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Short communication

Urocortin, a corticotropin-releasing factor-related mammalian peptide, inhibits edema due to thermal injury in rats

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Abstract

Urocortin is a recently characterized mammalian peptide which appears to be an endogenous ligand for corticotropin-releasing factor (CRF) receptors, in particular CRF receptor type 2. The effect of rat urocortin on protein extravasation and edema, produced by immersion of the paws of anesthetized rats in 58°C water for 5 min, was compared to that of rat/human CRF. Urocortin administered i.v. dose-dependently inhibited the leakage of Evans blue dye into the skin of the footpad and the increase in paw weight, with a potency 6.6–6.7 times greater than CRF. α-Helical CRF-(9–41), a more potent antagonist of type 2 than type 1 CRF receptors, completely reversed the inhibition of edema produced by either CRF or urocortin, at a dose (200 nmol/kg) that did not affect adrenocorticotropin secretion induced by either peptide. These data indicate that urocortin is a potent inhibitor of heat-induced edema, and that this action is mediated by CRF receptors, most likely CRF receptor type 2.

Keywords: Sauvagine; Urotensin I; CRF (corticotropin-releasing factor) receptor; Extravasation; Inflammation; ACTH (adrenocorticotropin)

1. Introduction

Corticotropin-releasing factor (CRF) is a mammalian neurohormone, best known as the major physiological regulator of pituitary adrenocorticotropin (ACTH) secretion. It belongs to a family of structurally related peptides (including amphibian sauvagine and piscine urotensin I), which produce diverse actions in mammalian systems (Lederis, 1987; Chadwick et al., 1993). The effects of CRF-related peptides are mediated by interaction with a number of distinct CRF receptors, which display discrete and fairly exclusive distributions. CRF receptor type 1 mRNA is expressed in the brain and pituitary (Potter et al., 1994). CRF receptor type 2 has at least two splice forms (Lovenberg et al., 1995a): 2α mRNA is found only in the brain (Lovenberg et al., 1995b), and 2β mRNA is present in both the brain and the periphery (Perrin et al., 1995; Kishimoto et al., 1995; Stenzel et al., 1995), but not pituitary (Lovenberg et al., 1995b). The distributions of receptors in the pituitary gland and central nervous system are consistent with the pivotal role of CRF in the integration of neuroendocrine, behavioral and autonomic responses to stress (Chadwick et al., 1993). CRF also influences a variety of cellular activities within peripheral tissues; in particular, CRF modulates inflammatory processes, and the inhibition of injury-induced edema by CRF-related peptides is well-documented (for review, see Wei and Thomas, 1993). Sauvagine, urotensin I and CRF exhibit similar rank orders of potencies for inhibiting edema (Wei and Thomas, 1993), and stimulating cAMP formation in cells overexpressing CRF receptor type 2 (Kishimoto et al., 1995; Lovenberg et al., 1995a; Vaughan et al., 1995). The recent identification and characterization of a second mammalian member of the CRF superfamily, urocortin, which possesses characteristics of an endogenous ligand for CRF receptor type 2 (Vaughan et al., 1995), prompted us to investigate the effects of this peptide on heat-induced paw edema and protein extravasation in the rat.

2. Materials and methods

2.1. Peptides

Rat urocortin, rat/human CRF and α-helical CRF-(9–41) were synthesized by solid-phase methodologies, and

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kindly provided by Dr. Jean Rivier (Salk Institute). Each peptide was dissolved in phosphate-buffered saline containing 0.1% bovine serum albumin and administered i.v. in a volume of 0.2 ml/100 g body weight.

2.2. Experimental procedure

Experiments were carried out on adult male Sprague-Dawley rats (240–260 g; Harlan-Sprague-Dawley, Indianapolis, IN) with the approval of the Salk Institute Animal Care and Use Committee. For i.v. injection and blood sampling, all animals were equipped with indwelling jugular venous catheters 48 h before experimentation. On the day of the experiment, the rats were anesthetized with a subcutaneous injection of ketamine (100 mg/kg)/acepromazine (4 mg/kg)/xylazine (10 mg/kg). 30 min later either vehicle, CRF or urocortin (0.45–36 nmol/kg) were administered (i.v.), followed 7 min later by 50 mg/kg Evans blue dye (Sigma, St Louis, MO) also injected i.v. Heat-induced protein extravasation and edema were produced 3 min later, and quantified as described previously (Wei and Thomas, 1993, 1994). Briefly, the right foot was immersed up to the ankle joint in water maintained at 58°C, for a total of 5 min. The animals were then killed by an overdose of pentobarbitone (i.v.), both paws were removed at the ankle joint and weighed, and the skin of the footpad of each paw was removed and placed in 4 ml of formamide for 3 days. Protein extravasation into the skin of the footpad was estimated by spectrophotometric quantification (at 640 nm) of the Evans blue dye extracted by the formamide, using the non-inflamed paw skin as the zero blank. The degree of edema was estimated as the difference in weight between the heated and unheated paw divided by the weight of the unheated paw (Wei and Thomas, 1994).

In separate experiments, we determined the effects of the CRF receptor antagonist, α-helical CRF-(9–41) (Rivier et al., 1984), on CRF and urocortin-induced adrenocorticotropin (ACTH) secretion and inhibition of edema. 30 min after induction of anesthesia, animals were pre-treated with either α-helical CRF-(9–41) (200 nmol/kg i.v.) or vehicle, and 2 min later injected (i.v.) with either CRF, urocortin or vehicle. 8 min later, a blood sample (0.3 ml) was drawn and animals subjected to paw immersion as described above. Plasma ACTH concentrations were determined by immunoradiometric assay (Allegro, Nichol's Institute, San Juan Capistrano, CA).

2.3. Data analysis

Data are presented as the mean ± S.E.M., and the numbers of animals in each experimental group are indicated in the figure legends. Linear regression analysis was performed using Cricket Graph III software, and the relative potencies [and 95% confidence intervals (CI)] of CRF and urocortin calculated using Bioprog software. Analysis of variance (ANOVA) followed by either Dunnett or Scheffé's multiple comparison tests (as appropriate) were used to statistically compare different groups. A two tailed probability of less than 5% (i.e. $P < 0.05$) was considered significant.

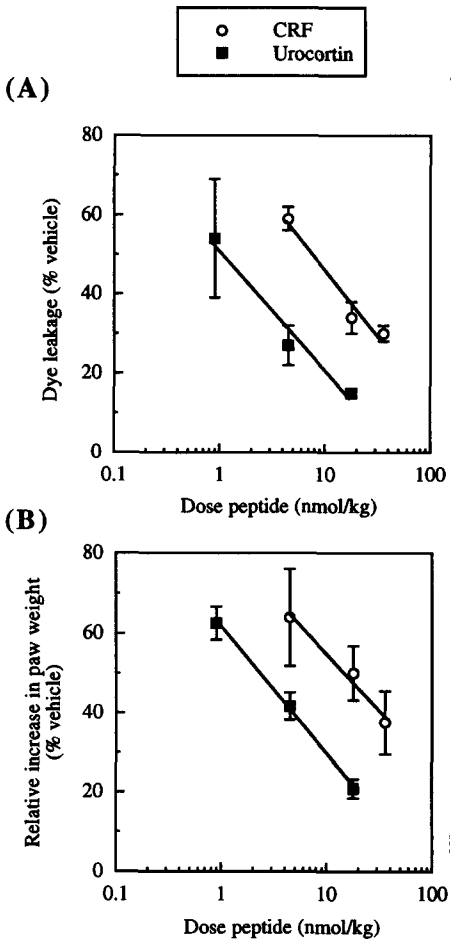

Fig. 1. The dose-related effects of the i.v. injection of rat/human CRF and rat urocortin on leakage of Evans blue dye into the skin of the footpad (A) and relative increase in whole paw weight (B), in rat paws after immersion in 58°C water for 5 min. Data are presented as percent of increase in vehicle-treated, control animals. Using the equations generated by linear regression analysis, the doses of CRF and urocortin producing 50% inhibition of dye leakage were 7.6 and 1.1 nmol/kg, respectively, and those inhibiting edema were 15.5 and 2.4 nmol/kg, respectively.

3. Results

Immersion of the paw in 58°C water for 5 min produced a marked leakage of dye (39.3 ± 6.4 μg) into the skin of the footpad and a pronounced edema (increase in paw weight of 59 ± 7% compared to contralateral, unheated paw). CRF or urocortin dose-dependently inhibited both dye leakage and edema (Fig. 1), an effect which was statistically significant ($P < 0.05$, Dunnett multiple comparison test) at all doses tested (lowest dose tested: uro-

cortin, 0.9 nmol/kg; CRF, 4.5 nmol/kg). Urocortin inhibited dye leakage with 6.6-fold (95% CI: 2.3–20.1), and edema with 6.7-fold (95% CI: 3.2–16.8) greater potency than CRF.

In a separate experiment, CRF (7.6 nmol/kg) and urocortin (1.1 nmol/kg) elicited significant and pronounced elevations in plasma ACTH concentrations 8 min after injection, and produced a marked inhibition of heat-induced edema (Fig. 2). The ability of α-helical CRF-(9–41) to antagonize these actions was tested using a dose of the antagonist of 200 nmol/kg. This dose is a 26-fold molar excess compared to CRF, a relative excess that completely abolishes CRF-induced cAMP accumulation in cells over-expressing CRF receptor type 2, but is totally ineffective at cells over-expressing receptor type 1 (Kishimoto et al., 1995). α-Helical CRF-(9–41) (200 nmol/kg) had no effect on either edema or plasma ACTH concentrations in animals injected with only vehicle (Fig. 2). In contrast, pre-treatment with α-helical CRF-(9–41) completely reversed CRF- and urocortin-induced inhibition of edema, while having no effect on elevations in plasma ACTH concentration induced by these peptides (Fig. 2).

4. Discussion

Rat urocortin is a 40 amino acid mammalian peptide which shares 63, 45 and 35% sequence homology with suckerfish urotensin I, rat/human CRF and frog sauvagine, respectively (Vaughan et al., 1995). Urocortin binds and activates CRF receptors with greater potency than CRF itself, and exhibits neuroanatomical and pharmacological characteristics consistent with a role for this peptide as an endogenous ligand at CRF receptor type 2 (Vaughan et al., 1995). The present study demonstrates that, in vivo, urocortin is a more potent inhibitor of heat-induced protein extravasation and edema than CRF, and that this activity of urocortin is mediated by CRF receptors sensitive to a CRF receptor antagonist, α-helical CRF-(9–41), which is more effective at the type 2 than the type 1 receptor (Kishimoto et al., 1995).

Wei and colleagues have demonstrated that CRF-related peptides inhibit edema and protein extravasation due to a variety of traumatic injuries to a number of different tissues, an action which is apparently not secondary to effects on either pituitary-adrenal activation or changes in systemic blood pressure (for review, see Wei and Thomas, 1993). The precise mechanism by which CRF-related peptides inhibit edema is unknown, but their greater potency when injected locally compared to intravenously, suggests that they interact with CRF receptors in the inflamed tissue to inhibit vascular leakage (Wei and Thomas, 1993). Indeed, specific binding of radiolabeled CRF is found within vascular tissues (Dashwood et al., 1987), and is elevated in inflamed tissues (Mousa et al., 1995).

Two distinct seven-transmembrane domain, G-protein-coupled receptors for CRF have been identified in several mammalian species. Present evidence indicates that CRF receptor type 1 mediates CRF-induced pituitary ACTH secretion, as mRNA for this receptor is co-localized with ACTH immunoreactivity in the anterior pituitary (Potter et al., 1994), and CRF, sauvagine and urotensin I are approximately equipotent at stimulating ACTH secretion from rat anterior pituitaries (Lederis, 1987) and cAMP formation in

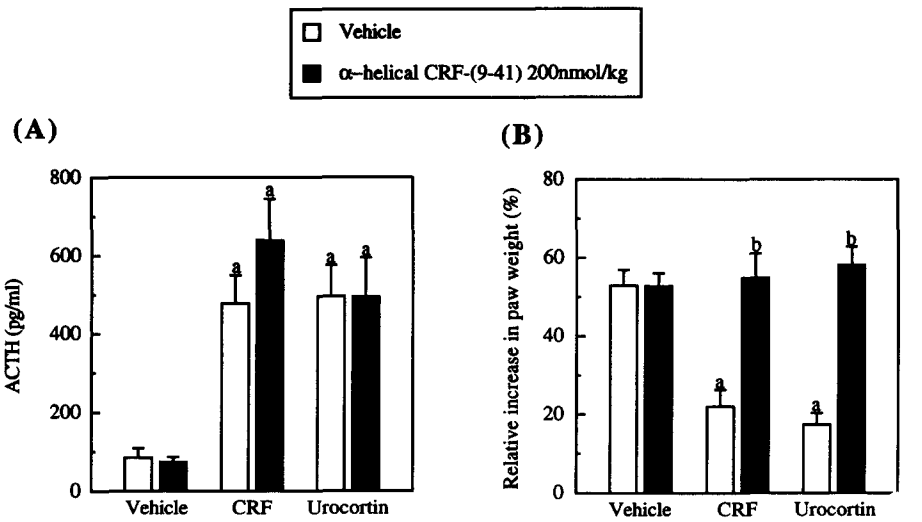

Fig. 2. The effects of α-helical CRF-(9–41) (200 nmol/kg i.v.) on elevations in plasma ACTH concentration (A), and inhibition of edema (B) produced by CRF (7.6 nmol/kg i.v.) and urocortin (1.1 nmol/kg i.v.). Data in each panel were derived from the same animals, and are presented as absolute ACTH concentrations, 8 min after injection of CRF or urocortin, and relative increase in paw weight (as a percentage of unheated paw) after immersion in 58°C water for 5 min. α-Helical CRF-(9–41) was administered 2 min before, and heat injury commenced 10 min after, injection of CRF/urocortin. [a] $P < 0.05$ compared to vehicle/vehicle-treated controls; [b] $P < 0.05$ compared to CRF/urocortin alone-treated animals (Scheffé's multiple comparison test). $n = 5–6$/group.

cells over-expressing CRF receptor type 1 (Kishimoto et al., 1995; Lovenberg et al., 1995a; Vaughan et al., 1995). On the other hand, neither known splice form of receptor type 2 is present in the pituitary (Lovenberg et al., 1995b), and the rank order of potency of sauvagine > urotensin I > CRF is apparent at this receptor subtype (Kishimoto et al., 1995; Lovenberg et al., 1995a; Vaughan et al., 1995). The observation that the relative orders of potency of sauvagine, urotensin I and CRF on heat-induced edema (Wei and Thomas, 1993) and CRF receptor type 2 (Kishimoto et al., 1995; Lovenberg et al., 1995a; Vaughan et al., 1995) are identical, and that CRF receptor type 2β is present in peripheral tissues (in particular in heart, where receptor mRNA is localized on arterioles, and in skeletal muscle) (Perrin et al., 1995; Lovenberg et al., 1995b), had suggested that this receptor subtype may mediate the anti-edema actions of CRF-related peptides. The present study provides support for this concept, and shows *direct* evidence for the involvement of CRF receptor type 2 in this action. α-Helical CRF-(9–41) displays differential antagonist activity in vivo, with an antagonist: agonist ratio of around 6 : 1 completely preventing CRF-induced hypotension, while a 1000 : 1 ratio is required to significantly reduce CRF-induced ACTH secretion (Fisher et al., 1991). It is now apparent that this differential antagonist activity is due to the far greater effectiveness of α-helical CRF-(9–41) at the type 2 compared to the type 1 CRF receptor (Kishimoto et al., 1995; Perrin, Sutton and Vale, unpublished observations). In the present experiments, α-helical CRF-(9–41) completely reversed the inhibition of edema produced by either CRF or urocortin, at a dose that had no impact on ACTH secretion induced by either peptide, strongly implying that a type 2-like CRF receptor mediates the anti-edema actions of CRF and related peptides. The present study therefore demonstrates that urocortin potently inhibits the development of edema via interaction with CRF receptors. The observation that the ACTH-releasing and anti-edema properties of CRF-related peptides can be dissociated by pretreatment with α-helical CRF-(9–41) indicates distinct functional roles for the different CRF receptors, and suggests that type 2 CRF receptor-selective ligands may be useful inhibitors of extravasation and associated inflammatory processes.

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