

Repeated inhalation of bradykinin attenuates adenosine 5'-monophosphate (AMP) induced bronchoconstriction in asthmatic airways

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ABSTRACT: Repeated bronchial challenges with inhaled bradykinin lead to a rapid loss of the bronchoconstrictor response and this has been suggested to be due to depletion of contractile neuropeptides from sensory nerve endings. If adenosine 5'-monophosphate (AMP), another potent bronchoprovocant in asthma, and bradykinin share a common pathway in inducing bronchoconstriction in asthmatic subjects, then repeated bradykinin bronchoprovocation tests should reduce the response to subsequent inhalation of AMP.

We examined this hypothesis in eight asthmatic subjects in a double-blind, randomized study. On the histamine study day, two consecutive concentration-response studies with inhaled histamine were followed by a third consecutive challenge with AMP and the provocation concentration producing a 20% fall in forced expiratory volume in one second from the post-diluent baseline value ($PC_{20}FEV_1$) for this agonist was calculated. On the bradykinin study day, two consecutive bronchoprovocation tests with bradykinin were followed by a third inhalation challenge to obtain the $PC_{20}AMP$ value. On a further occasion, the asthmatic subjects underwent two consecutive concentration-response studies with inhaled bradykinin followed by a third consecutive challenge with histamine.

On the histamine study day, the geometric mean $PC_{20}AMP$ value was $28.1 \text{ mg}\cdot\text{ml}^{-1}$, whilst on the bradykinin study day, the fifteenfold reduction in bradykinin responsiveness after the second bradykinin challenge was accompanied by a significant reduction of the airway responsiveness to AMP, the $PC_{20}AMP$ being $59.8 \text{ mg}\cdot\text{ml}^{-1}$. A similar reduction in bradykinin responsiveness failed to alter the airway response to a subsequent inhalation with histamine.

We conclude that repeated challenge of the airways with bradykinin attenuates a component of the responsiveness to AMP, suggesting a shared mechanism of refractoriness, possibly involving a reduced neural component consequent upon neuropeptide depletion.

Eur Respir J, 1992, 5, 700-706.

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Keywords: Adenosine 5'-monophosphate
bradykinin
bronchoconstriction

Received: July 12 1991
Accepted after revision January 13 1992

Bradykinin (Bk) is a naturally occurring inflammatory nonapeptide which is generated as a cleavage product from the action of kallikreins on plasma kininogen [1]. When inhaled by asthmatic subjects, Bk causes bronchoconstriction which reaches maximum 5-10 min after inhalation and resolves within 45-60 min [2, 3]. Bradykinin produces its many biological effects by stimulating specific receptors, which have been classified as B_1 or B_2 according to the ability (B_1) or not (B_2) of (desArg⁹)-bradykinin to evoke a response [1]. Although *in vivo* structure activity studies have suggested that the bronchoconstrictor response of the asthmatic airways to inhaled Bk may result from a specific pharmacological interaction compatible with the stimulation of B_2

receptors [3], the mechanism(s) of action of Bk in causing bronchoconstriction is not known. We have recently observed that both the selective histamine H_1 -receptor antagonist, terfenadine, and the potent inhibitor of cyclooxygenase, flurbiprofen, have only minimal effects on bronchoconstriction produced by inhaled Bk in asthma [4]. FULLER *et al.* [2] have shown that Bk-induced bronchoconstriction is attenuated by prior inhalation of the cholinergic antagonist, ipratropium bromide, suggesting that an important part of its constrictor effect is mediated *via* vagal reflexes.

In asthma, repeated provocation of the airways with Bk leads to a progressive loss of bronchoconstrictor response without altering the airway response to histamine [5]. Since functional antagonism

secondary to the local production of protective prostanoids is not involved [5], we proposed that loss of Bk response in asthma may represent a depletion of sensory tachykinins. Such a hypothesis would be compatible with recent findings of Bk inducing the release of tachykinins in perfused guinea-pig lung [6].

Adenosine and its related nucleotide, adenosine 5'-monophosphate (AMP), also cause bronchoconstriction when inhaled by atopic [7, 8] and non-atopic [7, 9] asthmatic subjects. Adenosine potentiates the release of the preformed mediators, β -hexosaminidase and histamine, from immunologically stimulated rodent [10] and human [11] mast cells *in vitro*, through an interaction with specific cell surface purinoceptors. That mast cell mediator release is a major cause of AMP-induced bronchoconstriction is supported by clinical studies with selective H_1 -receptor antagonists, such as terfenadine and astemizole, which are effective inhibitors of the response [8, 9, 12]. Evidence that neural reflexes also participate in the airway response to inhaled purines stems from experiments in rabbits where adenosine potentiates the airway constrictor response to transmural nerve stimulation [13], and in inbred rats where atropine has a small but definite inhibitory effect on bronchoconstriction provoked by intravenous adenosine [14]. In asthmatic subjects, OKAYAMA *et al.* [15] have reported some reversal of an established adenosine bronchoconstriction with inhaled atropine and lignocaine. In a more recent study, we have demonstrated [16] a small but definite inhibitory effect of inhaled ipratropium bromide against AMP-induced bronchoconstriction. In common with Bk, repeated challenge of the airways with AMP leads to the rapid development of tolerance to its bronchoconstrictor effect [17].

In the present study, we have investigated whether a common mechanism underlies the loss of responsiveness to both bronchoconstrictor stimuli by observing whether cross-refractoriness develops between Bk and AMP using histamine as a control.

Methods

Subjects

Eight subjects (6 male and 2 female) with a mean (\pm SEM) age of 25 (\pm 3) yrs participated in the study. All subjects were nonsmokers with atopic asthma as defined by positive skin prick tests (>2 mm wheal response) to five common aeroallergens: *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, mixed grass pollen, tree pollen and cat fur (Bencard, Brentford, Middlesex, UK). Their baseline forced expiratory volume in one second (FEV_1) was $>75\%$ of their predicted values and none were receiving oral corticosteroids or theophylline (table 1). Inhaled broncodilators were discontinued for at least 8 h prior to each visit to the laboratory, although subjects were allowed to continue inhaled corticosteroids as usual. Patients were not studied within 4 weeks of an upper respiratory tract infection or exacerbation of their asthma and all visits to the laboratory were carried out at the same time of day. The subjects gave their written informed consent and the study was approved by the Southampton University and Hospitals Ethics Subcommittee.

Bronchial provocation

Pulmonary function was measured as FEV_1 by means of a dry wedge spirometer (Vitalograph, Buckingham, UK), the higher of two consecutive measurements being recorded. Histamine acid phosphate (BDH Chemicals, Poole, Dorset, UK) and AMP (Sigma Chemical Co., St. Louis, MO, USA) were dissolved in 0.9% sodium chloride to produce a concentration range of 0.03–16 $mg \cdot ml^{-1}$ (0.1–52 $mmol \cdot l^{-1}$) (pH range 5.2–4.2; osmolarity range 274–385 mOsm) and 0.39–400 $mg \cdot ml^{-1}$ (4.48–1,151.6 $mmol \cdot l^{-1}$) (pH range: 5.6–5.7; osmolarity range 273–1,650 mOsm),

Table 1. — Characteristics of subjects studied

| Subject no. | Sex | Age yrs | Baseline FEV_1 % predicted | $PC_{20}H$ $mg \cdot ml^{-1}$ | $PC_{20}AMP$ $mg \cdot ml^{-1}$ | $PC_{20}Bk$ $mg \cdot ml^{-1}$ |
|-------------|-----|---------|------------------------------|-------------------------------|---------------------------------|--------------------------------|
| 1 | M | 44 | 75 | 0.55 | 11.8 | 0.23 |
| 2 | M | 19 | 87 | 0.52 | 5.9 | 0.05 |
| 3 | F | 30 | 121 | 2.1 | 79.8 | 0.31 |
| 4 | M | 19 | 115 | 1.8 | 80.2 | 0.88 |
| 5 | F | 20 | 104 | 0.40 | 14.1 | 0.07 |
| 6 | M | 23 | 90 | 0.25 | 98.3 | 0.89 |
| 7 | M | 23 | 83 | 1.3 | 22.6 | 0.29 |
| 8 | M | 22 | 86 | 2.4 | 54.3 | 0.14 |
| Mean | | 25 | 95 | 0.87* | 30.9* | 0.23* |
| SEM | | ± 3 | ± 5.8 | (0.25–2.39) | (5.86–98.3) | (0.05–0.89) |

*: geometric mean (range). FEV_1 : forced expiratory volume in one second; AMP: adenosine monophosphate; H: histamine; Bk: bradykinin; PC_{20} : provocative concentration of agonist producing a 20% fall in FEV_1 from the post-diluent baseline value.

respectively. Bradykinin triacetic acid (Nova Biochem Ltd, Nottingham, UK) was freshly prepared in 10% ethanol in 0.9% sodium chloride to produce a stock solution of 8 mg·ml⁻¹ and then diluted with its appropriate diluent to produce a concentration range of 0.0037–8 mg·ml⁻¹ (0.0035–7.54 mmol·l⁻¹) (pH range 5.4–5.5; osmolarity range 1,698–1,885 mOsm). To avoid loss of Bk through oxidation and adherence to plastic surfaces, the stock solution was stored at 4°C prior to use and bronchoprovocation was performed within 30 min of preparing the dilutions. The solutions were administered as aerosols generated from a starting volume of 3 ml in a disposable Inspiron Mini-nebulizer (C.R. Bard International, Sunderland, UK) driven by compressed air at 8 l·min⁻¹. Under these conditions, the nebulizer generates an aerosol with a mass median particle diameter of 4.7 µm [18]. Subjects inhaled the aerosolized solutions in five breaths from end-tidal volume to full inspiratory capacity via a mouthpiece as described by CHAI *et al.* [19]. Subjects were trained to take 3 s to reach full inspiratory capacity.

Study design

The study was divided into three phases.

Phase 1. This initial phase of the study comprised three separate non-randomized visits to the laboratory, at least 72 h apart, to undertake concentration-response studies with inhaled histamine, AMP and Bk. On each occasion, after 15 min of rest, three baseline measurements of FEV₁ were recorded at 3 min intervals. Subjects then inhaled nebulized vehicle diluent solution and FEV₁ measurements were repeated at 1 and 3 min, the higher value being recorded. Provided that the FEV₁ did not fall by >10% of the baseline value, bronchial provocation test with one of the three agents was carried out. After administration of each concentration of agonist, FEV₁ was measured at 1 and 3 min, the higher of the two values being recorded. Increasing concentrations of histamine, AMP or Bk were inhaled at 5 min intervals until FEV₁ had fallen by >20% of the post-diluent baseline value, or until the highest concentrations of agonist had been administered. The percentage decrease in FEV₁ from post-diluent baseline was plotted against the cumulative concentration of agonist administered and the provocative concentration of agonist required to produce a 20% fall in FEV₁ from the post-diluent baseline value (PC₂₀FEV₁) was determined by linear interpolation.

Phase 2. This consisted of two visits, separated by at least 5 days, during which 2 consecutive concentration-response studies with either inhaled Bk or histamine (as a control challenge) were undertaken in a double-blind, randomized manner. An initial bronchial provocation test with Bk or histamine was performed until FEV₁ fell to >20% of post-diluent baseline value. The airways were then allowed to recover spontaneously until the FEV₁ had returned to within 5% of their post-diluent baseline value.

On achieving this (approximately 50–75 min later) a second agonist concentration-response challenge was undertaken until FEV₁ fell >20% of the original post-diluent value, or the highest concentrations of agonist had been administered. Once the FEV₁ after the second inhalation test with either Bk or histamine had returned to within 5% of the post-diluent baseline value a third challenge was undertaken, this time with inhaled AMP, and PC₂₀ values for this agonist were derived as described previously.

Phase 3. This was carried out to determine the specificity of the Bk effect on subsequent nonspecific contractile stimuli. Subjects attended the laboratory on a single occasion to undertake a concentration-response study with inhaled histamine after receiving two consecutive inhalation challenges with Bk administered in an identical fashion to that described in phase 2.

Data analysis

Results are expressed as mean±SEM unless otherwise stated and the p<0.05 was accepted as the level of significance. Baseline FEV₁ values prior to bronchial challenges were compared between study days by analysis of variance (ANOVA). The airways response to histamine, AMP and Bk at each agonist concentration was expressed as the percentage change in FEV₁ from the post-diluent baseline value. The repeatability of the AMP challenge procedure was determined according to the method described by ALTMAN and BLAND [20], of plotting the difference against the mean of the logarithmically transformed PC₂₀ values obtained on the histamine and open study days. The mean and standard deviation (SD) of the difference between these values were then derived and used to calculate the coefficient of repeatability (CR) between the results of the two study days. Similarly, we assessed the repeatability of the loss of Bk responsiveness obtained in phases 2 and 3. The slopes of the AMP concentration-response curves after repeated exposure with Bk and histamine were determined by least squares linear regression analysis and compared by Student's t-test.

In two subjects (nos 4 and 6) in phase 2, and in one subject (no. 4) in phase 3, a minimal 20% fall in FEV₁ could not be achieved when the maximum concentration of Bk was administered during the second challenge. In these subjects, an estimate of the PC₂₀ was used as the cumulative concentration beyond the top dose administered. Because of this censored data the values for the first and second Bk challenge were compared for significance excluding subjects nos 4 and 6 in phase 2 and no. 4 in phase 3, using Student's t-test for paired data and including the minimal estimated values for these subjects using Wilcoxon's signed rank test.

Values of PC₂₀ AMP following consecutive Bk and histamine challenges were logarithmically transformed, which normalizes their distribution, and compared by the Student's t-test for paired data. Logarithmically

transformed PC_{20} histamine values obtained following maximally developed tachyphylaxis to Bk were compared with the PC_{20} values obtained on the histamine open study day using the paired Student's *t*-test.

Results

There was no significant difference in baseline values of FEV_1 between any of the study days (4.0 ± 0.3 l on histamine study day of phase 2, 3.9 ± 0.4 l on Bk study day of phase 2 and 3.9 ± 0.4 l on control study day of phase 3). The challenge procedure with AMP in this group of patients was found to be repeatable, with a CR of 2.5 fold difference and for 7 of the 8 subjects to within a twofold difference.

These findings were consistent with the repeatability data obtained in previous studies with the AMP challenge [12, 16].

Phase 1. Inhaled histamine, AMP and Bk all produced concentration-related falls in FEV_1 . The geometric mean (range) of $PC_{20} FEV_1$ values obtained were 0.87 (0.25–2.4), 30.9 (5.9–98.3) and 0.23 (0.05–0.89) $mg \cdot ml^{-1}$ for histamine, AMP and Bk, respectively, (table 1).

Phase 2. In all subjects there was no significant difference in the slopes of the concentration-response curves to both Bk and histamine on repeated challenge. Following recovery from the first dose-response challenge with Bk, the airways showed a substantially

Table 2. — Effects of repeated inhalations with bradykinin and histamine on airway AMP responsiveness (phase 2)

| Subject no. | $PC_{20} Bk/1$ $mg \cdot ml^{-1}$ | $PC_{20} Bk/2$ $mg \cdot ml^{-1}$ | $PC_{20} AMP$ $mg \cdot ml^{-1}$ | $PC_{20} H/1$ $mg \cdot ml^{-1}$ | $PC_{20} H/2$ $mg \cdot ml^{-1}$ | $PC_{20} AMP$ $mg \cdot ml^{-1}$ |
|------------------------|--------------------------------------|--------------------------------------|-------------------------------------|-------------------------------------|-------------------------------------|-------------------------------------|
| 1 | 0.14 | 3.0 | 21.3 | 0.79 | 0.76 | 24.1 |
| 2 | 0.03 | 2.5 | 13.2 | 0.27 | 0.56 | 4.3 |
| 3 | 0.71 | 9.0 | 123.0 | 1.5 | 1.5 | 82.8 |
| 4 | 0.91 | 15.1 | 96.0 | 0.98 | 1.3 | 67.7 |
| 5 | 0.06 | 0.33 | 15.5 | 0.56 | 0.63 | 7.5 |
| 6 | 0.63 | 15.1 | 345.0 | 0.63 | 1.1 | 59.3 |
| 7 | 0.44 | 0.99 | 62.1 | 1.3 | 1.9 | 29.4 |
| 8 | 0.09 | 1.8 | 148.2 | 1.1 | 0.95 | 49.7 |
| Geometric mean (range) | 0.21 (0.03–0.91) | 3.1 (0.33–15.13) | 59.8 (13.15–344.95) | 0.80 (0.27–1.52) | 1.00 (0.56–1.88) | 28.1 (4.33–82.81) |

For abbreviations see legend to table 1.

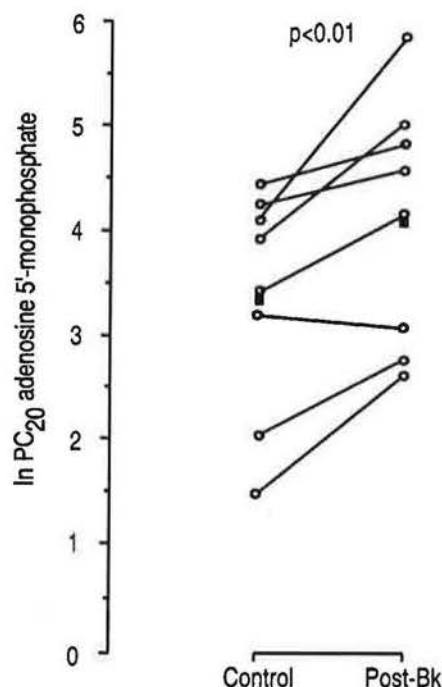


Fig. 1. — Individual $\ln PC_{20} AMP$ values obtained on baseline condition (control) and after having maximally induced Bk-tachyphylaxis (post-Bk). Closed squares represent geometric mean values. Bk: bradykinin; AMP: adenosine monophosphate; PC_{20} : provocative concentration of agonist producing a 20% fall in forced expiratory volume in one second from post-diluent baseline level.

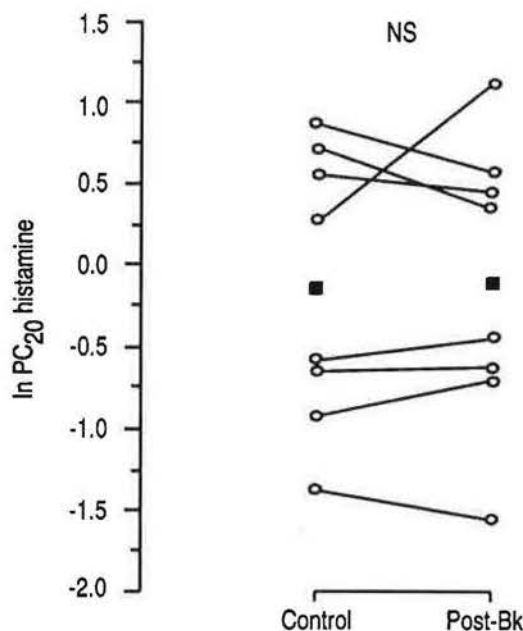


Fig. 2. — Individual $\ln PC_{20}$ histamine values obtained on baseline condition (control) and after having maximally induced Bk-tachyphylaxis (post-Bk). Closed squares represent geometric mean values. For abbreviations see legend to figure 1.

reduced response to a second dose-response challenge with the same agonist. Thus, the geometric mean PC_{20} (range) Bk for the group during the first challenge of 0.21 (0.03–0.91) increased fifteenfold to 3.1 (0.33–15.1) $mg \cdot ml^{-1}$ after the second challenge ($p < 0.01$) (table 2). Consecutive histamine provocation tests showed no significant change in responsiveness from the first to the second challenge, the geometric mean PC_{20} (range) values being 0.80 (0.27–1.52) and 1.00 (0.56–1.88) $mg \cdot ml^{-1}$, respectively, (table 2). Once the airways had recovered from the second Bk challenge, provocation with AMP also showed a reduced response, the geometric mean PC_{20} (range) value increasing from 30.9 to 59.8 (13.15–345.95) $mg \cdot ml^{-1}$ ($p < 0.01$) (table 2, fig. 1). In contrast, the PC_{20} (range) value for AMP obtained after the second histamine challenge of 28.1 (4.33–82.81) $mg \cdot ml^{-1}$ was not significantly different from that measured during phase 1 (table 1). In only one out of the eight subjects was some significant loss of airways responsiveness to histamine reported, supporting our view that it is difficult to uniformly demonstrate histamine tachyphylaxis in asthma [21]. By contrast, loss of the airway response to Bk was observed in all of the subjects studied.

Phase 3. Repeated challenge of the airways with Bk during this phase produced an almost identical fourteenfold loss of responsiveness, the $PC_{20}FEV_1$ (range) Bk value increasing from 0.18 (0.02–1.2) to 2.5 (0.95–15.1) $mg \cdot ml^{-1}$ ($p < 0.01$). However, on this occasion the airway response to a subsequent inhalation with histamine remained unchanged, the geometric mean PC_{20} (range) value of 0.90 (0.21–3.1) $mg \cdot ml^{-1}$ not being significantly different from that of 0.87 (0.25–2.4) $mg \cdot ml^{-1}$ obtained on the open study day (fig. 2). Analysis according to the method of ALTMAN and BLAND [20] showed that the degree of the loss of Bk responsiveness obtained in phase 2 and phase 3 was repeatable with a CR of 3.8 fold and for 5 of the 8 subjects to within twofold.

Discussion

This study confirms our previous findings that inhaled Bk and AMP cause concentration-related bronchoconstriction in asthmatic subjects [3, 10–12]. Sequential bronchial provocation with Bk resulted in a repeatable loss of responsiveness to this peptide, whilst in the same subjects, tachyphylaxis to histamine was difficult to demonstrate, as reported previously [21]. We have extended these observations by showing that repeated bronchial challenge with Bk produces a small but significant reduction in the bronchoconstrictor response to AMP without influencing the underlying level of "nonspecific" bronchial responsiveness when measured by histamine provocation.

The results of the present study indicate that some form of interreaction between the mechanism(s) responsible for the bronchoconstrictor activity of

inhaled Bk and AMP in asthma is present. Our hypothesis, that a common pathway for the two agonists exists at some point in the development of tachyphylaxis, would have been strengthened by demonstrating some reduction in airway responsiveness to Bk after repeated bronchial challenge with AMP. However, this step could not be considered in the protocol of the present study because of the impossibility of rendering asthmatic subjects with highly reactive airways tachyphylactic to AMP [17, 22]. Indeed, our subjects were selected on the basis of their bronchial hyperreactivity, because highly reactive asthmatics usually give repeatable dose response curves with inhaled Bk [4], but, on the other hand, they are less likely to develop AMP-tachyphylaxis [17, 22]. In looking for a shared mechanism accounting for some cross-refractoriness between the two stimuli, it is appropriate to examine how these stimuli provoke airway narrowing in asthma.

Although the mechanisms of Bk tachyphylaxis may be different from that causing airway obstruction, there are some similarities. We have previously shown that in asthmatic individuals repeated challenge with inhaled Bk leads to a loss of response, which appears to be agonist specific, and differs from that reported after exercise [23] and after histamine challenge [24], which are considered secondary to the production of protective prostanoids such as prostaglandin E_2 or I_2 (PGE_2 or PGI_2) [5]. The mechanism of tachyphylaxis to inhaled Bk in asthma is poorly understood. In the dog, Bk-induced bronchoconstriction [25] and increased mucus secretion [26] are associated with selective stimulation of C-fibre afferent nerve endings. There is also some evidence in animal models that this peptide can release sensory tachykinins such as substance P (SP), neurokinin A (NKA) and calcitonin gene-related peptide (CGRP), from sensory nerve endings [27, 28]. Moreover, SARIA *et al.* [6] have recently reported that in perfused guinea-pig lung Bk causes dose-dependent release of sensory tachykinins. In asthmatic subjects the ability of sodium cromoglycate and nedocromil sodium to attenuate the response of asthmatic airways to Bk [2, 29] has been interpreted as an effect mediated at the level of C-fibres [30]. These findings support the view that Bk tachyphylaxis in asthma may represent a down-regulation of Bk receptor function on sensory nerve endings or depletion of sensory tachykinins.

Adenosine, a naturally occurring purine nucleotide, acts on specific cell surface receptors to either decrease (*via* A1-receptors) or increase (*via* A2-receptors) intracellular levels of cyclic 3',5'-AMP [31]. The parent purine nucleotide, AMP, is thought to have similar actions following its transformation to adenosine [31]. The action of AMP in inducing bronchoconstriction in asthmatic subjects is suggested to be subsequent to histamine release from airway mast cells, as adenosine potentiates the release of preformed mediators from immunologically stimulated rodent [10] and human [11] mast cells *in vitro* and histamine H_1 -receptor antagonists are particularly

effective in inhibiting the AMP-induced bronchoconstriction [8, 9, 12]. However, enhancement of mast cell mediator release may not be the only mechanism accounting for the bronchoconstriction provoked by inhaled purines. There is some evidence to indicate that neural reflexes may contribute to the contractile airway response to this autacoid in asthma. The modulatory effects of adenine nucleotides and nucleosides on synaptic transmission were first demonstrated by GINSBORG and HIRST [32]. Since then, a number of studies have extended this initial observation by showing that the presynaptic modulation occurs both in peripheral nerves and in the central nervous system [33, 34]. In isolated rabbit airways adenosine enhances the constrictor response to transmural nerve stimulation [13], and in inbred rats atropine attenuates the changes in airway response provoked by intravenous adenosine [14]. In asthmatic subjects, we [16] and others [15] have reported some inhibition of the bronchoconstrictor response to inhaled purines with anticholinergic drugs such as ipratropium bromide and atropine, suggesting involvement of sensory neurones in the response.

There is lack of information on whether or not adenosine modulates synaptic transmission of peptidergic nerves in the airways. If the hypothesis that Bk tachyphylaxis in asthma results from the depletion of sensory tachykinins is correct, then the results of the present study may be interpreted as neural peptidergic pathways being shared both by Bk and AMP in the induction of a bronchoconstrictor response. Support for sensory nerve pathways being involved in adenosine- and Bk-induced bronchoconstriction is gained from observing that at least some of the protection afforded by sodium cromoglycate and nedocromil sodium against bronchoconstriction provoked by AMP [35], adenosine [36] and Bk [29] is due to the ability of these drugs to inhibit peptidergic neural reflexes in addition to their known effects on mast cells [37].

Further explanation for the attenuation of the airways response to AMP following repeated challenge with Bk is tachyphylaxis occurring at the receptor level. Although Bk tachyphylaxis *in vitro* may result from an alteration in receptor affinity following repeated exposure to the peptide [38], it is difficult to envisage that any specific desensitization of the Bk B₂ receptors could also alter adenosine receptor function without some fundamental change occurring in the contractile properties of the airways smooth muscle. Indeed LYON *et al.* [39] have recently demonstrated that specific B₂-Bk receptor binding in membrane homogenates from sheep nasal turbinates was not affected by the addition of either adenine or guanine nucleotides.

In conclusion, we have shown that repeated exposure of the airways of asthmatic subjects to Bk not only results in the development of a profound loss of response to this agonist but also in some attenuation of the subsequent response to inhaled AMP. By demonstrating some degree of cross-refractoriness we

suggest that a common pathway is implicated. A possible, albeit speculative, explanation is that Bk is acting through specific receptors to enhance neuropeptide release from sensory nerve endings and that it is at this end that AMP loss of responsiveness occurs. Whatever the mechanism involved, it is clear that further work is required to clarify whether neuropeptides serve an important role in the pathophysiology of adenosine- and Bk-induced bronchoconstriction in human asthma.

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