

Pituitary adenylate cyclase-activating polypeptide, effects in the human nose

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Summary

Background Pituitary adenylate cyclase-activating peptide (PACAP) is a neuropeptide with strong vaso- and bronchodilator capacity. There is recent evidence that PACAP decreases the release of proinflammatory cytokines and we have previously shown that PACAP inhibits neutrophil chemotaxis *in vitro*, but little is known about the effects of PACAP in human upper and lower airways.

Objective To investigate the effects of PACAP in the human upper respiratory tract focusing on vasodilatation/nasal airway resistance (NAR), neutrophil recruitment, plasma extravasation and endogenous production of IL-1-related mediators.

Methods Surgical specimens from five patients (aged 19–55 years), obtained in conjunction with nasal surgery, were used for immunohistochemical localization of PACAP in the nasal mucosa. In seven, healthy, non-allergic, non-smoking subjects (aged 19–45 years), NAR was measured with rhinomanometry. Nasal lavage was performed, before and after intranasal application of PACAP (200 µL of a 1 µM PACAP solution in each nasal cavity), with and without the addition of histamine. Cells, albumin and IL-1-related mediators were analysed in nasal lavage. In addition, the effects on pulse, blood pressure, ECG and pulmonary function were evaluated.

Results In the nasal mucosa, PACAP-like immunoreactive nerve fibres were seen close to blood vessels and seromucous glands. Application of PACAP in the nasal cavity increased NAR and augmented the increase in NAR induced by histamine. In addition, PACAP inhibited histamine-induced recruitment of neutrophils, increased plasma leakage and reduced the level of IL-1RA (an endogenously produced IL-1 receptor antagonist) in nasal lavage. Cardiovascular and pulmonary parameters were not affected.

Conclusion These results imply that PACAP is an important endogenous mediator in human upper airways, with a potential role as a regulator of vascular smooth muscle, secretion, plasma extravasation, neutrophil recruitment and cytokine activity.

Keywords human airway, *in vivo*, nasal lavage, nasal resistance, neutrophil, PACAP

Submitted 3 January 2003; revised 10 March 2003; accepted 9 April 2003

Introduction

Pituitary adenylate cyclase-activating peptide (PACAP) is a 38-amino-acid peptide discovered in 1989 as a hypothalamic factor that potently increased cAMP levels in pituitary cells [1]. Later, it was found to be widely expressed in the central and peripheral nervous systems. The amino-acid sequence of PACAP is 70% homologous to vasoactive intestinal peptide (VIP), both members of a peptide family that also includes secretin and glucagon [1]. In the human lung, PACAP is found in association with bronchial and vascular smooth muscle and around seromucous glands [2, 3]. We have previously demonstrated that PACAP is a potent dilator of isolated human airways and pulmonary vessels, and that it causes relaxation of contracted guinea-pig airways *in vivo*

[3, 4]. PACAP is also found in the microenvironment of lymphoid organs and increasing evidence suggests that PACAP, as well as VIP, plays a role in the immune system [5–7]. One of the most interesting actions of PACAP in the immune system is the anti-inflammatory function, which is exerted by its capacity to decrease proinflammatory cytokines like TNF- α and IL-6 and by the increase of anti-inflammatory cytokines, i.e. IL-10 [8–10]. PACAP activity is closely connected to IL-1 [11, 12], a cytokine suggested to contribute to glandular secretion in the inflammatory nose [13]. Like other cytokines, IL-1 activity is modulated by soluble cytokine receptors, i.e. a soluble IL-1 receptor (IL-1sR2) in the fluid phase can prevent IL-1 from interacting with the functional IL-1 receptor on the plasma membrane [14]. In addition, we have previously reported that high concentrations of the natural IL-1 receptor antagonist, IL-1RA, can be found in nasal lavage fluids (M Benson, G Wennergren, M Fransson, LO Cardell: unpublished data). Neutrophils might play a role in inflammatory airways, i.e. asthma and rhinitis

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[15]. PACAP and VIP inhibit neutrophil migration and both PACAP and VIP take part in neutrophil activation, although via different pathways [16–18]. Based on the above-described bronchodilator and anti-inflammatory effects of PACAP, the use of PACAP and PACAP analogues has been suggested in the treatment of asthma [19]. However, PACAP has never been tested in the human airways. The aim of the current study was to investigate the effects of PACAP in the human upper respiratory tract, focusing on vasodilatation/nasal airway resistance, neutrophil recruitment, plasma extravasation and changes in the amount of IL-1-related mediators. Systemic effects of intranasal PACAP on pulse, blood pressure, ECG and pulmonary function were also evaluated.

Methods

Immunohistochemistry

Specimens from human nasal mucosa were obtained in conjunction with nasal surgery (resection of the inferior turbinate) from five patients (age range 19–55 years) with no history of allergic disease. Two of the patients displayed nasal deviation following trauma, and the surgery was primarily performed to correct the nasal septum. The other three patients had problems with nasal breathing due to enlargement of the inferior turbinate. Two of these patients reported a non-specific nasal hyper-reactivity.

The specimens were immersed in an ice-cold fixative solution composed of 2% formaldehyde and 0.2% picric acid, buffered to pH 7.2 with 0.1 M phosphate buffer. After 12 h, the specimens were rinsed for 48 h in a Tyrode solution containing 10% sucrose, frozen on dry ice and sectioned in a cryostat at 10 µm thickness. The sections were processed for immunocytochemical studies of PACAP using indirect immunofluorescence. The PACAP antiserum (code no. B57-1, Eurodiagnostica, Malmö, Sweden) was raised in a rabbit against ovine PACAP and used in a dilution of 1:640. The sections were exposed to the peptide antiserum for 48 h at 4 °C in a moist chamber. The site of the antigen–antibody reaction was revealed by application of fluorescein isothiocyanate (FITC)-labelled antibodies against immunoglobulin G (IgG) (Dakopatts, Copenhagen, Denmark) in a dilution of 1:320 for 1 h at room temperature. Control sections were exposed to primary antiserum that had been pre-absorbed with excess amounts of the antigen. In addition, absorption tests showed that the PACAP antiserum does not cross-react with VIP, peptide histidine isoleucine, helodermin, helospectin, bombesin or substance P [20]. However, cross-reaction with still other peptides or proteins sharing amino-acid sequences with the examined peptide cannot be excluded. Therefore, it is appropriate to refer to the immunoreactive material as PACAP-like.

Subjects

Two separate groups were included in the functional studies: one subjected to rhinomanometric investigations and nasal lavages, and one for characterization of effects induced on heart rate, blood pressure and lung function. The first group included seven and the second group five healthy, non-

smoking subjects. The median ages were 35 (range 19–45) and 33 (range 30–53) years, respectively. Five subjects in the first group and two in the second group were women. All participants were symptom-free, had no history of allergic rhinitis or any other atopic disease, and had negative skin prick tests against a panel of common airborne allergens. Patients with aspirin-induced airway symptoms were excluded from the study along with subjects with a history of concurrent purulent nasal infection in the 6 weeks before the study or any kind of nasal surgery during the last year. None of the participating patients suffered from asthma.

The study was approved by the local Ethics Committee of the University of Lund and an informed written consent was obtained from all subjects. Subjects were recruited through advertisements.

Rhinomanometry

Nasal airway resistance (NAR) was determined by active anterior rhinomanometry [21]. A pressure catheter was connected to one nostril. The flow was measured by a pneumotachograph via an anaesthetic mask held air-tight with as little pressure as possible over the nose and mouth. Each nasal passage was measured separately and a total resistance was calculated. The recording of a pressure-flow curve was presented on a computer screen and total NAR was expressed as $V_{2\text{tot}}$ representing the resistance of nasal airflow according to the polar co-ordinate system of Broms et al. [22]. This method delivers adequate comparable resistance data even during periods of severe nasal obstructions. Before the measurements all subjects rested in a sitting position close to the equipment.

Nasal lavage and cell quantification

Nasal lavage fluids were collected as previously described [23]. Briefly, after clearing excess mucous by forceful exsufflation, a sterile saline solution of room temperature was aerosolized into both nostrils, alternately. The fluids were allowed to return passively and collected in a graded test tube, until 7 mL was recovered. The total number of cells/mL was computed in a Bürker chamber. The fluids were then centrifuged for 10 min at 1300 g at 4 °C. The supernatant was separated from the pellet. The pellet was used to prepare slides that were stained according to the May-Grünwald and Giemsa method for morphological assessment of cells in the fluid. A differential count was performed including epithelial cells, eosinophils, neutrophils and mononuclear cells (100 cells/slide). The total number of each cell type was also estimated (cells/mL). All fluid samples were processed within 30 min after the lavage (M Benson, G Wennergren, M Fransson, LO Cardell: unpublished data).

Mediators in nasal lavage fluid

The nasal lavage fluids were analysed for levels of albumin (Pharmacia Albumin RIA, Pharmacia Diagnostics, Uppsala, Sweden), IL-1β, IL-1 receptor (IL-1sR2) and IL-1 receptor antagonist (IL-1RA) using ELISA kits (Quantikine HS) from R&D Systems (Abingdon, UK).

Study design

After 15 min of supine rest, the NAR was measured and a nasal lavage was performed. Then 200 µL of a 1 µM PACAP solution was sprayed in each nasal cavity. NAR was measured after 5 min and a second nasal lavage was performed after 10 min. A third nasal lavage was performed 4 h later. After 7–10 days, the protocol was repeated replacing the PACAP solution with vehicle (0.9% NaCl).

Since the sensitivity of rhinomanometry is limited at low levels of NAR (normal conditions), the effect of PACAP pretreatment on histamine-induced increase of NAR was also evaluated. After an initial resting period, rhinomanometry and nasal lavage were performed followed by the application of PACAP in the nasal cavity (same dose and application procedure as above). NAR was measured after 5 min. Then solutions with rising concentrations of histamine (0.1, 0.9, 3, 7 and 10 mg/mL) were sprayed in both nasal cavities with an interval of 5 min between doses. Higher concentrations of histamine than 10 mg/mL could not be used due to systemic side-effects. NAR was measured before each new dose of histamine. A final nasal lavage was performed at 4 h. After 7–10 days, the protocol was repeated replacing the PACAP solution with vehicle (0.9% NaCl).

The effects of nasal application of PACAP on blood pressure, heart rate and pulmonary functions were evaluated in separate experiments (see below).

ECG, blood pressure and spirometry

The effects on the cardiovascular system and the function of the lower airways after PACAP application in the nose were evaluated in separate experiments. Initially the subjects rested for 20 min in a supine position. Then, with the subjects remaining in a supine position, an ECG (MAC 6, Marquette Electronics Inc., Milwaukee, WI, USA) was recorded, and pulse and blood pressure were measured. These proceedings were followed by spray application of 200 µL of PACAP (1 µM) in each nasal cavity. The pulse and blood pressure were subsequently recorded after 2, 5, 10, 15, 20, 25 and 30 min, and an ECG was recorded at 2 and 30 min. A couple of weeks later, the PACAP application procedure was repeated, but this time followed by measurement of the lung function using spirometry (Master Scope, Erich Jaeger GmbH, Höchberg, Germany) after 15 min and 3 h. Any subjective discomfort following the PACAP application was noticed.

Solutions and drugs

PACAP 38 (Peninsula Laboratories, San Carlos, CA, USA) and histamine (Sigma, St Louis, MO, USA) were used. All drugs were dissolved in 0.9% NaCl.

Statistics

Results are expressed as mean $\pm$ SEM in text and median in figures (if not otherwise stated). Statistical comparisons were made by Student's *t*-test and *P*-values less than 0.05 were considered to be statistically significant.

Results

Thin, varicose, PACAP-like containing nerve fibres were observed close to the smooth muscle of arteries and veins, and close to seromucous glands in the nasal turbinates from all investigated patients (Fig. 1). There was no principal difference in the PACAP innervation pattern between patients.

PACAP (1 µM) increased the $V_{2\text{tot}}$ from $14.9 \pm 1.8^\circ$ (vehicle) to $18.1 \pm 2.2^\circ$ (PACAP) 10 min after the application ($n = 7$, $P < 0.05$) (Fig. 2a). The cumulative application of histamine resulted in a dose-dependent increase of $V_{2\text{tot}}$. Pretreatment with PACAP (1 µM) augmented the effects induced by higher concentrations of histamine (7 and 10 mg/mL) ($n = 7$) (Fig. 2b).

Nasal lavage fluids were obtained on three occasions: before, 10 min and 4 h after the application of PACAP or vehicle. With the exception of epithelial cells, the neutrophils were the dominating cells in the nasal lavage fluid. A differential count revealed that more than 97% of the cells were neutrophils and about 1% consisted of eosinophils. This relation was not altered following the application of histamine (98/1% and 96/4% at 10 min, and 4 h, respectively).

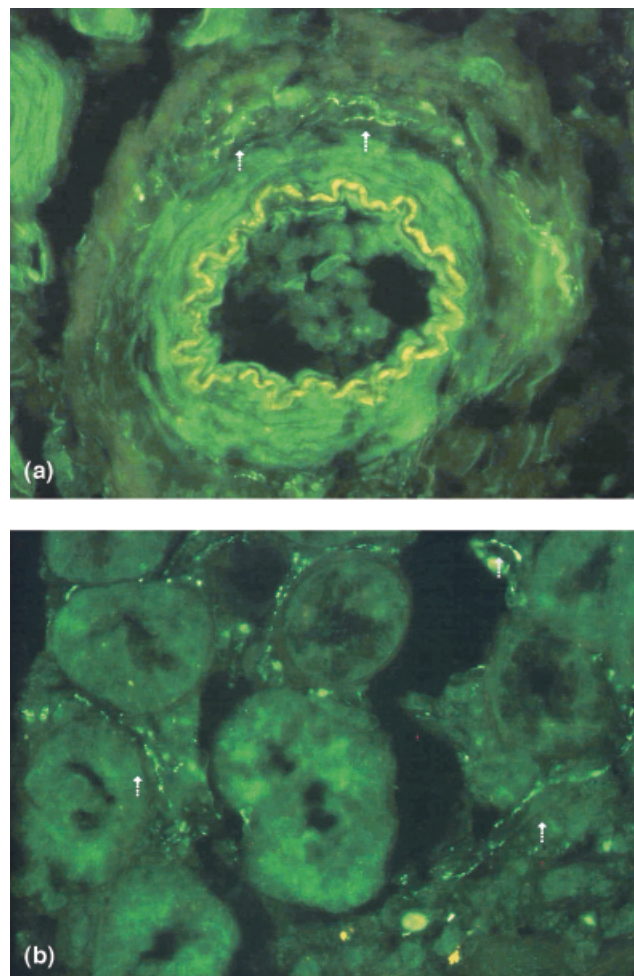


Fig. 1. PACAP-like immunoreactive nerve fibres in human nose (a), thin PACAP-like nerve fibres close to nasal blood vessels (arrows) (b), and in connection with seromucous glands (arrows) ($\times 300$).

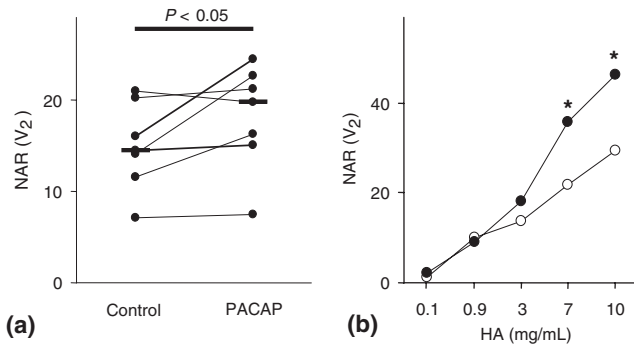


Fig. 2. (a) Nasal application of PACAP and rhinomanometric evaluation of nasal airway resistance (NAR); 400 μ L of vehicle (control) or a 1 μ M PACAP solution was sprayed into the nasal cavity and NAR was measured 5 min later. Data are expressed as $V_{2\text{tot}}$ values, horizontal bars represent the median values $n = 7$. (b) Effect of PACAP pretreatment on histamine-induced increases of NAR; 400 μ L of vehicle (control, $\circ$) or a 1 μ M PACAP 38 solution ($\bullet$) was sprayed into the nasal cavity followed by spray application of histamine solutions with rising concentrations with an interval of 5 min. NAR was measured 5 min after each histamine application. Data are expressed as increases in $V_{2\text{tot}}$ values following the histamine application, horizontal bars represent the median values, $n = 7$. * $P < 0.05$.

Neither was the relation significantly affected by PACAP (PACAP only; 86/0% and 91/3% at 10 min and 4 h, respectively; PACAP in combination with histamine; 98/0% and 97/0% at 10 min and 4 h, respectively). However, PACAP tended to decrease the total amount of cells found in the lavage fluid at 4 h from $1.8 \pm 0.6 \times 10^4$ cells/mL (vehicle) to $1.1 \pm 0.6 \times 10^4$ cells/mL (PACAP) (Fig. 3a). The inhibitory effect of PACAP became more evident with histamine. Histamine increased the number of neutrophils with $4.9 \pm 2.3 \times 10^4$ cells/mL at 4 h. This increase was reduced to $0.8 \pm 0.8 \times 10^4$ cells/mL following PACAP pretreatment ($n = 7$, $P < 0.05$) (Fig. 3b). No increase in the amount of cells was obtained at 10 min after the application of either PACAP or vehicle.

The amount of albumin in nasal lavage fluid increased following the application of PACAP, from 16 ± 5 mg/L (vehicle) to 26 ± 9 mg/L (PACAP) at 10 min ($n = 7$, $P < 0.05$) (Fig. 3). An increase was seen when histamine was administered to the nose after PACAP pretreatment; vehicle+histamine: 71 ± 19 mg/L and PACAP+histamine: 107 ± 14 mg/L ($n = 7$, $P < 0.05$). An increase was still evident at 4 h; vehicle:

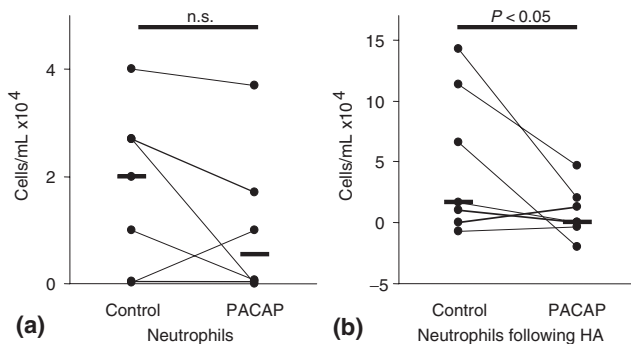


Fig. 3. (a) Nasal application of PACAP and neutrophils in nasal lavage fluid; 400 μ L of vehicle (control) or a 1 μ M PACAP solution was sprayed into the nasal cavity and nasal lavage was performed 4 h later. Data are expressed as neutrophils per mL, horizontal bars represent the median values, $n = 7$. (b) Effect of PACAP pretreatment on histamine-induced increase of neutrophils; 400 μ L of vehicle (control) or a 1 μ M PACAP solution was sprayed into the nasal cavity followed by spray application of histamine (10 mg/mL). Nasal lavage was performed 4 h later. Data are expressed as mean increase in the number of neutrophils following the histamine application, horizontal bars represent the median values, $n = 7$, n.s. = not significant.

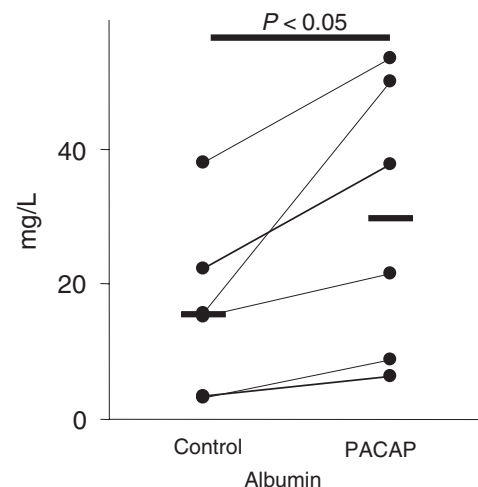


Fig. 4. Nasal application of PACAP and effects on albumin in nasal lavage fluid; 400 μ L of vehicle (control) or a 1 μ M PACAP solution was sprayed into the nasal cavity and nasal lavage was performed 4 h later. Albumin levels were analysed using ELISA kits. Data are expressed as gram albumin per liter, horizontal bars represent the median values, $n = 7$.

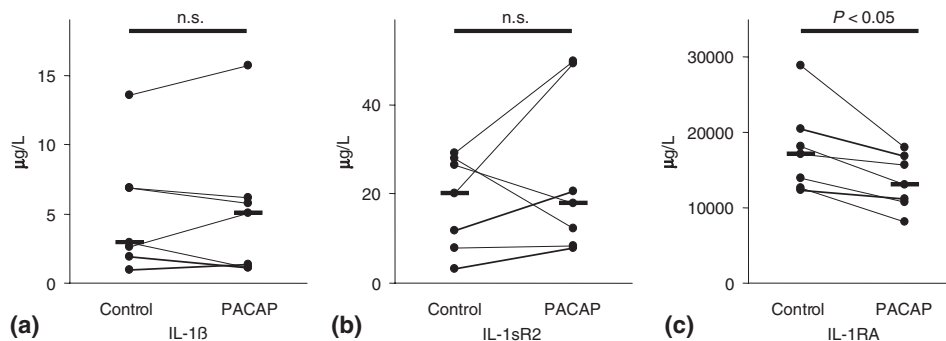


Fig. 5. Nasal application of PACAP and effects on cytokine-related mediators in nasal lavage; 400 μ L of vehicle (control) or a 1 μ M PACAP solution was sprayed into the nasal cavity and nasal lavage was performed 4 h later. (a) IL-1 β . (b) Soluble IL-1 receptor (IL-1sR2). (c) IL-1 receptor antagonist (IL-1RA). Mediator levels were evaluated using ELISA kits. Data are expressed as mediator levels per mL, horizontal bars represent the median values, $n = 7$.

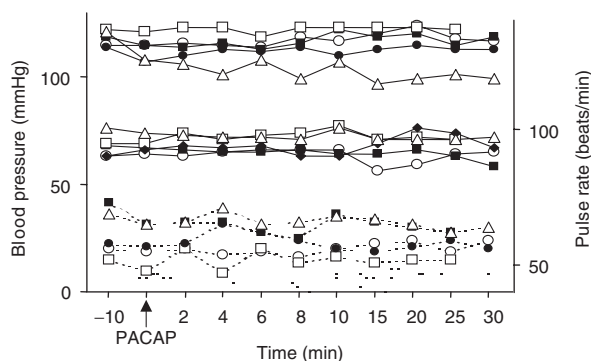


Fig. 6. Nasal application of PACAP and effects on blood pressure and heart rate; after 20 min of rest in the supine position, blood pressure and pulse were checked followed by the application of 400 μ L of a 1 μ M PACAP solution (arrow) in the nasal cavity. Blood pressure and pulse were continuously controlled after the application. Data are expressed as mmHg (systolic and diastolic pressure, left axis) and beats per minute (pulse, right axis), horizontal bars represent the median values, $n = 5$.

Table 1. PACAP-induced effects on blood pressure, heart rate and pulmonary functions

	0 min	2 min	15 min	25 min	3 h
Syst (mmHg)	113 $\pm$ 2	114 $\pm$ 2		114 $\pm$ 2	
Diast (mmHg)	68 $\pm$ 3	69 $\pm$ 2		69 $\pm$ 2	
Pulse (beats/min)	58 $\pm$ 2	60 $\pm$ 2		58 $\pm$ 2	
FEV ₁ (L/1 s)	4.3 $\pm$ 0.4		4.3 $\pm$ 0.4		4.3 $\pm$ 0.3
VC (L)	4.8 $\pm$ 0.4		4.9 $\pm$ 0.4		4.8 $\pm$ 0.4
FEV ₁ %	86 $\pm$ 0.5		84 $\pm$ 1.8		86 $\pm$ 2.0

Systolic (Syst) and diastolic (Diast) blood pressure, pulse; 1-s forced expiratory volume (FEV₁), vital capacity (VC) and FEV₁% were measured before, 2 min, 25 min and 3 h after spraying 400 μ L of a 10⁻⁶ M PACAP solution into the nasal cavity. Data are expressed as mean $\pm$ SEM for seven individuals.

17 $\pm$ 4 mg/L, PACAP: 43 $\pm$ 19 mg/L and vehicle+histamine: 71 $\pm$ 19 mg/L, PACAP+histamine: 75 $\pm$ 18 mg/L ($n = 7$, $P < 0.05$) (Fig. 4).

Previous work has demonstrated that the IL-1 receptor antagonist IL-1RA exists in relatively high concentrations in nasal lavage (M Benson, G Wennergren, M Fransson, LO Cardell: unpublished data). Thus, IL-1 related ligands became the focus for our cytokine measurements. The levels of IL-1 β and its soluble receptor IL-1sR2 were not affected by PACAP application, whereas the levels of IL-1RA were reduced ($n = 7$, $P < 0.05$) (Fig. 5).

Heart rate and blood pressure were not affected by nasal PACAP application ($n = 5$) (Fig. 6), neither was the ECG ($n = 5$, not shown). In accordance, no change in VC, FEV₁, FEV₁% or FVC were seen at 15 min and 4 h after the PACAP application (Table 1). None of the tested subjects, with the exception of a mild sensation of nasal secretion and blockage, reported any discomfort following the PACAP treatment.

Discussion

PACAP-like immunoreactive nerve fibres were seen in the nasal mucosa, close to vessels and seromucous glands.

Application of PACAP in the nasal cavity increased NAR and augmented the increase in NAR induced by histamine. In addition, PACAP inhibited histamine-induced recruitment of neutrophils, increased plasma leakage and reduced the level of the IL-1RA in nasal lavage.

PACAP has a wide distribution in the central and peripheral nervous system [24, 25]. In the present study, the human nasal mucosa was shown to contain a moderate number of PACAP-immunoreactive nerve fibres, which is in line with a previous report on the upper airways [26]. PACAP-immunoreactive fibres in the superficial layer of the dorsal horns of the spinal cord and in the epithelium have been reported in the rat [20]. This together with the findings of PACAP-immunoreactive neurons in sensory ganglia and the observation that capsaicin treatment markedly reduces the number of PACAP-immunoreactive nerve fibres, has suggested a role for PACAP as a sensory peptide [20, 27]. However, in airways, the majority of PACAP-immunoreactive nerves have been described in association with parasympathetic neurons [28, 29], indicating a role for PACAP in secretion and vasodilatation.

PACAP has been reported to produce a potent, long-lasting vasodilatation *in vitro* as well as *in vivo* [30, 31]. Local application of PACAP in the nose resulted in an increase of NAR. This reflects an increased thickness of the nasal mucosa, as a result of augmented blood content and oedema due to vasodilatation. In analogy, we have previously demonstrated that PACAP is a dilator of isolated human pulmonary arteries [3]. As dilators of pulmonary arteries, PACAP and VIP appear to induce identical effects [3], suggesting that the activity is mediated by a receptor subtype with high affinity for both peptides (VPAC₁ or VPAC₂) [1]. In contrast, the bronchodilator effects of PACAP in human tissue appear to be more consistent than the effects induced by VIP [3, 32], indicating an involvement of a receptor with higher affinity for PACAP than VIP (PAC₁) [1, 17].

In vivo experiments on guinea-pig have demonstrated that inhalation of PACAP has no effect on airway tonus *per se*, but inhibits histamine- and allergen-induced bronchoconstriction [4, 33]. Similar data can be obtained with isolated airways, where a pre-contraction is necessary in order to demonstrate PACAP- and VIP-induced relaxations [3, 31]. So far, *in vivo* studies of PACAP have not been performed in humans airways, but an inhibitory effect of VIP on histamine-induced bronchoconstriction has been demonstrated in asthmatic subjects [34]. In the present study, nasal application of PACAP did not seem to affect pulmonary functions. The values for FEV₁, VC and FEV₁% remained unchanged during the 3-h testing period. These results were not unexpected since airway precontraction *in vivo* as well as *in vitro* is a prerequisite in order to demonstrate a bronchodilatory effect of PACAP [3, 33].

The patients reported a mild sensation of nasal secretion following nasal application of PACAP. Nasal secretion is a mixture of mucus from goblet cells, fluid secretion from seromucous glands and watery secretion from anterior serous glands. In addition, there is a contribution from transudated blood plasma [35, 36]. PACAP has been reported to induce secretion of saliva from all major salivary glands in rat [37]. The presence of PACAP-like immunoreactivity in nerve fibres

surrounding seromucous glands, in conjunction with the described secretory sensation, might reflect a similar role for PACAP in humans. Albumin is a well-known marker of plasma extravasation [38]. The amount of albumin in nasal lavage increased following the application of PACAP. In addition, the increase in albumin induced by histamine was further augmented by PACAP pretreatment. These results are in concordance with previous findings in rat and rabbit skin [39, 40]. Neutrophils were the dominating cells in the nasal lavage in all test situations. This is a reflection of the continuous migration of neutrophils into the nasal cavity. The relatively high amounts of neutrophils is a well-known phenomenon often seen as 'physiological inflammation' in the mucosa, induced by continuous exposure to microorganisms and inhaled particles [41]. Eosinophils, which are quite common in the connective tissue of the lamina propria, appear under normal conditions in relatively low numbers both in the epithelium and in nasal secretions [42, 43]. In the present study, we demonstrated that PACAP pre-treatment decreased histamine-induced influx of neutrophils into the nasal cavity. The relation between cells in the differential count was not changed, implying a down-regulation also of eosinophil migration. However, due to the low levels of eosinophils this could not be statistically proven. A tendency for PACAP to reduce the normal, physiological influx of cells to the nasal cavity was also seen. The neutrophil data are in line with results derived from recent Boyden chamber experiments; PACAP as well as VIP were reported to inhibit neutrophil chemotaxis [16]. We have recently demonstrated an increased activation of neutrophils following migration into the nasal lumen during allergic rhinitis, an effect that is paralleled by a marked increase of the total amount of neutrophils found in the nasal cavity, suggesting a role for the neutrophils in the local allergic reaction [15]. The neutrophil data therefore support the idea of an anti-inflammatory role of PACAP. However, this interpretation is not entirely in line with the finding of an enhanced activity of neutrophils following PACAP application *in vitro* [17]. The reduced levels of IL-1RA in nasal lavage, along with unaltered levels of IL-1 and IL-1sR2, might argue against an anti-inflammatory role of PACAP. The IL-1 family consists of two agonists, IL-1 α and IL-1 β , two receptors, biologically active IL-1R1 and inert IL-1R2, and a specific receptor antagonist, IL-1RA. IL-1R2 also exists as a soluble receptor (IL-1sR2) in the fluid phase, preventing IL-1 from interacting with the functional IL-1R1 on the plasma membrane. The balance between IL-1 and IL-1RA in local tissues is suggested to play an important role in the susceptibility to and severity of many diseases, including airway inflammation [44]. The concentration of IL-1RA is reported to be about 1000 times higher than the concentrations of most other cytokines and soluble cytokine receptors in nasal lavage (M Benson, G Wennergren, M Fransson, LO Cardell: unpublished data), and the present analysis confirms the unique position of IL-1RA in this aspect. A lowered ratio of IL-1RA to IL-1, following PACAP application, is generally associated with airway disease [44]. A reduction of this ratio is seen in nasal lavage in conjunction with symptomatic intermittent allergic rhinitis (M Benson, G Wennergren, M Fransson, LO Cardell: unpublished data), and similar findings are seen in

bronchoalveolar lavage fluid from patients with status asthmaticus [45].

The IL-1RA findings, together with the previous report about PACAP-induced neutrophil activation, could be interpreted as contradicting an anti-inflammatory effect of PACAP in airways. However, evidence for an anti-inflammatory role of PACAP can be derived from a long series of experiments on macrophages. For instance, PACAP inhibits the release of TNF- α and increases IL-6 production in unstimulated macrophages, through activation of PKA and PKC. In lipopolysaccharide-stimulated macrophages, PACAP enhances the production of IL-10 and inhibits the release of IL-6, IL-12 and TNF- α , thereby acting as a protective agent regulating excessive release of pro-inflammatory cytokines [9, 10, 46–48].

It is known from experiments in animals that PACAP administered as a bolus intravenously increases heart rate and induces systemic hypotension [17]. Presently, nasal application of PACAP in concentrations inducing local vascular and secretory effects had no effect on systemic blood pressure, heart rate or ECG pattern. These findings are in line with data obtained after airway administration of PACAP to animals, where only mild cardiovascular side effects were noticed [4, 33]. Furthermore, infusion of PACAP in man has been reported to result in only negligible effects on heart rate and blood pressure [49, 50].

In summary, the results strengthen the case for PACAP as an important endogenous mediator in human upper airways, with a potential role as a regulator of vascular smooth muscle, secretion, plasma extravasation, neutrophil recruitment and cytokine activity. In addition, the dual effects of PACAP on airway inflammation are emphasized.

Acknowledgements

This work was supported by grants from the Swedish Medical Research Council, the Swedish Society of Medicine, the Swedish Society of Medicine Research, the Swedish Heart Lung Foundation, the Harald Jeansson Foundation, the Konsul Th C Bergh foundation, the Österlund Foundation and the University of Lund. The authors would also like to thank Ann Reutherborg, Bodil Persson, Helena Gunnlaugsdottir and Ingegerd Larsson for skilful technical assistance during the course of this study.

References

- 1 Vaudry D, Gonzalez BJ, Basille M, Yon L, Fournier A, Vaudry H. Pituitary adenylate cyclase-activating polypeptide and its receptors: from structure to functions. *Pharmacol Rev* 2000; 52:269–324.
- 2 Luts A, Uddman R, Alm P, Basterra J, Sundler F. Peptide-containing nerve fibers in human airways: distribution and coexistence pattern. *Int Arch Allergy Immunol* 1993; 101:52–60.
- 3 Kinhult J, Andersson JA, Uddman R, Stjarne P, Cardell LO. Pituitary adenylate cyclase-activating peptide 38 a potent endogenously produced dilator of human airways. *Eur Respir J* 2000; 15:243–7.
- 4 Linden A, Yoshihara S, Chan B, Nadel JA. Inhibition of bronchoconstriction by pituitary adenylate cyclase activating polypeptide (PACAP 1–27) in guinea-pigs *in vivo*. *Br J Pharmacol* 1995; 115:913–6.

- 5 Palmer JB, Cuss FM, Warren JB, Blank M, Bloom SR, Barnes PJ. Effect of infused vasoactive intestinal peptide on airway function in normal subjects. *Thorax* 1986; 41:663–6.
- 6 Bellinger DL, Lorton D, Broukhon S, Felten S, Felten DL. The significance of vasoactive intestinal polypeptide (VIP) in immunomodulation. *Adv Neuroimmunol* 1996; 6:5–27.
- 7 Ganea D, Delgado M. Neuropeptides as modulators of macrophage functions. Regulation of cytokine production and antigen presentation by VIP and PACAP. *Arch Immunol Ther Exp (Warsz)* 2001; 49:101–10.
- 8 Delgado M, Munoz-Elias EJ, Gomariz RP, Ganea D. Vasoactive intestinal peptide and pituitary adenylate cyclase-activating polypeptide enhance IL-10 production by murine macrophages: *in vitro* and *in vivo* studies. *J Immunol* 1999; 162:1707–16.
- 9 Delgado M, Martinez C, Pozo D et al. Vasoactive intestinal peptide (VIP) and pituitary adenylate cyclase-activation polypeptide (PACAP) protect mice from lethal endotoxemia through the inhibition of TNF- α and IL-6. *J Immunol* 1999; 162:1200–5.
- 10 Delgado M, Pozo D, Martinez C et al. Vasoactive intestinal peptide and pituitary adenylate cyclase-activating polypeptide inhibit endotoxin-induced TNF- α production by macrophages: *in vitro* and *in vivo* studies. *J Immunol* 1999; 162:2358–67.
- 11 Gottschall PE, Tatsuno I, Arimura A. Regulation of interleukin-6 (IL-6) secretion in primary cultured rat astrocytes: synergism of interleukin-1 (IL-1) and pituitary adenylate cyclase activating polypeptide (PACAP). *Brain Res* 1994; 637:197–203.
- 12 Sekiya K, Nagasaki H, Ozaki N, Suzuki A, Miura Y, Oiso Y. Pituitary adenylate cyclase-activating polypeptide prevents cytokine-induced cytotoxicity via inhibition of inducible nitric oxide synthase expression in beta TC cells. *Biochem Biophys Res Commun* 2000; 278:211–6.
- 13 Roca-Ferrer J, Mullol J, Xaubet A et al. Proinflammatory cytokines and eosinophil cationic protein on glandular secretion from human nasal mucosa: regulation by corticosteroids. *J Allergy Clin Immunol* 2001; 108:87–93.
- 14 Benson M, Adner M, Cardell LO. Cytokines and cytokine receptors in allergic rhinitis: how do they relate to the Th2 hypothesis in allergy? *Clin Exp Allergy* 2001; 31:361–7.
- 15 Kinhult J, Egesten A, Benson M, Uddman R, Cardell LO. Increased expression of surface activation markers on neutrophils following migration into the nasal lumen during allergic rhinitis. *Clin Exp Allergy* 2003; in press.
- 16 Kinhult J, Uddman R, Laan M, Linden A, Cardell LO. Pituitary adenylate cyclase-activating peptide inhibits neutrophil chemotaxis. *Peptides* 2001; 22:2151–4.
- 17 Kinhult J, Egesten A, Uddman R, Cardell LO. PACAP enhances the expression of CD11b, CD66 and CD 63 in human neutrophils. *Peptides* 2002; 28:1735–9.
- 18 Pedrera C, Lucas M, Bellido L, Lopez-Gonzalez MA. Receptor-independent mechanisms are involved in the priming of neutrophil's oxidase by vasoactive intestinal peptide. *Regul Pept* 1994; 54:505–11.
- 19 Linden A, Cardell LO, Yoshihara S, Nadel JA. Bronchodilation by pituitary adenylate cyclase-activating peptide and related peptides. *Eur Respir J* 1999; 14:443–51.
- 20 Moller K, Zhang YZ, Hakanson R et al. Pituitary adenylate cyclase activating peptide is a sensory neuropeptide: immunocytochemical and immunochemical evidence. *Neuroscience* 1993; 57:725–32.
- 21 Welinder R, Cardell LO, Uddman R, Malm L. Reduced nasal airway resistance following uvulopalatoplasty. *Rhinology* 1997; 35:16–8.
- 22 Broms P, Jonson B, Lamm CJ. Rhinomanometry. II. A system for numerical description of nasal airway resistance. *Acta Otolaryngol* 1982; 94:157–68.
- 23 Benson M, Strannegard IL, Strannegard O, Wennergren G. Topical steroid treatment of allergic rhinitis decreases nasal fluid TH2 cytokines, eosinophils, eosinophil cationic protein, and IgE but has no significant effect on IFN- γ , IL-1 β , TNF- α , or neutrophils. *J Allergy Clin Immunol* 2000; 106:307–12.
- 24 Arimura A, Somogyvari-Vigh A, Miyata A, Mizuno K, Coy DH, Kitada C. Tissue distribution of PACAP as determined by RIA: highly abundant in the rat brain and testes. *Endocrinology* 1991; 129:2787–9.
- 25 Kivipielto L, Absood A, Arimura A, Sundler F, Hakanson R, Panula P. The distribution of pituitary adenylate cyclase-activating polypeptide-like immunoreactivity is distinct from helodermin- and helospectin-like immunoreactivities in the rat brain. *J Chem Neuroanat* 1992; 5:85–94.
- 26 Hauser-Kronberger C, Hacker GW, Albegger K et al. Distribution of two VIP-related peptides, helospectin and pituitary adenylate cyclase activating peptide (PACAP), in the human upper respiratory system. *Regul Pept* 1996; 65:203–9.
- 27 Chaudhary P, Baumann T. Expression of VPAC2 receptor and PAC1 receptor splice variants in the trigeminal ganglion of the adult rat. *Brain Res Mol Brain Res* 2002; 104:137.
- 28 Uddman R, Luts A, Arimura A, Sundler F. Pituitary adenylate cyclase-activating peptide (PACAP), a new vasoactive intestinal peptide (VIP)-like peptide in the respiratory tract. *Cell Tissue Res* 1991; 265:197–201.
- 29 Sundler F, Ekblad E, Absood A, Hakanson R, Koves K, Arimura A. Pituitary adenylate cyclase activating peptide: a novel vasoactive intestinal peptide-like neuropeptide in the gut. *Neuroscience* 1992; 46:439–54.
- 30 Warren JB, Donnelly LE, Cullen S et al. Pituitary adenylate cyclase-activating polypeptide: a novel, long-lasting, endothelium-independent vasorelaxant. *Eur J Pharmacol* 1991; 197:131–4.
- 31 Cardell LO, Uddman R, Luts A, Sundler F. Pituitary adenylate cyclase activating peptide (PACAP) in guinea-pig lung: distribution and dilatory effects. *Regul Pept* 1991; 36:379–90.
- 32 Foda HD, Sharaf HH, Absood A, Said SI. Pituitary adenylate cyclase-activating peptide (PACAP), a VIP-like peptide, has prolonged airway smooth muscle relaxant activity. *Peptides* 1995; 16:1057–61.
- 33 Linden A, Cardell LO, Yoshihara S, Stjarne P, Nadel JA. PACAP 1-38 as an inhaled bronchodilator in guinea pigs *in vivo*. *Peptides* 1998; 19:93–8.
- 34 Morice A, Unwin RJ, Sever PS. Vasoactive intestinal peptide causes bronchodilation and protects against histamine-induced bronchoconstriction in asthmatic subjects. *Lancet* 1983; 2:1225–7.
- 35 Baraniuk JN, Kaliner MA. Neuropeptides and nasal secretion. *J Allergy Clin Immunol* 1990; 86(Part 2):620–7.
- 36 Brittebo EB. Metabolism-dependent activation and toxicity of chemicals in nasal glands. *Mutat Res* 1997; 380:61–75.
- 37 Mirfendereski S, Tobin G, Hakanson R, Ekstrom J. Pituitary adenylate cyclase activating peptide (PACAP) in salivary glands of the rat: origin, and secretory and vascular effects. *Acta Physiol Scand* 1997; 160:15–22.
- 38 Svensson C, Baumgarten CR, Pipkorn U, Alkner U, Persson CG. Reversibility and reproducibility of histamine induced plasma leakage in nasal airways. *Thorax* 1989; 44:13–8.
- 39 Cardell LO, Stjarne P, Wagstaff SJ, Agusti C, Nadel JA. PACAP-induced plasma extravasation in rat skin. *Regul Pept* 1997; 71:67–71.
- 40 Warren JB, Larkin SW, Coughlan M, Kajekar R, Williams TJ. Pituitary adenylate cyclase activating polypeptide is a potent vasodilator and oedema potentiator in rabbit skin *in vivo*. *Br J Pharmacol* 1992; 106:331–4.
- 41 Mygind N. Nasal inflammation and anti-inflammatory treatment. Semantics or clinical reality. *Rhinology* 2001; 39:61–5.
- 42 Mygind N, Thomsen J. Cytology of the nasal mucosa. A comparative study between a replica-method and a smear-method. *Arch Klin Exp Ohren Nasen Kehlkopfheilkd* 1973; 204:123–9.

- 43 Mygind N, Bretlau P. Scanning electron microscopic studies of the human nasal mucosa in normal persons and in patients with perennial rhinitis. II. Secretion. *Acta Allergol* 1974; 29:261–80.
- 44 Arend W. The balance between IL-1 and IL-1Ra in disease. *Cytokine Growth Factor Rev* 2002; 13:323.
- 45 Tillie-Leblond I, Pugin J, Marquette CH et al. Balance between proinflammatory cytokines and their inhibitors in bronchial lavage from patients with status asthmaticus. *Am J Respir Crit Care Med* 1999; 159:487–94.
- 46 Delgado M, Munoz-Elias EJ, Gomariz RP, Ganea D. VIP and PACAP inhibit IL-12 production in LPS-stimulated macrophages. Subsequent effect on IFN γ synthesis by T cells. *J Neuroimmunol* 1999; 96:167–81.
- 47 Martinez C, Delgado M, Pozo D et al. Vasoactive intestinal peptide and pituitary adenylate cyclase-activating polypeptide modulate endotoxin-induced IL-6 production by murine peritoneal macrophages. *J Leukoc Biol* 1998; 63:591–601.
- 48 Martinez C, Delgado M, Pozo D et al. VIP and PACAP enhance IL-6 release and mRNA levels in resting peritoneal macrophages: *in vitro* and *in vivo* studies. *J Neuroimmunol* 1998; 85:155–67.
- 49 Hammond PJ, Talbot K, Chapman R, Ghatei MA, Bloom SR. Vasoactive intestinal peptide, but not pituitary adenylate cyclase-activating peptide, modulates the responsiveness of the gonadotroph to LHRH in man. *J Endocrinol* 1993; 137:529–32.
- 50 Chiodera P, Volpi R, Capretti L, Coiro V. Effects of intravenously infused pituitary adenylate cyclase-activating polypeptide on arginine vasopressin and oxytocin secretion in man. *Neuroreport* 1995; 6:1490–2.