Bilateral regulatory action of corticotropin-releasing hormone on immune-mediated inflammation

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In trauma, infection and hemorrhagic shock derived stress, primary and secondary injury may result in severe derangement in the internal environment. The abnormal changes of immune-mediated inflammation interfere its pathogenesis and development directly. In recent years, various aspects of neuroendocrine responses, especially the regulatory effects of hypothalamic-pituitary-adrenal and sympathethico-adrenomedullary axes in inflammatory diseases have been the focus of research. Most importantly, corticotropin-releasing hormone (CRH) acts as a key player in the regulation of interactions between neuroendocrine and immunity both directly and indirectly. The paper summarized the recent development of CRH in the immune-mediated inflammation.

**Key words:** Corticotropin-releasing hormone; Adrenal glands; Inflammation; Immunity; Wounds and injuries

C orticotropin-releasing hormone (CRH), the key regulatory factor in posttraumatic stress, plays a bilateral role via activation of global stress response and peripheral immunoregulatory response. Hypothalamus-pituitary-adrenal (HPA) and sympathethico-adrenomedullary (SAM) axes are the most important for the CRH mediated neuroendocrine responses in trauma. The stress-induced release of hypothalamic CRH leads to systemic secretion of glucocorticoid, epinephrine and norepinephrine, involved in the regulation of immune inflammatory process. Otherwise, the cytokines from immune cells could initiate the CRH secretion through various routes. The extensive distribution of CRH and its receptors in the centrum and periphery indicates the comprehensive effects in the neuroendocrine immunoregulatory network. There is growing evidence that the CRH medi- ated neuroendocrine responses have reasonable immunosuppressive or immunoenhancing actions depending on their concentration, timing and immune parameters.1,2 This review summarized the regulatory role of CRH in immune inflammatory responses in view of the neuroendocrine immune network. Present evidences demonstrate that CRH links neuroendocrine and immune system, forming a coheseive and integrated early host defense system to trauma and injury, which is of great importance for the controlling of uncontrolled inflammatory responses.

**Structure and gene locus of CRH**

CRH is initially discovered from the hypothalamus extracts by Saffran and Schally. Vale et al separated the 41 peptide CRH and identified its amino acid sequence in ovine hypothalamus. The successive researches confirmed that different species, such as rat, mice, humans, have similar CRH molecular structure. The mouse CRH gene is located on chromosome 3, a region syntenic with the human CRH locus 8q12-13, including two exons, separated by an intron in its 5-untranslated region, similar to that in rats and sheep. The human CRH promoter comprises a CAMP response element. Its intron has restriction element-1/ neuron restriction silencing element (RE-1/NRSE) sequence. Alignment of the CRH sequence of human, rats and sheep have revealed the highest degree of homology in

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the 330 base pair (bp) long proximal segment of the 5'-flanking region, suggesting that it may play a very important role in CRH gene regulation for survival. The regulatory elements of human CRH gene include CAMP-responsive enhancer, CREB enhancer sequence/PKA activation phorbol ester, Phorbol ester, 12-O-tetradecanoyl phorbol-13-acetate (TPA)-response element (TRE) or AP-1 site/PKC activation, glucocorticoid response element (GRE), estrogen response element (GRE), TATA box, etc. The newly discovered CRH related peptide family, urocortins, is also involved in the integration of various stress responses. CRH and urocortins have function via G-protein coupled receptors, CRHR1 and CRHR2 with different affinity.

Expression and distribution of CRH

CRH is extensively distributed in central nervous system (CNS) and peripheral sites including immune tissues and inflammatory sites. The hypothalamus is the main origin of CNS CRH. CRH is located in the small cells in paraventricular nucleus (PVN) and other nuclei, with coexistence of many other neuropeptides, such as arginine vasopressin, oxytocin, dynorphin, neotensin. Other neurons releasing CRH are mainly in the neocortex, limbic system, brain stem related to the regulation of automatic nervous system, and intermediate lobe, with the highest expression in amygdale, bed nucleus of the stria terminalis, central gray, dorsal tegmental nucleus, nucleus ceruleus and parabrachial nuclei. The cytokines, TNF-α, IL-1 and IL-6 released in immune cells could immediately stimulate the CRH secretion in hypothalamus. Meanwhile, peripheral CRH and designated immunoreactive CRH are distributed in circulating leukocytes, thymocytes, splenic cells, adrenal cortex and medulla, Leydig cells, gut derived epithelial cells, nerve fiber, some endocrine cells, skin, lungs, testis, placenta, etc. The normal CRH level in plasma is only 6.2 ng/L, while it is elevated in inflammation. In the early phase of inflammatory responses, the immuoreactivity of CRH in dorsal root ganglion, posterior horn, sympathtoblast and sympathetic ganglion indicates that the peripheral CRH is mainly located in peripheral nerve. The immune stimuluss with LPS, ConA, PHA or 12-O-tetradecanoylphorbol-13-acetate could all enhance the secretion of CRH in lymphocytes. The CRH in skin displays differential expression, depending on cell type, physiological or pathological status.

Glucocorticoid plays a key role in the synthesis and releasing of CRH. The expression of CRH mRNA and peptide is downregulated with glucocorticoid treatment. The AtT-20 cells stably transfet human CRH gene, which can also be negatively regulated. The expression of CRH mRNA is inhibited in PVN of the dexamethasone-treated adrenalectomized rats. Otherwise, CRH gene expression is also regulated in human placenta and amygdale central nucleus with glucocorticoid administration.

Central CRH in the crossroad of neuroendocrine axes

The posttraumatic stress can initiate the CRH secretion in hypothalamus, followed by the release of adenocorticotropic hormone (ACTH) in pituitary gland and adrenal hormones. Glucocorticoid may inhibit the ACTH release and even the CRH secretion. ACTH can also limit the CRH secretion of hypothalamus. Meanwhile, CRH can work in a glucocorticoid independent manner, which is related to the central stimulation of the efferent activities of adrenergic nerve. The immune system could be coincidently involved in the release of these hormones and in turn responses.

The stimulation of CRH is the principal route in immune activation of HPA axis. The CRH deficiency can reduce the secretion of glucocorticoid and enhance the inflammation in respiratory tract and lung dysfunction. The intracerebroventricular administration of CRH can inhibit the LPS-induced margination, adherence, emigration, chemotaxis and the expression of adherence factor ICAM-1. But the exogenous glucocorticoid supplement fails to reach the down-regulatory effect induced by CRH, indicating the existence of the glucocorticoid independent mechanism. The research has showed that the levels of serum ACTH and corticosterone both increase in adult CRH knockout (KO) mice and their wild-type (WT) littermates after intraperitoneal injection of LPS, although the level is blunted to some extent in CRH KO mice. The results indicate that the cytokines released after LPS stimulation could act on pituitary and adrenal gland directly to compensate the CRH deficiency and repair the HPA axis responsiveness.

CRH may not be necessary in corticosterone secretion in inflammation. CRH KO mice have a cellular inflammatory response 50% greater than that of WT mice after administrating carrageenin subcutaneously.
However, their level of inflammatory response is similar after adrenalectomy plus glucocorticoid supplement. Hence, the effect of CRH deficiency on immune function is mainly due to the reduction of glucocorticoid secretion. Furthermore, adrenalectomy leads to a 7-fold fall in the inflammatory response of CRH KO mice whereas it has no effect in WT mice. The results demonstrate that the presence of pro-inflammatory factor within the adrenal gland has not been eliminated. The injection of CRH receptor antagonist, antalarmin, could reduce the acute inflammatory response. But after adrenalectomy, the inflammatory response is the same to these two types of mice. Thus, the genetic and pharmacological CRH deficiency could reduce inflammatory responses in combination with adrenalectomy. Finally, the stimulation of acute response is confirmed to be included in the actions of epinephrine.

Peripheral CRH as an immunoreactive neuropeptide

The peripheral CRH, immunoreactive CRH, is involved in regulating immune inflammatory responses as an autocrine or paracrine inflammatory cytokine. The contribution of peripheral CRH has synergistic pro-inflammatory effect with epinephrine in acute inflammatory responses. The administration of rabbit antiserum to CRH causes the suppression of both inflammatory exudate volume and cell concentration. The CRH receptor antagonist can reduce the carrageenin-induced inflammation and adjuvant-mediated arthritis. In the early phase of inflammation, the antiserum to CRH is shown to alleviate the uveitic symptom in mice. The administration of CRH causes the peripheral vasodilatation, congestion and hypotension due to the fluid accumulation of third space in primate and human. Similarly, intravenous injection of CRH can promote the leukocyte margination, adherence and chemotaxis, and stimulate the immune responses. The subcutaneous administration of CRH can induce the increase in the capillary permeability and degranulation in mast cells in a dose-dependent manner. The effect seems more evident as compared with secretagogue of mast cells. The degranulation in mast cells of stress rats’ brain is completely suppressed after the pretreatment of the antiserum to CRH, indicating the essential pro-inflammatory role in peripheral CRH in mast cells.

In the course of initiation, transmission and regulation of inflammatory responses, high concentration of CRH is shown to stimulate the secretion of IL-1 in monocytes, IL-2 in lymphocytes and IL-6 and chemokines in mononuclear cells, and the expression of IL-2 receptor and oxygen-derived free radicals in macrophages. It is also involved in the opiate-like substance-induced analgesia. In the psychological and physiological stress, the local CRH in skin or peripheral nerves could mediate the interactions between HPA-like system and central HPA axis.

According to the previous researches, the CRH in peripheral nerves constitute the axon reflex loop with immune cells. The pro-inflammatory mediators from peripheral immune cells further result in the recruitment Immune cells in inflammation locus and activate the local immune helper cells in the regulation of inflammation.

Researches in vivo and in vitro demonstrated that CRH could enhance the responsiveness to LPS stimulation in either RAW264.7 macrophages or thioglycolate-induced peritoneal macrophages. The amount of cytokines, TNF-α, IL-1β and IL-6 increased. CRH receptor antagonist, antalarmin, could inhibit these courses, and prolong the survival time in endotoxia mice, especially in the early phase of endotoxic shock. Hence, CRH might directly regulate the immune functions at the macrophage levels. The acute inflammatory responses induced by carrageenin are more serious in CRH KO mice compared to WT mice, partly due to the reduction of glucocorticoid. But the persistent exposure to glucocorticoid is shown to alleviate the inflammation in CRH KO mice compared to WT mice. Thus, the pro-inflammatory effect of peripheral CRH is abolished after CRH knockout. In addition, the proliferation of splenocytes is suppressed in CRH deficiency. The reduction of TNF-α and IL-1β after LPS stimulation is probably due to the lower DNA binding capacity of NF-κB.

Concerning the peripheral CRH in inflammation, the anti-inflammatory effect is also viewed. The reason is that the up-regulation of CRH in inflammatory locus is found to relate to peripheral analgesic effect of opium. CRH could also reduce the 5-HT-induced edema, inhibit the carrageenin-induced inflammation in rats, and reduce the excretion of CD11b+ cells and local hyperalgesia. The paradoxical effect of CRH is postulated to be related to the specific state of leukocytes. Slominski et al found that CRH could stimulate the secretion of IL-1 in resting monocytes while plays inhibitory effects after
its activation. The inflammatory locus has plenty of immunoreactive CRH, mainly distributed in immune helper cells and inflammatory exudates. The neutralizing antibody of CRH exerts inhibitory effect, similar to that of TNF-α, in immune inflammatory responses.

**CRH related immune inflammatory diseases**

The excessive responses of HPA axis in inflammatory responses may simulate severe stress. In such condition, the body sensitivity to infections increases with the enhanced resistance to inflammatory disease. Otherwise, the blunted responses in HPA axis may imitate the deficiency of glucocorticoid which results in the resistance to infections and enhanced susceptibility to inflammatory diseases. The CRH neurons in hypothalamus of Lewis rats show low responsiveness to neurotransmitters. The whole body responses to stress of inflammatory diseases are decreased, due to the low level of CRH secretion in hypothalamus. Likewise, patients with active rheumatoid arthritis have low or normal levels of serum ACTH and cortisol although the serum IL-1β and IL-6 are elevated. These patients have showed low reactivity to the stress of surgical intervention. The concentration of immunoreactive CRH increases in the inflammatory joints of these patients. In addition, the excessive immune-mediated inflammatory responses also result from the glucocorticoid resistance in target tissues. In traumatic brain injury (TBI), the gene expression of CRH is increased quickly in PVN and amygdala. The intracerebroventricular administration of CRH antagonist, D-Phe CRH(12-41), significantly alleviates the injury of the rats’ cortex, which indicates that CRH is directly involved in the pathogenesis of TBI.

In severe sepsis, the levels of serum cortisol in death cases are significantly lower than those alive after single intravenous injection of 100 mg CRH. Such low reactivity to CRH stimulation might indicate dysfunction of the endocrine organs. P substance, the inhibitors of CRH secretion in hypothalamus, is elevated possibly on account of the inhibition of CRH neurons in inflammation. Otherwise, the increase in inflammatory cytokines and interferon (IFN)-γ may restrict the HPA axis through blocking the effect of CRH and ACTH, seen in sepsis patients. The adrenal responses to the stress-induced or exogenous CRH and ACTH are damaged.

The central reduction of CRH and increase in peripheral immunoreactive CRH is related to the enhancement of sensitivity to autoimmune disease. The sensitivity and extent to acute inflammatory responses in female, including the expression of immunoreactive CRH, is reduced more significantly than those in male. The estrogen could stimulate the CRH expression. CRH knockout mice are showed to have resistance to experimental autoimmune encephalomyelitis (EAE). The clinical scoring and inflammatory infiltration in central nervous system in CRH KO mice are alleviated compared to WT mice. Furthermore, Antigen-specific responses of primed T cells as well as anti-CD3/anti-CD28 TCR costimulation decrease in CRH KO mice with decreased production of Th1 cytokines and increased secretion of Th2 cytokines. WT mice treated in vivo with a CRH antagonist have showed a decrease in IFN-γ production by primed T cells in vitro. This effect of CRH is independent of its ability to increase in corticosterone production because adrenalectomized wild-type mice have similar disease course and severity as control mice. Also, the IκB phosphorylation induced by TCR cross-linking is decreased in CRH deficiency T cells. These data demonstrate that peripheral immunoreactive CRH plays pro-inflammatory responses through selectively enhancing the Th1 response. Our laboratory recently found that the CRH KO mice possessed inhibited chemotaxis in peritoneal macrophages, and enhanced bacterial translocation as compared to WT mice after thermal injury. These results provide strong evidence for the bilateral regulatory actions of CRH in immune inflammatory process.

**Prospects**

The biosystem, sophisticated open system in interior structure, keeps their homeostasis in multilateral circumstance via the interactions of neuroendocrine and immune systems. CRH acts as a checkpoint in these courses. The necessity of further investigation of CRH in the basic research of trauma and injury is overwhelming. Recent evidence has shown that the regulatory pathway of CRH on immune inflammatory responses at least consists of the stimulus of the releasing of ACTH and glucocorticoid with CRH alone or concomitance with AVP, and the receptors on the surface of immune cells. Hence, different levels (animals, tissues, cells molecules and genes) of interference of CRH regulation might be considered with traditional and advanced techniques. In the research of posttraumatic stress around the CRH mediated neuroendocrine
pathway, the functional inactivation of CRH in the specific brain areas will help to disclose the compensatory or concomitant effects in the lack of CRH and adrenal hormones. Furthermore, the strategy of exogenous supplement and endogenous excitation is reasonable for the consonance of neuroendocrine regulated immune inflammatory responses, which might be of great importance for the abundance of trauma theory and improvement of its remedy.

REFERENCES


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