

Cross refractoriness between bradykinin and hypertonic saline challenges in asthma

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Background: Repeated inhalation of bradykinin and hypertonic saline leads to refractoriness of the bronchoconstrictor response in asthma. It is not known whether cross-refractoriness exists between these stimuli.

Objective: We postulated that repeated bradykinin and hypertonic saline bronchial challenges might reduce the airway response to subsequent hypertonic saline and bradykinin challenges, respectively.

Methods: Eleven atopic asthmatic subjects underwent two concentration-response studies, separated by 1 hour, with either inhaled histamine or bradykinin. After recovery, a hypertonic saline challenge was performed. During the next phase, nine subjects underwent two concentration-response studies, separated by 1 hour, with hypertonic saline. After recovery, a bradykinin challenge was performed.

Results: On the histamine study day, the mean provocative volume of agonist required to produce 20% drop in forced expiratory volume in 1 second (PD_{20}) hypertonic saline was 220.7 L (± 42.7 L) and this was not significantly different from that measured at baseline. On the bradykinin study day, the geometric mean provocative concentration of agonist required to produce a 20% drop in forced expiratory volume in 1 second (PC_{20}) was 0.39 mg/ml (0.01 to 11.73 mg/ml) for the first test and significantly higher at 1.38 mg/ml (0.01 to >16.0 mg/ml) for the second test ($p = 0.006$). The hypertonic saline PD_{20} increased significantly from a baseline of 159.2 L (± 27.3 L) to 377.6 (± 64.7 L) ($p = 0.003$). On the hypertonic saline study day, the mean PD_{20} was 152.8 L for the first test, and 337.7 L for the second test ($p = 0.01$). PC_{20} bradykinin increased significantly from a baseline of 0.57 to 2.56 mg/ml ($p = 0.02$). A significant correlation was found between loss of response to bradykinin and to hypertonic saline (r_s , 0.63 and 0.76).

Conclusion: Refractoriness produced by repeated exposure of the airways to bradykinin and hypertonic saline results in loss of responsiveness to hypertonic saline and bradykinin respectively, suggesting a shared mechanism for refractoriness produced by these stimuli. (J ALLERGY CLIN IMMUNOL 1995;96:502-9.)

Key words: Tachyphylaxis, bradykinin, hypertonic saline, cross refractoriness

Bradykinin is a potent vasoactive nonapeptide formed as cleavage product from the action of plasma kallikrein on high molecular weight kininogen. When inhaled by asthmatic subjects, bradykinin causes bronchoconstriction.¹⁻³ Bradykinin pro-

Abbreviations used

FEV ₁ :	Forced expiratory volume in 1 second
HS:	Hypertonic saline
PC ₂₀ :	Provocative concentration of agonist required to produce 20% drop in FEV ₁
PD ₂₀ :	Provocative volume of agonist required to produce 20% drop in FEV ₁

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duces many of its effects by interacting with specific receptors, designated β_2 . Although in vivo structure-activity studies have suggested that bradykinin produces bronchoconstriction by stimulating β_2 receptors,² the physiologic mechanism(s) involved are not clearly understood. Although both the

TABLE I. Characteristics of subjects studied

Subject no.	Age (yr)	Sex	Baseline FEV ₁ (% predicted)	PC ₂₀ bradykinin (mg/ml)	PD ₂₀ HS (L)	Medication
1	25	M	125.0	3.6	310.0	S, <i>p.r.n.</i>
2	25	F	91.5	0.04	191.2	S, <i>p.r.n.</i> ; Bs, 400 µg <i>b.d.</i>
3	34	F	123.8	0.02	38.4	S, <i>p.r.n.</i>
4	24	F	98.0	3.8	218.0	S, <i>p.r.n.</i>
5	24	F	76.0	0.28	75.9	S, <i>p.r.n.</i> ; B, 500 µg <i>b.d.</i>
6	36	M	99.0	3.1	290.4	S, <i>p.r.n.</i> ; B, 200 µg <i>b.d.</i>
7	33	M	94.3	5.61	226.0	S, <i>p.r.n.</i> ; B, 500 µg <i>b.d.</i>
8	19	M	86.1	0.63	133.0	S, <i>p.r.n.</i> ; B, 100 µg <i>b.d.</i>
9	26	F	81.8	0.04	43.8	S, <i>p.r.n.</i> ; B, 500 µg <i>b.d.</i>
10	18	M	81.3	0.05	170.0	S, <i>p.r.n.</i> ; B, 500 µg at night
11	31	M	108.0	0.08	65.0	S, <i>p.r.n.</i> ; B, 100 µg <i>b.d.</i>
Mean (± SEM)	26.8 (±1.7)		96.8 (±4.73)	0.33*	159.2 (±27.3)	

S, Inhaled salbutamol; B, inhaled beclomethasone; Bs, inhaled budesonide; *b.d.*, twice daily; *p.r.n.*, as needed.

*Geometric mean.

selective histamine H₁-receptor antagonist, terfenadine, and the potent inhibitor of cyclooxygenase, flurbiprofen, exert only minimal protective effects on bradykinin-induced bronchoconstriction in asthma,³ ipratropium bromide, a selective muscarinic antagonist, has been shown to be much more effective.¹ This suggests an important role for vagal reflexes in the airway response to this kinin. Adding further support to neural mechanisms, Ichinose et al.⁴ have shown that FK 224, a neurokinin1 (NK1) antagonist, afforded eightfold protection against bradykinin-induced bronchoconstriction in asthma. Furthermore, both nedocromil sodium and sodium cromoglycate, two drugs known to interfere with sensory nerve fiber discharge, also afford protection against this mediator.⁵

Hypertonic saline (HS) also causes bronchoconstriction when inhaled by asthmatic subjects.⁶⁻⁸ The mechanisms by which this stimulus achieves its effect are thought to involve mediator release from mucosal mast cells⁸ and stimulation of neurogenic reflexes, two factors that will be facilitated by epithelial damage known to occur in asthma.⁹ Evidence that neural reflexes contribute to the airway response with HS in asthma stems from experiments showing attenuation by atropine, ipratropium bromide, and lidocaine.^{10, 11}

Repeated provocation of asthmatic airways with inhaled bradykinin and HS leads to a progressive loss of the constrictor response, whereas responsiveness to histamine and methacholine are maintained.^{7, 11-13} The mechanism(s) of refractoriness induced by both stimuli are not clearly understood.

Because both of these constrictor stimuli exhibit refractoriness, this study was undertaken to inves-

tigate whether a common mechanism underlies the loss of responsiveness to both stimuli by observing whether cross refractoriness exists between bradykinin and HS challenges in asthma.

METHODS

Subjects

Eleven asthmatic subjects (six men) with a mean (±SEM) age of 26.8 (±1.71) years participated in the study. All subjects were nonsmokers with atopic asthma as judged by at least one wheal greater than 3 mm on skin prick testing with *Dermatophagoides pteronyssinus*, house dust, mixed grass pollen, cat fur and feathers (Bencard, Brentford, Middlesex, U.K.). Their baseline forced expiratory volume in 1 second (FEV₁) was greater than 75% of their predicted values, and none were receiving oral corticosteroids or theophylline (Table I). Treatment with inhaled β₂-agonists was stopped at least 8 hours before each visit to the laboratory, although subjects were allowed to continue use of inhaled corticosteroids as usual. Patients were not studied within 4 weeks of an upper respiratory tract infection or exacerbation of asthma, and all visits to the laboratory were carried out at the same time of day. The study was approved by the Southampton University and Hospitals ethical subcommittee, and written informed consent was given by all subjects involved in the study.

Bronchial provocation tests

Bronchial challenge with histamine and bradykinin. Histamine acid phosphate (BDH Chemicals, Poole, Dorset, U.K.) was made up in 0.9% sodium chloride in a series of doubling concentrations, ranging from 0.03 to 16 mg/ml (0.1 to 52.1 mmol/L). Bradykinin triacetic acid (Nova Biochem, Nottingham, U.K.) was dissolved in phosphate-buffered saline (Sigma, Poole, U.K.) to produce a concentration range of 0.015 to 16 mg/ml (0.014

to 15.09 mmol/L). To avoid loss of bradykinin through oxidation and adherence to plastic surfaces, the stock solution was stored at 4°C before use, and broncho-provocation was performed within 30 minutes of preparing the dilutions. The solutions were administered as aerosols generated from a starting volume of 3 ml in a Cirrus Mininebulizer (Intersurgical, Middlesex, U.K.) driven by compressed air at a rate of 8 L/min. Under these conditions, the nebulizer generates an aerosol with a mass median particle diameter of 4.1 μ m and has an output of 62%.¹⁴

Bronchial challenge was performed with a technique modified from that of Chai et al.¹⁵ Measurements of FEV₁ were made with a dry wedge spirometer (Vitalograph, Buckinghamshire, U.K.). Before challenge, after 15 minutes' rest, three baseline measurements of FEV₁ were made at 3-minute intervals, and the highest value was recorded. Subjects then inhaled continuously nebulized 0.9% sodium chloride (saline solution), taking 5 slow breaths from functional residual capacity to full inspiration. Two FEV₁ measurements were performed at 1 and 3 minutes, and the higher value was recorded. If the FEV₁ value was 10% of the initial FEV₁, then bronchial provocation with histamine or bradykinin was undertaken. Increasing concentrations of agonists were inhaled at 5-minute intervals until the FEV₁ had fallen by 20% or more of the post-diluent baseline value or until the highest concentrations of agonist had been administered. The percentage fall in FEV₁ from postdiluent baseline was plotted against the cumulative concentration of agonist administered, and the provocative concentration of agonist required to produce a 20% fall in FEV₁ (PC₂₀) from the postdiluent baseline was derived by linear interpolation of the last 2 points.

Bronchial challenge with HS. Hypertonic saline (3.6%) was aerosolized with a DeVilbiss model 65 ultrasonic nebulizer (DeVilbiss, Feltham, Middlesex, U.K.) with an output of 10.8 ml per 100 L of air at the chosen nebulizer setting. We have shown previously that the output of HS to the mouthpiece is linearly related to the volume of air drawn through the nebulizer.⁸ The nebulizer chamber was connected to a Parkinson Cowan gas meter (P. K. Morgan, Chatham, Kent, U.K.) by wide-bore corrugated tubing with an internal diameter of 4 cm. The signal from the gas meter was input to a BBC microcomputer (Acorn Computers, U.K.), which converted the reading to biometric and temperature pressure saturated and displayed the volume in real time on a monitor. The output port of the nebulizer was connected to the mouthpiece via a one-way valve by a 60 cm length of the same wide-bore tubing. The mouthpiece was in turn connected to a two-way valve so the expired air could be ducted out of the laboratory. Thus when the nebulizer was in operation, the subject inhaled air containing aerosolized HS, and the cumulative volume of air inhaled was displayed.

The HS challenge was undertaken in a dose-response manner. After 15 minutes' rest, three measurements of FEV₁ were made for each subject, and the higher

reading was recorded as baseline. With the nebulizer switched off, the subject was then asked to breathe at tidal volume through the mouthpiece until 25 L of air had been respired. Three further measurements of FEV₁ were recorded at 30 and 90 seconds and at 3 minutes, and the higher value was recorded as the new baseline. If this value was within 10% of the initial FEV₁, then the challenge test proceeded. The nebulizer was switched on and the subject, wearing a noseclip, breathed quietly through the mouthpiece. After respiring 5 L of HS-laden air, single recordings of FEV₁ were made at 30 and 90 seconds and at 3 minutes. If the FEV₁ had fallen by 20% or more from the post-air baseline value at any of the three time points, then the test was terminated; otherwise, additional volumes of HS were respired and the procedure was repeated. Volumes of 5, 10, 15, 20, 20, 25, 35, 40, 40, 50, and 50 L (biometric and temperature pressure saturated) HS-laden air were administered in this sequence until a fall of greater than 20% in FEV₁ occurred or a total maximum volume of 310 L of HS-laden air had been respired. The dose of HS administered was expressed as the volume of air drawn from the nebulizer and plotted on a linear scale against the percentage fall in FEV₁. The provocative volume of HS-laden air required to induce a 20% fall in FEV₁ (PD₂₀HS) was derived by linear interpolation of the last 2 points or, when not possible, by linear extrapolation. If the fall in FEV₁ after 310 L was too small to permit extrapolation, then the PD₂₀HS was estimated as 620 L.^{8, 10}

Study design

The study was divided into three phases, which were separated by at least 7 days.

Phase 1 (open study day). The initial phase comprised three separate nonrandomized visits to the laboratory at least 72 hours apart to undertake dose-response studies with inhaled histamine, bradykinin, and HS in order to establish the baseline level of response.

Phase 2 (bradykinin and histamine study days). The second phase consisted of two visits separated by at least 7 days, during which two consecutive concentration-response studies with inhaled bradykinin on one occasion and two with histamine on another occasion were undertaken in a double-blind, randomized manner. An initial bronchial provocation test with either bradykinin or histamine was performed until FEV₁ fell $\geq 20\%$ of postdiluent baseline value. The airways were then allowed to recover spontaneously until FEV₁ had returned to within 5% of the postdiluent baseline value. On achieving this (1 hour later), a second agonist concentration-response challenge was undertaken until the FEV₁ fell $\geq 20\%$ of the original postdiluent value or until the highest concentration of either agonist had been administered. On completion of the second agonist challenge, after an interval of 45 minutes, a bronchial provocation test with HS-laden air was then carried out until the FEV₁ fell $\geq 20\%$ of the postdiluent value or until the highest volume of agonist had been administered.

PC₂₀ values for bradykinin and histamine and PD₂₀ values for HS were derived as described previously.

Phase 3 (hypertonic saline study day). The third phase consisted of one visit, during which two consecutive concentration-response studies with HS were undertaken. An initial bronchial provocation test with HS was performed until FEV₁ fell $\geq 20\%$ of postdiluent baseline value. The airways were then allowed to recover spontaneously until FEV₁ had returned to within 5% of the postdiluent baseline value. On achieving this (1 hour later), a second HS dose-response challenge was undertaken until the FEV₁ fell $\geq 20\%$ of the original postdiluent value or until the highest volume of agonist had been administered. On completion of the second agonist challenge, after an interval of 45 minutes, bronchial provocation with bradykinin was then carried out until the FEV₁ fell $\geq 20\%$ of the postdiluent value or until the highest concentration of agonist had been administered. PC₂₀ values for bradykinin and PD₂₀ values for HS were derived as described previously.

Data analyses

For FEV₁ the lowest of the values recorded with each inhaled concentration or volume of agonist was used for analysis. Baseline FEV₁ measurements before bronchial challenges were compared between study days by two-way analysis of variance. The airway response to histamine, bradykinin, and HS at each agonist concentration was expressed as the percentage change in FEV₁ from the postdiluent baseline value. Values of PC₂₀ bradykinin and histamine were logarithmically transformed and compared by means of Student's *t* test for paired data. However, in three subjects (Nos. 4, 7, and 8) a minimal 20% fall in FEV₁ could not be achieved when the maximum concentration of bradykinin was administered during the second challenge. In these subjects an estimate of the PC₂₀ was used as the cumulative concentration beyond the highest dose administered (32 mg/ml). Because of this censored data, the values for the first and second bradykinin challenges were compared by using Wilcoxon's signed-rank test. Similarly, Wilcoxon's signed-rank test was used to compare PD₂₀ values for HS. To quantify bradykinin refractoriness, an index of refractoriness was calculated by dividing the second bradykinin PC₂₀ by the first bradykinin PC₂₀. For each subject, an index of refractoriness to HS after repeated bradykinin challenge was obtained by calculating the ratio of the PD₂₀HS values derived on the bradykinin challenge day by the PD₂₀HS values obtained on the baseline visit. Similarly, for each subject, an index of refractoriness to bradykinin after repeated HS challenge was also obtained by calculating the ratio of the bradykinin PC₂₀ values derived on the HS challenge day by the bradykinin PC₂₀ values obtained on the baseline visit. The relationship between these two indices was assessed by Spearman's rank correlation. Results are expressed as means ($\pm$ SEM) unless otherwise stated, and a *p* value of less than 0.05 was accepted as the level of significance.

RESULTS

All subjects exhibited bronchial hyperreactivity to histamine, bradykinin, and HS. On the open study day (phase 1), the geometric mean PC₂₀ values for histamine and bradykinin were 0.9 mg/ml (0.03 to 5.75 mg/ml) and 0.33 mg/ml (0.02 to 5.61 mg/ml), respectively (Table I). The mean PD₂₀ value for HS was 159.2 ± 27.3 L (Table I). Baseline values of FEV₁ did not differ significantly among any of the 3 study days; the mean values were 3.54 ± 0.33 L, 3.38 ± 0.21 L, 3.31 ± 0.21 L, 3.35 ± 0.21 L, 3.4 ± 0.24 L, and 3.45 ± 0.25 L for open HS, histamine, and bradykinin study days and for repeated bradykinin, histamine, and HS study days, respectively.

Repeated challenge of the airways with bradykinin resulted in loss of responsiveness, and for the group as a whole, the geometric mean bradykinin PC₂₀ increased from 0.39 mg/ml (0.01 to 11.73 mg/ml) after the first challenge to 1.38 mg/ml (0.01 to >16.0 mg/ml) after the second challenge (*p* = 0.006) (Table II), producing a concentration ratio of 3.54. The mean bradykinin PC₂₀ after the second challenge is a minimal estimate, because a PC₂₀ was not achieved in subjects 4, 7, and 8, for whom the PC₂₀ tabulated is one dose of concentration beyond the last. Consecutive histamine challenges showed no significant changes in responsiveness between the first and second dose-response exposures, the geometric mean (range) of histamine PC₂₀ values being 1.01 (0.04 to 8.41) and 0.91 (0.04 to 6.78) mg/ml, respectively (*p* > 0.05) (Table II).

On the bradykinin study day, after recovery from the second bradykinin challenge, provocation of the airways with HS-laden air also showed a reduced response. When compared with the open HS study day, the mean PD₂₀HS increased by more than twofold to 377.6 ± 64.7 L (*p* = 0.003) (Tables I and II). In contrast, the PD₂₀HS value obtained on the histamine study day was not significantly different from that measured on the open visit day, the mean value being 220.7 ± 42.7 L (*p* > 0.05) (Tables I and II). The PD₂₀HS value obtained on the bradykinin challenge day was also greater than that obtained on the histamine challenge day (*p* = 0.02). When loss of responsiveness was compared with bradykinin and HS, a significant correlation was observed (Spearman's correlation coefficient, 0.63; *p* = 0.02) (Fig. 1, A); however, no significant correlation could be established between baseline bradykinin PC₂₀ and the fold shift in reactivity to bradykinin ($[\text{PC}_{20}\text{BK}_2 - \text{PC}_{20}\text{BK}_1]/\text{PC}_{20}\text{BK}_1$) or HS ($[\text{PD}_{20}\text{HS}_2 - \text{PD}_{20}\text{HS}_1]/$

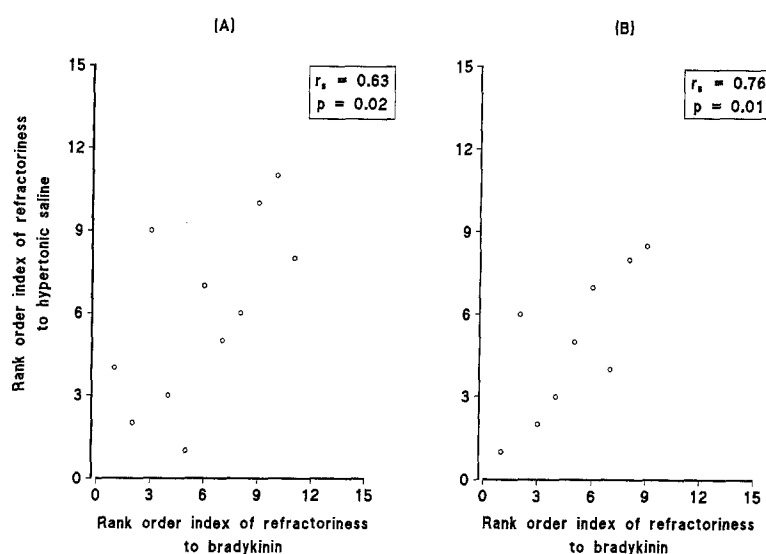


FIG. 1. Correlation between (A) HS (PD₂₀HS on bradykinin day/PD₂₀HS on open day) and bradykinin (PC₂₀BK₂/PC₂₀BK₁) and (B) bradykinin (PC₂₀BK on HS day/PC₂₀BK on open day) and HS (PD₂₀HS₂/PD₂₀HS₁) induced refractoriness to bronchoconstriction.

TABLE II. Effects of repeated inhalations with bradykinin and histamine on airway HS responsiveness in 11 subjects (phase 2)

Subject no.	PC ₂₀ BK ₁	PC ₂₀ BK ₂	PD ₂₀ HS	PC ₂₀ H ₁	PC ₂₀ H ₂	PD ₂₀ HS
1	1.57	10.3	620.0	1.57	1.87	377.9
2	0.02	0.07	289.2	5.79	4.9	459.3
3	0.01	0.01	47.3	0.16	0.08	39.2
4	11.73	32.0	620.0	8.41	6.78	452.6
5	2.27	2.25	110.0	0.04	0.04	127.3
6	5.4	7.63	394.9	2.88	2.25	289.9
7	8.2	32.0	496.0	1.31	3.21	371.2
8	0.68	32.0	378.0	1.0	0.52	115.0
9	0.03	0.06	50.0	1.26	0.92	41.7
10	0.04	0.66	601.9	0.42	0.32	151.7
11	0.08	1.48	552.0	1.2	1.56	120.0
Geometric mean	0.39	1.38	377.6 (±64.7)*	1.01	0.91	*220.7 (±42.7)

BK₁, Baseline bradykinin reactivity; BK₂, repeat bradykinin reactivity 1 hour later; H₁, baseline histamine reactivity; H₂, repeat histamine reactivity 1 hour later.

*Mean.

PD₂₀HS₁) (Spearman's correlation coefficient $r_s = 0.23$; $p > 0.05$) (Fig. 1, B).

Two subjects declined to take part in the third phase of the study. On the open visit day, for the nine subjects the geometric mean baseline bradykinin PC₂₀ was 0.57 mg/ml (0.04 to 5.61 mg/ml). Repeated challenge of the airways with HS resulted in loss of responsiveness, and for the group as a whole the mean PD₂₀HS increased from 152.8 ± 20.3 L after the first challenge to 337.7 ± 67.0 L after the second challenge ($p = 0.01$) (Table III), producing a concentration ratio of 2.17. The mean PD₂₀HS after the second

challenge is a minimal estimate, because a PD₂₀ was not achieved in three subjects for whom the PD₂₀ tabulated is 620 L. On recovery from the second HS challenge, provocation of the airways with bradykinin also showed a reduced response. When compared with open bradykinin study day, the mean bradykinin PC₂₀ increased by more than 4.47-fold to 2.56 mg/ml (0.04 to 10.22 mg/ml) ($p = 0.02$) (Table III). A significant correlation was also observed between loss of responsiveness to HS and bradykinin (Spearman's correlation coefficient $r_s = 0.76$; $p = 0.01$) (Fig. 1, B).

DISCUSSION

This study confirms previous findings that both inhaled bradykinin and HS cause dose-related bronchoconstriction in asthmatic subjects.^{1, 2, 6, 8} It has also been demonstrated that repeated exposure of asthmatic airways to bradykinin and HS leads to a progressive loss of constrictor response to these agonists.^{7, 12, 13} We have now extended this observation by demonstrating that repeated bronchial challenge with bradykinin and HS substantially inhibits the subsequent bronchoconstrictor response to HS and bradykinin, respectively. Within the group a significant relationship existed between the extent of bradykinin and HS refractoriness and the protection afforded against HS and bradykinin challenges, respectively. These data suggest that a common mechanism may account for refractoriness observed with both stimuli.

Previous work in our department has shown that the repeatability of bradykinin challenge when expressed as PC₂₀ lies within a single doubling dilution.^{2, 3, 12} If refractoriness is defined as greater than one doubling dilution of repeated bradykinin challenges, subjects 1, 2, 4, 7, 8, 10, and 11 were refractory on the bradykinin study day. Similarly, we have previously shown that for HS challenges, the repeatability of PD₂₀ values lies within 1.5 doubling volumes.⁸ If refractoriness is defined as greater than 1.5 doubling volumes of repeated HS challenges, subjects 2, 4, 7, 8, 10, and 11 were refractory on the HS study day. Bradykinin inhalation, followed by repeated histamine challenge, was not included in our study, because we have previously shown that repeated histamine inhalation does not influence bradykinin reactivity.¹² Furthermore, this study has also shown that only two subjects (1 and 7) showed any diminished responsiveness to repeat histamine challenges. Because PC₂₀ was not achieved in three subjects (Nos. 4, 7, and 8), the level of refractoriness calculated for bradykinin is likely to be an underestimation. Similarly, PD₂₀ was not achieved in three subjects, and the level of refractoriness calculated for HS is also likely to be an underestimation. Although with repeated histamine challenge five of the 11 subjects exhibited an apparent reduction in HS responsiveness, this failed to reach statistical significance.

Although the development of refractoriness to repeated bradykinin and HS challenges appears to be specific to these agonists in that cross-refractoriness with histamine has not been demonstrated,^{7, 13} the mechanism for the loss of response is not clearly understood. It is unlikely that direct end

TABLE III. Effects of repeated inhalations with HS on airway bradykinin responsiveness in nine subjects (phase 3)

Subject no.	PC ₂₀ HS ₁	PC ₂₀ HS ₂	PC ₂₀ bradykinin
1	280	280	9.39
2	210	566.2	6.93
4	180	310	10.2
6	210	233.3	4.31
7	114.5	174.1	2.96
8	60	200	2.76
9	40.7	35.3	0.04
10	180	620	0.7
11	180	620	9.39
Mean	152.8 (±20.3)	337.7 (±67.0)	*2.56

HS₁, Baseline hypertonic saline reactivity; HS₂, repeat HS reactivity 1 hour later.

*Geometric mean.

organ effects can result in refractoriness, because histamine, which is known to exert direct bronchoconstrictor effect, does not lead to refractoriness.¹⁶ Functional antagonism caused by the local production of protective prostanoids, such as prostaglandins E₂ and I₂, have been excluded in view of the lack of effect of cyclooxygenase inhibitors on refractoriness.¹² Another possible mechanism is a reduction in the capacity of sensory neurons to release tachykinins. In the microvasculature of the rat trachea while under neural regulation, repeated stimulation of sensory nerves results in a progressive loss of response measured in terms of protein leakage,¹⁷ a mechanism that is compatible with the observation in the guinea pig lung and in asthmatic patients, showing that bradykinin-induced bronchoconstriction is tachykinin-dependent.^{4, 18} Thus refractoriness to bradykinin with repeated inhalations might be explained on the basis of a downregulation of bradykinin function on sensory nerve endings either at the level of the receptor or by depleting neurons of their neuropeptides.

Refractoriness to HS can not be attributed to protective catecholamines,¹⁹ a reduction of non-specific airway responsiveness after the initial challenge,²⁰ or mediator release from mast cells.²¹ Furthermore, indomethacin had a variable effect on the refractory period induced by HS,²² suggesting other contributory mechanisms. In rabbits, HS has been shown to cause neurogenic inflammation in the eye,²³ and in rats HS-induced vascular permeability is potentiated by the neutral endopeptidase inhibitor phosphoramidon, indicating

that changes in vascular permeability are secondary to the release of neuropeptides from the sensory neurons.²⁴ Furthermore, in the same model capsaicin pretreatment has been shown to completely inhibit the vascular permeability changes induced by HS, but the response to substance P was unaffected, indicating that the ability of the vessels to respond to neuropeptides remained intact. This illustrates that the lack of increase in vascular leakage after airway challenge with HS is probably due to depletion of neuropeptides from sensory neurons.²⁴ According to animal studies, refractoriness to HS can be explained on the basis of depletion of neuropeptides from sensory neurons after repeated inhalation.

Although it is possible that refractoriness induced by both bradykinin and HS challenges may involve different mechanisms, this does not easily explain why repeated bradykinin inhalation should protect against the bronchoconstrictor effect of HS and vice versa. The plausible explanation might be that both these stimuli cause refractoriness via a very similar, if not identical, final common pathway. Repeated bradykinin and HS challenges, which had induced significant reduction in bronchial responsiveness to the same stimuli, have resulted in a similar level of protection against bronchoconstriction provoked by HS and bradykinin, respectively. On the other hand, repeated histamine challenge, which induced no obvious refractoriness, has no effect on subsequent HS challenge. The view of a shared mechanism is further strengthened by the observation of a significant correlation between the loss of responsiveness to the two stimuli ($r_s = 0.63$ and 0.76 , $p < 0.05$). However, the absence of significant correlation between baseline bradykinin PC₂₀ and fold increase in bradykinin or HS reactivity indicates that the amount of refractoriness is not related to the dose of agonist inhaled.

In conclusion, we have demonstrated that repeated exposure of the airways of atopic subjects with asthma to bradykinin and HS results in the development of cross-refractoriness to HS and bradykinin, respectively. By demonstrating cross-refractoriness between the stimuli, we suggest that bradykinin and HS produced a refractory period through a very similar, if not identical, final common pathway.

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