion, or an endogenous myometrial CRH acting locally, or a combination of both (60). Placental CRH is synthesized in syncytiotrophoblast cells, placental deciduas, and fetal membranes (61, 62) and is secreted into the maternal circulation during gestation. Its concentrations increase exponentially as pregnancy progresses (63). Non-pregnant human myometrium expresses three CRH receptor subtypes, namely 1α , 1β , and 2β . With the progression in the pregnancy, the myometrium starts to express the 2α receptor. In addition, the myometrium expresses the $1c$ and $1d$ receptor subtypes, indicating a possible functional role for these receptor subtypes at the end of pregnancy (59). The syncytiotrophoblasts of the placenta and the fetal membranes express the 1α , $1c$, $1d$, and 2β subtypes (64) and the fetal adrenal glands express both the 1α and 2α subtypes (65).

It appears that the effects of CRH in the reproductive tract are carried out mainly via its pro-inflammatory properties as in the case of ovulation, luteolysis (52, 53), and blastocyst implantation (56). The abundant expression of the gene encoding CRH and CRH-R1 in mature follicles compared with that in small antral follicles indicates that the CRH–CRH-R1 system in the thecal cells may play autocrine and paracrine roles in steroidogenesis and follicular maturation. Several studies have reported that CRH suppresses ovarian steroidogenesis *in vitro* (66–68). Both Calogero *et al.* (66) and Ghizzoni *et al.* (67) demonstrated that CRH

exerts an inhibitory effect on estrogen and progesterone production in human granulosa–luteal cells isolated from the follicular fluid upon oocyte retrieval (66–68). Likewise, Erden *et al.* demonstrated that CRH inhibited LH-stimulated DHEA and androstenedione production in isolated follicular thecal cells. Recently, CRH and CRH receptors were shown to be predominantly expressed in luteinized thecal cells of the early degenerating corpus luteum, which were losing their steroidogenic function (69). This finding may be one of the pieces of evidence suggesting that CRH could be linked to the processes of follicular atresia and luteolysis.

Endometrial CRH participates in the regulation of intrauterine inflammatory processes, such as stroma decidualization, blastocyst implantation, and early maternal tolerance (70). The progesterone-induced decidualization is modulated by locally produced inflammatory factors. Epithelial and stromal CRH affects decidualization of stromal cells by regulating local modulators, i.e., prostanoids (PGE_2) and cytokines (IL-1 and IL-6). The net effect of its actions is the fine-tuning of the decidualizing effect of progesterone (71). The blastocyst may modulate the expression of endometrial CRH through IL-1 and/or PGE_2 secretion. Subsequently, endometrial CRH, in association with other local factors, may participate in a local inflammatory response at the site of implantation, rendering the endometrial surface adhesive for the attachment of the blastocyst (72). In line with this hypothesis, a significantly higher concentration (3.5-fold) of the CRH transcript and its peptide product were shown at the early implantation sites of pregnant rats compared with the interimplantation uterine regions (56). Furthermore, it has been suggested that CRH participates in the nidation of the fertilized egg by inhibiting the local maternal immune response to the implanted embryo. Indeed, CRH of maternal (decidua) and fetal (trophoblast) origin acts in an autocrine/paracrine fashion, through CRH-R1, to stimulate FasL protein expression and to potentiate the ability of these cells to induce apoptosis of the surrounding maternal T lymphocytes activated by the presence of the embryo (73). Abnormalities of maternal tolerance pointing at inadequate CRH-mediated self-induction of FasL in extravillous trophoblasts and decidual cells may have deleterious consequences for the developing fetus. In line with these findings, female rats treated with a CRH antagonist in the first 6 days of gestation had a dose-dependent decrease of endometrial implantation sites and markedly diminished FasL protein expression (74).

Placental CRH, which is upregulated by both fetal and maternal F, may participate in the physiology of pregnancy and the onset of parturition. By mid-gestation, CRH levels are correlated inversely with gestational length making this peptide a unique candidate for regulation of fetal endocrine systems associated with the onset of labor (75, 76). In addition, the placental CRH/CRH-R system has been associated

with the pathological mechanisms leading to pre-eclampsia. A reduction of both CRH-R1 and CRH-R2 might result in a disturbance of the balance controlling vascular tone toward vasoconstriction. Although very little is known regarding regulation of expression of CRH-Rs in intrauterine tissues, it is possible that chronic exposure to elevated levels of placental CRH in pre-eclampsia or intrauterine growth retardation might downregulate its own receptors (77). It is not surprising that cumulative impact of enduring stress, such as economic position and racism may condition or unmask neuroendocrine mechanisms 'priming' the likelihood of preterm delivery. Low family income has been associated with high maternal CRH (78). In addition, reported findings from the Black Women Health Study show an impact of individually directed racism on the risk of preterm delivery (79). Additionally, infection and inflammation of the maternal genital tract, believed to account for 20–30% preterm deliveries, are more prevalent among black women (80).

CRH receptor antagonists-perspectives

Multiple research groups have demonstrated during the last decade the expression of CRH and their receptors in several components of the immune system and their participation in the regulation of inflammatory phenomena, suggesting that the administration of CRH antagonists/inhibitors might improve the clinical profile of such conditions. Antalarmin, an *N*-butyl-*N*-ethyl-[2,5,6-trimethyl-7-(2,4,6-trimethylphenyl)-7H-pyrrolo[2,3-d]pyrimidin-4-yl] amine has been synthesized recently as a therapeutic tool for both CNS and inflammatory disorders associated with central and peripheral CRH hypersecretion respectively (81). Regarding the anti-inflammatory properties of this CRH-R1 antagonist on peripheral CRH activity, antalarmin has ameliorated carrageenin-induced aseptic inflammation in rats, and acute and chronic streptococcal cell wall- and adjuvant-induced arthritis in Lewis rats (81, 82). In addition, antalarmin prolonged survival of mice subjected to LPS-induced septic shock by lowering pro-inflammatory cytokine levels (25).

Given the ability of peripheral CRH to degranulate mast cells, CRH-R1 antagonist could be considered for the treatment of allergic conditions, such as asthma, eczema, urticaria, or even stress-induced brain inflammatory disorders that increase blood–brain barrier permeability (83, 84). In the gastrointestinal tract, these compounds open new therapeutic options in the treatment of lower-GI inflammatory diseases associated to CRH, such as the chronic inflammatory bowel syndromes, irritable bowel disease, and ulcerative colitis (85, 86). In human endometrium, CRH-R1 antagonists may be used as anti-implantation agents interfering with the inflammatory phenomena taking place during implantation (86). Administration of antalarmin to

early pregnant rats (day 1 of pregnancy) results in a 70% reduction in implantation sites (74, 87). These examples illustrate the potential therapeutic significance of CRH in regulating inflammatory phenomena without affecting the rest of the immune system.

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