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Short communication

Corticotropin-releasing hormone inhibits lowering of interstitial pressure in rat trachea after neurogenic inflammation

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Abstract

Increased negativity of interstitial fluid pressure (P_{if}) is a key determinant of edema formation after tissue injury. In this study, we addressed the question of whether the anti-inflammatory effects of corticotropin-releasing hormone (CRH) shown by others are mediated by changes in interstitial fluid pressure. CRH, 25 to 50, but not 5 and 11 μg/kg s.c., administered 45 min before antidromic stimulation of the vagal nerve inhibited the lowering of interstitial fluid pressure in rat trachea produced by nerve stimulation. This inhibitory effect of CRH was blocked by pretreatment with the CRH receptor antagonist, α-helical CRH-(9–41), 0.15 mg/kg i.v., administered 5 min before CRH. These results suggest that CRH receptors modulate the structural integrity of the extracellular matrix in rat trachea for its response to inflammatory stimuli.

Keywords: CRH (corticotropin-releasing hormone); Neurogenic inflammation; Trachea

1. Introduction

Corticotropin-releasing hormone (CRH), a 41-residue peptide, reduces plasma protein extravasation and edema formation in various models of injury to skin, upper and lower respiratory tract, skeletal muscle and brain (Wei et al., 1993). These anti-inflammatory effects are independent of adrenal activation as the actions of CRH on skin, muscle and brain were demonstrated in adrenalectomized animals. In humans, periorbital edema after blepharoplasty may be reduced by a single intravenous dose of CRH at 8 μg/kg, but the results were not conclusive (Schendel and Stephanides, 1996). In the rat trachea, CRH reduced the extravasation of dye-labeled protein, obtained by antidromic stimulation of the vagal nerve, by exposure to formaldehyde vapors, or by subcutaneous injection of substance P (Wei et al., 1993; Yoshihara et al., 1995). We now investigated the mechanism of the anti-edema actions of CRH on rat trachea. Previous studies have shown that a primary driving force for tracheal edema formation and protein extravasation after antidromic vagal stimulation was an increased negativity of interstitial fluid pressure (P_{if}) (Woie et al., 1993). P_{if} is normally maintained at −1.0 to −1.5 mmHg in the tracheal interstitium. After nerve stimulation, P_{if} becomes more negative, to about −4.5 to −5 mmHg, and this pressure gradient then facilitates egress of fluid and proteins from capillaries. This is a substantial rise in net transcapillary filtration pressure which is normally 0.5 to 1 mm Hg (Rodt et al., 1994). For comparison, the capillary filtration coefficient ('water permeability') increases only by a factor of two to three in acute inflammation and tissue injury, even in injuries such as burn injury to the skin (Reed et al., 1997). Agents which suppress edema may do so by attenuating the increased negativity of P_{if} seen after inflammatory stimuli (Rodt et al., 1994). Development of increased negativity of P_{if} involves perturbation of the cellular adhesion molecules towards extracellular matrix receptors, the β_1-integrins (Reed et al., 1997); the $\alpha_2\beta_1$-integrins being particularly involved in the development of increased negativity of P_{if} (Rodt et al., 1996). This is important mainly because modulation of the cell-matrix connections in vivo will alter P_{if} and thereby the transcapillary fluid flux, and via this mechanism influence interstitial fluid volume and transcapillary protein flux. Thus, the observation that CRH and its related peptides have an effect on the development of increased negativity of P_{if} in

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acute inflammation is evidence that these agents influence the integrity and normal function of the extracellular matrix.

2. Materials and methods

Female Wistar rats, weighing 200 to 250 g, were anesthetized with sodium pentobarbital 50 mg/kg, i.p. and saline or CRH dissolved in saline (American Peptides Systems, Sunnyvale, CA, USA) was injected 5, 11, 25 or 50 μg/kg s.c. in the groin region at a volume of 0.1 ml/100 g body weight. In a second series of experiments, saline or 0.15 mg/kg of α-helical CRH-(9-41), a synthetic CRH receptor antagonist, (Rivier et al., 1984, synthesized by Dr. Nicholas Ling, Neurocrine Biosciences, La Jolla, CA, USA), was injected i.v. 5 min before saline or CRH. In all experiments at 45 min after s.c. injection of saline or CRH, cardiac arrest was induced by i.v. injection of 0.5 ml saturated KCl. Immediately afterwards, the trachea was surgically exposed and the left vagus was isolated and placed on stimulating electrodes. The trachea was then covered with mineral oil. Sharpened glass capillaries (4 to 10 μm), connected to a servocontrolled counterpressure system (Woie et al., 1993), were used to take initial readings of P_{if}, after which electrical stimulation of the vagus was commenced (stimulation parameters: 20 V, 20 Hz, 0.5 ms for 5 or 15 min) (Woie et al., 1993). The experiments lasted for 60 min after cardiac arrest, the averaged values of P_{if} measurements being recorded before and after electrical stimulation. Measurements were made after circulatory arrest because edema associated with acute inflammation will elevate interstitial fluid volume and pressure, and thereby cause underestimation of the negative interstitial fluid pressure. Measurements of P_{if} were accepted when the following criteria were fulfilled: (1) Feedback gain could be altered without changing the recorded pressure. (2) After fulfilment of criterion 1, fluid communication between pipette and tissue was verified by applying suction to the servocontrolled pump. When fluid could be moved into the pipette, this was seen as increased electrical resistance in the pipette due to lower tonicity of the fluid entering the pipette. (3) Zero measurement was unaltered from the prepuncture value after measurement had been performed. Zero measurement was performed in a cup filled with saline at the level of the puncture site. Statistical analysis was done with a one-way ANOVA (analysis of variance), using the change in P_{if} from before to after stimulation, change in P_{if} = (P_{if} before stimulation) − (P_{if} after stimulation), and comparing all groups in the same analysis. The one-way ANOVA was followed by subsequent Bonferroni and *t*-tests. The procedures described in this article were carried out with the approval of and in accordance with the recommendations of the Norwegian State Commission for Laboratory Animals.

3. Results

The results are given in Table 1. The baseline P_{if} of animals receiving saline but no electrical stimulation averaged -1.6 ± 0.2 (S.D.) mmHg and remained stable during the 60-min experimental period. In a second group, the P_{if} of saline-injected animals averaged -1.4 ± 0.8 mmHg before electrical stimulation. In agreement with the results of previous studies (Woie et al., 1993), P_{if} became more negative, to about -5 mmHg after electrical stimulation ($P < 0.01$, Fig. 1). CRH produced a dose-dependent sup-

Table 1
Effects of corticotropin-releasing hormone (CRH) on interstitial fluid pressure (P_{if}) in rat trachea before and after antidromic electrical stimulation of the vagal nerve (ES)

Series	CRH (μg/kg) s.c.	ES	*n*	P_{if} before ES (mmHg)	P_{if} after ES mmHg)	Effect of ES, Bonferroni *P*-value[a]	Inhibition by CRH, Bonferroni *P*-value[b]
Controls	–	no	4	-1.6 ± 0.2	–	–	–
Controls	–	yes	10	-1.4 ± 0.8	-4.9 ± 0.9	0.01	–
CRH	5	yes	8	-1.3 ± 0.6	-5.1 ± 1.0	0.01	n.s.
CRH	11	yes	10	-1.8 ± 0.6	-4.2 ± 0.4	0.01	n.s.
CRH	25	yes	6	-1.8 ± 0.6	-2.4 ± 0.9	n.s.	0.01
CRH	50	yes	14	-1.4 ± 0.7	-1.4 ± 0.6	n.s.	0.01
α-Helical CRH 0.15 mg + saline	–	yes	8	-1.7 ± 0.5	-5.6 ± 1.5	0.01	n.s.
α-Helical CRH 0.15 mg + CRH	50	yes	10	-2.2 ± 0.4	-4.5 ± 1.1	0.01	n.s.
ANOVA *P*-value						0.0000	0.0000

CRH injected 45 min before cardiac arrest. α-Helical CRH-(9–41) injected 5 min before CRH.
n = Number of animals used. Values are expressed as means ± S.D.
Statistical comparison: [a]One-way ANOVA with subsequent Bonferroni and *t*-tests comparing the change in P_{if} from before to that after stimulation (change in P_{if} = (P_{if} before stimulation) − (P_{if} after stimulation)) simultaneously between all the groups listed in the table. 'Control, no CRH and no ES' as control group. [b]One-way ANOVA with subsequent Bonferroni and *t*-tests comparing the change in P_{if} from before to that after stimulation as described above, 'Control, no CRH + ES' as control group. Not significant is n.s.

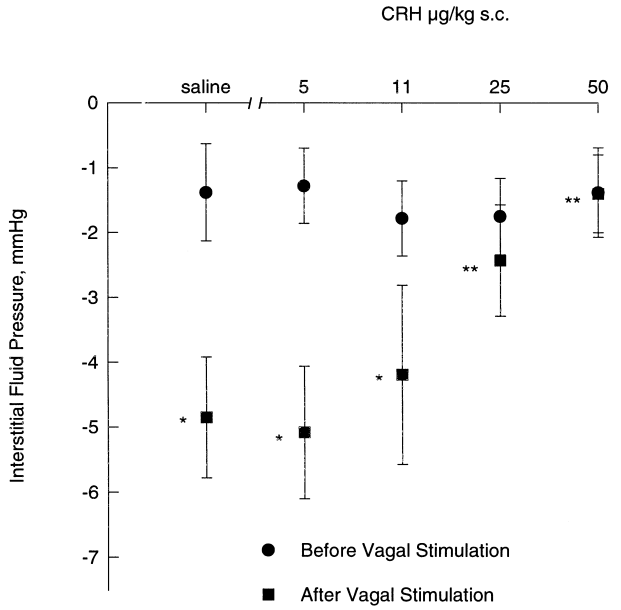

Fig. 1. Corticotropin-releasing hormone (CRH) inhibits development of increased negative interstitial fluid pressure (P_{if}) in anesthetized rat trachea after antidromic stimulation of the vagus. Values are means ± S.D. $*\ P < 0.01$, represents one-way ANOVA with subsequent Bonferroni's comparison of the change in P_{if} from before to after stimulation (change in P_{if} = (P_{if} before stimulation) − (P_{if} after stimulation)). ** Represents significant inhibition of fall in P_{if}.

pression of the increased negativity in P_{if} produced by electrical stimulation, the attenuation being clearly significant at doses of 25 and 50 µg/kg s.c. Alone, α-helical CRH-(9–41), 0.15 mg/kg i.v., did not affect the baseline P_{if} or the change towards a more negative P_{if} produced by electrical stimulation. The ability of CRH (50 µg/kg s.c.) to attenuate the increased negativity of P_{if} was blocked by pretreatment with α-helical CRH-(9–41), administered 5 min before CRH. α-Helical CRH-(9–41) together with CRH resulted in an average P_{if} of −2.2 mmHg, before stimulation, and fell to an average −4.5 mmHg after stimulation. This is a response similar to that of the nontreated (vehicle) controls.

4. Discussion

The results reported here show that CRH pretreatment attenuated a key determinant for the transcapillary pressure gradient that generates edema formation in inflamed tissue, namely, the increased negativity of P_{if}. The effects of CRH shown here may involve the extracellular matrix components and/or the β_1-integrin system because we have observed that blocking of the β_1-integrin system in vivo causes development of an increased negativity with a time course and magnitude similar to those observed in several inflammatory reactions (Reed et al., 1992; Rodt et al., 1994, 1996). However, the exact manner by which the CRH peptides may affect P_{if} via the integrin system and intracellular signalling pathway is unclear. It should be noted that control P_{if} was not affected by hemodynamic effects of CRH the most predominant one being a long-lasting lowering of arterial blood pressure.

Several earlier studies have demonstrated an anti-inflammatory effect of CRH on airways including inhibition of edema formation (Wei and Kiang, 1987; Gao et al., 1991; Wei et al., 1993; Yoshihara et al., 1995). In this study we have shown that one mechanism by which CRH affects edema is by attenuating the lowering of P_{if}. Furthermore, we have tried to find whether this effect is mediated via the CRH_2 receptor by using α-helical CRH-(9–41) which is a nonselective CRH receptor antagonist. In human skin there is evidence of gene expression of CRH and its receptor (Slominski et al., 1995), and also that melanocytes produce CRH peptides (Slominski et al., 1996). Thus, local production of this peptide may participate in the regulation of the physiology of the integument. Two major types of CRH receptors have been cloned, with CRH_2 receptors being the predominant form in peripheral tissues (Turnbull and Rivier, 1997). The ability of the nonselective CRH receptor antagonist, α-helical CRH-(9–41), to block both the anti-inflammatory (Wei and Gao, 1991) and the attenuating effects of CRH on P_{if}, indicates that CRH_2 receptors may modulate peripheral tissue responses to injury. Functional and binding studies have shown the presence of CRH receptors on vascular smooth muscle and endothelium. Furthermore, mRNA coded for CRH_2 receptors has been localized around brain arterioles. Autoradiographic studies have revealed binding of CRH to epithelial cells in close proximity to sites of induced vascular leakage (Gao et al., 1991).

It was first thought that, in skin and mucous membranes, the inhibitory effect of CRH on plasma protein extravasation and edema formation was situated at the postcapillary venules (Gao et al., 1991; McDonald, 1990). However, this idea was rejected since CRH is also effective to inhibit the pulmonary edema induced by epinephrine injection (Serda and Wei, 1992) and the brain edema induced by freeze lesion (Wei and Gao, 1991). Also, the vascular beds of the postcapillary venule in lungs and brain are known to be insensitive to, among others, histamine and serotonin, yet CRH is active to suppress edema in these tissues. The precise mechanisms and the intracellular pathways which link CRH receptors to an effect on P_{if} and response to transmural edematogenic forces are not, however, clear. In this context, it is of interest to note that a highly conserved sequence of CRH family peptides, i.e., CRH-(4–16) and sucker fish urotensin I(4–16), –PPISLDLTFHLLR– exhibit strong homology to human fibronectin (1289–1301) –PPPSIDLTNFLVR–. Fibronectin is a protein of the extracellular matrix important for cell adhesion and the maintenance of tissue integrity. It is possible that fibronectin fragments and CRH receptors may interact to influence the development of increased negativity of P_{if} in inflammation.

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