Cross-tachyphylactic airway response to inhaled bradykinin, kallidin and [desArg⁹]-bradykinin in asthmatic subjects


ABSTRACT: Kinins are oligopeptides that may act as mediators in the pathogenesis of bronchial asthma by interacting with specific cell surface receptors designated B₁ and B₂. When administered by inhalation to asthmatic subjects, bradykinin and kallidin, but not [desArg⁹]-bradykinin, provoke potent bronchoconstriction, thus suggesting a specific effect compatible with the stimulation of B₁ receptors. To characterize further the receptor(s) mediating this bronchospastic response we have carried out cross-tachyphylactic studies with inhaled bradykinin, kallidin, and [desArg⁹]-bradykinin, administered in a randomized double-blind fashion in a group of 10 asthmatic subjects.

Inhalation of bradykinin and kallidin, but not [desArg⁹]-bradykinin, elicited concentration-related falls in forced expiratory volume in one second (FEV₁) in all the subjects studied. The geometric mean provocation concentrations of inhaled agonists reducing FEV₁ by 20% of baseline (PC₂₀) were 0.12 and 0.28 mg·ml⁻¹ for bradykinin and kallidin, respectively. When inhaled at concentrations up to 10.62 mg·ml⁻¹, [desArg⁹]-bradykinin failed to provoke any significant fall in FEV₁ from baseline in any of the subjects studied. Following recovery from the second bradykinin challenge, provocation with kallidin revealed a reduced response to this agonist, the PC₂₀ value increasing from 0.28 to 1.23 mg·ml⁻¹. Similarly, once the airways had recovered from the second kallidin challenge, provocation with bradykinin also showed a reduced response, the PC₂₀Bk increasing from 0.12 to 0.94 mg·ml⁻¹. Surprisingly, despite failing to cause bronchoconstriction, repeated exposures with inhaled [desArg⁹]-bradykinin reduced the airway response to bradykinin, the PC₂₀Bk increasing from 0.12 to 0.41 mg·ml⁻¹. The airway response to inhaled histamine was not significantly changed after bronchial provocation with any of the kinins tested.

These findings suggest a complex action of kinins on asthmatic airways, probably involving more than one receptor subtype.

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Kinins are naturally occurring inflammatory autacoids, which are potent vasoactive peptides formed as cleavage products from the action of kallikreins on high and low molecular weight kininogens [1]. In the upper airways, kinin generation occurs, and correlates with symptoms, in the immediate [2], and late [3] responses to allergen challenge. Kinins have also been measured in the lower airways of asthmatic subjects, both spontaneously [4], and following allergen challenge of the bronchial mucosa [5, 6]. These findings, together with their known abilities to increase vascular permeability [7, 8] and to provoke bronchoconstriction when administered by inhalation in asthmatic subjects [9], support the view that these peptides may be important mediators of the pathogenesis of airway obstruction in bronchial asthma.

Bradykinin is a nonapeptide, generated as cleavage product from the action of kallikreins on high molecular weight kininogen (HMWK). An additional substrate for the kallikreins is low molecular weight kininogen (LMWK), cleavage of which generates lys-bradykinin (kallidin). Once generated, both kinins may undergo enzymatic cleavage of the N-terminal arginine residue, forming [desArg⁹]-bradykinin and [desArg¹⁰]-kallidin [1]. Two main kinin receptor subtypes have been described, designated B₁ and B₂, on the basis of studies of differing agonist potencies in separate tissue preparations [1]. In cat ileal preparations [10], and in isolated canine tracheal strips [11], both bradykinin and kallidin induce responses via an action on the B₁-receptors, whilst the B₂-agonist [desArg⁹]-bradykinin is without effect. In rabbit aorta the situation is reversed, with [desArg⁹]-bradykinin inducing contraction, whereas bradykinin and kallidin are inactive [1].

We have recently shown that both bradykinin and kallidin, but not [desArg⁹]-bradykinin, are potent bronchoconstrictor stimuli when administered by inhalation in...
asthmatic subjects [9]. Because bradykinin and kallidin are agonists of B₂-receptors and [desArg⁹]-bradykinin is an agonist for B₁-receptors [1], these in vivo structure activity studies suggest that this potent bronchoconstrictor action may result from a specific pharmacological effect, compatible with the stimulation of B₂-receptors. Confirmation of a common receptor for bradykinin and kallidin would also be obtained if cross-tachyphylaxis between the two could be shown. To characterize further the receptor mediating the bronchoconstrictor response to inhaled kinins, we have carried out crossed bronchoprovocation tests with bradykinin, kallidin and [desArg⁹]-bradykinin in a group of 10 asthmatic subjects.

Methods

Subjects

Ten asthmatic subjects (8 males, 2 females) with a mean±SEM age of 29±3 yrs, who were all nonsmokers, participated in the study (table 1). All subjects were atopic, as defined by positive skin prick tests (>2 mm weal response) to two or more of five common aeroallergens (Dermatophagoides pteronyssinus, Dermatophagoides farinae, mixed grass pollens, cat fur, dog hair (Bencard, Brentford, Middlesex, UK)). Their baseline forced expiratory volume in one second (FEV₁) was >70% of their predicted values, and none had received oral corticosteroids or theophylline within the preceding 3 weeks. Inhaled bronchodilators were discontinued for at least 8 h prior to each visit to the laboratory, although subjects were allowed to continue inhaled corticosteroids as usual. Subjects 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.

Table 1. - Details of the asthmatic subjects studied

<table>
<thead>
<tr>
<th>Pt no.</th>
<th>Sex</th>
<th>Age yrs</th>
<th>Baseline FEV₁ % pred</th>
<th>PC₂₀H mg·ml⁻¹</th>
<th>PC₂₀Bk mg·ml⁻¹</th>
<th>Medications</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>35</td>
<td>94</td>
<td>2.12</td>
<td>0.96</td>
<td>T, B</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>30</td>
<td>83</td>
<td>1.12</td>
<td>0.07</td>
<td>S, B</td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>42</td>
<td>79</td>
<td>0.57</td>
<td>0.05</td>
<td>S, B</td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>26</td>
<td>74</td>
<td>0.35</td>
<td>0.02</td>
<td>T, B</td>
</tr>
<tr>
<td>5</td>
<td>M</td>
<td>44</td>
<td>71</td>
<td>0.52</td>
<td>0.11</td>
<td>S, B</td>
</tr>
<tr>
<td>6</td>
<td>M</td>
<td>19</td>
<td>88</td>
<td>0.95</td>
<td>0.09</td>
<td>S</td>
</tr>
<tr>
<td>7</td>
<td>F</td>
<td>25</td>
<td>99</td>
<td>1.35</td>
<td>0.30</td>
<td>S</td>
</tr>
<tr>
<td>8</td>
<td>M</td>
<td>23</td>
<td>90</td>
<td>1.09</td>
<td>0.43</td>
<td>S</td>
</tr>
<tr>
<td>9</td>
<td>M</td>
<td>22</td>
<td>86</td>
<td>0.63</td>
<td>0.02</td>
<td>S, B</td>
</tr>
<tr>
<td>10</td>
<td>M</td>
<td>23</td>
<td>95</td>
<td>0.38</td>
<td>0.16</td>
<td>S</td>
</tr>
</tbody>
</table>

Mean ±SEM

|             | 29±2.7 | 85.9±2.9 | 0.78±(0.35–2.12) | 0.11±(0.02–0.96) |

*: geometric mean (range). T: terbutaline; B: beclomethasone 250 µg-puff⁻¹; S: salbutamol; FEV₁: forced expiratory volume in one second; PC₂₀: provocative concentration producing a 20% fall in FEV₁; H: histamine; Bk: bradykinin.

Bronchial provocation

Airway calibre was recorded as FEV₁. This measurement was derived from flow-volume curves produced on a rolling seal, flow-dependent spirometer (Morgan Spiroflow, P.K. Morgan Ltd, Kent, UK) connected to an 85B desk top computer via an 82940A GP-10 interface (Hewlett Packard, Wokingham, Berkshire, UK).

Histamine acid phosphate (BDH Chemicals, Poole, Dorset, UK) was dissolved in 0.9% sodium chloride to produce a stock solution of 16 mg·ml⁻¹ (52 mmol·l⁻¹). On each study day, bradykinin triacetic acid (Nova Biochem Ltd, Nottingham, UK), kallidin triacetic acid (Sigma Chemical Co., St. Louis, USA) and [desArg⁹]-bradykinin (Nova Biochem Ltd, Nottingham, UK) were freshly prepared in 10% ethanol in 0.9% sodium chloride to produce stock solutions of 8 mg·ml⁻¹. Each stock solution was then diluted with its respective diluent, to produce a concentration range of 0.03–8 mg·ml⁻¹ (0.1–26 mmol·l⁻¹) for histamine, and 0.0037–4 mg·ml⁻¹ (0.0035–3.77 mmol·l⁻¹, 0.0031–3.37 mmol·l⁻¹, 0.0041–4.43 mmol·l⁻¹) for bradykinin, kallidin and [desArg⁹]-bradykinin, respectively.

The purity of the synthetic kinins used was confirmed by high performance liquid chromatography (HPLC), using a solvent system of 1% trifluoroacetic acid in water and 1% trifluoroacetic acid in acetonitrile, after extraction through a C-18 cartridge column (Novapak, Waters, Milford, USA). Bradykinin, kallidin and [desArg⁹]-bradykinin were eluted as single peaks, as identified by optical density at 210 nm, confirming their purity. To avoid loss of kinins through oxidation and adherence to plastic surfaces, the stock solutions were 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 had an output of 0.48 ml·min⁻¹ and
generated an aerosol with a mass median particle diameter of 4.7 μm [12]. 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. [13]. Subjects were trained to take 3 s to reach full inspiratory capacity.

Study design

The study was divided into three phases (fig. 1). In the first phase, subjects attended the laboratory on two separate occasions, at least 48 h apart, to undertake concentration-response studies with inhaled histamine and bradykinin. On the first occasion, after 15 min rest, three baseline measurements of FEV\(_1\) were made at intervals of 3 min, followed by inhalation of the corresponding diluent solution and further FEV\(_1\) measurements repeated at 1 and 3 min, the higher value being recorded. Provided FEV\(_1\) had not fallen by more than 10% of the baseline value, a histamine concentration-response study was carried out. After administration of each histamine concentration, FEV\(_1\) was measured at 1 and 3 min. Increasing doubling concentrations of histamine were inhaled at 5 min intervals until FEV\(_1\) had fallen by more than 20% of the post-saline value, and the corresponding PC\(_{20}\)FEV\(_1\) values derived. On the second occasion, a bronchial provocation test with inhaled bradykinin was undertaken, according to a previously described protocol [9]. In brief, increasing fourfold concentrations of bradykinin were inhaled at approximately 5 min intervals, until FEV\(_1\) had fallen by >20% of the post-diluent value, and the corresponding PC\(_{20}\)FEV\(_1\) values derived.

In the second phase of the study, subjects attended the laboratory on three occasions, separated by at least 5 days, during which two consecutive concentration-response studies with inhaled bradykinin, kallidin and [desArg\(_9\)]-bradykinin were undertaken in a double-blind, randomized manner (fig. 1). During this phase, an initial bronchial provocation test with bradykinin or kallidin was performed until FEV\(_1\) fell by >20% of post-diluent baseline value. The initial inhalation test with [desArg\(_9\)]-bradykinin was carried out until its maximal concentration had been administered, and the second challenge with this kinin was carried out after a fixed wait of 45 min, in order to maintain double-blindness. Following the initial bronchoprovocation test with bradykinin or kallidin, the airways were then allowed to recover spontaneously, until FEV\(_1\) had returned to within 5% of post-diluent baseline value. On achieving this, after approximately 35–60 min, a second kinin bronchoprovocation was undertaken with the same agonist, until FEV\(_1\), fell by >20% of the original post-diluent value, or the highest concentrations of each kinin had been administered. Again, once the FEV\(_1\) after the second inhalation test had returned to within 5% of the post diluent baseline value, a third concentration-response study was undertaken, this time with kallidin on the sequential bradykinin-bradykinin day, and with bradykinin on the kallidin-kallidin and [desArg\(_9\)]-bradykinin-[desArg\(_9\)]-bradykinin days (fig. 1). When possible, the PC\(_{20}\) values for each of the kinin challenges were derived.

The final phase of the study was carried out to determine the specificity of the three kinins on subsequent nonspecific contractile stimuli. Six subjects (nos 1–6) attended the laboratory on three further visits, at least 5 days apart, to undertake a single-blind concentration-response study with inhaled histamine, after receiving two consecutive inhalation challenges with bradykinin, kallidin and [desArg\(_9\)]-bradykinin administered in an identical fashion to that described in the second phase (fig. 1).

Data analysis

Figures refer to the mean±SEM unless otherwise stated, and the p<0.05 level of significance was accepted. Baseline FEV\(_1\) values prior to bronchial challenges were compared between study days by analysis of variance (ANOVA).

Concentration-response curves were constructed by plotting the percentage change in FEV\(_1\) from the post-diluent baseline value against the cumulative concentration of the agonist administered on a logarithmic scale and the concentration of agonist required to produce a 20% fall in FEV\(_1\), from the post-diluent baseline value (PC\(_{20}\)FEV\(_1\)) determined by interpolation. The repeatability of the kinin challenge procedure was determined according to the method described by Altman and Bland [14], of plotting the difference against the mean of the logarithmically transformed PC\(_{20}\) values. The mean and standard deviation (sd) of the difference between these values were derived and used to calculate their coefficient of repeatability (CoR). Similarly, we assessed the

**Fig. 1.** Schematic flow diagram for the protocol adopted in the three phases of the study. The order of days 3, 4 and 5 was randomized and double-blind. H: concentration-response study with histamine; Bk: concentration-response study with bradykinin; K: concentration-response study with kallidin; des: concentration-response study with [desArg\(_9\)]-bradykinin.
repeatability of the loss of kinin responsiveness obtained in phases 2 and 3 of the study.

In two subjects (nos 1 and 5) in phase 2, and in one subject (no. 1) in phase 3, a 20% fall in FEV1 could not be achieved when the maximum concentration of kinin was administered. In addition in all subjects in both phase 2 and 3, a 20% fall in FEV1 could not be achieved when the maximum concentration of [desArg9]-bradykinin was administered. In these cases, an estimate of the PC20 was used as the next cumulative concentration beyond the top dose administered. Because of this censored data, these values were analysed for significance excluding subject nos 1 and 5 in phase 2 and no. 1 in phase 3, using Student's t-test for paired data and, when including the estimated values for these subjects, using Wilcoxon's signed rank test.

Values of PC20 bradykinin, kallidin and histamine following consecutive kinin challenges were logarithmically transformed to normalize their distribution, and compared by the Student's t-test for paired data. Any relationship between the airway responses to histamine and the kinines tested was examined by least-squares linear regression analysis of the logarithmically transformed values, and a relative potency derived in molar terms.

Results

There was no significant difference in baseline values of FEV1 between and within any of the study days, with means±SEM values ranging from 3.32±0.27 to 3.43±0.28 l. The challenge procedure with bradykinin and kallidin in this group of patients was found to be repeatable, with a CoR of 1.7 (for 8 of the 10 subjects to within a single doubling dilution) and 1.6 (for 4 of the 6 subjects to within a single doubling dilution) doubling dilutions, respectively. These findings were consistent with the repeatability data obtained in previous studies from our laboratory with bradykinin bronchoprovocation tests [15, 16].

In phase 1, inhaled histamine and bradykinin produced concentration-related falls in FEV1. The geometric mean (range) of PC20 values obtained were 0.78 (0.35-2.12) and 0.11 (0.02-0.96) mg·ml⁻¹ for histamine and bradykinin, respectively, (table 1). A weak but significant correlation was obtained between PC20 values for histamine and bradykinin (r=0.71; p<0.05; n=10) with bradykinin in molar terms being 2.3 (range 7.6-108) times more potent than histamine.

In phase 2, inhalation of bradykinin, kallidin but not [desArg9]-bradykinin elicited concentration-related falls in FEV1. For the group as a whole, the geometric mean PC20 values were 0.12 (0.01-1.59) mg·ml⁻¹ and 0.28 (0.01-4.62) mg·ml⁻¹ for bradykinin and kallidin, respectively (table 2). A significant correlation was obtained between the PC20 values for bradykinin and kallidin (r=0.77; p<0.01; n=10), but in molar terms, bradykinin was 2.1 (range 0.6-30.9) times more potent than kallidin. When [desArg9]-bradykinin was administered up to a cumulative concentration of 10.62 mg·ml⁻¹, a 20% fall in FEV1 could not be achieved in any of the asthmatic subjects studied.

Following recovery from the first concentration-response challenge with bradykinin or kallidin, the airways showed a substantially reduced response to a second challenge with the same agonist. Thus, the geometric mean PC20 values for bradykinin and kallidin during the first challenge of 0.12 and 0.28 mg·ml⁻¹ increased after the second challenge 4.3 and 2.8 fold to 0.52 (0.02-10.62) (p<0.01) and to 0.79 (0.03-10.62) (p<0.01) mg·ml⁻¹ respectively (table 2). Consecutive inhalation tests with [desArg9]-bradykinin showed no apparent change from the lack of a response in all the subjects studied.

Once the airways had recovered from the second bradykinin challenge, provocation with kallidin also revealed a reduced response to this agonist, the geometric mean PC20 value increasing 4.4 fold from 0.28 at baseline to 1.23 (0.03-10.62) mg·ml⁻¹ post-bradykinin (p<0.01) (table 2).

Table 2. – PC20 FEV1 values for bradykinin (Bk) and kallidin (K) after sequential concentration-response challenges in phase 2

<table>
<thead>
<tr>
<th>Study</th>
<th>Day 3</th>
<th></th>
<th>Study</th>
<th>Day 4</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Pt no.</td>
<td>PC20Bk/1 mg·ml⁻¹</td>
<td>PC20Bk/2 mg·ml⁻¹</td>
<td>PC20K/3 mg·ml⁻¹</td>
<td>PC20K/1 mg·ml⁻¹</td>
<td>PC20K/2 mg·ml⁻¹</td>
</tr>
<tr>
<td>2</td>
<td>0.03</td>
<td>0.16</td>
<td>1.21</td>
<td>1.03</td>
<td>3.63</td>
</tr>
<tr>
<td>3</td>
<td>0.03</td>
<td>0.07</td>
<td>0.14</td>
<td>0.02</td>
<td>0.04</td>
</tr>
<tr>
<td>4</td>
<td>0.01</td>
<td>0.02</td>
<td>0.03</td>
<td>0.01</td>
<td>0.03</td>
</tr>
<tr>
<td>5</td>
<td>0.09</td>
<td>2.24</td>
<td>2.87</td>
<td>0.32</td>
<td>1.97</td>
</tr>
<tr>
<td>6</td>
<td>1.15</td>
<td>1.77</td>
<td>3.20</td>
<td>0.34</td>
<td>1.56</td>
</tr>
<tr>
<td>7</td>
<td>0.40</td>
<td>0.81</td>
<td>2.62</td>
<td>0.32</td>
<td>1.12</td>
</tr>
<tr>
<td>8</td>
<td>0.64</td>
<td>1.67</td>
<td>4.00</td>
<td>0.63</td>
<td>2.08</td>
</tr>
<tr>
<td>9</td>
<td>0.05</td>
<td>0.13</td>
<td>0.76</td>
<td>0.26</td>
<td>0.30</td>
</tr>
<tr>
<td>10</td>
<td>0.22</td>
<td>0.93</td>
<td>2.04</td>
<td>0.49</td>
<td>0.99</td>
</tr>
<tr>
<td>GM</td>
<td>0.12</td>
<td>0.52</td>
<td>1.23</td>
<td>0.28</td>
<td>0.79</td>
</tr>
<tr>
<td>(range)</td>
<td>(0.01-1.59)</td>
<td>(0.02-10.62)</td>
<td>(0.03-10.62)</td>
<td>(0.01-4.62)</td>
<td>(0.03-10.62)</td>
</tr>
</tbody>
</table>

Bk (bradykinin and K (kallidin) were given as challenges according to the protocol outlined in figure 1. Day 3, Day 4: separate day, order randomized; GM: geometric mean. For abbreviations see legend to table 1.
CROSS-TACHYPHYLAXIS WITH INHALED KININS IN ASTHMA

Fig. 2. - Individual responses of FEV₁ to increasing cumulative concentrations of inhaled bradykinin obtained on day three of the study (○), and on day five after sequential exposure to [desArg²]-bradykinin (●) in 10 subjects with asthma. FEV₁: forced expiratory volume in one second.

Table 3. - Effects of sequential concentration-response studies with bradykinin, kallidin and [desArg⁹]-bradykinin on airway histamine responsiveness (n=6)

<table>
<thead>
<tr>
<th>Study days</th>
<th>Bradykinin</th>
<th>Kallidin</th>
<th>[desArg⁹]-Bk</th>
</tr>
</thead>
<tbody>
<tr>
<td>PC₂₀ histamine pre-kinins</td>
<td>0.78 (0.35–2.12)*</td>
<td>0.78 (0.35–2.12)</td>
<td>0.78 (0.35–2.12)</td>
</tr>
<tr>
<td>PC₂₀ kinin 1</td>
<td>0.08 (0.02–0.98)</td>
<td>0.23 (0.02–4.01)</td>
<td>&gt;10.62</td>
</tr>
<tr>
<td>PC₂₀ kinin 2</td>
<td>0.68 (0.05–10.62)</td>
<td>0.96 (0.05–10.62)</td>
<td>&gt;10.62</td>
</tr>
<tr>
<td>PC₂₀ histamine post-kinins</td>
<td>0.77 (0.25–1.57)</td>
<td>0.89 (0.29–1.88)</td>
<td>0.75 (0.30–2.51)</td>
</tr>
</tbody>
</table>

Data are presented as geometric mean and range in parenthesis. PC₂₀: histamine pre-kinins is the PC₂₀ value for histamine obtained on study day 1. For abbreviations see legend to table 1.

Similarly, once the airways had recovered from the second kallidin challenge, provocation with bradykinin also showed a reduced response, the geometric mean PC₂₀ value increasing 7.8 fold from 0.12 to 0.94 (0.02–10.62) mg·ml⁻¹ (p<0.01) (table 2). Surprisingly, repeated exposure of the airways with the inactive B₂-agonist, [desArg⁹]-bradykinin, reduced the response to inhaled bradykinin in 8 out of 10 subjects (particularly in subjects nos 2 and 3) and the geometric mean PC₂₀ value increased 3.4 fold from 0.12 to 0.41 (0.01–2.00) mg·ml⁻¹ (p<0.01) (fig. 2).

In phase 3 of the study, repeated challenge of the airways with bradykinin and kallidin produced an almost identical loss of kinin responsiveness (8.5 and 4.2 fold, p<0.01) (table 3) respectively, to that observed with consecutive challenges with the same agonists in phase 2. Thus, the geometric mean PC₂₀ value increased from 0.08 (0.02–0.98) to 0.68 (0.05–10.62) mg·ml⁻¹ (p<0.01), and from 0.23 (0.02–4.01) to 0.96 (0.05–10.62) mg·ml⁻¹ (p<0.01) for bradykinin and kallidin, respectively, (table 3). However, in the presence of reduced kinin responsiveness the airway response to a subsequent inhalation with histamine remained unchanged, the geometric mean PC₂₀ histamine pre-kinins value of 0.78 mg·ml⁻¹ not being significantly different from that of 0.77 (0.25–1.57) mg·ml⁻¹ and of 0.89 (0.29–1.88) mg·ml⁻¹ obtained on the bradykinin and kallidin study day, respectively, (table 3). The PC₂₀ value for histamine obtained after the second exposure of the airways with [desArg⁹]-bradykinin of 0.75 (0.30–2.51) mg·ml⁻¹ was also not significantly different from that measured initially (table 3), or from the PC₂₀ histamine values obtained when the airways developed maximal tachyphylaxis to bradykinin or kallidin.

Discussion

The results of the present study confirm our previous findings that bradykinin and kallidin, but not [desArg⁹]-bradykinin, cause concentration-related bronchoconstriction in asthmatic subjects [9], and that sequential bronchial provocation with bradykinin results in a repeatable loss of responsiveness to this peptide [16, 17]. We have
extended these observations by showing tachyphylaxis to kallidin inhalation and cross-tachyphylaxis between the different kinins tested. Of particular interest, is the demonstration that repeated exposures with [desArg9]-bradykinin, a selective agonist for B1-receptor systems, produces a significant reduction in the bronchoconstrictor response to bradykinin, a B2-agonist, without itself producing any direct constrictor responses. These findings suggest a complex action of kinins on asthmatic airways, probably involving more than one receptor subtype.

The general criteria used to characterize receptors include comparison of order of potency of agonists, the use of desensitization experiments, and evaluation of the efficacy of potent and specific antagonists. In asthmatic airways, in vivo structure activity studies suggested that the kinin receptor subtype responsible for the bronchoconstrictor response is of the B2 variety [9]. Since no specific and competitive B2-receptor blockers are as yet available for in vivo studies in humans, the only criterion that we could use in order to extend our previous findings, and to find out whether bradykinin and kallidin act on the same or on different receptors in asthma, was to establish whether cross-tachyphylaxis could be shown between the two.

On the basis of what is known about the receptor subclasses for kinins, a rank potency order of bradykinin > kallidin >> [desArg9]-bradykinin is consistent with the view that the constrictor response is mediated through B2-receptors [1]. A similar potency order has also been observed in the present study, and parallels that obtained for cat ileum in vitro [10], canine tracheal preparations [11], and nasal airways in atopic rhinitis and in normal volunteers [18].

Two consecutive concentration-response challenges with bradykinin and kallidin resulted in a repeatable loss of responsiveness to these peptides, without influencing the underlying level of "nonspecific" bronchial reactivity, when measured by histamine provocation. The mechanism of tachyphylaxis to inhaled kinins in asthma is poorly understood. Although secondary release of protective prostaglandins with functional effects such as prostaglandins E2 and L1 (PGE2 and PGL1) could explain the phenomenon, we [16] and others [19] have failed to show that loss of kinin responsiveness was sensitive to blockade by prior treatment with cyclooxygenase inhibitors. An additional possibility is that kinins, in being potent vasodilators [20], could enhance bronchial blood flow to increase the transepithelial clearance of subsequently administered agonists. However, if functional antagonism mediated by these mechanisms operated in the case of kinin tachyphylaxis, then a parallel loss or histamine responsiveness might be anticipated, but was not observed. Taken together, these data suggest that refractoriness to these peptides is a receptor-specific mechanism, and may represent a down-regulation of B2-receptor function or impaired neuropeptide release from sensory nerves.

Sequential bronchial provocation with bradykinin produced a significant reduction in the bronchoconstrictor response to kallidin and vice versa. Although functional antagonism cannot be excluded, these effects were obtained without influencing the underlying level of "nonspecific" bronchial responsiveness when measured by histamine provocation, and could be interpreted as an interaction between the mechanism(s) responsible for the bronchoconstrictor activity of the two kinins in asthma occurring at the same receptor level. Although this is insufficient to allow us to draw definitive conclusions about the identity of the receptors stimulated by bradykinin and kallidin, our data offer some support to the view that both bradykinin and kallidin are acting via the same B2-receptors.

When bradykinin concentration-response studies followed repeated inhalations with the B2-receptor agonist, [desArg9]-bradykinin, we were surprised to see that the dose-response curves were shifted to the right in 8 of the 10 subjects studied, without affecting their underlying level of "nonspecific" bronchial responsiveness. However, whilst this response was particularly manifest in two individuals (nos 2 and 3), it was not observed in all the subjects studied. There is no obvious explanation for this discrepancy, as we could not pinpoint any relationship with their baseline airway calibre, their clinical status, their use of anti-asthma medications, or their level of bronchial responsiveness. In four out of 10 sets of experiments, we confirmed the purity of the synthetic kinins administered throughout phase 2 by HPLC, in order to exclude contamination by their breakdown products (data not shown). Although this bizarre kinin interaction gives rise to a novel observation, it introduces a confounding factor in the interpretation of these data. It is difficult to explain the effects of [desArg9]-bradykinin in attenuating the airway response to bradykinin, but functional antagonism is a possibility. Although not causing any overt airway response, exposure with [desArg9]-bradykinin may still be capable of causing subsequent release of inhibitory prostaglandins, such as PGE2 and PGI2, from the epithelium and other airway cells [1, 21], which may be acting as functional antagonists. In addition, [desArg9]-bradykinin might have acted as a local vasodilator [1], thus enhancing bronchial blood flow and, therefore, altering the kinetics of the clearance of the inhaled kinins. However, if this accounted for loss of airways response to [desArg9]-bradykinin, then a parallel reduction in histamine responsiveness might be anticipated, but was not seen. Although functional antagonism cannot be discarded, another possibility is that [desArg9]-bradykinin might have acted as a partial antagonist at the B2-receptor level. In a variety of pharmacological models, it has been shown that naturally occurring kinins are relatively non-selective in acting on both B1 and B2 receptors [22]. It has also been demonstrated that B1 receptors can be induced during pathological states [23]. Therefore, although symptoms induced by bronchial challenge with kinins are not due to stimulation of B1-receptors [9], we cannot rule out the possibility that, in the inflamed airways of asthmatic subjects, repeated exposure with [desArg9]-bradykinin might induce B1-receptors, which could interfere with the sensory neural pathways activated by B2-receptor stimulation, possibly presynaptically, as described for many other pharmacological stimuli [1]. Furthermore, it is conceivable that different subsets of B2-receptors are expressed in inflamed asthmatic airways.
which, although fully responsive to the classical B2-receptor agonists, may also interact with [desArg9]-bradykinin. Recent findings [24, 25] indicate that multiple B2, kinin receptors may exist. Characterization of kinin receptors by the simple criteria of the order of potency of agonists and of the cross-tachyphylactic response to B2-agonists provides limited information. Only with the availability of a potent and selective antagonist of kinin receptors will it become possible to help to identify additional kinin receptors subtypes.

In conclusion, the present study provides additional evidence for the existence of a B2 bradykinin receptor in the airways of asthmatic subjects, that mediates a constrictor response with both inhaled bradykinin and kallidin. We have also provided evidence that repeated exposure to bradykinin or kallidin results in tachyphylaxis with cross-reactivity occurring between the two peptides. Finally, by showing no direct agonist but a potent antagonist property of [desArg9]-bradykinin against the constrictor response to bradykinin, we suggest that this kinin may have effects on the neuroeffector mechanism of bronchoconstriction, which are more complex than initially proposed, and possibly involve different receptor subtypes.

References