Inhibition of bronchoconstriction by pituitary adenylate cyclase activating polypeptide (PACAP 1-27) in guinea-pigs *in vivo*

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1 We studied the inhibitory effect of pituitary adenylate cyclase activating polypeptide (PACAP 1-27) on the increase in total pulmonary resistance (R_L) caused either by allergen or histamine in anaesthetized, ventilated guinea-pigs.

2 PACAP 1-27 given via i.v. infusion (0.045 – 4.5 nmol kg⁻¹ min⁻¹) dose-dependently reduced the increase in R_L caused by inhaled ovalbumin and histamine. At the highest dose, PACAP 1-27 prevented the increase in R_L caused by ovalbumin and histamine completely. Infusion of PACAP 1-27 and the β_2 -adrenoceptor agonist, salbutamol (0.045 – 4.5 nmol kg⁻¹ min⁻¹) inhibited the increase in R_L similarly, but salbutamol increased the heart rate more than PACAP 1-27.

3 PACAP 1-27 and salbutamol given via inhaled aerosol (0.1 mM, 20 breaths) significantly reduced the increase in R_L caused by histamine infused i.v., whereas aerosolised sterile saline did not. Both PACAP 1-27 and salbutamol caused bronchodilator effects within 1 min of drug inhalation and these effects remained throughout the 20 min of study.

4 Because PACAP 1-27 produced significant bronchodilatation and rapid onset of sustained action *in vivo* and without pronounced cardiovascular side effects, we conclude that this peptide may have therapeutic potential as a bronchodilator.

Keywords: Bronchodilator; histamine; inhalation; intravenous infusion; ovalbumin; PACAP; pulmonary resistance; salbutamol

Introduction

Immunoreactivity for pituitary adenylate cyclase activating polypeptide (PACAP) is present in airway smooth muscle of guinea-pigs and man (Cardell *et al.*, 1991; Luts *et al.*, 1993). *In vitro*, PACAP 1-38 relaxes guinea-pig and rabbit tracheal smooth muscle precontracted by histamine and by acetylcholine (Cardell *et al.*, 1991; Kanemura *et al.*, 1993). PACAP 1-38 also increases adenosine 3':5'-cyclic monophosphate (cyclic AMP) in tracheal smooth muscle, providing a possible mechanism for the relaxant effect of PACAP 1-38 (Kanemura *et al.*, 1993). However, the extent to which PACAP 1-27, the N-terminal residue of PACAP 1-38, relaxes airway smooth muscle is unknown and the PACAPs have not been tested against bronchoconstriction *in vivo*.

We assessed PACAP 1-27 as a bronchodilator and compared it with a β_2 -adrenoceptor agonist, salbutamol, in anaesthetized, ventilated guinea-pigs. The two bronchodilators were administered both by i.v. infusion and by inhaled aerosol. Increase in total pulmonary resistance (R_L) was induced by ovalbumin inhalation and by histamine both by inhalation and i.v. infusion. Heart rate was monitored as an indicator of cardiovascular side effects during i.v. infusion of the bronchodilators.

Methods

Animals

Eighty-two male Hartley guinea-pigs (Simonsen Lab. Inc., Gilroy, CA, U.S.A.) were maintained pathogen-free (mean $\pm$ s.d. weight: 420 ± 54 g) in a temperature-controlled environment with sterile laboratory food and water freely available.

Sensitization procedure

For ovalbumin challenge, animals were sensitized by intraperitoneal injection of ovalbumin (70 mg) dissolved in

sterile saline (1.5 ml) (Bertrand *et al.*, 1993). This injection was given twice, at one week intervals, and the animals were challenged two weeks after the second injection.

Measurement of R_L

Animals were initially anaesthetized with pentobarbitone (45 mg kg⁻¹, i.p.) and then ventilated (85×3 ml min⁻¹) via a tracheal cannula connected to a constant-volume ventilator (model 683 Harvard Apparatus, South Natick, MA, U.S.A.). As previously described (Bertrand *et al.*, 1993), airflow was monitored continuously with a pneumotachygraph (A. Fleisch Medical, Richmond, VA, U.S.A.) connected to a differential pressure transducer (model DP45; Validyne Engineering, Northridge, CA, U.S.A.). A fluid-filled catheter was introduced into the oesophagus to measure local pressure as an approximation of pleural pressure. Intratracheal pressure was measured with another catheter connecting the tracheal tube to the pneumotachygraph. Transpulmonary pressure was measured with a differential pressure transducer (model DP7; Validyne Engineering, Northridge, CA). Output signals representing transpulmonary pressure and airflow were amplified (model CD19; Validyne Engineering, Northridge, CA) and recorded on a polygraph recorder (model 1508 B Visicorder; Honeywell, Denver, CO). R_L was calculated as previously described (Dusser *et al.*, 1988). Heart rate was monitored via an electrocardiogram recorded on the polygraph recorder. A catheter (22 GA IIN Angiocath; Becton Dickinson Vascular Access, Sandy, UT, U.S.A.) was inserted in the right jugular vein for delivery of drugs. Finally, a supplemental dose of pentobarbitone (10 mg kg⁻¹, i.v.) was followed by hyperinflation of the lungs.

Protocol for bronchodilators via i.v. infusion

In experiments with histamine, the muscarinic antagonist, atropine (1 mg kg⁻¹, i.v.) was given to inhibit cholinergic neural reflexes. Twenty min later, either ovalbumin (10 mg ml⁻¹, 5 breaths) or histamine (3.1 mg ml⁻¹, 5 breaths) was given by aerosol (0.12 ml min⁻¹) using an ultrasonic nebuliser

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(Pulmo-Sonic model 25; DeVillbiss, Somerset, PA, U.S.A.). These R_L responses reached 90% of maximum R_L caused by histamine given as a bolus i.v. (3.1 mg kg^{-1}). In experiments with histamine alone, histamine challenges were administered twice at the same dose. One hundred min after the first challenge, which served as a reference, pentobarbitone (3 mg kg^{-1} , i.v.) and atropine (1 mg kg^{-1} , i.v.) were given, followed by lung hyperinflation; 20 min later, a second challenge was given.

PACAP-27 (0.045 to $4.5 \text{ nmol kg}^{-1} \text{ min}^{-1}$), salbutamol (0.045 to $4.5 \text{ nmol kg}^{-1} \text{ min}^{-1}$) or sterile saline was given i.v. (1.0 ml) via an infusion pump (model 600-000; Harvard Apparatus), starting 5 min before and continuing 10 min after airway challenge. In experiments using histamine aerosol, i.v. infusion was given during the second aerosol challenge.

Pretreatment with pyrilamine (10 mg kg^{-1} , i.p.) and cimetidine (10 mg kg^{-1} , i.p.), a histamine H_1 - and H_2 -antagonist, respectively, reduced the ovalbumin-induced increase in R_L from $0.92 \pm 0.07 \text{ cmH}_2\text{O ml}^{-1} \text{ s}^{-1}$ in control animals to $0.03 \pm 0.02 \text{ cmH}_2\text{O ml}^{-1} \text{ s}^{-1}$ in pretreated animals ($P < 0.0001$, $n = 4-5$). After pretreatment with atropine alone, the corresponding increase in R_L was $0.91 \pm 0.03 \text{ cmH}_2\text{O ml}^{-1} \text{ s}^{-1}$ ($P = 0.98$, $n = 3-5$), thereby confirming the dominant role of histamine in the responses to ovalbumin (Barnes *et al.*, 1988).

Protocol for bronchodilators via inhaled aerosol

As in the experiments on bronchodilators delivered i.v., atropine was given initially. Twenty min later, an increase in R_L was established by infusion of histamine i.v. ($20 \text{ } \mu\text{g kg}^{-1} \text{ min}^{-1}$) during 30 min (2.0 ml), producing an R_L response reaching 30% of maximum R_L caused by histamine given as a bolus i.v. (3.1 mg kg^{-1}). When the R_L increase stabilized after an additional 7 min, either PACAP 1-27 salbutamol (0.1 mM , 20 breaths) or sterile saline was given by aerosol inhalation. R_L was then recorded for another 20 min.

Drugs

Atropine sulphate, histamine diphosphate, chicken egg albumin (grade V, ovalbumin), pyrilamine maleate salt and salbutamol hemisulphate were purchased from Sigma, St. Louis, MO, U.S.A. Pituitary adenylate cyclase-activating peptide (PACAP 1-27) was provided by American Peptide Co, Sunnyvale, CA, U.S.A. Sodium pentobarbitone was obtained from Anthony Products Co., Arcadia, CA, U.S.A. and cimetidine hydrochloride was purchased from SmithKline Beecham Pharmaceuticals, Philadelphia, PA, U.S.A. Pentobarbitone and cimetidine were used as commercially available buffer solutions; all other drugs were dissolved and diluted in sterile saline (NaCl 0.9%).

Statistics

Correlation factors were evaluated by Spearman's non-parametric rank correlation. Slopes of dose-response data were compared by multiple linear regression. Single measure points were compared by Student's two-tailed t test (Colton, 1974).

Results

Bronchodilators via i.v. infusion

Ovalbumin challenge PACAP 1-27 prevented the peak increase in R_L caused by inhaled ovalbumin in a dose-dependent fashion ($r = -0.87$, $P < 0.0001$, $n = 21$) (Figure 1). The highest dose of PACAP 1-27 completely abolished the increase in R_L caused by inhaled ovalbumin (Figures 1 and 2). The two highest doses of PACAP 1-27 produced sustained and significant inhibition during the 20 min of observation that followed after the i.v. infusion of PACAP 1-27 was completed (Figure 2). Baseline R_L values were similar in all groups compared (data not shown).

Figure 1 Inhibitory effect of PACAP 1-27 (15 min infusion i.v.) on the peak increase in total pulmonary resistance (R_L) induced by inhaled ovalbumin (10 mg ml^{-1} , 5 breaths) in sensitized guinea-pigs. Mean values with s.e.mean ($n = 4-5$).

Figure 2 Time course for the inhibitory effect of PACAP 1-27 (15 min infusion, i.v.) on increase in total pulmonary resistance (R_L) induced by inhaled ovalbumin (10 mg ml^{-1} , 5 breaths) in sensitized guinea-pigs. PACAP 1-27 was given in 0.45 (●) or 4.5 (▲) $\text{nmol kg}^{-1} \text{ min}^{-1}$ and was compared with saline (■). Mean values with s.e.mean. ** $P \leq 0.01$ compared with saline (Student's t test, $n = 4$).

Figure 3 Inhibitory effect of PACAP 1-27 (●) or salbutamol (▲) (15 min infusion, i.v.) on the peak increase in total pulmonary resistance (R_L) induced by inhaled histamine (3.1 mg ml^{-1} , 5 breaths) in guinea-pigs. The histamine-induced increase in R_L is presented as a percentage (% of reference) of a preceding reference response to inhaled histamine (3.1 mg ml^{-1} , 5 breaths), given prior to the bronchodilator. Mean values with s.e.mean ($n = 4-5$).

Table 1 Baseline levels (mean $\pm$ s.d.) of total pulmonary resistance (R_L : $\text{cmH}_2\text{O ml}^{-1} \text{ s}^{-1}$) and heart rate (HR: beats min^{-1})

Route of delivery		Drug		
Infusion i.v.	R_L	PACAP 1-27	Salbutamol	Saline
		0.17 ± 0.03	0.16 ± 0.02	0.16 ± 0.02
	HR	189 ± 11	186 ± 20	188 ± 7
	n	17*	17*	5
Inhalation	R_L	0.16 ± 0.01	0.16 ± 0.01	0.16 ± 0.02
	n	5	5	5

*Each value represents the average of all doses given of this drug ($n=4-5$ at each dose).

Figure 4 Effect of PACAP 1-27 (●), salbutamol (▲) or saline (■) on heart rate during the first 5 min of bronchodilator infusion i.v. in guinea-pigs. Mean values with s.e.mean ($n=3-5$).

Figure 5 Effect of inhaled PACAP 1-27 (●) or salbutamol (▲) (0.1 mM) given by aerosol ($85 \times 3 \text{ ml min}^{-1}$, 20 breaths) on histamine-induced ($20 \mu\text{g kg}^{-1} \text{ min}^{-1}$, i.v.) increase in total pulmonary resistance (R_L) in anaesthetized guinea-pigs. The effect of inhaled saline (■) is also presented. The data are presented as percentages of the histamine-induced increase in R_L immediately prior to the inhalation of aerosol. Mean values with s.e.mean. ** $P \leq 0.01$ compared with saline (Student's t test, $n=4-5$).

Histamine challenge Both PACAP 1-27 ($r = -0.87$, $P < 0.0001$, $n = 22$) and salbutamol ($r = -0.88$, $P < 0.0001$, $n = 22$) prevented the peak increase in R_L caused by inhaled histamine dose-dependently (Figure 3). Multiple linear regression analysis showed no statistically significant difference in the slopes of the dose-response curves for PACAP 1-27 and salbutamol ($P = 0.61$, $n = 17$). The highest dose of PACAP 1-27 and salbutamol completely abolished the increase in R_L caused by inhaled histamine (Figure 3). However, the histamine response during saline infusion lasted only 10 min, and therefore it was not possible to determine the duration of inhibitory action for PACAP 1-27 and salbutamol (data not shown).

The increase in R_L caused by the histamine reference challenge was 0.77 ± 0.05 and $0.75 \pm 0.03 \text{ cmH}_2\text{O ml}^{-1} \text{ s}^{-1}$ (mean $\pm$ s.e.mean), in the PACAP 1-27 and salbutamol groups respectively, and were not significantly different ($P = 0.82$, $n = 17$). Baseline R_L values were similar in all groups (Table 1).

Salbutamol ($r = 0.84$, $P = 0.0002$, $n = 21$) but not PACAP 1-27 ($r = 0.40$, $P = 0.07$, $n = 22$) increased heart rate (HR) dose-dependently during the first 5 min of i.v. infusion (Figure 4). Multiple linear regression analysis proved the difference between the slopes of the dose-response curves to be statistically significant ($P = 0.03$, $n = 16-17$). Baseline HR values were similar in all groups (Table 1).

Bronchodilators via inhaled aerosol

When PACAP 1-27 or salbutamol was given by inhalation they inhibited increase in R_L caused by histamine infused i.v., with a peak inhibitory effect (% of histamine-induced R_L) of $39 \pm 6\%$ and $66 \pm 3\%$ (mean $\pm$ s.e.mean) respectively. The corresponding peak effect of inhaled saline (control) was $-3 \pm 7\%$ which was significantly different from the effect of either PACAP 1-27 ($P = 0.002$, $n = 5$) or salbutamol ($P < 0.0001$, $n = 5$). The time from drug delivery until peak drug response was 3.6 ± 1.1 and 5.4 ± 1.3 min (mean $\pm$ s.e.mean), for PACAP 1-27 and salbutamol, respectively; this difference was not statistically significant ($P = 0.32$, $n = 5$). Both PACAP 1-27 and salbutamol produced a significant inhibitory effect 1 min after drug delivery, and this effect was sustained throughout the 20 min of R_L recording (Figure 5).

The level of histamine-induced R_L immediately before drug inhalation was 0.24 ± 0.01 and $0.23 \pm 0.03 \text{ cmH}_2\text{O ml}^{-1} \text{ s}^{-1}$ (mean $\pm$ s.e.mean) in the PACAP 1-27 and salbutamol groups respectively and were not significantly different ($P = 0.72$, $n = 5$). Baseline R_L values were similar in all groups (Table 1).

Discussion

PACAP 1-27 given by i.v. infusion abolished the increase in R_L caused by inhaled ovalbumin and histamine in guinea-pigs *in vivo* without any significant increase in heart rate. When given by inhalation, PACAP 1-27 inhibited the increase in R_L caused by histamine infused i.v.

Intravenous delivery of PACAP 1-27 and salbutamol inhibited a nearly maximum increase in R_L caused by inhaled

histamine dose-dependently. There was no significant difference in this relaxant effect between PACAP 1-27 and the β_2 -adrenoceptor agonist, salbutamol. Because salbutamol effectively inhibits bronchoconstriction clinically (Wallin *et al.*, 1993), our findings suggest that PACAP 1-27 given systemically has a significant potency and efficacy as a bronchodilator. This idea is also supported by our experiments with ovalbumin-induced increase in R_L because PACAP 1-27 given i.v. also dose-dependently inhibited this near maximum increase in R_L . Although the effects on R_L were similar, salbutamol increased heart rate dose-dependently, while PACAP 1-27 did not.

Inhaled aerosols of PACAP 1-27 and salbutamol also inhibited the increase in R_L caused by histamine. This suggests that PACAP 1-27 may be effective as a bronchodilator not only for systemic treatment but also for inhalation. Vasoactive intestinal polypeptide (VIP) is structurally similar to PACAP 1-27 and acts via the same receptor (Arimura, 1992). However, in contrast to our findings with PACAP 1-27, inhaled VIP is reported to have no bronchodilator effect on bronchoconstriction in guinea-pigs *in vitro* (O'Donnell *et al.*, 1994). Furthermore, inhaled VIP has only a weak bronchodilator effect in asthmatic subjects (Barnes & Dixon, 1984). It is possible that VIP is more easily cleaved in the airways by local peptidases such as neutral endopeptidase (Nadel, 1992). The fact that bronchodilatation with PACAP 1-27 lasted throughout the 20 min of study also suggests that this peptide is not susceptible to local peptidases.

In separate experiments, we confirmed a direct, potent relaxant effect of PACAP 1-27 on primate bronchial smooth muscle precontracted by carbachol *in vitro*, comparable to the effect of salbutamol (data not shown). Furthermore, our *in vivo* data indicated inhibition of bronchoconstrictor responses caused by histamine directly and those caused by antigen (ovalbumin). Thus, PACAP 1-27 exerts functional antagonism of histamine at the level of smooth muscle but additional pathways of action, such as inhibition of histamine release from mast cells, may also play a role in allergic bronchoconstriction.

In conclusion, this study indicates that PACAP 1-27 produces significant and sustained bronchodilatation *in vivo*, both when given i.v. and by inhalation. PACAP 1-27 does not cause pronounced cardiovascular side effects when given i.v. and it has a rapid onset of action when given by inhalation. PACAP 1-27, or its structural analogues, may therefore have therapeutic potential as bronchodilator drugs.

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References

- ARIMURA, A. (1992). Pituitary adenylate cyclase activating polypeptide (PACAP): discovery and current status of research. *Regul. Pept.*, **37**, 287–303.
- BARNES, P., CHUNG, K.F. & PAGE, C.F. (1988). Inflammatory mediators and asthma. *Pharmacol. Rev.*, **40**, 49–84.
- BARNES, P. & DIXON, C.M. (1984). The effect of inhaled vasoactive intestinal polypeptide on bronchial reactivity to histamine in humans. *Am. Rev. Respir. Dis.*, **130**, 162–166.
- BERTRAND, C., NADEL, J.A., GRAF, P. & GEPPETTI, P. (1993). Capsaicin increases airflow resistance in guinea-pigs *in vivo* by activating both NK2 and NK1 tachykinin receptors. *Am. Rev. Respir. Dis.*, **148**, 909–914.
- CARDELL, L.-O., UDDMAN, R., LUTS, A. & SUNDLER, F. (1991). Pituitary adenylate-cyclase activating peptide (PACAP) in guinea pig lung: distribution and dilatory effects. *Regul. Pept.*, **36**, 379–390.
- COLTON, T. (1974). *Statistics in Medicine*. 1st Edition. Boston, MA, U.S.A.: Little, Brown & Co.
- DUSSER, D.J., UMENO, E., GRAF, P.D., DJOKIC, T.D., BORSON, D.B. & NADEL, J.A. (1988). Airway neutral endopeptidase-like enzymes modulate tachykinin-induced bronchoconstriction *in vivo*. *J. Appl. Physiol.*, **65**, 2585–2591.
- KANEMURA, T., TAMAOKI, J., CHIYOTANI, A., TAKEYAMA, K., SAKAI, N., TAGAYA, E. & KONNO, K. (1993). Role of Na^+/K^+ -ATPase in airway smooth muscle relaxation by vasoactive intestinal peptide and pituitary adenylate cyclase activating peptide. *Res. Commun. Chem. Pathol. Pharmacol.*, **79**, 11–22.
- LUTS, A., UDDMAN, R., ALM, P., BASTERRA, J. & SUNDLER, F. (1993). Peptide-containing nerve fibers in human airways: distribution and coexistence pattern. *Int. Arch. Allergy Immunol.*, **101**, 52–60.
- NADEL, J.A. (1992). Neurogenic inflammation in airways and its modulation by peptidases. *Ann. N.Y. Acad. Sci.*, **664**, 408–414.
- O'DONNELL, M., GARIPPA, R.J., RINALDI, N., SELIG, W.M., TOCKER, J.E., TANNU, S.A., WASSERMAN, M.A., WELTON, A. & BOLIN, D.R. (1994). Ro-25-1553: a novel, long-acting vasoactive intestinal peptide agonist. Part II: Effect on *in vitro* and *in vivo* models of pulmonary anaphylaxis. *J. Pharmacol. Exp. Ther.*, **270**, 1289–1294.
- WALLIN, A., SANDSTROM, T., ROSENHALL, L. & MELANDER, B. (1993). Time course and duration of bronchodilatation with formoterol dry powder in patients with stable asthma. *Thorax*, **48**, 611–614.

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