

Comparative nasal effects of bradykinin and histamine: influence on nasal airways resistance and plasma protein exudation

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Abstract

Background—Bradykinin may contribute to the pathogenesis of allergic rhinitis. Like histamine, nasal challenge with bradykinin induces rhinorrhoea, nasal blockage, and plasma protein leakage. Their comparative nasal potencies have not, however, been fully elucidated.

Methods—Three double blind, randomised, placebo controlled and crossover studies were undertaken to compare objectively the nasal effects of bradykinin, histamine, and vehicle.

Results—Both bradykinin and histamine produced dose dependent increases in nasal airways resistance (NAR). There was no significant difference in the effects of bradykinin and histamine on NAR at any dose level. On a molar basis, however, bradykinin was 6.98 times more potent than histamine in inducing a 50% increase in NAR. Nasal challenge with bradykinin and histamine also induced significant rhinorrhoea compared with vehicle. The amount of rhinorrhoea induced by histamine was significantly greater than that induced by bradykinin at any dose level. Bradykinin and histamine induced dose dependent nasal pain and nasal itch respectively. When administered as single doses both bradykinin (1.9 μ mol) and histamine (1.9 μ mol) induced significant rhinorrhoea compared with the vehicle. The volume of rhinorrhoea secretions induced by histamine was 29% greater than that induced by bradykinin. In contrast, although NAR was increased significantly more by histamine than by the vehicle, the effect of bradykinin on NAR was significantly greater than histamine and vehicle in both magnitude and duration of effect. The incremental effect of bradykinin on lavage albumin levels was also significantly greater than both histamine and vehicle.

Conclusions—This study shows that the nasal vascular effects of histamine are less prominent than its actions on rhinorrhoea, and that the greater obstructive effect of bradykinin than histamine on NAR may contribute to the relative lack of efficacy of H₁ antihistamines on nasal blockage in clinical disease.

Allergic rhinitis is a common condition characterised by symptoms of nasal itch, sneezing, rhinorrhoea, and nasal blockage. As the local release of histamine has been identified in both the immediate^{1,2} and late³ nasal response to allergen challenge, and nasal insufflation with histamine induces nasal itch, sneeze, rhinorrhoea, and transient nasal blockage,^{4,6} it has been implicated in the pathophysiology of rhinitis. While the local release of histamine from activated mast cells or basophils within the nasal mucosa can explain many of the symptoms of rhinitis,⁴ the involvement of non-histamine mediators in this disease is suggested by the incomplete therapeutic efficacy of H₁ antihistamines, particularly with respect to nasal blockage.⁴ A prominent candidate among the non-histamine mediators for a major role in rhinitis is bradykinin, a potent vasoactive nonapeptide formed as a cleavage product from the action of plasma kallikreins on high molecular weight kininogen.

Kinin activity is present in increased amounts in nasal lavage fluid after early⁷ and late⁸ phases of nasal allergen provocation. Its activity was also found to be increased in naturally occurring seasonal allergic rhinitis⁸ and during natural⁹ and induced¹⁰ rhinoviral colds. Nasal insufflation with bradykinin elicits many of the features of rhinitis including nasal blockage, rhinorrhoea, and increased plasma vascular leakage.¹¹⁻¹⁴ As the release of kinins into nasal lavage fluid is coincidental with the onset of symptoms of rhinitis after local allergen challenge, and as these kinins are known to exhibit a range of proinflammatory actions,¹⁵ they have also been causally implicated in the pathogenesis of allergic rhinitis.

There have been few studies directly comparing the nasal effects of bradykinin with other mediators identified in association with rhinitis, and only one study comparing the nasal effects of bradykinin and histamine has been published.¹³ This study did not, however, attempt to match the agonists on a molar basis and did not investigate the comparative effects of these agonists on plasma protein leakage. We have therefore undertaken a double blind, randomised study to compare the nasal effects of bradykinin and histamine by investigating their effects on nasal airways resistance (NAR), rhinorrhoea, sensory nasal symptoms of pain and itch, and plasma protein exudation.

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Methods

SUBJECTS

Ten non-rhinitic (asymptomatic), non-smoking, non-atopic healthy volunteers (six men and four women) with a mean age of 27 years participated in the study. All subjects refrained from taking any form of medications for one month before and throughout the study period. None of the subjects had a history of recent upper respiratory tract infection, nasal polyps, infective rhinitis, nasal surgery, or nasal deformities, and all gave written informed consent. The study was approved by the Southampton Hospitals and University joint ethical subcommittee.

CHALLENGE SOLUTIONS

Bradykinin triacetic acid (Nova Biochem Ltd, Nottingham, UK) and histamine acid phosphate (Sigma, Poole, Dorset, UK) were dissolved in 10% ethanol in 0.9% sodium chloride and 0.9% sodium chloride respectively to produce a concentration range of 0.77, 3.85, and 7.7 mg/ml (0.73, 3.63, and 7.26×10^{-3} mol/l and 2.0, 4.0, and 8.0 mg/ml (6.5, 13.0, and 26.0×10^{-3} mol/l) respectively. These doses were chosen from previous dose-response studies.^{4,5,12} For the second and third phases of the study bradykinin and histamine were dissolved in the relevant diluents to produce concentrations of 7.7 mg/ml (7.26×10^{-3} mol/l) and 2.25 mg/ml (7.32×10^{-3} mol/l) respectively. The purity of the synthetic bradykinin was confirmed by high performance liquid chromatography as described previously.¹²

STUDY DESIGN

The study was conducted in three phases.

Phase 1: Dose-response study

The subjects attended on three occasions at the same time of day to receive nasal challenge with incremental doses of either bradykinin (0.19 μ mol (0.2 mg), 0.95 μ mol (1.0 mg), and 1.9 μ mol (2.0 mg)), histamine (1.7 μ mol (0.52 mg), 3.4 μ mol (1.04 mg), and 6.8 μ mol (2.08 mg)), or vehicle placebo in a double blind, randomised, crossover fashion. Each visit was separated by at least one week. After a 15 minute rest, measurements were made of baseline NAR (mean of three measurements) by active posterior rhinomanometry using a Mercury NR6 Rhinomanometer (Mercury Instruments, Glasgow, UK) as described previously.¹² This method of measurement of NAR has a coefficient of variation of repeated measurements of 10.7%.¹² Subjects then underwent nasal challenge with the vehicle followed by incremental doses of bradykinin or histamine at 15 minute intervals until the top challenge dose had been achieved. Nasal challenge was undertaken bilaterally using a hand held pump spray delivering 0.13 ml/activation with a coefficient of variation of output of 8.4%. The spray was placed in one nostril while occluding the other and activated once during quiet inspiration. The procedure was then repeated in the opposite nostril. The doses of

the agonist were equally divided between nostrils. After each challenge repeat measurements of NAR were made at 3, 5, 10, and 15 minutes and subjects recorded the nasal pain or discomfort and nasal itch on a 10 cm visual analogue scale. Rhinorrhoea was measured 45 seconds before each measurement of NAR by collecting blown secretions on preweighed tissues. The tissue towels were then immediately reweighed and the weight of secretion calculated by subtraction. The symptom of sore throat was recorded as present or absent and the number of sneezes was counted.

Phase 2: Time course study

Each of the 10 subjects attended on three occasions at the same time of day. At each visit, separated by at least one week, measurements were made of baseline NAR by active posterior rhinomanometry after a 15 minute rest. Subjects then underwent nasal challenge with a single dose of bradykinin (1.9 μ mol), histamine (1.9 μ mol), or vehicle placebo in a double blind, randomised, crossover fashion. The dose of histamine was selected on an equimolar basis to the selected dose of bradykinin. Repeat measurements of NAR were made at 3, 5, 10, 15, 20, 25, 30, 35, and 40 minutes after the challenge and subjects recorded the nasal pain or discomfort and nasal itch on a 10 cm visual analogue scale. Rhinorrhoea was measured as described above.

Phase 3: Nasal lavage study

Eight subjects (five men) attended on a further three occasions having been randomly allocated to receive nasal challenge with the same single dose of bradykinin, histamine, or vehicle in a double blind, crossover design. Subjects were instructed to tilt their heads backwards about 30° from the horizontal while in the sitting position, to hold their breath, and to refrain from swallowing. Two and a half ml of 0.9% prewarmed saline were then instilled into each nostril and after 10 seconds the subjects flexed their necks and expelled the mixture of mucus and saline into a collection vessel. Samples were immediately sieved to remove mucus plugs and stored on ice until the conclusion of the experiment and then frozen at -20°C for protein analysis. At each attendance sequential nasal lavage was performed bilaterally five times, at one minute intervals, before nasal insufflation challenge with the fifth sample which was used for baseline analysis. Repeated saline nasal lavages were performed at 3, 5, 10, 15, 20, 25, 30, 35, and 40 minutes after the challenge.

TOTAL PROTEIN AND ALBUMIN MEASUREMENT

Total protein concentrations were measured in 0.25 ml replicates of nasal lavage fluid by the method of Bradford¹⁶ using Coomassie Blue G-250 reagent (Pierce, Rockford, Illinois, USA) as indicator. Absorbency read at 595 nm was compared with a standard curve constructed with bovine serum albumin

(Sigma, Poole, UK) to give absolute protein concentrations. Albumin levels were measured by rocket immunoelectrophoresis¹⁷; 3 μ l aliquots of nasal lavage fluid were introduced into wells cut in agarose gel and subjected to electrophoresis (4 V/cm) for 20 hours into a "window" of agarose containing 1.18 μ l/ml rabbit antihuman albumin antibody (Nordic, Tilburg, Netherlands). Immunoprecipitates were stained with Coomassie Brilliant Blue. Albumin levels were quantified by comparison with standard curves constructed using human albumin (Sigma, Poole, UK). The sensitivity of the albumin assay was 1 μ g/ml and the coefficient of variation of repeated measurements was 5%.

DATA ANALYSES

Baseline measurements of NAR ($\text{Pa cm}^{-3} \text{ s}$), lavage albumin, and total protein on the different challenge days were compared between days using the Friedman's two way ANOVA test. To compare the dose-response changes in NAR, the maximum increase in NAR for each subject was plotted against the dose of agonist on a logarithmic scale and the provocation dose causing a 50% increase in NAR (PD_{50}) was derived by linear interpolation and geometric mean values calculated for each group. The nasal response to each agonist was compared as PD_{50} values with the Student's paired *t* test after logarithmic transformation of the data. The time dependent changes in NAR, albumin, and total protein following nasal challenge with single dose of bradykinin, histamine, and vehicle placebo were compared both within groups and between groups by Friedman's test, as the changes at single time points for each agonist were not normally distributed. To assess differences between treatments, the mean area under the curve (AUC) for each challenge period of observation was calculated and compared with the Wilcoxon's signed rank test for paired data. The Wilcoxon's signed rank test was also used for comparisons of the effects of bradykinin, histamine, and vehicle placebo challenges on the quantity of nasal secretions, the number of sneezes, and the visual analogue scores for nasal pain and nasal itch. Values were expressed as mean (SE) or median values with ranges as appropriate. A *p* value of <0.05 was accepted as the level of significance.

Results

PHASE 1

There were no significant differences between the baseline NAR measurements ($\text{Pa cm}^{-3} \text{ s}$) on any of the three challenge days (*p* > 0.05). Nasal challenge with incremental doses of both bradykinin and histamine induced a dose dependent increase in NAR in all 10 subjects (*p* < 0.001) while vehicle placebo was without significant effect (fig 1). At the doses selected there were no significant differences in the effects of bradykinin and histamine on NAR at any dose level (*p* > 0.05) whether assessed as peak response or AUC.

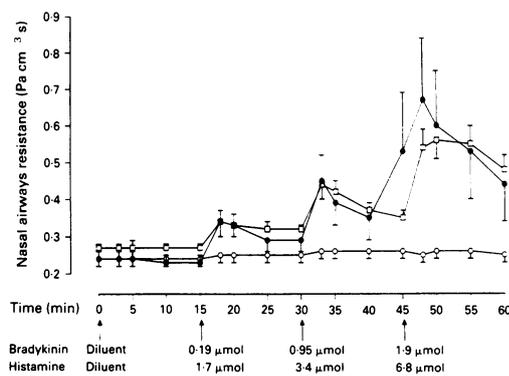


Figure 1 Dose dependent changes in nasal airways resistance ($\text{Pa cm}^{-3} \text{ s}$) following nasal challenge with incremental doses of bradykinin (\square), histamine (\bullet) and 10% ethanol saline (\circ). Each result is mean (SE) of observations in 10 subjects.

The mean PD_{50} value for bradykinin was 637.3 μ g and this did not differ significantly from the corresponding value with histamine of 1287.3 μ g. Comparison on a molar basis, however, identified PD_{50} values of 0.6 μ mol and 4.2 μ mol for bradykinin and histamine respectively (table) (*p* < 0.01), with bradykinin being 6.98 (0.56–59.1) times more potent than histamine in inducing the changes in NAR. Similarly analysis of the AUC data on a molar basis identified bradykinin to be 5.4 (0.72–17.43) times more potent than histamine in inducing nasal obstruction. No PD_{50} could be calculated after nasal challenge with the vehicle as no increase in NAR occurred. Both bradykinin and histamine also induced significantly greater rhinorrhoea than the vehicle (*p* < 0.05) with median secretion weights of 212, 260, and 280 mg for bradykinin and 360, 390, and 440 mg for histamine (fig 2). Histamine induced rhinorrhoea was significantly greater than that induced by bradykinin at all dose levels (*p* < 0.05).

Bradykinin induced significant nasal pain (*p* < 0.05) with median values of 2, 3, and 3 cm on the visual analogue scale (fig 3A). Maximal nasal pain was felt 3–5 minutes after the challenge and lasted for 10 minutes. Histamine and vehicle did not induce nasal pain. Histamine, however, induced significant nasal itch with median values on the visual

PD_{50} values for bradykinin and histamine

Subject no.	PD_{50} BK (μ mol)	PD_{50} H (μ mol)	Molar ratio of PD_{50} (PD_{50} H/ PD_{50} BK)
1	<0.19	<1.69	8.98
2	<0.19	11.15	59.10
3	3.02	<1.69	0.56
4	1.03	11.86	11.47
5	1.11	<1.69	1.53
6	<0.19	4.92	26.10
7	1.07	3.69	3.44
8	<0.19	6.42	34.03
9	2.03	6.78	3.34
10	0.65	3.31	5.11
Geometric mean	0.60	4.19	6.98

BK—bradykinin; H—histamine.

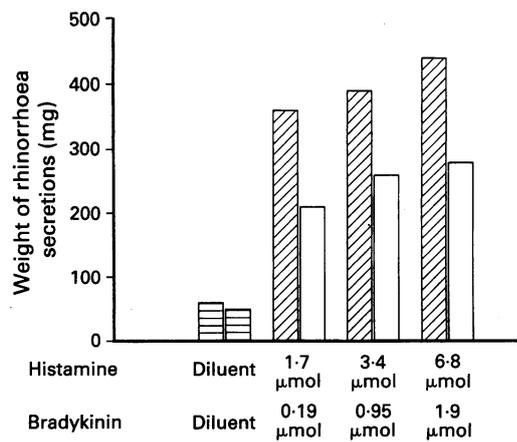


Figure 2 Changes in weight of rhinorrhoea secretions following nasal challenge with incremental doses of bradykinin (open bars), histamine (hatched bars), and vehicle placebo (horizontal hatched bars). Each column represents the median values from 10 subjects.

analogue scale of 1, 2, and 2 cm (fig 3B). Maximal nasal itch was felt three minutes after the challenge. Bradykinin and vehicle were without significant effect. Eight subjects sneezed more than twice in response to histamine challenge while no such response was observed after challenge with bradykinin or vehicle. Four subjects complained of a sore throat lasting up to 15 minutes after bradykinin challenge.

PHASE 2

There were no significant differences between baseline NAR measurements on any of the three challenge days ($p > 0.05$). Both bradykinin and histamine produced significant increases in NAR compared with the vehicle, with mean maximal percentage increments three minutes after the challenge of 88% ($p < 0.001$) and 25% ($p < 0.05$) respectively.

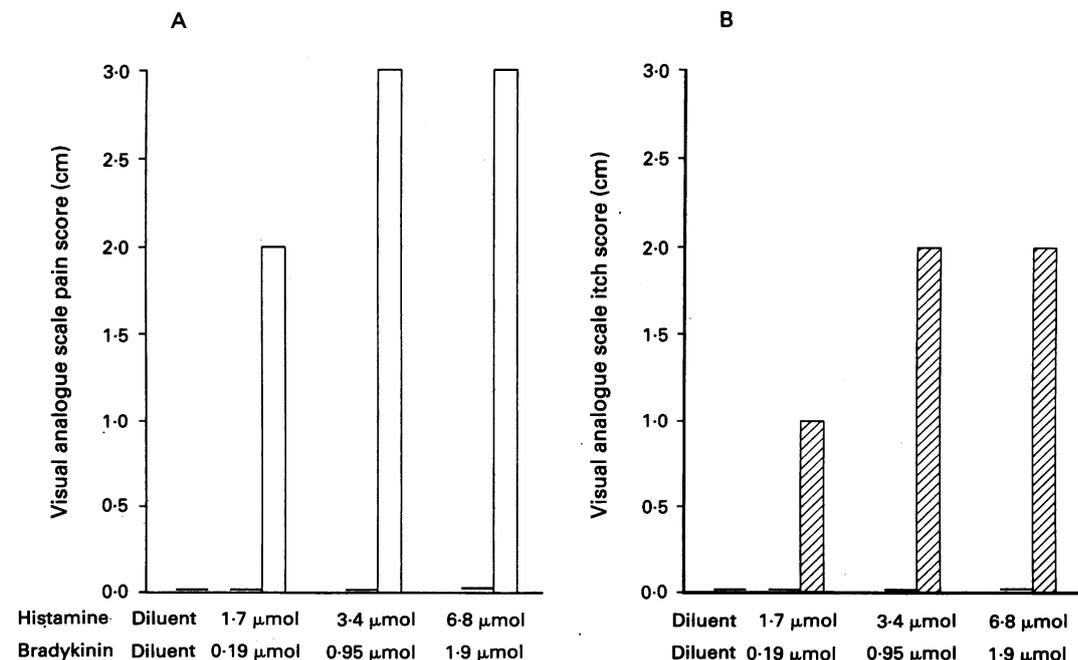
The effect of histamine was transient (less than five minutes) while the increase in NAR with bradykinin was maintained throughout the 40 minute period of observation. The AUC for the time response plot for bradykinin on NAR was significantly greater than for either histamine ($p < 0.05$) or the vehicle ($p < 0.005$). Bradykinin and histamine induced significant rhinorrhoea compared with the vehicle ($p < 0.05$) with median secretion weights of 376 (range 83–597) mg and 484 (range 96–1700) mg respectively. The volume of rhinorrhoea secretions induced by histamine was significantly greater than that induced by bradykinin ($p < 0.05$).

Bradykinin induced significant nasal pain ($p < 0.05$) with median values of 2.8 (range 0.4–7.2) cm on the visual analogue scale. Histamine, however, induced significant nasal itch with a median value of 2.2 (range 1.1–4) cm. Three subjects complained of sore throat lasting for up to 25 minutes after bradykinin challenge. Four subjects sneezed more than twice in response to histamine challenge. No such response was observed after bradykinin and vehicle nasal challenges.

PHASE 3

The mean volume of nasal lavage fluid recovered was 3.2 ml (64%). There were no significant differences between the fifth baseline levels of nasal lavage albumin and total protein in the eight subjects on any of the three challenge days (fig 4). Vehicle challenge had no significant effect on these levels (fig 4). In contrast, both bradykinin ($p < 0.01$) and histamine ($p < 0.05$) challenges produced significant elevations in lavage albumin and total protein levels (fig 4), with mean maximal levels of albumin and total protein of 193.9 and 343.3 $\mu\text{g/ml}$ for bradykinin, and 122.8 and 304.4 $\mu\text{g/ml}$ respectively for histamine. The albumin levels induced by bradykinin were significantly greater than those induced by histamine ($p < 0.05$).

Figure 3 Reporting of (A) nasal pain and (B) nasal itch following nasal challenge with incremental doses of bradykinin (open bars), histamine (hatched bars), and vehicle placebo (horizontal hatched bars). Each column represents the median values from 10 subjects.



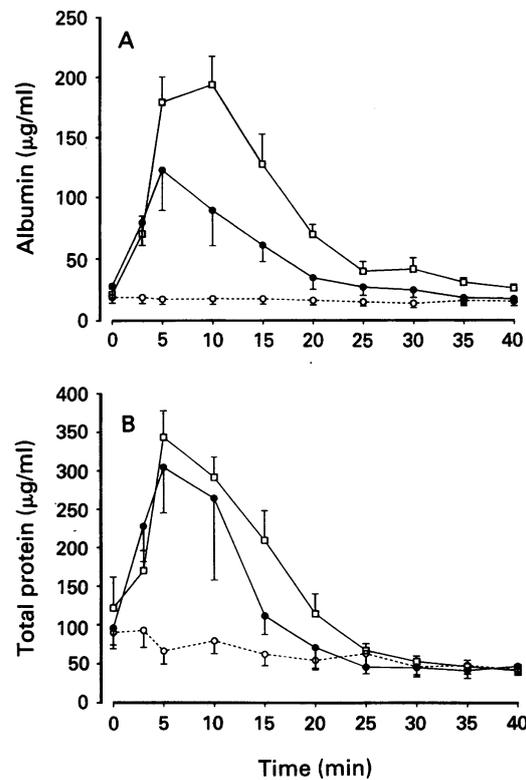


Figure 4 Effects of bradykinin 1.9 µmol (□), histamine 1.9 µmol (●), and 10% ethanol saline (○) on (A) nasal lavage albumin and (B) total protein. Each result is the mean (SE) of observations in eight subjects.

Discussion

By performing both a dose-response comparison and comparison of single, equimolar dose challenges in the same subjects, it has been possible to relate the vascular and non-vascular actions of bradykinin and histamine. This study shows that bradykinin is more potent than histamine in inducing nasal vascular changes and neurally mediated nasal pain, whereas histamine has a greater effect on neurally mediated rhinorrhoea and sneeze. Although these investigations were undertaken in healthy volunteers and it is possible that bradykinin and histamine may have differing comparative nasal effects in subjects with symptomatic allergic rhinitis, previous studies in rhinitic and normal subjects have not indicated consistent differences in the nasal response between subject groups,^{11 12 14} in contrast to the distinct disease related differences identified in asthma with inhalation challenge.¹⁸

The investigations of both NAR and nasal lavage protein and albumin measurements in the present study has allowed appraisal of the effects of bradykinin and histamine on both the nasal capacitance vessels and the superficial fenestrated capillaries within the nose. From the dose-response study it was possible to determine the PD₅₀ value for both agonists. This showed that bradykinin was 6.98 times more potent than histamine on a molar basis in inducing nasal blockage. Consistent with this, single dose nasal challenge with hista-

mine produced an increase in NAR that was significantly less than that induced by an isomolar dose of bradykinin, both in magnitude and duration, and only exceeded that of the vehicle placebo at three minutes after the challenge. This weak and transient effect of histamine on NAR supports previous findings.¹⁹ Our study therefore identifies within the confines of our experimental setup that bradykinin is more potent than histamine on a molar basis in inducing nasal blockage. The order of potency for bradykinin and histamine in influencing nasal airflow contrasts, however, with the findings reported by Doyle *et al* in the only other comparative study of these agonists within the nose.¹³ Their study indicated that histamine, when given in incremental doses, was more potent than bradykinin in inducing nasal blockage. While this might visually appear so at the highest doses investigated in the present study (fig 1), there was no significant difference between the two agonists at any time after the challenge. It is of interest, however, that histamine challenge has been shown to produce elevated kinin levels in nasal lavage specimens,²⁰ and an element of nasal blockage after repeated challenge with histamine could relate to an additional action of kinins rather than the action of histamine itself. This and the methods of comparison may explain the dichotomous results of Doyle *et al*.¹³ They compared the cumulative dose effect (mg for mg) of these agonists; the doses were not compared on an equimolar basis as in the present study and no comparative time course study using equimolar doses of bradykinin and histamine was performed. As the molecular weight of histamine is 3.45 times less than that of bradykinin, the molar dose of histamine was in fact greater than that of bradykinin and their results are thus not inconsistent with the findings presented in this paper. Furthermore, Doyle *et al* used average nasal conductance values at three and 10 minutes for comparison,¹³ in contrast to the more global AUC or specific PD₅₀ values used in the present study. As subjects differ in their time to peak nasal response after challenge, the selection of isolated time points for analysis may give rise to inaccuracies. The use of all time points as in AUC analysis or individual peak response with frequent measurements after challenge minimises this source of error.

In addition to the influence of agonists on nasal blockage, the comparative effect of bradykinin and histamine on plasma protein exudation was also investigated within the nose. The major change in lavage proteins after challenge was an increase in albumin, the increase being significantly greater with bradykinin than histamine (fig 4A). The present study therefore shows that bradykinin is more potent than histamine in inducing plasma protein leakage from the superficial mucosal vasculature, in addition to its effects on the nasal capacitance vessels. This was only quantified in a single isomolar dose challenge so no mathematical estimate of the

relative potencies can be ascertained for this parameter. This action on vascular protein exudation is likely to be of relevance both to extravascular fluid accumulation in mucosal oedema and to the volume of luminal secretions. In contrast to the relative nasal vascular effects, histamine had a greater effect on rhinorrhoea than bradykinin (fig 2). Although it is possible that long lasting nasal blockage may have reduced the recovery of anterior nasal secretions after bradykinin, it is more likely that histamine, unlike bradykinin,²¹ induces reflex mediated glandular secretion in addition to inducing vascular permeability changes. Consistent with this, histamine induced a comparable increase in total nasal lavage protein to bradykinin (fig 4B) in the absence of a comparable increase in nasal lavage albumin (fig 4A). The increase in non-albumin proteins is suggestive of glandular secretion rather than extravasation of proteins from the vascular component.²²

Evidence for relevant sensory neural stimulation with histamine was apparent in this study, with both itch and sneeze reported after challenge. Neither of these neurally mediated responses occurred with bradykinin challenge and we have previously shown that the rhinorrhoea associated with bradykinin is not inhibited by a muscarinic antagonist²³ indicating a non-cholinergic pathway. This, together with the nasal pain and discomfort reported with bradykinin challenge, suggests that bradykinin stimulates a different neural sensory mechanism within the nose from that stimulated by histamine, potentially non-cholinergic, non-adrenergic pathways.

This study therefore provides a potential explanation for the relative lack of effect of H₁ antihistamines in the treatment of nasal blockage in rhinitis since bradykinin, which has been found in increased amounts in nasal lavage fluid after allergen challenge in vivo, is more potent than histamine in inducing both nasal obstruction and plasma protein exudation. The development of potent and selective β_2 receptor kinin antagonists will allow further evaluation of kinins in nasal airflow obstruction and potentially enhance the treatment of allergic rhinitis.

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1 Naclerio RM, Proud D, Peters SP, Silber G, Sobotka AK, Adkinson NF, *et al.* Inflammatory mediators in nasal secretions during induced rhinitis. *Clin Allergy*

- 1986;16:101-10.
- 2 Naclerio RM, Meier HL, Sobotka AK, Adkinson NF, Meyers DA, Norman PS, *et al.* Mediator release after nasal airway challenge with allergen. *Am Rev Respir Dis* 1983;128:597-602.
 - 3 Naclerio RM, Proud D, Togias AG, Adkinson F, Meyers DA, Sobotka AK, *et al.* Inflammatory mediators in late antigen-induced rhinitis. *N Engl J Med* 1985;313:65-70.
 - 4 Howarth PH. Allergic rhinitis: a rational choice of treatment. *Resp Med* 1989;83:179-88.
 - 5 Corrada OJ, Gould CAL, Kassab JY, Davies RJ. Nasal responses of rhinitic and non-rhinitic subjects to histamine and methacholine; a comparative study. *Thorax* 1986;41:863-8.
 - 6 Borum P, Gronberg H, Brofeldt S, Mygind N. Nasal reactivity in rhinitis. *Eur J Respir Dis* 1983;64(Suppl 128):65-71.
 - 7 Proud D, Togias A, Naclerio RM, Crush SA, Norman PS, Lichtenstein LM. Kinins are generated in vivo following nasal airway challenge of allergic individuals with allergen. *J Clin Invest* 1983;72:1678-85.
 - 8 Svennsson C, Andersson M, Persson CGA, Venge P, Alkner U, Pipkorn U. Albumin, bradykinin and eosinophil cationic protein on the nasal mucosal surface in patients with hay fever during natural allergen exposure. *J Allergy Clin Immunol* 1990;85:828-33.
 - 9 Proud D, Naclerio RM, Gwaltney JM, Hendley JO. Kinins are generated in nasal secretions during natural rhinovirus colds. *J Infect Dis* 1990;161:120-3.
 - 10 Naclerio RM, Proud D, Lichtenstein LM, Sobotka AK, Hendley JO, Sorrentino J, *et al.* Kinins are generated during experimental rhinovirus colds. *J Infect Dis* 1988;157:133-42.
 - 11 Proud D, Reynolds CJ, Lacapra S, Sobotka AK, Lichtenstein LM, Naclerio RM. Nasal provocation with bradykinin induces symptoms of rhinitis and a sore throat. *Am Rev Respir Dis* 1988;137:613-6.
 - 12 Rajakulasingam K, Polosa R, Holgate ST, Howarth PH. Comparative nasal effects of bradykinin, kallidin and [des-arg⁷]-bradykinin in atopic rhinitic and normal volunteers. *J Physiol* 1991;437:577-87.
 - 13 Doyle WJ, Boehm S, Skoner DP. Physiologic responses to intranasal dose-response challenges with histamine, methacholine, bradykinin and prostaglandin in adult volunteers with and without nasal allergy. *J Allergy Clin Immunol* 1990;86:924-35.
 - 14 Holmberg K, Bake B, Pipkorn U. Vascular effects of topically applied bradykinin on the human nasal mucosa. *Eur J Pharmacol* 1990;175:35-41.
 - 15 Maraceu F, Leissier A, Regoli D, Girroud JP. Pharmacology of kinins: their relevance to tissue injury and inflammation. *Gen Pharmacol* 1983;14:209-29.
 - 16 Bradford MM. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 1976;72:248-54.
 - 17 Weeke B. Rocket immunoelectrophoresis. *Scand J Immunol* 1973;1:37-46.
 - 18 Townley RJ, Hopp RJ. Inhalation methods for the study of airway responsiveness. *J Allergy Clin Immunol* 1987;80:111-24.
 - 19 Howarth PH, Wilson S, Lau L, Rajakulasingam K. The nasal mast cell and rhinitis. *Clin Exp Allergy* 1991;21:3-8.
 - 20 Svennsson C, Baumgarten CR, Pipkorn U, Alkner U, Persson CGA. Reversibility and reproducibility of histamine induced plasma leakage in nasal airways. *Thorax* 1989;44:13-18.
 - 21 Baraniuk JN, Lundgren JD, Mizoguchi H, Peden D, Gawin A, Merida M, *et al.* Bradykinin and respiratory mucous membranes. *Am Rev Respir Dis* 1990;141:706-14.
 - 22 Raphael GD, Meredith SD, Baraniuk JN, Druce HM, Banks SM, Kaliner MA. The pathophysiology of rhinitis 2. Assessment of sources of protein in histamine-induced nasal secretions. *Am Rev Respir Dis* 1989;139:791-800.
 - 23 Rajakulasingam K, Polosa R, Lau LCK, Holgate ST, Howarth PH. Contribution of terfenadine and ipratropium bromide alone and in combination to bradykinin induced rhinitis and plasma vascular leakage. *Clin Exp Allergy* 1992;22:717-23.