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Effect of passive sensitization on the mechanical activity of human isolated bronchial smooth muscle induced by substance P, neurokinin A and VIP

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1 The effect of passive sensitization on the mechanical activity of human isolated bronchial smooth muscle induced by the following neuropeptides substance P (SP), neurokinin A (NKA) and vasoactive intestinal peptide (VIP) was studied both in the absence and in the presence of the neutral endopeptidase (NEP) inhibitor, phosphoramidon.

2 Cumulative concentration-response curves (CCRC) to these neuropeptides were constructed in human passively sensitized isolated bronchial rings and compared to those in paired controls. Passively sensitized human isolated bronchial rings were tissues incubated overnight in serum from asthmatic patients atopic to *Dermatophagoides pteronyssinus* and paired controls were tissues originating from the same lung specimens but incubated overnight in serum from healthy donors.

3 In the absence of phosphoramidon, passive sensitization significantly increased the amplitude of the contractile responses to SP and NKA including that to the maximal concentration given from 50 ± 5% to 76 ± 6% ($n = 5$, $P < 0.05$) and from 70 ± 7% to 101 ± 6% ($n = 5$, $P < 0.05$) of the maximal response to acetylcholine, respectively. Passive sensitization significantly shifted to the left the CCRC for both tachykinins as measured by the geometric means dose-ratios which were 8.5 (95% confidence limits (CL): 3.1–13.9) and 7.3 (95% CL: 4.2–10.3) for SP and NKA, respectively.

4 In the presence of phosphoramidon (10 μM), passive sensitization still increased significantly the amplitude of the contractile responses to SP and NKA including that to the maximal concentration given from 74 ± 4% to 115 ± 7% ($n = 5$, $P < 0.05$) and from 104 ± 9% to 146 ± 16% ($n = 5$, $P < 0.05$) of the maximal response to acetylcholine, respectively. Passive sensitization still significantly shifted to the left the CCRC for both tachykinins as measured by the dose-ratios which were 9.0 (95% CL: 4.3–13.6) and 5.4 (95% CL: 2.9–7.9) for SP and NKA, respectively.

5 The relaxant response to the maximal concentration of VIP given in tissues precontracted with histamine (0.5 mM) was significantly reduced by passive sensitization from 41 ± 4% to 25 ± 3% ($n = 5$, $P < 0.05$) of the amplitude of the precontraction in the absence of phosphoramidon and from 72 ± 1% to 49 ± 4% ($n = 5$, $P < 0.05$) in the presence of phosphoramidon (10 μM). Passive sensitization significantly shifted to the right the CCRC for VIP as measured by the dose-ratios which were 10.4 (95% CL: 6.6–14.1) and 6.4 (95% CL: 3.0–9.8) in the absence and in the presence of phosphoramidon, respectively.

6 We conclude that passive sensitization enhances the mechanical response to neuropeptides which contract human isolated bronchial smooth muscle and reduces that to a neuropeptide which relaxes it. The mechanism of passive sensitization-induced changes in the mechanical activity appears to be independent of a decrease in NEP activity since these changes persist in the presence of the NEP inhibitor, phosphoramidon.

Keywords: Substance P; neurokinin A; vasoactive intestinal peptide; phosphoramidon; human isolated bronchial smooth muscle; passive sensitization; asthma

Introduction

There is now accumulating evidence that many different neuropeptides such as substance P (SP), neurokinin A (NKA) and vasoactive intestinal peptide (VIP) may play an important role in the control of airway calibre. These peptides have been localized in airway tissues of several species, including man (Richardson & Beland, 1976; Barnes, 1987a). Tachykinins (SP and NKA) have potent contractile effects in airways both *in vivo* and *in vitro* (Advenier *et al.*, 1987; Ewans *et al.*, 1988; Shore *et al.*, 1988; Naline *et al.*, 1989) and several recent investigations have shown that, in human airways, NKA is considerably more potent than SP (Palmer & Barnes, 1987; Black *et al.*, 1988). VIP, a 28 amino acid peptide that was found in cholinergic efferent fibres, causes relaxation of bronchial smooth muscle (Diamond *et al.*, 1983; Barnes & Dixon, 1984; Palmer *et al.*, 1986).

Like neurotransmitters, neuropeptides are physiologically degraded by enzymes and mostly by neutral endopeptidase (NEP, also called 'enkephalinase') which is heavily concentrated within airway epithelium and smooth muscle (Johnson *et al.*, 1985). Removal of epithelium from tracheal or bronchial strips potentiates the contractile response to SP and NKA by inhibiting tachykinin cleavage and inactivation (Devillier *et al.*, 1988; Frossard *et al.*, 1989). In addition, pretreatment of airway tissue with phosphoramidon, a potent inhibitor of NEP, significantly enhances tachykinin-induced contraction of the smooth muscle (Black *et al.*, 1988; Lötvall *et al.*, 1991).

Although the alteration in the epithelial layer often seen *in vivo* in chronic severe asthma could likewise enhance tachykinin reactivity, to date, results about the potential contribution of neuropeptides to human airway obstruction are controversial. Indeed, Joos and coworkers (1986) demonstrated that inhalation of NKA induced bronchoconstriction in asthmatic subjects. A recent study of Cheung *et al.* (1991),

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showing that inhalation of thiorphan (an inhibitor of NEP) potentiated tachykinin-induced bronchoconstriction in non-asthmatic subjects, supported the hypothesis that a decrease in NEP activity could contribute to bronchial hyperresponsiveness to NKA. Moreover, pharmacological evidence suggests that tachykinins could participate in the pathogenesis of airflow obstruction induced by isocapnic dry gas hyperpnoea in human subjects with asthma. Indeed, sodium cromoglycate which inhibits C-fibres discharge (Dixon *et al.*, 1980) and thus the release of tachykinins or the tachykinin receptor antagonist, SCH 37224 (Solway & Leff, 1991) blunt hyperpnoea-induced broncho-obstruction in asthma. Similarly, it has been suggested that VIP and related peptides released from airway cholinergic nerves may also play a role in asthma (Barnes, 1989). Joos and coworkers (1989), however, found a relatively small degree of tachykinin aerosol-induced bronchoconstriction in asthmatic patients. Moreover, no evidence for an impaired nonadrenergic noncholinergic (NANC) bronchodilator reflex has been observed in patients with mild asthma (Michoud *et al.*, 1988; Lammers *et al.*, 1989).

Passive sensitization produces hyperresponsiveness of human isolated airways (Black *et al.*, 1989; Marthan *et al.*, 1992) and provides the opportunity to study the interaction between allergic factors and smooth muscle behaviour. As far as we know, no studies have attempted to determine whether responses to neuropeptides are altered in human passively sensitized airway tissue. Therefore, we have examined the effect of passive sensitization on cumulative concentration-response curves to SP, NKA and VIP in the human isolated bronchus and, by the use of phosphoramidon, investigated the role of NEP activity in this effect.

Methods

Tissue preparation

Human lung was obtained at thoracotomy as previously described (Savineau *et al.*, 1991) from 34 patients. These patients (22 males and 12 females aged from 37 to 68 years) were current or ex-smokers and underwent resection for pulmonary carcinoma. As in previous studies (Marthan *et al.*, 1992; Villanove *et al.*, 1992), specimens were selected from patients whose lung function was within the normal range i.e. whose forced expiratory volume in one second (FEV_1) measured, before surgery, in our Lung Function Testing Laboratory in the Hospital was above 80% of predicted. Moreover, analysis of the medical record of the patients revealed that none of them had a history of atopy. After resection, the specimens were immediately transferred to the laboratory in an ice-cold oxygenated Krebs-Henseleit solution (composition in mM: NaCl 118.4, KCl 4.7, $CaCl_2.2H_2O$ 2.5, $MgSO_4.7H_2O$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25.0, D-glucose 11.1). From a macroscopically tumour-free part of each of the specimens, segments of human bronchi (3rd and 4th generation) 30–40 mm in length and 3–5 mm in internal diameter were carefully dissected from surrounding parenchyma without damaging the airway membrane. After removal of adhering fat and connective tissue, bronchi were cut into rings measuring 4–5 mm in length.

Passive sensitization

Passive sensitization was carried out as previously described (Black *et al.*, 1989; Marthan *et al.*, 1992) once it was checked that each of the lung specimens was not spontaneously sensitized. This was tested by the fact that administration of 0.05 ml i.e. 300 units of two different allergens (house dust mite and grass pollen) to a single bronchial ring did not elicit a contractile response (see Mechanical recording). The remaining rings were then divided into two paired groups. One group was passively sensitized by incubation overnight at room temperature in a non-diluted serum pool from atopic asthmatic subjects whose concentration of both total IgE and specific IgE to *Dermatophagoides pteronyssinus* (*D. pter.*) was above 1000 International Units (iu) ml^{-1} and 17.5 PRU ml^{-1} (i.e. 4 + RAST titre), respectively. The other group of bronchial rings was similarly incubated overnight in a non-diluted serum pool from non-asthmatic non-atopic subjects whose total IgE concentration was below 10 iu ml^{-1}.

Mechanical recording

In the morning of the next day, the paired rings of bronchus were mounted in vertical organ baths of an automatic system (IOS_1 isolated organ system, Celaster, Lusignan, France) (Mahe *et al.*, 1989) filled with 20 ml of Krebs-Henseleit solution, bubbled with 95% O_2 in CO_2 at pH 7.4, and maintained at 37°C. Each ring was suspended by means of two stainless clips passed through the lumen. One clip was anchored to the bottom of the organ chamber and the other was connected to an isometric force transducer (Celaster). The resting tension was ajusted to a 1.5 g load. This load was selected on the basis of a separate series of experiments conducted on 4 different lung specimens in which we constructed length-tension curves using repeated administration of single doses of acetylcholine (ACh 1 mM). It was found that optimal length was achieved for these rings when they were stretched to equilibrate against a load of 1.5 g whether the contraction was expressed as raw value of force or force normalized to dry tissue weight (De Jongste *et al.*, 1985) (Figure 1).

Protocol

When stable tone had been established, for experiments using tachykinins (SP and NKA), a supramaximal dose of ACh (1 mM) was first administered to the bath to all of the rings and the contractile response (reference response) allowed to

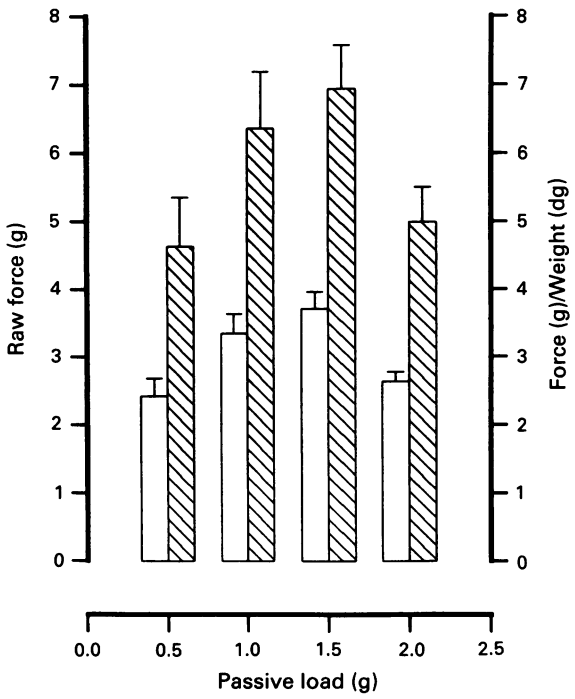

Figure 1 Optimal passive resting load in human isolated bronchial smooth muscle rings. Raw values of force (left axis, open column) as well as values of force normalized to dry tissue weight in response to iterative stimulation with a supramaximal concentration of acetylcholine (ACh, 1 mM bolus) are plotted versus passive preload values achieved by stretching the ring to increasing initial lengths. Data are means ± s.e.mean for 16 rings originating from 4 different lung specimens.

plateau (< 5 min) before the ACh was removed from the baths by repeated washing of the tissues. When stable resting tone returned to baseline, a cumulative concentration-response curve (CCRC) was obtained to the tachykinins in each ring either sensitized or non-sensitized. For experiments using VIP, tissues were precontracted with histamine (0.5 mM) and the CCRC for VIP was obtained at the plateau of the histamine-induced contraction. Only one peptide was tested in one patient and thus, this series of experiments was conducted on 10 sets of paired rings for each neuropeptide (i.e. 5 sets in the absence of phosphoramidon and 5 sets in the presence of phosphoramidon).

At the end of each experiment, when baseline tone had re-established on repeated washout of the tissue, 0.05 ml (300 u) of *D. pter.* was administered to all of the bronchial rings to verify that it induced a contractile response only in those that were passively sensitized. The mean (± s.d.) amplitude of antigen-induced contraction was 95 ± 11% of the maximal ACh-induced contraction in the passively sensitized tissues.

Data analysis

Data were acquired on-line with MOISE software and processed with the ANAMOISE programme (Mahe *et al.*, 1989). The contractile response in each ring to SP and to NKA was expressed as a percentage of the maximal contractile response to ACh in that ring and relaxation response to VIP was expressed as a percentage of the histamine-induced precontraction. Both ACh-induced contraction and histamine precontraction were always elicited in the reference solution i.e. in the absence of phosphoramidon. CCRC relating the response to the logarithm of the concentration of the neuropeptide were constructed for each tissue and for each peptide. From each of these curves, the concentration-ratio, so-called dose-ratio, was calculated from the concentrations of each neuropeptide that produced an arbitrarily determined response for each pair of tissues, i.e. sensitized and non-sensitized, which was on the linear parallel portion of the log concentration-response curves (Black *et al.*, 1981). Such a condition for paired CCRC in a single experiment was always achieved for a response in the range of 30 to 40% of the maximal response to ACh for SP and NKA in the absence and in the presence of phosphoramidon, respectively. Similarly, dose-ratios were calculated for a response in the range of 10 and 30% of histamine-induced precontraction for VIP in the absence and in the presence of phosphoramidon, respectively. Sensitization-induced changes in the potency of neuropeptides were assesed in this way because the true maximal response is difficult to obtain and requires very large concentrations of peptides. Since in each experiment, duplicate bronchial rings were studied for each peptide in both the passively sensitized and the non-sensitized tissues, a mean CCRC was calculated for each type of tissue (i.e. sensitized and non-sensitized) to be representative of the lung specimen. Overall mean CCRC were constructed for all experiments. Mechanical responses are given as means ± s.e.mean and dose-ratios as geometric means with 95% confidence limits (CL). Statistical analysis was carried out only between pairs of tissues dissected from the same lung specimens using both analysis of variance (ANOVA) and paired two-tailed Student's *t* test to compare results for sensitized and control (non-sensitized) tissues, either in the absence or in the presence of phosphoramidon. Dose-ratios were compared to unity which is the figure found when the potency of an agonist is the same whatever the two experimental conditions. Differences were considered as having statistical significance when a P value of less than 0.05 was found.

Drugs

The following drugs were used: substance P, neurokinin A, vasoactive intestinal peptide, acetylcholine, histamine acid phosphate, phosphoramidon (N-(α-L-rhamnopyranosyloxy-hydroxyphosphinyl)-L-leucyl-L-tryptophan) (from Sigma, St Louis, MO, U.S.A.) and house dust mite (*Dermatophagoides pteronyssinus*), grass pollen (*Phleum pratense*) 1:100 wt/vol, 10000 protein nitrogen units per milliliter (from Institut Pasteur, Paris, France). All drugs were dissolved in distilled water to make 1 ml aliquots of stock solutions which were stored frozen and further diluted in the Krebs-Henseleit solution on the day of experimentation. Since neuropeptides were to be used in these experiments, the automatic system IOS_1 was equipped with plastic organ baths.

Results

Effect of passive sensitization on responses to substance P

In the absence of phosphoramidon, contractile responses induced by SP were recorded over the concentration range 1 nM to 3 μM. In a set of experiments conducted on 5 different lung specimens, passive sensitization produced significant increases in the contractile response over the complete concentration-range tested (Figure 2a). Passive sensitization increased the response to the maximal concentration of SP given and shifted to the left the CCRC as measured by the mean dose-ratio (Table 1). The mean increase in the maximal isometric force ΔF_{max} i.e. the difference in the maximal response in sensitized and non-sensitized tissues expressed as a percentage of the maximal response in non-sensitized tissues was 53.2 ± 7.9%.

In the presence of phosphoramidon added to the organ

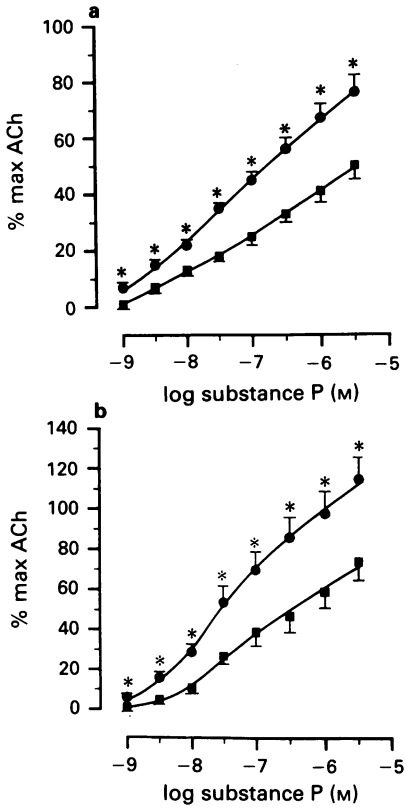

Figure 2 Mean cumulative concentration-response curves (CCRC) for substance P in paired non-sensitized (■) and passively sensitized (●) human isolated bronchial smooth muscle rings. (a) In the absence of phosphoramidon. Each symbol represents the mean value ± s.e.mean ($n = 5$ different lung specimens) of contractile force expressed as a percentage of the maximal contraction to acetylcholine (ACh). $^*P < 0.05$. (b) In the presence of phosphoramidon (10 μM) added to the bath 20 min prior to the CCRC. Legend as in (a).

Table 1 Effect of passive sensitization on responses to substance P (SP), neurokinin A (NKA) and vasoactive intestinal peptide (VIP) in human isolated bronchial smooth muscle rings in the absence of phosphoramidon

		Non-sensitized		*Passively-sensitized*
SP	F_{max} (%)	50 (5)*		76 (6)*
	Dose-ratio		8.5 (3.1–13.9)°	
NKA	F_{max} (%)	70 (7)*		101 (6)*
	Dose-ratio		7.3 (4.2–10.3)°	
VIP	R_{max} (%)	41 (4)*		25 (4)*
	Dose-ratio		10.4 (6.6–14.1)°	

F_{max}: maximal contractile response to the largest concentration of SP or NKA given expressed as a percentage of the maximal contraction to ACh; R_{max}: maximal relaxant response to the largest concentration of VIP given expressed as a percentage of the precontraction to histamine; figures in parentheses indicate s.e.mean for F_{max} or R_{max} and 95% confidence limits for dose-ratios.
Values of maximal response significantly different (*$P<0.05$) from each other; dose-ratio significantly (°$P<0.05$) different from 1.

bath 20 min before the CCRC at a concentration of 10 μM which inhibits NEP activity (Black *et al.*, 1988), contractile responses to SP were greater than those in the absence of phosphoramidon whatever the allergic status of the lung i.e. passively or non-sensitized. However, a direct statistical comparison was not made since experiments were conducted on a different set of 5 lung specimens in each case (see Data analysis). As was the case in the absence of phosphoramidon, passive sensitization significantly increased the contractile response over the complete concentration-range tested (Figure 2b). Passive sensitization increased the response to the maximal concentration of SP given and shifted to the left the CCRC as measured by the mean dose-ratio (Table 2). The mean increase in the maximal isometric force ΔF_{max} was 57.6 ± 11.5%.

Effect of passive sensitization on responses to neurokinin A

CCRC to NKA were obtained beteween 1 nM and 3 μM. In the absence of phosphoramidon, as was the case for SP, passive sensitization increased the amplitude of the contractile response to NKA and shifted to the left the CCRC (Figure 3a and Table 1). ΔF_{max} was 48.8 ± 8.9%.

Table 2 Effect of passive sensitization on responses to substance P (SP), neurokinin A (NKA) and vasoactive intestinal peptide (VIP) in human isolated bronchial smooth muscle rings in the presence of phosphoramidon

		Non-sensitized		*Passively-sensitized*
SP	F_{max} (%)	74 (4)*		115 (3)*
	Dose-ratio		9.0 (4.3–13.6)°	
NKA	F_{max} (%)	104 (9)*		146 (16)*
	Dose-ratio		5.4 (2.9–7.9)°	
VIP	R_{max} (%)	72 (1)*		49 (4)*
	Dose-ratio		6.4 (3.0–9.8)°	

F_{max}: maximal contractile response to the largest concentration of SP or NKA given expressed as a percentage of the maximal contraction to ACh; R_{max}: maximal relaxant response to the largest concentration of VIP given expressed as a percentage of the precontraction to histamine; figures in parentheses indicate s.e.mean for F_{max} or R_{max} and 95% confidence limits for dose-ratios.
Values of maximal response significantly different (*$P<0.05$) from each other; dose-ratio significantly (°$P<0.05$) different from 1.

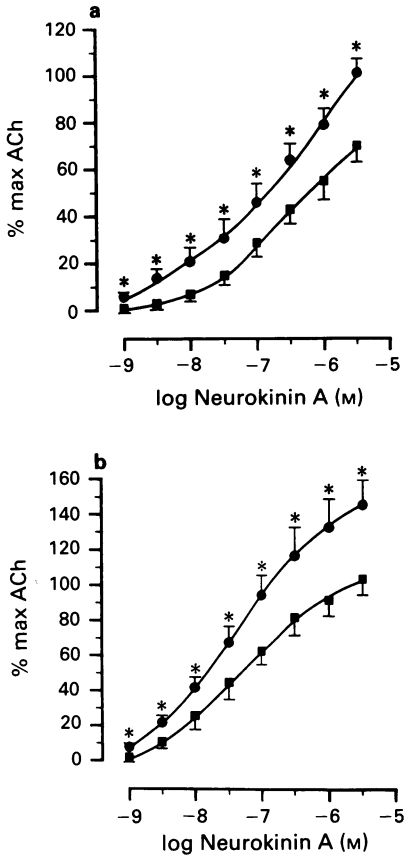

Figure 3 Mean CCRC for neurokinin A in paired non-sensitized (■) and passively sensitized (●) human isolated bronchial smooth muscle rings. (a) In the absence of phosphoramidon. Each symbol represents the mean value ± s.e.mean ($n = 5$ different lung specimens) of contractile force expressed as a percentage of the maximal contraction to acetylcholine (ACh). *$P<0.05$. (b) In the presence of phosphoramidon (10 μM) added to the bath 20 min prior to the CCRC. Legend as in (a).

Pretreatment with phosphoramidon performed on another set of 5 lung specimens led to modifications in the NKA-induced response that resembled those observed with SP: contractile responses appeared greater and passive sensitization still increased the amplitude of the contractile response to NKA and shifted to the left the CCRC (Figure 3b and Table 2). ΔF_{max} was 40.2 ± 6.3%.

Effect of passive sensitization on responses to vasoactive intestinal peptide

In order to measure relaxant responses in the absence of supramaximal tone, VIP-induced relaxation was studied in tissues precontracted with histamine at a concentration (0.5 mM) which is maximal but not supramaximal in human airway smooth muscle (Marthan *et al.*, 1987). When phosphoramidon was to be used, it was added to the plateau of the histamine precontraction, 20 min before the CCRC for VIP. Phosphoramidon induced no changes in the amplitude or maintenance of histamine-induced tone. Passive sensitization reduced the amplitude of the relaxation to VIP i.e. passively sensitized tissues relaxed less than did non-sensitized both in the absence of phosphoramidon (Figure 4a) ΔF_{max} being − 40.2 ± 6.5% and in the presence of phosphoramidon (Figure 4b) ΔF_{max} being − 31.2 ± 4.8%. Passive sensitization also shifted to the right the CCRC as assessed by the dose-ratios both in the absence (Table 1) and in the presence (Table 2) of the NEP inhibitor.

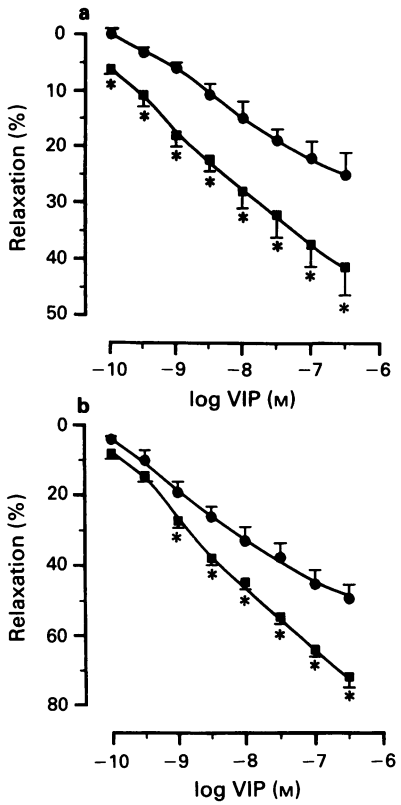

Figure 4 Mean CCRC for vasoactive intestinal peptide (VIP) in paired non-sensitized (■) and passively sensitized (●) human isolated bronchial smooth muscle rings. (a) In the absence of phosphoramidon. Each symbol represents the mean value ± s.e.mean ($n = 5$ different lung specimens) of relaxation response expressed as a percentage of the precontraction to histamine. $^*P < 0.05$. (b) In the presence of phosphoramidon (10 μM) added to the bath 20 min prior to the CCRC. Legend as in (a).

Discussion

This study shows that passive sensitization alters the mechanical activity in response to stimulation by neuropeptides in human isolated airways. It enhances the contractile properties of tachykinins (SP and NKA) and reduces relaxant responses to VIP. The mechanism of passive sensitization-induced changes in the mechanical activity appears to be independent of a decrease in NEP activity since these changes persist in the presence of the NEP inhibitor, phosphoramidon.

Since actual maximal responses to neuropeptides are difficult to obtain owing to the large concentrations required, the effect of passive sensitization had to be assessed in a manner different from the conventional one i.e. in terms of true maximal response and EC_{50} (the concentration producing 50% of the maximal response). This is why we used the dose-ratios to estimate the changes in the potency of these peptides induced by passive sensitization. For each experiment, in each pair of tissues, a dose-ratio was calculated from the two concentrations of the peptide which produced an arbitrarily determined response on the linear and parallel portion of the concentration-response curves (Black *et al.*, 1981).

Our data on the effect of neuropeptides in human non-sensitized airway smooth muscle can be compared to those of previous studies in this tissue. Overall, our results are in general agreement with those of Black *et al.* (1988) and Naline *et al.* (1989) for tachykinins, and those of Palmer *et al.* (1986) for VIP. Nevertheless, we found that the amplitude of the response to identical concentrations of SP was greater than that found by Black *et al.* (1988) and Naline *et al.* (1989). We also found that the amplitude of the reponse to identical concentrations of NKA was greater than that reported by Black *et al.* (1988) but similar to that reported by Naline *et al.* (1989). Using the same agonist to precontract human airways as that used by Palmer *et al.* (1986) (i.e. histamine) although at a higher concentration to standardize the level of tone, we found VIP to be as potent but less efficacious than in the latter investigation. These slight differences could be for several reasons: (i) responses to neuropeptides may vary according to the size and localization of the airway preparation in the respiratory tract (Palmer *et al.*, 1986); (ii) the biological activity of commercially available neuropeptides is batch-dependent (Palmer *et al.*, 1986); in the present study, each of the neuropeptides used was from the same batch; (iii) since our study was designed to assess the effect of passive sensitization, control tissues were processed in a different manner from that in the above cited studies in that they were incubated overnight in serum from healthy donors.

Previously we have reported that, as was the case for airway tissues from actively sensitized animals, passively sensitized bronchial preparations isolated from human lung specimens exhibited increased contractile responses to a variety of pharmacological agonists (Black *et al.*, 1989). Recently, we observed that passive sensitization also reduced responses to relaxant compounds (Villanove *et al.*, 1992). It decreased relaxation responses which depend upon modulation of ion channels (i.e. responses to calcium channel antagonists and to potassium channel openers) but not those which depend upon activation of β-adrenoceptors by isoprenaline. The results of the present study show that responses to neuropeptides are similarly altered i.e. passive sensitization increases the response to neuropeptides which contract human isolated airways and reduces that to a neuropeptide which relaxes it.

The question which then arises deals with the mechanism of the effect of passive sensitization on the response to neuropeptides. Since the airway mechanical activity induced by neuropeptides depends on their inactivation by NEP, we investigated the role of this enzyme in the effect of sensitization. The experiments performed in the presence of the NEP inhibitor, phosphoramidon, have revealed that passive sensitization-induced alterations in the mechanical activity were similar to those observed in the absence of phosphoramidon. Although a direct statistical comparison between experiments conducted with and without phosphoramidon cannot be made since they were performed on different sets of lung specimens in each case, both the increase in force induced by the maximal concentration of each of the neuropeptides given (ΔF_{max}) and the dose-ratio for each neuropeptide were similar whether phosphoramidon was present or not. Therefore, the effect of passive sensitization was independent of changes in NEP activity. Alternative mechanisms related to the coupling efficiency of neuropeptides should thus be evoked to account for the effect of sensitization. Complementary experiments investigating the coupling mechanisms of neuropeptides in human airway smooth muscle are required. Such information would help to clarify the cellular pathways via which passive sensitization alters responses to neuropeptides and to explain the differential effect of sensitization on SP and NKA on one hand and VIP on the other hand.

The results of the present study provide some *in vitro* evidence that responses to neuropeptides may be altered under conditions of sensitization of the airways in man. In this connection, our data lend some support to the hypothesis that neuropeptide release by axon reflexes account for, at least part of, the pathophysiological aspects of asthma (Barnes, 1986). However, one should be cautious in extrapolating these findings to the *in vivo* situation for patients suffering from bronchial hyperresponsiveness. In particular, although we found that hyperresponsiveness to neuropeptides occurred in the absence of alteration in NEP activity, such an alteration may play a role in the *in vivo* situation. Passive

sensitization does not alter the epithelium of human isolated airways, at least when examined histologically, whereas NEP activity may decrease in relation to the shedding of the epithelial layer in asthmatic bronchi (Barnes, 1987b).

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