Pituitary adenylate cyclase activating peptide regulates neurally mediated airway responses

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Pituitary adenylate cyclase activating peptide regulates neurally mediated airway responses. M. Shigyo, H. Aizawa, H. Inoue, K. Matsumoto, S. Takata, N. Hara. ©ERS Journals Ltd 1998.
ABSTRACT: To clarify the protective effects of pituitary adenylate cyclase activating peptide (PACAP) on airway narrowing, we examined the effects of PACAP on smooth muscle contraction and plasma extravasation in guinea-pig airways.

Smooth muscle contraction evoked by electrical field stimulation (EFS) or exogenously applied acetylcholine (ACh) or substance P (SP) was measured before and after PACAP *in vitro*. The effect of PACAP on airway plasma extravasation was also measured *in vivo*.

In trachea, PACAP (10^{-9}–10^{-7} M) significantly suppressed smooth muscle contraction evoked by EFS without affecting ACh sensitivity, suggesting that PACAP inhibits cholinergic neuroeffector transmission. In the main bronchi, PACAP (10^{-9}–10^{-8} M) significantly suppressed the contraction evoked by EFS without affecting SP sensitivity in the presence of atropine, suggesting that PACAP inhibits SP release from excitatory nonadrenergic noncholinergic (eNANC) nerves. In animals treated with atropine and propranolol, PACAP attenuated the increase in plasma extravasation induced by electrical vagus stimulation or by SP.

These results suggest that pituitary adenylate cyclase activating peptide may play a role in modulation of airway responses through inhibition of cholinergic and noncholinergic mechanisms.
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Pituitary adenylate cyclase activating peptide (PACAP) is a vasoactive intestinal peptide (VIP)-like peptide that has been purified from the ovine hypothalami [1]. PACAP has the same potency as VIP in relaxing airway and vascular smooth muscle [2–5]. Recently, it has been demonstrated that PACAP induced bronchodilatation in animals treated with histamine or allergen [6]. However, the pathophysiological roles of PACAP in mammalian airways have not been fully elucidated. The structural and functional homology between PACAP and VIP makes it likely that PACAP may participate in the modulation of airway responses in a similar manner to VIP.

VIP immunoreactivity is localized to the vagus nerve in the smooth muscle of mammalian airways [7–9]. It has been reported that VIP inhibits cholinergic neuroeffector transmission by suppressing acetylcholine (ACh) release from vagal nerve termini [10]. PACAP may have the same effects on cholinergic nerves. It has also been reported that VIP suppresses the smooth muscle contraction evoked by substance P (SP) [11]. SP belongs to the tachykinin family and is released from bronchial C-fibres, causing smooth muscle contraction and plasma extravasation [12].

Increased plasma extravasation and smooth muscle contraction mediated by the vagus nerve may contribute to the airway hyperresponsiveness and inflammation observed in asthma. However, it is not known whether SP and PACAP interact.

For these reasons, in the present study, we examined the roles of PACAP involved in airway smooth muscle response and plasma extravasation induced by cholinergic and excitatory nonadrenergic noncholinergic (eNANC) nerves in guinea-pigs.

Methods

Immunohistochemical examination

Lungs were dissected out from three guinea-pigs after sacrifice. The specimens were immersed for 12 h in an ice-cold fixative solution composed of 2% formaldehyde buffered to pH 7.2 with 0.1 M phosphate buffer and 0.2% picric acid. They were then rinsed in a Tyrode solution containing 10% sucrose for 48 h, frozen on dry ice, and sectioned by a cryostat at a thickness of 10 μm. Then, they were immersed in sucrose-enriched Tyrode solution for 24–48 h, briefly rinsed in 0.1 M phosphate buffer (pH 7.4), and stretched on chrome-alum subbed glass slides as whole mounts. Cryostat sections and whole mounts were processed for immunohistochemical demonstration of PACAP using an avidin-biotin complex method. The PACAP antiserum (Yanaihara Institute Inc., Shizuoka, Japan) was raised in a rabbit against human PACAP 27 or PACAP 38 and used at a dilution of 1:12,000. The sections were exposed to the peptide antiserum for 4 h at room temperature. The antigen-antibody reaction was demonstrated by application of biotin-labelled anti-rabbit immunoglobulin G (IgG)

for 1 h at room temperature. For control incubation, rabbit anti-human PACAP 27 or PACAP 38 serum was replaced by normal rabbit serum.

Absorption tests showed that the PACAP 27 antiserum does not cross-react with PACAP 38, VIP, peptide histidine isoleucine (PHI), secretin or helodermin. The PACAP 38 antiserum does not crossreact with PACAP 27, glucagon, VIP, PHI, or secretin. However, crossreaction with 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 or VIP-like.

Effect of PACAP on smooth muscle contraction

Forty guinea-pigs of either sex, weighing 500–800 g, were anaesthetized with pentobarbital sodium (50 mg·kg^{-1} *i.p.*), sacrificed by exsanguination, and the trachea and lungs were removed. The segments of trachea and main bronchus were opened longitudinally through the anterior aspects, and a dorsal strip of trachea or a whole strip of main bronchus was cut transversely to a length of 2–3 mm and a width of 1–1.5 mm. The mucosa was carefully removed. To measure the mechanical change, the strip was mounted in a 3.5-mL organ bath filled with Krebs-Henseleit solution aerated with 95% O_2 and 5% CO_2 and kept at 37°C. The composition of Krebs solution was as follows (in mM): Na^+, 131.4; K^+, 5.9; Mg^{2+}, 1.2; Ca^{2+}, 2.5; Cl^-, 134.0; $H_2PO_4^-$, 1.2; HCO_3^-, 15.5; and glucose, 11.5. Krebs solution was changed every 20 min from a reservoir by a peristaltic pump (MP-3B, Eyela, Tokyo, Japan) set at flow of 10 mL·min^{-1}. The strip was placed vertically in the bath and its ends were tied with silk thread. One end of the strip was tied to an isometric transducer (TB-612T, Nihon Kohden Ltd, Tokyo, Japan) and the other end to a hook at the bottom of the bath. The strip was set with an initial tension of 0.5 g, which was determined in a preliminary study to be optimal for this size strip. The strip was equilibrated over 1–2 h with Krebs solution, and isometric tension was recorded continuously with a pen recorder (WT-687G, Nihon Kohden Ltd, Tokyo, Japan).

Study 1. To study the relaxant activity of PACAP, tracheal strips were precontracted by infused acetylcholine (ACh) (10^{-4} M). After the contraction reached a plateau, an infusion of VIP or PACAP (10^{-6} M) was initiated. After 15 min, VIP or PACAP was stopped, but the infusion of ACh was maintained without interruption throughout the study. Isometric tension was continuously recorded.

Study 2. To investigate the effects of PACAP on the cholinergic nerve-mediated contraction, electrical field stimulation (EFS) was applied through a pair of electrodes consisting of silver-silver chloride plates 5 mm apart, and placed so that the current pulse would pass transversely across the tracheal strip. Repetitive stimulation at 20 Hz was applied every 3 min using a current pulse of 0.8 ms and 20 V. Intensity-response curves were obtained from the contractions evoked by different intensities of EFS (5, 10, 20, and 40 pulses).

To avoid the effects of prostaglandins and sympathetic nerves, each study was carried out in the presence of both indomethacin (10^{-6} M) and guanethidine (10^{-6} M).

The first intensity-response curve to EFS was measured as a control after equilibration with Krebs solution for 90 min, when the resting tone of the muscle strip was stable. After the first intensity-response curve was completed, PACAP (10^{-9} to 10^{-7} M) was added to the bath. After 5 min, the second intensity-response curve was obtained.

To measure the ACh sensitivity of the smooth muscle, a cumulative ACh concentration-response curve was generated by administering serial 10-fold increasing concentrations. Each additional dose was given after the response to the previous dose had reached a plateau. After equilibration for 90 min with Krebs solution, the smooth muscle was contracted by exposure to ACh (10^{-5} M). Sixty minutes after ACh was eliminated, PACAP was added to the bath, and then the concentration-response curve to ACh (10^{-8}–10^{-5} M) was obtained after 5 min. A control concentration-response curve was obtained from the vehicle-treated group.

Study 3. To investigate the effects of PACAP on eNANC nerve-mediated contraction of the main bronchus, EFS at 20 Hz was applied every 3 min using a current pulse of 0.8 ms and 20 V (5, 10, 20, and 40 pulses) in the presence of indomethacin (10^{-6} M), guanethidine (10^{-6} M), and atropine (10^{-6} M).

The first intensity-response curve to EFS was measured as a control after equilibration with Krebs solution for 90 min. After the first intensity-response curve was completed, PACAP (10^{-9}–10^{-7} M) was added to the bath, and the second intensity-response curve was obtained 5 min later.

To measure the sensitivity of the smooth muscle to substance P (SP), a cumulative SP concentration-response curve was generated by administering serial 10-fold increasing concentrations. Each additional dose was given after the response to the previous dose had reached a plateau. After equilibration for 90 min with Krebs solution, the smooth muscle was contracted by exposure to SP (10^{-8} M). Sixty minutes after SP was eliminated, PACAP was added to the bath, and then the concentration-response curve to SP (10^{-10} M to 10^{-8} M) was obtained after 5 min. A control concentration-response curve was obtained from the vehicle-treated group.

Effect of PACAP on airway plasma extravasation

Airway plasma extravasation was assessed using Evans blue dye, as employed in many previous studies [13–16].

Briefly, guinea-pigs were anaesthetized with pentobarbital sodium (50 mg·kg^{-1}, *i.p.*) and mechanically ventilated with a tidal volume of 7 mL·kg^{-1} and a frequency of 60 breaths·min^{-1} (Harvard Apparatus, Model, South Natick, MA, USA). A catheter was introduced into the jugular vein for drug administration.

Vehicle, PACAP, or VIP was administered intravenously 6 min before either vagus nerve stimulation (5 V, 5 ms, 5 Hz, 150 s) or intravenously administered SP (1 µg·kg^{-1}) in the presence of atropine (1 mg·kg^{-1}) and propranolol (1 mg·kg^{-1}). Evans blue dye, dissolved in 0.9% saline at a concentration of 20 mg·mL^{-1}, was intravenously administered

1 min before each stimulus at a dose of 1 mL·kg⁻¹. Five minutes after the Evans blue injection, animals were disconnected from the respirator, the thorax was opened, and a cannula was inserted into the ascending aorta through the left ventricle.

The animals were perfused with 500 ml of 0.9% saline at a pressure of 16 kPa (120 mmHg) to remove intravascular dye from the bronchial circulation, and the main bronchi were dissected. All tissues were weighed wet and incubated in 1 mL of formamide overnight at room temperature. The Evans blue dye concentration in the formamide extracts was measured by light absorbance at 620 nm, using a spectrophotometer (Model UV-2200A, Shimadzu Scientific Instruments, Tokyo, Japan) and calculated from a standard curve of dye concentrations in the range of 0.1–10 µg·mL⁻¹. The amount of dye extravasated into the tissues was expressed in ng·mg⁻¹ of wet weight.

Drugs

The following drugs were used in this study: pentobarbital sodium, indomethacin, guanethidine, acetylcholine hydrochloride, atropine sulphate, propranolol, substance P, PACAP and VIP (Sigma, St. Louis, MO, USA).

Statistical analysis

The results are expressed as the arithmetic mean and the standard error of the mean (SEM). In the EFS experiments, the values are expressed as a percentage of the maximum contraction evoked by the intensity of 40 pulses in each control measurement. Two-way analysis of variance (ANOVA) was used to evaluate differences between the control (vehicle) intensity-response curve and those obtained in the presence of PACAP and VIP. Each statistical comparison was assessed by Scheffe's methods.

In the experiments with exogenously applied ACh or SP, values are expressed as a percentage of the contraction evoked by 10^{-5} M ACh or 10^{-8} M SP during the first contraction of each measurement. A repeated measures analysis was used to evaluate the differences between the concentration-response curves of the second measurements.

A $p<0.05$ value was considered statistically significant.

Results

Distribution of PACAP

PACAP immunoreactivity was observed in nerve fibres among bundles of smooth muscle, beneath the epithelium in the guinea-pig bronchial wall (fig. 1). Only occasionally were fibres seen around seromucous glands and blood vessels, and none was present in the epithelium. All specimens from three guinea-pigs stained for both PACAP-27 and PACAP38 antibodies almost equally.

Effect of PACAP on smooth-muscle relaxation

The relaxation evoked by PACAP lasted longer than that by VIP. The tracheal tone returned to the baseline within 23.5±1.5 min after the application of VIP, whereas relaxation induced by PACAP was not reversed at the end of the observation period (at least 2 h) (fig. 2, n=4).

Effects of PACAP on tracheal smooth muscle contraction

The effects of PACAP on the contractions of tracheal smooth muscle evoked by EFS are shown in figure 3. EFS

Fig. 2. – Relaxations induced by the same concentration of pituitary adenylate cyclase activating peptide (PACAP; ●) and vasoactive intestinal peptide (VIP; ○) (10^{-6} M) in the trachea contracted by acetylcholine (10^{-4} M).
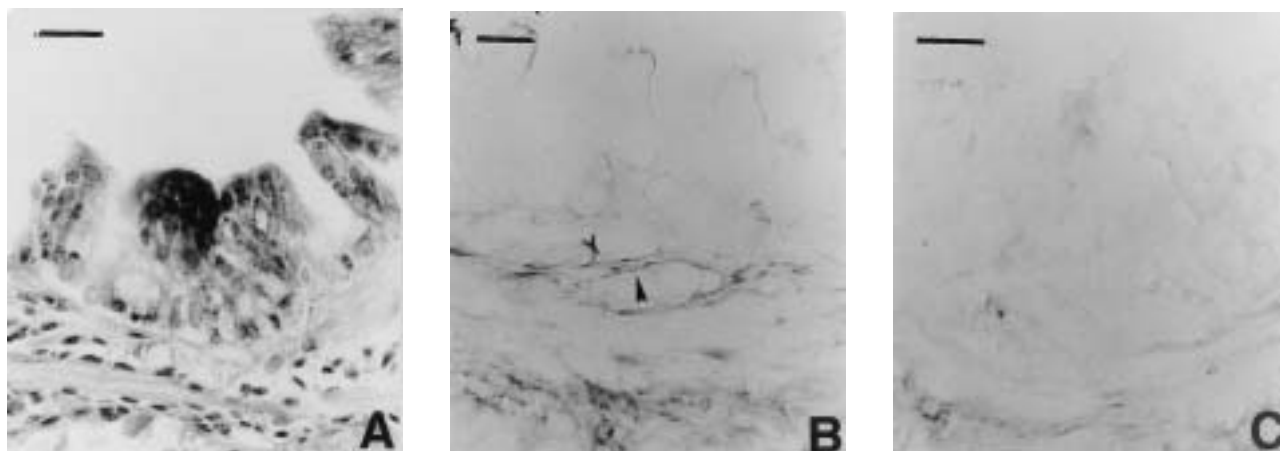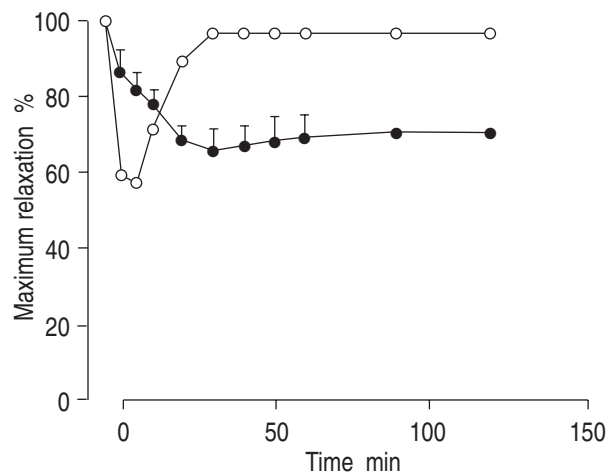
Fig. 1. – A) Guinea-pig bronchial sections, stained with haematoxylin and eosin. B) Pituitary adenylate cyclase activating peptide (PACAP) immunoreactivity observed in nerve fibres among bundles of smooth muscle (arrow), beneath the epithelium (arrow head). C) No positive signal was detected when specific rabbit serum was replaced by normal rabbit serum. (Internal scale bar=50 µm).
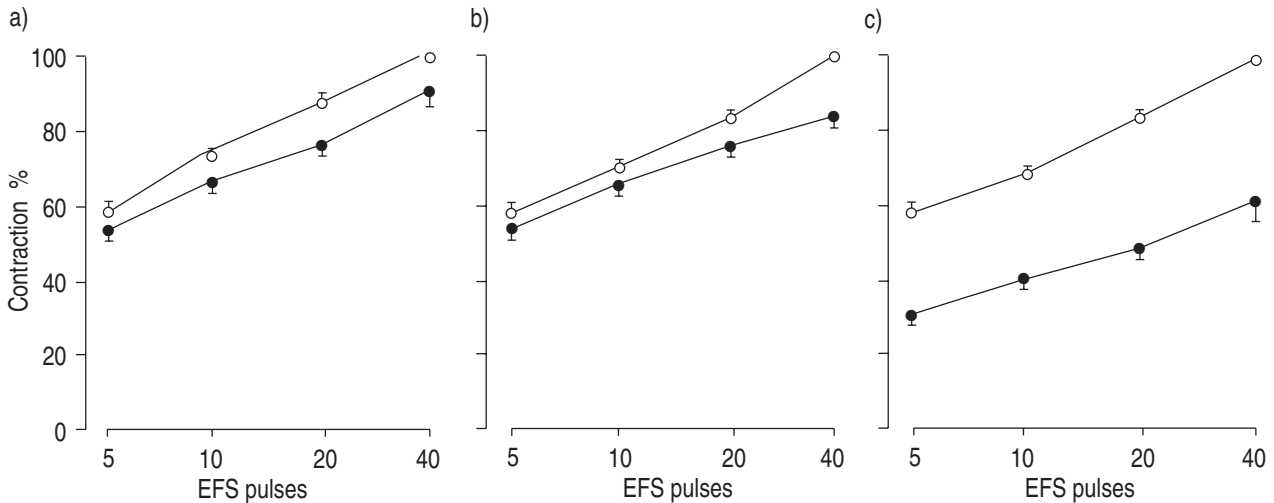
Fig. 3. – Effect of pituitary adenylate cyclase activating peptide (PACAP; ●) at: a) 10^{-9} (n=10); b) 10^{-8} (n=8); and c) 10^{-7} (n=8) M on the contractions of tracheal smooth muscle evoked by electrical field stimulation (EFS) (20 V, 20 Hz, 0.8 ms). The amplitude of the contraction is expressed as a percentage of the contraction evoked by 40 pulses of EFS in the absence of PACAP (control; ○). (All p<0.01).

Table 1. – Effect of pituitary adenylate cyclase-activating peptide (PACAP) on the amplitude of contractions induced by exposure to acetylcholine (ACh)

ACh M	Control (n=5)	PACAP 10^{-9} M (n=5)	PACAP 10^{-8} M (n=5)	PACAP 10^{-7} M (n=5)
10^{-8}	6.4±1.9	2.3±2.3	3.7±3.7	0
10^{-7}	25.2±5.3	29.7±4.8	40.7±10.2	1.7±1.7
10^{-6}	76.0±6.8	76.0±5.0	91.7±5.8	59.7±8.8
10^{-5}	131.0±7.5	116.0±0.6	136.3±7.1	127.3±6.3
		p>0.05	p>0.05	p>0.05

The p-values shown are those compared with the response in the control state. The amplitude of the contraction is expressed as a percentage of the first contraction evoked by 10^{-5} M ACh.

caused the smooth muscle contractions in an intensity-dependent manner. PACAP significantly suppressed the EFS-induced contractions at concentrations of 10^{-9} M,

10^{-8} M, and 10^{-7} M, which did not change the basal tone (n=8–10, p<0.01).

In contrast, PACAP at these concentrations did not affect the amplitude of the contractions evoked by ACh as shown in table 1 (n=5). There was no statistical significance between the control and PACAP by ANOVA.

Effects of PACAP on bronchial smooth muscle contraction

The effects of PACAP on the contractions evoked by EFS are shown in figure 4. EFS caused smooth muscle contraction of main bronchus in the presence of atropine (10^{-6} M), guanethidine (10^{-6} M), and indomethacin (10^{-6} M). PACAP significantly suppressed the EFS-induced contractions at concentrations of 10^{-9} M, 10^{-8} M, and 10^{-7} M (n=5, p<0.01). Exogenously applied SP also caused bronchial smooth muscle contraction in the presence of atropine, guanethidine, and indomethacin. PACAP significantly
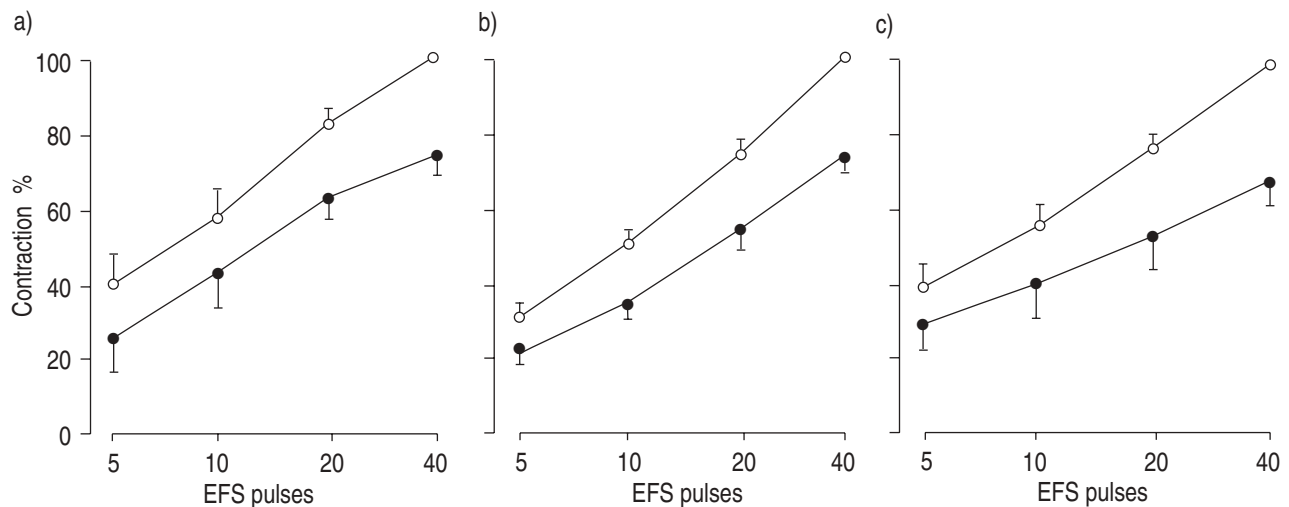

Fig. 4. – Effect of pituitary adenylate cyclase activating peptide (PACAP; ●) at: a) 10^{-9}; b) 10^{-8}; and c) 10^{-7} M on the contractions of bronchial smooth muscle evoked by electrical field stimulation (EFS) (20 V, 20 Hz, 0.8 ms). The amplitude of the contraction is expressed as a percentage of the contraction evoked by 40 pulses of EFS in the absence of PACAP (control; ○). (All n=5, p<0.01).

suppressed the SP-induced contractions at a concentration of 10^{-7} M, but did not affect them at 10^{-9} and 10^{-8} M (table 2, n=5).

Table 2. – Effect of pituitary adenylate cyclase-activating peptide (PACAP) on the amplitude of contractions induced by exposure to substance P (SP)

SP M	Control (n=5)	PACAP 10^{-9} M (n=5)	PACAP 10^{-8} M (n=5)	PACAP 10^{-7} M (n=5)
10^{-10} M	5.8±12.8	28.9±10.1	48.4±16.0	9.3±3.8
10^{-9} M	81.2±19.7	48.8±20.1	79.5±25.8	17.3±7.7
10^{-8} M	211.8±29.4	183.9±97.9	173.1±30.7	45.7±8.9
		p>0.05	p>0.05	p<0.05

The p-values shown are those compared with the response in the control state. The amplitude of the contraction is expressed as a percentage of the first contraction evoked by 10^{-8} M of SP.

Effect of PACAP on plasma extravasation

Stimulation of the vagus nerve (5 V, 5 ms, 5 Hz, 150 s) significantly increased the leakage of Evans blue dye in the main bronchi in the presence of atropine (1 mg·kg^{-1}) and propranolol (1 mg·kg^{-1}). PACAP (10^{-7} and 10^{-6} mol·kg^{-1} i.v.) significantly inhibited the increase in the leakage of dye induced by vagus nerve stimulation (fig. 5).

VIP (10^{-6} mol·kg^{-1} i.v.) did not inhibit the increase in the leakage of dye induced by vagus nerve stimulation (fig. 6).

Intravenously administered SP (1 µg·kg^{-1}) significantly increased the Evans blue dye leakage. Although the increase in dye leakage was not inhibited by VIP (10^{-6} mol·kg^{-1} i.v.) (fig. 6), PACAP (10^{-6} mol·kg^{-1} i.v.) significantly inhibited the increase in the leakage of dye induced by SP (fig. 5).

The effect of PACAP or VIP on mean systemic blood pressure is shown in table 3. Neither VIP nor PACAP affected blood pressure at the doses of 10^{-8} and 10^{-7} mol·kg^{-1}, but they significantly suppressed blood pressure at 10^{-6} mol·kg^{-1} (p<0.05, n=4).
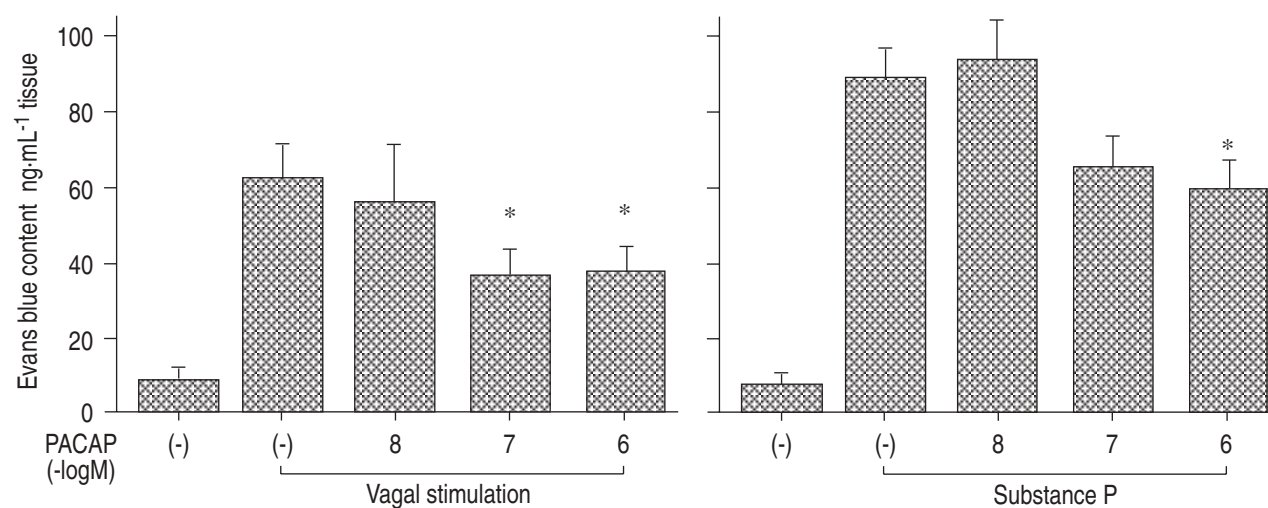

Fig. 5. – Effect of pituitary adenylate cyclase-activating peptide (PACAP) (10^{-8}, 10^{-7} and 10^{-6} mol·kg^{-1}) on the plasma extravasation induced by vagus nerve stimulation (5 V, 5 Hz, 5 ms, 150 s) or by substance P (1 µg·kg^{-1} i.v.) in the main bronchi. PACAP (10^{-7} and 10^{-6} mol·kg^{-1} i.v.) significantly inhibited the increase in the leakage of Evans blue dye induced by the vagus nerve stimulation. PACAP (10^{-6} mol·kg^{-1} i.v.) significantly inhibited the increase in the leakage of dye induced by substance P (mean+SEM) n=5, *: p<0.05).
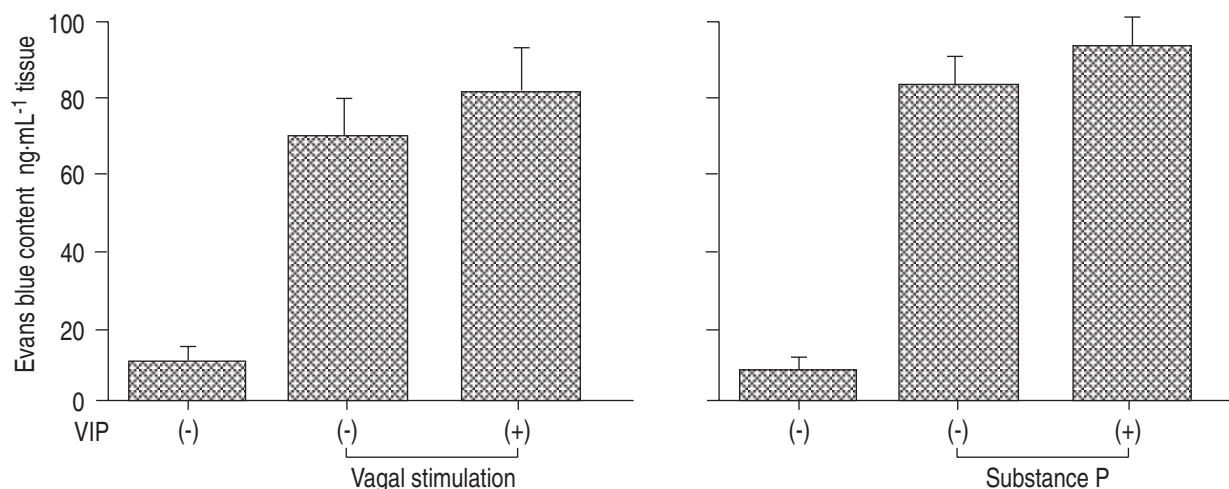

Fig. 6. – Effect of vasoactive intestinal peptide (VIP) (10^{-6} mol·kg^{-1}) on the plasma extravasation induced by vagus nerve stimulation or by substance P (1 µg·kg^{-1}, i.v.) in the main bronchi. VIP (10^{-6} mol·kg^{-1}, i.v.) did not affect the increase in the leakage of dye induced by the vagus nerve stimulation or by substance P in the main bronchi, (mean+SEM, NS).

Table 3. – Effect of vasoactive intestinal peptide (VIP) or pituitary adenylate-activating peptide (PACAP) on mean systemic blood pressure

	Pretreatment	PACAP	Pretreatment	VIP
10^{-8} mol·ng^{-1}	79.1±9.6	79.1±9.6	66.0±12.1	76.0±8.7
10^{-7} mol·ng^{-1}	77.5±5.2	63.0±11.1	83.8±8.1	77.6±16.7
10^{-6} mol·ng^{-1}	79.3±14.1	40.8±74*	106.0±13.2	50.6±7.5*

Values are presented as mean±SEM (mmHg). *: $p<0.05$ versus pretreatment values.

Discussion

This study demonstrated that PACAP-like immunoreactivity is contained in the guinea-pig airway, and exogenously applied PACAP attenuates smooth muscle contractions and plasma extravasation mediated by cholinergic and eNANC nerves.

PACAP belongs to a peptide family that contains VIP, peptide histidine isoleucine (PHI), helodermin and helospectin, and has been shown to localize in the mammalian airway (including the human airway) [3, 17, 18]. The present data show a rich supply of PACAP-immunoreactive nerve fibres among bundles of smooth muscle, beneath the epithelium, and occasional fibres around seromucous glands and blood vessels in the bronchi of guinea-pigs, which confirmed previous observations.

Relaxation induced by PACAP remained after the discontinuation of infusion, and this action was significantly more prolonged than that of VIP. The effect of PACAP in relaxing smooth muscle is 5–10 times longer than that of VIP in human airways [19]. The sustained effect of PACAP in relaxing airway smooth muscle may be explained by a reduced susceptibility to degradation compared to VIP [20].

In trachea, PACAP suppressed smooth muscle contraction evoked by EFS. In our previous studies, the contraction evoked by EFS was completely abolished by atropine or by tetrodotoxin in the trachea [21–23], and, therefore, EFS was considered to induce smooth muscle contraction by stimulation of the cholinergic component of the vagus nerve. ACh is considered to contract smooth muscle directly [24]. As PACAP significantly suppressed the amplitude of contractions evoked by EFS without altering the ACh sensitivity of smooth muscle in the present study, it appeared that this peptide suppressed smooth muscle contraction pre-junctionally, presumably by reducing the release of ACh from vagal nerve termini.

Similarly, in the main bronchi, PACAP is likely to inhibit the smooth muscle contraction induced by the eNANC component of the vagus nerve, pre-junctionally at low concentrations (10^{-9}–10^{-8} M), and pre- and post-junctionally at a higher concentration (10^{-7} M). The contraction induced by EFS in the presence of atropine and guanethidine is considered to be eNANC-mediated. PACAP suppressed the eNANC-mediated contraction without affecting SP sensitivity at 10^{-9} and 10^{-8} M, which suggested that PACAP inhibited SP release from eNANC. By contrast, PACAP inhibited SP sensitivity at 10^{-7} M. Although we did not employ direct measurements of ACh or SP in the present study, the release of ACh or SP was inferred from a comparison of the contractile responses to EFS and to exogenous ACh or SP as for the previous reports [25–28], and it was concluded that PACAP is likely to suppress the release of ACh or SP from nerve termini.

Stimulation of the vagus nerve or intravenously administered SP caused a significant increase in plasma extravasation in the presence of atropine and propranolol. PACAP significantly inhibited the increase in plasma extravasation mediated by the eNANC nerve. PACAP also significantly inhibited the increase in plasma extravasation induced by SP at a high dose.

PACAP may suppress blood pressure and decrease the blood perfusion of airways, which in turn may decrease the plasma extravasation into the airway. In the present study, the low doses of PACAP or VIP (10^{-8}, 10^{-7} mol·kg^{-1}, i.v.) did not affect the blood pressure, suggesting that blood pressure does not affect plasma extravasation. At a high dose (10^{-6} mol·kg^{-1} i.v.), PACAP and VIP significantly decreased blood pressure to a similar extent, whereas PACAP, but not VIP, inhibited the increase in plasma extravasation. These results suggest that PACAP seems to have an effect in preventing plasma extravasation by mechanisms other than the suppression of blood pressure.

Although PACAP and VIP have been reported to have similar biological activities, VIP did not inhibit the increase in plasma extravasation induced by eNANC or SP. It has been shown that VIP potentiates plasma extravasation induced by SP [29], and suggested that vasodilation induced by VIP leads to potentiation of extravasation induced by other stimuli. The reasons for different actions of VIP and PACAP on plasma extravasation are unknown. PACAP may have an additional action on endothelial postcapillary venules in preventing plasma extravasation. Alternatively, it is possible that PACAP may have an inhibitory effect on the release of SP from eNANC nerves as observed in bronchial smooth muscle.

The pathophysiological roles of PACAP in airway diseases are not understood. Disorder of the autonomic nervous system is involved in airway hyperresponsiveness. Such abnormalities include enhanced cholinergic and eNANC mechanisms [26, 30, 31], or reduced β-adrenergic [32] and inhibitory NANC mechanisms [33]. PACAP directly relaxed the airway smooth muscle, and in addition, inhibited the release of ACh or SP from nerve termini in this study. It has been demonstrated that VIP and ACh coexist in the same nerves in the feline trachea [7–9], and VIP released simultaneously may protect against broncho-constriction mediated by the cholinergic nerve via both direct and indirect mechanisms. PACAP may have similar physiological roles in protecting against bronchoconstriction and plasma extravasation mediated by cholinergic and eNANC nerves. The lack of inhibitory mechanisms in the output of ACh or SP from the nerve terminal may be a factor in the enhanced cholinergic and eNANC activity. In addition, recently, PACAP or PACAP analogues have been reported to produce significant bronchodilatation in guinea-pig, which suggests that this peptide may have a therapeutic potential as a bronchodilator [6, 34–36].

In summary, pituitary adenylate cyclase-activating peptide-immunoreactive nerve fibres exist in airways, and it inhibits smooth muscle contraction induced by cholinergic and excitatory nonadrenergic noncholinergic nerves, and plasma extravasation induced by the latter. Thus, pituitary adenylate cyclase-activating peptide may be involved

in regulating airway responsiveness through these actions. Additional investigations are needed to clarify the physiological roles of pituitary adenylate cyclase-activating peptide in the airways, as well as its potential for treating obstructive airway disease.

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