CORTICOTROPIN RELEASING HORMONE AND THE IMMUNE/INFLAMMATORY RESPONSE

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ABSTRACT

The immune/inflammatory (I/I) response is a reaction of the vascularized connective tissue, characterized by the accumulation of fluid and leukocytes in extravascular tissues. In this process cellular (leukocytes and lymphocytes T, B, NK) and extra-cellular elements participate in a complex co-operative network. The balance between Th1 and Th2 is important for the immune system homeostasis. Glucocorticoids and catecholamines have a significant effect on this balance. The (I/I) response is influenced by the brain *via* regulation of peripheral nervous system functions and endocrine responses. The hypothalamic-pituitary-adrenal (HPA) axis is particularly involved in this regulation. Hypothalamic corticotropin-releasing hormone (CRH) is pivotal in the HPA axis response to stress while it acts indirectly in an anti-inflammatory fashion because it leads to cortisol production, an anti-inflammatory hormone. CRH in plasma is bound to a high-affinity binding protein (CRH-BP) which limits its distribution and activity. The biological effects of CRH are mediated by CRH-Receptor (R)1 and CRH-R2.

Receptors for a number of hormones, neurotransmitters and neuropeptides are carried by cells of the immune system. In their turn, immune cells produce CRH and corticotropin (ACTH) which act locally as autacoids during both the early and late stages of the I/I process. This locally produced CRH, so-called 'peripheral CRH', is found in the adrenal medulla, the testes, the ovaries, the cardiovascular system, the gastrointestinal tract, the pancreas, the lung, the spinal cord, the endometrium and the placenta, as well as in diverse inflammatory sites. In the latter it acts in a pro-inflammatory fashion while most of the CRH effects in the female reproductive tract seem to be pro-inflammatory as well. This is the case in ovulation, luteolysis and blastocyst implantation. Ovarian CRH is found in the theca and stroma and in the cytoplasm of the oocyte. CRH suppresses ovarian steroidogenesis *in vitro.* Endometrial CRH participates in the early maternal tolerance of the semiallograft embryo. Placental CRH is synthesized in syncytiotrophoblast cells, in placental decidua and fetal membranes and is secreted into the maternal circulation during gestation. Its concentrations increase as pregnancy progresses and it participates in the physiology of pregnancy and the onset of parturition. The placental CRH/CRH-R system has been associated with the pathological mechanisms leading to preeclampsia.

The expression of CRH and CRH-Rs in several components of the immune system and their participation in the regulation of inflammatory phenomena led researchers to suggest CRH antagonists/inhibitors as potential therapeutic agents of such conditions. Antalarmin, has been proposed as a therapeutic tool for both CNS and inflammatory disorders associated with central and peripheral CRH hypersecretion. Astressin B, a nonspecific CRH receptor antagonist, accelerates the return to normal cyclicity. Thus, it emerges as a potential therapeutic agent in stress-related endocrine dysfunction, including the functional hypothalamic chronic anovulation syndrome or the persistent inadequate luteal phase syndrome, and therefore in the treatment of infertility. CRH-R1 antagonists could be considered for the treatment of allergic conditions (asthma, eczema, urticaria) and in the treatment of lower gastro-intestinal inflammatory diseases associated to CRH (chronic inflammatory bowel syndromes, irritable bowel

disease and ulcerative colitis). For complete coverage of this and related topics, please visit www.endotext.org.

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, like corticotropin-releasing hormone (CRH) and corticotropin (ACTH) which act locally as autacoids during both the early and late stages of the I/I process (3). This locally produced CRH, is subsequently called 'peripheral CRH'.

CENTRAL CRH AND THE HPA AXIS. PERIPHERAL CRH

CRH is a 41-amino-acid peptide that plays a central role in organizing the HPA axis and the systemic response to stress (4). It shows significant interspecies homology at the amino terminal region and acts as the main physiologic ACTH stimulator (5, 6). CRH is a member of a family of peptides, such as sauvagine, urotensin I, and urocortin (UCN) that show similar activity (7, 8, 9). The single CRH and UCN genes are located on human chromosomes 8 and 2, respectively. At first, CRH is synthesized as a larger precursor molecule (191 amino acid in humans) from which it is cleaved at flanking basic amino acid pairs (10). CRH is synthesized by parvocellular neurons of the hypothalamic paraventricular nucleus (PVN) and is secreted in the hypophyseal portal blood *via* projecting axons to the median eminence (10). These neurons also secrete other hormones that are potent stimulators of ACTH secretion, such as arginine vasopressin (AVP) which acts synergistically with CRH and is also secreted by magnocellular neuron terminals at the posterior pituitary (11). The magnocellular PVN contain CRH-synthesizing neurons that project to the posterior pituitary (12). In addition, CRH is distributed in the brain and the spinal cord. The plasma half-life of CRH in humans is four minutes (13). ACTH released by CRH leads to secretion of cortisol and other adrenal steroids such as DHEA and, transiently, aldosterone (14). Pituitary adenylate cyclase activating polypeptide (PACAP) increases CRH mRNA levels in the parvocellular region of the PVN of the hypothalamus, suggesting that this polypeptide is involved in the positive regulation of CRH gene expression. Interleukin (IL)-6, produced in the PVN stimulates CRH gene expression both directly and indirectly. Forskolin and PACAP stimulate IL-6 mRNA and protein levels in the hypothalamic cells while the stimulatory effects of PACAP on CRH promoter activity are inhibited by treating hypothalamic cells with anti-IL-6 monoclonal antibody. Therefore, endogenous IL-6 production would be involved in the PACAP-induced CRH gene transcription in an autocrine manner in hypothalamic cells. Hypothalamic parvocellular neurons in the PVN are known to express glucocorticoid receptors (GC). The latter regulate CRH

gene transcription and expression levels directly in this nucleus (15). IL-6 is co-expressed with CRH and AVP in the supraoptic and PVN neurons (16). It plays an important role in regulating both CRH and AVP in the hypothalamus and it increases CRH gene expression and secretion in the PVN (17,18). IL-6 may be important to sustain the activity of CRH and AVP genes. Estradiol may enhance the activation of CRH gene expression in response to stress (15).

There have not been noted any sex or age differences in plasma ACTH or cortisol responses to CRH (19). ACTH response to CRH is not influenced by the hour of the day. However, the corresponding cortisol response is maximized late in the afternoon due to cortisol receptor change of affinity (20). Inputs from higher centers regulate the HPA axis activity. The effects of the circadian pacemaker, stress and glucocorticoid negative feedback are superimposed. This negative feedback acts at pituitary, hypothalamic and higher levels, such as the hippocampus (1). Interestingly, chronic corticosterone treatment increases the expression of CRH mRNA at the central nucleus of the amygdala in adrenally intact rats (21).

CRH plays a central role in the activation hormonal and neuronal signaling cascade in vertebrates as a response to stress. Expression of brain CRH is subject to strong, brain region-specific glucocorticoid hormones-regulated and neurogenic intracellular signals. It is hypothesized that steroid receptor coactivator 1 (SRC-1), a transcriptional coregulator of the glucocorticoid receptor, is involved in the sensitivity of CRH regulation by stress related factors, as it was shown after experiments in the brains of SRC-1 knockout mice. It was also suggested that CRH gene is positively and negatively regulated by SRC-1 showing the importance of this coactivator in the adaptive capacity to stress (22).

Peripheral CRH has also been found in the adrenal medulla, the testes, the ovaries, the cardiovascular system, the gastrointestinal tract, the pancreas, the lung, the spinal cord, the endometrium and the placenta (20, 23-27), as well as in diverse inflammatory sites (1, 28, 29). Peripheral sensory afferent type C fibers and postganglionic sympathetic nerves also express CRH (30) and have been suggested as an additional source of the immune CRH (1). Another peripheral organ where CRH is locally produced is the skin where CRH receptor type 1 (CRH-R1) isoforms are expressed in keratinocytes (31). 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 (SP) and TNF, including their down-regulation by GCs (32).

The majority of the plasma CRH is of non-hypothalamic origin. That is because hypothalamic CRH is rapidly enzymatically decomposed at the pituitary level. However, under certain circumstances, such as insulin-induced hypoglycemia and pregnancy, hypothalamic CRH release leads to increments in plasma CRH concentration. Uniquely to humans, CRH in plasma is bound to a high-affinity binding protein (37 kilodaltons) (CRH-BP) (33). This protein has been characterized and is expressed in the brain, the pituitary and the peripheral tissues (liver, kidney, spleen). It plays a major role in limiting the distribution or activity duration of CRH (34).

CRH RECEPTORS

The biological effects of CRH are mediated by two different receptors, CRH-R1 and CRH-R2 that belong to the G-protein coupled receptor superfamily and typically

are positively coupled to adenylate cyclase (35, 36). CRH-R1 and CRH-R2 share an approximately 70% homology of their amino acid sequence but exhibit unique pharmacologic profiles. They are differentially expressed and appear to mediate selective actions of CRH at different tissues. The affinity that CRH exhibits towards CRH-R1 is ten times higher than towards CRH-R2 (35). These two receptors are products of separate genes. CRH-R1 is encoded by human chromosome 17q21 while CRH-R2 is encoded by human chromosome 7p14 (37, 38).

The CRH-R1 gene expresses nine subtypes (α, β, c, d, e, f, g, h and v_1) produced by alternative splicing of exons 3-6 and 10-13. The CRH-R2 gene expresses three known subtypes (α, β, and γ) that are produced by the use of alternate 5' exons (39,40,41). In most mammals, the fully active CRH-R1 receptor protein arises from transcription of 13 exons present within the CRH-R1 gene sequence. However, the human type 1 CRH-R gene contains an additional exon (exon 6) that has to be spliced out in order to generate the fully active CRH-R1α. CRH-R1α contains 415 amino acids. It mediates CRH and UCN 1 actions and is widely expressed throughout the body. Transcription of all 14 exons results in a CRH-R1 variant (CRH-R1β), a 444-amino acid heptathelial protein receptor with an extended first intracellular loop that exhibits impaired agonist binding and signaling properties. CRH-R1β can be regarded as a "pro-CRH-R1" receptor isoform without knowing its biological actions (42). CRH-R1β is expressed in a tissue specific manner and is present at the anterior pituitary (43), at reproductive tissues such as myometrium, endometrium and chorion trophoblast cells and mast cells but not in the placenta, adrenal and synovium (21). No tissue has been found to express CRH-R1β alone, until now, suggesting that the splicing mechanism is closely related to the mechanism regulating CRH-R1 gene expression in native tissues.

Another CRH-R1 variant is CRH-R1d, containing 14 amino acids missing from the putative seventh transmembrane domain due to exon 13 deletion, a splicing event which is common in the other members of the B1 family of G protein-coupled receptors. In human embryonic kidneys the CRH R1d variant is mainly retained in the cytoplasm in contrast to CRH-R1α, although some cell membrane expression is evident, too. Membrane expressed CRH-R1d contains an extracellular C terminus. Co-expression studies between CRH-R1d and CRH-R2β showed that the CRH-R2β could partially rescue CRH-R1d membrane expression. This fact is associated with important attenuation of UCN 2-induced cAMP production and ERK1/2 and p38MAPK activation, suggesting that CRH-R1d might specifically induce heterologous impairment of CRH-R2 signaling responses. Accelerated CRH-R2β endocytosis seems to be involved in this mechanism (44). Glucocorticoid exerts its negative effect on CRH gene transcription in a glucocorticoid dependent manner, as it was demonstrated after examination of human BE(2)C neuronal cell line (45).

A stomach variant has also been identified for CRH-R2 (46). CRH-R1 expression is highest in the cerebral cortex, striatum, amygdale and cerebellum. On the other hand, CRH-R2 is mostly present in subcortical structures such as the lateral septal nucleus, several nuclei of the hypothalamus and the choroid plexus (47). CRH receptors mRNA and protein are also detectable in adipose tissue. CRH-R2 expression in fat tissue is comparable with its expression in heart, where the highest expression of CRH-R2 is detected. At the periphery, CRH-R2 is widely expressed in the gastrointestinal tract, the lung, skeletal muscle, arteries and the heart muscle. Indeed, peripherally injected CRH augments gastrointestinal motility, inhibits gastric secretion, lowers blood pressure and affects heart output and decreases inflammatory reactions. In mice CRH-R2 has anti-angiogenic activity during postnatal development. Studies focus on the role of CRH system in the modulation of blood

vessel formation and cardiovascular function aiming at the development of new anti-angiogenic therapies (48). CRH-R2 is also expressed in the colon of patients with ulcerative colitis. CRH-R2 down-regulation in distal/sigmoid biopsies of ulcerative colitis patients is indicative of change in CRH-R2 signaling associated with the process of inflammation (49). Increased serum CRH with decreased lesional skin CRH-R1 gene expression was observed in patients with psoriasis and atopic dermatitis, suggesting their involvement in stress-induced worsening of symptoms (50). Substance P, another factor involved in inflammatory diseases, can stimulate mast cells and increase the expression of functional CRH-R1. CRH induces NK-1 gene expression, explaining CRH-R1 and NK-1 expression in lesional skin of psoriatic patients (51).

CRH-R1 is also detected in the ovarian stroma and the theca and in the cumulus oophorus of the graafian follicle (37, 52). CRH-R1α is present on both epithelial and stromal cells of the human endometrium (53). CRH-R1/CRH-R2 ratio varies according to adipose tissue type. CRH-R1 expression is higher in subcutaneous than in visceral fat in contrast to CRH-R2 expression which is the opposite. Urocortin and stresscopin, ligands of the CRH-R, are also expressed in fat tissue, having metabolic and potent anorexic effects. CRH-R1 and CRH-R2 expression is down regulated by CRH in isolated adipocytes (54). CRH down-regulates adipose 11β HSD-1 activity and the availability of cortisol for intracrine effect in mature human subcutaneous adipocytes *in vitro*. Additionally, it reduces lipolysis in differentiated human adipocytes (55). However, antalarmin, a CRH-R1 antagonist did not block the effect of CRH on 11β HSD-1 in fat tissue cells, suggesting a predominant action through CRH-R2 in human fat (54).

Both CRH receptors are probably involved in some coordinate ways to express the totality of the physiological responses to stress including behavioral responses (34). Acute stress induces bladder vascular permeability and vascular endothelial growth factor (VEGF) release that is dependent on CRH-R2, as it was shown by experiments in mice (56). These findings suggest that CRH and VEGF might participate in the pathogenesis of interstitial cystitis/painful bladder syndrome and thus, they could provide new therapeutic targets (56). CRH, CRH-R1 and CRH-R2 have been reported in several types of carcinoma. Sato et al, have reported that intratumoral CRH and CRH-R1 signaling plays an important role in the progression of endometrial carcinoma and that CRH-R1 is a potent prognostic factor in patients with this disease (57).

In the anterior pituitary, CRH binds to CRH-R1 of the corticotrophs (58). The ACTH response can be modulated by the number of CRH-R in these cells. Immobilization, stress, adrenalectomy, administration of CRH, AVP or GCs, reduce the number of CRH-R in the anterior pituitary but not in the brain (59). Binding of CRH to the receptor results in adenylcyclase activation, leading to an increase in intracellular cAMP concentration. Cyclic AMP (cAMP)-protein kisase A (PKA) pathway is the main stimulator of CRH gene transcription. PKA inhibitor strongly blocks the forskolin-induced CRH promoter activity (60). The classical effects of CRH on anterior pituitary corticotrophs are mediated by the PKA signaling pathway (61). The coupling of CRH receptor and G protein may also stimulate other cAMP pathway events in a variety of brain-derived and peripheral cell lines. These events include the cross-talk between CRH receptor-initiated signal transduction and activation of nuclear factor-κB (NF-κB) in T cell. The molecular mechanism of this effect has been established through experiments using cultures of mouse thymocytes (62). It was shown that the CRH-induced activation of the NF-κB was mediated by two protein kinase systems, PKA and Protein Kinase C (PKC) (63, 64). CRH affects the PKC

signaling pathway in the pituitary, Leydig cells, adrenals, placenta, immunocytes, myometrium and hippocampus. In human epidermoid cells, CRH induces the activity and the translocation of the conventional PKC isoenzymes (61). It is very interesting that UCN exerts its neuroprotective effect on cultured hippocampal neurons by the PKC pathway *via* activation of the CRH-R1 receptor (65). Activation of the PKC pathway may modify the sensitivity to CRH since it affects the number of CRH-Rs (66, 67). CRH increases the concentration of cytosolic calcium ions in calcium rich and in calcium free media. In both situations, PKA inhibitor [myristoylated PKA (Myr-GRTGR-RNAI-NH2)] abolishes the effect of CRH. However, PKC inhibitor [pseudo-substrate myristoylated alanine-rich PKC (Myr-N-FARK-GALRQ- NH2)] abolishes the effect of CRH only in calcium free conditions. As a result, the PKA signaling pathway mediates both effects of CRH while on the other hand the PKC signaling pathway mediates only the CRH-induced mobilization of calcium ions from intracellular stores, possibly *via* P-type calcium ion channel (68, 69, 70). CRH -R1α and -R1β exhibit differential responses to PKC-induced phosphorylation. This might represent an important mechanism for functional regulation of CRH signaling in target cells. PKC activation increases internalization of CRH-R1β but not CRH-R1α in a β-arrestin-independent manner, although both CRH-R1 variants are susceptible to homologous desensitization and internalization following treatment with CRH (71).

It was shown that CRH-R2 and its high affinity ligand, UCN 1, are key mediators of the endoplasmatic reticulum stress response in a murine model of acute pancreatic inflammation (72). Urocortin is known to participate in inflammation. The increased expression of intracellular adhesion molecule 1 (ICAM1) plays important role in inflammation and immune responses. Wan et al, have shown that UCN 1 significantly enhances the expression of ICAM1. Urocortin 1increases ICAM1 expression *via* cPLA2-NFkB and cPLA2-COX2-PGE2-PKA-CREB pathways by means of CRH-R2 in human umbilical vein endothelial cells (73).

THE IMMUNE/INFLAMMATORY RESPONSE

The I/I response is a reaction of the vascularized connective tissue, characterized by the accumulation of fluid and leukocytes in extravacular tissues. It is considered to be a protective mechanism aiming to rid the organism of both the initial cause of cell injury (such as micro-organisms, toxins, antigens) and its consequences (e.g. necrotic cells and tissues). In this process cellular and extra-cellular elements participate in a complex co-operative network. The cellular components of the I/I response include leukocytes such as monocytes-macrophages, polymorphonuclear neutrophils, eosinophils and basophils, platelets, dentritic cells, mast cells, epithelial and endothelial cells and fibroblasts (innate immune system) as well as lymphocytes T, B, NK (adaptive immune system). These cells co-operate using molecular signals, including cytokines [ILs, colony-stimulating factors (CSFs), interferons (IFNs), TNF, transforming growth factor (TGF), epidermal growth factor (EGF), chemokines], vasoactive amines (histamine, serotonine), plasma proteases (kinine system, complement system), arachidonic acid metabolites (prostaglandins, leukotrienes, lipoxins), platelet-activating factor (PAF), nitric oxide (NO) and neuropeptides. The first step in the initiation of the I/I response is the activation of the innate immune system. This nonspecific response serves to locate the injurious agent, restrict the tissue damage and eliminate the harmful agent, initiate the adaptive immune system and determine the path that will be followed (cellular / humoral response).

In this procedure mast cells play an initiative role, early affecting the microvascular response. Mast cells are necessary for allergic reactions, but are increasingly implicated in acquired immunity and inflammatory diseases worsened by stress. Mast cells consist of two populations, distinguished by their enzyme content. The T (tissue) mast cells contain trypsin alone and are also termed mucosal mast cells due to their location near mucosal surfaces. The TC (connective tissue) mast cells contain both trypsin and chymotrypsin. Rapidly responding to any foreign stimulus substance, mast cells are activated leading to their degranulation, releasing several pro-inflammatory mediators. The activation and degranulation of mast cells is regulated by several mediators such as IL-3 and stem cell factor (SCF). TNF seems to be an important mast cell survival factor whereas IFN-γ is a potential inducer of mast cell apoptosis (74, 75).

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 dentritic cells, interstitial and Langerhans dentritic 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, NK lymphocytes) and humoral (B lymphocytes, plasma cells, antibodies) immunity. 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. T-helper cells produce interleukin 17, a cytokine that acts as a potent mediator in delayed-type reactions by increasing chemokine production in various tissues to recruit monocytes and neutrophils to the site of inflammation. IL-17 is induced by IL–23 which results in destructive tissue damage in delayed-type reactions. IL-17 acts synergistically with TNF and IL-1. The balance between Th1 and Th2 is important for the homeostasis within the immune system. Glucocorticoids and catecholamines, the stress hormones, have a significant effect on this balance. Glucocorticoids suppress production of IL-12 by monocytes/macrophages, the main inducer of Th-1 responses (76, 77) 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 up-regulating their IL-4, IL-10 and IL-13 production (77, 78). On the other hand, the two major catecholamines, norepinephrine (NE) and epinephrine potently inhibit the production of IL-12 by APCs thus suppressing the development of Th1 type cells (76, 79, 80).

CRH AND THE IMMUNE/INFLAMMATORY RESPONSE

Central CRH And The Immune/Inflammatory Response

oThe immune response balances between pro- and anti- inflammatory actions (81). Hypothalamic CRH has been considered to act indirectly in an anti-inflammatory fashion, since the final product of the HPA axis stimulation is cortisol, known for its anti-inflammatory actions. Interleukin 6, a known proinflammatory cytokine, is a potent stimulator of the HPA axis and a secretagogue of magnocellular AVP. The subcutaneous administration of IL-6 in humans causes considerable elevations in

plasma ACTH, cortisol and AVP (82). It has been proven that IL-6 levels are increased in patients with head trauma (an aseptic inflammation) and the syndrome of inappropriate secretion of antidiuretic hormone. In this case IL-6 levels are strongly correlated with increased AVP levels (83). Cortisol secretion after dynamic stimulation is deficient in a subset of critically ill patients with moderate to severe head injury. This disorder is associated with prior vasopressor dependency and higher IL-6 levels (84).

However, disturbances in the HPA axis activity affect the I/I response. An excessive HPA response (for example a state of stress or relative hypercortisolemia) can increase susceptibility to infectious agents and tumorogenesis but enhance resistance to autoimmune or aseptic inflammatory diseases. On the other hand, a defective HPA axis response (for example relative glucocorticoid deficient state) diminishes susceptibility to infections and tumorogenesis but increases susceptibility to autoimmune or aseptic inflammatory diseases. Indeed, these suggestions 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 glucocorticoid production and enhances allergen-induced airway inflammation and lung mechanical dysfunction in CRH knock-out mice. Thus inherited or acquired CRH deficiency could increase asthma severity in human subjects (85). Hypofunction of the HPA axis was also found in patients with Sjogren's syndrome (86) and sarcoidosis (87). Interestingly, high plasma levels of cortisol, CRH and ACTH that have been found in centenarians indicate an activation of the entire stress axis, likely counteracting the systemic inflammatory process occurring with age. This activation fits with the hypothesis that lifelong low-intensity stressors activate ancient, hormonal defense mechanisms, favoring healthy aging and longevity (88). On the other hand, the relative hypercortisolemia that accompanies ageing has been suggested to result from an age-related HPA axis resilience.

Peripheral CRH And The Immune/Inflammatory Response

The presence of CRH receptors in human lymphocytes secreting POMC-derived peptides (ACTH and β-endorphin) supports the direct involvement of CRH in the I/I response (89). By employing the rat air pouch model of carrageenin-induced acute aseptic chemical inflammation, immunoreactive (Ir) CRH was detected in the inflamed area but not in the systemic circulation. Corticotropin releasing hormone produced in peripheral inflammatory sites, in contrast to its systemic indirect immunosuppressive effects, acts as an autocrine or paracrine inflammatory cytokine (32). Immunoreactive CRH was found in the synovial lining cell layers and blood vessels from the joints of patients with rheumatoid arthritis and osteoarthritis (90), whereas high CRH levels were found in the synovial fluids of the former patients (91). In addition IrCRH was found in immune accessory cells from uveitic retinas and corpora vitrea from Lewis rats with experimentally induced autoimmune uveitis (29, 92). 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 (93). Thus, CRH in inflammatory sites seems to be involved in the activation of the Fas/FasL system. On the other hand, studies of immunoneutralization *in vivo* with a highly specific anti-CRH polyclonal antiserum resulted in a major suppression of the inflammatory

response (32). Somatostatin analogues have significant anti-inflammatory effects *in vivo*, associated with suppression of proinflammatory cytokines and neuropeptides. Corticotropin-releasing hormone levels at experimentally induced inflammatory sites are lowered in the presence of somatostatin analogues (94). Furthermore, locally produced somatostatin mediates the anti-inflammatory actions of GCs (95).

Regarding studies on models of septic inflammation, CRH-deficient mice had reduced ileal secretion, histological damage and inflammation in response to clostridium difficile (toxin A). In addition, the content of SP (a sensory neuropeptide with pivotal role in the mediation and amplification of toxin A-induced inflammatory signal) at the inflammatory sites is CRH-dependent. These results revealed the major proinflammatory role of CRH in the pathophysiology of toxin A-mediated inflammatory diarrhea and indicate a SP-linked pathway (96). Recent findings support the idea that increased peripheral CRH mediates the enhanced visceral nociception in rats recovered from experimental colitis (97). Furthermore, IrCRH and CRH mRNA as well as CRH binding sites are present in inflammatory cells of rat joint tissue with streptococcal cell wall- and adjuvant- induced arthritis (90, 98). Data exist showing that CRH enhances the expression of nitric oxide synthase (NOS III) to promote NO production from CRH-R1α expressing cells. These results confirm a role for CRH-R-mediated responses in regulating vascular changes associated with chronic synovitis (99). Corticotropin-releasing hormone acting through both receptors induces a significant increase of reactive oxygen species (ROS) content, catalase activity and superoxide dismutase activity, accompanied by a simultaneous significant decrease of endothelial NO activity and NO levels as well as a significant increase in nitrotyrosine (peroxynitrite) levels (100). These data indicate that CRH may act as a regulator of pro-inflammatory mechanisms inducing adaptation of endothelial cell function to local oxidative stress (100).

Interestingly, CRH-deficient mice are resistant to experimental autoimmune encephalomyelitis (32). This effect of peripheral CRH is independent of its ability to increase corticosterone production, because adrenalectomized wild-type mice had similar disease course and severity as control mice. Thus, it seems that peripheral CRH exerts a proinflammatory 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 (MS), a demyelinating autoimmune disease characterized by inflammation of the central nervous system (101). Substantial evidence indicates that stress can precipitate or worsen symptoms of inflammation in multiple sclerosis (MS). However, the exact mechanism of how stress affects MS is not well understood. Karagkouni et al, proposed that neuropeptides secreted under stress, such as CRH and neurotensin, activate microglia and mast cells to release inflammatory molecules, leading to maturation and activation of T17 autoimmune cells, disruption of the blood-brain barrier and T cell entry into the central nervous system, thus promoting brain inflammation and contributing to MS pathology. Reduction of stress and inhibition of these processes by selected flavonoids could provide novel therapeutic approaches (102).

Peripheral CRH exerts proinflammatory effects, possibly through mast cell activation. 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 (103). 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 (104). Finally, CRH can induce secretion of VEGF selectively (105).

Stress participates and worsens not only asthma and atopic dermatitis but also acute coronary syndromes (ACSs) which are associated with coronary inflammation. Activation of coronary mast cells by stress through CRH and other neuropeptides, contributes to coronary inflammation and coronary artery disease. Therefore, inhibition of cardiac mast cells may be a novel treatment approach (106).

Periodontal disease involves inflammation of the gingival tissues, caused by microbial pathogens. Recent research suggests that emotional stress worsens periodontal disease. Papathanasiou et al, proposed that stress-induced CRH secretion stimulates gingival mast cells and other neuropeptides and cytokines as well to secrete pro-inflammatory molecules that contribute to periodontal pathology. Stress reduction and/or mast cell inhibition may provide additional therapeutic approaches (107).

Septic shock is associated with decreased ACTH synthesis in humans and in rats, which is not compensated by its two natural secretagogues, AVP and CRH. Polito et al, suggested that one underlying mechanism might be increased expression of iNOS in hypothalamic parvocellular neurons (108). In septic patients during cardiovascular deregulation, increased levels of POMC derivatives have been found. Majetec et al, found that after CRH administration, heart rate, cardiac index and stroke index increased and the systemic vascular resistance index decreased. Moreover, a positive correlation between ACTH concentration and stroke index as well as an inverse correlation between α-melanocyte stimulating hormone (αMSH) concentration and systemic vascular resistance index was observed. CRH and ACTH may have opposite effects on the blood pressure. Therefore, POMC derivatives seem to have influences on patients hemodynamic during sepsis (109). In patients with septic shock, the up-regulation of mHLA-DR expression after CRH infusion is independent of POMC derivatives release. From the positive correlation between plasma concentration of αMSH and mHLA-DR expression they concluded that in this group of patients, the down-regulation of mHLA-DR expression is accompanied by the loss of αMSH release by monocytes into the cardiovascular compartment (110).

It has been shown previously that CRH induces NF-κB DNA-binding activity in mouse and human leukocytes. Karalis et al., demonstrated that in the human monocytic P-1 cells, CRH activates the PI3K/Akt and ERK1/2 pathways. CRH-R2 mediates these CRH effects as suggested by their abolishment following treatment with the specific CRH-R2 antagonist, Astressin 2B. The CRH-mediated PI3K/Akt activation induces cell survival as suggested by the stimulation of the anti-apoptotic factor Bcl-2. ERK1/2 activation results in upregulation of IL-8 expression, an effect inhibited by the CRH-induced activation of PI3K/Akt. These studies demonstrate novel effects of CRH in human monocytes mediated by the activation of PI3K/Akt and they reveal pathway-specific effects of the CRH/CRH-R2 system in chemokine activation and cell survival which is important for the development of new therapeutic agents for inflammatory diseases (111).

CRH In The Female Reproductive System

Peripheral CRH and its receptors have been identified in most female reproductive tissues, including ovary, uterus and placenta (20, 37, 38, 53, 112, 113).

Most of the CRH effects in the reproductive tract seem to be carried out *via* its proinflammatory properties as in the case of ovulation, luteolysis (37, 38) and blastocyst implantation (114).

Ovarian CRH and CRH receptors

Ovarian CRH is primarily found in the theca and stroma and also in the cytoplasm of the oocyte (37, 38). Corticotropin releasing hormone R1 receptors are detected in the ovarian stroma and the theca and in the cumulus oophorus of the graafian follicle (37, 52). Granulosa cells are devoid of the expression of CRH and CRH-R1 genes and peptides (52). The abundant expression of the gene encoding CRH and CRH-R1 in mature follicles compared to that in small antral follicles indicates that the CRH-CRH-R1 system in the theca cells may play autocrine and paracrine roles in steroidogenesis and follicular maturation. Several studies have reported that CRH suppresses ovarian steroidogenesis *in vitro* (115-117). Corticotropin releasing hormone exerts an inhibitory effect on estrogen and progesterone production in human granulosa-lutein cells isolated from the follicular fluid upon oocyte retrieval (115-117). Likewise, Erden et al. demonstrated that CRH inhibited LH-stimulated DHEA and androstenedione production in isolated follicular theca cells. Corticotropin releasing hormone and CRH receptors were shown to be predominantly expressed in luteinized theca cells of the early degenerating corpus luteum, which were losing their steroidogenic function (118). This finding may be one of the pieces of evidence suggesting that CRH could be linked to the processes of follicular atresia and luteolysis. The addition of CRH has an inhibitory effect on *in vitro* fertilized oocytes, resulting from cultured preantral follicles at all stages of preimplantation embryo development (119). In addition, the presence of CRH in the culture medium inhibits steroidogenesis by preantral mouse follicles cultured *in vitro* (119). CRH-R1 is present in stages of mouse follicle growth, whereas 10^{-9}, 10^{-7} and 10^{-6} mol/L CRH inhibits oocyte maturation *in vitro*, an effect reversed by antalarmin addition (120). CRH, UCN and CRHR gene expression is higher during the regression of the human corpus luteum than in the earlier stages of the luteal phase (118). This discrepancy may result from the difference between species, methods for classifying stages of the luteal phase and /or techniques employed. Xiao et al., have also shown that systemic administration of astressin in the rhesus monkeys accelerates the return to normal luteal function, presumably by restoring normal neuroendocrine regulation of gonadotropin secretion (121). This could be CRH-receptor mediated at the level of GnRH pulse generator. It is known that CRH inhibits the latter in rodents and primates. Stresscopin/urocortin 3 system is present in human ovaries (SCP/Ucn3 and CRH-R2 mRNAs and proteins are expressed in cultured human granulosa-lutein cells). Treatment with SCP/Ucn3 inhibits progesterone production in these cells through interaction with CRH-R2, a phenomenon reversed by the CRH antagonist antisauvagine-30 (122).

Uterine CRH and CRH receptors

Epithelial cells of the human endometrium and differentiated stromal cells also express the CRH gene (53, 114, 123). In addition, CRH-R1 is present in both epithelial and stromal cells of human endometrium and myometrium (124-126). Although it is known that CRH is expressed in myometrial smooth muscle cells with CRH transcript and Ir 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 endogenous myometrial CRH acting locally, or a combination of both (127, 128). Non-pregnant human myometrium expresses three CRH-R subtypes, namely CRH -R1α, -R1β and -R2β. Endometrial CRH participates in the regulation of intrauterine inflammatory processes such as stroma decidualization, blastocyst implantation and early maternal tolerance (129). 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 (PGE2) and cytokines (IL-1 and IL-6). The net effect of its actions is the fine–tuning of the decidualizing effect of progesterone (130). The blastocyst may modulate the expression of endometrial CRH through IL-1 and/or PGE2 secretion.

Endometriosis is considered as an aseptic inflammatory disease, characterized by the presence of ectopic endometrium-like tissue. Corticotropin-releasing hormone -R1β and -R2α are expressed in endometriotic sites and they are more strongly expressed in eutopic endometrium of women with endometriosis compared to healthy women endometrium at the mRNA and protein level (131). Corticotropin-releasing hormone, UCN, CRH -R1 and -R2 mRNAs and proteins are also more highly expressed in ectopic rather than eutopic endometrium in women with endometriosis. These findings indicate that CRH and UCN might play an immunoregulatory role in endometriotic sites by affecting reproductive functions such as decidualization and implantation in women with endometriosis (131).

Placental CRH and CRH receptors

Placental CRH is synthesized in syncytiotrophoblast cells, in placental decidua and fetal membranes (132, 133) and is secreted into the maternal circulation during gestation. Its concentrations increase exponentially as pregnancy progresses (134). The presence in plasma and in amniotic fluid of a CRH-binding protein (CRHbp) that reduces the bioactivity of circulating CRH by binding is unique to humans (135). Placental CRH may participate in the physiology of pregnancy and the onset of parturition. Placental trophoblasts express both CRH -R1 and -R2 (136). The syncytiocytotrophoblasts of the placenta and the fetal membranes express the CRH -R1α, -R1c, -R1d and -R2β subtypes (137). By contrast to the hypothalamic CRH system, the production of placental CRH is positively regulated by both fetal and maternal GCs (138). This positive feed-forward system is another unique feature of placental CRH, and indicates a distinct role in pregnancy (139). Placental CRH production is also modified by estrogen, progesterone and NO which are inhibitory and by a range of neuropeptides which are stimulatory (140). CRH increases estrogen biosynthesis in cultured human placental cells (141).

Role of CRH in Pregnancy (pre- and post- partum conditions)

As pregnancy progresses, the myometrium starts to express CRH-R2α. In addition, at term, the myometrium expresses the CRH -R1c and -R1d subtypes, indicating a possible functional role for these receptor subtypes at the end of pregnancy (142). CRH-R1 maintains myometrial quiescence whereas CRH-R2 promotes smooth muscle contractility. The protein level of CRH-R1 in upper segment myometrium is significantly down-regulated in pregnancy, with a further decrease at

the onset of labor. However, the expression of CRH-R1 in lower segment myometrium remains unchanged during pregnancy and labor. No significant changes are observed in the expression of CRH-R2 in upper or lower segment myometrium (143).

Endometrial CRH may also participate in early maternal tolerance of semiallograft embryo. 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 (144). 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 to the interimplantation uterine regions (114). 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 by extravillous trophoblasts and decidual cells and to potentiate the ability of these cells to induce apoptosis of the surrounding maternal T lymphocytes activated by the presence of the embryo (145). Abnormalities of maternal tolerance pointing at inadequate CRH-mediated self induction of FasL in extravillous trophoblasts (EVTs) and decidual cells may have deleterious consequences for the developing fetus. In line with these findings, female rats treated with a CRH antagonist (antalarmin) in the first six days of gestation had a dose-dependent decrease of endometrial implantation sites and markedly diminished FasL protein expression (146). Makrigiannakis et al., have studied the hypothesis that the expression of the pro-apoptotic Fas/FasL system at the implantation site is impaired in abortions. They reported that abortive deciduas contain leukocytes that are positive for FasL and EVTs, which show increased rates of apoptosis and increased expression of Fas, in contrast to normal pregnancies (147).

By mid gestation, CRH levels are correlated inversely with gestational length making this peptide a unique candidate for regulation of fetal endocrine systems (135, 148). In addition, the placental CRH/CRH-R system has been associated with the pathological mechanisms leading to preeclampsia. 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 preeclampsia or IUGR might downregulate its own receptors (149). In fetuses with fetal growth restriction umbilical cord CRH is also elevated (150). Interestingly, the observed down-regulation of CRH-R in the pre-eclamptic placenta is not an isolated finding: several hormonal signals that regulate NOS expression and activity have attenuated effects due to down-regulation of their respective receptors, including CRH -R1 and -R2. This down-regulation results in compromised responses and reduced relaxation in feto-placental vessels from pre-eclamptic placentas (151).

Near term the human fetal adrenals initiate production of cortisol, which promotes organ maturation and acts to increase placental CRH biosynthesis and therefore participates in the endocrine cascade that is involved in the timing of human parturition (152). The adrenal glands represent the largest endocrine glands in the fetus and at term they weigh the same as adult adrenal glands. Late in gestation, the definite zone expresses the enzymes needed for the production of aldosterone, the transitional zone those for cortisol, and throughout gestation the fetal zone expresses enzymes and cofactors needed for high levels of DHEAS

production (153). The ability of CRH to stimulate both cortisol and DHEAS is highly significant because it would allow the fetal adrenal to work with the placenta to create a feed-forward endocrine cascade that would not end until the separation of the fetus from the placenta at delivery. Especially, if CRH can stimulate fetal adrenals to produce cortisol, then the fetal-derived cortisol can stimulate placental CRH production *via* the unique glucocorticoid-positive feedback system seen in the human placenta. The placental effect may involve activation of different CRH-R subtypes or the initiation of a different cascade of events after binding to the receptor (154). This positive feedback cascade results in rising placental CRH and fetal cortisol production in the last trimester of human gestation (152).

It is not surprising that cumulative impact of enduring stress such as low economic position and racism may condition or unmask neuroendocrine mechanisms 'priming' the likelihood of preterm delivery. A US study reported an association between low family income and high maternal CRH (155). In addition reported findings from the Black Women Health Study show an impact of individually directed racism on the risk of preterm delivery (156). Additionally, infection and inflammation of the maternal genital tract believed to account for 20-30% of preterm deliveries are more prevalent among black women (157). The pathogenesis of preterm delivery is not clear, although inflammation/infection play a major role. Corticotropin-releasing hormone and UCN are involved in the pathogenesis of preterm delivery. Corticotropin-releasing hormone, UCN2 and CRH-R1 mRNA expression is higher, while UCN and CRH-R2 mRNA expression is lower in placentas with premature rupture of membranes (pPROM) with chorioamnionitis that in preterm deliveries and pPROM (158). Urocortin 3 mRNA expression was lower in pPROM with and without chorioamnionitis than in preterm delivery. These findings showed a significant association of pPROM with chorioamnionitis on placental CRH peptides and receptors, suggesting that placental expression of stress-related pathways is activated in infectious processes (158). Taking into account that placental CRH plays a central role in modulating the effects of hypoxia, infections, possible decidual haemorrhage and psychosocial stress *per se* on prematurity-related outcomes, it is likely that placental CRH levels are related to the length of gestation and fetal growth either directly by participating in physiologic processes involved in parturition and fetal maturation, or indirectly by bringing to light anterpartum conditions priming for prematurity. Prostaglandin administration for labor induction increases bioactive maternal CRH *via* reduction of circulating CRH-BP levels, as well as increase of maternal CRH levels. This positive feedback of prostaglandin on maternal CRH release might contribute to the development of active labor (159). Women with preterm labor, or those destined to have premature delivery, have higher mid-pregnancy plasma CRH level than those who delivered at term (150). Corticotropin releasing hormone, UCN, tryptase and IL-8 levels are significantly increased in products of conception of women with spontaneous abortions and these levels are highest in patients with at least two spontaneous abortions (150). IL-8, an inflammatory cytokine, may play an important role in the process of protease-induced neurogenic inflammation, contributing to stress-induced proinflammatory effects of mast cells in aborted products of conception, by recruiting neutrophils and lymphocytes in the endometrium (160). In some women with idiopathic preterm labor, CRH levels increase up to 10 weeks before the development of any symptoms. The biological purpose of CRH secretion is not yet known; it might reflect the increased stress of the feto-maternal unit and the role of CRH is to integrate the homeostatic mechanisms that allow the mother (and the fetus) to adapt to the increasingly stressful environment, thus acting as a "true" stress hormone, or it might actively be involved in the mechanism leading to the onset of labor, or both (139). Women presenting with preterm labor have higher levels of IL-1β and CRH than women at the same week of gestation who delivered at term. There might be a positive

relationship between IL-1β and placental CRH which may lead to enhanced production of the latter and facilitating, as a result, the onset of labor. It seems, however, that critical levels of CRH must be achieved for the onset of labor (161).

IL-6 and CRH are both secreted in a pulsatile function during the active phase of term human labor. The time-integrated concentrations of the two hormones are positively correlated, with IL-6 leading CRH secretion (162). It appears, thus, that proinflammatory mediators may be direct and/or indirect promoters of placental CRH release. Furthermore, the secretion of IL-6, which is a myokine, seems to be associated positively with uterine contractility. Additional studies are needed to elucidate the combined effect of inflammation, placental CRH release and/or CRH-Rs in parturition (162). Corticotropin-releasing hormone induces the production of chemokines and cytokines in myometrium at term and subsequently participates in the cascade of inflammation in uterus (163). The CRH-induced inflammation can lead to activation of the uterus (163).

Pregnancy is known to be a state of transient hypercortisolemia (135). Increased levels of unbound placental CRH may be responsible for the hypercortisolism of the second half of pregnancy. This hypercortisolism is followed by a transient suppression of hypothalamic CRH secretion in the postpartum period. This may explain the depressive states frequently observed in the postpartum period (164).

CRH RECEPTOR ANTAGONISTS. PERSPECTIVES

'Peripheral CRH', which is identical to central CRH, found in immune organs and inflammatory sites is present at high levels in many sites of experimental inflammation in the rat (93, 96) and mouse (32, 104) and in a variety of aseptic or septic inflammatory sites examined thus far in humans. The latter include the inflamed joints of patients with rheumatoid arthritis and osteoarthritis (90).

Multiple research groups have demonstrated 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 administration of CRH antagonists/inhibitors might improve the clinical profile of such conditions. Antalarmin, 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 as a therapeutic tool for both CNS and inflammatory disorders associated with central and peripheral CRH hypersecretion, respectively (165). 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 (165, 166). In addition, antalarmin prolonged survival of mice subjected to LPS-induced septic shock by lowering pro-inflammatory cytokine levels (36).

Astressin B, a nonspecific CRH receptor antagonist is shown to have a different antagonist profile than antalarmin. Astressin B is unlikely to have access to the CNS following systemic administration in contrast to antalarmin which exerts its activity on the CNS. The differences between antalarmin and astressin B may be due either to non-CRH receptor-mediated effects of antalarmin or to a complex interaction of antalarmin effects at both central and peripheral CRH-Rs (167). Another study demonstrated that antalarmin and astressin exert different effects on prostaglandin biosynthetic enzymes and thereby modulate the output of prostaglandins from placenta which would be important for controlling pregnancy and parturition (168).

Xiao et al., showed that astressin B treatment accelerates the return to normal cyclicity, by restoring the normal neuroendocrine regulation of gonadotropin secretion. This effect of CRH receptor antagonist happens although astressin B does not blunt the peripheral HPA axis response to the stressor. The ability to restore normal luteal phase places astressin B or any other CRH antagonist in a very important position in the research of a potential therapeutic agent in stress-related endocrine dysfunction, including the functional hypothalamic chronic anovulation syndrome or the persistent inadequate luteal phase syndrome, and therefore in the treatment of infertility (121).

Given the ability of peripheral CRH to degranulate mast cells, CRH-R1 antagonists 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 (169, 170). In the GI 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 (171, 172). In human endometrium, CRH-R1 antagonists may be used as anti-implantation agents interfering with the inflammatory phenomena taking place during implantation (172). Administration of antalarmin to early pregnant rats (day 1 of pregnancy) results in a 70% reduction in implantation sites (146, 173). These examples illustrate the potential therapeutic significance of the CRH in regulating inflammatory phenomena without affecting the rest of the immune system.

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