Neuronal mechanisms contribute to corticotropin-releasing factor-induced anti-oedema effect in the rat hind paw

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Summary The present study is designed to elucidate the involvement of neuronal mechanisms in corticotropin-releasing factor (CRF)-induced anti-oedema effects. Oedema was induced in the rat hind paw by subcutaneous injection of 3 nmol of serotonin (5-HT). A single dose of CRF (9.4, 37.5 or 75 pmol) was given either ipsilaterally or contralaterally 30 min before 5-HT injection and oedema formation was subsequently measured every 30 min for 5.5 h. Compared to saline pre-treatment CRF (37.5 pmol) reduced oedema formation for 3.5 h when given ipsilaterally, and at 1.5 h (9.33, 37.5 and 75 pmol) when injected contralaterally. Administration of CRF along with CRF receptor antagonist, alpha-helical CRF, abolished the anti-oedema effects of CRF. Sciatic nerve ligation on the injected side attenuated the ipsilateral CRF-induced anti-oedema effect when compared with saline pre-treatment and sham-operated rats. Ipsilateral pre-treatment with 37.5 pmol of CRF caused a reduction in hind paw temperature compared to treatment with saline. Results of the present study indicate that the nervous system contributes to CRF effects in 5-HT-induced oedema formation. © 2000 Harcourt Publishers Ltd

INTRODUCTION

Corticotropin-releasing factor (CRF) is a major regulator of the hypothalamo-pituitary-adrenal (HPA) axis (Harris, 1948; Saffran and Schally, 1955). In the HPA axis, especially under stressful conditions, CRF is synthesized in the paraventricular nuclei of the hypothalamus and released into the median eminence. CRF regulates the production of adrenocorticotropic hormone through the hypophysial portal system (Sawchenko, et al., 1993). CRF has also been identified in both the neurons of rat dorsal root ganglia (Skofitsch, et al., 1985) and in the dorsal horn of the spinal cord (Merchenthaler, et al., 1983). Immunohistochemical studies have shown the presence of CRF in the synovia tissue (Crofford, et al., 1993; Crofford, et al., 1995), skin (Schäfer, et al., 1996; Slominski, et al., 1995; Roloff, et al., 1998), human blood leukocytes (Stephanou, et al., 1990) and T-lymphocytes (Ekman, et al., 1993).

Recent findings indicate the presence of CRF in the peripheral nervous system of the rat. CRF positive nerve fibres were identified in the immune organs and knee joint synovial tissue (Bileviciute, et al., 1997), predominantly in and around the blood vessels, suggesting a potent vasoactive function of the peripheral neuronal CRF. Previous studies show that CRF administered i.v. specifically inhibits neuronal plasma extravasation (Kiang and Wei, 1985). Correa and collaborators demonstrated that CRF injected into the hind paw inhibits inflammatory oedema formation (Correa, et al., 1997; Thomas, et al., 1993). However, the mechanisms behind CRF induced vasoconstriction are not established.

The aim of the present study is to investigate if the peripheral nervous system contributes to CRF-induced vasoconstriction following oedema formation in the rat hind paw.
MATERIALS AND METHODS

Experimental protocol

Experiments, performed on male albino Sprague Dawley rats weighing 200–250 g (B&K Universal AB, Sollentuna, Sweden), were approved by the Karolinska Institutet local ethical committee. All rats were accustomed to the testing conditions three times daily for 3 days before the experiment was run. On the day of the experiment the left and the right hind paw were measured (basal values) and then either the left (ipsilateral) or the right hindpaw (contralateral) was injected with either saline or human/rat CRF (9.4, 37.5 and 75 pmol; Phoenix Pharmaceuticals, USA). The doses of CRF were selected according to pilot studies, where higher and lower doses did not show an anti-inflammatory effect. Thirty minutes afterwards 3 nmol of 5-HT (Sigma Chemical Company, USA) was injected into the left hind paw. All injections were given in a volume of 50 μl and according to a blinded protocol. 37.5 pmol of alpha-helical CRF (Phoenix Pharmaceuticals, USA) was used to test the specificity of CRF action. Control animals had 2 injections of saline only. The volume of both hind paw volumes of both left and right hind paws were measured every 30 min for 5.5 h by using a plethysmometer (UGO Basile, type 7150, Italy). There measurements were carried out on each occasion and the average value was used for statistical analysis. To confirm the anti-inflammatory effect of CRF 37.5 pmol, the temperature of the hind paw was measured following the ipsilateral injection by using the thermometer with an infrared light in a separate experiment with animals trained 3 days before.

Sciatic nerve ligation

To investigate the contribution of the peripheral nervous system to CRF induced vasoconstriction, the left hind paw was denervated by cutting the left common sciatic nerve under anaesthesia with intraperitoneal chloralhydrate (350 mg/kg). Control rats had sham operations. One week after the denervation, training was started for the experiment. Both ligated and sham-operated rats received either saline or 37.5 pmol of CRF into the left hind paw followed by 3 nmol of 5-HT into the same hind paw.

Statistical analysis

The left hind paw values and the left skin temperature expressed in percentage changes from basal values from the start of the experiment are presented. The basal value of the left hind paw was 1.76±0.01 ml (mean±SEM), n=117, and the skin temperature before ipsilateral injections was 31.26±0.13°C (mean±SEM), n=15. The hind paw volume and the skin temperature of the right hind paw did not show significant changes following ipsilateral CRF administration in comparing different injections and the data is not presented. Statistical analysis is carried out using the SPSS software (release 6). Either ANOVA or t-test for independent samples was used to test the differences between the different treatments in hind paw volume.

RESULTS

Ipsilateral pre-treatment with CRF

Injection of saline into saline pre-treated control rats enhanced hind paw volume after 30 min by 10% and injection of 3 nmol of 5-HT by approximately 30% (Fig. 1). The significant difference between controls and saline pre-treated rats lasted for 3.5 h. Pre-treatment of the rat hind paw with 37.5 pmol CRF significantly reduced 5-HT induced oedema formation for 3.5 h compared to the saline (16% vs 30%) (Fig. 1). Pre-treatment with either 9.4 or 75 pmol of CRF did not affect 5-HT-induced oedema formation in comparison with saline (Fig. 1). Significant differences between 37.5 pmol vs 9.4 and 75 pmol lasted for 5.5 h (Fig. 1). Pre-treatment with 18.7 pmol of CRF did not induce significant changes compared with both saline and 37.5 pmol (not shown).

Concomitant administration of CRF 37.5 pmol and CRF receptor antagonist alpha-helical CRF reversed CRF 37.5 pmol-mediated reduction in 5-HT-induced oedema

Fig. 1 Changes in 5-HT-induced hind paw oedema preceded by ipsilateral pre-treatment of either saline or CRF. Results are presented in percentage value as mean±SEM (vertical axis). Horizontal axis indicates hours following injection. (□, hatched lines) saline, (■) CRF 37.5 pmol, (+) CRF 75 pmol, (▲) CRF 9.33 pmol and (○) control group (saline+saline). *significant difference saline vs CRF 37.5 pmol.+ significant difference CRF 37.5 pmol vs CRF 9.33 and 75 pmol. § significant difference control vs saline pre-treated 5-HT group. P<0.05; ANOVA test; n=6–11.
formation (Fig. 2) and prolonged the oedema formation over the last 3 h as compared with saline (Fig. 2). Pretreatment with alpha-helical CRF (37.5 pmol) alone prolonged the oedema formation from 1.5 to 5.5 h as compared to CRF (Fig. 2) and at 5.5 h as compared with saline (differences not indicated).

As shown in Figure 3, denervation of the hind paw compared to sham-operation abolished the CRF 37.5 pmol effect for 4.5 h in 5-HT-induced oedema formation. No difference in oedema formation was found between denervated rats pre-treated with saline and those with CRF 37.5 pmol (27 vs 28% respectively).

Ipsilateral pre-treatment with CRF 37.5 pmol in comparison with saline reduced hind paw temperature for 5.5 h following 5-HT injection (Fig. 4). This effect was not abolished by concomitant administration with alpha-helical CRF (not shown).

**Contralateral pre-treatment with CRF**

Contralateral pre-treatment with CRF (9.4, 37.5 and 75 pmol) significantly reduced the 5-HT-induced oedema formation at 1.5 h compared to saline (Fig. 5). The effect was abolished by concomitant administration of CRF 37.5 pmol with alpha-helical CRF (Fig. 5).

**DISCUSSION**

At the present time CRF is suggested to exert both pro- and anti-inflammatory effects (Karalis, et al., 1997). The anti-inflammatory effects are mediated through the hypothalamo-pituitary axis (HPA), resulting in the release of glucocorticoids as final anti-inflammatory substances acting at the periphery (Karalis, et al., 1997). The pro-inflammatory effect of CRF is suggested to be mediated directly at the site of inflammation, mainly through the secretion of CRF from the immune cells (Crofford,
et al., 1992). Indeed, an antagonist of CRF receptors reduces carrageenan-induced inflammation (Webster et al., 1996). On the other hand, an up-regulation of CRF at the site of inflammation (Mousa et al., 1996) was demonstrated to be involved in peripheral opioid analgesia (Schäfer et al., 1996), indicating that local up-regulation of CRF during inflammation has an anti-inflammatory action. Indeed, to our knowledge, there is no study showing that direct administration of physiological doses of CRF peripherally or at the site of inflammation augments the inflammatory response. Moreover, a number of studies indicate that CRF administered into the periphery exerts anti-inflammatory (Thomas et al., 1993) and antinoceptive (Hargreaves et al., 1987) effects. Wei et al. (1986) demonstrated that i.v. CRF specifically inhibits neuronal inflammation and Kiang and Wei (1987) reported that CRF administered either i.v. or into the skin inhibits thermal injury. CRF administered systemically is shown to induce hypotension (Hargreaves et al., 1987; Vale et al., 1981). However, the anti-inflammatory effect of CRF cannot be explained by the hypotensive action of CRF on the vascular bed (Wei et al., 1986). Therefore, it is suggested that the CRF effect on oedema formation is mediated through the direct action of CRF on endothelial cells or through the inhibition of the release of neuronal and cellular pro-inflammatory substances which act on the endothelial cells and postcapillary venules (Correa et al., 1997; Gao, 1991; Kiang, 1987; Wei, 1993).

However, none of these hypotheses are accepted or proven. The results of the present study indicate that 37.5 pmol of CRF administered as a pre-treatment inhibits 5-HT-induced oedema formation by acting through CRF type 2 receptors, as also demonstrated by Turnbull et al. (1996). The effect of CRF in the present study was not dose dependent and the administration of higher doses (300 pmol) induces oedema formation (unpublished results), indicating that CRF might also act through the cellular mechanisms (Correa et al., 1997). These findings are in line with a present report that i.v. administration of urocortin, a CRF-related peptide, in doses in the nmol range induces 6.6–6.7 more potent effect on oedema formation (Turnbull et al., 1996) and mast cell degranulation independently on the peripheral nerve supply (Singh et al., 1999).

Pre-treatment with 37.5 pmol of CRF also reduced the 5-HT-induced rise in skin temperature increase but this effect was not blocked by the CRF-receptor antagonist. Nor was the temperature response parallel to the oedema formation, indicating the different mechanisms involved and a possible role of CRF in skin cells metabolism. Recently, Slominski et al. (1995) demonstrated CRF expression in human melanocytes, cells of the basal epidermis and nerve bundles of growing murine skin (Roloff et al., 1998). According to their findings, CRF induces a rapid dose-dependent increase in intracellular Ca2+ in melanoma cells, an effect abolished by CRF receptor antagonist (Fazal et al., 1998). Furthermore, dexamethasone was found to inhibit the expression of CRF and CRF type 1 receptor in cultured skin cells (Slominski et al., 1998). These findings indicate an important role of CRF in dermal cell metabolism. It might be postulated that, by acting on Ca2+ turnover in dermal cell, CRF has an inhibitory effect on cell functions, thereby reducing inflammation.

Akoev et al. (1996) demonstrated that serotonin activates both afferent nerve fibres and mast cells, where, by acting on 5-HT1- and 5-HT2-receptors, it induces plasma extravasation (Khalil et al., 1989, Khalil et al., 1990, Richardson, 1990, Taiwo, 1992). In the present study the 5-HT-induced oedema formation is suppressed by pretreatment with CRF, also demonstrated previously (Kiang and Wei, 1985; Correa et al., 1997; Kiang et al., 1987). Results of the present study show that CRF is an important mediator involved in the regulation of inflammatory responses since concomitant administration of CRF with alpha-helical CRF prolonged 5-HT-induced oedema formation on the ipsilateral side.

Recently, we demonstrated that contralateral treatment with local anaesthetics reduces carrageenan-induced hind paw inflammation (Bileviciute, 1998; Bileviciute, 1999). This contralateral effect was due to the inflammatory stimulus or increased

![Fig. 5 Changes in 5-HT-induced hind paw oedema preceded by contralateral pre-treatment of either saline, or CRF, or αCRF + CRF. Results are presented in percentage value as mean±SEM (vertical axis). Horizontal axis indicates hours following injection. (□) saline pre-treated group, (■) CRF 37.5 pmol, (●) CRF 75 pmol, (▲) CRF 9.33 pmol, (○) αCRF + CRF (37.5 pmol). *significant difference between saline vs CRF 9.33, 37.5 and 75 pmol. **P<0.05; ANOVA test; n=7–14. + significant difference between CRF (37.5 pmol) and αCRF + CRF (37.5 pmol). P<0.05; t-test; n=6–10.](image-url)
nerve activity and was abolished by sciotic nerve ligation and injection of saline intrathecally, indicating a neuronal/spinal cord involvement. In the present study contralateral CRF administration specifically reduced 5-HT oedema formation at 1.5 h and the effect of CRF was not dose dependent. Our unpublished results indicate that 3 nmol of 5-HT administered into the hind paw did not produce nociception. In the present study the short-lasting effect of contralateral CRF on oedema formation might be explained by the milder pro-inflammatory stimulus given ipsilaterally which did not induce such long-lasting bilateral nerve activity as carrageenan (Bileviciute, 1998; Bileviciute, 1999).

Recently, Turnbull et al. (1996) demonstrated dose dependent anti-oedema effects from intravenous CRF or urocortin in rats and showed the anti-oedema effect induced by physiological concentrations of CRF (in pmol ranges) not to be mediated through activation of pituitary ACTH. However, in the present study CRF did not exert a dose dependent effect, perhaps due to activation of mast cells at higher doses of CRF following local administration.

Altogether, the results of the present study indicate that the anti-oedema effects of CRF in 5-HT-induced oedema formation are dependent on an intact peripheral nerve supply and may act bilaterally. An important role for CRF in vasoregulation and inflammation mediated through peripheral neuronal mechanisms is indicated.

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