British Journal of Pharmacology (1997) 121, 1730–1734

Long lasting smooth muscle relaxation by a novel PACAP analogue in guinea-pig and primate airways *in vitro*

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1 We compared the relaxant effect of pituitary adenylate cyclase activating peptide (PACAP) 1–27 with that of a newly developed PACAP 1–27 analogue, [Arg^{15,20,21}Leu^{17}]-PACAP-Gly-Lys-Arg-NH_2, in the guinea-pig trachea and primate bronchi *in vitro* ($n=4-5$).

2 In the guinea-pig trachea precontracted by a submaximally effective carbachol concentration (0.1 μM), cumulative administration of PACAP 1–27 and the β_2-adrenoceptor agonist salbutamol (3 nM–3 μM) caused significant and concentration-dependent smooth muscle relaxation, with salbutamol being approximately one log-step more potent in this model. However, in primate bronchi precontracted by carbachol (0.1 μM), cumulative administration of PACAP 1–27 and salbutamol caused concentration-dependent smooth muscle relaxation with very similar potencies and maximum relaxant effects.

3 In the guinea-pig trachea, non-cumulative administration of the PACAP 1–27 analogue and the original PACAP 1–27 (0.3–3 μM) caused concentration-dependent relaxation with a very similar maximum relaxant effect and potency. However, the onset and offset of action was markedly slower for the PACAP 1–27 analogue than for the original PACAP 1–27 (>90% versus <10% of peak relaxation remaining 6 h after administration). Separate experiments confirmed that the PACAP 1–27 analogue also caused significant relaxation with slower onset and offset of action than did the original PACAP 1–27 in primate bronchi.

4 Peptidase inhibition by captopril (10 μM) and phosphoramidon (1 μM) significantly increased the maximum relaxant effect and duration of action of PACAP 1–27 but not of the PACAP 1–27 analogue, during the 3 h of observation in the guinea-pig trachea.

5 We conclude that [Arg^{15,20,21}Leu^{17}]-PACAP-Gly-Lys-Arg-NH_2 produces significant, concentration-dependent and sustained airway smooth muscle relaxation *in vitro*. The sustained relaxant effect is due, at least in part, to the PACAP 1–27 analogue being less susceptible to cleavage by peptidases than the original peptide PACAP 1–27.

Keywords: Bronchodilator; guinea-pig airways; pituitary adenylate cyclase activating peptide (PACAP); primate airways; smooth muscle relaxation; salbutamol

Introduction

Two endogenous derivatives of the pituitary adenylate cyclase activating peptide (PACAP) have been identified, PACAP 1–38 and PACAP 1–27 (Arimura, 1992). PACAP 1–38 is present in the airways of several species including man (Cardell *et al*., 1991; Luts *et al*., 1993). Recent studies have shown that both PACAP 1–27 and 1–38 relax the rodent trachea *in vivo* (Cardell *et al*., 1991; Araki & Takaghi, 1992; Kanemura *et al*., 1993). PACAP 1–27 also causes bronchodilatation *in vivo* (Lindén *et al*., 1995). There is data on the guinea-pig trachea suggesting that proteases do not cleave PACAP 1–38 (Bhogal *et al*., 1994). However, for PACAP 1–27 no such information is available. Because *in vitro* data on the guinea-pig trachea (Araki & Takaghi, 1992) indicate that PACAP 1–27 is significantly shorter-acting than PACAP 1–38, this raises the possibility that PACAP 1–27 is susceptible to peptide degrading enzymes. But at present there is no information on the enzymatic degradation of PACAP 1–27. Until now, the duration of action of the PACAPs has not been studied carefully in airways.

PACAP 1–27 produces less cardiovascular effects than PACAP 1–38 and vasoactive intestinal peptide (VIP) on rats and dogs (Nandha *et al*., 1991; Ishizuka *et al*., 1992) and less than salbutamol in guinea-pigs (Lindén *et al*., 1995). Therefore, we chose PACAP 1–27 as the basis for developing a potent, efficacious and long-acting bronchodilator. A N- and C-terminally modified PACAP 1–27 molecule, [Arg^{15,20,21}Leu^{17}]-PACAP-Gly-Lys-Arg-NH_2, was thus produced (Figure 1) and its relaxant effect was compared with the relaxant effect of primate and guinea-pig airway smooth muscle *in vitro*. The role of peptidases in determining the duration of action for PACAP 1–27 and the novel PACAP 1–27 analogue was also evaluated.

Methods

Tissue preparation

Tracheal preparations were obtained from male Dunkin-Hartley guinea-pigs (Simonsen Laboratories Inc., Gilroy, CA, 0.3–0.5 kg, $n=30$) which were anaesthetized by an intraperitoneal injection (2.0 ml) of sodium pentobarbitone (1st grade, 64.8 mg ml^{-1}, Anpro Pharmaceuticals, Arcadia, CA) and exsanguinated as described previously (Lindén *et al*., 1993). Bronchial preparations were obtained from male monkeys (Macaca Fascicularis, 2.5–9.9 kg, $n=6$) via the tissue-sharing programme of University of California (San Francisco). The

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                1                5                10
PACAP27         H-His-Ser-Asp-Gly-Ile-Phe-Thr-Asp-Ser-Tyr-

PACAP27         H-His-Ser-Asp-Gly-Ile-Phe-Thr-Asp-Ser-Tyr-
analogue

               11               15                20
                Ser-Arg-Tyr-Arg-Lys -Gln-Met- Ala-Val- Lys-

                Ser-Arg-Tyr-Arg-Arg-Gln-Leu-Ala-Val-Arg-

               21               25
                Lys- Tyr-Leu-Ala-Ala-Val-Leu-NH2

                Arg-Tyr-Leu-Ala-Ala-Val-Leu-Gly-Lys-Arg-NH2
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Figure 1 Amino acid sequence of the original PACAP 1–27 and a novel PACAP 1–27 analogue, [Arg^{15,20,21}Leu^{17}]-PACAP-Gly-Lys-Arg-NH_2.

monkeys were killed by an intravenous injection (1.0 ml) of sodium pentobarbitone (6th grade, 388.8 mg ml^{-1}, Anpro Pharmaceuticals, Arcadia, CA). After removal, the airway tissue was placed in a 100 ml oxygenated (95% O_2; 5% CO_2) dissection bath and washed repeatedly with Krebs-Ringer solution (mM: NaCl 118, KCl 5.9, $CaCl_2$ 2.5, $MgSO_4$ 1.2, NaH_2PO_4 1.2, $NaHCO_3$ 25.5 and glucose 5.6) at room temperature.

Airway rings were cut transversely from the tracheae and bronchi, each approximately 5 mm in diameter. These airway rings were opened longitudinally along the most cartilaginous part and connected to steel hooks and unfolded as isolated strips. The preparations were mounted in temperature-controlled (37°C) and oxygenated 10 ml custom-made glass organ baths, as described previously (Lindén *et al*., 1993).

Tension recordings

The isometric tension was recorded via force transducers (Grass ET03, Grass Instrument Co., Quincy, MASS) connected to a six-channel signal recorder (Grass Model 7 Polygraph). The difference in tension between the precontraction induced by carbachol (0.1 μM) and the level during a final maximum theophylline-induced relaxation (1 mM) was regarded as 100% active tension (precontraction).

Experimental protocol

In all experiments, the inherent tone of the tracheal smooth muscle was initially abolished by adding indomethacin (10 μM, Sigma Chem Co, St Louis, MO, U.S.A.) which was continuously present in the Krebs-Ringer solution. The applied, passive tension was then adjusted to 1.4 g. After a moderate decline during 5 min of equilibration, the tension was readjusted to 1.4 g. Muscarinic precontraction was established after a 45 min equilibration period by flushing (0.3 ml min^{-1}) the organ baths with Krebs-Ringer solution containing carbachol (0.1 μM, Sigma Chem Co, St Louis, MO), causing approximately 70% of the maximum carbachol-induced contraction (data not shown). The induced precontraction stabilized over 20 min and the flushing was then stopped. At the end of all experiments, the remaining active tension was abolished by a maximally effective concentration of theophylline (0.1 mM, Sigma Chem Co, St Louis, MO).

Paired concentration-response experiments for PACAP 1–27 (provided by the American Peptide Co., Sunnyvale, CA, U.S.A.) and salbutamol (purchased from Sigma Chem Co, St Louis, MO) were conducted to compare the maximum relaxant effect and the potency of PACAP 1–27 and salbutamol. In these experiments, the concentration of PACAP 1–27 and salbutamol was increased cumulatively as soon as the peak drug response was observed. Paired time course experiments were conducted separately to compare the potency and onset and offset of action for PACAP 1–27 and its analogue [Arg^{15,20,21}Leu^{17}]-PACAP-Gly-Lys-Arg-NH_2. Because of the slow onset of action of the PACAP 1–27 analogue, these experiments were conducted non-cumulatively, with one concentration of each drug being used for each airway preparation. The onset and offset of relaxation was recorded for 6 h in each experiment. Corresponding experiments were conducted with and without peptidase inhibition by captopril (10 μM, Sigma Chem Co, St Louis, MO, U.S.A.) and phosphoramidon (1 μM, Sigma Chem Co, St Louis, MO, U.S.A.). These experiments were used to examine the importance of peptidases in determining magnitude and onset and offset of relaxation for a submaximally effective concentration of PACAP 1–27 and its analogue for 3 h.

Statistical analysis

All results are presented as the mean ± s.e.mean. Spearman's rank correlation was used to determine the correlation between bronchodilator concentration and relaxant effect. Slopes of concentration-response curves were compared by use of multiple linear regression. Single measurement points were compared by means of Student's *t* distribution (one- or two-tailed, paired or unpaired). EC_{50} values were determined by means of linear interpolation from each individual cumulative concentration-response curve. $P<0.05$ was considered statistically significant, *n* equals the number of guinea-pigs or monkeys included in each treatment group.

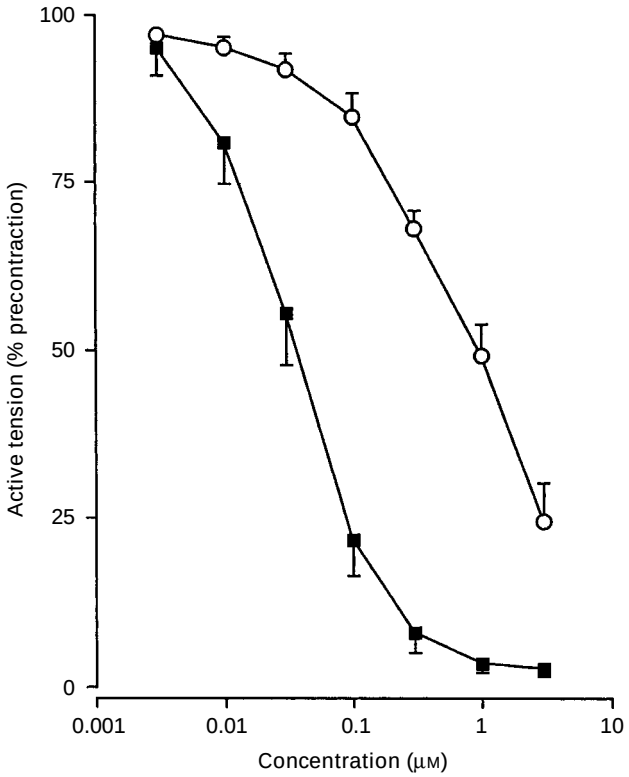

Figure 2 Cumulative concentration-response curves for PACAP 1–27 (○) and the β_2-adrenoceptor agonist salbutamol (■) in the guinea-pig trachea *in vitro*. The minimum active tension after addition of each bronchodilator concentration is presented as a percentage (% precontraction) of the difference in active tension between the precontraction level induced by carbachol (0.1 μM) and the tension in the presence of theophylline (1 mM). Mean values with s.e.mean (vertical lines) $n=4$) are shown. The mean ± s.d. precontraction was 2.73 ± 0.56 g and 3.06 ± 0.50 g, for preparations treated with PACAP 1–27 and salbutamol, respectively. −log EC_{50} (M) was 6.05 ± 0.12 and 7.45 ± 0.16, for PACAP 1–27 and salbutamol, respectively ($P<0.01$, $n=4$).

Results

Concentration-response experiments for PACAP 1–27 and salbutamol

PACAP 1–27 produced concentration-dependent ($r=-0.9$, $P<0.0001$, $n=5$) smooth muscle relaxation, as did salbutamol ($r=-0.9$, $P<0.0001$, $n=4$) in the guinea-pig trachea (Figure 2). In this model, multiple linear regression proved salbutamol to be more potent than the original PACAP 1–27 ($P<0.0001$, $n=4-5$). However, in primate bronchi the concentration-dependent relaxant effect of PACAP 1–27 ($r=-0.8$, $P<0.0001$, $n=4$) and salbutamol ($r=-0.9$, $P<0.0001$, $n=4$) were similar as determined by multiple linear regression ($P<0.95$, $n=4$) (Figure 3). Student's t test showed no significant difference in maximum relaxant effect ($P>0.05$, $n=4$) for the two bronchodilators in primate bronchi.

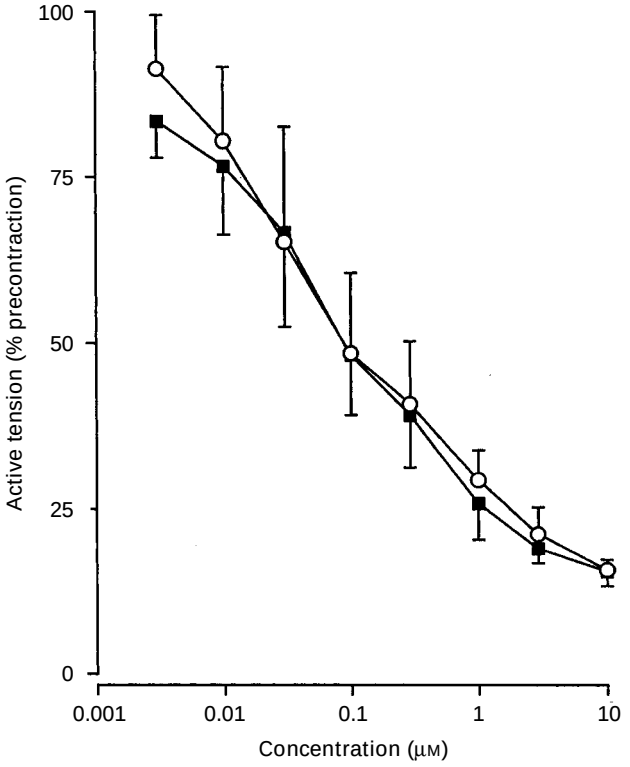

Figure 3 Cumulative concentration-response curves for PACAP 1–27 (○) and the β_2-adrenoceptor agonist salbutamol (■) in primate bronchi *in vitro*. The minimum active tension after addition of each bronchodilator concentration is presented as a percentage (% precontraction) of the difference in active tension between the precontraction level induced by carbachol (0.1 μM) and the tension in the presence of theophylline (1 mM). Mean values with s.e.mean (vertical lines) ($n=4$) are shown. The mean ± s.d. precontraction was 1.63 ± 0.81 g and 1.39 ± 0.43 g, for preparations treated with PACAP 1–27 and salbutamol, respectively. −log EC_{50} (M) was 6.69 ± 0.45 and 7.03 ± 0.38, for PACAP 1–27 and salbutamol, respectively $n=4$).

Table 1 Peak relaxant effect (% of precontraction) of PACAP 1–27 and [$Arg^{15,20,21}Leu^{17}$]-PACAP-Gly-Lys-Arg-NH_2 (PACAP 1–27 analogue) in non-cumulative time course experiments in the guinea-pig trachea *in vitro*

	Bronchodilator concentration			
	0.3 μM	1.0 μM	3.0 μM	r
PACAP 1–27	23.9 ± 5.4	40.6 ± 12.9	67.0 ± 8.4	–0.7
PACAP 1–27 analogue	29.4 ± 3.5	43.1 ± 9.9	61.8 ± 4.8	–0.6

[1]As determined by Spearman's rank correlation ($P<0.05$, $n=13$). Multiple linear regression showed no significant difference ($P=0.42$, $n=13$) in the slope of these concentration-response curves. The mean ± s.d. precontraction was 2.17 ± 0.58 g and 1.95 ± 0.57 g, for preparations treated with PACAP 1–27 and the PACAP 1–27 analogue, respectively.

Concentration-response and time course experiments for PACAP 1–27 and [$Arg^{15,20,21}Leu^{17}$]-PACAP-Gly-Lys-Arg-NH_2

In the guinea-pig trachea, both the original PACAP 1–27 and its analogue [$Arg^{15,20,21}Leu^{17}$]-PACAP-Gly-Lys-Arg-NH_2 produced concentration-dependent tracheal smooth muscle relaxation without any significant difference (Table 1). However, the duration of action was significantly longer for the PACAP

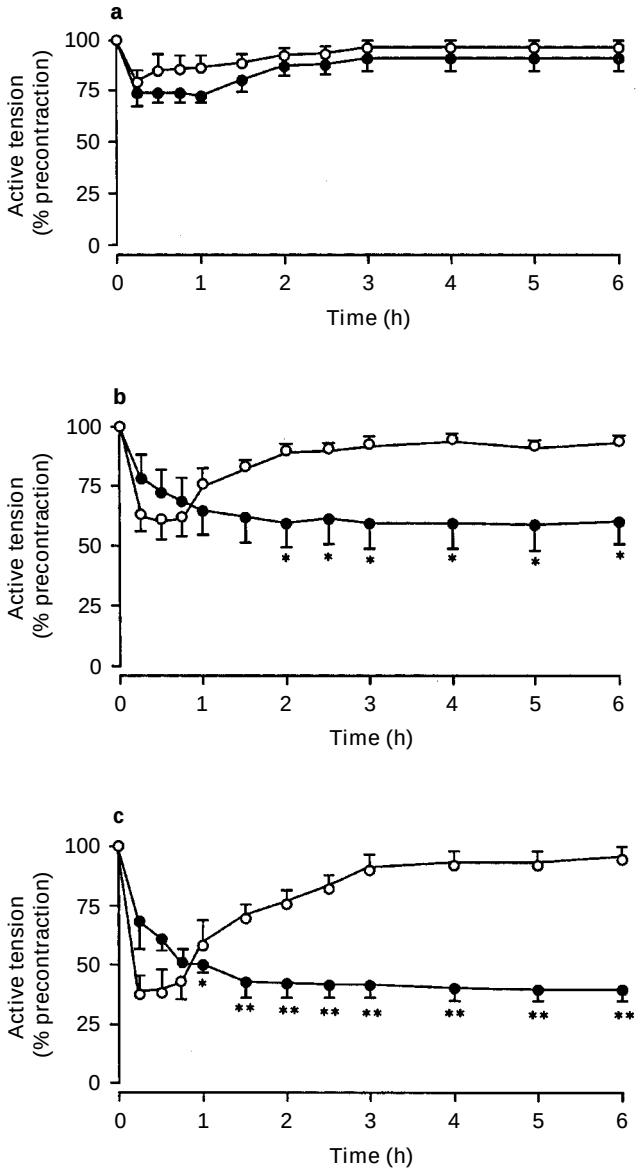

Figure 4 Time course of relaxant effect for the PACAP 1–27 analogue [$Arg^{15,20,21}Leu^{17}$]-PACAP-Gly-Lys-Arg-NH_2 (●) and the original PACAP 1–27 (○) in the guinea-pig trachea *in vitro*. The active tension after addition of each of three bronchodilator concentrations (a) 0.3, (b) 1 and (c) 3 μM, is presented as a percentage (% precontraction) of the difference in active tension between the precontraction level induced by carbachol (0.1 μM) and the tension in the presence of theophylline (1 mM). Mean values with s.e.mean (vertical lines) ($n=5$) are shown. $^*P<0.05$, $^{**}P<0.01$ (unpaired Student's t test).

1–27 analogue than for the original PACAP 1–27 (Figure 4). Six hours after administration, the PACAP 1–27 analogue still produced more than 90% of its peak relaxation, whereas the effect of the original PACAP 1–27 was no longer present. In primate bronchi, the duration of action was also significantly longer for the PACAP 1–27 analogue than for the original PACAP 1–27 (Figure 5).

Time-course experiments with and without peptidase inhibition for PACAP 1–27 and [Arg^{15,20,21}Leu^{17}]-PACAP-Gly-Lys-Arg-NH$_2$

In the guinea-pig trachea, peptidase inhibition by captopril and phosphoramidon significantly increased the magnitude and the duration of relaxant effect for the original PACAP 1–27 but not for the analogue [Arg^{15,20,21}Leu^{17}]-PACAP-Gly-Lys-Arg-NH$_2$ (Figure 6).

Discussion

This study showed that a novel PACAP 1–27 analogue, [Arg^{15,20,21}Leu^{17}]-PACAP-Gly-Lys-Arg-NH$_2$, causes longer acting, concentration-dependent smooth muscle relaxation than does the original PACAP 1–27 in airway smooth muscle *in vitro*. Without peptidase inhibition, the potency and maximum relaxant effects were similar for these two peptide molecules. The study also showed that the PACAP 1–27 analogue is less susceptible to degradation by peptidases than is the original PACAP 1–27, implying that this may contribute to the long duration of action.

Previous studies on VIP as a bronchodilator (Morice *et al*., 1983; Barnes & Dixon, 1984; O'Donnel *et al*., 1994) have demonstrated severe cardiovascular side effects during systemic treatment and the bronchodilator effect of inhaled VIP is weak. However, PACAP 1–27 causes significant bronchodilatation in guinea-pigs *in vivo*, when given intravenously and when given by inhalation (Lindén *et al*., 1995), although it acts via a similar receptor type and increases adenosine 3′:5′-cyclic monophosphate (cyclic AMP), as does VIP (Arimura, 1992; Kanemura *et al*., 1993). Previously, we demonstrated that the potency of PACAP 1–27 in guinea-pigs *in vivo* is similar to that of the clinically utilized β_2-agonist salbutamol, but PACAP 1–27 causes less cardiovascular side effects. Data from the present study indicated that PACAP 1–27 and salbutamol also have a similar potency in primate bronchi.

The novel PACAP 1–27 analogue, [Arg^{15,20,21}Leu^{17}]-PACAP-Gly-Lys-Arg-NH$_2$, produced significant, concentration-dependent smooth muscle relaxation in the guinea-pig trachea and its relaxant effect was confirmed in primate bronchi. Without peptidase inhibition, this PACAP 1–27

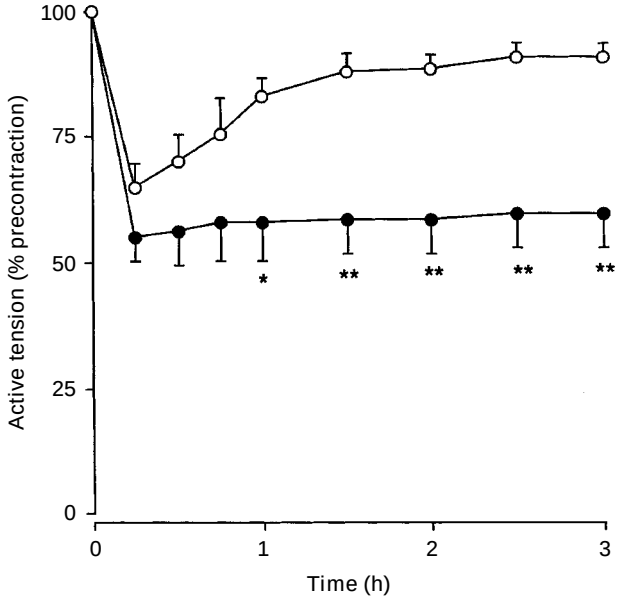

Figure 5 Time course of relaxant effect for the PACAP 1–27 analogue [Arg^{15,20,21}Leu^{17}]-PACAP-Gly-Lys-Arg-NH$_2$ (0.1 μM, ●) and the original PACAP 1–27 (0.1 μM, ○) in primate bronchi *in vitro*. The active tension after addition of each bronchodilator is presented as a percentage (% precontraction) of the difference in active tension between the precontraction level induced by carbachol (0.1 μM) and the tension in the presence of theophylline (1 mM). Mean values with s.e.mean (vertical lines) ($n=4$) are shown. $^*P<0.05$, $^{**}P<0.01$ (unpaired Student's t test). The mean ± s.d. precontraction was 1.20 ± 0.32 g and 1.38 ± 0.29 g, for preparations treated with PACAP 1–27 and the PACAP 1–27 analogue, respectively.

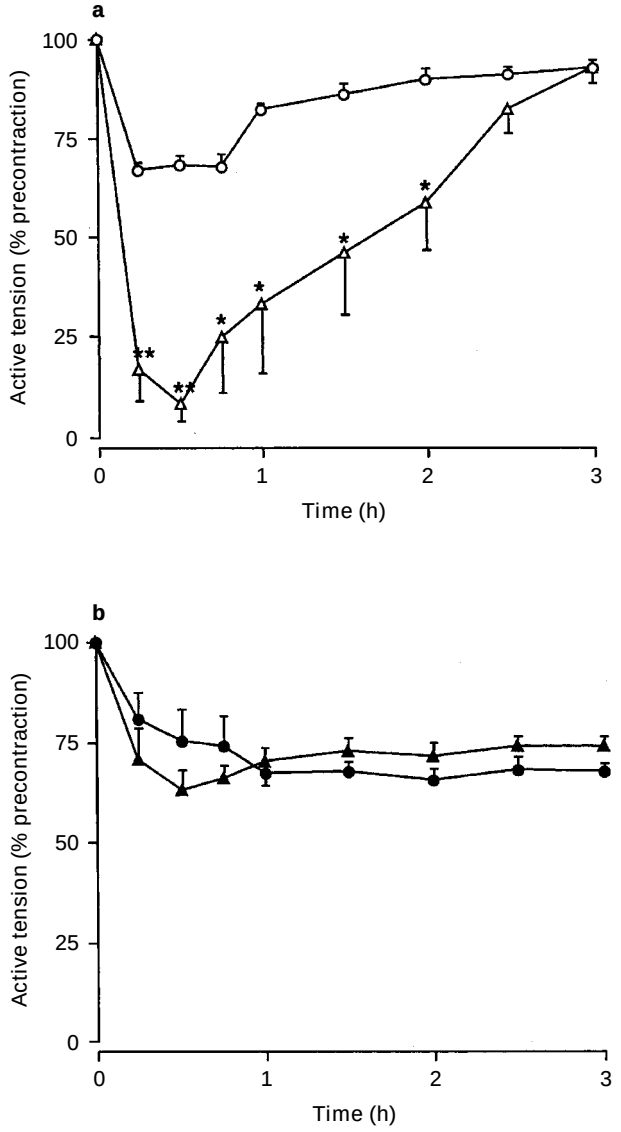

Figure 6 Time course of relaxant effect for (a) the original PACAP 1–27 1 μM and (b) the PACAP 1–27 analogue [Arg^{15,20,21}Leu^{17}]-PACAP-Gly-Lys-Arg-NH$_2$ 1 μM with (triangles) and without (circles) peptidase inhibition by captopril (ACE, 10 μM) and phosphoramidon (Phos 1 μM) in the guinea-pig trachea *in vitro*. The active tension after addition of each bronchodilator is presented as a percentage (% precontraction) of the difference in active tension between the precontraction level induced by carbachol (0.1 μM) and the tension in the presence of theophylline (1 mM). Mean values with s.e.mean (vertical lines) ($n=5$) are shown. $^*P<0.05$, $^{**}P<0.01$ (unpaired Student's t test). The mean ± s.d. precontraction was 2.37 ± 0.53 g and 2.07 ± 0.67 g, for preparations treated with PACAP 1–27 and the PACAP 1–27 analogue, respectively.

analogue appeared to be as potent as is the original PACAP 1–27 in the guinea-pig trachea, whereas the original PACAP is more potent in the presence of peptidase inhibition. In primate bronchi, the original PACAP 1–27 and the β_2-agonist salbutamol have similar potencies. For these reasons and also because the two PACAP molecules had a similar effect in single concentration experiments in primate bronchi, our data indirectly suggest that the potency of the PACAP 1–27 analogue is in the same order of magnitude as salbutamol.

The PACAP 1–27 analogue produced significant relaxation within less than half and hour after administration and this was observed in airway smooth muscle precontracted by a submaximally effective concentration of the postjunctionally acting muscarinic agonist, carbachol. The sustained action of the PACAP 1–27 analogue displayed no trend towards a reduced response during the entire experiment, lasting three and six hours in primate and guinea-pig airways, respectively. In contrast, the relaxation produced by the original PACAP 1–27 decreased within less than one hour in all experiments. Thus, the PACAP 1–27 analogue appears to be markedly longer acting than the original PACAP 1–27, producing airway smooth muscle relaxation for at least five hours in airways *in vitro*.

The present results suggest that the mechanism underlying the sustained action of the PACAP 1–27 analogue may be related to the differences in enzymatic degradation: For the original PACAP 1–27, peptidase inhibition by captopril and phosphoramidon significantly increased its duration of action in the guinea-pig trachea *in vitro*. However, this was not the case for the PACAP 1–27 analogue. The sustained relaxation of the PACAP 1–27 analogue may thus be due, at least in part, to this molecule being less susceptible to peptidases such as neutral endopeptidase and angiotensin converting enzyme than the original PACAP 1–27. However, our results do not completely rule out additional involvement of proteases other than neutral endopeptidase.

The molecular modelling of the original PACAP 1–27 included both N- and C-terminal modifications (Figure 1 and Kashimoto *et al*., 1995). As a result, the PACAP 1–27 analogue has a more basic nature than does the original PACAP 1–27, which is likely to account for its more sustained action (Kashimoto *et al*., 1995). The basis for this molecular modelling was the previous observation that the endogenous analogue of PACAP 1–27, PACAP 1–38, includes a longer C-terminal amino acid sequence, with a more basic nature, than does PACAP 1–27, and this longer C-terminal is accompanied by more sustained cardiovascular effects (Nandha *et al*., 1991; Ishizuka *et al*., 1992; Kashimoto *et al*., 1995).

In conclusion, the novel PACAP 1–27 analogue [Arg ^{15,20,21}Leu^{17}]-PACAP-Gly-Lys-Arg-NH_2 produces significant, concentration-dependent and sustained airway smooth muscle relaxation *in vitro*. The long-lasting relaxant effect is in part due to the PACAP 1–27 analogue being less susceptible to cleavage by peptidases than the original peptide PACAP 1–27. For these reasons, the novel PACAP 1–27 analogues deserves further characterization of its potential as a bronchodilator *in vivo*.

The technical assistance of B. Chan, B.S. is gratefully acknowledged. The authors thank Drs P. Goldsmith, Department of Surgery, H. Goodies and J. White, Department of Dentistry for generously providing primate airways via the University of California San Francisco Tissue Sharing Program. This study was supported in part by NIH PPG Grant HL-06285. Dr A Lindén was funded by Blanceflor Boncompagni-Ludovisi's Fund, Hermann Krefting's Fund, King Oscar II's Jubilee Fund, the Sweden-America Foundation, the Swedish Medical Research Council and the Swedish Society of Medicine.

References

ARAKI, N. & TAKAGHI, K. (1992). Relaxant effect of pituitary adenylate cyclase-activating polypeptide on guinea pig tracheal smooth muscle. *Eur. J. Pharmacol.*, **216,** 113–117.

ARIMURA, A. (1992). Pituitary adenylate cyclase activating polypeptide (PACAP): discovery and current status of research. *Reg. Peptides*, **37,** 287–303.

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.

BHOGAL, R., SHELDRICK, R.L.G., COLEMAN, R.A., SMITH, D.M. & BLOOM, S.R. (1994). The effects of PACAP and VIP on guinea pig tracheal smooth muscle in vitro. *Peptides*, **15,** 1237–1241.

CARDELL, L-O., UDDMAN, R., LUTS, A. & SUNDLER, F. (1991). Pituitary adenylate-cyclase activating peptide (PACAP) in guinea pig lung: distribution and dilatory effects. *Reg. Peptides*, **26,** 379–390.

ISHIZUKA, Y., KASHIMOTO, K., MOCHIZUKI, T., SATO, K., OHSHIMA, K. & YANAIHARA, N. (1992). Cardiovascular and respiratory actions of pituitary adenylate cyclase-activating polypeptides. *Reg. Peptides*, **40,** 29–39.

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.

KASHIMOTO, K., NAGANO, Y., SUITANI, Y., HAMANAKA, K., MIZUMOTO, T., TOMIZAKI, K., TAKAHATA, H., NAGAMOTO, A., OHATA, A., YOSHIHARA, S. & ICHIMURA, T. (1996). Structure-activity relationship studies of PACAP 27 and VIP analogue. 2nd International symposium on VIP, PACAP and Related Peptides. *Ann. New York Acad. Sci.* (in press).

LINDÉN, A., BERGENDAL, A., ULLMAN, A., SKOOGH, B-E. & LÖFDAHL, C-G. (1993). Salmeterol, formoterol, and salbutamol in the isolated guinea pig trachea: differences in maximum relaxant effect and potency but not in functional antagonism. *Thorax*, **48,** 547–553.

LINDÉN, A., YOSHIHARA, S., CHAN, B. & NADEL, J.A. (1995). Inhibition of bronchoconstriction by pituitary adenylate-cyclase activating peptide (PACAP 1–27) in guinea-pigs *in vivo*. *Br. J. Pharmacol.*, **115,** 913–916.

LUTS, A., UDDMAN, R., ALM, P., BASTERRA, J. & SUNDLER, F. (1993). Peptide-containing nerve fibres in human airways: distribution and coexistence pattern. *Int. Arch. Allergy Immunol.*, **101,** 52–60.

MORICE, A., UNWIN, R.J. & SEVER, P.S. (1983). Vasoactive intestinal peptide causes bronchodilation and protects against histamine-induced bronchoconstriction in asthmatic subjects. *Lancet*, **2,** 1225–1226.

NANDHA, K.A., BENITO-ORFILA, M.A., SMITH, D.M., GHATEI, M.A. & BLOOM, S.R. (1991). Action of pituitary adenylate-cyclase-activating polypeptide and vasoactive intestinal polypeptide on the rat vascular system: effects on blood pressure and receptor binding. *J. Endocrinol.*, **129,** 69–73.

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.

(Received April 14, 1997
Accepted May 12, 1997)