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Review

Immunomodulatory Role of the Corticotropin-Releasing Factor

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Abstract. Corticotropin-releasing factor (CRF) was originally identified as a hypothalamic peptide which stimulates secretion of the hypophyseal adrenocorticotropin hormone. CRF exhibits its actions through G protein-dependent seven membrane domain receptors. Two subtypes of CRF receptors (CRF-R1 and CRF-R2) have been characterized thus far. CRF and its receptors were found in a number of brain regions, where they function by neuromodulation and also in several peripheral organs. Besides CRF, another naturally occurring CRF-like peptide, urocortin, has been characterized. In the immune system, CRF and CRF-R1 were so far detected at both mRNA and protein levels in several lymphoid organs and at sites of inflammation. Locally injected CRF was shown to modulate the severity of inflammation. This effect was not only a result of hemodynamic changes known to be induced by CRF or by activation of the hypothalamo-pituitary-adrenal axis, as CRF-binding sites were also found on immune cells. CRF was shown to directly modulate secretion of cytokines and neuropeptides, proliferation, chemotaxis and degranulation of purified macrophage and lymphocyte populations *in vitro*. Functional CRF-R was more recently demonstrated also on polymorphonuclear cells and significant amounts of CRF were shown to be produced in lymphoid organs, or delivered to lymphoid organs by peripheral nerves. Taken together, the experimental results obtained so far strongly point to the importance of CRF as a signaling molecule in lymphoid tissues and at the sites of inflammation.

Key words: corticotropin-releasing factor; inflammation; neutrophil; stress; immune system.

Introduction

Stress is associated with activation of the hypothalamic-pituitary-adrenal (HPA) axis, and this activation has been proposed as a defining endocrine feature of the stress response. The primary mediator of this response is corticotropin-releasing factor (CRF), a 41-residue peptide[45] accepted as the principal stimulus for stress-induced secretion of the hypophyseal adrenocorticotropic hormone[50] (ACTH). ACTH in turn regulates the production of glucocorticoids by the adrenal cortex. The action of CRF is exhibited through CRF receptors (CRF-R) which belong to a G protein-coupled receptor superfamily with seven transmembrane domains[10] and

Abbreviations used: HPA axis – hypothalamic-pituitary-adrenal axis, CRF – corticotropin-releasing factor, ACTH – adenocorticotropic hormone, CRF-R1 – CRF receptor type 1, CRF-R2 – CRF receptor type 2, cAMP – cyclic adenosine monophosphate, IL-1 – interleukin-1, CNS – central nervous system, LPS – lipopolysaccharide.

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are expressed as different subtypes, CRF-R1 and CRF-R2. A CRF-related mammalian peptide, which was named urocortin, has been identified recently[51].

Immunomodulatory CRF Effects Mediated by the HPA Axis

It is well established that CRF derived from hypothalamus exerts suppressive effects of stress on the immune system indirectly through activation of the HPA axis[24]. Conversely, in response to inflammation, the immune system is activated and can stimulate the HPA axis by increased CRF levels in the hypophyseal portal circulation[38]. Mediators of such HPA axis stimulation by the immune system are mainly interleukin 1 (IL-1), tumor necrosis factor α (TNF-α) and interleukin 6[5, 38] (IL-6). Taken together, injury and inflammation result in immediate secretion of cytokines from immune cells into the local tissue and circulation[15]. This leads to mobilization of immune defense mechanisms to fight the intrusion of foreign material and, at the same time, to an activation of the HPA axis which prevents those mechanisms from overshooting and thus becoming harmful[9, 46, 55]. Additionally, it has been proposed that CRF may mediate its immunosuppressive effects by enhancement of the central sympathetic out-flow[8, 24].

Effects of Locally Injected CRF on the Signs of Inflammation

There is ample evidence relating CRF to the inflammatory response[25, 33, 34]. Both pro- and anti-inflammatory local paracrine actions of CRF were reported at the site of inflammation. In a rat model of acute inflammatory reaction induced by carrageenan, systemic pre-treatment with anti-CRF antibodies reduces the inflammatory response[25], thus suggesting a pro-inflammatory action of CRF. On the other hand, CRF was reported to suppress experimental autoimmune encephalomyelitis in rats[33] and to suppress the formation of edema (reviewed in[53]). We have shown that CRF exerts either a pro- or anti-inflammatory effect on carrageenan-induced rat paw edema, depending on its concentration[12]. Low doses of CRF injected directly into the hind paws exert anti-inflammatory, whereas high doses result in pro-inflammatory effects[12].

In addition to its immunomodulatory effects, CRF was also reported to induce the secretion of β-endorphin from immune cells in inflamed tissue, resulting in inhibition of pain[29].

Direct Effects of CRF on Immune Cells

In vivo immunomodulatory effects of CRF injected at sites of inflammation may have been largely a result of its vasodilatory effect and increase of vascular permeability[28, 47, 48] which promote edema. Additionally, it is possible that effects of CRF injected locally at the site of inflammation were exerted by CRF entering the systemic circulation and binding to CRF receptors in the pituitary. Thus, through activation of the HPA axis an increase of the level of corticosterone, the potent immunomodulatory hormone, may result. However, *in vitro* effects of CRF on purified leukocyte populations strongly suggest that immune cells also bear CRF receptors. CRF promotes proliferation of rat splenocytes[31], cAMP production in human monocytes[42], IL-6 secretion by human mononuclear cells[3], chemotaxis by human mononuclear leukocytes[17], enhances production of oxygen radicals by rabbit macrophages[26], induces degranulation of mast cells from rat[47] and ACTH production in leukocytes of chicken[22]. CRF was also found to cause a bipolar-shape response of human neutrophils in some individuals[23]. Thus, chemotactic properties of CRF are suggested.

Taken together, effects of CRF were almost exclusively examined in the mononuclear cell fraction, but not in polymorphonuclear cells (neutrophils), which express higher levels of CRF-R1 than mononuclear immune cells in mouse[34, 35]. We have recently shown that CRF inhibits the secretion of IL-1β from mature neutrophils purified from spleens of mice injected with lipopolysaccharide[34] (LPS; Fig. 1). On the basis of an

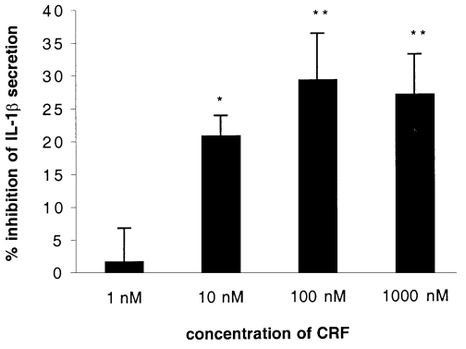

Fig. 1. Inhibition of IL-1β production by ovine CRF. Comparison of the concentrations of IL-1β in the supernatants of splenic neutrophils incubated for 5 h with different concentrations of ovine CRF. Neutrophils were purified from spleens of mice that were injected with 100 μg LPS 12 h earlier as described[34]. Results are expressed as mean values ±SEM of the percent of reduction of IL-1β concentration in comparison to cells incubated without CRF. Statistically significant differences determined by ANOVA: *$p<0.05$; **$p<0.01$, in comparison to cells incubated without ovine CRF. A representative experiment of a total of 5 experiments is presented

experiment with the endotoxic shock model[34], it has been proposed that CRF may exert a protective effect in sepsis by acting as a negative feedback to limit IL-1β secretion from CRF-R1$^+$ neutrophils. On the other hand, CRF did not exhibit a direct effect on the production of superoxide radicals nor on exocytosis, nor did it modulate these anti-microbial functions of neutrophils when they were induced by stimulation with formyl hexapeptide or phorbol myristate acetate[34]. IL-1 has been demonstrated to be one of the principal mediators of LPS-induced toxicity on the basis of IL-1 receptor blockade experiments[2]. Neutrophils should be considered as a major source of IL-1β in view of the finding that following intratracheal LPS injection, most of the IL-1β RNA from bronchoalveolar lavages is attributable to polymorphonuclear, as opposed to mononuclear cells[49]. Similarly, after intravenous infusion of LPS into rats, most of the IL-1β RNA was detected in a fraction enriched with polymorphonuclear leukocytes harvested from the pulmonary vasculature[54]. Others have found inhibitory effects of CRF on IL-1 and IL-6 expression induced by LPS in human monocytes[20]. The inhibitory effects of glucocorticoids and CRF on the secretion of IL-1β from human mononuclear cells were shown to be additive[20]. In contrast, CRF increases the IL-1 production[42] of the same human immune cell type. More recent findings suggest that the ability of CRF to either enhance or inhibit the IL-1 production by monocytes depends on the level of monocyte activation[32]. Thus, CRF increases the production of IL-1 and IL-1 receptor antagonist in resting cells, whereas it acts by inhibition on activated monocytes.

CRF Receptors on Immune Cells

CRF-binding sites of immune cells, identified by binding of radioactively labeled CRF, have been localized to murine adherent splenocytes[52], or human monocytes and T lymphocytes[43]. In contrast to these reports, using autoradiographic detection methods[43, 52] or functional CRF-dependent assays[4, 37, 40, 42], we could not detect CRF-R1 on a significant number of mature murine mononuclear cells by use of immunohistochemical analysis of CRF-R1 immunoreactivity in the spleen[34, 35]. Thereby, CRF-R1$^+$ cells from spleens of naive mice were identified as mature and immature neutrophils[34]. However, the studies mentioned[4, 37, 40, 42, 43, 52] did not determine the type of the CRF receptor. Therefore, the effects of human/rat CRF could have been caused by its binding to CRF-R2[16] or to an unknown CRF-R subtype. Alternatively, this discrepancy may be due to differences between species.

Interestingly, CRF did not induce an increase of intracellular cAMP in CRF-R1$^+$ splenic neutrophils (RADULOVIC and SPIESS, unpublished results). On the basis of this result, it is suggested that CRF-R1 on neutrophils uses a different signalling pathway than the pituitary CRF-R1[19].

mRNA coding for CRF-R1 in spleens of naive animals was found to be identical in nucleotide sequence to pituitary CRF-R1 (RADULOVIC et al, unpublished results). On the basis of the lack of expression of CRF-R1 mRNA in major neutrophil pools in the absence of immunologic challenge it was concluded that CRF-R1 was not constitutively expressed by all mature or immature mouse neutrophils. Although most of the CRF--R1$^+$ spleen cells of naive mice were identified as neutrophils, CRF-R1 mRNA could not be detected in the main neutrophil pools outside of the spleen, such as bone marrow or peripheral blood leukocytes[34]. The evaluation of the amount of mRNA coding for CRF-R1 has been extended here to thymus and lymph nodes by means of semi-quantitative PCR (Fig. 2). CRF-R1 mRNA was much less abundant in thymus than in the spleen, whereas it was berely detectable in mesenterial lymph nodes (Fig. 2). The relative amounts of CRF-R1 mRNA in peripheral lymphoid organs of naive animals positively correlated with the abundance of CRF-R1$^+$ cells in these lymphoid organs (Table 1 and Fig. 2).

The number of CRF-R1$^+$ cells in the spleen is dramatically upregulated following the induction of acute

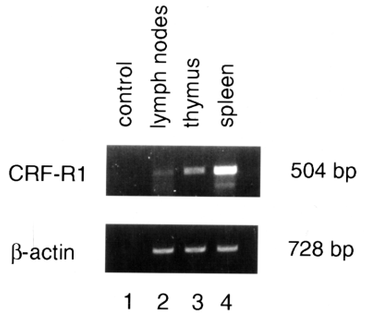

Fig. 2. Expression of CRF-R1 mRNA in lymphoid organs of a naive mouse analyzed by RT-PCR. Lane 1 – 20 ng of splenic RNA, lane 2 – mesenteric lymph node, lane 3 – thymus, lane 4 – spleen. RT-PCR was performed using specific primer sets for CRF--R1 or β-actin. The primers used for PCR were 5'-TACTTCCACGTAACCAACTTCTTCTGGATG-3' (mouse CRF-R1, sense); 5'-AACAATAGAACACAGACACGAAGAAGCCC-3' (mouse CRF-R1, antisense). Total RNA was obtained from organs of the same naive animal as described[34]. Samples were amplified for 33 cycles (CRF-R1 primer set) or 17 cycles (β-actin primer set). Data are representative of seven naive C57BL/6J mice

Table 1. Abundancy of CRF-R1$^+$ cells in lymphoid organs of naive C57BL/6J mice

Number of cells[a]		
lymph nodes	thymus	spleen
1.5±0.7	4.2±0.4	39.0±7.4

[a]Expressed as mean of absolute number ± SEM of CRF-R1$^+$ cells per 10^6 μm^2. Calculation was performed on the basis of data obtained from four animals per data point from a representative experiment. The findings have been reproduced in two additional experiments.

or chronic inflammation[34]. Interestingly, acute and chronic inflammation are linked to the upregulation of CRF-R1 on neutrophils at distinct stages of maturation[34]. Following an acute inflammation CRF-R1 is selectively upregulated on mature neutrophils, while in a chronic inflammatory model CRF-R1-bearing cells are mostly immature neutrophils[34].

The number of CRF-R1$^+$ mature neutrophils in mouse is also increased even following a non-immunological stimulus, such as immobilization stress[35]. Interestingly, this effect is specific for the spleen as the number of CRF-R1$^+$ cells following immobilization was reduced in thymus and unchanged in lymph nodes. It is therefore conceivable that the observed increase of CRF-R1 was either initiated or directly mediated by some of the messengers of the CNS response to stressors such as catecholamines, corticosterone or endogenous opioids[14, 27]. This suggestion is in agreement with the results of BROUXHON et al. [7] showing close apposition of noradrenergic nerve fibers with CRF$^+$/ /CRFmRNA$^+$ cells in lymphoid organs. Endotoxin and immobilization stress were previously shown to stimulate CRF-R1 gene transcription in the hypothalamic paraventricular nucleus[36] with a time course similar to that of the appearance of CRF-R1 protein in the spleen[34, 35]. The simultaneous increase of the production of CRF-R1 in selective brain areas and in the spleen is suggestive of a common underlying mechanism that triggers CRF-R1 production. However, the specific stimulus responsible for the induction of CRF-R1 remains unknown. Experiments aimed to define the stimulus that induces CRF-R1 in neutrophils are currently underway.

The fraction of total mature splenic neutrophils producing CRF-R1 is maximally 46%[34, 35]. CRF-R1 can therefore be regarded as a marker of a distinct subpopulation of mature neutrophils. Antigenically distinguishable populations of neutrophils were also observed by use of other antibody preparations[39].

The Origin of Ligand(s) Acting on CRF Receptors on Immune Cells

As hypothalamic CRF acts through portal blood over a very short distance, its concentration in peripheral blood is very low[18] (approx. 27 pM) and it is therefore unlikely that hypothalamic CRF could act on CRF--R1$^+$ immune cells in the periphery. Therefore, actions of CRF at peripheral sites are expected to be mediated by CRF that is locally produced, or delivered by peripheral nerves. Accordingly, it was shown that cytokines released in response to inflammation not only stimulate the expression of CRF in the hypothalamus, but also at the site of inflammation[21]. In human inflamed synovial tissues CRF is present in the same concentrations as in hypophyseal portal venous blood[13]. CRF is produced by mononuclear leukocytes, tissue fibroblasts and vascular endothelial cells at inflamed areas in rats[25], rat thymus and spleen[1] and mouse immune cells in the spleen, thymus and lymph nodes[7]. In contrast, others have found CRF immunostaining only in nerve fibers in the splenic capsule and trabeculae of the rat[6]. The demonstration of CRF-like immunoreactivity in the dorsal horn of the spinal cord and dorsal root ganglia of rats[30, 44] agrees with the hypothesis that CRF detected in immune organs and at sites of inflammation may be not only produced by immune cells, but also delivered by peripheral nerve endings.

The observation of low levels of CRF in immune organs[11] has implied that locally produced CRF exerts only short-range actions. This assumption is compatible with the observation of the similar anatomical distribution of CRF[7] and CRF-R1 producing cells in spleen, thymus and lymph nodes[35]. CRF-containing cells may thus provide a paracrine and/or autocrine source of ligand for binding to CRF-R1 on neutrophils in the spleen.

In summary, experimental results of many laboratories strongly point to the importance of CRF as a signaling molecule in lymphoid tissues and at the sites of inflammation. CRF may also mediate the effects of stress on the immune system in mouse by directly binding to CRF-R1$^+$ neutrophils.

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