Autoradiographic localisation of VIP receptors in human lung

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Localisation and pharmacological properties of the VIP receptor in human lung sections are described. The receptor density determined by saturation analysis using ^{125}I-VIP is approx. 100 fmol/mg protein, with a K_d of approx. 600 pM. Inhibition of ^{125}I-VIP binding with VIP and related peptides gives a rank order of potency: VIP > peptide histidine methionine > secretin. Light microscope autoradiography reveals specific VIP binding sites, with a high density over the pulmonary artery smooth muscle and the alveolar walls and with a lower density over the bronchial epithelium.

Hormone receptor *(Human lung)* *Autoradiography* *Vasoactive intestinal peptide*

1. INTRODUCTION

Vasoactive intestinal peptide (VIP) is a potent bronchodilator in man [1] and other species [2,3], and also relaxes mammalian pulmonary vascular smooth muscle [4]. VIP-immunoreactive nerves have been demonstrated in association with mammalian airways and pulmonary vessels [5,6], suggesting a possible physiological role for VIP in these locations. A high-affinity VIP receptor coupled to adenylate cyclase has been described in lung membrane preparations of the rat, mouse and guinea pig [7,8]. Recently, a method for preparing human lung membranes free of carbon contamination has been described, and using this technique a high-affinity VIP binding site in the human lung has been characterised [9]. The localisation of VIP receptors by in vitro autoradiography has been reported in the rat lung, showing a high density of binding over the alveoli and the epithelium and muscular tunica of bronchi [10]. We now report the autoradiographic localisation of specific ^{125}I-VIP binding sites in human lung sections, and the pharmacological characteristics of these binding sites.

2. MATERIALS AND METHODS

2.1. *Tissue preparation*

Human lung specimens were obtained either from surgical resection or post-mortem (within 12 h), insufflated with tissue-embedding medium (Tissue-Tek OCT compound) diluted 1:4 with phosphate-buffered saline, and rapidly frozen in isopentane cooled in liquid nitrogen. Lung sections were cut in a cryostat (Slee, London) set at −20°C, and thaw-mounted onto microscope slides which had previously been coated with gelatine and immersed in 0.3% polyethyleneimine overnight. This was to reduce the high degree of non-specific VIP binding to the gelatine. After mounting, tissue sections were stored at −20°C until required.

2.2. *Biochemical studies*

Saturation data was obtained by incubating 20 μm sections for 2 h at room temperature with 100–1500 pM ^{125}I-VIP in 25 mM Tris-HCl, 5 mM $MgCl_2$, 0.5% bovine serum albumin and 0.1% bacitracin (pH 7.4). Non-specific binding was defined by the presence of 1 μM unlabelled VIP. After incubation, the slides were washed for

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2 × 15 min in cold buffer (25 mM Tris-HCl, 5 mM $MgCl_2$, pH 7.4) and wiped onto glass fibre filters (Whatman, GF-C) to be counted by conventional γ-counting techniques. Preliminary studies established the optimal incubation and washing times. K_d and B_{max} values were calculated by non-linear regression analysis. Representative sections were sonicated and protein content was determined according to Lowry et al. [11], with bovine serum albumin as the protein standard.

The inhibition of ^{125}I-VIP binding by VIP, peptide histidine methionine (PHM) and secretin was studied by incubating sections with 250 pM ^{125}I-VIP and varying amounts of the competing peptide. K_i values were calculated by the Cheng and Prusoff equation [12].

2.3. *Autoradiography*

12 μm sections were incubated with 400 pM ^{125}I-VIP and washed as described above. Non-specific binding was defined by the presence of 3 μM PHM. After washing, the slides were rapidly dried in a stream of cold air and stored with desiccant under vacuum overnight. Autoradiography was performed by a modification of the method described by Young and Kuhar [13]. Emulsion (Ilford K.2) coated coverslips were attached to the slides and left in light-tight boxes for an exposure period of 3 days. The coverslips were then partially separated from the sections so that the emulsion could be developed (Ilford Phenisol) and the sections fixed with Carnoy's solution and lightly stained with 1% pyronine Y. The coverslips were mounted onto the slides in their former positions and viewed with both light- and dark-field microscopy.

2.4. *Drugs and chemicals*

^{125}I-VIP was obtained from Amersham (England); unlabelled VIP, secretin, bovine serum

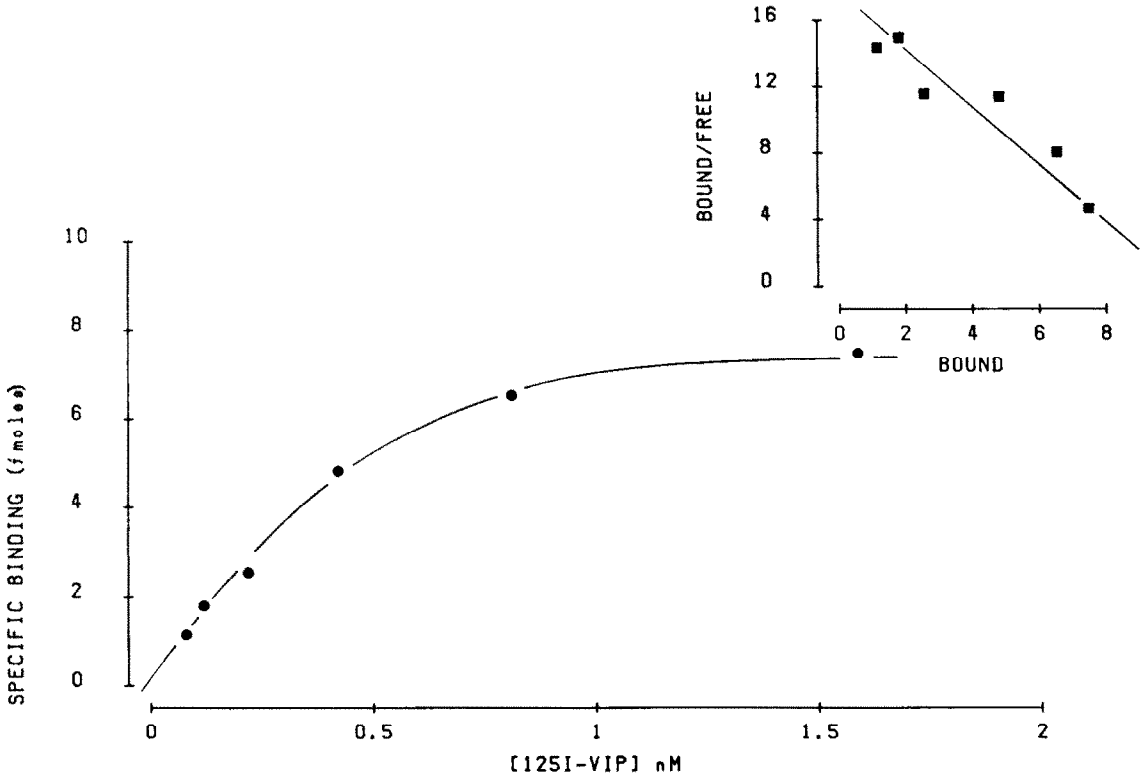

Fig.1. Typical saturation binding curve of specific ^{125}I-VIP binding to human lung sections ($n = 3$, lungs from different individuals). Specific binding is not corrected for protein content of the sections. B_{max} range 93–111 fmol/mg protein; K_d range 480–730 pM. (Inset) Scatchard plot: Abscissa, specific ^{125}I-VIP binding (fmol); ordinate, the ratio of specific ^{125}I-VIP binding (fmol) to the free ligand concentration (nM).

albumin, bacitracin, polyethyleneimine from Sigma (Poole, England); PHM from Peninsula Laboratories (St. Helens, England); Tissue-Tek OCT compound from Raymond A. Lamb, London.

3. RESULTS

3.1. *Biochemical studies*

At room temperature the time course ^{125}I-VIP binding to human lung sections was slow, reaching equilibrium at 2 h (not shown). Specific binding was saturable and of high affinity with a K_d of 480–730 pM ($n=3$) and a receptor density of 93–111 fmol/mg protein ($n=3$) (fig.1). Over the concentration range studied, Scatchard analysis [14] described a single population of binding sites; however, at higher concentrations the Scatchard plot became curvilinear indicating the presence of a second population of binding sites of low affinity (not shown). At low concentrations of ^{125}I-VIP the non-specific binding as defined by 1 μM unlabelled VIP was 15–20% of the total binding.

Displacement curves for VIP and related peptides are shown in fig.2. The order of potency of these peptides was VIP (K_i 0.88–1.86 nM, $n=3$)> PHM (K_i 19.3–75.7 nM, $n=3$)>secretin (0.62–5.54 μM, $n=3$). The Hill slope factors in all cases were less than 1 (nH VIP and PHM = 0.75, nH secretin = 0.55), indicating that these peptides are agonists at the VIP receptor.

3.2. *Autoradiography*

Autoradiography revealed specific ^{125}I-VIP binding sites throughout human lung. After incubation with ^{125}I-VIP alone, binding was concentrated over tissue with no elevation of grain density above background in the interstitial spaces. It was thought preferable to use a ligand other than VIP to demonstrate non-specific binding and displacement studies showed that 3 μM PHM inhibited VIP binding to the same extent as 1 μM VIP (fig.2). Adjacent sections incubated with ^{125}I-VIP in the presence of 3 μM PHM showed relatively few autoradiographic grains and in general grain density did not conform to the pattern of the

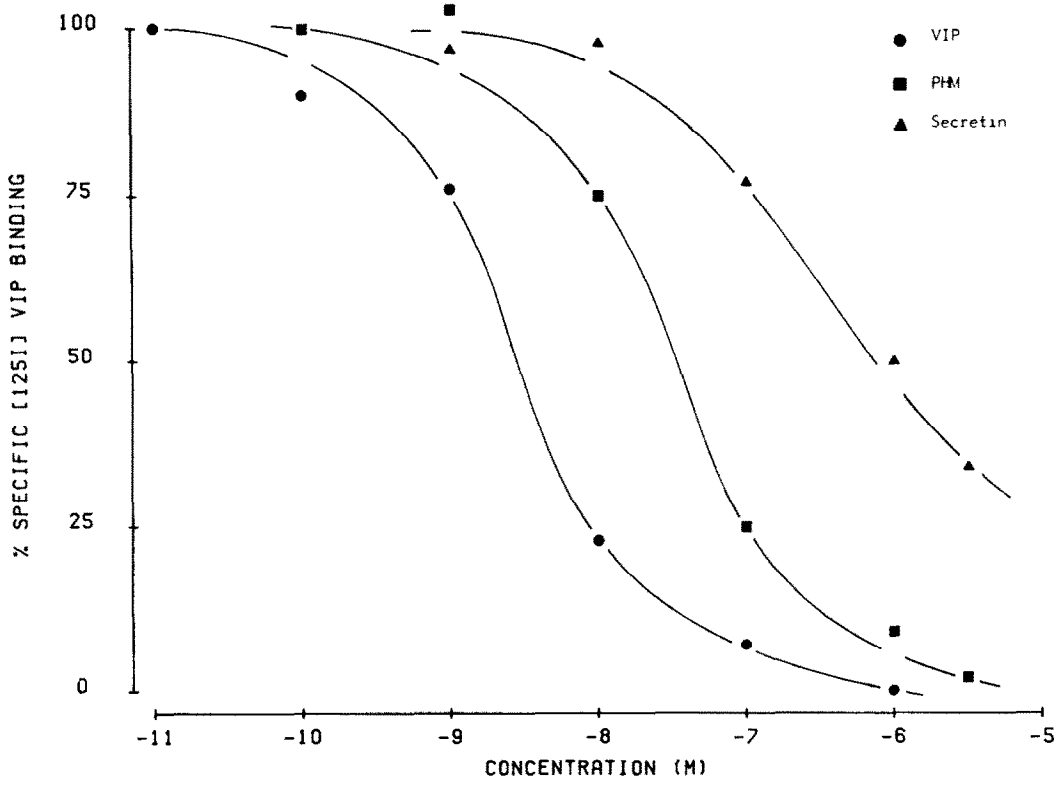

Fig.2. Inhibition of ^{125}I-VIP binding by unlabelled VIP, PHM and secretin. Typical curves from $n=3$. K_i ranges: VIP, 0.88–1.86 nM; PHM, 19.3–75.7 nM; secretin, 0.62–5.54 μM.

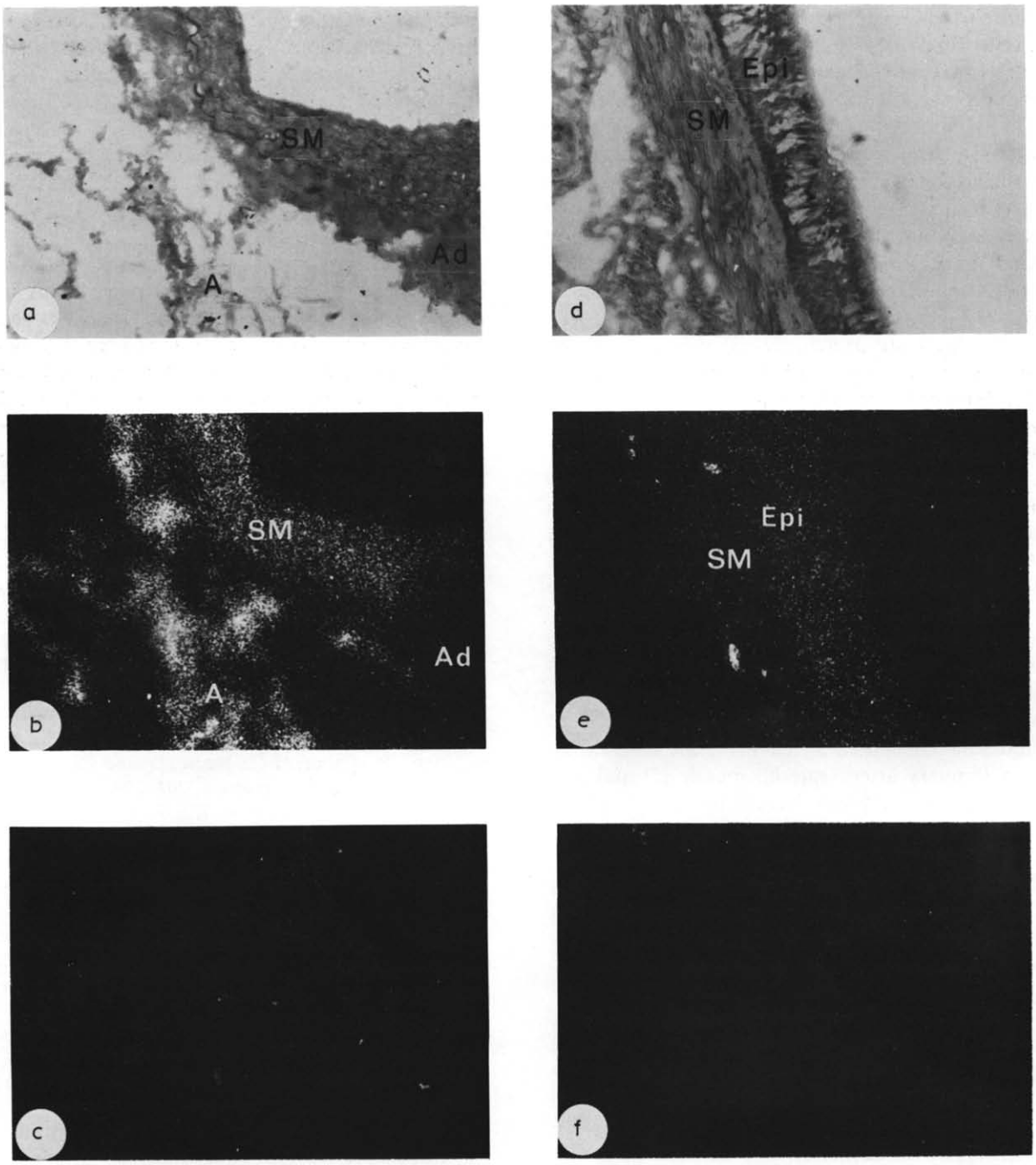

Fig.3. Distribution of ^{125}I-VIP binding in human lung. (a) Light-field photomicrograph of a section of human lung showing pulmonary artery wall (internal diameter 2 mm) and alveoli. (b) Dark-field autoradiograph of the same area incubated with 400 pM ^{125}I-VIP showing dense labelling of arterial smooth muscle and alveoli. (c) Adjacent section incubated with ^{125}I-VIP in the presence of 3 μM PHM, showing no specific labelling. (d) Photomicrograph of a section of bronchial wall (internal diameter 5 mm). (e) Dark-field autoradiograph of the same area showing ^{125}I-VIP binding over the epithelium. (f) Adjacent section incubated with ^{125}I-VIP and 3 μM PHM. SM, smooth muscle; Ad, adventitia; A, alveoli; Epi, epithelium.

underlying tissue. Exceptions to this were non-displaceable ^{125}I-VIP binding over bronchial cartilage, mucous glands and carbon particles.

Specific grain density was highest over alveoli and pulmonary artery smooth muscle, irrespective of artery size, with fewer grains present over the bronchi (fig.3). Within the bronchi, grains were localised to the epithelium with no specific binding to bronchial smooth muscle. This was observed in airways of all sizes, including extrapulmonary bronchus.

4. DISCUSSION

Specific ^{125}I-VIP binding to mounted sections of human lung within the concentration range studied demonstrated a single saturable binding site of high affinity. This was similar to that previously described in human lung membranes [9]. Competition of ^{125}I-VIP binding with unlabelled peptides produced the same rank order of potency that we and others have previously described for the human VIP receptor [9,15,16].

The high density of VIP receptors in pulmonary artery smooth muscle demonstrated by autoradiography is in keeping with the potent relaxant effect of this peptide [4]. Immunocytochemical techniques have localised a rich VIP-ergic innervation to pulmonary artery smooth muscle [5] and it is possible that VIP may have a major role in the regulation of pulmonary blood flow.

The presence of VIP receptors over the epithelium of bronchi would be predicted from the known action of VIP in promoting active transport of ions across airway epithelium [17] and VIP may have a role in regulating the composition of bronchial mucous.

Perhaps the most surprising finding of this study is the lack of specific binding over bronchial smooth muscle. Bronchodilation produced by VIP has been demonstrated both in vivo and in vitro in man and other species [1–3,18]. However, whilst exogenously administered VIP is very potent in these studies, the magnitude of relaxation observed is small [1], and it is therefore possible that receptor density is too low for autoradiographic visualization.

The demonstration of high grain density over the alveolar walls was surprising in view of the sparse VIP-ergic innervation of peripheral lung tissue [6]. It is possible that these binding sites may represent a receptor for circulating VIP [10] or may be important in its metabolism [19].

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