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ACCELERATED COMMUNICATION

Evidence for a Pulmonary B_3 Bradykinin Receptor

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SUMMARY

We have examined pulmonary effects of bradykinin (Bk) *in vivo* and *in vitro* in guinea pigs and their potential inhibition by antagonists of Bk B_1 and B_2 receptors. Bk was a potent bronchoconstrictor *in vivo* and caused contractions of isolated, epithelium-denuded trachealis. D-Arg[Hyp^3,D-Phe^7]-Bk (NPC567) and D-arg[Hyp^3,Thi^{5,8},D-Phe^7]-Bk (NPC349), B_2 receptor antagonists, were weak inhibitors of Bk-induced bronchoconstriction *in vivo* and were virtually inactive as antagonists of Bk-induced airway smooth muscle contraction. Several other B_2 antagonists as well as B_1 antagonist, des-Arg^9-[Leu^8]-Bk, did not inhibit Bk-induced tracheal contraction. The B_1 receptor agonist des-Arg^9-Bk was without effect on tracheal tone. Tracheal responses to Bk were unaffected by antagonists of muscarinic, histamine, serotonin, and catecholamine receptors. The inability of the antagonists to inhibit Bk is unlikely to be due to their degradation, because NPC567 was only weakly active in the presence of inhibitors of kininase I (EC 3.4.11.2), kininase II (EC 3.4.15.1), and neutral endopeptidase (EC 3.4.24.11). These studies were corroborated by ligand binding experiments in guinea pig and ovine airways. In [^{3}H]Bk binding, the Bk antagonists had no effect in guinea pig trachea, slightly displaced [^{3}H]Bk in ovine trachea, and inhibited approximately 60% of total specific binding in lung. des-Arg^9-[Leu^8]-Bk and several other agents, including atropine, neurokinin A, substance P, and vasoactive intestinal peptide, had no effect on lung Bk binding. Bk and its analogs were not degraded during the binding assay. These data suggest that pulmonary tissue, particularly in the large airways, contains a novel Bk binding site, a B_3 receptor, which may be involved in Bk-induced bronchoconstriction.

Bk and related kinins, formed in tissues and fluids during inflammation, exert many physiological effects mediated by two receptor subtypes (1–3). B_2 receptors are activated by Bk but not des-Arg^9-BK, whereas B_1 receptors are much more sensitive to des-Arg^9-Bk than to Bk. Several Bk analogs are specific antagonists at B_2 receptors (3, 4), whereas others such as des-Arg^9-[Leu^8]-Bk are specific B_1 antagonists (1, 3).

The involvement of kinins in bronchial asthma has been a subject of speculation for several years. Bk has been implicated in the pathogenesis of asthma, in that bronchoalveolar kinin levels are elevated following allergen challenge in allergic asthmatics (5). Moreover, Bk is a potent bronchonstrictor in asthmatics but not in nonasthmatics (6, 7), and elevated kinin levels have been detected in plasma of asthmatic patients (8). Although there are several reports of the pulmonary effects of Bk on airways *in vivo* and *in vitro* in animals and humans (6–11), there is a paucity of information on the nature of Bk receptors mediating those effects. Indeed, to our knowledge there are no studies investigating the nature of the receptors mediating Bk-induced bronchoconstriction. The recent availability of specific B_2 antagonists (3) has enhanced knowledge of the role of the peptide in many systems. For example, several [D-Phe^7]-substituted analogs of Bk, such as NPC349 or NPC567, are selective antagonists of Bk-induced responses in ileal and uterine smooth muscle (4, 12) and 3T3 fibroblasts (13), and of Bk-induced pain (4). The present study set out to examine the pulmonary effects of Bk and various B_1 and B_2 antagonists in anesthetized guinea pigs and in isolated airway smooth muscle. In addition, we have examined the binding of [^{3}H]Bk and its displacement by several antagonists in membrane preparations of both guinea pig and ovine tracheal smooth muscle and lung parenchyma.

Materials and Methods

Studies *in vivo*. PIP, as an index of intrathoracic airway resistance, was determined in guinea pigs as described elsewhere (14). Briefly, guinea pigs were anesthetized with ketamine and xylazine (30 mg·kg^{-1} and 0.6 mg·kg^{-1}, respectively, IV) and mechanically ventilated with a Harvard Rodent Ventilator via a tracheal cannula (70 strokes·min^{-1} with 10 ml·kg^{-1} air). PIP, measured from a lateral port in the afferent limb of the ventilation circuit with a Gould P50 pressure transducer, was displayed on a Gould 2400S chart recorder. Where appropriate, Bk was administered IV (via a jugular catheter), with 10 min elapsing

ABBREVIATIONS: Bk, bradykinin; NPC349, D-Arg[Hyp^3,Thi^{5,8},D-Phe^7]-bradykinin; NPC361, [D-Phe^7]-bradykinin; NPC414, Lys-Lys[Hyp^{2,3},Thi^{5,8},D-Phe^7]-bradykinin; NPC431, [Thi^{5,8},D-Phe^7]-bradykinin; NPC567, D-Arg[Hyp^3,D-Phe^7]-bradykinin; MGTPA, DL-2-mercaptomethyl-3-guanidinoethylthiopropionic acid; PIP, pulmonary inflation pressure; IV, intravenous; TES, *N*-tris[hydroxymethyl]methyl-2-aminoethanesulfonic acid.

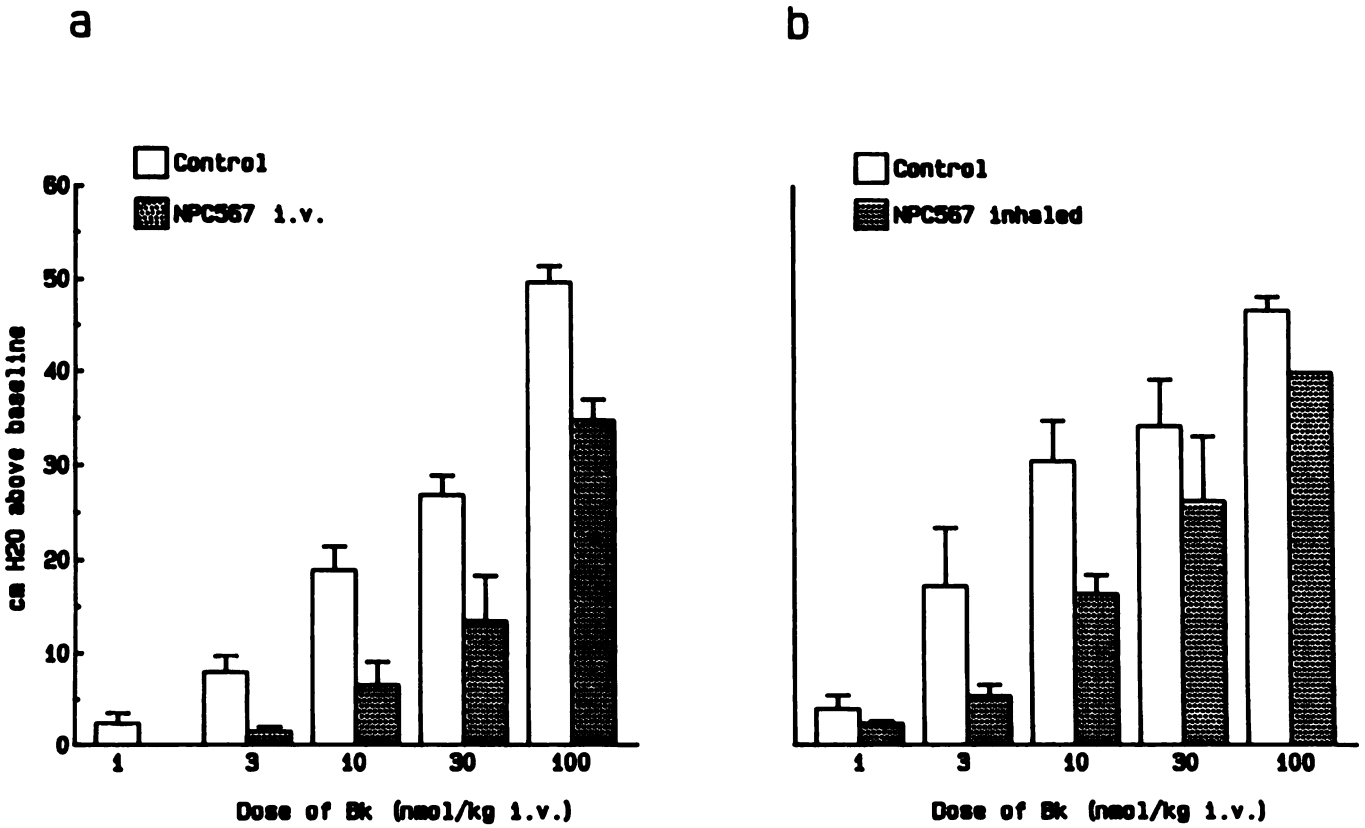

Fig. 1. Effect of NPC567 on Bk-induced bronchoconstriction. a, Bk was administered intravenously with 10 min elapsing between successive doses. Effect of NPC567 (100 $\mu g \cdot kg^{-1}$) was examined by injection 30 sec before Bk. Each *point* is mean ± standard error of five observations. b, Effect of inhaled NPC567 on intravenous Bk-induced bronchoconstriction. A solution of NPC567 (100 $mg \cdot ml^{-1}$) was used to generate an aerosol mist in an ultrasonic nebulizer.

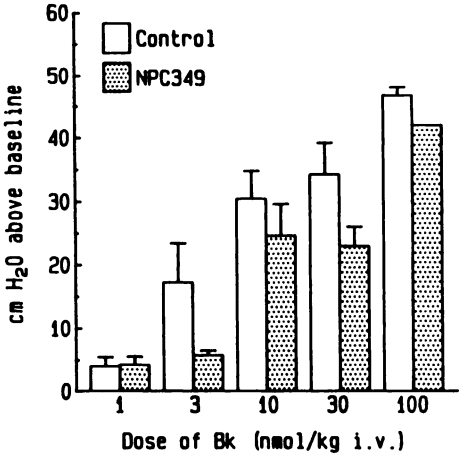

Fig. 2. Effect of inhaled NPC349 on intravenous Bk-induced bronchoconstriction. A solution of NPC349 (100 $mg \cdot ml^{-1}$) was used to generate an aerosol mist in an ultrasonic nebulizer. Each *point* is mean ± standard error of four observations.

between successive doses. The effects of NPC567 were examined by injection 30 sec before Bk. To examine the effect of inhaled NPC349 or NPC567 on IV Bk-induced bronchoconstriction, a DeVilbiss Pulmosonic Nebulizer was used to generate an aerosol from antagonist solutions. The aerosol was inhaled, via the tracheal cannula, for 1 min before and during Bk infusion. In some experiments, Bk and NPC567 were both administered inhalationally. Aerosolized Bk was generated from solutions containing 1, 10, and 30 $mg \cdot ml^{-1}$ and aerosolized NPC349 and NPC567 from solutions containing 10 and 30 $mg \cdot ml^{-1}$.

Isolated smooth muscle studies. Strips of guinea pig isolated trachea and lung parenchyma were prepared for recording of isometric tension using standard methods (15, 16). Transverse strips of trachea, containing or lacking an intact epithelium, or strips of peripheral lung parenchyma were suspended in jacketed tissue baths that contained modified Krebs-Henseleit solution (composition, in mM: NaCl, 118; KCl, 4.7; $CaCl_2$, 2.5; KH_2PO_4, 1.2; $MgSO_4$, 1.2; $NaHCO_3$, 25.0; glucose, 10.0), gassed with 5% CO_2 in O_2, and maintained at 37°. Preparations were allowed to equilibrate for 60 min, with the bath fluid being changed every 15 min. Concentration-response curves for Bk were generated following the addition of the peptide to the tissue bath, and pD_2 values ($-\log EC_{50}$) were calculated by regression analysis of logit-transformed data. In our initial experiments Bk was added to the bath in a cumulative fashion. For reasons discussed later, however, we conducted most of the concentration-response experiments noncumulatively, with Bk being washed out between successive applications and 15–20 min elapsing between each exposure.

The abilities of the following analogs to inhibit Bk-induced responses were examined: NPC361, NPC349, NPC414, NPC431, and NPC567.

Because the effects of Bk or its antagonists, in isolated tissues, may be influenced by endogenous peptidase enzymes, we conducted a series of experiments in the presence of MGTPA, captopril, and DL-thiorphan, each at 10 μM, to inhibit kininase I, kininase II, and neutral endopeptidase, respectively. In these studies we examined the ability of NPC567 (3, 10, and 30 μM) to antagonize Bk-induced contractions of epithelium-denuded trachea.

Bradykinin binding. Binding of [^{3}H]Bk was performed using previously described methods (12). Parenchymal lung, tracheal smooth muscle, and ileum were obtained from male Hartley guinea pigs (150–450 g). Because of the limited amount of tissue (approximately 50 mg) in a single guinea pig, we dissected the smooth muscle free from the

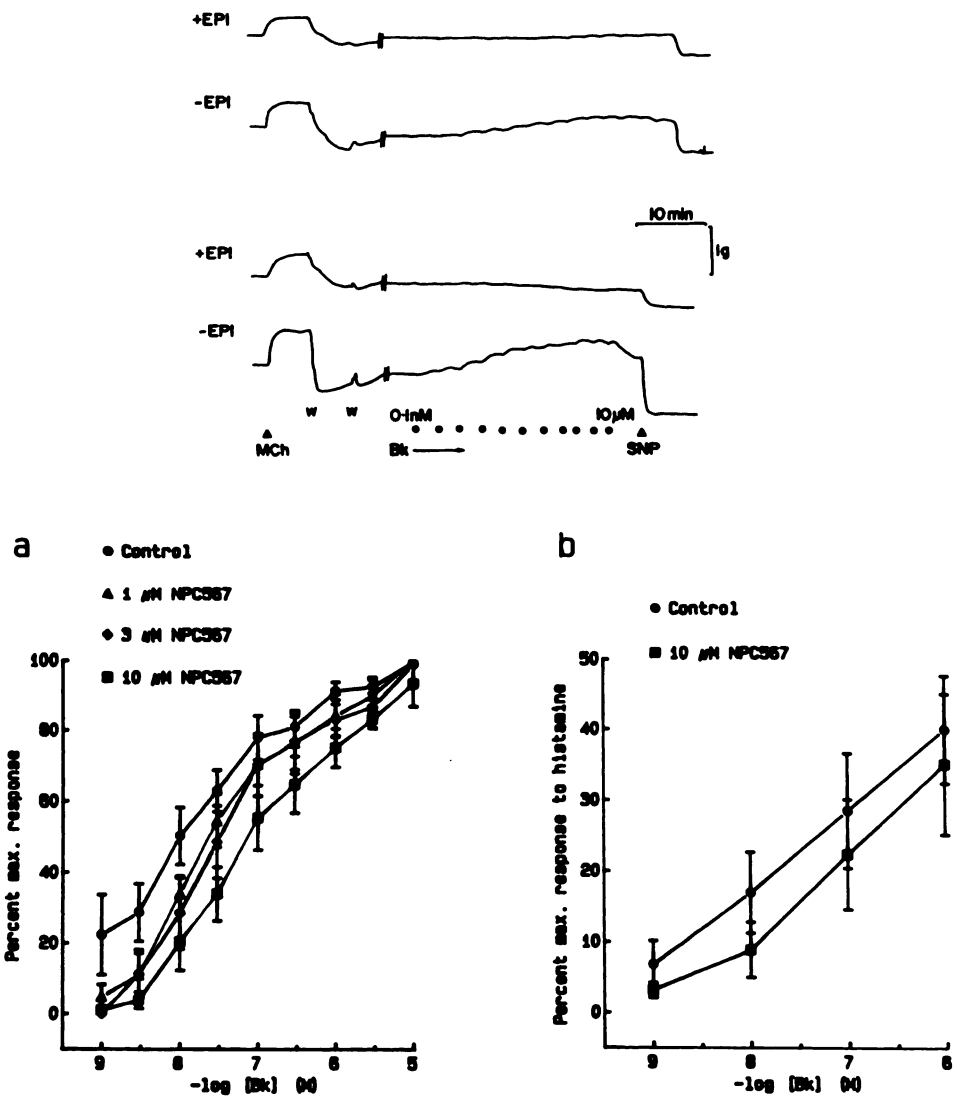

Fig. 3. Effect of NPC567 on Bk-induced contraction of isolated airways. *Top*, Representative tracings of responses of tracheal smooth muscle to Bk. *+Epi*, preparations with intact epithelium; *−Epi*, tissues in which the epithelial layer was removed (15). *MCh* indicates a reference response to methacholine (*+Epi*, 2 μM; *−Epi*, 1 μM). Sodium nitroprusside (*SNP*) (30 μM) was added to the bath at the end of each experiment to ascertain baseline tension. *w* denotes wash. *Bottom*, *a*, Concentration-response curves for Bk in epithelium-denuded trachea, in the presence and absence of NPC567. Each point is mean ± standard error of at least six observations. *b*, Effect of NPC567 (10 μM) on Bk-induced contraction of lung parenchymal strips. Each *point* is derived from five observations.

tracheae of 20 animals for each tracheal binding study. These were pooled, minced finely, weighed, placed in 20 volumes of ice-cold buffer A (25 mM TES, pH 6.8, containing 0.2 $mg \cdot ml^{-1}$ 1,10-phenanthroline), and homogenized using a Polytron Tissumizer at setting 6 for 15 sec. An entire lung or a segment of terminal ileum was blotted, diced, weighed, and treated in a manner similar to trachea.

Binding studies were also performed with ovine lung and trachealis. These tissues, obtained locally from freshly slaughtered, 1-year-old lambs, were treated exactly as described for guinea pig. Homogenates were centrifuged at 50,000 × *g* for 10 min, the supernatant was discarded, and the pellets were resuspended in ice-cold buffer A. Each type of tissue was homogenized and centrifuged three times and was finally resuspended in buffer A that contained bovine serum albuminn (1 $mg \cdot ml^{-1}$), bacitracin (140 $\mu g \cdot ml^{-1}$), and captopril (10 μM), to a final volume of 170 $ml \cdot g^{-1}$ (original wet weight) for lung or ileum, 50 $ml \cdot g^{-1}$ for guinea pig trachea, and 25 $ml \cdot g^{-1}$ for sheep trachea.

The binding assay was performed in triplicate in 12 × 75 mm polypropylene tubes and contained 50 μl of [^{3}H]Bk (20,000 dpm, approximately 0.3 nM, for competition displacement and 200 to 400,000 dpm for saturation binding), 100 μl of displacing ligand in buffer A, 750 μl of tissue, and additional buffer A plus albumin and bacitracin, to bring the final volume to 1 ml. To determine nonspecific binding, triplicate tubes contained 1 μM unlabeled Bk. For saturation binding, at each concentration of label triplicate tubes containing 1 μM Bk were included. For competition studies, 11 concentrations of each displacing ligand were used and, for saturation binding, 12 concentrations of unlabeled Bk were used. Tubes were incubated at ambient temperature for 90 min, and the assay was terminated by filtration over Whatman GF/B glass fiber filters that were pretreated for 2 hr with polyethyleneimine (2 $mg \cdot ml^{-1}$), using a Brandel Tissue Harvester. This was followed by washing four times with 1-ml aliquots of ice-cold Tris (50 mM, pH 7.4). Filters were dissolved overnight in Ready-Safe Fluor (Beckman). K_d and K_i values were determined using EBDA (17) followed by LIGAND (18).

Drugs. Atropine sulfate, pyrilamine maleate, prazosin hydrochloride, DL-propranolol hydrochloride, DL-thiorphan, and indomethacin were obtained from Sigma Chemical Co. (St. Louis, MO). Bk, des-Arg^9-Bk, and des-Arg^9-[Leu^8]-Bk (acetate salts) were obtained from either Sigma or Bachem (Torrance, CA). The Bk analogs NPC414 and NPC431 were synthesized by J. M. Stewart and R. J. Vavrek (University of Colorado Medical School, Denver, CO). NPC567 was synthesized by Abbott Laboratories (North Chicago, IL). NPC349 was purchased from Bachem Biosciences Inc. (Switzerland) and NPC361 from Bachem. MGTPA (Plummer's Inhibitor) was purchased from Calbiochem (La Jolla, CA), captopril from Squibb Pharmaceuticals Inc. (Princeton, NJ), and cyproheptadine hydrochloride from Aldrich Chemical Co. (Milwaukee, WI). Unless otherwise stated, all drugs were dissolved immediately before use in distilled water or saline. Indomethacin was dissolved, as a 30 mM stock solution, in 100 mM Na_2CO_3 solution and stored at −20° until use. DL-Thiorphan was solubilized in a few drops of dimethylsulfoxide, and subsequent dilutions made in saline.

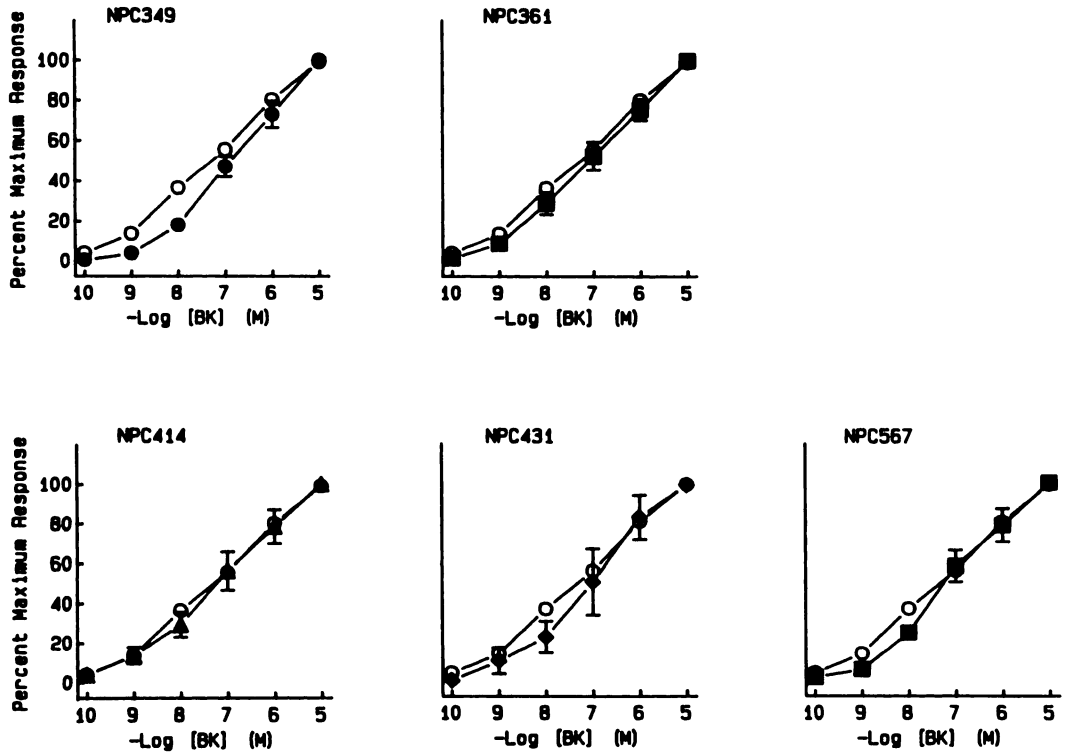

Fig. 4. Effect of five peptide analogs (each at 10 μM) on Bk concentration-response curves in epithelium denuded trachea. *Open symbols*, controls; *filled symbols*, in presence of antagonist. Each *point* represents mean ± standard error of 26 (control), 7 (NPC349), 6 (NPC361), 4 (NPC414), 4 (NPC431), or 5 (NPC567) experiments.

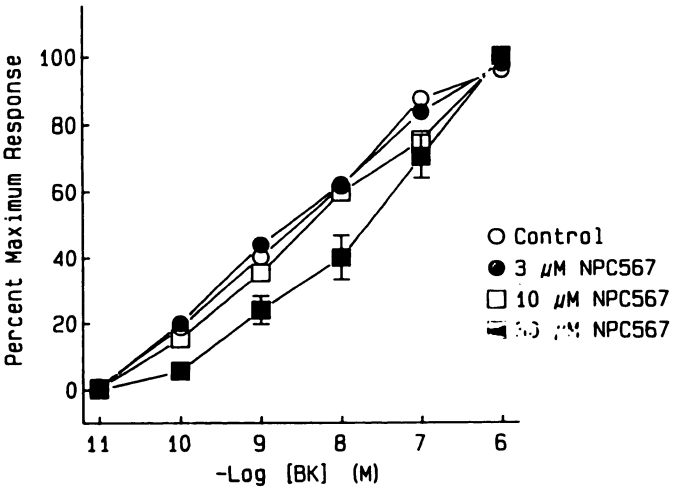

Fig. 5. Effect of NPC567 on Bk-induced contraction of epithelium-denuded trachea in the presence of captopril, thiorphan, and MGTPA (10 μM). Each *point* represents the mean of at least 12 observations. For clarity, some of the *error bars* are omitted.

Results

Effects of Bk and NPC567 on Airway Resistance *In Vivo*

Bk (1–100 $\mu g \cdot kg^{-1}$ IV) caused a marked, dose-dependent increase in PIP, which was partially inhibited by NPC567 (100 $\mu g \cdot kg^{-1}$, IV; Fig. 1a). A lower dose of NPC567 was without effect, whereas a higher dose (300 $\mu g \cdot kg^{-1}$) caused no further inhibition. Inhaled NPC567 also partially inhibited bronchoconstriction caused by IV Bk (Fig. 1b), although the antagonist was not potent. Likewise, inhaled NPC349 had minimal effects on Bk-induced bronchoconstriction (Fig. 2). Inhaled Bk (1, 10, and 30 $mg \cdot ml^{-1}$) caused dose-dependent bronchoconstriction that was not blocked by inhaled NPC567 (10 and 30 $mg \cdot ml^{-1}$, five experiments; data not shown). Neither NPC349 nor NPC567, at the doses studied, had any effect on basal PIP.

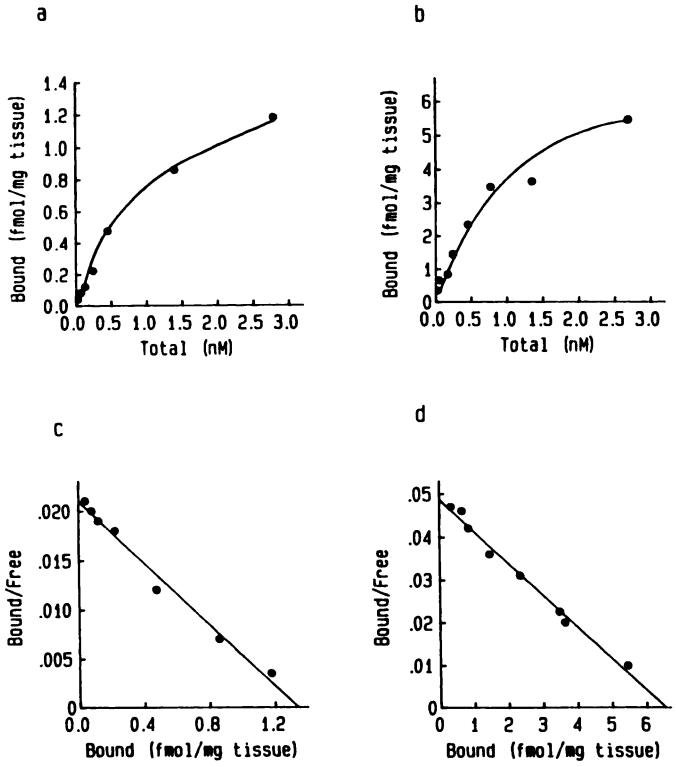

Fig. 6. Saturation binding of Bk to guinea pig trachea and lung. a, Saturation in trachea; b, saturation in lung. c, Scatchard transformation in trachea; d, Scatchard transformation in lung, with lines of best fit calculated by LIGAND (18).

Effects of Bk and NPC567 on Isolated Airway Smooth Muscle

In isolated trachealis, Bk caused contraction only in preparations denuded of epithelium (Fig. 3). In tissues with an intact epithelium, Bk either caused small relaxations or, more usually,

TABLE 1

Binding parameters for Bk in guinea pig tissues

Numbers in parentheses represent number of observations. In each observation for trachea, data were obtained from tissues pooled from 20 animals (see Materials and Methods for explanation).

Tissue	K_d	B_{max}	Hill Coefficient
	pM	fmol · mg of tissue^{-1}	
Ileum	92 ± 22 (10)	38.0 ± 9.0	0.94 ± 0.05
Lung	302 ± 78 (8)	7.5 ± 0.9	1.02 ± 0.03
Trachea	284 ± 28 (3)	1.1 ± 0.3	1.01 ± 0.01

had very little effect (Fig. 3). All subsequent experiments were carried out, therefore, in tracheal preparations lacking an intact epithelium. The excitatory effect of Bk in trachea was only slightly affected by NPC567 (Fig. 3a). Similarly, NPC567 (10 μM) did not antagonize Bk-induced contraction of lung strips (Fig. 3b). Antagonists (each at 1 μM) of the following receptors had no effect on tracheal sensitivity to Bk: muscarinic (atropine), serotonin and histamine (cyproheptidine), histamine (pyrilamine), α-adrenoceptors (prazosin), and β-adrenoceptors (propranolol). Bk-induced contractions were attenuated by indomethacin (3 μM), confirming that they are mediated by excitatory prostanoids. The pD_2 value of 8.10 ± 0.17 (seven experiments) for Bk in controls was significantly greater ($p < 0.001$) than that of 6.10 ± 0.12 (seven experiments) in the presence of indomethacin. In the presence of indomethacin, NPC567 (10 μM) was without effect on Bk-induced tracheal contraction (control pD_2, 6.10; pD_2 in presence of NPC567, 6.08 ± 0.17). The B_1-selective agonist des-Arg^9-Bk (10 μM) had no effect on tracheal or lung strip tone (three experiments).

The effects of several Bk analogs on Bk-induced contractions of denuded trachea are shown in Fig. 4. Unlike the experiments described above, these curves were obtained noncumulatively because less variable responses resulted. The reasons for this observation are unclear. Bk causes relaxation of trachea that has been precontracted, and it is possible that the response to a given concentration of Bk, in preparations that already have a degree of Bk-induced tone, is the additive effect of qualitatively different responses. Thus, Bk-induced contraction and relaxation may functionally antagonize each other. When experiments were conducted noncumulatively, basal tone was achieved between each application of Bk. It is clear that Bk-induced tracheal contractions were unaffected by NPC361, NPC414, NPC431, or NPC567, whereas NPC349 may have caused a slight rightward shift (Fig. 4).

Effect of Peptidase Inhibitors

As described in Materials and Methods, we conducted a series of experiments in the presence of MGTPA, captopril, and DL-thiorphan, each at 10 μM, to inhibit kininases I and II and neutral endopeptidase, respectively. The pD_2 value for Bk was 8.10 ± 0.17 (seven experiments) in controls and 8.62 ± 0.18 (14 experiments) in the presence of the peptidase inhibitors. Thus, inhibition of kininases I and II as well as neutral endopeptidase caused a small, 3.3-fold, leftward shift in the Bk concentration-response curve, which was statistically significant ($p < 0.05$) by Student's t test. In the presence of peptidase inhibitors, NPC567 had minimal effects on Bk-induced contraction (Fig. 5). Only at a concentration of 30 μM did NPC567 cause a rightward shift in the Bk concentration-response curve. When the pD_2 value for Bk of 8.62 ± 0.18 in controls was compared (Student's t test) with that of 8.09 ± 0.18 (12 experiments) in the presence of 30 μM NPC567, the p value of 0.05 indicated

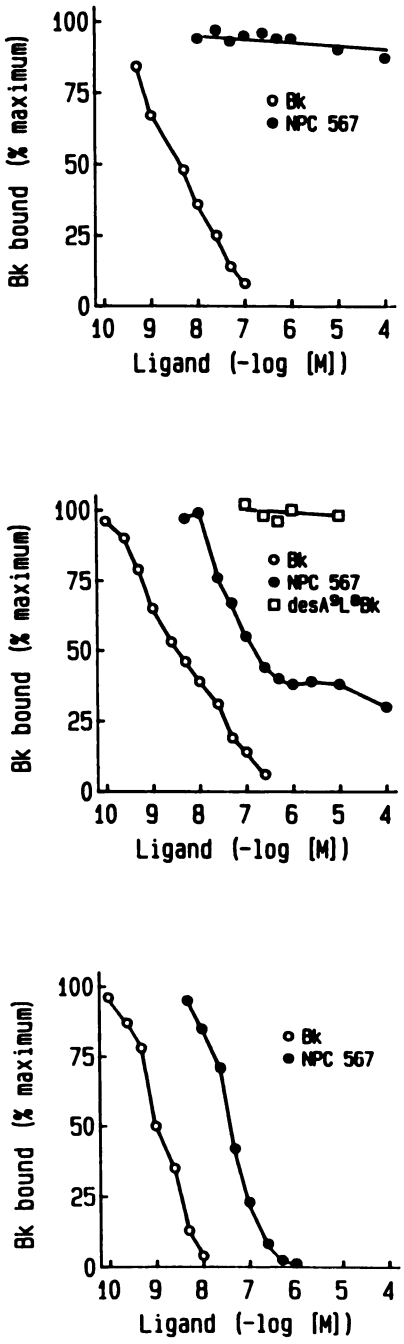

Fig. 7. Effect of NPC567 on Bk binding in guinea pig tissues, trachea (*top*), lung (*middle*), and ileum (*bottom*). [^{3}H]Bk was 0.2 nM, and LIGAND corrected for Bk when determining K_i values. The K_i value for NPC567 in lung was determined using LIGAND set for a two-site model (18) and was 31 ± 11 nM (four experiments) for the high affinity site and greater than 100 μM for the low affinity site. The K_i value for des-Arg^9-[Leu^8]-Bk was in excess of 100 μM both in trachea and lung. The K_i value for NPC567 in trachea was also greater than 100 μM. In ileum, NPC567 caused 100% displacement of [^{3}H]Bk with a K_i of 29 ± 4 nM (eight experiments).

marginal statistical significance. Thus, in the presence of peptidase inhibitors, NPC567 (30 μM) caused a 3.4-fold rightward shift in the Bk concentration-response curve. The B_1 receptor antagonist des-Arg^9-[Leu^8]-Bk (10 μM) did not antagonize Bk-induced responses (pD_2 for Bk in the presence of B_1 antagonist, 8.99 ± 0.15; three experiments). In the presence of the peptidase inhibitor cocktail, higher concentrations of the Bk antagonists

TABLE 2

Bk binding displacement in guinea pig airways by Bk analogs

Numbers in parentheses represent number of observations ± standard error.

Analog (10 μM)[a]	Displacement	
	Lung	Trachea
	%	
NPC349	63 ± 2 (3)	8
NPC361	56 ± 4 (3)	6
NPC414	59 ± 1 (3)	12
NPC431	64 ± 4 (3)	21
NPC567	64 ± 4 (8)	12
des-Arg^9-Bk	−10 ± 1 (3)	−15
des-Arg^9-[Leu^8]-Bk	−11 ± 2 (3)	−8

[a] Per cent inhibition of [^{3}H]Bk was the same at 1 and 10 μM for each displacing ligand.

TABLE 3

Binding parameters for Bk in sheep pulmonary tissues

Numbers in parentheses represent number of observations.

Tissue	K_d	B_{max}	Hill Coefficient
	pM	fmol · mg of tissue^{-1}	
Lung	128 ± 60 (2)	5.2 ± 2.4	0.94 ± 0.04
Trachea	342 ± 54 (5)	0.8 ± 0.3	1.01 ± 0.02

often had effects themselves, consisting either of a transient contraction or a transient relaxation. This was not a concentration-dependent phenomenon and diminished with successive applications.

Ligand Binding Studies

Guinea pig tissues. As seen (Fig. 6, a and b), guinea pig trachea and lung membranes both contain saturable binding sites for Bk, for which Scatchard analyses indicated single affinity binding sites (Fig. 6, c and d). Specific binding of Bk (0.3 nM) was 65, 75, and greater than 95% in guinea pig trachealis, lung, and ileum, respectively; binding parameters for Bk in guinea pig tissues are summarized in Table 1. NPC567 was able to displace 64 ± 4% (seven experiments) (Table 2) of specifically bound Bk in lung membranes but had no significant effect on binding of [^{3}H]Bk to trachea (Fig. 7). In contrast, [^{3}H]Bk binding in guinea pig ileum was displaced 100% by NPC567 (Fig. 7). The B_1 antagonist des-Arg^9-[Leu^8]-Bk (100 μM) had no effect on [^{3}H]Bk binding in lung or trachea (Fig. 7).

Binding of Bk in lung and trachea was not influenced by captopril (up to 300 μM), suggesting that the NPC567-resistant Bk binding site is not kininase II. Indeed, [D-Phe^7]-substituted analogs are not substrates for kininase II (19). To ascertain the ability of bacitracin, captopril, and 1,10-phenanthroline in the binding medium to inhibit Bk degradation, trichloroacetic acid (final concentration, 10%) was added at the end of the 90-min incubation period. Bk was measured using high pressure liquid chromatography with radioactivity detection, as described elsewhere (19). As reported previously in ileum (12, 20), no metabolism of Bk occurred in guinea pig airway tissue binding media. In the presence of NPC567 (100 μM), lung Bk binding was not displaced further by various agents, including substance K, substance P, angiotensin II, neurotensin(1–8), neurotensin(1–11), somatostatin(1–14), arginine-vasopressin, vasoactive intestinal peptide, and atropine (each at 10 μM).

NPC349, NPC361, NPC414, and NPC431 each exhibited binding activity in guinea pig lung and trachealis similar to that described for NPC567, and the data are summarized in Table 2. These agents caused 55–65% displacement of labeled Bk in lung membranes but had no significant effect in trachealis. Neither des-Arg^9-Bk nor des-Arg^9-[Leu^8]-Bk had any effect on Bk binding in guinea pig pulmonary tissues (Table 2).

Sheep tissues. Bk binding was examined in sheep tissues to determine whether the apparently unique binding site observed in guinea pig pulmonary tissue is specific to that species. In addition, sheep provided more adequate quantities of trachealis smooth muscle for binding. In sheep tissue, specific binding was 84% in lung and 75% in trachealis. Sheep lung and trachealis exhibited binding parameters similar to those in guinea pig (Table 3). In sheep trachea NPC567 (10 μM) displaced 28 ± 4% (five experiments) and in lung 73 ± 9% of bound Bk (Fig. 8). In lung it appeared that NPC567 may compete with the second, low affinity binding site, although this was evident only at 100 μM NPC567 (Fig. 8).

Discussion

To our knowledge, there are no previous reports of the effects of Bk antagonists on bronchoconstriction *in vivo* or *in vitro*.

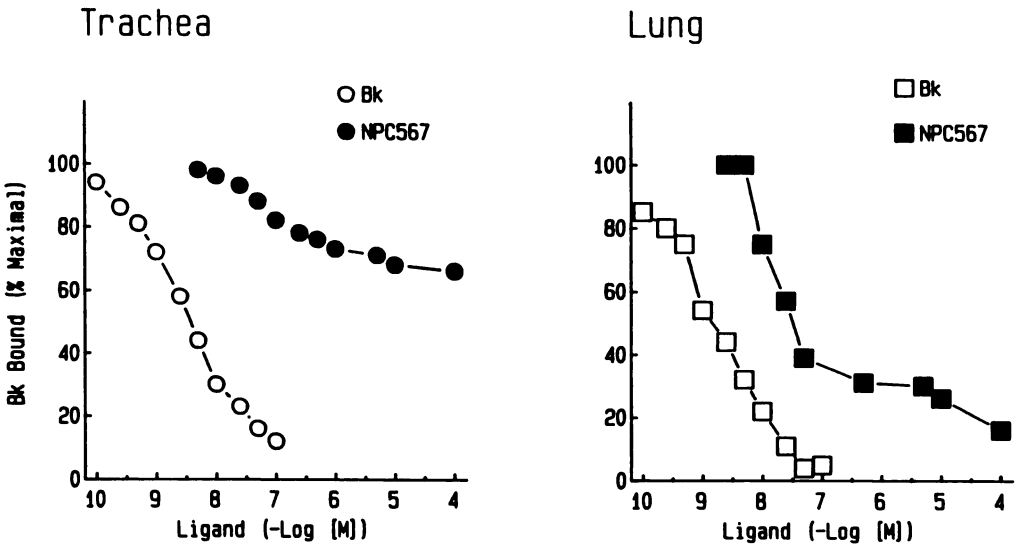

Fig. 8. Effect of NPC567 on Bk binding in sheep trachealis and lung. [^{3}H]Bk was 0.2 nM, and LIGAND corrected for Bk when determining K_i values. The K_i value for NPC567 in lung was determined using LIGAND set for a two-site model (18) and was 30 nM (two experiments) for the high affinity site. In sheep tracheal binding, the K_i value for the first component was 29 ± 5 nM (four experiments), although in another two tissue samples LIGAND was unable to provide K_i values.

NPC349 and NPC567, selective B_2 receptor antagonists, were relatively weak inhibitors of Bk-induced increases in PIP *in vivo* in anesthetized guinea pigs (Figs. 1 and 2). Although the present data provide no information on differential effects of Bk on airway resistance as opposed to compliance, a previous report demonstrated that Bk (IV) increases airway resistance and decreases compliance to a similar degree (21). Moreover, that measurements of airway overflow pressure provide an index of intrathoracic resistance is well established (14, 21). It is clear that the Bk-induced bronchoconstriction, in the present study, was only poorly inhibited by the two B_2 receptor antagonists. Furthermore, studies with isolated airway smooth muscle demonstrated that several specific B_2 antagonists (3, 4, 12, 13) were inactive against Bk-induced contractile responses (Figs. 3 and 4).

Although it is possible that the weak activity of the antagonists *in vivo* may be due to metabolism, it is unlikely that this could explain their inability to antagonize Bk in isolated tissues. The cocktail of peptidase inhibitors caused a modest potentiation in sensitivity to Bk, indicating that endogenous kininase I, kininase II, and neutral endopeptidase do exert some influence on responses to Bk. However, in trachea NPC567 exhibited only slight antagonist activity at a concentration of 30 μM, even in the presence of the peptidase inhibitors (Fig. 5). The effects of the inhibitors on sensitivity to Bk in the present study are qualitatively similar, although smaller in magnitude, to observations in ferret airway smooth muscle (9). In addition, NPC567 did not antagonize the effects of Bk in lung parenchymal strip (Fig. 3).

This latter observation implies that the NPC567-sensitive bronchoconstriction *in vivo* results from activation of cell types different than those contributing to contraction of isolated trachea or parenchymal strips. Bk-induced bronchoconstriction probably results both from direct effects on airway smooth muscle and via a vagal reflex following stimulation of afferent C-fibers (7, 22). The lung parenchymal strip is an anatomically complex tissue and several cell types, including airway and vascular smooth muscle, as well as alveolar interstitial cells contribute to its contractile responses (23).

The B_1 agonist des-Arg^9-Bk was without effect in guinea pig trachea and the B_1 antagonist des-Arg^9-[Leu^8]-Bk did not inhibit Bk-induced contractions, indicating the absence of this receptor subtype. Finally, antagonists of several autonomic receptors did not affect tracheal responses to Bk. Thus, the pulmonary effects of Bk in guinea pigs are mediated by specific receptors that are of neither the B_1 nor the B_2 subtype.

Ligand binding studies corroborate the isolated tissue studies. Whereas Bk binding parameters in pulmonary tissues were similar to those previously described in ileum (12), the analogs caused virtually no displacement of [^{3}H]Bk in guinea pig trachealis. In contrast, the analogs displaced approximately 60% of total specific binding in lung parenchyma. These data further suggest that the Bk binding sites in guinea pig trachea and 40% of those in lung are not B_2 receptors. In addition, because a B_1 agonist and antagonist did not displace Bk, the binding site is not a B_1 receptor. Finally, several other bioactive peptides, including substance P, neurokinin A, angiotensin II, and vasoactive intestinal peptide, did not displace lung Bk binding.

The Bk binding site described in guinea pig airways appears not to be unique to this species, because studies in ovine pulmonary tissues indicated that, whereas NPC567 displaced approximately 70% of labeled Bk in lung, only 28% was displaced in trachea. It is interesting that no displacement of [^{3}H]-Bk by antagonists was evident in guinea pig tracheal smooth muscle, yet there apparently was a small population of B_2 receptors in sheep trachea. This discrepancy may represent a true species difference. Alternatively, relatively greater quantities of sheep tracheal smooth muscle are available for binding analyses. Because the B_{max} in trachealis was only around 1 fmol·mg of tissue^{-1}, we would require greater than 20% of receptors to be sensitive to NPC567 in order to achieve greater than the available counting efficiency in our assay (3%). A small population of B_2 receptors in guinea pig trachealis, undetectable in the binding assay, may have been responsible for the modest effects of NPC567 (Fig. 3) and NPC349 (Fig. 4) on Bk-induced tracheal contractions.

The inability of the Bk antagonists to displace Bk is unlikely to be due to their being degraded in the binding assay, for several reasons. Firstly, we have previously reported that several Bk analogs are not metabolized under identical binding conditions (12) and are not substrates for kininase II (19). Moreover, the binding medium contains the enzyme inhibitors bacitracin, 1,10-phenanthroline, and captopril, which inhibit Bk degradation (12, 20).

The variable effects of Bk in tracheal strips containing an intact epithelium may be due to the known inhibitory influence of the airway epithelium on the effect of several bronchoconstrictors (24). It is possible that Bk releases smooth muscle inhibitory prostanoids from epithelial cells in this tissue, as it does in canine tracheal epithelium (25). Preliminary reports indicate that the epithelium has an inhibitory effect on responses of guinea pig tracheal smooth muscle to Bk (26, 27).

In conclusion, the bronchoconstrictor effect of BK appears to be mediated in part by a novel receptor subtype, which we propose be classified 'B_3'. It is possible that NPC567-resistant bronchoconstriction may represent the effect of Bk on these receptors and that there is a heterogeneity of Bk receptor distribution in the airways, with a predominance of B_2 receptors in peripheral airways and B_3 receptors in large airways. Further characterization of the B_3 receptor, using different Bk antagonists as well as other novel analogs of Bk, may be relevant to our understanding of the role of Bk in the pulmonary system and its involvement in inflammatory airway diseases such as bronchial asthma.

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