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# Role of kinins in anaphylactic-induced bronchoconstriction mediated by tachykinins in guinea-pigs

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**1** In the present study, we have investigated the role of kinins in allergen-induced bronchoconstriction.

**2** Anaesthetized guinea-pigs were sensitized to ovalbumin, ventilated artificially, pretreated with atropine (1.4 $\mu$mol kg$^{-1}$, i.v.) and total pulmonary resistance ($R_L$) measured. In preliminary studies in the presence of the neutral endopeptidase inhibitor, phosphoramidon (4.5 $\mu$mol kg$^{-1}$, i.v.), the bradykinin $B_2$ receptor antagonist Hoe 140 (0.1 $\mu$mol kg$^{-1}$, i.v.) completely abolished the increase in $R_L$ following aerosolized bradykinin (1 mM, 40 breaths), but had no effect on the increase in $R_L$ following aerosolized neurokinin A (NKA, 10 $\mu$M, 40 breaths). On the other hand, a combination of the $NK_1$ (CP-96,345, 2 $\mu$mol kg$^{-1}$, i.v.) and $NK_2$ (SR 48968, 0.3 $\mu$mol kg$^{-1}$, i.v.) tachykinin receptor antagonists abolished completely the increase in $R_L$ produced by NKA and partially inhibited the increase in $R_L$ produced by bradykinin. These results confirm previous studies that suggest that bradykinin induces the release of tachykinins from sensory nerves in guinea-pig airways.

**3** Aerosolized ovalbumin (0.5%, 5 breaths) increased $R_L$ in sensitized guinea-pigs pretreated with atropine (1.4 mmol kg$^{-1}$, i.v.), an effect that began within 2 min and reached a maximum within 5 min; $R_L$ remained above baseline at 20 min. Pretreatment with the bradykinin $B_2$ receptor antagonist, Hoe 140, decreased the bronchoconstrictor effect of ovalbumin markedly at 10 to 20 min. In the presence of phosphoramidon (4.5 $\mu$mol kg$^{-1}$, i.v.) the inhibition induced by Hoe 140 was apparent earlier and remained over the 20 min period of study.

**4** Pretreatment with a combination of $NK_1$ (CP-96,345) and $NK_2$ (SR 48968) tachykinin receptor antagonists also markedly inhibited ovalbumin-induced bronchoconstriction; addition of the bradykinin $B_2$ receptor antagonist to the $NK_1$ and $NK_2$ tachykinin receptor antagonists had no additional inhibitory effect on antigen-induced bronchoconstriction.

**5** These findings confirm that activation of sensory nerves to release tachykinins in guinea-pig airways contribute to antigen-induced bronchoconstriction, and provide evidence that tachykinin release is due to kinins generated during the allergic response.

**Keywords:** Kinins; tachykinins; receptors; neurogenic inflammation; allergen challenge; bronchoconstriction; sensory nerves; Hoe 140; SR 48968; CP-96,345.

## Introduction

Stimulation of a subpopulation of sensory nerves causes the release of neuropeptides, including the tachykinins substance P (SP) and neurokinin A (NKA), from their peripheral terminals. This release produces a series of effects collectively referred to as 'neurogenic inflammation', which in the airways includes plasma extravasation and bronchconstriction (Lundberg & Saria, 1983; Lundberg *et al.*, 1984; Barnes, 1986; Nadel, 1991). Plasma extravasation and bronchconstriction are effects produced by exposure to antigen in sensitized animals. Recently, we showed that tachykinins released from sensory nerve endings (via activation of $NK_2$ and $NK_1$ tachykinin receptors) play an important role in antigen-induced plasma extravasation and bronchoconstriction in sensitized guinea-pigs (Bertrand *et al.*, 1993a,b). As there is no evidence that the antigen-antibody complex *per se* activates sensory nerves, we reasoned that mediators released from tissue, inflammatory cells or plasma may be involved in the antigen-evoked stimulation of sensory nerves.

Kinins, including bradykinin, derived from a plasma precursor, and kallidin released from a tissue precursor, have been proposed to take part in the allergic inflammatory response because increased kinin levels have been detected in immediate and late phase reactions in experimental animals (Erjefalt *et al.*, 1993) and in man (Christiansen *et al.*, 1992). Bradykinin antagonists have been found to reduce the late bronchial response (Abraham *et al.*, 1991) and the hyperresponsiveness (Farmer *et al.*, 1992) that occurs after exposure to antigen. Bradykinin potently stimulates primary sensory neurones (Kaufman *et al.*, 1980) and releases neuropeptides from their peripheral endings in different tissues, including the airways (Geppetti *et al.*, 1988; Saria *et al.*, 1988; Geppetti, 1993). Kinins released upon exposure to antigen play an important role in the activation of sensory nerves, which contribute substantially to the antigen-evoked plasma extravasation in guinea-pigs (Bertrand *et al.*, 1993d).

In the present study, we investigated whether bronchoconstriction in response to antigen and mediated by activation of sensory nerves is due to release of kinins. To test this hypothesis, we used Hoe 140, a selective and potent peptide antagonist of $B_2$ bradykinin receptors (Hock *et al.*, 1991), because this compound completely prevents the bronchoconstriction evoked by aerosolized or intravenously injected bradykinin in guinea-pigs (Wirth *et al.*, 1993). The effect of Hoe 140 on antigen-induced bronchoconstriction was compared to the effect produced by the combination of nonpeptide antagonists of $NK_2$ (SR 48968) (Emonds-Alt *et al.*, 1992) and $NK_1$ (CP-96,345) (Snider *et al.*, 1991) tachykinin recep-

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tors. The combination of $NK_1$ and $NK_2$ tachykinin receptor antagonists was used because this combination is necessary to block completely the bronchoconstriction induced by tachykinins released by capsaicin in guinea-pigs *in vivo* (Bertrand *et al.*, 1993c). Some experiments were performed in animals pretreated with phosphoramidon, because previous studies (Bertrand *et al.*, 1993a,b) showed that blockade of neutral endopeptidase (NEP) activity by phosphoramidon increases the sensory nerve-mediated responses to antigen.

## Methods

### *Animals*

Male Hartley guinea-pigs (Simonsen Laboratories Inc., Gilroy, CA, U.S.A) that weighed 250–300 g at the time of housing, were used in this study. They were kept in a temperature-controlled environment with standard laboratory food and water freely available.

### *Sensitization procedure*

Animals were sensitized according to a protocol described previously (Dunn *et al.*, 1988; Bertrand *et al.*, 1993a). Sensitization consisted of two separate injections of 70 mg ovalbumin (Grade V) in 1.5 ml 0.9% NaCl intraperitoneally, with an interval of one week between the injections. The animals were studied two weeks after the second injection. Non-sensitized (control) animals were injected twice with 1.5 ml 0.9% NaCl.

### *Measurement of total pulmonary resistance ($R_L$)*

Animals were anaesthetized using sodium pentobarbitone (45 mg $kg^{-1}$, i.p.; Anthony Product Corp., Arcadia, CA, U.S.A.) and then ventilated artificially with a tracheal cannula, using a constant-volume ventilator (model 683; Harvard Apparatus Co., Inc., South Natick, MA, U.S.A.) at a frequency of 80 breaths $min^{-1}$. The tidal volume was adjusted to maintain normal arterial blood gases as described previously (Dusser *et al.*, 1988). Airflow was monitored continuously with a pneumotachograph (A. Fleisch, Medical Inc., Richmond, VA, U.S.A.) connected to a differential pressure transducer (model DP45; Validyne Engineering Corp., Northrige, CA, U.S.A.). A fluid-filled polyethylene catheter was introduced into the oesophagus to measure the oesophageal pressure as an approximation of pleural pressure. Intratracheal pressure was measured with a polyethylene catheter inserted into a short tube connecting the tracheal cannula to the pneumotachograph. The transpulmonary pressure (defined as the pressure difference between the intratracheal and oesophageal pressures) was measured with a differential pressure transducer (model DP7; Validyne Engineering Corp.). Output signals representing transpulmonary pressure and airflow were amplified with an amplifier (model CD19; Validyne Engineering Corp.) and recorded on a polygraph recorder (model 1508 B Visicorder; Honeywell, Inc., Denver, CO, U.S.A.). $R_L$ was calculated as described previously (Dusser *et al.*, 1988). The right jugular vein and the left carotid artery were cannulated to permit administration of drugs and to withdraw a sample of blood for arterial blood gas measurement.

### *Experimental design*

Baseline $R_L$ remained stable for at least 2 h, and no significant changes were produced by aerosols (5 breaths or 40 breaths) or i.v. injection (1 ml $kg^{-1}$) of 0.9% NaCl after a stabilization period of 30 min. Aerosols of saline or antigen (ovalbumin 0.5%; 5 breaths) were generated by an ultrasonic nebulizer (model 25, DeVilbiss, Somerset, PA) and were delivered into the airways by the respirator via the tracheal cannula (aerosol delivery rate, 0.2 ml $min^{-1}$). All animals were pretreated with atropine (1.4 μmol $kg^{-1}$, i.v., 15 min before the stimulus). Phosphoramidon (4.5 μmol $kg^{-1}$, i.v.) was given 5 min before the stimulus.

### *Drugs*

Ovalbumin, atropine and histamine were obtained from Sigma Chemical (St. Louis, MO, U.S.A.). Phosphoramidon, bradykinin and NKA were purchased from Peninsula Laboratories, Inc. (Belmont, CA, U.S.A.). CP-96,345 ((2S,3S) cis-2-(diphenylmethyl)-N-((2-methoxy-phenyl)-methyl)-1-azabicyclo[2.2.2]octan-3-amine) and SR 48968 (S)-N-methyl-N([4-(-acetyl-amino-4-phenylpiperidino)-2-(3,4dichlorophenylbutyl] benzamide) were kindly provided by Dr J.A. Lowe III (Pfizer Inc, Groton, CT, U.S.A.) and Dr X. Emonds-Alt (Sanofi Recherche, Montpellier, France), respectively. Hoe 140 (D-Arg-[$Hyp^3$,$This^5$,D-$Tic^7$,$Oic^8$]-bradykinin) was a kind gift of Dr F.J. Hock (Hoechst AG, Frankfurt, Germany). Drugs were dissolved in 0.9% saline or in dimethyl sulphoxide (Hoe 140 and SR 48.968). Further dilutions were made in 0.9% saline.

### *Statistical analysis*

Values in the text and figures are the mean ± standard error of the mean (s.e.mean). Statistical comparisons were performed by a one way analysis of variance and Dunnett's test or bilateral unpaired Student's *t* test, when appropriate. In all cases, a P value of less than 0.05 was considered significant.

## Results

Aerosolized bradykinin (1 mM, 40 breaths) increased $R_L$ approximately 2 fold above baseline value, and the bradykinin $B_2$ receptor antagonist Hoe 140 (0.1 μmol $kg^{-1}$, i.v., 15 min before the stimulus) abolished the bronchoconstriction completely (Figure 1b) in guinea-pig pretreated with atropine (1.4 μmol $kg^{-1}$) and phosphoramidon (4.5 μmol $kg^{-1}$). A combination of $NK_2$ (SR 48968, 0.3 μmol $kg^{-1}$, i.v., 15 min before the stimulus) and $NK_1$ (CP-96,345, 2 μmol $kg^{-1}$ i.v., 5 min before the stimulus) tachykinin receptor antagonists decreased the bradykinin-induced increase in $R_L$ by approximately 60% (Figure 1b). Aerosolized NKA (10 μM, 40 breaths) increased $R_L$ more than 3 fold above baseline value, an effect that was not changed by pretreatment with Hoe 140 (0.1 μmol $kg^{-1}$, i.v.), but was abolished completely by the combination of $NK_2$ (SR 48968, 0.3 μmol $kg^{-1}$ i.v.) and $NK_1$ (CP-96,345, 2 μmol $kg^{-1}$ i.v.) tachykinin antagonists (Figure 1a).

Aerosols of the vehicle of ovalbumin (0.9% NaCl, 5 breaths) did not increase $R_L$ significantly over the 20 min period of study in guinea-pigs sensitized to ovalbumin (data not shown). However, aerosolized ovalbumin (0.5%, 5 breaths) increased $R_L$ approximately 2 fold above baseline value in the sensitized guinea-pigs: the increase in $R_L$ was evident within 2 min after exposure, reached a maximum within 5 min, and $R_L$ was still increased above baseline after 20 min (Figure 2a). Pretreatment with the bradykinin $B_2$ receptor antagonist Hoe 140 (0.1 μmol $kg^{-1}$, i.v.) decreased the bronchoconstrictor effect of aerosolized ovalbumin at 10, 15 and 20 min after the delivery of ovalbumin. When the guinea-pigs were pretreated with phosphoramidon (4.5 μmol $kg^{-1}$, i.v.), a selective inhibitor of NEP, the increase in $R_L$ after inhalation of ovalbumin was not significantly greater than the increase obtained in the absence of phosphoramidon (Figure 2a,b). In the presence of phosphoramidon Hoe 140 still decreased markedly the ovalbumin-induced increase in $R_L$ (Figure 2b): a significant inhibition by Hoe 140 of the ovalbumin-induced bronchoconstriction was found as early as 3 min and remained at 20 min (Figure 2b).

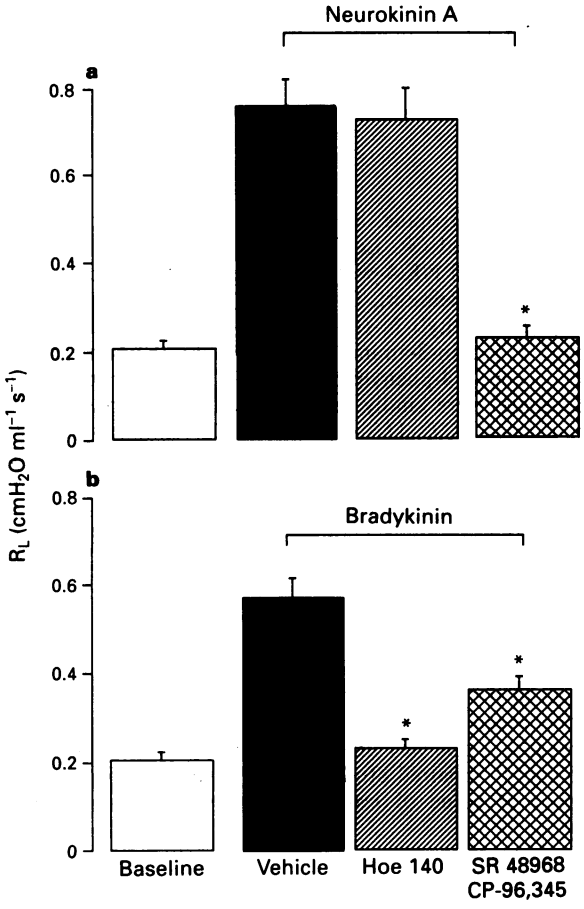


**Figure 1** Effect of the bradykinin $B_2$ receptor antagonist (Hoe 140; 0.1 μmol $kg^{-1}$, i.v., given 15 min before the stimulus) (hatched column), or the combination of tachykinin $NK_2$ (SR 48968; 0.3 μmol $kg^{-1}$, i.v., given 15 min before stimulus) and $NK_1$ (CP-96,345; 2 μmol $kg^{-1}$, i.v., given 5 min before stimulus) receptor antagonists (cross-hatched column) on the maximal increase in total pulmonary resistance ($R_L$ evoked) by aerosolized bradykinin (b) or aerosolized neurokinin A (a). Animals were pretreated with phosphoramidon (4.5 μmol $kg^{-1}$, i.v., given 5 min before stimulus) and atropine (1.4 μmol $kg^{-1}$, i.v., given 15 min before stimulus). Vehicle (filled columns) indicates animals pretreated with the vehicle of Hoe 140 and SR 48968 (10% dimethyl sulphoxide in 0.9% saline) 15 min before the stimulus. Baseline (open columns) indicates values measured before the beginning of the aerosolization of the stimulus. Each column is the mean ± s.e.mean of at least 5 experiments. $^*P<0.01$ versus control.

In another series of experiments in the presence of phosphoramidon, we studied the effect of adding Hoe 140 to the tachykinin receptor antagonists SR 48968 (0.3 μmol $kg^{-1}$, i.v.) and CP-96,345 (2 μmol $kg^{-1}$, i.v.): the increase in $R_L$ produced by aerosolized ovalbumin was decreased significantly by pretreatment with the combination of SR 48968 and CP-96,345 (Figure 3). In animals whose pretreatment included SR 48968 and CP-96,345 plus Hoe 140, no further reduction in the ovalbumin-induced effects occurred compared to the inhibitory effects of the $NK_2$ and $NK_1$ tachykinin antagonists alone (Figure 3).

## Discussion

The major finding in the present study is that a selective bradykinin $B_2$ receptor antagonist decreases markedly oval-

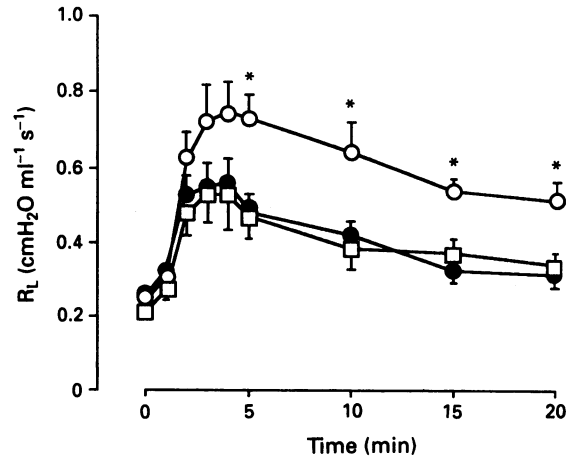


**Figure 3** Effect of the combination of antagonists on $NK_2$ (SR 48968, 0.3 μmol $kg^{-1}$, i.v., 15 min before stimulus) and $NK_1$ (CP-96,345, 2 μmol $kg^{-1}$, i.v., 5 min before stimulus) tachykinin receptor (●) or the combination of these two antagonists plus the bradykinin $B_2$ receptor antagonist, Hoe 140 (0.1 μmol $kg^{-1}$, i.v., 15 min before stimulus) (□), on the increase in total pulmonary resistance ($R_L$) evoked by aerosolized ovalbumin (0.5%, 5 breaths) in sensitized guinea-pigs. Anaesthetized animals were pretreated with atropine (1.4 μmol $kg^{-1}$, i.v., 15 min before stimulus) and phosphoramidon (4.5 μmol $kg^{-1}$, i.v., 5 min before stimulus). Controls (○) received the vehicle of Hoe 140 and SR 48968 (10% dimethyl sulphoxide in 0.9% saline) 15 min before the stimulus. Each point is the mean ± s.e.mean of 7 experiments. $^*P<0.05$ versus control.

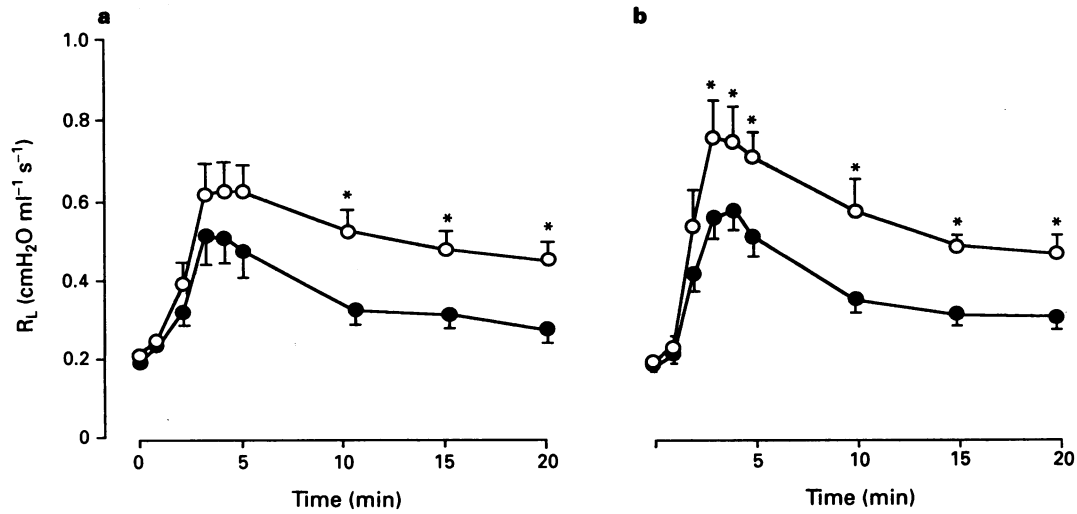


**Figure 2** Effect of Hoe 140 (0.1 μmol $kg^{-1}$, i.v., 15 min before the stimulus) (●) and its vehicle (10% dimethyl sulphoxide in 0.9% saline) (○) on the increase in total pulmonary resistance ($R_L$) evoked by aerosolized ovalbumin (0.5%, 5 breaths) in sensitized guinea-pigs. Experiments were performed in the absence (a) or in the presence of phosphoramidon (4.5 μmol $kg^{-1}$, i.v., 5 min before stimulus) (b). Each point is the mean ± s.e.mean of 8 experiments. $^*P<0.05$ vs. Hoe 140.

bumin-induced bronchoconstriction in sensitized guinea-pigs, thus implicating kinins in this allergic response. Present and previous (Wirth *et al.*, 1993) findings show that $B_2$ bradykinin receptors mediate bradykinin-induced bronchoconstriction in guinea-pigs. Bradykinin is known to be released into bronchoalveolar lavage fluid in both sensitized guinea-pigs (Erjefalt *et al.*, 1993) and in asthmatic human subjects (Christiansen *et al.*, 1992) after inhalation of antigen, adding credibility to the present finding that a bradykinin $B_2$ antagonist inhibits antigen-induced bronchoconstriction to a considerable extent.

The present studies also provide data on the importance of tachykinins in the antigen-induced bronchoconstriction in guinea-pigs. These results, which confirm previous findings (Bertrand *et al.*, 1993b), indicate that a combination of $NK_1$ and $NK_2$ receptor antagonists inhibit considerably antigen-induced bronchoconstriction in sensitized guinea-pigs. Furthermore, present results provide evidence that kinins released into airways as a result of antigen-antibody interactions and the subsequent release of tachykinins from the sensory nerves in the airways acts as a 'cascade' to produce allergic bronchoconstriction. This conclusion is based on the following: first, bradykinin is known to stimulate sensory nerves and to release sensory neuropeptides from several tissues, including airways (Geppetti, 1993). When bradykinin is applied topically to the airways, most of its effects are mediated by the stimulation of sensory nerves with the subsequent release of tachykinins. In the present studies, bradykinin-induced bronchoconstriction was markedly attenuated by pretreatment with $NK_1$ and $NK_2$ receptor antagonists, confirming previous studies in which capsaicin pretreatment was used to deplete tachykinins from sensory nerves (Ichinose *et al.*, 1990). Second, in the present study, pretreatment with a combination of $NK_1$ and $NK_2$ receptor antagonists markedly reduced antigen-induced bronchoconstriction. Furthermore, addition of a bradykinin $B_2$ receptor antagonist to the tachykinin antagonists did not further reduce antigen-induced bronchoconstriction.

From these results, we suggest the following sequence of events: the antigen-antibody response in airways causes the release of mediators, including kinins. The kinins activate sensory nerves to release tachykinins which act on their receptors on airway smooth muscle. Of course, one might hypothesize that the opposite sequence occurs (i.e., that antigen-antibody interactions in airways cause sensory nerves to release tachykinins, with the subsequent release of kinins). Several pieces of information mitigate against this sequence: first, to our knowledge, tachykinins are not reported to release kinins in any tissue. Second, in the present study, tachykinin (NKA)-induced bronchoconstriction was not inhibited by pretreatment with a bradykinin $B_2$ receptor antagonist.

Ovalbumin also increases vascular permeability in airways in sensitized guinea-pigs, and a similar cascade of antigen-antibody interaction is hypothesized leading to kinin activation in airways, which in turn causes the release of tachykinins from sensory nerves, which act on receptors in the postcapillary venules to increase vascular permeability (Bertrand *et al.*, 1993d). Thus, in guinea-pigs, similar cascades involving kinin activation and tachykinin release appear to play a role in both antigen-induced airway smooth muscle contraction and plasma extravasation.

We showed previously that phosphoramidon, an NEP inhibitor, potentiates the mild bronchoconstriction induced by an aerosol of 0.1% ovalbumin, but not the marked response induced by a higher dose of ovalbumin (0.5%) (Bertrand *et al.*, 1993b). The present results showing that bronchoconstriction induced by 0.5% ovalbumin was not increased by blockade of NEP are in agreement with these previous data. However, NEP appears to be involved in the break down of kinins and/or tachykinins which are released after the inhalation of 0.5% ovalbumin and which mediate the bronchoconstriction in response to this dose of antigen. This conclusion is suggested by the observation that inhibition of ovalbumin-induced bronchoconstriction by Hoe 140 is evident earlier in animals pretreated with phosphoramidon than in animals not pretreated with phosphoramidon.

If the present mechanisms found in guinea-pigs also exist in human subjects, we suggest that bradykinins and/or tachykinin receptor antagonists may be of value in reducing symptoms in allergic states where kinins and tachykinins appear to be involved in the pathophysiological responses.

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