PEPTIDE HISTIDINE VALINE, A NOVEL MEMBER OF THE VIP FAMILY, IS A POTENT RELAXANT OF TRACHEAL SMOOTH MUSCLE

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ABSTRACT

Peptide histidine valine (PHV), a 42-residue peptide belonging to the vasoactive intestinal peptide (VIP) family, is contained within the VIP precursor protein, pre-pro VIP, and its sequence includes that of the peptide histidine methionine (PHM), a partial VIP agonist. PHV, PHM, or both, may be synthesized with VIP in the lung and other tissues. The objectives of this study were: 1) to characterize the airway-relaxant action of PHV, in relation to those of VIP and PHM; 2) to examine whether PHV could prevent or reverse the tracheal contractile effect of the bronchoconstrictor peptides neurokinin A (NKA), neurokinin B (NKB), and endothelin-1 (ET-1); and 3) to compare the binding sites of PHV and VIP in the lung. PHV relaxed isolated perfused guinea-pig tracheal strips dose-dependently at a minimal effective concentration of 1.8×10^{-8} M and an EC_{50} of 4.1×10^{-8} , and was at least as potent as VIP. PHV also totally reversed tracheal smooth muscle contraction caused by NKA (9×10^{-8} – 4.4×10^{-7} M), NKB (8×10^{-8} – 8×10^{-7}), and ET-1 (4×10^{-7}). PHV and VIP displaced ¹²⁵I-VIP binding to rat alveolar macrophages with equal potency, suggesting that PHV binds to VIP receptors on these cells. These results indicate that PHV is a potent and effective relaxant of guinea-pig trachea, binds to VIP receptors in the lung, and may act with VIP and PHM to modulate airway smooth muscle tone.

Peptide histidine valine (PHV), a 42-residue peptide belonging to the vasoactive intestinal peptide (VIP) family of peptides, was recently isolated and partially characterized (21, 22). Synthesized from the VIP precursor protein, pre-pro VIP, along with VIP itself, PHV has a sequence corresponding to pre-pro VIP 81–122 (2). During post-translational

processing, PHV may be formed with VIP in different tissues, including the lung (8, 21). Alternatively, peptide histidine methionine (PHM), the human counterpart of peptide histidine isoleucine (PHI), or both PHM and PHV, may be synthesized together with VIP (Fig. 1) (4, 9). Strong evidence suggests that VIP may be the major peptide transmitter of the nonadrenergic, noncholinergic inhibitory component of autonomic innervation of the lung (15, 16). The pulmonary effects of this peptide include relaxation of airway and vascular smooth muscle, and stimulation of bronchial water, chloride and macromolecular secretion (15). Our objectives in this study were: 1) to characterize the relaxant action of PHV on airway smooth muscle, compared to that of VIP and PHM; 2) to examine the ability of PHV to prevent or reverse the tracheal contractile effects of the bronchoconstrictor

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PROBABLE POSTTRANSLATIONAL PROCESSING OF PRE-PRO VIP

Fig. 1 Schema of alternate post-translational processing of pre-pro VIP to yield PHM plus VIP, or PHV plus VIP

peptides neurokinin A (NKA), neurokinin B (NKB), and endothelin-1 (ET-1); and 3) to investigate whether PHV and VIP share binding sites in the lung. For the latter goal we used rat alveolar macrophages as target cells because: a) rat VIP is identical to human and porcine VIP, b) rat alveolar macrophages can be easily obtained in large numbers, and c) the alveolar macrophage is an important and well-characterized cell of the lung that expresses VIP receptors (10, 17).

METHODS

Smooth Muscle Preparation

Isolated, perfused guinea-pig tracheal strips were prepared as described previously (19). Hartley strain male guinea pigs (300–500 g) were euthanized with intraperitoneal pentobarbital (60 mg/kg). The tracheas were removed, freed of connective tissue and vasculature, and cut spirally into 2 mm × 2 cm strips weighing approximately 50 mg. The strips were suspended in 5-ml organ baths and perfused with Krebs-Ringer solution (in mM; NaCl 117.6, KCl 5.4, MgSO₄ 1.2, NaHCO₃ 25.0, NaH₂PO₄ 1.0, glucose 11.1, CaCl₂ 2.5) that was equilibrated with 95% O₂-5% CO₂, maintained at 37°C, and circulated continuously at 3 ml/min by an infusion pump (Cole-Parmer). The lower end of the tracheal strip was fixed to the floor of the organ bath, and the upper end was tied to an isometric force transducer (Harvard Apparatus, model 2671). Changes in tracheal smooth muscle tension were recorded graphically on a chart recorder (Beckman type R Dynograph). The tissues were allowed to stabilize for 1 h at an initial load of 1 g, which we previously found to provide a suitable tone for measuring changes in

tension with relaxation and contraction. The test peptides were always compared on the same tissue at the same tone to minimize variations in responses due to variations in tissue tensions.

Materials

Porcine VIP (identical to human and rat VIP) was obtained from Professor Viktor Mutt, Karolinska Institute, Stockholm, Sweden; PHV and other peptides from Peninsula Laboratories, Belmont, CA; and all other chemicals from Sigma Chemical Company, St. Louis, MO.

Comparing the Airway Relaxant Action of PHV, VIP and PHM

To test the dose-response relationships of the three peptides, their effects were always compared on the same tissue strips. Cumulative doses of each peptide, at final concentrations in the bath ranging from 1.8×10^{-8} to 2.9×10^{-7} M, were added in aliquots of 50 μ l. After the last (highest) dose of the peptide was given, the tissue tone was allowed to return to baseline tone. A period of approximately 60 min elapsed between the testing of two peptides, and the sequence of peptide administration was randomized: in some experiments, VIP was administered before PHV and PHM, while in other experiments PHM or PHV was given first, followed by the other two peptides. The degree of reduction in baseline tone was reported as a percent of maximal relaxation caused by isoproterenol (3.8×10^{-6} M). In another group of experiments, we compared the duration of equal relaxations induced by PHV (7×10^{-8} M) and VIP (3×10^{-7}

M), by measuring the time needed for the tissue tone to return from full relaxation to baseline. The tracheal response to PHV was also tested in the absence and presence of the β -adrenoceptor blocker propranolol (7.7×10^{-6} M).

Reversal of Tracheal Contraction

To test the ability of PHV to reduce or reverse tracheal smooth muscle contraction, we used three potent endogenous peptides known to contract airway smooth muscle (1, 5, 18): NKA, at concentrations of 9×10^{-8} M and 4.5×10^{-7} M; NKB, at concentrations of 8×10^{-8} and 8×10^{-7} M; and ET-1 at a concentration of 4×10^{-7} M. Tissue perfusion was interrupted after each of these peptides was added to the organ bath. When the contraction had reached a plateau, PHV was added at concentrations ranging from 2×10^{-8} M to 2×10^{-7} M.

Preparation of Rat Alveolar Macrophages

Virus-free Sprague-Dawley rats (350–450 g) were anesthetized with intraperitoneal pentobarbital (50 mg/kg) and exsanguinated. The lungs were isolated and the trachea cannulated. Broncho-alveolar lavage (BAL) was performed with approximately ten 12-ml aliquots of phosphate buffer solution (PBS), for a total return of approximately 100 ml of fluid from each rat. The BAL fluid was kept on ice and centrifuged at $500 \times g$ for 10 min at 4°C . The cell pellet was washed with PBS twice, then resuspended in 2 ml of PBS and counted in a hemocytometer.

VIP and PHV Binding to Alveolar Macrophages

Cells ($3.1\text{--}3.5 \times 10^{-5}$) were incubated with ^{125}I -VIP (50,000 cpm/tube) in Roswell Park Memorial Institute (RPMI) medium 1640 containing 1% bovine serum albumin and the protease inhibitors aprotinin (Trasylol, 125 units/ml), phosphoramidon (10^{-5} M) and phenylmethylsulfonyl fluoride (50 μM), for 1 h at 37°C . At the end of the incubation, native VIP or PHV (10^{-6} M) was added. The tubes were re-incubated for 5, 15, 30, 60 and 90 min at 15° or 37°C , then placed in an ice bath to terminate the reaction. The tubes were then centrifuged at $500 \times g$ for 2 min at 4°C ; and the pellet was counted in a gamma-counter (Beckman Gamma 5500). The percent of ^{125}I -VIP bound to alveolar macrophages after the addition of native peptides

was calculated for each time interval.

Analysis of Data

All data were expressed as means $\pm$ SEM, and were compared using Student's paired t test in the dose-response experiments and unpaired t test in the receptor-binding experiments. The concentration of peptide that caused 50% of maximal relaxation (EC_{50}) was measured from the response curves for each tissue, and the mean for the whole group was then calculated. Differences at $P < 0.05$ were considered significant.

RESULTS

Dose-Dependent Tracheal Relaxation by PHV, Compared to VIP and PHM

PHV relaxed guinea-pig tracheal strips in a dose-dependent manner, at a minimal effective concentration of 1.8×10^{-8} M. The tracheal-relaxant activity of PHV was unaffected by β -adrenoceptor blockade with propranolol (7.7×10^{-6} M). As a tracheal relaxant, PHV was slightly more potent than VIP ($P < 0.01$) and PHM ($P < 0.01$), with an EC_{50} of $4.1 \times 10^{-8} \pm 0.5$ M, compared to $6.6 \times 10^{-8} \pm 0.5$ M for VIP and $8.6 \times 10^{-8} \pm 1.0$ M for PHM (Fig. 2). The efficacy of all three peptides was nearly identical (Fig. 2).

The equipotent concentrations of PHV (7×10^{-8} M) and VIP (3×10^{-7} M) produced relaxations of similar duration ($n = 6$).

Reversal of Airway Smooth Muscle Contraction by PHV

PHV, in concentrations from 2×10^{-8} M to 2×10^{-7} M, dose-dependently reversed airway smooth muscle contraction caused by NKA and NKB. At a concentration of 7.2×10^{-8} M, PHV promptly and completely reversed the contraction of guinea-pig tracheal strips caused by NKA (4.4×10^{-7} M) and NKB (8×10^{-7} M) (Fig. 3). PHV (10^{-7} M) also reversed the tracheal strip contraction caused by ET-1 (4×10^{-7} M) (Fig. 3). Against the latter peptide, higher concentrations of PHV (2×10^{-7} M) not only reversed the increased tracheal tone, but even reduced it below baseline levels.

Binding Sites for PHV and VIP on Alveolar Macrophages

PHV and VIP were equally effective in displacing

Fig. 2 Concentration-relaxation responses of guinea-pig tracheal strips to PHV, VIP and PHM. Relaxations are expressed % of maximal relaxation induced by isoproterenol (3.8×10^{-6} M). Means $\pm$ SEM, $n = 6$

Fig. 3 Tracings of guinea-pig tracheal smooth muscle tension, illustrating prompt and complete reversal by PHV (7.2×10^{-8} M) of contractions caused by NKA (4.4×10^{-8} M) and NKB (8.8×10^{-8} M) and reversal by PHV (2×10^{-7} M) of contraction caused by ET1 (4×10^{-7} M, $n = 4$)

Fig. 4 Dissociation of ^{125}I -VIP bound to rat alveolar macrophages over time, on addition of PHV (5×10^{-6} M) or VIP (5×10^{-6} M), at 15°C and 37°C . Binding is % of total bound ^{125}I -VIP at 0 time. Means $\pm$ SEM ($n = 3$)

min, and $20.1 \pm 5.2\%$ at 90 min. The displacement of bound ^{125}I -VIP by both PHV and VIP was similar and temperature-dependent; displacement was markedly less at 15°C than at 37°C (Fig. 4).

bound ^{125}I -VIP from rat alveolar macrophages at 37°C (Fig. 4). Five min after adding cold VIP, $64.6 \pm 2.9\%$ of the ^{125}I -VIP was bound, and at 90 min the binding was only $18.4 \pm 0.3\%$. In the presence of PHV, $63.9 \pm 7.3\%$ of ^{125}I -VIP was bound at 5

DISCUSSION

Little is known about the actions and the physiological significance of PHV, but its derivation from the same precursor as VIP and PHM (or PHI), and

especially the structural similarity of these three peptides, suggest that they may have similar biological activity. Our results show that PHV is at least as potent as VIP and twice as potent as PHI in relaxing guinea-pig tracheal strips. This smooth-muscle relaxing ability was independent of β -adrenergic receptors. Our data also show that PHV, like VIP (1, 5, 15), can reverse tracheal smooth muscle contraction caused by the bronchoconstrictor peptides NKA, NKB and endothelin. These findings need to be verified *in vivo* in intact animals and human subjects. Our results agree with those of Yiangou *et al.*, who reported that PHV is more potent than VIP and PHM in relaxing isolated guinea-pig trachea (21). However, they did not test the ability of PHV to reverse pharmacologically evoked contractions of the airways, and did not examine whether PHV and VIP shared similar binding sites. Another report found that PHV, injected intravenously in normal subjects before a histamine challenge, did not prevent the histamine-induced reduction in expiratory flow rates (3). Our binding studies in alveolar macrophages suggest that PHV probably binds to VIP receptors in the lung. If the slightly higher potency of PHV relative to VIP as tracheal relaxant is confirmed, this would raise the possibility that PHV-preferring may be present on airway smooth muscle. We had expected PHV to have a more prolonged duration of action than VIP in relaxing airway smooth muscle because of its extended sequence at the C-terminus, making it similar in length to helodermin and pituitary adenylate cyclase-activating peptide (PACAP₃₈), both of which produce considerably more sustained airway relaxation compared to VIP (6, 7). The nearly equal duration of action of PHV and VIP may mean that PHV and VIP are approximately equally susceptible to degradation by airway peptidases. Helodermin and PACAP₃₈ appear to be more resistant to degradation by these peptidases (11).

The similarity between the relaxant actions of VIP and PHV on the airways, along with their generation from the same precursor, suggest that PHV may play a complementary role to VIP. It is possible that PHV is released, together with VIP and PHM (or PHI), during electrical field stimulation of airways (12, 20), and that PHV participates in mediating nonadrenergic, noncholinergic relaxation (14). By the same token, our results suggest a possible role for PHV in the pathogenesis of bronchial asthma. Asthmatic airways have been reported to be deficient in immunoreactive VIP nerve fibers

(13), lending support to the view that VIP deficiency may be a factor in the increased airway hyperactivity in asthma (3). Because of the common derivation of PHV and VIP, it is reasonable to speculate that a deficiency of VIP based on impairment of its synthesis may also be associated with deficiency of PHV (as well as of PHM), and that these combined deficiencies may contribute to the increased airway responsiveness characteristic of bronchial asthma. In conclusion, PHV, a neuropeptide belonging to the VIP family, is a potent endogenous relaxant of airway smooth muscle. PHV may play a physiologic role in modulating human airway tone, and may prove useful as a therapeutic agent in the relief of bronchoconstriction.

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