Br. J. clin. Pharmac. (1991), **31**, 287–294 ADONIS 0306525191000577

A competitive kinin receptor antagonist, [DArg^0, Hyp^3, DPhe^7]-bradykinin, does not affect the response to nasal provocation with bradykinin

JACQUELINE A. PONGRACIC, ROBERT M. NACLERIO, CURT J. REYNOLDS & DAVID PROUD
Department of Medicine, Division of Clinical Immunology, and Department of Otolaryngology, Johns Hopkins University School of Medicine, Baltimore, MD 21224, USA

1 In two double-blind, placebo controlled studies, we tested the effects of intranasal administration of 500 μg of a competitive kinin receptor antagonist, [DArg^0, Hyp^3, DPhe^7]-bradykinin (NPC 567), on the response to nasal provocation with 20 μg of bradykinin. Nasal lavage was performed before and after provocation, and subjects recorded symptom scores. Lavages were assayed for albumin and TAME-esterase activity (indicators of vascular permeability).

2 In our initial study, 12 subjects received NPC 567 or placebo 5 min before bradykinin. After placebo, bradykinin challenge resulted in values (mean ± s.e. mean) for albumin, TAME-esterase activity and total symptom scores of 275 ± 51 μg ml^{-1}, 32.1 ± 7.2 counts min^{-1} × 10^{-3}, and 1.8 ± 0.5, respectively. After NPC 567, bradykinin challenge resulted in values of 317 ± 99 μg ml^{-1}, 31.4 ± 6.9 counts min^{-1} × 10^{-3}, and 2.6 ± 0.4 for these parameters. No significant difference was observed between placebo and drug treatment for any parameter.

3 To evaluate if the lack of drug effect was due to its enzymatic degradation prior to bradykinin administration, a second study was performed in which NPC 567 was coadministered with bradykinin (n = 8). After placebo-bradykinin challenge, values of 168 ± 42 μg ml^{-1}, 11.3 ± 4.0 counts min^{-1} × 10^{-3}, and 2.8 ± 0.6 were recorded for albumin, TAME-esterase activity, and symptom scores, respectively, while following NPC 567-bradykinin challenge, these values were 174 ± 51 μg ml^{-1}, 12.3 ± 4.1 counts min^{-1} × 10^{-3}, and 3.1 ± 0.7. Again, no significant differences were noted.

4 We conclude that NPC 567 had no effect upon the response to nasal provocation with bradykinin. Possible explanations for the lack of effect of NPC 567 include low potency of the drug or the presence of different kinin receptors in the nasal mucosa.

Keywords bradykinin rhinitis kinin antagonist airway inflammation

Introduction

In recent years, evidence has accumulated to support the hypothesis that kinins may play a role in the pathogenesis of several inflammatory diseases of the airways. It has been demonstrated that these potent vasoactive peptides are generated in airway secretions during allergic reactions in both the upper and lower airways (Christiansen *et al.*, 1987; Naclerio *et al.*, 1985; Proud *et al.*, 1983). Moreover, kinins are the only mediators detected to date that are generated in nasal secretions during experimental and natural rhinovirus colds (Naclerio *et al.*, 1987; Proud *et al.*, 1990). In each of these inflammatory responses, kinin generation correlates with the onset of symptoms. Further support for a role of kinins in airway inflammation has been provided by provocation studies in which the nonapeptide, bradykinin, was administered to the mucosal surface of the airways. In the lower airways, it has been demonstrated that this peptide induces retrosternal discomfort and cough in all subjects (Fuller *et al.*, 1987) and is a potent bronchoconstrictor in asthmatics (Fuller *et al.*, 1987;

Correspondence: Dr David Proud, Johns Hopkins Asthma and Allergy Center, Clinical Immunology Unit Office 2, 301 Bayview Boulevard, Baltimore, MD 21224, USA

Herxheimer & Streseman, 1961; Varonier & Panzani, 1968), while we have shown that nasal provocation with bradykinin induces nasal obstruction, rhinorrhea and a sore throat regardless of the atopic status of the subject (Proud *et al.*, 1988). The ability of bradykinin to induce nasal symptoms has recently been confirmed by others (Holmberg *et al.*, 1990).

Despite these observations, definitive proof that kinins play a role in the pathogenesis of inflammatory diseases of the airways has been lacking, due to the inability to specifically and effectively block their actions and monitor a concomitant effect on symptoms. The recent development of competitive kinin receptor antagonists, therefore, seemed to provide the first opportunity for such interventive studies. These novel compounds are generally characterized by replacement of the proline residue at position 7 of the bradykinin sequence with a D-phenylalanine residue (Vavrek & Stewart, 1985). One such compound, [DArg^0, Hyp^3, DPhe^7]-bradykinin (NPC 567), inhibits bradykinin induced contraction of guinea pig ileum and rat uterus *in vitro*, with pA_2 values of 5.9 and 6.5, respectively (Steranka *et al.*, 1988a). *In vivo*, NPC 567 inhibits both bradykinin and urate induced hyperalgesia in the rat paw (Steranka *et al.*, 1987, 1988a) and inhibits bradykinin induced increased vascular permeability in rabbit skin (Steranka *et al.*, 1988b). More recently, it has been demonstrated, in sheep, that NPC 567 blocks bradykinin-induced bronchoconstriction, as well as inhibiting the antigen induced inflammatory response and increase in airway responsiveness that occurs 2 h after challenge (Soler *et al.*, 1990). In light of these properties, we were interested in determining if this compound may be useful as a tool to delineate the role of kinins in airway inflammation in humans. We, therefore, evaluated the ability of topically applied NPC 567 to inhibit the response of the human nasal mucosa to provocation with bradykinin.

Methods

Subjects

Healthy, male volunteers between the ages of 18 and 55 years were recruited without regard to atopic status. All subjects refrained from taking any antiinflammatory medications for a period of at least 4 weeks before entering any of the studies and no subject had had an upper respiratory tract infection within 4 weeks of challenge. All volunteers had previously demonstrated a positive response to nasal provocation with 20 μg of bradykinin; this response was defined as a two-fold increase in human serum albumin (HSA) levels in nasal lavages as compared with a control (diluent) challenge. Before, during and after completion of the study, subjects underwent a complete medical history and physical examination, including blood and urine analysis, and electrocardiogram (ECG). The studies were approved by the Joint Committee for Clinical Investigation of Johns Hopkins University, and written consent was obtained from all subjects before participation.

Materials

Bradykinin was obtained from Peninsula Laboratories, Belmont, CA. Sterile lactated Ringer's solution (American Hospital Supply Corp., Irvine, CA) was used as the diluent for nasal provocation with bradykinin. Each challenge day, a 10 mg ml^{-1} stock solution of bradykinin was prepared in lactated Ringer's containing an additional 1.5 g l^{-1} of sodium bicarbonate in order to buffer the peptide preparation to a final pH of 6.4. A 0.1 mg ml^{-1} solution for provocation was prepared by further dilution in normal lactated Ringer's. After challenge, the concentration of each bradykinin stock was verified by radioimmunoassay (Proud *et al.*, 1983). The kinin antagonist, [DArg^0, Hyp^3, DPhe^7]-bradykinin (NPC 567), and a placebo consisting of the diluent in which the antagonist was dissolved were provided by NOVA Pharmaceutical Corporation (Baltimore, MD). All administrations of bradykinin, NPC567, placebo and diluent were from metered pump spray bottles that delivered 0.1 ml per actuation. Sterile, isotonic saline used to perform lavages was obtained from the hospital pharmacy.

Study protocols

Rising dose tolerance studies Preliminary dose escalation studies were performed in 32 subjects to establish safety and tolerability of NPC 567 by intranasal administration. These studies were conducted in a double-blind, placebo controlled, random serial design fashion in which four successively increasing dose levels (0.01, 0.1, 1.0 or 4.0 mg) of antagonist were tested. For each dose of antagonist, eight subjects were recruited. Six subjects from each group received drug, while two received placebo. The protocol involved an initial visit, during which a medical history was taken and a physical examination, including a complete otolaryngological examination, ECG, blood and urine analysis were performed. Subjects who satisfied entrance criteria returned to the laboratory for the nasal challenge procedure. After noting initial symptom scores, a series of five nasal lavages with 2.5 ml of saline per nostril were performed at 2 min intervals to reduce resting protein levels in nasal secretions to a stable baseline. Subjects then received a known diluent challenge and noted symptom scores 5 min after challenge. A nasal lavage was performed 10 min after the diluent challenge. A second diluent challenge and lavage was performed and subjects then received either drug or placebo into both nostrils, such that half the total dose indicated above was administered into each nostril. Symptom scores, blood pressure and heart rate were noted 5 min after challenge and nasal lavages were performed 10 and 20 min after challenge. An ECG and otolaryngological examination were then performed and blood pressure and heart rate again recorded. At the end of the challenge procedure, nasal lavages were processed as previously described for the subsequent determination of albumin and *N*-α-tosyl-L-arginine methyl ester (TAME)-esterase activity (Baumgarten *et al.*, 1986). A follow up physical examination, ECG and blood and urine analysis was performed one week after the challenge procedure. Drug safety was based on otolaryngological and general physical examin-

ation as well as the results of blood and urine analysis. Partial agonist activity and tolerability was assessed based on symptom scores and upon measurement of markers of vascular permeability (albumin and TAME-esterase activity) that have previously been shown to increase in nasal lavage after nasal provocation with bradykinin (Proud *et al.*, 1988). Following the demonstration of safety and tolerability at one dose of drug, the protocol was repeated on eight different subjects using the next highest dose of drug.

NPC 567 preadministration study Twelve subjects with normal blood and urine chemistries, who satisfied physical and otolaryngological examinations were recruited and participated in a randomized, double-blind, placebo controlled crossover challenge. Each visit consisted of three stages, and visits were separated by 7–9 days. The first, or baseline stage, of the protocol involved conducting an otolaryngological examination and recording blood pressure and heart rate. A series of initial lavages was performed as described above, and the first and fifth lavages were retained. The second stage of the protocol involved two challenges with diluent, performed at 12 min intervals. Diluent was delivered to the right nostril via two actuations of the metered pump spray. Symptom scores were recorded 5 min after each challenge and nasal washes were performed 10 min after each diluent challenge. The final stage of the protocol involved administration of 500 μg of NPC 567 or placebo delivered by two actuations of the metered pump spray to the right nostril. Symptom scores were noted 2 min after delivery of drug or placebo and 20 μg of bradykinin was administered to the same nostril 5 min after receipt of drug or placebo. Symptom scores and vital signs were recorded 5 min after bradykinin challenge, while a nasal lavage was performed 10 min after challenge. Symptom scores were, again, noted and a lavage performed 15 and 20 min after bradykinin administration, respectively. At the conclusion of the experiment, nasal lavages were processed and stored at −70° C for subsequent measurements of albumin and TAME-esterase activity.

The doses of bradykinin and NPC 567 used in these studies were selected to favor any potential drug effect. It has been reported that NPC 567 inhibits bradykinin induced hyperalgesia in the rat when used at concentrations of 10 times that of bradykinin (Steranka *et al.*, 1987). We chose to employ a dose of bradykinin (20 μg) that would induce modest symptomatic responses and a clear increase in vascular permeability in all subjects and to attempt to inhibit the actions of this dose of bradykinin with a 25 fold excess of antagonist.

NPC 567 coadministration study Because of concerns for the possibility of rapid metabolism and clearance of the drug before kinin challenge, we performed a second study to evaluate the effects of simultaneous administration of NPC 567 and bradykinin. Eight subjects participated in a randomized, double-blind, placebo controlled crossover study with 7–9 day intervals between visits. The protocol was identical to that described for the preadministration study except for the fact that the bradykinin challenge solution was prepared in the drug or placebo solution and was coadministered with drug or placebo.

Additional single blind studies To study further the effects of NPC 567, additional single-blind, placebo controlled crossover studies were performed in a limited number of patients. In three subjects we evaluated the effect of increasing the administered dose of NPC 567 to 2 mg delivered into the right nostril 5 min prior to challenge with 20 μg of bradykinin, following the protocol described above for the preadministration study.

In addition, because it had been reported that NPC 567 inhibited bradykinin-induced bronchoconstriction optimally when preadministered 30 min prior to kinin challenge in sheep (Soler *et al.*, 1990), we examined the effect, in three subjects, of administering 2 mg of NPC 567 into the right nostril 30 min prior to challenge with 20 μg of bradykinin. Other than the interval between drug or placebo administration and bradykinin provocation, all aspects of the protocol were as described above for the preadministration study.

Assessment of symptoms

As indicated above, subjects recorded symptom scores several times throughout the challenge procedure. A 4 point scale ranging from 0 to 3 (0 = no symptoms, 3 = severe symptoms) was used for the subjective evaluation of the following individual symptoms: nasal obstruction, irritation, burning, pruritus, pain, rhinorrhea and sore throat.

Mediator assays

Human serum albumin (HSA) was measured using a specific, competitive radioimmunoassay sensitive to 1 ng of albumin ml^{-1} (Baumgarten *et al.*, 1985).

Enzymes with arginine esterase activity were assayed by the method of Imanari *et al.* (1976), which is based on the liberation of tritiated methanol from the synthetic substrate [^{3}H]-*N*-α-tosyl-L-arginine methyl ester (TAME). The activity of 40 μg of recovered lavage is reported as thousands of counts per minute (counts $min^{-1} \times 10^{-3}$).

Statistical analysis

Responses to bradykinin challenge between treatment groups were compared nonparametrically using the Wilcoxon two-tailed matched pairs signed rank test. For each parameter (symptom scores, albumin and TAME-esterase activity) responses were analyzed as increase above baseline by subtracting the mean of the values seen after the two diluent challenges from values after bradykinin challenge. For symptom scores, increases above diluent 5 and 15 min after bradykinin challenge for the two groups were compared individually, and the sum of the increases at the two time points were also compared. Similarly, for albumin and TAME-esterase activity increases above diluent 10 and 20 min after bradykinin challenge were compared between treatment groups using each time point individually, as well as using the sum of the increases at the two time points. All analyses were performed using a Macintosh Plus computer (Apple Computer, Cupertino, CA) using Statview software (Brainpower, Inc., Calabasas, CA). Significance was presumed to be achieved for two tailed P values of < 0.05.

Results

In our preliminary rising dose tolerance studies, NPC 567 induced no side effects at any dose used. Similarly, even at doses of 4 mg administered, the drug displayed no partial agonist activity, as assessed by its inability to induce symptoms or to cause any increases above diluent challenge in the levels of albumin and TAME-esterase activity in nasal lavages.

In the study in which placebo or 500 μg of NPC 567 was administered 5 min prior to bradykinin challenge there was, again, no increased symptomatic response, compared with diluent, associated with administration of the drug (Figure 1). Following pretreatment with placebo, bradykinin provocation led to a total symptom score of 1.8 ± 0.5 (mean ± s.e. mean) 5 min after challenge, a value significantly ($P < 0.05$) greater than that induced by diluent challenge. A significant ($P < 0.005$) increase in symptom score, to a value of 2.6 ± 0.4, was also seen 5 min after bradykinin provocation following drug pretreatment. There was no significant difference in symptom scores, expressed as increase above diluent, between drug and placebo pretreatment. Similarly, no differences between the two treatments were seen when increases in symptom scores 15 min after challenge were analyzed, or when the sum of increases at the two time periods were compared.

When the levels of albumin and TAME-esterase activity in nasal lavages were examined as indices of increased vascular permeability, there was, again, no evident partial agonist activity associated with NPC 567 (Figure 2). Although the relatively low dose of bradykinin used in our experiments induced only modest increases in subjective symptom scores, this dose of bradykinin was associated with clearly measurable increases in these objective indices of vascular permeability. Following placebo pretreatment, bradykinin provocation increased the level of HSA in lavages to 275 ± 50.7 μg ml^{-1} (Figure 2a), compared with levels after diluent challenge of 11.1 ± 2.2 μg ml^{-1} ($P < 0.005$). Bradykinin challenge also led to a significant ($P < 0.005$) increase in albumin levels, to a value of 317 ± 99 μg ml^{-1}, on the drug pretreatment day. There was no significant difference in the increase above diluent challenge for drug compared with placebo pretreatment. Results for TAME-esterase activity paralleled those for HSA (Figure 2b). Bradykinin challenge significantly ($P < 0.005$, in each case) increased TAME-esterase activity to 32.1 ± 7.2 counts min^{-1} × 10^{-3} after placebo pretreatment and to 31.4 ± 6.9 counts min^{-1} × 10^{-3} after pretreatment with NPC 567. Again, there was no significant difference between the increases above diluent following placebo or drug treatment.

Because NPC 567 is susceptible to metabolism by peptidases, it was possible that the failure of this compound to block the effects of a bradykinin challenge may have been due to its rapid degradation by peptidases in the 5 min prior to kinin provocation. Alternatively,

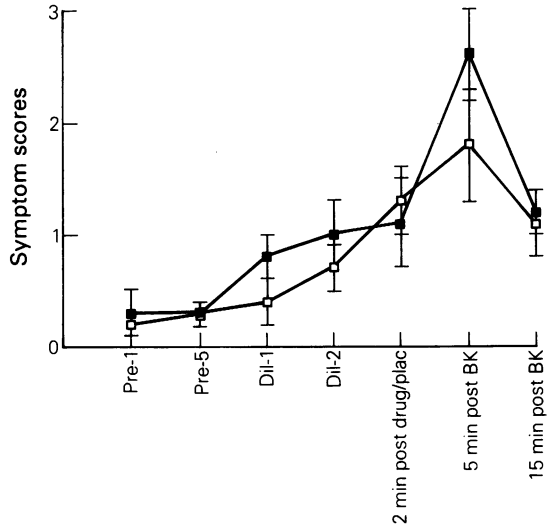

Figure 1 Symptom scores induced upon bradykinin provocation following pretreatment with NPC 567 (■) or placebo (□). Results are expressed as mean ± s.e. mean. The protocol is shown on the abscissa. There were no significant differences between the results obtained with the two pretreatments. BK, bradykinin.

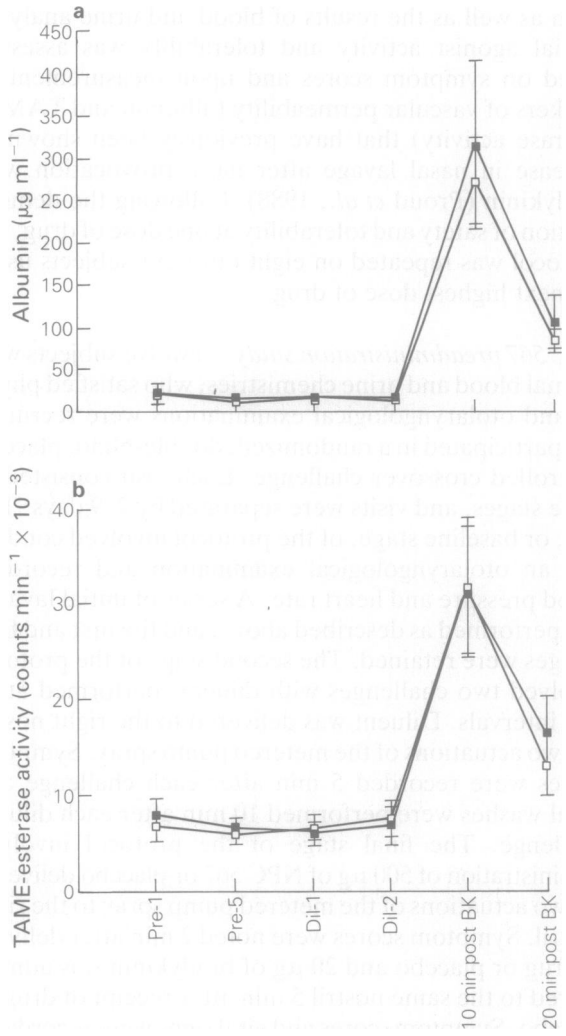

Figure 2 Levels of albumin and TAME-esterase activity in nasal lavages in response to bradykinin provocation following pretreatment with placebo (□) or NPC 567 (■). Results are expressed as mean ± s.e. mean. There were no significant differences for either parameter between the results obtained with the two pretreatments. Panel a shows results for albumin. Panel b shows results for TAME-esterase activity.

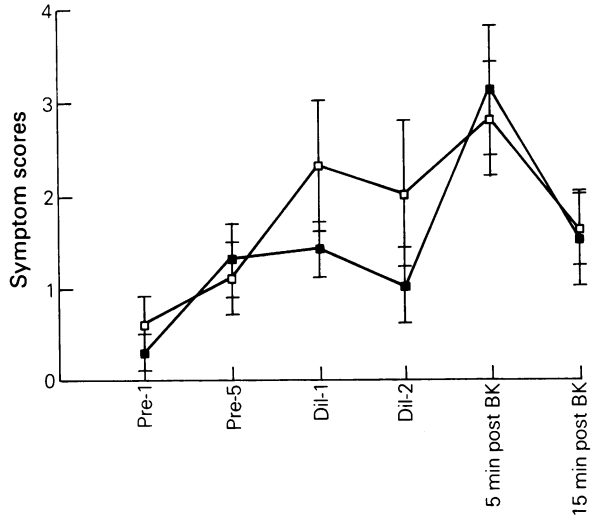

Figure 3 Symptom scores induced upon coadministration of bradykinin with placebo (□) or NPC 567 (■). Results are expressed as mean ± s.e. mean. The protocol is shown on the abscissa. There were no significant differences between results for drug and placebo administration.

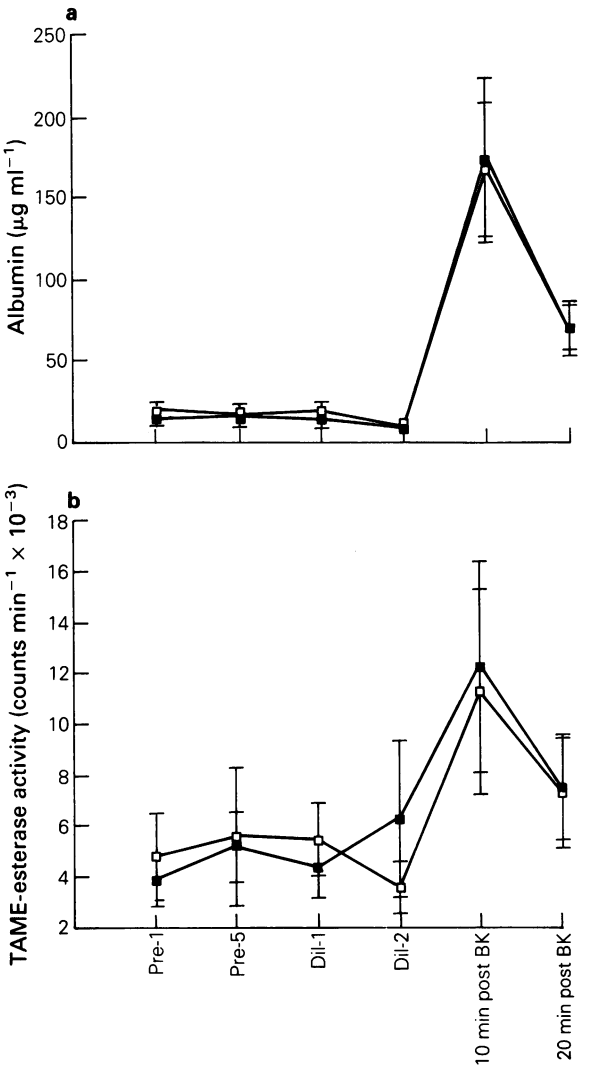

Figure 4 Levels of albumin and TAME-esterase activity in nasal lavages following coadministration of bradykinin with either placebo (□) or NPC 567 (■). Results are expressed as mean ± s.e. mean. There were no significant differences for either parameter between the results obtained with the two treatments. Panel a shows results for albumin. Panel b shows results for TAME-esterase activity.

mucociliary clearance mechanisms could, conceivably, have removed the drug from the nasal cavity. To evaluate these possibilities, we performed a second study in which drug or placebo was coadministered with bradykinin. Coadministration of bradykinin and placebo led to a modest, but significant ($P < 0.05$) increase in total symptom score to a value of 2.8 ± 0.6 5 min after challenge (Figure 3). A similar, significant ($P < 0.05$) increase, to a level of 3.1 ± 0.7 was seen 5 min after coadministration of bradykinin and NPC 567. No differences were seen between drug and placebo days for increases above diluent. As expected, bradykinin/placebo provocation also led to increased vascular permeability, as evidenced by an increase in HSA levels to 168 ± 42 μg ml^{-1} (Figure 4a). A similar increase, to a level of 174 ± 51 μg ml^{-1}, was also seen, however, following coadministration of bradykinin and NPC 567. TAME-esterase activity was also increased to similar levels on both challenge days (Figure 4b) and there were no significant differences between placebo and drug days for increases above diluent levels for either parameter.

The failure of NPC 567 to affect the response to bradykinin provocation in these two studies was perplexing in light of the efficacy of this compound in animal models. To evaluate if this lack of effect could be overcome by other modifications in our protocol we performed two additional single-blind studies in three subjects each. In the first study we employed an identical protocol to that used in our initial preadministration trial but increased the dose of NPC 567 used to 2 mg, the maximum dose per nostril used in our rising dose tolerance studies. The second study also used this higher dose of drug but, because studies in sheep had suggested that NPC 567 reduced the airway response to bradykinin when given 30 min prior to kinin challenge (Soler *et al.*, 1990), we also used a 30 min preadministration of drug or placebo before bradykinin provocation. The results of these two trials are summarized in Table 1. Although

Table 1 Results of single-blind studies using 2 mg of NPC 567

Study	*Symptom scores* NPC 567	Placebo	*Albumin* (μg ml^{-1}) NPC 567	Placebo	*TAME-esterase* (*counts* $min^{-1} \times 10^{-3}$) NPC 567	Placebo
1	2.0 ± 1.3	1.3 ± 1.4	102 ± 84	187 ± 138	26.2 ± 20.5	30.0 ± 20.8
2	2.8 ± 1.4	3.3 ± 1.9	107 ± 8	63 ± 26	13.1 ± 1.0	5.5 ± 1.5

Study 1: Placebo or 2 mg of NPC 567 administered 5 min prior to kinin challenge.
Study 2: Placebo or 2 mg of NPC 567 administered 30 min prior to kinin challenge.
Results are expressed as mean ± s.e. mean ($n = 3$).

the limited number of subjects used prevents the use of nonparametric statistics, it is clear that increasing the dose of drug to 2 mg had no obvious effect on the response to a bradykinin provocation performed 5 min later. Similarly, in the study in which the drug was administered 30 min prior to kinin challenge, there was no apparent effect on symptom scores, while levels of HSA and TAME-esterase activity were actually higher on the drug day in all three subjects.

Discussion

Although the known pharmacologic properties of kinins, together with the demonstration that they are formed during a variety of inflammatory events, have led to the suggestion that these peptides may be important mediators of inflammatory diseases in humans (Marceau *et al.*, 1983; Proud & Kaplan, 1988), the inability to interfere specifically with the actions of kinins has not permitted definitive evaluation of their role in inflammatory events. The present study represents the first attempt to use a kinin receptor antagonist to demonstrate specific inhibition of the effects of bradykinin in humans.

Our understanding of the pharmacology of kinin receptors is still evolving but Regoli & Barabe (1980) suggested that the actions of kinins are mediated via two types of receptors. On the B_1 receptor, the compound Leu^8-des-Arg^9-bradykinin is an effective antagonist and the kinin metabolite, des-Arg^9-bradykinin is a more effective agonist than the parent peptide. Indeed, it has recently been shown that the apparent ability of bradykinin to stimulate B_1 receptors is actually dependent upon local hydrolysis to des-Arg^9-bradykinin by tissue carboxypeptidases (Regoli *et al.*, 1986). On the B_2 receptor, bradykinin and lysylbradykinin are equally potent, while des-Arg^9-bradykinin is essentially inactive. In humans, the majority of actions of bradykinin appear to be mediated via B_2 receptors (Bathon & Proud, 1991). The series of receptor antagonists developed by Vavrek & Stewart (1985) are the first specific antagonists of the actions of bradykinin at the B_2 receptor, although it has recently been demonstrated that these compounds become B_1 receptor antagonists upon metabolism by carboxypeptidase N (Regoli *et al.*, 1986).

The compound selected for *in vivo* studies, [DArg^0, Hyp^3, DPhe^7]-bradykinin (NPC 567), has been shown to be capable of antagonizing the actions of bradykinin in a variety of animal models (Soler *et al.*, 1990; Steranka *et al.*, 1987, 1988a,b) and has shown no appreciable partial agonist activity in isolated tissue preparations (Steranka *et al.*, 1989). Consistent with this latter observation, our present studies clearly demonstrate that this compound displayed no partial agonist activity, at the doses used, when applied to the human nasal mucosa. There was no symptomatic response to NPC 567 even at an administered dose of 2 mg per nostril, nor was there any increase in vascular permeability. Unfortunately, it was also clear that administration of a 25 fold excess of antagonist 5 min prior to bradykinin provocation had no effect on the response to kinin challenge. Not only did NPC 567 fail to reduce the subjective symptomatic response to kinin challenge, but the striking similarities between drug and placebo days for the objective measurements of albumin and TAME-esterase activity (Figure 2) are a clear indication that NPC 567 had no effect on bradykinin-induced increases in vascular permeability.

The decision to administer the drug 5 min before kinin challenge was based on giving the compound the opportunity to occupy receptors before bradykinin was introduced. A possible explanation for the failure of NPC 567 to antagonize the effects of bradykinin provocation was that the antagonist was being removed from the nasal cavity, either by mucociliary clearance or by degradation, prior to the administration of bradykinin. Although it has been demonstrated that all of the DPhe^7-bradykinin analogues so far tested are resistant to metabolism by angiotensin converting enzyme (Togo *et al.*, 1989), they are still readily degraded by other enzymes, such as carboxypeptidase N (Regoli *et al.*, 1986). Moreover, at least during allergic inflammation, it has been shown that carboxypeptidase N is one of the major enzymes involved in kinin degradation on nasal secretions (Proud *et al.*, 1987). The possibility that clearance or degradation was responsible for a lack of functional antagonism seems unlikely, however, in light of the results obtained in our second study, in which bradykinin was coadministered with drug. Coadministration of bradykinin with either placebo or drug induced increases in symptoms and vascular permeability that were comparable to those seen in response to the same dose of bradykinin in our first study population. Thus, even under conditions where peptidases would have simultaneous access to bradykinin and NPC 567, the drug was completely ineffective in blocking the response to bradykinin.

An obvious explanation for the lack of effect of NPC 567 in these studies would be that the drug was not administered in an adequate dose to function effectively. In an attempt to address this issue we performed a pilot study in three subjects using the maximum dose of drug (2 mg) that had been considered practical for testing in our rising dose tolerance study. Even at this 100 fold excess of NPC 567 compared with the dose of bradykinin used, however, there was no apparent inhibition of the response to kinin challenge. Because it had been reported that NPC 567 inhibited bradykinin-induced bronchoconstriction when administered 30 min prior to kinin challenge, we also examined the effect of this higher dose of drug when administered 30 min prior to nasal challenge with bradykinin. Again, no apparent inhibition was observed. Although the pA_2 values obtained for NPC 567 in isolated animal tissue preparations suggest that a 10 to 100 fold excess of the compound should be sufficient to induce inhibition of bradykinin's effects, to our knowledge there has been no *in vitro* testing of the ability of this compound to inhibit the effects of bradykinin on human cell or tissue preparations. It is feasible, therefore, that species differences may lead to a requirement for higher doses of antagonist to compete effectively at the receptor level than those used in our present studies. Alternatively, it may be that the structural changes introduced into the bradykinin sequence to confer antagonist properties may make it more difficult for the antagonist to gain access to the relevant receptors

when administered *in vivo*. Finally, it is possible that the actions of bradykinin in the nasal mucosa may be mediated via a receptor subtype at which NPC 567 is not an antagonist. It has recently been demonstrated in the guinea pig, for example, that NPC 567 is a very weak inhibitor of bradykinin-induced bronchoconstriction *in vivo* and that this compound is essentially ineffective at inhibiting bradykinin-induced tracheal smooth muscle contraction *in vitro* (Farmer *et al.*, 1989). Moreover, these authors have also demonstrated the presence, in guinea pig trachealis, of specific bradykinin binding that could not be displaced by NPC 567, and have suggested that the actions of bradykinin in this tissue may be mediated via a putative B_3 receptor subtype (Farmer *et al.*, 1989).

Regardless of whether its lack of effect is due to insufficient potency, poor bioavailability or to the presence of a different receptor subtype, it is clear from the present studies that NPC 567 is not a suitable compound to delineate the role of kinins in inflammatory diseases of the upper airways in humans. Although these results are disappointing in this regard, the development of kinin antagonists is an area of considerable activity in several laboratories and more potent compounds are already being produced. The present studies clearly indicate that it would be wise to test the efficacy of such compounds in relevant target tissues, particularly in human tissues and cells, before proceeding to *in vivo* studies. With appropriate caution, however, it seems likely that the use of selective, more potent kinin receptor antagonists will provide important insights into the involvement of these vasoactive peptides in airway inflammation.

This work was supported by Grants HL 32272, HL 37119 and DC 00320 from the National Institutes of Health and by a Grant in Aid from Nova Pharmaceutical Corporation.

References

Bathon, J. M. & Proud, D. (1991). Bradykinin antagonists. *Ann. Rev. Pharmac. Tox.*, (in press).

Baumgarten, C. R., Nichols, R. C., Naclerio, R. M., Lichtenstein, L. M., Norman, P. S. & Proud, D. (1986). Plasma kallikrein during experimentally-induced allergic rhinitis: Role in kinin formation and contribution to TAME-esterase activity in nasal secretions. *J. Immunol.*, **137**, 977–982.

Baumgarten, C. R., Togias, A. G., Naclerio, R. M., Lichtenstein, L. M., Norman, P. S. & Proud, D. (1985). Influx of kininogens into nasal secretions after antigen challenge of allergic individuals. *J. clin. Invest.*, **76**, 191–197.

Christiansen, S. C., Proud, D. & Cochrane, C. G. (1987). Detection of tissue kallikrein in the bronchoalveoiar lavage fluids of asthmatic subjects. *J. clin. Invest.*, **79**, 188–197.

Farmer, S. G., Burch, R. M., Meeker, S. A. & Wilkins, D. E. (1989). Evidence for a pulmonary B_3 bradykinin receptor. *Mol. Pharmac.*, **36**, 1–8.

Fuller, R. W., Dixon, C. M. S., Cuss, F. M. C. & Barnes, P. J. (1987), Bradykinin-induced bronchoconstriction in humans. Mode of action. *Am. Rev. resp. Dis.*, **135**, 176–180.

Herxheimer, H. & Stresemann, E. (1961). The effect of bradykinin aerosol in guinea pigs and man. *J. Physiol.* (London), **158**, 38–39.

Holmberg, K., Bake, B. & Pipkorn, U. (1990). Vascular effects of topically applied bradykinin on the human nasal mucosa. *Eur. J. Pharmac.*, **175**, 35–41.

Imanari, T., Kaizu, T., Yoshida, H., Yates, K., Pierce, J. V. & Pisano, J. J. (1976). Radiochemical assays for human urinary, salivary and plasma kallikreins. In *Chemistry and biology of the kallikrein-kinin system in health and disease*, eds Pisano, J. J. & Austen, K. F., pp. 205–213. Washington, D.C.: DHEW Publication No. (NIH) 76–791.

Marceau, F., Lussier, A., Regoli, D. & Giroud, J. P. (1983). Pharmacology of kinins: their relevance to tissue injury and inflammation. *Gen. Pharmac.*, **14**, 209–229.

Naclerio, R. M., Proud, D., Lichtenstein, L. M., Kagey-Sobotka, A., Hendley, J. O., Sorrentino, J. & Gwaltney, J. M., Jr. (1987). Kinins are generated during experimental rhinovirus colds. *J. infect. Dis.*, **157**, 133-142.

Naclerio, R. M., Proud, D., Togias, A. G., Adkinson, N. F., Jr., Meyers, D. A., Kagey-Sobotka, A., Plaut, M., Norman, P. S. & Lichtenstein, L. M. (1985). Inflammatory mediators in late antigen-induced rhinitis. *New Engl. J. Med.*, **313**, 65–70.

Proud, D. & Kaplan, A. P. (1988). Kinin formation: Mechanisms and role in inflammatory disorders. *Ann. Rev. Immunol.*, **6**, 49–83.

Proud, D., Baumgarten, C. R., Naclerio, R. M. & Ward, P. E. (1987). Kinin metabolism in human nasal secretions during experimentally-induced allergic rhinitis. *J. Immunol.*, **138**, 428–434.

Proud, D., Naclerio, R. M., Gwaltney, J. M., Jr. & Hendley, J. O. (1990). Kinins are generated in nasal secretions during natural rhinovirus colds. *J. infect. Dis.*, **161**, 120–123.

Proud, D., Reynolds, C. J., LaCapra, S., Kagey-Sobotka, A., Lichtenstein, L. M. & Naclerio, R. M. (1988). Nasal provocation with bradykinin induces symptoms of rhinitis and a sore throat. *Am. Rev. resp.* Dis., **137**, 613–616.

Proud, D., Togias, A., Naclerio, R. M., Crush, S. A., Norman, P. S. & Lichtenstein, L. M. (1983). Kinins are generated in vivo following nasal airway challenge of allergic individuals with allergen. *J. clin. Invest.*, **72**, 1678–1685.

Regoli, D. & Barabe, J. (1980). Pharmacology of bradykinin and related kinins. *Pharmac. Rev.*, **31**, 1–46.

Regoli, D., Drapeau, G., Rovero, P., Dion, S., Rhaled, N-E., Barabe, J., D'Orleans-Juste, P. & Ward, P. E. (1986). Conversion of kinins and their antagonists into B_1 receptor activators and blockers in isolated vessels. *Eur. J. Pharmac.*, **127**, 219–224.

Soler, M., Sielczak, M. W. & Abraham, W. M. (1990). A bradykinin antagonist blocks antigen-induced airway hyperresponsiveness and inflammation in sheep. *Pul. Pharmac.*, **3**, 9–15.

Steranka, L. R., DeHaas, C. J., Vavrek, R. J., Stewart, J. M., Enna, S. J. & Snyder, S. H. (1987). Antinociceptive effects of bradykinin antagonists. *Eur. J. Pharmac.*, **136**, 261–262.

Steranka, L. R., Farmer, S. G. & Burch, R. M. (1989). Antagonists of B_2 bradykinin receptors. *FASEB J.*, **3**, 2019–2025.

Steranka, L. R., Manning, D. C., DeHaas, C. J., Ferkany, J. W., Borosky, S. A., Connor, J. R., Vavrek, R. J., Stewart, J. M. & Snyder, S. H. (1988a). Bradykinin as a pain mediator: Receptors are localized to sensory neurons, and antagonists have analgesic actions. *Proc. Nat. Acad. Sci. USA.*, **85**, 3245–3249.

Steranka, L. R., Rodriguez, R. A. & DeHaas, C. J. (1988b). Effects of bradykinin antagonists on vascular permeability in rat skin. *Pharmacologist*, **30**, A30.

Togo, J., Burch, R. M., DeHaas, C. J., Connor, J. R. & Steranka, L. R. (1989). D-Phe^7-substituted peptide bradykinin antagonists are not substrates for kininase II. *Peptides*, **10**, 109–112.

Varonier, H. S. & Panzani, R. (1968). The effect of inhalation of bradykinin on healthy and atopic (asthmatic) children. *Int. Arch. Allergy*, **34**, 293–296.

Vavrek, R. J. & Stewart, J. M. (1985). Competitive antagonists of bradykinin. *Peptides*, **6**, 161–164.

(Received 21 August 1990, accepted 18 October 1990)