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Airway effects of inhaled bradykinin, substance P, and neurokinin A in sheep

W. M. Abraham, PhD,* A. Ahmed, BVSC,* A. Cortes, BS,* M. Soler, MD,** S. G. Farmer, PhD,*** L. E. Baugh, PhD,**** and S. L. Harbeson, PhD****
Miami Beach, Fla., Basel, Switzerland, Baltimore, Md., and Cincinnati, Ohio

*The effects of inhaled bradykinin (BK), substance P (SP), and neurokinin A (NKA) on pulmonary resistance and airway responsiveness to carbachol were studied in conscious allergic sheep. Inhaled BK (20 breaths, 0.1 to 5.0 mg · ml⁻¹) caused dose-dependent increases in pulmonary resistance. Neither inhaled SP nor NKA (20 breaths, 0.1 to 1.0 mg · ml⁻¹) produced significant bronchoconstriction in allergic sheep. However, the response to SP could be enhanced (p < 0.05) by pretreatment with the neutral endopeptidase inhibitor, thiorphan (40 breaths, 1 mg · ml⁻¹). Sheep that were allergic to *Ascaris suum* antigen were 5.9 times (p < 0.05) more sensitive to the constrictor effects of BK than nonallergic sheep. BK-induced bronchoconstriction was blocked in a dose-dependent fashion by the BK β_2 -receptor antagonist, NPC 567 (D-arginine{hydroxyproline³, D-phenylalanine⁷}BK). Atropine (0.2 mg · kg⁻¹, intravenously) and nedocromil sodium (1 mg · kg⁻¹ in 3 ml of saline, aerosolized) significantly inhibited the BK-induced bronchoconstriction by 97% and 43%, respectively. Chlorpheniramine (2 mg · kg⁻¹, intravenously) had no effect. NKA caused a transient increase in airway responsiveness in allergic sheep, producing a mean 1.9-fold leftward shift in dose-response curves to aerosolized carbachol (p < 0.05). This hyperresponsiveness was not evident 24 hours after NKA challenge. Neither SP nor BK changed airway responsiveness. Thus, in allergic sheep, inhaled BK caused a more pronounced bronchoconstriction than that observed in nonallergic sheep. The bronchoconstriction was blocked by a BK-receptor antagonist and appeared to be partially mediated via cholinergic reflexes. The sensory neuropeptides, unlike BK, were not potent bronchoconstrictors when they were administered by inhalation, but NKA may play a role in modulating airway responsiveness. (J ALLERGY CLIN IMMUNOL 1991;87:557-64.)*

From *Mount Sinai Medical Center, Miami Beach, Fla.; **Medical University Klinik, Basel, Switzerland; ***Nova Pharmaceutical Corp., Baltimore, Md.; and ****Merrell Dow Research Institute, Cincinnati, Ohio.

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Reprint requests: William M. Abraham, PhD, Department of Research, Mount Sinai Medical Center, 4300 Alton Road, Miami Beach, FL 33140.

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Abbreviations used

BK:	Bradykinin
SP:	Substance P
NKA:	Neurokinin A
R_L :	Pulmonary resistance
SR_L :	Specific lung resistance
V_{tg} :	Thoracic gas volume
PD_{400} :	Dose of carbachol causing a 400% increase in SR_L
ED_{200} :	Dose of BK causing a 200% increase in R_L
SG_{aw} :	Specific airway conductance
NPC 567:	D-Arginine(hydroxyproline ³ , D-phenylalanine ⁷) bradykinin

BK is a potent inflammatory peptide that is generated from kininogens by plasma and tissue kallikrein.¹ There is some evidence that BK may play a role in the pathogenesis of asthma, but the exact mechanism is unclear. BK causes bronchoconstriction in subjects with asthma but not in normal volunteers,^{2,4} and there is evidence for BK release after antigen challenge.^{5,6} Furthermore, a BK antagonist, NPC 567,⁷⁻¹⁰ can modify some of the pathophysiologic events associated with antigen challenge, including antigen-induced hyperresponsiveness.¹¹ BK also stimulates sensory neurons in the airways, causing the release of the neuropeptides, SP and NKA. These peptides coexist in capsaicin-sensitive sensory neurons (nonmyelinated afferent nerves or C fibers)¹² and are potent constrictors of airway smooth muscle in vitro.¹³ Therefore, stimulation of these sensory neurons could influence airway tone. There is additional evidence that these peptides may influence airway responsiveness as well. Tachykinins have been implicated in the airway hyperresponsiveness induced by toluene diisocyanate in guinea pigs,^{14,15} and intravenously administered BK and SP have been reported to increase airway responsiveness in this species.¹⁶

Therefore, to understand better the roles of these peptides in the control of airway tone and airway responsiveness, the effects of inhaled BK, SP, and NKA on these variables were compared in sheep allergic to *Ascaris suum* antigen. Specifically, the bronchoconstrictor effects of these agents, the pharmacology of this bronchoconstriction, and the influence of these peptides on airway responsiveness was studied.

MATERIAL AND METHODS**Animal preparation**

Twelve adult sheep (mean weight, 33 kg; range, 25 to 52 kg) were used in these experiments. Seven animals were

defined as allergic and five as nonallergic, based on the presence or absence of a bronchoconstrictor response to a standard inhalation challenge with *Ascaris suum* antigen.¹⁷ Animals were conscious throughout the procedures, of which all were approved by the Mount Sinai Medical Center Animal Research Committee.

Measurement of airway mechanics

These methods have been described in detail previously.¹⁸ The unsedated sheep were restrained in a cart in the prone position with their head immobilized. After topical anesthesia of the nasal passages with 2% lidocaine solution, a balloon catheter was advanced through one nostril and placed in the lower esophagus. The animals were then intubated with a cuffed endotracheal tube through the other nostril with fiberoptic bronchoscopy. Pleural pressure was estimated with the esophageal balloon catheter, filled with 1 ml of air, which was positioned in the lower esophagus, where the end expiratory pressure ranged between -2 and -5 cm H₂O. Lateral pressure in the trachea was measured with a side-hole catheter, advanced through and positioned distal to the endotracheal tube. Transpulmonary pressure was obtained from the pressure difference between the esophageal pressure and tracheal pressure, as measured by a differential pressure transducer (type P-7D, Pace Engineering, Hollywood, Calif.). This pressure transducer catheter system revealed no phase shift between pressure and flow up to a frequency of 9 Hz. For the measurement of R_L , the proximal end of the endotracheal tube was connected to a pneumotachograph (Fleisch, Dyna Sciences, Inc., Blue Bell, Pa.). While the animals were breathing spontaneously, the signals of flow and transpulmonary pressure were recorded on-line with a PDP-11 digital computer (Digital Equipment, Maynard, Mass.) for calculation of R_L from transpulmonary pressure, respiratory volume (obtained by flow integration), and flow, with midtidal volume technique.¹⁹ Analysis of at least eight breaths was used to determine R_L in cm H₂O · L⁻¹ · sec. In some studies, V_{tg} gas volume was also measured in a constant volume body plethysmograph as previously described.¹⁷ When V_{tg} was measured, we computed specific lung resistance ($SR_L = R_L \times V_{tg}$) in L · cm H₂O · L⁻¹ · sec.

Aerosol delivery

All aerosols were generated with a disposable medical nebulizer (Raindrop, Puritan-Bennett, Lenexa, Kan.) that provided an aerosol with a mass median aerodynamic diameter of 3.6 μ m (geometric SD, 1.9), as determined by an Andersen (Andersen, Inc., Atlanta, Ga.) cascade impactor. The nebulizer was connected to a dosimeter system consisting of a solenoid valve and a source of compressed air (20 psi). The output of the nebulizer was directed into a plastic T piece, one end of which was connected to the inspiratory port of a Harvard (Harvard Apparatus, Inc., South Natick, Mass.) respirator. The solenoid valve was activated for 1 second at the beginning of the inspiratory cycle of the respirator. All aerosols were delivered at a tidal volume of 500 ml and a rate of 20 breaths per minute. This method of aerosol challenge has been used extensively in

the past and has no adverse or nonspecific effects with regards to the physiologic end points measured (i.e., pulmonary mechanics).^{20, 21}

Dose-response curves to inhaled carbachol

Dose-response curves were performed as previously described for this model.²² To assess airway responsiveness, we performed cumulative dose-response curves to carbachol by measuring SR_L immediately after inhalation of buffer and after each administration of 10 breaths of increasing amounts of carbachol (percent weight per volume in buffered saline: 0.25, 0.5, 1.0, 2.0, and 4.0). Bronchoprovocation was stopped when SR_L increased >400% from the postsaline value or after the highest carbachol concentration had been delivered. Airway responsiveness was determined by computing the cumulative carbachol dose (in breath units) that increased SR_L by 400% over the postsaline value (PD_{400}) by interpolation from the dose-response curve: SR_L (ordinate) versus cumulative carbachol dose in breath units (abscissa).²² One breath unit of carbachol was defined as one breath of an aerosolized solution containing 1.0% carbachol.

Material

BK acetate salt, SP acetate salt, DL-thiorphan, and carbachol chloride were obtained from Sigma Chemical Co. (St. Louis, Mo.). BK and SP were dissolved in 0.9% NaCl solution (saline) containing 10% ethanol. Thiorphan was dissolved in saline at a concentration of $1 \text{ mg} \cdot \text{ml}^{-1}$. NKA, synthesized (by S. H.) at the Merrell Dow Research Institute, was dissolved in 0.2% acetic acid and diluted in buffered saline. The BK receptor antagonist, NPC 567 (Nova Pharmaceutical Corp., Baltimore, Md.), was dissolved in buffered saline at concentrations of 2, 5, and $10 \text{ mg} \cdot \text{ml}^{-1}$. The histamine H_1 -antagonist, chlorpheniramine maleate (USP injection, Schering Corp., Kenilworth, N.J.) was administered undiluted ($2 \text{ mg} \cdot \text{kg}^{-1}$). Atropine sulphate solution (Elkin-Sinn, Inc., Cherry Hill, N.J.) was diluted with saline to a final concentration of $0.2 \text{ mg} \cdot \text{kg}^{-1}$. Nedocromil sodium ($1 \text{ mg} \cdot \text{kg}^{-1}$) (Fisons plc., Loughborough, U.K.) was diluted in 3 ml of buffered saline and delivered as an aerosol. Doses of atropine, chlorpheniramine, and nedocromil were based on previous studies in which these agents blocked muscarinic agonist-, histamine agonist-, and antigen-induced bronchoconstriction, respectively, in conscious sheep.²³⁻²⁵ *Ascaris suum* extract (Greer Diagnostics, Lenoir, N.C.) was diluted with buffered saline to a concentration of 82,000 protein nitrogen units $\cdot \text{ml}^{-1}$ and delivered as an aerosol.

Experimental protocol

Bronchoconstrictor effects of inhaled BK, SP, and NKA

In five allergic sheep, measurements of R_L were obtained before and immediately after each administration of 20 breaths of increasing concentrations of peptide solutions (0.1, 0.5, and $1.0 \text{ mg} \cdot \text{ml}^{-1}$). In a separate experiment, these five sheep were pretreated with 40 breaths of thiorphan

($1 \text{ mg} \cdot \text{ml}^{-1}$) 30 minutes before inhalation challenge with a single dose of SP (20 breaths, $0.5 \text{ mg} \cdot \text{ml}^{-1}$).

The time course of the BK-induced bronchoconstriction was determined in seven allergic sheep. Baseline measurements of R_L were obtained before and after 20 breaths of the BK diluent. The sheep were then challenged with 20 breaths of $0.5 \text{ mg} \cdot \text{ml}^{-1}$ of BK. R_L was measured immediately after challenge and for every 5 minutes thereafter until 20 minutes after challenge.

In a separate series of experiments, dose-response curves to inhaled BK were performed in six allergic sheep. Measurements of R_L were obtained before and immediately after each administration of 20 breaths of increasing doses of BK (0.1, 0.5, 1.0, and $5 \text{ mg} \cdot \text{ml}^{-1}$). Bronchoprovocation was stopped after challenge with the $1.0 \text{ mg} \cdot \text{ml}^{-1}$ solution if the increase in R_L was >200% over baseline. Otherwise, the sheep were challenged with the $5 \text{ mg} \cdot \text{ml}^{-1}$ of aerosol. Airway responses were assessed by calculating the log dose of BK in cumulative breath units that increased R_L by 200% (log ED_{200}). One breath unit was defined as one breath of $0.1 \text{ mg} \cdot \text{ml}^{-1}$ of BK. Dose-response curves to inhaled BK were obtained in five nonallergic sheep for comparison with those obtained in allergic sheep. The allergic status of the sheep had been determined previously by measuring R_L before and immediately after the animals had been challenged with *Ascaris suum* antigen (400 breaths).

Pharmacology of BK-induced bronchoconstriction

When it was established that the maximum response to BK occurred immediately after challenge (see above), on different days, the sheep ($n = 6$) were treated 30 minutes before BK challenge with an aerosol of nedocromil sodium ($1 \text{ mg} \cdot \text{kg}^{-1}$ in 3 ml of buffer), atropine ($0.2 \text{ mg} \cdot \text{kg}^{-1}$, intravenously) or chlorpheniramine ($2 \text{ mg} \cdot \text{kg}^{-1}$, intravenously). R_L was measured before and after drug treatment and immediately after inhalation challenge with 20 breaths of $0.5 \text{ mg} \cdot \text{ml}^{-1}$ of BK.

The effect of the BK antagonist, NPC-567, on the dose-response curves to inhaled BK was determined in six allergic sheep. Dose-response curves to inhaled BK were obtained under control conditions and 30 minutes after treatment with 20 breaths of aerosol NPC 567 (final concentrations, 2, 5, and $10 \text{ mg} \cdot \text{ml}^{-1}$). Log ED_{200} was determined in the presence and absence of the antagonist. If R_L did not increase by 200% after challenge with the highest concentration of BK ($5 \text{ mg} \cdot \text{ml}^{-1}$), then for statistical purposes, this value was used to determine the cumulative breath units.

Effects of peptides on airway responsiveness

Carbachol dose-response curves were performed in five allergic sheep to establish baseline values for airway responsiveness. Then, on separate occasions, sheep were challenged with 20 breaths of $0.5 \text{ mg} \cdot \text{ml}^{-1}$ of aerosols of either SP, BK, or NKA. SR_L was measured before and immediately after challenge and then 30 minutes after challenge to ensure that there was no residual bronchoconstriction before

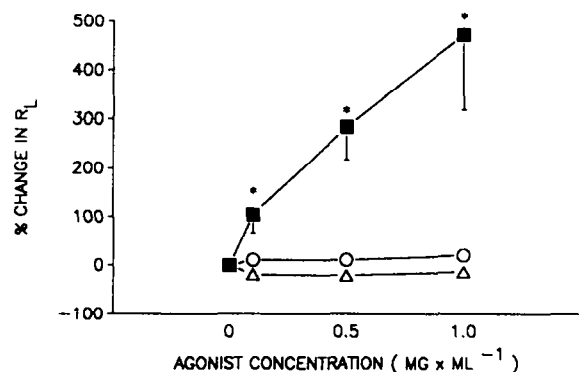


FIG. 1. Changes in R_L induced by inhaled BK (■), SP (○), and NKA (◇) in allergic sheep. Each point is the mean $\pm$ SE mean of five observations; * $p < 0.05$ versus baseline (0). Where not indicated, SE mean lies within the symbol.

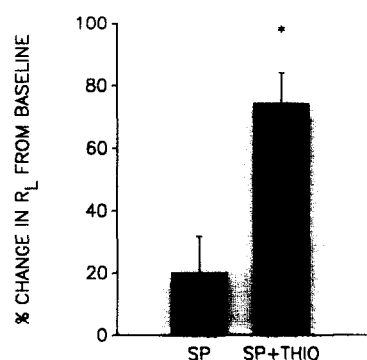


FIG. 2. Effect of thiorphan (Thio) on SP-induced increases in R_L in allergic sheep. Each point is the mean $\pm$ SE mean of five observations; * $p < 0.05$ versus SP alone.

the start of the carbachol dose-response curve. Then, the postchallenge carbachol dose-response curves were performed. Because we observed that NKA increased airway responsiveness, we also performed dose-response curves in these sheep 24 hours after NKA challenge.

Statistics

All data are expressed as mean $\pm$ SE mean. Friedman's analysis of variance (block design) followed by a post hoc pair-wise comparison of treatment blocks was used for all statistical comparisons except comparisons made between allergic and nonallergic sheep. The Mann-Whitney U test was used for the latter comparisons because only two groups were involved. The effects of the peptides on airway responsiveness were also analyzed (i.e., in addition to Friedman's analysis of variance) by computing the difference in the logs of the PD₅₀ before and after challenge with the peptides. A log difference (i.e., log baseline PD₅₀ - log PD₅₀ after challenge) of zero indicates that airway responsiveness is unchanged, whereas an increase in the log difference reflects an increase in airway responsiveness. Analysis of these log differences was made by two-way analysis

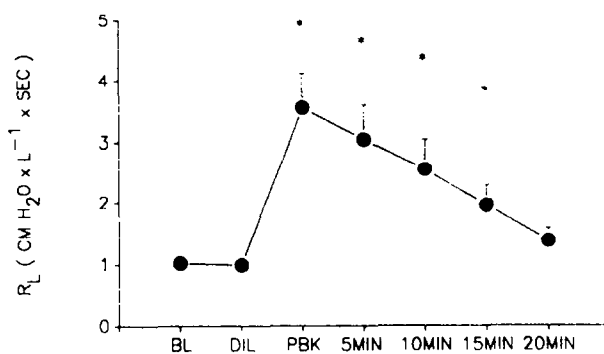


FIG. 3. Time course of BK-induced bronchoconstriction in allergic sheep. Each point is the mean $\pm$ SE mean of seven observations; * $p < 0.05$ versus baseline (BL); diluent (DL); immediately postbradykinin inhalation (PBK). Where not indicated, SE mean lies within the symbol.

TABLE I. Differences in sensitivity to inhaled BK in allergic and nonallergic sheep

	% Change in R_L after <i>Ascaris</i>	ED ₂₀₀ (log BK BU)
Allergic (6)	231% $\pm$ 31%*	1.82 $\pm$ 0.29*
Nonallergic (5)	9% $\pm$ 7%	2.59 $\pm$ 0.24

BU, Breath units of inhaled BK.

Values are mean $\pm$ SE mean percent change from baseline. Baseline values of R_L before challenge in allergic and nonallergic sheep were not different (1.04 ± 0.05 and 1.02 ± 0.06 cm H₂O $\cdot$ L⁻¹ $\cdot$ sec, respectively). The ED₂₀₀ was calculated from BK dose-response curves (see METHODS). Numbers in parentheses represent number of observations.

* $p < 0.05$ versus nonallergic (by Mann-Whitney U test).

of variance. Significance was accepted as $p < 0.05$ with a two-tailed analysis.

RESULTS

Effects of inhaled BK, SP, and NKA

The dose-response relationship among SP, NKA, and BK in allergic sheep is compared in Fig. 1. Only BK produced a significant bronchoconstriction over the range of doses used. To determine if the bronchial responses to SP could be increased, the sheep were pretreated with the neutral endopeptidase inhibitor, thiorphan ($1 \text{ mg} \cdot \text{mL}^{-1}$). The bronchial response to SP was significantly enhanced in the presence of thiorphan (Fig. 2).

The time course of BK-induced bronchoconstriction in allergic sheep is illustrated in Fig. 3. A single dose of inhaled BK caused an immediate response that began to decline rapidly, returning to baseline values by 20 minutes after challenge.

The bronchial response to BK was enhanced in

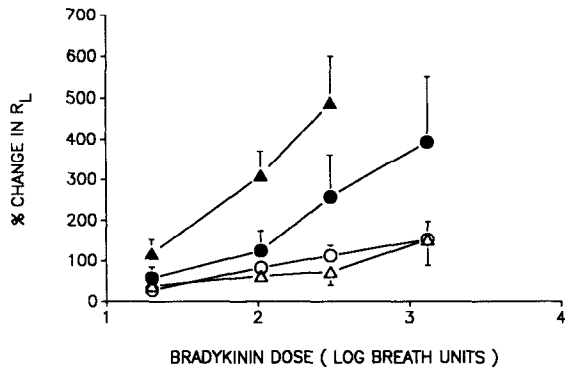


FIG. 4. Dose-response curve to inhaled BK in allergic sheep after placebo (▲) and after 20 breaths of 2 mg · ml⁻¹ (●), 5 mg · ml⁻¹ (△), and 10 mg · ml⁻¹ (○) of NPC 567. Each point is the mean ± SE mean of six observations. Where not indicated, SE mean lies within the symbol.

TABLE II. Effect of NPC-567 on BK-induced bronchoconstriction in allergic sheep

Treatment	ED ₂₀₀ (log BK BU)	Mean converted BU
Control	1.82 ± 0.29	66
NPC 567		
2 mg · ml ⁻¹	2.50 ± 0.28*	316
5 mg · ml ⁻¹ †	2.96 ± 0.25*‡	912
10 mg · ml ⁻¹	3.06 ± 0.15*‡	1148

BU, Breath units of inhaled BK.

Values are mean ± SE mean for six sheep. Drug levels refer to nebulizer concentration. Twenty breaths of NPC 567 were delivered 30 minutes before beginning of BK challenge.

**p* < 0.05 versus control.

†Four of six sheep in the 5 mg · ml⁻¹ trial and five of six sheep in the 10 mg · ml⁻¹ trial failed to achieve a 200% increase in R_L. Therefore, the highest number of breath units delivered to the animal was used for statistical purposes.

‡*p* < 0.05 versus 2 mg · ml⁻¹ of NPC 567 (by Friedman's analysis of variance).

allergic sheep as compared to nonallergic sheep (Table I). Allergic sheep, that is, sheep that responded with a bronchoconstriction to inhaled *Ascaris suum* antigen, were 5.9 times more sensitive to inhaled BK than nonallergic sheep (nonresponders to antigen).

Pharmacology of BK-induced bronchoconstriction

Dose-response curves to inhaled BK alone and in the presence of differing concentrations of the BK receptor antagonist are illustrated in Fig. 4. Inhaled BK produced dose-dependent increases in R_L that were blocked by NPC 567. For these studies, NPC

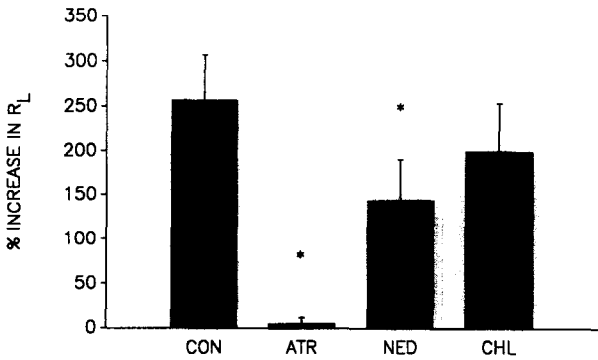


FIG. 5. Effect of different drugs on BK-induced bronchoconstriction in allergic sheep. Atropine (ATR, 0.2 mg · kg⁻¹ intravenously), nedocromil sodium (NED, 1 mg · kg⁻¹ in 3 ml of saline aerosol), and chlorpheniramine (CHL, 2 mg · kg⁻¹ intravenously). Each point is the mean ± SE mean of six observations; **p* < 0.05 versus control (CON).

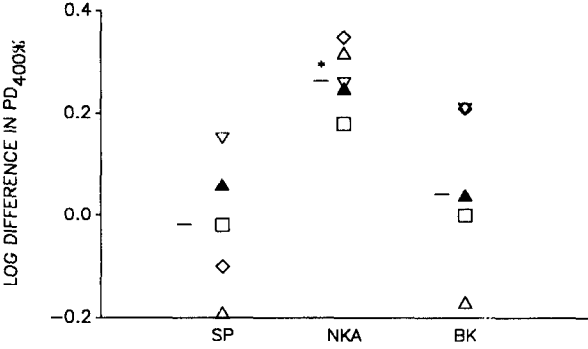


FIG. 6. Effect of SP, NKA, and BK on airway responsiveness. Values are expressed as the difference in the log PD₄₀₀ baseline and log PD₄₀₀ after challenge for the individual sheep. A log difference of zero indicates no change in responsiveness, whereas an increase in the log difference signifies airway hyperresponsiveness. The solid line represents the mean value of the log differences; **p* < 0.05 versus baseline, SP, and BK.

567 at 2, 5, and 10 mg · ml⁻¹ caused an increase in the mean log ED₂₀₀ by 0.68, 1.14, and 1.24, which corresponds to 4.8-fold, 13.8-fold, and 17.4-fold reductions, respectively, in responsiveness to BK (Table II). The drug itself had no effect on baseline airway tone. The anticholinergic agent, atropine, provided almost complete protection (97% ± 2%) against BK-induced bronchoconstriction. The anti-inflammatory agent, nedocromil sodium, also provided partial (43% ± 17%), but significant, protection. The histamine H₁-antagonist, chlorpheniramine, had no protective effect (Fig. 5).

TABLE III. Effects of BK, NKA, and SP on airway responsiveness to carbachol in allergic sheep

	Control	BK	NKA	SP
PD ₄₀₀	40.1 ± 3.9*	42.2 ± 4.8*	21.4 ± 2.3†	35.5 ± 4.1*

Values are mean ± SE mean for five sheep. PD₄₀₀ was obtained by interpolation from individual dose-response curves

* $p < 0.05$ versus NKA (by Friedman's analysis of variance).

† $p < 0.05$ versus control.

Effects on airway responsiveness

Individual differences in log PD₄₀₀ to inhaled carbachol before and after inhalation of BK, SP, and NKA are illustrated in Fig. 6, and mean PD₄₀₀ values are presented in Table III. BK and SP did not significantly affect airway responsiveness to carbachol (log difference not different from zero). However, NKA significantly increased airway responsiveness. NKA caused a mean 1.9-fold increase in responsiveness ($p < 0.05$). This increased responsiveness was short-lived, however, since 24 hours later, airway responsiveness had returned to prechallenge values (62.6 ± 16.4 breath units). Baseline SR_L values were not different among the four trials.

DISCUSSION

Our findings indicate that inhaled peptides have variable effects in sheep airways. BK caused bronchoconstriction that was more severe in allergic than in nonallergic animals but did not in itself alter airway responsiveness. Alternatively, NKA, at the doses used in this study, had no bronchoconstrictor effects but did cause a short-lived increase in airway responsiveness to inhaled carbachol. SP produced only a modest bronchoconstriction, only after the animals were pretreated with the neutral endopeptidase inhibitor, thiorphan.

The observation that allergic sheep were more sensitive to inhaled BK than nonallergic sheep is consistent with data obtained in human subjects.⁴ That inhaled BK caused bronchoconstriction in both groups of sheep is consistent with findings of Simonsson et al.,²⁶ but not with those of Fuller et al.,⁴ who found no bronchoconstriction in normal subjects. In the present study, however, the ED₂₀₀ in normal sheep was between 1 and 5 mg · ml⁻¹, which is higher than the largest dose administered in the human studies.

Fuller et al.⁴ found that the bronchoconstriction caused by inhaled BK was blocked either by the anticholinergic agent, ipratropium bromide, or the antiallergic agent, cromolyn sodium. Since cromolyn sodium has been demonstrated to have a protective effect on both unmyelinated and myelinated sensory nerves in the airways^{27, 28} and can inhibit other bron-

choconstrictor challenges having a neural reflex component,^{29, 30} these investigators concluded that BK-induced bronchoconstriction is mediated, in part, through reflex cholinergic mechanisms. Likewise, Simonsson et al.²⁶ found that the BK-induced bronchoconstriction was inhibited by atropine. In the present study, the BK-induced bronchoconstriction was blocked by atropine and partially suppressed by the anti-inflammatory agent, nedocromil sodium. Recently, nedocromil sodium has also been demonstrated to be effective in blocking BK-induced bronchoconstriction in man.³¹ The protection by nedocromil sodium in the present study against the BK-induced bronchoconstriction did not result from inhibition of histamine release because no protective effects of the H₁-histamine antagonist, chlorpheniramine, were observed. However, because nedocromil sodium has been demonstrated to be superior to cromolyn sodium in protecting against reflex-induced bronchoconstriction,^{31, 32} our findings suggest a role for BK acting through cholinergic reflex-mediated mechanisms in allergic sheep similar to that described in humans.

That the BK β₂-receptor antagonist, NPC 567,¹⁰ blocked BK-induced bronchoconstriction in sheep airways is the first report that inhalation of this agent blocked bronchoconstriction caused by inhaled BK. A recent study in guinea pigs demonstrated aerosolized NPC 567 to be ineffective against inhaled BK, although the effects of intravenous BK were inhibited.⁹ Similarly, NPC 349 (D-arginine-{hydroxyproline³, β-(2-thienyl)-alanine^{5, 8}, D-phenylalanine⁷}BK), another BK antagonist, inhibited intravenous BK-induced bronchoconstriction in this species.^{9, 33} Our present observations indicate that there are functional BK β₂-receptors in sheep airways as has been suggested from ligand-binding studies.⁹ Based on physiologic data, it is possible that the BK β₂-receptors in the airways may be associated with sensory nerves.

There is evidence that some of the effects of BK may be mediated by sensory neuropeptides. For example, BK-induced contraction of isolated iris sphincter smooth muscle was mediated by SP,^{13, 34} and in perfused guinea pig lung, levels of SP and NKA (as

measured by radioimmunoassay) were increased after perfusion with 1 $\mu\text{mol/L}$ of BK.³⁵ Both SP and NKA are potent constrictors of airway smooth muscle in vitro with NKA being the more potent of the two. Studies in subjects with asthma have demonstrated that inhalation challenge with both SP and NKA cause bronchoconstriction.^{36, 37} Joos et al.³⁷ found that inhaled SP and NKA caused no change in SG_{aw} in normal subjects. Likewise, SP, up to the highest concentration of $10^{-6} \text{ mol} \cdot \text{ml}^{-1}$, did not change SG_{aw} in the subjects with asthma. However, $5 \times 10^{-7} \text{ mol} \cdot \text{ml}^{-1}$ NKA caused a 48% fall in SG_{aw} in the subjects with asthma. The failure of the subjects with asthma to respond to SP in the study by Joos et al.³⁷ may have been dose related because Crimi et al.³⁶ reported 20% falls in FEV_1 at doses of SP between 0.09 and 0.37 mg ($\sim 6 \times 10^{-5}$ mole to 2.7×10^{-4} mole) in subjects with asthma. Therefore, it was of interest to study the bronchoconstrictor effects of these neuropeptides in allergic sheep and compare the responses to effects observed with BK. Neither of the neuropeptides, at the doses used, produced significant bronchoconstriction in allergic sheep. Since the doses of SP and NKA used were within the range of doses reported to cause bronchoconstriction in subjects with asthma,^{36, 37} it may indicate that the sheep airways are less sensitive to these neuropeptides. Part of this insensitivity may be related to the peptidase activity in the sheep airways because the airway response to inhaled SP was enhanced if the neutral endopeptidase inhibitor, thiorphan, was administered before challenge. Thus, it is possible that the peptidase activity in the airways of allergic sheep, even when they are unstimulated, is sufficient to protect against the concentrations of these agonists used.

Effects on airway responsiveness

Studies in guinea pigs indicated that toluene diisocyanate-induced airway hyperresponsiveness was mediated by tachykinins,^{14, 15} and our own studies in sheep suggested a modulatory role for BK in antigen-induced airway hyperresponsiveness.¹¹ Therefore, we examined directly the effects of these substances on airway responsiveness to inhaled carbachol. We found that only NKA produced a consistent and significant shift in airway responsiveness. SP and BK did not significantly affect airway responsiveness in the group. The failure of BK to increase airway responsiveness to carbachol in the sheep is consistent with inability of BK to increase airway responsiveness to histamine in subjects with asthma.⁴ The hyperresponsiveness produced by NKA was short-lived since it was resolved by 24 hours. The airway hyperresponsiveness found in these experiments cannot be attrib-

uted to differences in baseline lung resistance and therefore indicate a true increase in responsiveness after inhalation of NKA. These findings provide direct evidence that neuropeptides may have a role in modulating acute changes in airway responsiveness.

Our findings are somewhat divergent from those findings recently reported in anaesthetized guinea pigs wherein intravenous BK and SP enhanced acetylcholine-induced bronchospasm.¹⁶ Although the guinea pigs used in that study were treated with indomethacin, it is also possible that the different routes of administration of the peptides and the constricting agents were responsible for the somewhat different results. Nevertheless, these studies reinforce the possibility that BK and tachykinins have roles in modulating airway tone and airway hyperresponsiveness.

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