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Effects of bradykinin receptor antagonists on antigen-induced respiratory distress, airway hyperresponsiveness and eosinophilia in guinea-pigs

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1 We examined effects of bradykinin (BK) receptor antagonists on airway hyperresponsiveness and eosinophilia in sensitized guinea-pigs that had been administered single, as well as repeated (chronic) challenges with inhaled ovalbumin. In addition, the effects of BK antagonists on antigen-induced respiratory distress during the chronic study were noted.

2 At 24 h following single antigen challenge, guinea-pigs exhibited airway hyperresponsiveness to the bronchoconstrictor effect of i.v. histamine, characterized by a left shift in the dose-response curve. In addition, responses to the maximum dose of histamine that could be used were significantly increased in hyperresponsive guinea-pigs. The percentages of bronchoalveolar fluid, eosinophil and neutrophils also increased.

3 A BK B_1 receptor antagonist, desArg^9-[Leu^8]-BK, significantly inhibited airway hyperresponsiveness induced by single antigen challenge. A B_2 receptor antagonist, D-Arg-[Hyp^3, Thi^{5,8},D-Phe^7]-BK (NPC 349) had a small, but statistically significant inhibitory effect on responsiveness to the highest histamine dose in challenged animals. DesArg^9-[Leu^8]-BK significantly inhibited the neutrophilia, whereas NPC 349 inhibited infiltration by both cell types.

4 Chronic antigen challenge also caused airway hyperresponsiveness to i.v. acetylcholine (ACh), distinguished by an increase in the slope of the dose-response curve. Thus, the magnitude of the bronchoconstrictor responses to the maximum dose of ACh that could be used was significantly increased. No change in sensitivity to ACh was evident. Marked eosinophilia was also noted in the trachea, bronchi and lung parenchyma.

5 Airway hyperresponsiveness and eosinophilia, induced by chronic antigen challenge, were markedly inhibited by the B_2 antagonists, D-Arg-[Hyp^3,D-Phe^7]-BK (NPC 567) or D-Arg-[Hyp^3,Thi^{5}D-Tic^7,Tic^8]-BK (NPC 16731). NPC 16731 also abolished antigen-induced cyanosis, and delayed the onset of dyspnoea, doubling the time taken for animals to exhibit respiratory distress.

6 The ability of BK receptor antagonists to inhibit antigen-induced airway hyperresponsiveness, in addition to eosinophilia, indicates an important role for endogenous kinins. Moreover, the abrogation of eosinophil infiltration suggests that BK has a significant function in maintaining allergic inflammation of the airways.

Keywords: Bradykinin; B_1 receptor; B_2 receptor; antagonist; airway; allergen challenge; hyperresponsiveness; eosinophil; respiratory distress; asthma

Introduction

Bradykinin (BK) has been implicated as an important mediator in inflammatory diseases of the upper and lower airways (see reviews by Farmer, 1991a,b; Pongracic *et al.*, 1991). For example, inhaled BK is a very potent bronchoconstrictor in asthmatic but not non-asthmatic subjects (Fuller *et al.*, 1987; Polosa & Holgate, 1990). Furthermore, allergic patients are reported to be more responsive to the effect of BK on microvascular leakage, following its topical application in the nasal mucosa, than are non-allergic subjects (Brunnée *et al.*, 1991). Increased levels of immunoreactive kinins are evident in the upper airways of allergic patients, following allergen challenge (Proud *et al.*, 1983). Similarly, in allergic asthmatics, bronchoalveolar lavage (BAL) levels of immunoreactive kinins, as well as kinin-generating enzymes, are significantly elevated following inhalation of antigen (Christiansen *et al.*, 1987). Thus, the kallikrein-kinin system, via generation of inflammatory kinins in the airways, may be involved in the pathogenesis of allergic asthma and rhinitis.

Similar to observations in man (Fuller *et al.*, 1987; Polosa & Holgate, 1990), sheep which are allergic to *Ascaris suum* antigen, are more responsive to the bronchoconstrictor effect of inhaled BK than non-allergic animals (Abraham *et al.*, 1991a). The potential involvement of endogenous kinins in experimental antigen-induced airway hyperresponsiveness and inflammation is supported by observations that aerosol administration of a B_2 receptor antagonist, D-Arg-[Hyp^3,D-Phe^7]-BK (NPC 567), prior to antigen challenge, inhibits both phenomena in allergic sheep (Solér *et al.*, 1990). In addition, NPC 567 abolishes the onset of late bronchial obstruction, as well as increased BAL levels of several inflammatory mediators (peptidoleukotrienes, leukotriene B_4, prostaglandins) in dual responding sheep, 4–8 h following challenge with *A. suum* (Abraham *et al.*, 1991b).

Sensitized guinea-pigs, repeatedly exposed to antigen, exhibit hyperresponsiveness to the bronchoconstrictor action of acetylcholine (ACh), and airway infiltration by eosinophils (Ishida *et al.*, 1989). In addition, we recently reported the induction of airway hyperresponsiveness to histamine 24 h

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after a single challenge with inhaled ovalbumin in sensitized guinea-pigs (Seeds *et al.*, 1991). Also in this species, airway hyperreactivity to ACh and 5-hydroxytryptamine (5-HT) was reported to occur as early as 60 min after single challenge with ovalbumin (Daffonchio *et al.*, 1989). Antigen challenge causes increased circulating levels of kinins and exposure of perfused lungs from sensitized guinea-pigs to antigen results in kallikrein production *in vitro* (Brocklehurst & Lahiri, 1962; Jonasson & Becker, 1966).

We have examined, therefore, the effects of three B_2 receptor antagonists, NPC 567, D-Arg-[Hyp^3,Thi^{5,8},D-Phe^7]-BK (NPC 349) (Farmer & Burch, 1991), and D-Arg-[Hyp^3,Thi^5,D-Tic^7,Tic^8]-BK (NPC 16731), recently described as a more potent BK antagonist (Farmer *et al.*, 1991a; Kyle *et al.*, 1991), on airway hyperresponsiveness and eosinophilia induced by acute and chronic inhalation of ovalbumin in sensitized guinea-pigs. In addition, the effects of desArg^9-[Leu^8]-BK, a B_1 receptor antagonist, on the acute effects of antigen on airway responsiveness and eosinophil infiltration were examined. Some of these data were presented to the British Pharmacological Society (Farmer *et al.*, 1991c).

Methods

Acute antigen-induced airway hyperresponsiveness

Male Dunkin-Hartley guinea-pigs (250–600 g) from Olac (Bicester, Oxon) were used for this aspect of the study. Each animal was injected i.p. with 40 μg ovalbumin (OA), dissolved in a suspension of $Al(OH)_3$ gel, that had been diluted 1:1 with sterile saline (0.9% w/v NaCl solution). Controls received $Al(OH)_3$ alone; 18–20 days later, all animals were exposed to a mist of OA, generated in a jet-type nebulizer (1 mg ml^{-1}, 8–10 ml h^{-1}), for 1 h in an exposure chamber. The BK receptor antagonists, NPC 349 or desArg^9-[Leu^8]-BK, were administered immediately before antigen inhalation. Doses of each antagonist were 400 μg kg^{-1} i.v., plus 600 μg kg^{-1}, s.c.

Twenty-four hours later, animals were anaesthetized with urethane (7 ml kg^{-1} i.p., 25% w/v solution), and the trachea cannulated and connected to a Harvard ventilator pump. Animals were ventilated (1 ml 100 g^{-1} body weight) with room air at a rate of 70 strokes per min. Pulmonary inflation pressure (PIP), as an index of intrathoracic airway resistance, was measured with a pressure transducer (Druck Ltd.) mounted to a side arm on the tracheal cannula. A jugular vein and carotid artery were also cannulated to allow administration of drugs and the monitoring of arterial blood pressure, respectively. Airway responsiveness was determined from dose-response curves to intravenously administered histamine. Doses of histamine (1–50 μg kg^{-1}) were administered at 5 min intervals.

Bronchoalveolar lavage

Bronchoalveolar lavage (BAL) was performed as described previously (Seeds *et al.*, 1991). In brief, animals were killed with an overdose of urethane, and 5 ml aliquots of saline were instilled and recovered from the lungs a total of five times. Total cell counts were obtained from the resultant cell suspension and, following adjustment to 5×10^5 cells ml^{-1}, cytospin preparations were prepared, and differential cell counts obtained by use of Lendrum's Stain (Lendrum, 1944) to visualize eosinophils.

Chronic antigen-induced airway hyperresponsiveness

Female Hartley guinea-pigs (200–250 g) (Hazelton, Denver, PA, U.S.A.) were sensitized to OA by a slight modification of the method of Ishida *et al.* (1989). Animals were injected i.p. with 10 μg OA dissolved in 0.5 ml saline containing 10 mg $Al(OH)_3$ in suspension. After two weeks the animals were challenged by inhalation of an aerosolized solution containing OA (0.5% w/v dissolved in normal saline). Aerosols were generated by an ultrasonic nebulizer (DeVilbiss Pulmo-Sonic Nebulizer). Inhalation of antigen was carried out twice a week for five weeks. All animals were given the histamine H_1 receptor antagonist, diphenhydramine (30 mg kg^{-1}, i.p.), 60 min before each antigen challenge.

Animals were exposed to antigen aerosol until the onset of laboured breathing and/or cyanosis was evident. The maximum exposure time was 60 min and, from weeks 3–5, the time taken for each animal to exhibit symptoms was recorded. After an interval of 24–48 h following the final challenge, animals were anaesthetized with urethane, and the trachea and jugular vein were cannulated for measurement of PIP, and i.v. drug administration, in a manner similar to that described for the acute antigen studies. Airway responsiveness was determined from dose-response curves to acetylcholine (ACh, 1–300 μg kg^{-1} i.v.).

Treatment groups

Animals were divided into six treatment groups as follows. Controls consisted of non-sensitized or sensitized guinea-pigs, both groups being challenged with vehicle for drug and OA (saline). As no difference in airway responsiveness between these groups was evident (data not shown), results from these animals were combined. The second group was of sensitized guinea-pigs, chronically challenged with OA. There were four drug treatment groups. NPC 567 or NPC 16731 were administered to controls and also to sensitized, challenged guinea-pigs. Aerosolized NPC 567 (30 mg ml^{-1}) or NPC 16731 (1.5 mg ml^{-1}) were administered by inhalation for 30 min before each antigen challenge. NPC 567 (6 mg ml^{-1}) or NPC 16731 (0.3 mg ml^{-1}) were also co-administered with antigen.

Histology

Airway histological sections were prepared from animals from each group. Anaesthetized guinea-pigs were killed by exsanguination, and the lungs inflated with glutaraldehyde solution (2.5% w/v in saline). The trachea and lungs were removed and placed in glutaraldehyde for at least 48 h. Sections (10 μm) of trachea, right main bronchus, right main lobar bronchus and lung parenchyma were prepared, mounted on slides and stained with haematoxylin and eosin. Eosinophils in 10 random fields were counted in a light microscope. Care was taken to include only airway mucosal and submucosal eosinophils, and not those seen in blood vessels.

Drugs

Urethane, acetylcholine chloride, histamine diphosphate, ovalbumin (Grade V, fatty acid-free) and desArg^9-[Leu^8]-BK were obtained from Sigma Chemical Co. (Poole, Dorset and St. Louis, MO, U.S.A.). $Al(OH)_3$ moist gel was obtained from FSA Laboratory Supplies (Loughborough). D-Arg-[Hyp^3,Thi^{5,8},D-Phe^7]-BK (NPC 349) was a gift from Dr J.M. Stewart, Department of Biochemistry, University of Colorado School of Medicine, Denver, Colorado, U.S.A. D-Arg-[Hyp^3,D-Phe^7]-BK (NPC 567) was synthesized by Abbott Laboratories (North Chicago, IL, U.S.A.), and D-Arg-[Hyp^3, Thi^5,D-Tic^7,Tic^8]-BK (NPC 16731), at Nova. All drugs were prepared in saline at the time of use.

Data analysis

Dose-response curves were compared by two-tailed *t* tests, and cell counts in different experimental groups were compared by one-way analysis of variance (ANOVA), performed by InStat (GraphPad Software, San Diego, California, U.S.A.). Where the *F* ratio generated a *P* value of <0.05, adjusted *t* tests, with *P* values corrected by the Bonferroni method, were

carried out between appropriate experimental groups. Probability (P) values of <0.05 were considered statistically significant. All data are expressed as mean ± s.e.mean.

Results

Acute studies

Twenty-four hours after single antigen challenge, guinea-pigs exhibited airway hyperresponsiveness to histamine. This was characterized by a leftward shift in the histamine dose-response curve, and a small increase in PIP to the maximum dose of histamine (Figure 1). Associated with the airway hyperresponsiveness, the percentages of BAL fluid eosinophils and neutrophils, but not monocytes, increased significantly 24 h after antigen challenge (Figure 2). The percentage of BAL eosinophils increased from 16.6 ± 3.0 in controls, to 35.0 ± 3.7 24 h after antigen challenge, although no significant alteration in total cell counts was apparent (282,000 ± 54,000 cells ml^{-1} in controls, and 422,000 ± 79,000 cells ml^{-1} following antigen challenge).

In guinea-pigs pretreated with NPC 349, antigen-induced eosinophil infiltration was significantly inhibited, whereas desArg^9-[Leu^8]-BK was without effect on eosinophil numbers (Figure 2). In contrast, the increase in the percentage of BAL neutrophils was inhibited by both antagonists. The small increase in bronchoconstrictor responses, in response to 50 $\mu g\,kg^{-1}$ histamine, was inhibited by NPC 349, while this peptide did not affect the leftward shift in the dose-response curve (Figure 1b). In contrast, desArg^9-[Leu^8]-BK significantly attenuated the antigen-induced leftward shift in the histamine dose-response curve (Figure 1c).

Chronic studies

Each OA challenge caused conspicuous respiratory distress. The animals exhibited laboured breathing, cyanosis and often collapsed. Several animals exhibited these symptoms within a few min and, despite being pretreated with an antihistamine, had to be quickly removed from the exposure chamber to prevent death by asphyxiation. An unexpected observation in the present study was that the time taken for the onset of laboured breathing in all animals increased with each challenge. Times to onset of respiratory distress were subsequently determined on weeks 3, 4 and 5 and, by week 5, they were significantly greater than at the first challenge of week 3 (Table 1).

Although dyspnoea occurred in the animals pretreated with NPC 567 or NPC 16731, cyanosis was rarely observed.

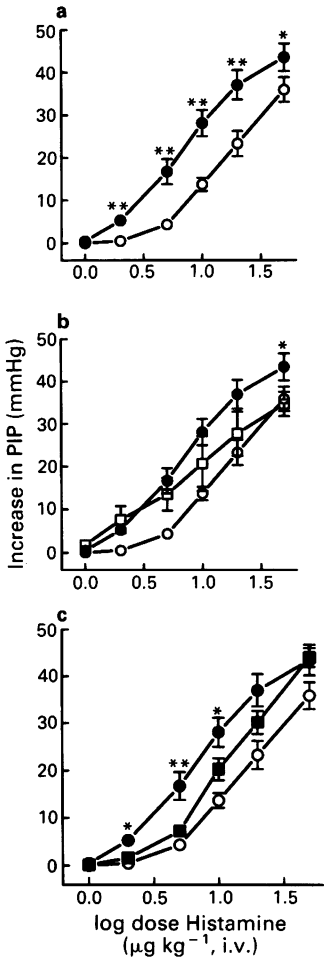

Figure 1 Dose-response curves for the bronchoconstrictor effect of intravenous histamine in anaesthetized guinea-pigs, previously sensitized to ovalbumin. (a) Data show airway hyperresponsiveness to histamine 24 h following acute antigen challenge: (○) non-sensitized controls; (●), antigen-challenged animals (**$P<0.01$ compared with controls). (b) (□), Effect of the bradykinin B_2 receptor antagonist, D-Arg-[Hyp^3,Thi^{5,8},D-Phe^7]-BK. The antagonist significantly decreased responses of hyperresponsive animals to 50 $\mu g\,kg^{-1}$ histamine (*$P<0.05$). (c) (■) Effects of the B_1 receptor antagonist, desArg^9-[Leu^8]-BK. This drug significantly decreased responses of hyperresponsive animals at 2, 5 and 10 $\mu g\,kg^{-1}$ histamine (*$P<0.05$; **$P<0.01$). Data are expressed as mean ± s.e.mean (vertical bars) of 6–9 experiments.

Table 1 Effect of the bradykinin B_2 receptor antagonists, D-Arg-[Hyp^3, D-Phe^7]-BK (NPC 567) and D-Arg-[Hyp^3,Thi^5,D-Tic^7,Tic^8]-BK (NPC 16731) on time to onset of dyspnoea induced by ovalbumin (OA) inhalation in chronically challenged, sensitized guinea-pigs

	Treatment group		
Challenge No.	*OA controls*	*NPC 567 + OA*	*NCP 16731 + OA*
Week 3, No. 1	2.9 ± 0.3	4.0 ± 0.5[1]	6.6 ± 1.0**
Week 3, No. 2	2.9 ± 0.2	2.9 ± 0.8[1]	6.3 ± 1.2*
Week 4, No. 1	3.3 ± 0.5	6.9 ± 1.1[1]	9.3 ± 1.9**
Week 4, No. 2	4.9 ± 0.8	5.8 ± 1.1[1]	11.4 ± 1.6**
Week 5, No. 1	5.8 ± 1.1	6.1 ± 1.2[1]	10.6 ± 1.2*
Week 5, No. 2	6.2 ± 1.5[2]	8.2 ± 1.4[1,2]	12.0 ± 1.7*[,2]

Values are in min before animals were observed to exhibit laboured breathing. Group data were compared by one-way ANOVA followed, where appropriate, by modified t tests.
*$P<0.05$; **$P<0.01$; [1]not significant, when compared to OA control.
[2]Values are significantly different (t test, $P<0.05$) from those of challenge on Week 3, No. 1.
OA and antagonists were administered by inhalation. See Methods for protocols. Data are expressed as mean ± s.e.mean of 6–8 observations.

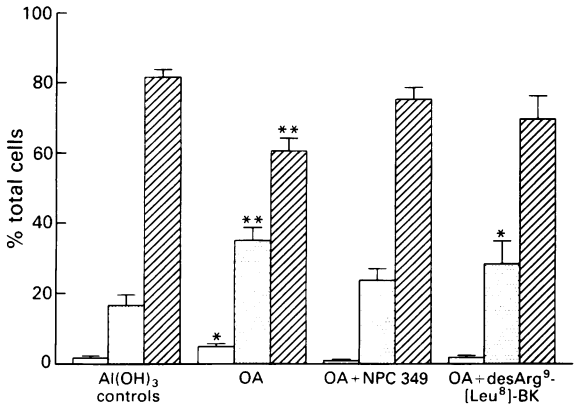

Figure 2 Effects of desArg^9-[Leu^8]-BK and D-Arg-[Hyp^3,Thi^{5,8},D-Phe^7]-BK on the percentage of neutrophils (open columns), eosinophils (stippled columns) and mononuclear cells (hatched columns) found in bronchoalveolar fluid of sensitized guinea-pigs, 24 h following acute challenge with ovalbumin (OA). $*P<0.05$, and $**P<0.01$ compared with Al(OH)$_3$ controls. Data are expressed as mean ± s.e.mean (vertical bars) of 6–9 experiments.

Moreover, these animals did not collapse, even after 60 min exposure and, upon their removal from the exposure chamber, they recovered relatively quickly. NPC 16731-treated guinea-pigs took approximately twice as long to exhibit signs of laboured breathing when exposed to antigen aerosol (Table 1). This was evident throughout the duration of these studies. NPC 567 had no significant effect on the onset of dyspnoea (Table 1).

Chronic challenges with OA caused airway hyperresponsiveness, characterized by an increase in the slope of the ACh dose-response curve, resulting in an approximately 50% increase (from 33.0 ± 3.8 to 49.6 ± 3.5 cmH_2O, $P<0.01$) in the response to the maximum dose that could be administered (Figure 3). There was no alteration in airway sensitivity to ACh following chronic antigen exposures. Thus the control $-\log ED_{50}$ ($\mu g\,kg^{-1}$) of 1.97 ± 0.16 was not different from the value of 1.81 ± 0.07 in antigen-challenged guinea-pigs. Neither NPC 567 nor NPC 16731 had any effect on airway responsiveness in control animals (Figure 3). Both antagonists abolished the development of airway hyperresponsiveness to ACh (Figure 3b and c).

Chronic exposure of sensitized guinea-pigs to inhaled antigen also resulted in significantly increased numbers of eosinophils in all airway regions examined (Figure 4). For example, the numbers of eosinophils observed in 10 fields rose from 3.2 ± 0.9 in control tracheal sections, to 16.7 ± 2.4 in sections obtained from antigen-challenged animals. Similar degrees of eosinophilia were observed in main and lobar bronchus, as well as in parenchyma (Figure 4).

Airway eosinophilia was attenuated to varying degrees by the BK antagonists. In parenchyma, for example, both NPC 567 and NPC 16731 abolished antigen-induced eosinophilia (Figure 4). In contrast, NPC 16731 had a greater effect on eosinophilia in main bronchus than did NPC 567 (Figure 4). The antagonists had no significant effects on eosinophil numbers in airway tissues from non-sensitized control animals (data not shown). Thus, the B_2 receptor antagonists inhibited chronic antigen-induced airway eosinophilia and hyperresponsiveness.

Discussion

These experiments confirm previous studies demonstrating airway hyperresponsiveness to bronchoconstrictors in sensitized guinea-pigs after a single challenge (Daffonchio *et al.*, 1988; 1989; Seeds *et al.*, 1991), or repeated challenges (Ishida *et al.*, 1989; Schellenberg *et al.*, 1991) with antigen. In addition, airway eosinophil infiltration after acute (Dunn *et al.*,

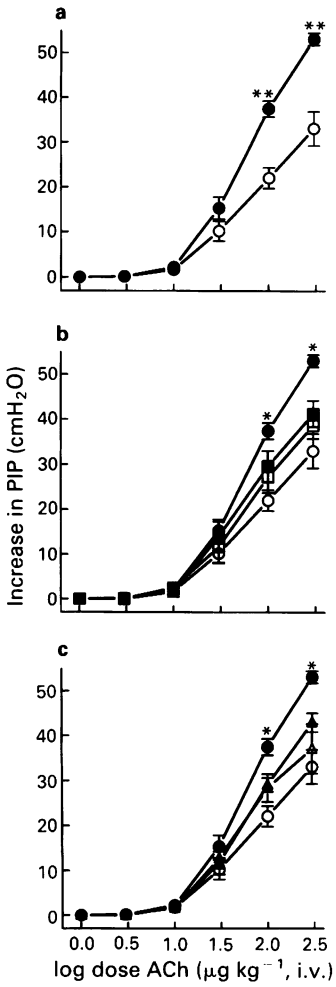

Figure 3 Dose-response curves for the bronchoconstrictor effect of intravenous acetylcholine (ACh) in anaesthetized guinea-pigs. These animals were sensitized to ovalbumin and challenged twice weekly for 4–5 weeks with inhaled ovalbumin. (a) Data show airway hyperresponsiveness to ACh 24–48 h following the final antigen challenge: (○) controls; (●) antigen-challenged animals. Ovalbumin challenges caused a significant increase in airway responsiveness ($**P<0.01$). (b) Effects of the bradykinin B_2 receptor antagonist, D-Arg-[Hyp^3,D-Phe^7]-BK (NPC 567): (□) in control animals, and (■), in animals chronically challenged with antigen. NPC 567 significantly inhibited the effect of ovalbumin challenge ($*P<0.05$). (c) Effects of the bradykinin B_2 receptor antagonist, D-Arg-[Hyp^3,Thi^5,D-Tic^7,Tic^8]-BK (NPC 16731): (△) in control animals, and (▲), in animals chronically challenged with antigen. NPC 16731 significantly inhibited the effect of ovalbumin challenge ($*P<0.05$). Data are expressed as mean ± s.e.mean (vertical bars) of 6–8 experiments.

1988; Hutson *et al.*, 1988; Richards *et al.*, 1989; Seeds *et al.*, 1991) and chronic (Ishida *et al.*, 1989; 1990) exposure to antigen has been reported.

Respiratory distress

As has been described by many investigators, antigen challenge induced acute respiratory distress. Interestingly, guinea-pigs took no longer to respond to each exposure to ovalbumin. Thus, during the third week, they took around 3 min to evince signs of distress, becoming cyanotic and exhibiting physical signs from mild dyspnoea to laboured breathing and anaphylactic collapse. By the final challenges, time to onset of respiratory symptoms had doubled. Although the reasons for this observation are not known, they perhaps involve immunological desensitization. Alternatively, the time to onset of respiratory distress may increase with the age of the animals.

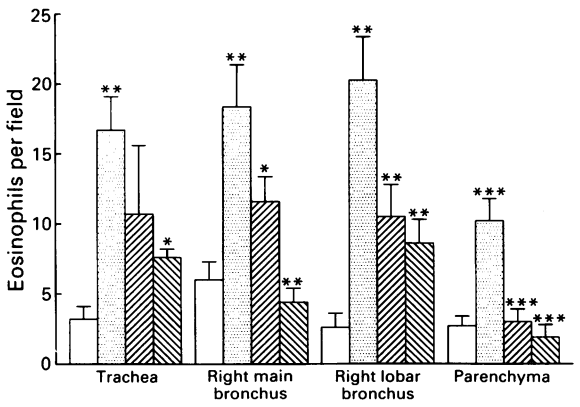

Figure 4 Effects of D-Arg-[Hyp^3,D-Phe^7]-BK (NPC 567) and D-Arg-[Hyp^3,Thi^5,D-Tic^7,Tic^8]-BK (NPC 16731), bradykinin B_2 receptor antagonists, on the numbers of eosinophils present in tissue sections from various airway regions of guinea-pigs. Open columns, control tissues; stippled columns, tissues from animals chronically challenged with ovalbumin (OA); left hatched columns, challenged animals treated with NPC 567; right hatched columns, challenged animals treated with NPC 16731. In all airway regions examined, OA exposures significantly increased tissue eosinophil numbers. NPC 16731 significantly inhibited or abolished OA-induced eosinophilia in all airway regions. NPC 567 had significant effects in main and lobar bronchi, and in parenchyma: $*P<0.05$; $**P<0.01$; $***P<0.001$ (ANOVA). Data are expressed as mean ± s.e.mean (vertical bars) of 6–9 experiments.

Administration of NPC 16731, prior to and simultaneously with inhaled antigen, significantly delayed the onset of respiratory distress in chronically challenged guinea-pigs. While difficult to quantify, these animals rarely exhibited cyanosis and appeared to have a less severe reaction to ovalbumin challenges. Albeit NPC 567 did not significantly affect the onset of antigen-induced dyspnoea, our subjective observations suggested that animals treated with the antagonist also seemed to have a milder reaction to ovalbumin than did untreated controls. The lack of effect of NPC 567 on antigen-induced respiratory distress may be a reflection of its lower potency as a B_2 receptor antagonist than NPC 16731 (Farmer *et al.*, 1991a).

While the mechanisms underlying the ability of NPC 16731 to delay antigen-induced respiratory distress can only be speculated upon, they may reflect the inhibition of airway hyperresponsiveness by B_2 receptor antagonists. As discussed here, chronic challenge with inhaled ovalbumin resulted in airway hyperresponsiveness to ACh. In the single challenge study, guinea-pigs were also hyperresponsive to the bronchoconstrictor action of histamine. Moreover, hyperresponsiveness to ACh and 5-HT has been reported under similar experimental conditions (Daffonchio *et al.*, 1988; 1989). It is feasible then, that the airways were also hyperresponsive to mast cell mediators released endogenously by inhaled antigen. Since BK antagonists inhibited nonspecific airway hyperresponsiveness, the degree of constriction induced by mast cell-derived mediators may have been reduced by NPC 16731, resulting in a delayed and less severe antigen-induced bronchoconstriction.

Antigen-challenge in sensitized guinea-pigs activates the lung kallikrein-kinin system, and releases kinins (Brocklehurst & Lahiri, 1962; Jonasson & Becker, 1966). Similarly, inhalation of allergen in sheep (Abraham *et al.*, 1991b) and allergic asthmatics (Christiansen *et al.*, 1992) dramatically elevates BAL levels of immunoreactive kinins. NPC 567, however, does not inhibit acute antigen-induced bronchoconstriction in sheep (Abraham *et al.*, 1991b) or guinea-pigs (S.G. Farmer, unpublished observation). It is unlikely, therefore, that endogenous kinins, formed in response to antigen challenge, contribute to anaphylactic bronchoconstriction in these species. Rather, our data with NPC 567 and NPC 16731 suggest a role for BK in airway hyperresponsiveness and eosinophilia.

Effects of acute antigen exposure

We have confirmed that, 24 h following single challenge with inhaled ovalbumin, sensitized guinea-pigs exhibited airway hyperresponsiveness, characterized by a left shift in the dose-response curve to i.v. histamine (Seeds *et al.*, 1991). Higher doses could not be employed due to the profound cardiovascular effects of this agonist. Associated with the acute hyperresponsiveness was an increase in the percentages of BAL eosinophil and neutrophil numbers.

Administration of BK antagonists inhibited airway hyperresponsiveness in different ways. Thus, desArg^9-[Leu^8]-BK, a B_1 antagonist, prevented the increase in pulmonary sensitivity (i.e. the left shift of the dose-response curve) to histamine. NPC 349, a B_2 receptor antagonist, prevented the increase in bronchoconstrictor responses to $50\,\mu g\,kg^{-1}$ histamine that occurred after single antigen challenge. However, the magnitude of the latter effect was small, and its 'biological significance' is dubious. The B_2 antagonist prevented antigen-induced increase in both neutrophils and eosinophils, whereas the B_1 antagonist inhibited only the airway neutrophilia. The effect of desArg^9-[Leu^8]-BK on airway supersensitivity and neutrophilia suggest that the two phenomena may be related, and involve endogenous kinins acting on B_1 receptors. Moreover, the small effect of NPC 349 may involve antagonism at B_1 receptors. Although the [D-Phe^7]-substituted analogues of BK, including NPC 349, are usually referred to as 'B_2 antagonists,' they are often nonselective for B_1 and B_2 receptors, probably due to their degradation by carboxypeptidases to their desArg-derivatives (see references in Farmer & Burch, 1991; Ward, 1991).

Although the effects of the BK antagonists on airway granulocyte numbers were statistically significant, they were modest. Moreover, the degree of airway hyperresponsiveness was also modest, being characterized by a less than two fold left shift in the histamine dose-response curve. Although this is considerably less than the magnitude of airway hyperresponsiveness in asthmatic patients (Boushey *et al.*, 1980), it is very similar to the shifts described by other investigators using sensitized guinea-pigs (Daffonchio *et al.*, 1987; 1989). In addition, NPC 567 inhibited airway hyperresponsiveness and neutrophila, induced by inhaled *Ascaris* antigen, in allergic sheep (Solér *et al.*, 1990). Thus, endogenous kinins may play a role in acute airway hyperresponsiveness in these models.

The observation that a B_1 antagonist had effects at all is surprising. DesArg^9-BK, a B_1 agonist, is not a bronchoconstrictor in guinea-pigs (Farmer *et al.*, 1989; Jin *et al.*, 1989), and has no effect on isolated airway smooth muscle (Farmer *et al.*, 1989). In addition, B_1 receptor ligands do not displace [^{3}H]-BK binding in trachea and lung (Farmer *et al.*, 1989; Mak & Barnes, 1991), indicating the absence of B_1 receptors in healthy guinea-pig airways.

However, in rabbit tissues, B_1 receptor expression may be induced by noxious stimuli, possibly as a homeostatic response to inflammation (DeBlois *et al.*, 1991; Farmer *et al.*, 1991b). Inflammatory changes, induced by antigen challenge, may bring about B_1 receptor induction in the airways, but their role in hyperresponsiveness or cell influx remains to be determined.

Effects of chronic antigen exposure on airway responsiveness and eosinophilia

As previously found by Schellenberg's group (Ishida *et al.*, 1989), repeated exposure of sensitized guinea-pigs to a mist of inhaled ovalbumin solution caused airways hyperresponsiveness to i.v. ACh, and eosinophilia. In the present study, administration of the B_2 receptor antagonists, NPC 567 or

NPC 16731, as well as their concomitant inhalation with ovalbumin, abolished hyperresponsiveness and, depending upon the airway region, partially or completely abrogated the infiltration by eosinophils. These data suggest that endogenous BK, formed in response to repeated antigen challenge, is involved in these phenomena. Our results are similar to results with the antiasthmatic drug, nedocromil, which inhibited both airway eosinophilia and hyperresponsiveness, in response to repeated antigen challenge (Schellenberg *et al.*, 1991).

Eosinophils and antigen-induced airway hyperresponsiveness

One interpretation of the data with BK antagonists or nedocromil might be that antigen-induced airway eosinophilia and hyperresponsiveness are interdependent. Nevertheless, other pharmacological interventions have contrasting effects on eosinophilia and hyperresponsiveness. For example, in the same guinea-pig model of repeated antigen challenge a platelet-activating factor (PAF) antagonist, while having no effect on airway eosinophilia, inhibited hyperresponsiveness (Ishida *et al.*, 1990). Similarly, we recently reported that eosinophil infiltration, induced by antigen, can occur at times when bronchial hyperresponsiveness is not evident (Seeds *et al.*, 1991).

Havill and colleagues (1990) also examined the effects of PAF antagonists in guinea-pigs that received three i.p. booster doses of ovalbumin followed, two to three weeks later, by single inhaled challenge. Neither WEB 2086 nor SDZ 64-412 had any effect on airway eosinophil numbers 24 h after antigen challenge, although both drugs abolished hyperresponsiveness. Taken together, these data indicate that PAF may be involved in antigen-induced airway hyperresponsiveness. In contrast, the lack of effect of these agents on eosinophil recruitment suggest that the presence of eosinophils alone is not a prerequisite to the development of allergen-induced airway hyperresponsiveness (Havill *et al.*, 1990; Ishida *et al.*, 1990).

Preteatment of guinea-pigs with capsaicin also differentiates between airway hyperresponsiveness and eosinophilia in response to repeated antigen challenge. Matsuse and co-investigators (1991) recently reported that, in animals depleted of sensory neuropeptides by capsaicin, airway hyperresponsiveness was abolished. However, infiltration of the airways by eosinophils was unaffected. Preliminary data indicate that capsaicin also inhibits airway hyperresponsiveness, but not eosinophilia, after single antigen challenge of sensitized guinea-pigs (Ladenius & Biggs, 1989). Thus, if eosinophils are significant to the development of allergic airway hyperresponsiveness, factors other than simply increasing their number are involved. It is possible that, in the inflammatory microenvironment of the airways, eosinophils trigger the release of neuropeptides, which in turn cause hyperresponsiveness. On the other hand, airway neuropeptides may stimulate the release of an eosinophil-derived factor that induces hyperresponsiveness (Koregel *et al.*, 1990).

Bradykinin and airway hyperresponsiveness

Many physiological effects of BK can be attributed, at least in part, to its ability to release sensory neuropeptides and/or PAF (see Farmer, 1991a,b). Thus, BK stimulates sensory nerve endings, and releases substance P and calcitonin gene-related peptide, in several tissues including those of the airways (Lundberg & Saria, 1983; Saria *et al.*, 1988; Geppetti *et al.*, 1990; Ray *et al.*, 1991). It has also been shown that capsaicin pretreatment markedly reduces the magnitude of bronchoconstriction to tracheal instillation of BK (Ichinose *et al.*, 1990). In addition, in vascular endothelial cells, smooth muscle and fibroblasts, BK and desArg^9-BK both stimulate the synthesis of PAF (Cahill *et al.*, 1988), and BK-induced prostacyclin synthesis is blocked by PAF antagonists (Stewart *et al.*, 1990). Moreover, the prolonged airway microvascular leakage in guinea-pigs, in response to BK, is likewise inhibited by PAF antagonists (Rogers *et al.*, 1990). It is also noteworthy that PAF can release substance P and neurokinin A from guinea-pig isolated, perfused lungs (Martins *et al.*, 1991).

The ability of BK antagonists to inhibit antigen-induced airway hyperresponsiveness in guinea-pigs is in concurrence with other studies. As mentioned, NPC 567 inhibits airway hyperresponsiveness, in addition to generation of several inflammatory mediators, and the late bronchial response following challenge with *Ascaris suum* antigen in sheep (Solér *et al.*, 1990; Abraham *et al.*, 1991b). Furthermore, BK itself was reported to produce airway hyperresponsiveness to ACh in anaesthetized guinea-pigs (Omini *et al.*, 1989) and, also, in perfused cat lungs (Kimura *et al.*, 1989).

In conclusion, our data with BK antagonists in sensitized guinea-pigs, associated with those in allergic sheep (Solér *et al.*, 1989, Abraham *et al.*, 1991b), demonstrate that endogenous kinins may play a pivotal role in the genesis of allergic airway hyperresponsiveness. The additional ability of NPC 567 and NPC 16731 to inhibit antigen-induced eosinophil infiltration also indicates potential antiinflammatory activity of the BK antagonists in guinea-pig airways.

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