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PHARMALOGICAL REVIEW

Bronchodilation by pituitary adenylate cyclase-activating peptide and related peptides

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Bronchodilation by pituitary adenylate cyclase-activating peptide and related peptides. A. Lindén, L-O. Cardell, S. Yoshihara, J.A. Nadel. ©ERS Journals Ltd 1999.

ABSTRACT: Pituitary adenylate cyclase-activating peptide (PACAP) is present in nerves in the vicinity of bronchial and vascular smooth muscle in the airways. At least one endogenous form of PACAP, PACAP 1-27, has high affinity binding sites in the lung, probably including cholinergic nerve terminals, bronchial smooth muscle, epithelial and mononuclear inflammatory cells.

The mechanism of action for PACAP 1-27 and 1-38 *in vivo* involves endogenous catecholamines, peptidases and nitric oxide, depending on tissue type. Intracellularly, cyclic adenosine monophosphate (cAMP) as well as calcium and sodium mobilization is probably involved. PACAP 1-27 and 1-38 inhibit airway smooth muscle tone *in vitro* and *in vivo*. The inhibitory effect of PACAP 1-38 is more sustained than that of PACAP 1-27, *in vitro* as well as *in vivo*. PACAP 1-38 also causes more sustained inhibition of bronchoconstriction after inhalation *in vivo*, than does vasoactive intestinal peptide (VIP). PACAP 1-27 given intravenously virtually abolishes allergen-induced bronchoconstriction *in vivo*. Novel synthetic analogues of PACAP 1-27 cause more sustained inhibition of airway smooth muscle tone *in vitro* and *in vivo* than do PACAP 1-27 or 1-38. Both PACAP 1-27 and 1-38 inhibit arterial smooth muscle tone but, administration of PACAP 1-27, 1-38 or a structural analogue of PACAP 1-27 in the airways, induces no cardiovascular side effects at doses inhibiting bronchoconstriction. PACAP 1-38 enhances phagocytosis in macrophages and inhibits the release of the pro-inflammatory cytokine interleukin-2 in lymphocytes, suggesting anti-inflammatory effects.

It is concluded that pituitary adenylate cyclase-activating peptide 1-27 and 1-38, or structurally related molecules, may be useful as bronchodilators but their effect on human bronchial smooth muscle and on human inflammatory cells is in need of evaluation.

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Two endogenous forms of pituitary adenylate cyclase-activating peptide (PACAP) are known; PACAP 1-27 and PACAP 1-38, where PACAP 1-27 constitutes the N-terminal portion of PACAP 1-38. Both forms of PACAP are amidated and share some of their biochemical properties with peptides such as vasoactive intestinal peptide (VIP), peptide histidine isoleucide, helospectrin and helodermin [1]. PACAP 1-27 and 1-38 were originally isolated from ovine hypothalami, and they increase intracellular cyclic adenosine monophosphate (cAMP) in pituitary cells a thousand-fold more potently than VIP [2, 3]. PACAP 1-27 and 1-38 display several biological activities that may be relevant to the treatment of obstructive airway disease such as asthma and chronic obstructive pulmonary disease. These activities include inhibition of airway [4–6] and vascular smooth muscle tone [7], as well as modulation of inflammatory cell activity [8–14].

This article reviews data suggesting that PACAP 1-38, or synthetic analogues of PACAP 1-27, may be useful as bronchodilators with potential anti-inflammatory properties. These molecules appear superior to VIP in terms of sustained bronchodilatory effect combined with lack of cardiovascular side effects *in vivo*. They may constitute an alternative strategy for the treatment of obstructive airway diseases, which includes anticholinergics, β-adrenoceptor agonists, glucocorticoids, and xanthines.

Pituitary adenylate cyclase-activating peptide and bronchodilation

PACAP in airway nerves

Endogenous PACAP 1-27 or 1-38 is present in nerves close to bronchial smooth muscle in primates and rodents, as indicated by studies on immunoreactivity [1, 4, 15]. Clusters of endocrine cells within the airways, so-called neuroepithelial bodies, also display immunoreactivity for PACAP [1]. In human bronchi, PACAP 1-27 or 1-38 appears to be more abundant than is VIP in the vicinity of nonvascular smooth muscle [15], suggesting a possible role in the endogenous control of bronchial smooth muscle tone. Apart from this, the neural distribution of PACAP 1-27 or 1-38 is similar to that of VIP and helospectrin [15].

PACAP receptors and mechanism of action in airway smooth muscle

The specific receptor subtypes for PACAP 1-27 and 1-38 in airway smooth muscle are not yet characterized in detail. However, it is known that PACAP 1-27 and PACAP 1-38 have high affinity binding sites in the rat lung [16–18], probably including neuromuscular sites pre- and post-junctionally in bronchial smooth muscle, as indicated by functional studies in guinea pig airways *in vitro* and *in vivo* [5, 19–21]. At these lung binding sites, the affinity for PACAP 1-27, 1-38 and VIP is fairly similar, which is compatible with the presence PACAP type II receptors [16–18, 22–25]. Transcripts from human lung tissue indicate the presence of PACAP type II receptors as well [26]. More extensive studies on this type of receptor binding are lacking, however. In the guinea pig trachea *in vitro*, PACAP-induced smooth muscle relaxation is probably caused by cAMP-mediated activation of Ca^{++}-dependent K^{+}-channels [19, 27, 28]. At present, it is not clear whether additional, intracellular mechanisms, such as the signal transduction *via* the glutamyl transpeptidase (GTP-ase) protein Ras and the cytoplasmic mitogen activated protein kinase Raf, contribute to the effect of the PACAPs in airway smooth muscle [29].

PACAP and airway smooth muscle

In the guinea pig trachea *in vitro*, PACAP 1-38 inhibits smooth muscle tone induced by acetylcholine, histamine or metacholine [4, 28, 30, 31] (fig. 1) with markedly longer duration of action than that of PACAP 1-27 or VIP, parallelled by a more sustained increase in cAMP by PACAP 1-38 [27, 31]. VIP is, however, slightly more potent than PACAP 1-27 or 1-38 in relaxing the guinea pig trachea *in vitro* [4, 30, 32]. In contrast, PACAP 1-38 at least, is more potent than VIP in causing an increase in cAMP in this type of airway smooth muscle [32]. Perhaps of greater relevance to human airways. PACAP 1-27 inhibits smooth muscle tone in primate bronchi precontracted by carbachol *in vitro* [5] (fig. 2). In this airway

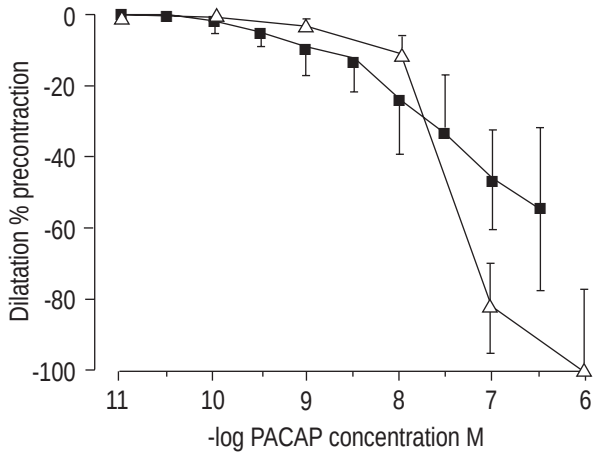

Fig. 1. – Concentration-response data for pituitary adenylate cyclase-activating peptide (PACAP) 1-38 (■), VIP (△) in the guinea-pig trachea *in vitro*. Data are presented as mean±SD percentage of histamine-induced (0.3 μM) precontraction (n=5–14). (Reproduced with permission from [4]).

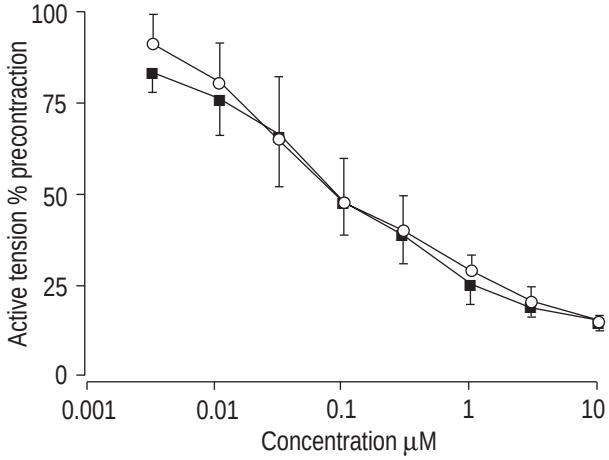

Fig. 2. – Concentration-response data for pituitary adenylate cyclase-activating peptide (PACAP) 1-27 (○) and salbutamol (■) in primate bronchi *in vitro*. Data are presented as mean±SEM percentage of histamine-induced (0.3 mM) precontraction (n=4). (Reproduced with permission from [6]).

model, PACAP 1-27 is equipotent to the clinically utilised β_2-adrenoceptor agonist salbutamol.

In guinea pigs *in vivo*, inhalation of PACAP 1-27 and 1-38 [5, 33] causes significant inhibition of histamine-induced bronchoconstriction (figs. 3 and 4a). This effect of inhaled PACAP 1-38 is markedly more sustained than that of PACAP 1-27 or VIP (fig. 4b), although the potency of these peptides appears to be nearly the same [20]. Intravenous administration of PACAP 1-27 and 1-38 inhibits carbachol- or histamine-induced bronchoconstriction and these effects are dose-dependent [5, 20, 33] (figs. 5 and 6). PACAP 1-38 given intravenously is also longer acting than PACAP 1-27 [33]. Interestingly, intravenous administration of PACAP 1-27 virtually abolishes bronchoconstriction caused by a near-maximally effective dose of allergen in sensitized guinea pigs [5] (fig. 7).

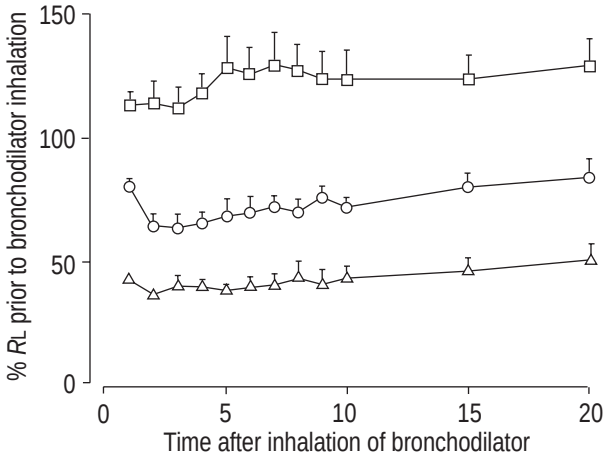

Fig. 3. – Time course of total pulmonary resistance (R_L) after inhalation (0.1 mM, 85 × 3 mL·min^{-1}, 20 breaths) of pituitary adenylate cyclase-activating peptide (PACAP) 1-27 (○) or salbutamol (△) or vehicle (□) in anaesthetized guinea pigs *in vivo*. Bronchoconstriction was induced by intravenous infusion of histamine (20 μg·kg^{-1}·min^{-1}) prior to aerosol inhalation. Data presented as mean±SEM percentage of $R_{0.8h>L}$ at baseline (n=4–5). (Reproduced with permission from [5]).

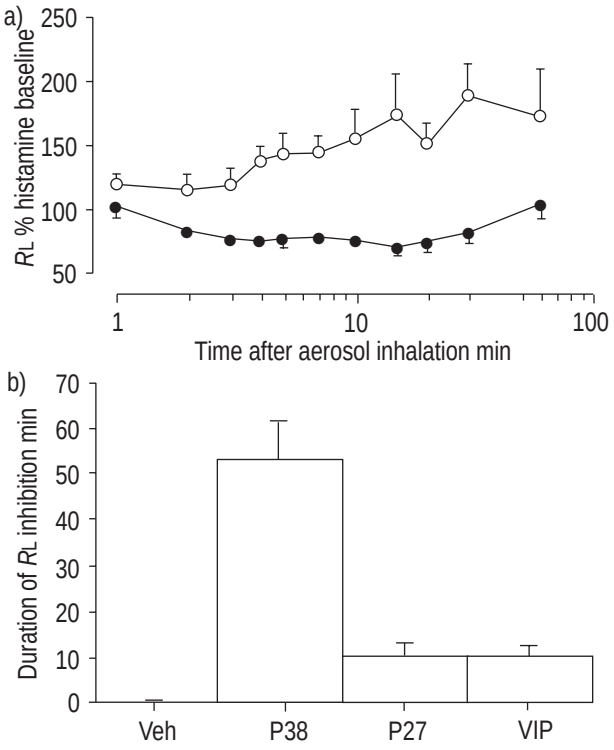

Fig. 4. – a) Time course of total pulmonary resistance (R_L) after inhalation (0.1 mM, 85 × 3 mL·min^{-1}, 20 breaths) of pituitary adenylate cyclase-activating peptide (PACAP) 1-38 (●) or vehicle (○) in anaesthetized guinea-pigs *in vivo*. b) Duration (min) of >10% inhibition of R_L after inhalation (0.1 mM, 85 × 3 mL·min^{-1}, 20 breaths) of PACAP 1-38 (P38), PACAP 1-27 (P27), vasoactive intestinal peptide (VIP) or vehicle (Veh). Bronchoconstriction was induced by intravenous infusion of histamine (3.3 µg·kg^{-1}·min^{-1} during 70 min) prior to aerosol inhalation. Data are presented as mean±SEM percentage of R_L immediately prior to aerosol inhalation (n=3–7). (Reproduced with permission from [20]).

PACAP and endopeptidases in airway smooth muscle

In the guinea pig trachea *in vitro*, phosphoramidon, a selective inhibitor of neutral endopeptidase (NEP) potentiates the inhibitory effect on tone induced by PACAP 1-27 [5, 6] (fig. 8). This effect of phosphoramidon is epithelium-dependent, indicating the involvement of epithelial NEP [32]. In contrast, a mixture of protease inhibitors including phosphoramidon, does not significantly potentiate the effect of PACAP 1-38 the same type of airway preparation [30]. However, phosphoramidon potentiates the cAMP response to PACAP 1-38 but not to PACAP 1-27 in human airway epithelial-like, adenocarcinoma cells *in vitro* [23] (fig. 9). Possibly, the referred discrepancy can be explained by the fact that BHOGAL *et al*. [30] utilised a "cocktail" of various protease-inhibitors, each of which may either potentiate or inhibit the end point signal, resulting in a "zero" net outcome.

In guinea pigs *in vivo*, NEP inhibition with phosphoramidon significantly potentiates the bronchodilator effect of PACAP 1-38 given intravenously [33], supporting a role of NEP in controlling airway effects of PACAP 1-38. In this context, there is no information available, to the authors′ knowledge, on NEP and PACAP 1-27 *in vivo*.

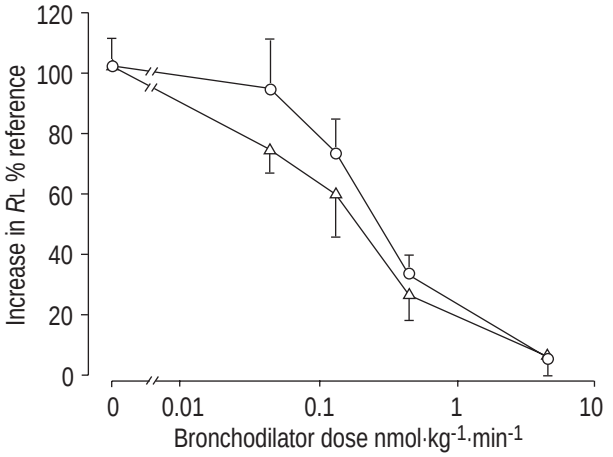

Fig. 5. – Dose-response data for pituitary adenylate cyclase-activating peptide (PACAP) 1-27 (○) and salbutamol (△) given during intravenous infusion (15 min) *versus* the peak increase in total pulmonary resistance (R_L) induced by inhaled histamine (3.1 mg·mL^{-1}, 5 breaths) in anaesthetized guinea-pigs *in vivo*. Data are mean±SEM percent of the histamine-induced increase in R_L (% of reference) of a preceding reference response to inhaled histamine (3.1 mg·mL^{-1}, 5 breaths), given prior to the bronchodilator (n=4–5). (Reproduced with permission from [5]).

PACAP analogues and airway smooth muscle

To extend the duration of its inhibitory action on airway smooth muscle tone, two novel structural analogues of PACAP 1-27 have been developed [6, 34, 35]. The two analogue molecules were produced by replacing key amino acids (fig. 10), resulting in the M and BM type of PACAP analogue. Recent data show that both of these peptide analogues cause a more sustained inhibition of smooth muscle tone in the guinea pig trachea *in vitro* than does the original PACAP 1-27 [6, 35] (figs. 11 and 12). The BM type of PACAP analogue, [Arg$^{15, 20, 21}$Leu^{17}]-PACAP-Gly-Lys-Arg-NH_2, also displays a sustained

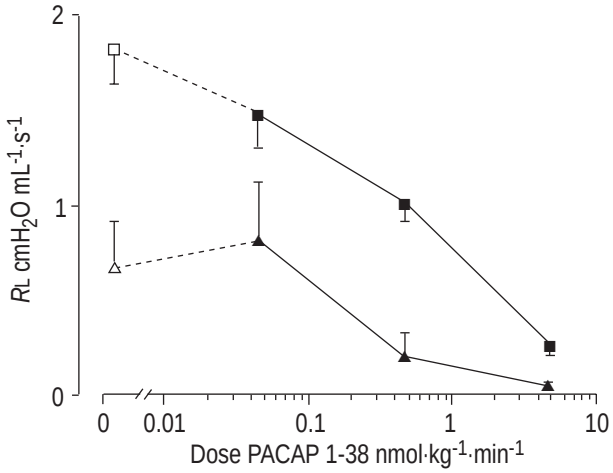

Fig. 6. – Dose-response data for pituitary adenylate cyclase-activating peptide (PACAP) 1-38 given during intravenous infusion (0.047, 0.47 or 4.7 nmol·kg^{-1}·min^{-1}, 15 min) *versus* the peak increase in total pulmonary resistance (R_L; cmH_2O·mL^{-1}·s^{-1}) caused by inhaled histamine aerosol (▲; 10 mM, 5 breaths) or infusion of carbachol intravenously (■: 0.1 mM, 0.5 mL) in anesthetized guinea-pigs *in vivo*. The R_L responses to histamine and carbachol after infusion of the vehicle (phosphate buffered saline) are also presented (□, △). Data are mean±SEM (n=3–4). (Reproduced with permission from [20]).

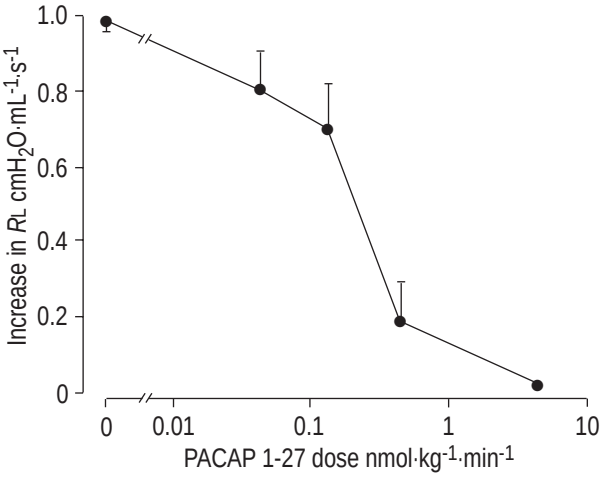

Fig. 7. – Dose-response data for pituitary adenylate cyclase-activating peptide (PACAP) 1-27 given during intravenous infusion (0.047, 0.47 or 4.7 nmol·kg^{-1}·min^{-1}, 15 min) *versus* the peak increase in total pulmonary resistance (R_L cmH_2O·mL^{-1}·s^{-1}) caused by inhaled ovalbumin (10 mg·mL^{-1}, 5 breaths) in sensitized, anesthetized guinea-pigs *in vivo*. Data are mean±SEM. (n=4–5). (Reproduced with permission from [5]).

action in primate bronchi *in vitro* [6] (fig. 13). The time to full onset of action is ~1 h for both PACAP analogues *in vitro* (figs. 11 and 12). Interestingly, both these analogues maintain their full effect throughout the 5 h of observation *in vitro*. In contrast to the effect of the original PACAP 1-27, the effect of the M and BM type of PACAP analogue is not potentiated by NEP inhibition with phosphoramidon [6, 35] (figs. 8 and 14). Thus, the sustained action of the PACAP analogues may at least in part be due to a reduced susceptibility to NEP.

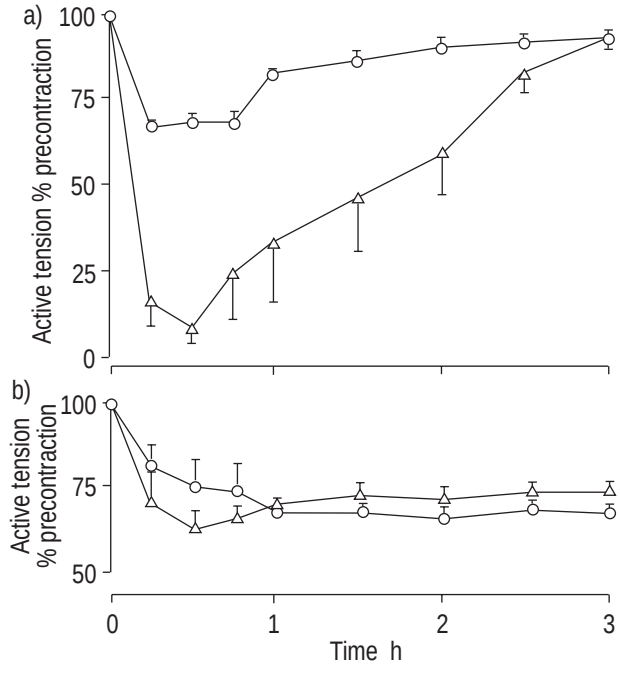

Fig. 8. – Time course of the relaxant effect of: a) pituitary adenylate cyclase-activating peptide (PACAP) 1-27 (1 μM); and b) the PACAP 1-27 BM analogue, [Arg^{15,20,21}Leu^{17}]-PACAP-Gly-Lys-Arg-NH_2 (1 μM) with (○) or without (□) peptidase inhibition by captopril (10 μM) and phosphoramidon (1 μM) in the guinea-pig trachea *in vitro*. Data are mean±SEM (n=5). (Reproduced with permission, from [6]).

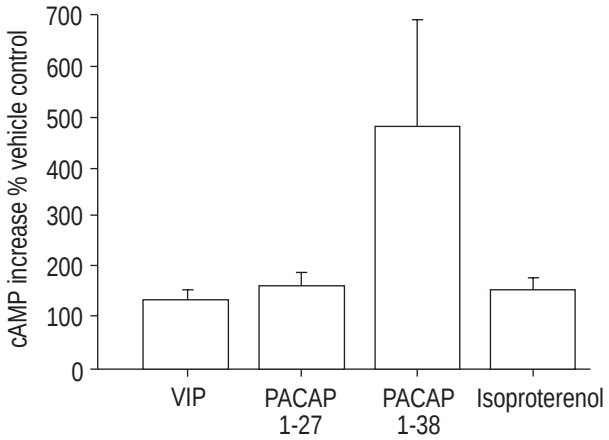

Fig. 9. – Effect of peptidase inhibition using pretreatment (15 min) with captopril (10 μM) and phosphoramidon (1 μM) on the increase in intracellular cyclic adenosine monophosphate (cAMP) caused by vasoactive intestinal peptide (VIP), pituitary adenylate cyclase-activating peptide (PACAP) 1-27, PACAP 1-38 and isoproterenol (0.1 μM during 15 min) in human airway epithelial-like (Calu-3) cells. Basal (inherent) cAMP was 0.19±0.04 pmol·μg deoxyribonucleic acid (DNA)$^{-1}$ after stimulation with vehicle (saline 0.9%). The control response was 3.6±1.2, 1.5±0.3, 2.2±1.2 and 12.0±3.5 pmol·μg DNA^{-1}, for VIP, PACAP 1-27, PACAP 1-38 and isoproterenol respectively. Data are mean±SEM percentage of the cAMP response to each peptide (% vehicle control) after pretreatment with the vehicle (saline 0.9%) (n=3–7). (Reproduced with permission from [24]).

In guinea pigs *in vivo*, intratracheal administration of the BM type of PACAP analogue decreases acetylcholine airway responsiveness significantly [36]. The onset of action is slow, requiring 4 h to produce a significant inhibitory effect on muscarinic responsiveness (fig. 15). However this protective effect is not diminished throughout 5 h of observation.

PACAP and inflammatory cells

PACAP and airway inflammation

At present, it is not known whether either PACAP 1-27 or 1-38 exert beneficial effects on airway inflammation *in vivo*. However, there is data on the effect of PACAP 1-27 and 1-38 on isolated inflammatory cells.

PACAP and lymphocytes

PACAP 1-38 given in nanomolar concentrations inhibits the spontaneous mobility of rat lymphocytes *in vitro*, although this effect is dependent upon the activation state of protein kinase C [10]. Costimulation with the cAMP-elevating agent forskolin inhibits chemotaxis of lymphocytes more potently than does PACAP 1-38 alone [8, 10]. A VIP receptor antagonist attenuates the referred effects in rat lymphocytes, indicating the involvement of PACAP type II receptors [10]. In humans, the PACAP type II receptor is predominant in peripheral B-lymphocytes, whereas the PACAP type I receptor is predominant in several human T-lymphocyte cell lines, as demonstrated by detection of receptor messenger ribonucleic acid (mRNA) for these receptors [37]. However, the expression of PACAP type II receptors is probably dependent upon the activation of lymphocytes [11].

Peptide	Amino acid sequence
	1 5 10 11 15 20 21 25
PACAP 27	H-His-Ser-Asp-Gly-Ile-Phe-Thr-Asp-Ser-Tyr-Ser-Arg-Tyr-Arg-Lys -Gln-Met- Ala-Val- Lys-Lys-Tyr-Leu-Ala-Ala-Val-Leu-NH_2
BM type	H-His-Ser-Asp-Gly-Ile-Phe-Thr-Asp-Ser-Tyr-Ser-Arg-Tyr-Arg-Arg-Gln-Leu-Ala-Val-Arg-Arg-Tyr-Leu-Ala-Ala-Val-Leu-Gly-Lys-Arg-NH_2
M type	H-His-Ser-Asp-Gly-Ile-Phe-Thr-Asp-Ser-Tyr-Ser-Arg-Tyr-Arg-Arg-Gln-Leu-Ala-Val-Arg-Arg-Tyr-Leu-Ala-Ala-Val-Leu-NH_2

Fig. 10. – Amino acid sequence for the pituitary adenylate cyclase-activating peptide (PACAP) 1-27 *versus* the PACAP 1-27 M analogue, [$Arg^{15,20,21}Leu^{17}$]-PACAP-NH_2, and the PACAP 1-27 BM analogue, [$Arg^{15,20,21}Leu^{17}$]-PACAP-Gly-Lys-Arg-NH_2. (Reproduced with permission from [6] and [35]).

PACAP 1-38 attenuates the production of the pro-inflammatory cytokine, interleukin (IL)-2 in murine T-lymphocytes *in vitro* [14]. This effect is mimicked by VIP and the cAMP-elevating agent forskolin. For VIP, this effect involves an inhibited transcription process *via* a cAMP-dependent transcription factor (nuclear factor of activated T-lymphocytes (NFAT)) as well as destabilization of IL-2 mRNA. However, in addition to its direct effect on IL-2, PACAP 1-38 also reduces the production of the anti-inflammatory cytokine IL-10, in murine T-lymphocytes [13]. This latter event occurs through the inhibition of transcription alone.

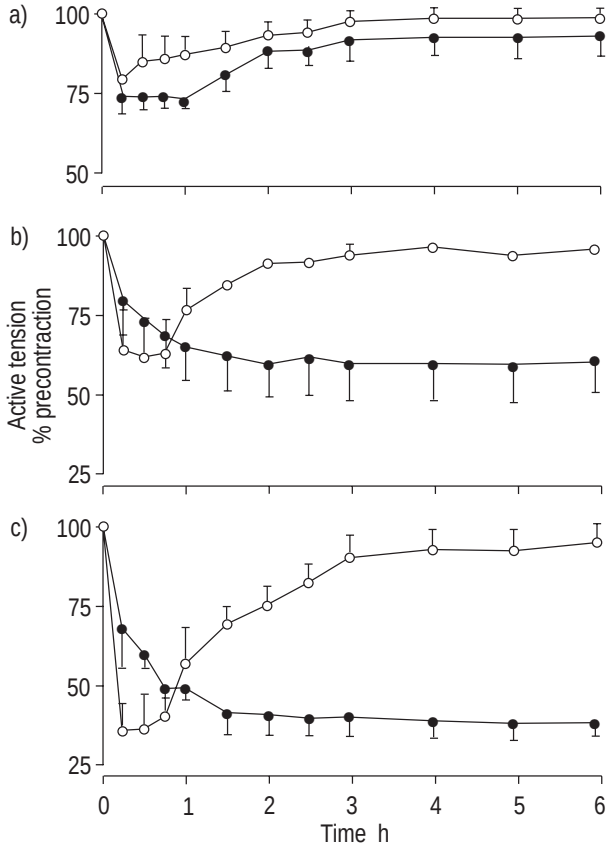

Fig. 11. – Time course of relaxant effect for the pituitary adenylate cyclase-activating peptide (PACAP) 1-27 BM analogue, [$Arg^{15,20,21}Leu^{17}$]-PACAP-Gly-Lys-Arg-NH_2 (●) and PACAP 1-27 (○) in the guinea-pig trachea *in vitro*. The active tension after addition of each of three bronchodilator concentrations: a) 0.3; b) 1; and c) 3 mM, is shown. The active tension is presented as the mean±SEM 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) (n=5). (Reproduced with permission from [6]).

PACAP and granulocytes

There are no published data, to the authors′ knowledge, on the effect of PACAP 1-27 or 1-38 on isolated granulocytes, either for eosinophils or for neutrophils. However, there is data suggestive of PACAP 1-27 acting on granulocytes. PACAP 1-27 given in micromolar concentrations attenuates the release of thromboxane B_2 caused by leukotriene D_4 in guinea pig lung strips *in vitro* [32]. This thromboxane may originate from granulocytes or thrombocytes.

PACAP and macrophages

PACAP 1-38 increases both adherence and mobility of isolated rat macrophages when given in nanomolar concentrations *in vitro*. These effects are dependent upon the activation state of protein kinase C [10]. The peptide is

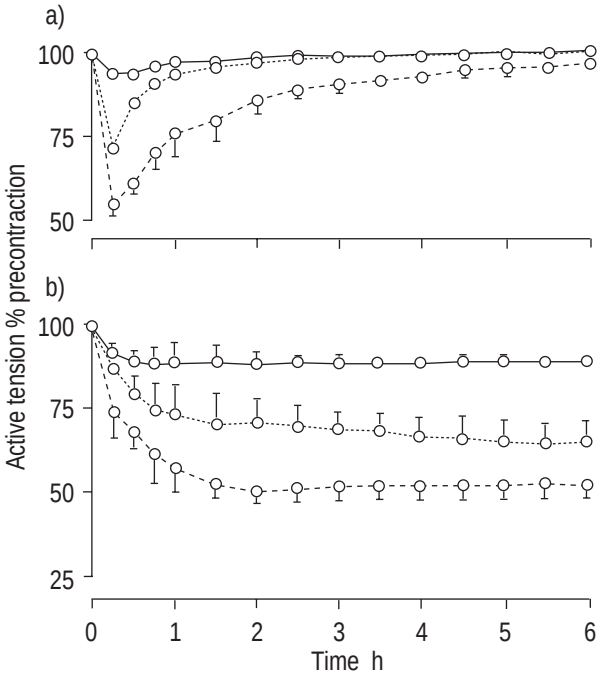

Fig. 12. – Time course of relaxant effect for: a) pituitary adenylate cyclase-activating peptide (PACAP) 1-27 (—— : 0.03 μM; ·····: 0.1 μM; - - - - : 0.3 μM); and b) the PACAP 1-27 M analogue, [$Arg^{15,20,21}Leu^{17}$]-PACAP-NH2 (—— : 0.3 μM; ·····: 1 μM; - - - - : 3 μM) in the guinea-pig trachea *in vitro*. The active tension after addition of each of the three bronchodilator concentrations is presented as the mean±SEM 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) (n=4–6). (Reproduced with permission from [35]).

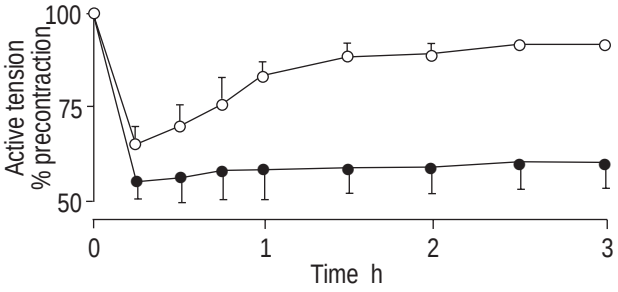

Fig. 13. – Time course of relaxant effect for the pituitary adenylate cyclase-activating peptide (PACAP) 1-27 BM analogue, [$Arg^{15,20,21}$ Leu^{17}]-PACAP-Gly-Lys-Arg-NH_2 (l) and PACAP 1-27 (m) (0.1 mM) in primate bronchi *in vitro*. The active tension after addition of each of the bronchodilators is presented as the mean±SEM 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) (n=4). (Reproduced with permission from [6]).

chemotactic *per se* for rat macrophages, and it counteracts the inhibitory effect of forskolin on macrophage chemotaxis [10]. The cytotoxic action of macrophages may be increased by PACAP 1-38, because it triggers superoxide anion production with higher potency than VIP in human monocytes [38]. Both PACAP 1-27 and 1-38 enhance phagocytosis in mouse macrophages *in vitro*, and they do this more potently than VIP, particularly PACAP 1-38 [12]. PACAP 1-38 also stimulates phagocytosis in rat macrophages *in vitro*, parallelled by a significant increase in cAMP, *via* a pathway independent of protein kinase C [9]. The role of cAMP as a second messenger is also supported by the associated increase in cAMP and superoxide production after stimulation with PACAP 1-38 in human monocytes [38]. It is likely that PACAP type I receptors mediate the effects on macrophages, because a PACAP type II receptor antagonist does not affect chemotaxis or phagocytosis induced by PACAP 1-38 [9, 10].

PACAP and mast cells

There are no functional studies on PACAP 1-27 or 1-38 in isolated mast cells, to the authors′ knowledge, but mRNA for PACAP type II receptors has been detected in a murine mast cell line [37]. The observation that a histamine antagonist inhibits plasma extravasation caused by PACAP 1-38 in rat skin indicates that mast cells may mediate effects of PACAPs by releasing histamine [39]. Another supporting observation is that PACAP 1-38 releases histamine from rat peritoneal cells [40]. VIP, which acts *via* PACAP type II receptors (equipotently to the PACAPs), releases histamine from mast cells, including mast cells in human skin [41, 42]. Thus, it is possible that at least PACAP 1-38 exerts actions on mast cells or basophils, although there is no direct evidence for this at present.

PACAP and the cardiovascular system

PACAP and vascular smooth muscle

In vitro, PACAP 1-38 inhibits smooth muscle tone in the human pulmonary artery [6]. This effect is endothelium-

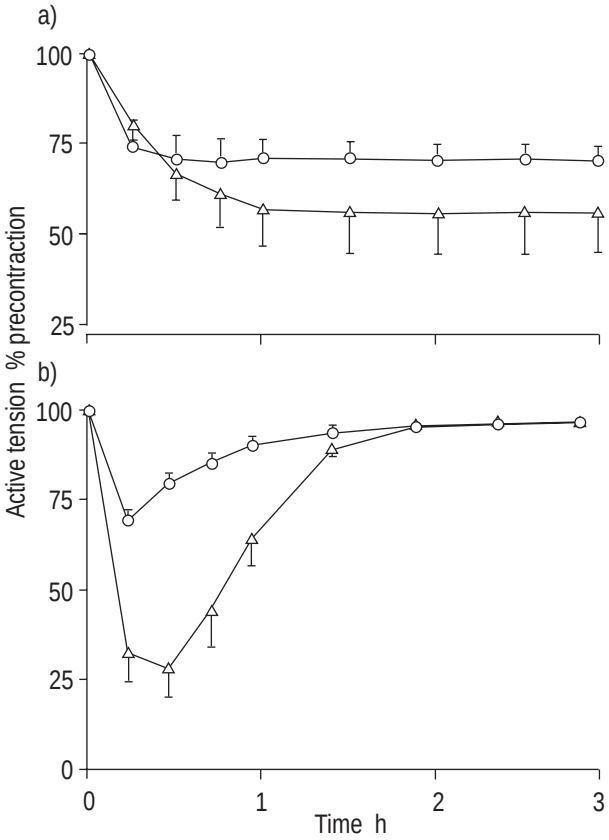

Fig. 14. – Time course of the relaxant effect of: a) the PACAP 1-27 M analogue, [$Arg^{15,20,21}Leu^{17}$]-PACAP-NH_2 (1 μM) and b) pituitary adenylate cyclase-activating peptide (PACAP) 1-27 (1 μM) with (△) or without (○) peptidase inhibition by captopril (10 μM) and phosphoramidon (1 μM) in the guinea-pig trachea *in vitro*. Data are mean±SEM (n=5). (Reproduced with permission from [35]).

and nitric oxide-dependent, which indicates a mechanism different from that of VIP (fig. 16). In intact human artery *in vitro*, the potency of PACAP 1-38 is fairly similar to that of VIP in causing smooth muscle relaxation. Similarly, PACAP 1-38 relaxes the guinea pig pulmonary artery *in vitro*, but there is conflicting data on whether this relaxation is endothelium-dependent [4, 7, 43]. The rat mesenteric artery *in vitro* is also relaxed by PACAP 1-38, which is slightly more potent than PACAP 1-27 or VIP [16]. PACAP 1-38 given as an intravenous bolus also causes significant vasodilation in human subjects [44]. PACAP type II receptors probably mediate the effects on vascular smooth muscle, as indicated by the similar affinity for PACAP 1-27 and 1-38 and VIP in the rat aorta, the femoral and iliac arteries and veins [16, 45].

PACAP and plasma extravasation

In high doses, PACAP 1-38 *per se* increases plasma extravasation more potently than PACAP 1-27 or VIP but slightly less potently than does substance P (SP) in rat skin *in vivo* [40]. However, in low doses, PACAP 1-38 does not cause plasma extravasation *per se*, but it does potentiate SP-induced extravasation in rabbit skin *in vivo* [48].

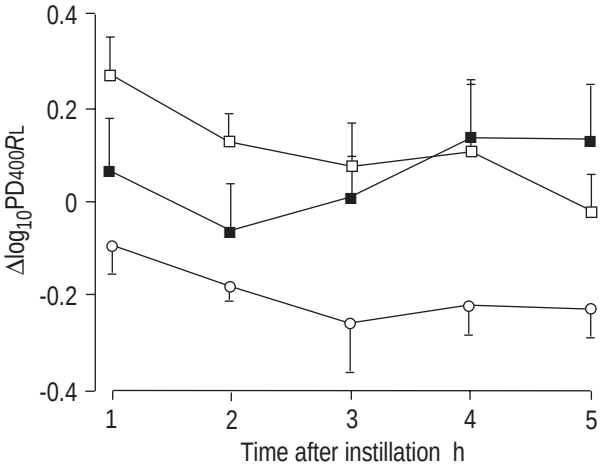

Fig. 15. – Time course of the within-animal shift in the dose of acetylcholine causing 400% increase in total pulmonary resistance above baseline ($\Delta\log_{10}PD_{400}RL$) after intratracheal instillation of the pituitary adenylate cyclase-activating peptide (PACAP) 1-27 BM analogue (350 nmol; ■), salbutamol (35 nmol; □) or vehicle (○). Data presented as mean±SEM (n=4–7). (Reproduced with permission from [36]).

PACAP and the heart

Cultured neonatal rat myocardiocytes respond to PACAP 1-27 and 1-38 (but not to VIP) by increasing the intracellular level of cAMP and the secretion of atrial natriuretic peptide [47], suggesting involvement of PACAP type I receptors. In support of this observation, PACAP 1-27 and 1-38 but not VIP increase myocardial inotropy in the neonatal pig heart *in vitro* [48, 49]. PACAP 1-27 is more potent than PACAP 1-38 in this model, approaching the potency of isoproterenol. PACAP 1-38 also increases right ventricular inotropy in the dog heart *in vivo* [50]. In the guinea pig ventricular strip *in vitro*, however, the inotropy is not affected by either PACAP 1-27 or 1-38, and chronotropy is decreased in the guinea pig atrium *in vitro* [51]. Similarly, inotropy is decreased in the denervated dog heart *in situ* and in the perfused dog heart *in vitro* while chronotropy is either increased or decreased by PACAP 1-38, depending on the dose [52, 53]. The increase in chronotropy that is caused by PACAP 1-38 may be due to a direct effect on sinus rate [53]. In contrast, VIP has no effect on chronotropy [51] and, therefore, PACAP type I receptors are probably involved in this response. Data showing a similar vasodilator activity of PACAP 1-27 and 1-38 and VIP suggests the presence of PACAP type II receptors in porcine coronary arteries [16, 49].

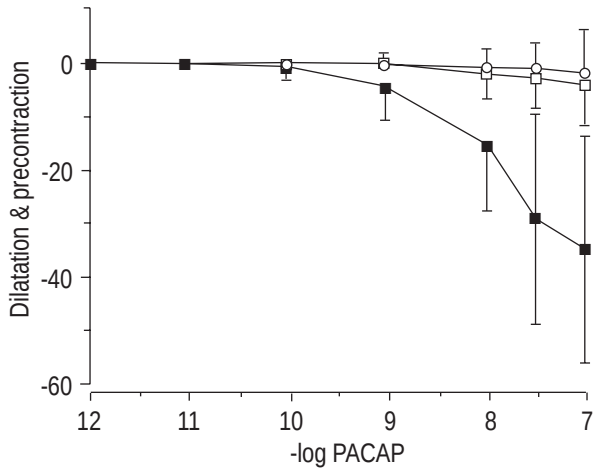

Fig. 16. – Concentration-response data for pituitary adenylate cyclase-activating peptide (PACAP) 1-38 with intact epithelium with (□) and without N^G-monomethyl-L-arginine (L-NMMA) (100 mM; ■), as well as without epithelium (○) in the human pulmonary artery *in vitro*. Data presented as mean±SD percentage of endothelin-1-induced (0.1 mM) precontraction (n=5–10). (Reproduced with permission from [7]).

In contrast to the effects on isolated hearts or heart cells, PACAP 1-38 has no effect on heart rate in human subjects when given as a slow intravenous infusion at a dose that increases endogenous arginine vasopressin [54]. Also, PACAP 1-27 causes less effect on heart rate than does the clinically utilized β_2-adrenoceptor agonist salbutamol, during slow intravenous infusion at doses causing bronchodilation in guinea pigs *in vivo* [5]. Inhalation of PACAP 1-38 does not have negative effects on heart rate when given in doses causing bronchodilation in guinea pigs *in vivo* [20]. However, when given as an intravenous bolus, PACAP 1-38 and 1-27 do affect heart rate; in dogs *in vivo*, PACAP 1-27 causes a dose-dependent increase in heart rate but it is not known how the doses given relate to doses required for bronchodilation [55]. Under the same conditions, PACAP 1-38 causes only a minor increase in heart rate, and it is unclear whether this effect is due to a reflex caused by hypotension [55]. In the cat *in vivo*, a bolus injection of PACAP 1-38 increases heart rate [50].

PACAP and blood pressure

When given as a slow intravenous infusion, in a dose that increases endogenous arginine vasopressin, PACAP 1-38 has virtually no effect on systemic blood pressure in human subjects [54]. Similarly, inhaled PACAP 1-38 does not decrease blood pressure, when given in doses causing bronchodilation in guinea pigs *in vivo* [20]. However, when given as an intravenous bolus, both PACAP 1-27 and 1-38 do cause systemic hypotension in rats *in vivo* [45]. Interestingly, in this rat model, PACAP 1-27 is less potent than PACAP 1-38, which is less potent than VIP. In dogs *in vivo*, PACAP 1-27 and 1-38 are clearly less potent than VIP in causing systemic hypotension when given as an intravenous bolus [55]. In dogs, the response is biphasic; an initial decreasing effect is followed by a moderate and more sustained increasing effect on systemic blood pressure [56]. Both PACAP 1-27 and 1-38 produce a moderate biphasic effect on systemic blood pressure in the cat *in vivo*, but PACAP 1-27 is the most potent [48, 50]. In contrast, VIP causes hypotension only in cats. The pressor effect of PACAP 1-27 or 1-38 on systemic blood pressure is mediated through catecholamines in cats and dogs, acting on α-adrenergic receptors in cats [48, 50]. PACAP 1-38 also increases the spinal sympathetic neural outflow in rats [57]. In cats *in vivo*, PACAP 1-27 and 1-38 either increase or decrease pulmonary artery pressure whereas VIP only decreases pulmonary artery pressure [48, 50].

Conclusions

Pituitary adenylate cyclase-activating peptide-related molecules potently inhibit airway smooth muscle tone in

several species both *in vitro* and *in vivo*. Pituitary adenylate cyclase-activating peptide 1-38 is clearly superior to vasoactive intestinal peptide after inhalation *in vivo* in terms of causing sustained bronchodilation without cardiovascular side effects. Pituitary adenylate cyclase-activating peptide 1-27 or 1-38 also inhibit chemotaxis and cytokine production of lymphocytes and may modulate the activity of macrophages and mast cells *in vitro*. Additional studies on human cells are required to determine whether pituitary adenylate cyclase-activating peptide-related molecules inhibit tone in bronchial smooth muscle and activity of inflammatory cells before these molecules should be tested for treatment of obstructive airways disease.

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