

## CRH and the immune system

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### Abstract

Inflammatory cytokines released during immune system activation can stimulate the hypothalamic–pituitary–adrenal axis and cause increased secretion of corticotropin-releasing hormone (CRH), adrenocorticotropin and glucocorticoids. Identification of CRH peptide and mRNA, as well as its receptors in immune tissues, suggested a role for this peptide as a mediator of the neuroendocrine–immune interactions. Experimental evidence suggests that CRH may modulate the immune and inflammatory responses via two pathways: an antiinflammatory one operated by centrally released CRH, most likely through stimulation of glucocorticoid and catecholamine release, and one proinflammatory, through direct action of peripherally released CRH. This review highlights these concepts. In addition preliminary data on immune activation and inflammatory response in CRH-deficient mice created in our laboratory are discussed.

*Keywords:* Corticotropin-releasing hormone; Neuroendocrine–immune interaction; Hypothalamic–pituitary–adrenal axis

### 1. Introduction

Communication between the nervous, endocrine and immune systems is achieved via ligands and receptors shared by all three systems (Payan and Goetzl, 1985; Bateman et al., 1989; Blalock, 1989; Saphier, 1989; Reichlin, 1993; Gaillard, 1994). The array of adaptative mechanisms elicited in response to threatened homeostasis constitutes the stress response. Immune activation together with the inflammatory process are elements of this adaptative response to stressors, which includes activation of the hypothalamic–pituitary–adrenal (HPA) axis (Chrousos and Gold, 1992). The HPA axis, via stimulation of hypothalamic release of corticotropin-releasing hormone (CRH) (Vale et al., 1981) and activation of the catecholaminergic system, is a major mediator of the stress response (Chrousos and Gold, 1992; Valentino et al., 1993). Cytokines stimulate the HPA axis directly, causing increased secretion of CRH, adrenocorticotropin (ACTH) and glucocorticoids (Leme and Schapoval, 1975; Besedovsky et al., 1986; Bateman et al., 1989; Rivier et al., 1989a), and indirectly by augmenting the release of other HPA axis

regulators, such as catecholamines and prostaglandins (Katsuura et al., 1988; Rivier et al., 1989b; Watanabe et al., 1990). The intriguing concept that excessive activation of the immune system is restrained by the HPA axis, and particularly by adrenal glucocorticoids, has been proposed (Munck et al., 1984; Kapcala et al., 1996). In this review we will focus on the role of CRH during the activation of the immune system as a potential mediator of neuroimmune–endocrine interactions.

### 2. Cytokines activate the HPA axis

Upon activation of the immune system, a cascade of cytokines, including  $\text{TNF}\alpha$ , IL-1, and IL-6, are produced by leukocytes and accessory cells of the inflammatory response. A plethora of studies has shown that cytokines can stimulate the HPA axis (Reichlin, 1993) and cause release of adrenal glucocorticoids, which then downregulate the expression of several components of inflammation (Fauci et al., 1976; Boumpas et al., 1993). Thus, a feedback system operates between activated adrenals and the immune cells that may prevent unrestrained inflammatory responses. This important function of the HPA axis during immune activation is demonstrated in adrenalectomized mice, which following lipopolysaccharide (LPS) administration have very high  $\text{TNF}\alpha$  and IL1 levels and greatly increased lethality (Bertini et al., 1988). Other studies have suggested that defects of HPA axis responsiveness might

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be implicated in the pathogenicity of several models of autoimmune diseases in rodents (Wilder, 1995). Whether physiologic adrenal glucocorticoid secretion modulates the immune responses to specific antigens has not yet been established.

The majority of *in vivo* studies suggest that direct stimulation of hypothalamic CRH by cytokines is the principal route by which immune activation stimulates the HPA axis (Besedovsky et al., 1986; Sapolsky et al., 1987; Suda et al., 1990; Harbuz et al., 1992). This process is prostaglandin-dependent, although in some studies adrenergic receptor-mediated pathways seem also to be involved (Matta et al., 1990; Rivier and Rivest, 1993). How cytokines reach areas of the brain protected by the blood–brain barrier remains unclear. Several possibilities have been raised, including (i) action of cytokines outside of the blood–brain-barrier, such as at the circumventricular organs, where IL1 receptors are expressed (De Souza, 1993), (ii) specific transport of cytokines into the brain (Banks et al., 1991), and (iii) a paracrine mode of action of IL1 produced in either brain blood vessels or within the CNS itself (Katsuura et al., 1990; Breder et al., 1994; Tilders et al., 1994). Direct effects of cytokines on the pituitary have also been reported, but require more prolonged and higher levels of cytokines compared to those needed for hypothalamic stimulation (Kehrer et al., 1988; Kovacs and Elenkov, 1995). Finally, cytokines such as  $\text{TNF}\alpha$ , IL1 and IL6 also can directly stimulate adrenal steroidogenesis, mainly through catecholaminergic pathways (Winter et al., 1990; Andreis et al., 1991; Gwosdow et al., 1992; O'Connell et al., 1994).

Interestingly, in chronic inflammatory states such as in adjuvant-induced arthritis in rats, activation of the pituitary–adrenal axis seems to be maintained by increased hypothalamic vasopressin (AVP) secretion, with elevated CRH expression found only during the initial stages of the disease (Chowdrey et al., 1995).

### 3. CRH in immune cells

A role for CRH expressed in peripheral tissues during activation of the immune system was postulated by identification of the peptide and its mRNA in immune tissues. Thus, immunoreactive CRH and CRH mRNA have been identified in human peripheral blood cells (Stephanou et al., 1990) and peripheral lymphocytes (Ekman et al., 1993). Bioactive and immunoreactive CRH is present in rat thymus and spleen (Redei, 1992; Aird et al., 1993). CRH mRNA has been found in rat thymus (Redei, 1992), and in mouse T lymphocytes (Muglia et al., 1994). A direct role for CRH in immune and inflammatory responses is further supported by binding of radiolabeled CRH to human peripheral blood lymphocytes and monocytes (Singh and Fudenberg, 1988; Audhya et al., 1991), and to resident mouse splenocytes (Webster and DeSouza, 1988; Webster et al., 1990).

Recent evidence suggests that neuropeptides synthesized and released by sensory afferent neurons and sympathetic nerve fibers are important modulators of inflammatory responses (Payan and Goetzl, 1985). CRH is expressed in sensory and sympathetic nerve fibers, and in dorsal root ganglia (Merchenthaler et al., 1983). Colocalization of CRH with substance P, a major regulator of inflammation in capsaicin sensitive fibers (Skofisch et al., 1984), further suggests a role for this peptide in the inflammatory process.

## 4. Effects of CRH on the immune system

### 4.1. *In vitro* actions

Several *in vitro* studies suggest an immunomodulatory action of CRH on immune cells, although findings have been contradictory. CRH has been shown to stimulate B- (McGillis et al., 1989) and T-lymphocyte proliferation and expression of IL2 receptors (Singh, 1989), enhance both lysis mediated by NK cells (Leu and Singh, 1991) and chemotaxis (Genedani et al., 1992), and enhance leukocyte IL1 and IL2 (Singh and Leu, 1990), and IL6 secretion (Leu and Singh, 1992). Other studies have shown CRH to inhibit gamma-interferon secretion (Angioni et al., 1993), IL2-induced splenocyte proliferation as well as LPS-mediated IL1 and IL6 production by peripheral blood monocytes (Hagan et al., 1992). CRH was able to promote induced antibody responses (Irwin, 1993). Stimulation of the production of leukocyte-derived ACTH and  $\beta$ -endorphin by CRH led to the hypothesis of a CRH–ACTH axis operating in leukocytes during immune activation (Smith et al., 1986). CRH has also been shown to stimulate acute-phase protein expression in hepatocytes (Hagan et al., 1993). CRH has also been reported to inhibit vascular permeability (Wei et al., 1993). In our preliminary studies we have also found that CRH, at physiologically relevant doses, is a potent stimulator of angiogenesis, a physiological process linked to inflammation (J. Arbiser et al., manuscript in preparation).

### 4.2. *In vivo* actions

Immunoreactive CRH has been found in peripheral tissues, during acute or chronic inflammatory states (Karalis et al., 1991; Crofford et al., 1992; Crofford et al., 1993; Skopa et al., 1994). As we have previously shown, CRH extracted from rodent and human inflamed tissues has the same electrophoretic profile with h/r CRH 1–41 (Karalis et al., 1991), is down-regulated by glucocorticoids, and its pattern of expression is similar to that of the inflammatory mediators substance P and  $\text{TNF}\alpha$  (Karalis et al., 1995a). Peripheral CRH seems to exert proinflammatory effects, since administration of CRH antiserum can significantly decrease acute inflammation *in vivo* in several models of

inflammation (Karalis et al., 1991; Castaghuolo et al., 1995).

Stimulation of the release of hypothalamic CRH during the inflammatory response leads to secretion of glucocorticoids, a strong antiinflammatory agent. Furthermore, central administration of CRH exerts immunosuppressive effects independent of glucocorticoid action, as shown by reduction of NK cell activity (Irwin et al., 1987) and inhibition of *in vivo* specific antibody responses (Irwin et al., 1988, Irwin et al., 1989). Furthermore, co-administration of a CRH antagonist blocks the immunosuppressive effects of CRH, suggesting that these effects are mediated by specific receptor mechanisms (Irwin, 1993). Peripheral administration of CRH by these same investigators was not found to exert any immunosuppressive effects (Irwin et al., 1990).

A natural paradigm of hyporesponsive central CRH neurons associated with decreased corticosterone secretion has been found during immune activation in the Lewis rat (Sternberg et al., 1989). Lewis rats show a higher propensity to develop severe inflammation following a variety of stimuli, as compared to histocompatible Fischer 344 rats, which have greater responses in CRH neurons following the same stimuli (Wilder, 1995). We have shown that administration of dexamethasone caused dose-dependent inhibition of acute aseptic inflammation in Lewis rats, while administration of the glucocorticoid antagonist RU 486, caused a dose-dependent increase of the inflammatory response of Fischer rats (Karalis et al., 1995b). Our findings suggest that manipulation of glucocorticoid levels may reverse the effects of central CRH deficiency on the inflammatory response. Interestingly, immunoreactive levels of peripheral CRH are increased in Lewis rats with experimental arthritis (Crofford et al., 1992), possibly a result of their glucocorticoid-deficient state, and suggests differential regulation of central and peripheral CRH.

The above suggest that CRH plays a dual, antithetical role in the control of the inflammatory response: a proinflammatory, direct action of peripheral CRH, and an anti-inflammatory, indirect role of central CRH, most likely through stimulation of glucocorticoid and catecholamine release.

## 5. The CRH-deficient mouse: Immune and inflammatory responses

A CRH-deficient (CRH<sup>-/-</sup>) mouse has been created in our laboratory by targeted gene deletion and homologous recombination in embryonic stem cells (Muglia et al., 1994; Muglia et al., 1995). CRH<sup>-/-</sup> mice have low basal and stimulated corticosterone blood levels. Despite their altered glucocorticoid status, life expectancy of CRH<sup>-/-</sup> mice does not differ from their wild-type (CRH<sup>+/+</sup>) littermates and is not complicated by spontaneous infections or any other sign of immune system incompetence. Administration of glucocorticoids to CRH<sup>-/-</sup>

homozygote matings is required for fetal lung development to allow for the postnatal survival of CRH<sup>-/-</sup> litters, but postnatal glucocorticoid supplementation is not necessary despite severe glucocorticoid deficiency. To study the consequences of CRH deficiency on immune system activation, we have used three experimental models of inflammation: a model of acute aseptic inflammation, acute endotoxemia, and high affinity T-cell receptor activation.

### 5.1. Acute inflammatory response

Our previous findings suggested a proinflammatory role for peripheral CRH during inflammation (Karalis et al., 1991). We used CRH<sup>-/-</sup> mice to study the consequences of CRH deficiency in the development and progress of carrageenin-induced aseptic acute inflammation. Carrageenin is a seaweed polysaccharide which, when injected locally, induces development of an acute aseptic inflammatory reaction characterized by accumulation of an exudate rich in polymorphonuclear cells and monocytes (Kumakura et al., 1988). The acute phase of this reaction peaks at 7 h and is inhibited by dexamethasone administration (Laue et al., 1988). We administered carrageenin to male CRH<sup>-/-</sup> mice and their CRH<sup>+/+</sup> littermates according to the experimental protocol used previously in rats (Karalis et al., 1991). The inflammatory response of CRH<sup>-/-</sup> mice was much higher (50% higher) compared to that of their wild type littermates, as shown mainly by the leukocyte concentration of the exudate. Carrageenin-induced inflammation stimulates a stress response, and thus causes increased secretion of glucocorticoids which down-regulate the expression of several mediators of the inflammatory response. CRH<sup>-/-</sup> mice have lower corticosterone levels compared to their wild type littermates following other stressful stimuli such as ether inhalation or restraint. We hypothesized that decreased corticosterone levels of CRH<sup>-/-</sup> mice during carrageenin-induced inflammation could cause their increased inflammatory response, compared to CRH<sup>+/+</sup> mice. In fact, corticosterone levels of CRH<sup>-/-</sup> mice measured at the peak of the inflammatory response were significantly lower (60% lower) compared to those of CRH<sup>+/+</sup> mice (Karalis et al., 1996). It is interesting that corticosterone levels of the inflamed CRH<sup>-/-</sup> mice were elevated approximately 10-fold above base-line levels, indicating that adreno-corticosteroid activation during inflammation can occur even with absolute CRH deficiency. To study the impact of the relative role of glucocorticoid versus CRH deficiency on the inflammatory response of CRH<sup>-/-</sup> mice, we simultaneously adrenalectomized both CRH<sup>-/-</sup> and CRH<sup>+/+</sup> mice and implanted a subcutaneous pellet containing a standard physiological dose of corticosterone (Akana et al., 1985). This procedure restores basal levels of blood corticosterone to approximately 5  $\mu\text{g}/\text{dl}$ , but, because it is released at a constant rate, corticosterone never reaches the peak levels physiologically attained during the circadian cycle or the stress

response. Following adrenalectomy and pellet replacement, corticosterone levels in CRH  $-/-$  and CRH  $+/+$  mice were similar, both before and during carrageenin-induced inflammation. Interestingly, the inflammatory response of adrenalectomized CRH  $-/-$  mice with corticosterone pellet implantation was much lower (65% lower) than that of similarly treated CRH  $+/+$  mice, as indicated by the leukocyte concentration of the inflammatory exudate (Karalis et al., manuscript in preparation). This suggests that when glucocorticoid levels are similar, CRH is required for the induction of the inflammatory response, supporting a proinflammatory role for peripheral CRH.

### 5.2. LPS-induced endotoxemia

LPS administration in experimental animals, widely used as a model of gram-negative bacteremia and septic shock, is a very potent stimulus of the HPA axis (Moberg, 1971; Perlstein et al., 1993; Rolih and Ober, 1995). LPS administration causes secretion of cytokines, such as TNF $\alpha$ , IL1, and IL6, that stimulate the HPA axis (Chensue et al., 1991). Most studies suggest that stimulation of the HPA axis following LPS is mediated by CRH (Sapolsky et al., 1987; Rivier et al., 1989a; Rivier et al., 1989b), which causes increased ACTH secretion with a peak response at 1½ to 2 h after the injection, followed by a more sustained elevation of glucocorticoid release. We measured ACTH and corticosterone levels in CRH  $-/-$  and CRH  $+/+$  mice 0, 1½, 4 and 6 h following intraperitoneal injection of 200  $\mu$ g of LPS. To our surprise, LPS administration caused a significant elevation of ACTH and corticosterone blood levels in CRH  $-/-$  mice throughout the time period studied. This response, although approximately 40% lower than that of CRH  $+/+$  mice, indicates that CRH is not required for LPS-induced activation of this axis. This suggests that either CRH is not required for stimulation of the pituitary–adrenal axis by LPS in the normal mouse, with cytokines acting directly at the level of pituitary and/or adrenal, or that in CRH-deficient mice, compensatory mechanisms not present in the normal mouse have developed, which restore HPA axis responsiveness following immune activation (Karalis et al., manuscript in preparation).

### 5.3. High affinity T cell receptor-induced thymocyte apoptosis

Clonal deletion of intrathymic T-lymphocytes by apoptotic mechanisms is believed to be a major component of the response of the immune system involved in modulation of self-recognition (Lo, 1992). An experimental paradigm mimicking high affinity T cell interaction and leading to T-lymphocyte apoptosis consists of administration of an antibody against the CD3-T cell receptor complex of CD4 + CD8 + thymocytes (Smith et al., 1989). Glucocorticoids have been implicated as regulators of apoptosis-induced programmed cell death (Compton et al., 1991; Gruber et

al., 1994). We monitored T cell distribution and apoptosis, along with blood corticosterone levels in CRH  $-/-$  and CRH  $+/+$  mice following administration of anti-CD3 $\epsilon$  antibody (2C11). To our surprise no differences in T cell subtype distribution or extent of apoptotic cell death, as shown by DNA fragmentation, were found between CRH  $+/+$  and CRH  $-/-$  mice given anti-CD3 $\epsilon$  antibody. Furthermore, the plasma corticosterone levels of treated CRH  $-/-$  deficient mice at the peak of the apoptotic process (12 h after administration of anti-CD3 $\epsilon$ ) were elevated to levels comparable to those of CRH  $+/+$  mice (Bae et al., 1996). This finding indicates that immune stimulation can activate adrenal glucocorticoid secretion by pathways that do not require CRH. In support of the absolute requirement for glucocorticoids during antiCD3 $\epsilon$ -induced T cell apoptosis, we found that adrenalectomized CRH  $-/-$  and CRH  $+/+$  mice, which are incapable of any endogenous glucocorticoid production, show no evidence of T-lymphocyte apoptosis following antibody administration (Bae et al., manuscript in preparation).

## 6. Summary

Although the regulation and function of CRH in various peripheral tissues are inadequately understood, experimental evidence strongly indicates that CRH is an important mediator of the communication between neuroimmune and endocrine systems during immune system activation. The effects of CRH on the inflammatory response are both stimulatory and inhibitory, and are likely exerted by peripheral and central CRH, respectively. CRH-deficient mice provide a unique model with which to study the role of CRH and endogenous glucocorticoids in the control of the inflammatory response. Furthermore, this model may help to more precisely identify the locus of CRH action in the inflammatory cascade, including its role in the regulation of inflammatory cytokine synthesis and release.

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