British Journal of Pharmacology (2000) 129, 525–532
www.nature.com/bjp

Involvement of kinins in hyperresponsiveness induced by platelet activating factor in the human nasal airway

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1 The aim of this study was to investigate the role of kinins in the development of nasal hyperresponsiveness induced by platelet activating factor (PAF) in normal human subjects.

2 Intranasal administration of PAF, 60 μg, induced an increased responsiveness to histamine, 200 μg per nostril, 6 h later. This effect was abolished by pretreatment with the bradykinin B_2 receptor antagonists icatibant and [1-adamantaneacetyl-D-Arg^0,Hyp^3,β-(2-thienyl)-Ala^{5,8},D-Phe^7]-bradykinin ([Ad]-BK), both at 200 μg, every 2 h following PAF administration.

3 In a separate experiment, utilizing the same protocol, nasal lavage was used to measure the release of mediators into the nasal cavity following treatment with PAF. PAF increased the levels of eosinophil cationic protein (ECP) and kinin detected in the lavage samples, compared with a saline control. The levels of these mediators were reduced by pretreatment with either icatibant or [Ad]-BK.

4 Administration of lyso-PAF, 60 μg intranasally, did not cause a rise in kinin or ECP levels in nasal lavage fluid.

5 Exogenous bradykinin, 500 μg, or a saline control, applied topically to the nasal mucosa every 30 min for 2 h, failed to cause hyperresponsiveness to histamine.

6 We conclude that bradykinin itself does not cause hyperresponsiveness, but is involved in the hyperresponsiveness induced by PAF in the human nasal airway.
British Journal of Pharmacology (2000) **129,** 525–532

Keywords: Bradykinin; platelet activating factor; human; nasal airway; icatibant; hyperresponsiveness; albumin; eosinophil cationic protein

Abbreviations: [Ad]-BK, [1-adamantaneacetyl-D-Arg^0,Hyp^3,β-(2-thienyl)-Ala^{5,8},D-Phe^7]-bradykinin; Amin, minimal cross-sectional area of the nasal airway; AUC, area under curve; BK, bradykinin; ECP, eosinophil cationic protein; Icat, icatibant; MBP, major basic protein; PAF, platelet activating factor

Introduction

One of the characteristic signs of allergic rhinitis is hyperresponsiveness of the nasal airway to challenge with a range of stimuli, including histamine and bradykinin. In non-atopic subjects, platelet activating factor (PAF) (1-O-hexadecyl-2-acetyl-*sn*-glycero-3-phosphocholine), but not its inactive precursor and metabolite, lyso-PAF, can be used to induce a nasal hyperresponsiveness similar to that caused by antigen in allergic rhinitis (Andersson & Pipkorn, 1988; Austin & Foreman, 1993). PAF is released into the nasal cavity following antigen challenge in allergic rhinitis (Shin *et al*., 1994; Tsai *et al*., 1995), indicating that PAF may have a role as a mediator in this condition.

PAF is a membrane-derived phospholipid with a range of biological effects. These include increasing vascular permeability, acting as a powerful chemoattractant for eosinophils and neutrophils, and inducing free radical production and leukotriene synthesis in these cells (O'Flaherty & Wykle, 1983). However, the mechanism by which PAF causes nasal hyperresponsiveness is unknown.

PAF causes the recruitment of eosinophils into the nasal airway, the subsequent activation of eosinophils and the increased production of eosinophil cationic protein (Tedeschi *et al*., 1994a). These events also occur in antigen-induced hyperresponsiveness (Knani *et al*., 1992). In the guinea-pig, major basic protein (MBP), an eosinophil-derived cationic protein, induces airway hyperresponsiveness which is dependent upon the generation of kinins (Coyle *et al*., 1995). Farmer *et al*. (1992) observed that bradykinin antagonists inhibit antigen-induced hyperresponsiveness, while in another study, bradykinin produced hyperresponsiveness to acetylcholine in guinea-pig airways (Omini *et al*., 1989). Our aim was to investigate the contribution of kinins to the development of human nasal airway hyperresponsiveness induced by PAF, using the bradykinin B_2 receptor antagonists icatibant and [1-adamantaneacetyl-D-Arg^0, Hyp^3, β-(-2-thienyl)-Ala^{5,8},D-Phe^7]-bradykinin ([Ad-BK]).

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Methods

Subjects

The study was approved by the local ethics committee of University College London, and all subjects gave their informed consent. In all experiments, normal, non-atopic, healthy volunteers with an age range of 19–48 years were used. Subjects with symptoms of nasal infection, or who were taking medication at the time of the study or within the previous 4 weeks, were excluded. Experiments were performed in a laboratory with a controlled temperature (21°C) and humidity.

Administration of drugs

Compounds were administered to the nasal cavity using a hand-held pump spray (Perfect-Vallois) which delivered a

volume of 100 μl per actuation. The dose administered was controlled by varying the concentration of the compound in the pump spray. Compounds were prepared in sterile saline (NaCl 154 mM), which also served as the control. In all experiments, compounds were delivered to both nostrils and the doses stated are the amounts delivered into each nostril.

Fresh solutions were made each day from stock solutions stored at -20°C, and were allowed to equilibrate with room temperature prior to administration. PAF was prepared in sterile saline at a concentration of 600 μg ml^{-1}, and histamine (as the diphosphate salt) at 2 mg ml^{-1}. Bradykinin was dissolved to give solutions of 2 and 5 mg ml^{-1}. The selective bradykinin B_2 receptor antagonists, icatibant and [Ad]-BK were prepared at a concentration of 2 mg ml^{-1} (Austin & Foreman, 1994a; Dear *et al*., 1996). The doses used were based on previous studies (Austin & Foreman, 1993; 1994a,b) and pilot experiments.

Measurement of nasal patency

Nasal patency was determined by acoustic rhinometry, as previously described (Austin & Foreman, 1994b). The parameter used to assess nasal patency was the minimal cross-sectional area (Amin.) of the nasal airway. For each determination of Amin., triplicate measurements were made on each side of the nasal airway.

Nasal lavage

The method used to perform nasal lavage was adapted from that used in previous studies by Naclerio *et al*. (1983) and Wihl *et al*. (1995). Prewarmed (37°C) sterile saline (5 ml) was instilled into each nostril, using a syringe to which was attached a nasal olive. Subjects were sitting with the neck flexed and the head at an angle of about 50° from the vertical, to prevent fluid from reaching the nasopharynx. To ensure adequate washing, the lavage fluid was passed slowly into the nasal cavity and then back into the syringe twice. Finally, the fluid was instilled back into the nasal cavity, and collected by the subject expelling the fluid into a collection vessel. Recovery was approximately 90%. This method was validated by constructing a dose-response curve to histamine, using albumin levels in the lavage fluid. The results were similar to those obtained by Naclerio *et al*. (1983).

In each experiment, three initial washes were performed to remove any pre-existing mediators, and the third wash retained and used as a baseline. Lavage samples were centrifuged at 4°C for 10 min at $1000 \times g$, following which the supernatants were separated and stored at -70°C until analysis.

Biochemical assays

The albumin content of the nasal lavage samples was determined using a commercially available radial immunodiffusion assay. An albumin standard was used to establish a calibration curve. ECP was measured by a commercially available radioimmunoassay. The radioisotope used was [^{125}I]-ECP, and the antiserum was raised in the rabbit. The detection limit of the assay was <2 μg l^{-1}, with no significant cross-reactivity with other proteins. The kinin content of the nasal lavage was determined with a commercially available bradykinin radioimmunoassay kit. The antiserum used for this assay was raised in the rabbit. [^{125}I-Tyr^{0}]-bradykinin was used as the radioisotope and the range of sensitivity of this assay was 1–128 pg of kinin per tube. This assay has equal sensitivity for bradykinin and kallidin, with no significant cross-reactivity with other peptides of a similar structure. In order to reduce degradation of kinins by kininases, EDTA was added to samples (at a final concentration of 40 mM) after centrifugation of the sample and prior to freezing (Proud *et al*., 1983).

Effect of bradykinin B_2 receptor antagonists on PAF-induced hyperresponsiveness

Each subject received either icatibant, 200 μg, or saline. The duration of action of icatibant in the nasal airways is about 2 h (Dear & Foreman, unpublished), so subjects received either saline or icatibant again 2 and 4 h after the initial administration. Two minutes after the first treatment with icatibant or saline, subjects received either PAF, 60 μg, or saline. Six hours later, subjects were challenged with histamine, 200 μg. Immediately prior to, and at 2, 5 and 10 min after histamine challenge, the subjects' Amin. was measured. The first measurement of Amin. immediately prior to histamine challenge formed the baseline value. Subsequent values of Amin., after challenge with histamine, are expressed as a percentage change in Amin. from baseline.

Each subject received, in a double-blind, randomized-block, cross-over design, the following four combinations of treatment: Saline/Saline, Saline/PAF, Icatibant/Saline and Icatibant/PAF. Each combination was administered on separate occasions, at least 2 days apart. Subjects were randomly assigned to the treatment protocols.

The experiment was repeated on a separate occasion, using [Ad]-BK, 200 μg, instead of icatibant, and again the response to histamine, 200 μg, was assessed as before. All subjects received all four possible combinations of treatment.

In a separate experiment, utilizing the same protocol, nasal lavage was carried out in order to assess changes in mediator production by cells associated with the nasal mucosa. Initially, three nasal lavages were performed, and the third sample was retained as a baseline. Subjects then received the appropriate combination of PAF or saline, with or without bradykinin B_2 antagonist. The subjects' nasal cavities were then lavaged at 2, 4 and 6 h later, prior to subsequent administrations of antagonist or saline control, as described above. The lavage samples were assayed for their albumin, kinin and ECP content.

In a further experiment, subjects received either saline, PAF, 60 μg, or lyso-PAF, 60 μg, Nasal lavage was carried out at 2, 4 and 6 h after the initial drug treatment, and the nasal lavages analysed as above.

Effect of bradykinin on the responsiveness of the human nasal airway to histamine

In this study, an initial value of Amin. was determined as described above, 5 min after administration of saline into each nostril. This value of Amin. was used only to compare with the baseline value determined later in the protocol, and to determine any change in the resting level of Amin. resulting from pretreatment of the nose with bradykinin or saline. Following this, subjects received five intranasal administrations of bradykinin, 5 mg ml^{-1}, or saline, at 30 min intervals. After each administration, Amin. was determined 5 min later. At 3, 4 and 6 h later, a baseline measure of Amin. was taken, followed by nasal provocation with histamine, 2 mg ml^{-1}. Three further values of Amin. were measured at 2, 5 and 10 min after histamine challenge. Each subject received both bradykinin and saline pretreatments on separate occasions, at

least 72 h apart. The experiment was repeated on a separate occasion, using a dose of bradykinin of 2 mg ml^{-1}.

Data analysis

The dimensions of the nasal airway vary between subjects and also within subjects from day to day, and so the data have been normalized by expressing changes in Amin. as the percentage decrease in Amin. from the baseline control value. The absolute values for the control measurements have been given together with s.e.means. For each determination of nasal patency in a subject, a response-time curve was constructed and the area under curve (AUC) calculated. Data from the determinations of nasal patency are expressed as mean AUC $\pm$ s.e.mean. Mediator levels in nasal lavage fluid do not fit a normal distribution, and so are given as median values together with the 80% central range of values. Analysis of baseline values was used to control for variation between experiments. The data were first analysed using non-parametric analysis-of-variance tests, followed by an appropriate, *post-hoc* test. In all cases, the comparisons were made between active treatment and saline control. The non-parametric statistical test is given with each data set. A value of $P<0.05$ is taken as significant.

Materials

Bradykinin, PAF (C_{16}) and lyso-PAF were obtained from Calbiochem Novabiochem, (Nottingham, U.K.). Histamine diphosphate was obtained from Sigma, (Poole, Dorset, U.K.). Icatibant was kindly supplied by Dr K. Wirth, Hoechst AG (Frankfurt, Germany). [Ad]-BK was purchased from Bachem AG, (Switzerland). The ECP assay kit was produced by Pharmacia Diagnostics (Uppsala, Sweden). The bradykinin radioimmunoassay kit was obtained from Peninsula Laboratories (St. Helens, U.K.). Radial immunodiffusion assay plates for albumin were purchased from Behring (Germany). All other substances used were of Analar or similar quality.

Results

Effect of bradykinin B_2 receptor antagonists on PAF-induced hyperresponsiveness

Histamine, 200 μg, caused a significant increase from baseline in AUC of Amin. against time, corresponding to a decrease in the patency of the nasal airway ($P<0.05$, Wilcoxon sign-rank test) (Figures 1 and 2). The increase in response to histamine following PAF was significantly altered following pretreatment with icatibant or [Ad]-BK, 200 μg ($P>0.05$, Friedman's test). Treatment with either antagonist, in the absence of PAF, did not change this response to histamine ($P>0.05$, Wilcoxon sign-rank test). Pretreatment with PAF, 60 μg, resulted in an increased nasal response to histamine (Figures 1 and 2) that was significantly greater than that induced by histamine following pretreatment with saline ($P<0.05$, Wilcoxon sign-rank test). This hyperresponsiveness to histamine was abolished when the nasal cavity was pretreated with icatibant, 200 μg ($P<0.05$, Wilcoxon sign-rank test (Figure 1). Similarly, [Ad]-BK also prevented the increase in nasal response to histamine caused by PAF ($P<0.05$, Wilcoxon sign-rank test) (Figure 2). There were no differences in the initial baseline values of Amin. between treatment groups in either experiment (0.64 ± 0.03 and 0.61 ± 0.03 cm^2 respectively: $P>0.05$, Friedman's test).

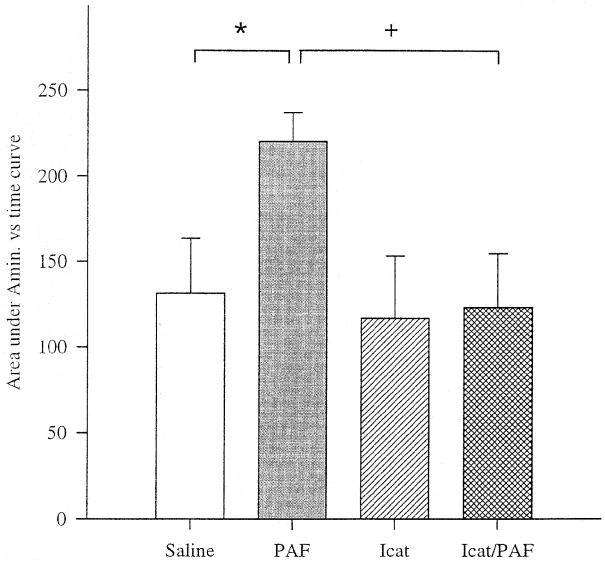

Figure 1 Area under Amin. against time curves (AUC), following challenge with histamine, 200 μg. The nasal cavity was pretreated with saline, icatibant (Icat), 200 μg, and/or PAF, 60 μg, as described in the text. Data are means from eight subjects. Vertical bars represent s.e.mean. The mean $\pm$ s.e.mean baseline value for Amin. was 0.64 ± 0.03 cm^2. *Significant difference in AUC following pretreatment with PAF compared to saline control ($P<0.05$, Wilcoxon sign-rank test). +Significant difference in AUC when pretreatment with PAF is compared to icatibant/PAF ($P<0.05$, Wilcoxon sign-rank test).

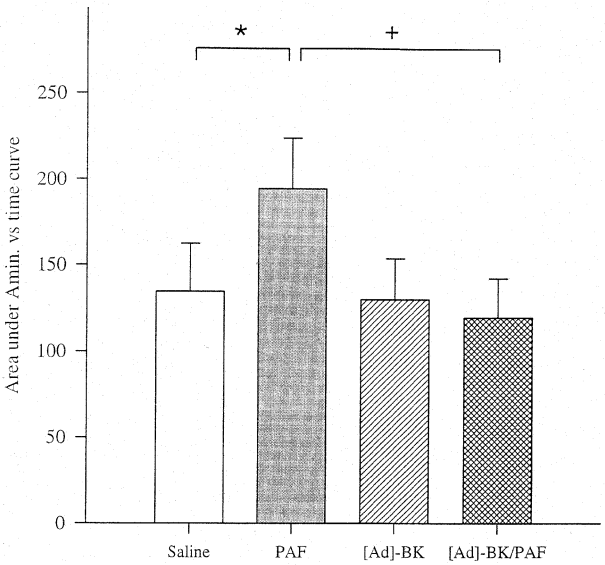

Figure 2 Area under Amin. against time curves (AUC), following challenge with histamine, 200 μg. The nasal cavity was pretreated with saline, [Ad]-BK, 200 μg, and/or PAF, 60 μg, as described in the text. Data are means from nine subjects. Vertical bars represent s.e.mean. The mean $\pm$ s.e.mean baseline value for Amin. was 0.61 ± 0.03 cm^2. *Significant difference in the AUC following saline/PAF pretreatment compared to saline control ($P<0.05$, Wilcoxon sign-rank test). +Significant difference in the histamine-induced decrease in AUC when pretreatment with saline/PAF is compared to [Ad]-BK/PAF ($P<0.05$, Wilcoxon sign-rank test).

Effect of bradykinin B_2 receptor antagonists on PAF-induced mediator release

Figure 3 shows the levels of ECP detected in the lavage samples immediately prior to, and 6 h after pretreatment with PAF,

60 μg, or saline, with or without treatment using icatibant (Figure 3a) or [Ad]-BK (Figure 3b). Administration of PAF caused an increase in the levels of ECP detected (80% range: 2–5.4 ng ml^{-1}) compared to saline control (80% range: 2–3.1 ng ml^{-1}) ($P<0.05$, Friedman's test; $P<0.01$, Wilcoxon sign-rank test). Icatibant alone did not alter ECP levels ($P>0.05$, Wilcoxon sign-rank test), but did significantly reduce the increase in ECP induced by PAF (80% range: 2–4.7 ng ml^{-1}) ($P<0.01$, Friedman's test; $P<0.01$, Wilcoxon sign-rank test). Pretreatment with [Ad]-BK did not reduce the PAF-induced increase in ECP ($P>0.05$, Wilcoxon sign-rank test).

In the 6 h following challenge with PAF, there was an overall increase in the concentration of kinins detected in nasal lavage fluid compared to saline controls ($P<0.01$, Wilcoxon sign-rank test) (Figure 4). The maximal increase in kinin concentration, which occurred at 2 h after PAF administration, was 504 pg ml^{-1} (80% range: 67.1–1284 pg ml^{-1}) compared with 137.5 pg ml^{-1} (80% range: 47.8–209.8 pg ml^{-1}) for the saline control: these values were significantly different ($P<0.01$, Wilcoxon sign-rank test). This increase in kinin release was significantly altered following pretreatment with icatibant ($P<0.01$, Friedman's test). Icatibant alone did not alter the kinin content of the nasal lavage samples ($P>0.05$, Wilcoxon sign-rank test). However, treatment with icatibant, 200 μg, every 2 h attenuated the increase in kinin caused by PAF: this effect was significant at 6 h after PAF administration ($P<0.05$, Wilcoxon sign-rank test) (Figure 4a). Following treatment with [Ad]-BK, PAF failed to cause an increase in the kinin content of nasal lavage fluid, compared to the saline control ($P>0.05$, Wilcoxon sign-rank test) (Figure 4b).

PAF did not cause a significant increase in the albumin content of the nasal lavages at any timepoint ($P>0.05$, Friedman's test) (data not shown). No significant correlations were observed between the albumin content of lavage fluid and kinin or ECP levels. Although the nasal lavage data showed a degree of variability, this was due to biological variation in the production of mediators between individuals. No differences were found in the initial levels of mediators between treatment groups ($P>0.05$, Friedman's test), confirming that this variability was not a significant confounding factor.

It has been previously demonstrated that lyso-PAF does not cause hyperresponsiveness in the human nasal airway (Austin

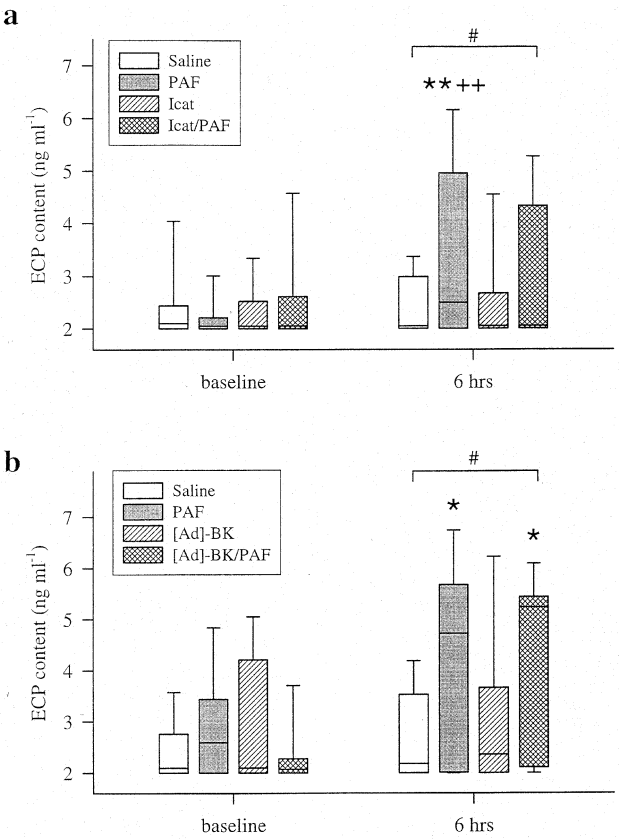

Figure 3 The ECP content of nasal lavage fluid, before (baseline) and 6 h after treatment of the nasal cavity with saline, a bradykinin B_2 receptor antagonist, 200 μg, and/or PAF, 60 μg, as described in the text. The bradykinin B_2 receptor antagonists used were icatibant (Icat) ($n=13$, graph a) or [Ad]-BK ($n=10$, graph b). Data are presented as medians, indicated by a horizontal line within the interquartile range of values for each median. Vertical bars represent the 80% central range of the values. #Significant difference in ECP levels following different pretreatments ($P<0.05$, Friedman's test). */**Significant increase in ECP levels for the pretreatment shown, compared to treatment with saline control (*$P<0.05$, **$P<0.01$, Wilcoxon sign-rank test). ++Significant difference in ECP content following pretreatment with PAF compared to icatibant/PAF ($P<0.01$, Wilcoxon sign-rank test).

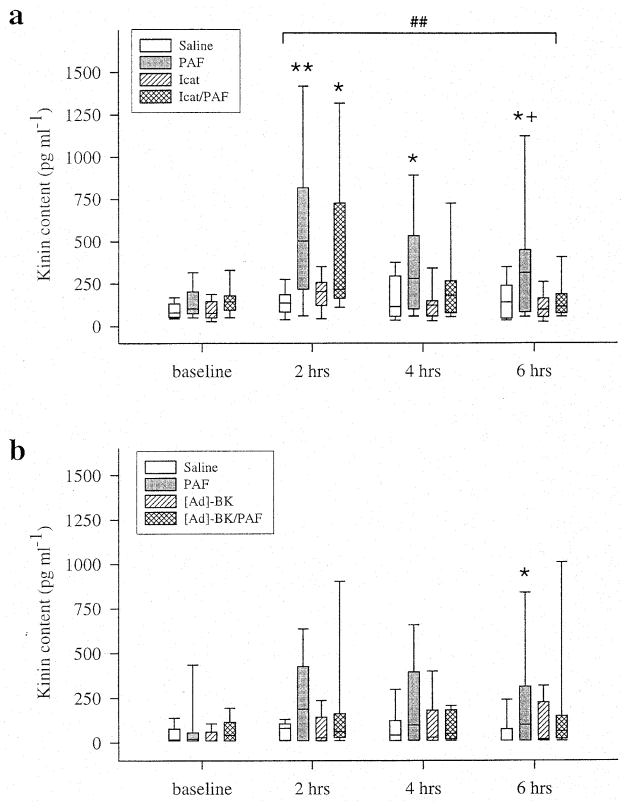

Figure 4 The kinin content of nasal lavage fluid, measured before (baseline) and 2, 4 and 6 h after treatment of the nasal cavity with saline, a bradykinin B_2 receptor antagonist, 200 μg, and/or PAF, 60 μg, as described in the text. The bradykinin B_2 receptor antagonists used were icatibant (Icat) ($n=13$, graph a) or [Ad]-BK ($n=10$, graph b). Data are presented as medians, indicated by a horizontal line within the interquartile range of values for each median. Vertical bars represent the 80% central range of the values. ##Significant difference in kinin levels following different pretreatments ($P<0.01$, Friedman's test). */**Significant increase in kinin levels for the pretreatment shown, compared to treatment with saline control (*$P<0.05$, **$P<0.01$, Wilcoxon sign-rank test). ++Significant difference in kinin content following pretreatment with PAF compared to icatibant/PAF ($P<0.01$, Wilcoxon sign-rank test).

Table 1 The ECP and kinin content of nasal lavage fluid before (baseline) and at various time intervals after treatment of the nasal cavity with saline, PAF, 60 µg, or lyso-PAF, 60 µg

	ECP (ng ml^{-1})		*Kinin (pg ml^{-1})*		
Treatment	*Baseline*	*6 h*	*Baseline*	*2 h*	*6 h*
Saline	2.1 (2.0–4.1)	2.0 (2.0–3.5)	68 (43–183)	107 (40–304)	124 (38–345)
PAF	2.0 (2.0–2.4)	3.0 (2.0–6.5)*	163 (69–333)	570 (60–1453)*	294 (55–676)*
Lyso-PAF	2.0 (2.0–3.6)	2.0 (2.0–4.0)	123 (52–215)	110 (91–260)	118 (80–198)

Each datum is the median (with 80% central range) from eight subjects. *Significant increase in mediator shown following treatment with PAF, compared to saline control ($P<0.05$, Wilcoxon sign-rank test).

& Foreman, 1993). To investigate whether the PAF-induced increases in mediator release were due to its action as a surfactant, eight subjects were also challenged with lyso-PAF. Lyso-PAF, 60 µg, did not increase the ECP or kinin content of lavage fluid at 6 h after administration ($P>0.05$, Wilcoxon sign-rank test) (Table 1), nor alter albumin levels ($P>0.05$, Wilcoxon sign-rank test) (data not shown).

Effect of bradykinin on the responsiveness of the human nasal airway to histamine

Pretreatment of the nasal airway with bradykinin, 5 mg ml^{-1}, did not alter the response to histamine compared to pretreatment with the saline control, at any timepoint ($P>0.05$, Wilcoxon sign-rank test) (Figure 5). Similarly, pretreatment with bradykinin, 2 mg ml^{-1}, also failed to induce a hyperresponsiveness to histamine ($P>0.05$, Wilcoxon sign-rank test; data not shown). The nasal response to bradykinin was not altered by prior administration of bradykinin ($P>0.05$, Friedman's test), thus providing no evidence of a reduction in the response to bradykinin by tachyphylaxis (data not shown).

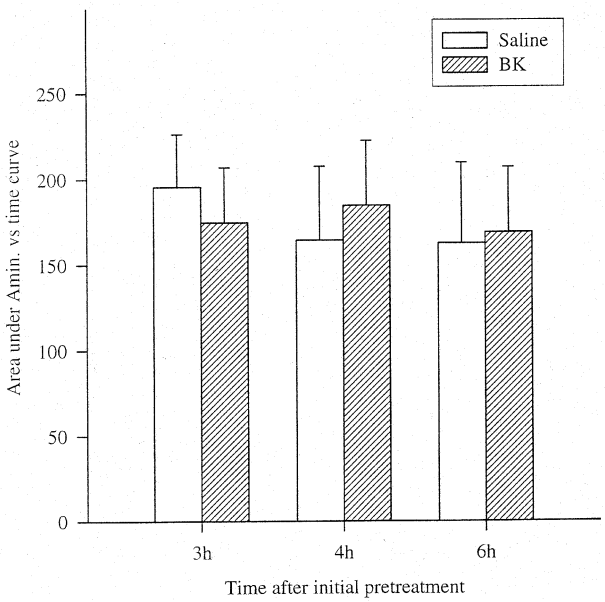

Figure 5 Area under Amin. against time curves (AUC), following challenge with histamine, 200 µg. The nasal cavity was pretreated with saline or bradykinin (BK), 500 µg, every 30 min for 2 h, as described in the text. Data are means from ten subjects. Vertical bars represent s.e.mean. The baseline Amin. values (mean ± s.e.mean) were 0.64 ± 0.02 cm^2 for saline and 0.65 ± 0.03 cm^2 for bradykinin.

Discussion

Histamine produced a reduction in the patency of the human nasal airway. Pretreatment of the nasal airway with icatibant or [Ad]-BK had no effect on the response to histamine challenge, demonstrating that the action of histamine is not mediated through the release of kinins, nor mediated through the bradykinin B_2 receptor. Pretreatment of the nasal airway with PAF enhanced the nasal action of histamine, as previously reported (Austin & Foreman, 1993).

The PAF-induced nasal hyperresponsiveness to histamine was abolished following pretreatment with the two selective B_2 receptor antagonists, icatibant and [Ad]-BK, suggesting that kinin generation, and activation of bradykinin B_2 receptors are required for PAF-induced hyperresponsiveness. This is further supported by the detection of increased levels of kinins in nasal lavage fluid at 2, 4 and 6 h following administration of PAF, but not lyso-PAF. The development of a nasal hyperresponsiveness by PAF, at the dose used in this study, is apparent at 6 h but not 2 h after administration (Austin & Foreman, 1993). In the current study, the PAF-induced increase in kinin production occurred within 2 h of administration. Therefore, kinin production by PAF appears to be an early event in the pathway which results in hyperresponsiveness. The assay used was equally sensitive to bradykinin and kallidin, so we could not distinguish between the production of these two peptides by PAF, or their relative roles in promoting hyperresponsiveness.

Interestingly, when the nasal airway was pretreated with icatibant or [Ad]-BK, the degree of kinin production by PAF was reduced. There is some evidence that icatibant inhibits kallikrein in an animal model, in addition to its action as an antagonist at the bradykinin B_2 receptor (Dr K. Wirth, unpublished). Furthermore, it has been shown that icatibant reduces the production of kinins *in vivo* following challenge with house dust-mite (Dear *et al*., 1996). PAF causes plasma extravasation in both the skin and lower airways (Barnes *et al*., 1988) and, at higher doses, in the human nasal airway (Leggieri *et al*., 1991). At the dose used in this study, PAF did not increase albumin levels in nasal lavage fluid, so the increase in kinin levels, and the effect of pretreatment with icatibant or [Ad]-BK, are likely to be due to a change in kinin production (and/or metabolism), rather than a consequence of altered vascular permeability.

Coyle *et al*. (1995) reported that MBP, released from activated eosinophils, produced hyperresponsiveness of the rat lower airways by a kinin-dependent mechanism that involved the bradykinin B_2 receptor. ECP can activate plasma kallikrein (Venge *et al*., 1979). Therefore, PAF may induce a hyperresponsiveness by recruiting eosinophils and releasing cationic proteins such as ECP and MBP which, in turn, activate kallikrein-like enzymes and generate kinins. However, at the dose used in this study, PAF did not cause an increase in albumin levels, which can be used as a marker of plasma extravasation (Naclerio *et al*., 1983). Therefore, it is unlikely

that the kinins were generated by the action of plasma kallikrein on kininogen from plasma. Although the levels of tissue kallikrein in nasal secretions are increased in allergic rhinitis (Baumgarten *et al*., 1986), this enzyme is not activated by cationic proteins (Coyle *et al*., 1995). Neutrophils, however, contain tissue kallikrein and bind plasma kallikrein, together with high and low molecular weight kininogen, on the cell surface (Gustafson *et al*., 1989; Henderson *et al*., 1994). Nasal challenge with antigen or PAF causes a neutrophilia in addition to eosinophil recruitment (Tedeschi *et al*., 1994b), and it is therefore possible that neutrophils provide the components needed for the generation of kinins by ECP and MBP.

However, this hypothesis does not explain the reduction in PAF-induced ECP production following pretreatment with icatibant, but not with [Ad]-BK. Whether this effect was due to a decrease in eosinophil activation or a consequence of the decrease in kinin production is unknown. Farmer *et al*. (1992) found that bradykinin antagonists reduced the eosinophilia caused by antigen challenge in the lower airway of the guinea-pig. Therefore, icatibant may have inhibited the eosinophilia caused by PAF and, consequently, the release of ECP. A number of antagonists at the histamine H_1 receptor possess additional anti-eosinophil properties which are independent of their action at histamine receptors. The reduction in ECP by icatibant, but not by [Ad]-BK, may have been due to an anti-eosinophil activity of icatibant, not shared by [Ad]-BK. Indeed, since both antagonists prevented the hyperresponsiveness, the data suggests that the development of a nasal hyperresponsiveness following PAF occurs independently of ECP release. It is interesting to note that intranasal PAF causes ECP release 2 h after administration, without an associated hyperresponsiveness at that timepoint (Austin & Foreman, 1993).

There are alternative models to explain the kinin dependency of PAF-induced nasal airway hyperresponsiveness. The production of kinins by PAF and the kinin-dependent development of PAF-induced hyperresponsiveness could result from an action of PAF which is independent of eosinophils. Antigen-induced hyperresponsiveness can occur without an obvious eosinophilia in both animals (Spina *et al*., 1991) and man (Klementsson *et al*., 1991). As previously mentioned, intranasal PAF induced an increase in kinins 2 h after administration, at a time when no hyperresponsiveness is detected (Austin & Foreman, 1993). Furthermore, pilot experiments indicated that bradykinin did not affect the immediate nasal response to challenge agents. Therefore, we decided to monitor the presence of a hyperresponsiveness to histamine at 3, 4 and 6 h after repeated administrations of bradykinin. However, we were unable to demonstrate that bradykinin alone could induce a hyperresponsiveness to histamine. This is in agreement with studies in animal models (Abraham *et al*., 1991; Coyle *et al*., 1995), though another study in guinea-pigs found that bradykinin could potentiate the response of the airway to acetylcholine (Omini *et al*., 1989). There are no reports of PAF mediating its actions through bradykinin generation (Sakamoto *et al*., 1992). It is, therefore, likely that PAF also caused the release of other mediators, particularly those derived from eosinophils and neutrophils, which are needed to generate kinins and induce hyperresponsiveness.

Icatibant and [Ad]-BK may have prevented the PAF-induced hyperresponsiveness by their ability to inhibit kinin production, rather than an action at B_2 receptors. Thus, the induction of hyperresponsiveness by PAF could be independent of bradykinin B_2 receptor activation. However, both icatibant and [Ad]-BK abolished the hyperresponsiveness even though kinin levels in nasal lavage samples remained above control levels. If the bradykinin antagonists inhibited the development of hyperresponsiveness purely by an effect on kallikrein, one would not expect kinin levels to remain elevated in the absence of a hyperresponsiveness. In addition, there is no evidence to suggest the involvement of the bradykinin B_1 receptor in the physiology of the nasal airways of normal, non-atopic subjects (Rajakulasingham *et al*., 1991; Austin & Foreman, 1994a). Therefore, the data imply that the induction of a nasal hyperresponsiveness by PAF is bradykinin B_2 receptor dependent.

The mechanism by which kinins may induce hyperresponsiveness remains unknown. Neuropeptide release may be important in the hyperresponsiveness induced by PAF in the lower airway of the guinea-pig (Spina *et al*., 1991), and cationic proteins in the lower airway of the rat (Coyle *et al*., 1994). Furthermore, neuropeptide depletion prevents antigen-induced airway hyperresponsiveness in animals (Ladenius *et al*., 1989; Matsuse *et al*., 1991). Bradykinin can stimulate sensory nerve endings, causing the release of substance P and other neuropeptides (Saria *et al*., 1988; Bertrand & Geppetti, 1996). Therefore, the development of hyperresponsiveness may depend on the kinin-mediated release of neuropeptides. Furthermore, Fox *et al*. (1996) reported that bradykinin can cause sensitization of C-fibres in the guinea-pig trachea, and a similar process may happen in the human nasal airway. Alternatively, the kinins may stimulate the production, by inflammatory cells and neurones, of cytokines such as IL-1, IL-6 and IL-8 (Ferreira *et al*., 1993), which may be involved in the development of hyperresponsiveness (Howarth, 1995). In addition, PAF and eosinophil-derived cationic proteins are cytotoxic to the epithelial lining of the airway (Ganbo & Hisamatsu, 1990; Ohashi *et al*., 1997), and this could increase the responsiveness of the nasal airway by exposing nerve endings. This might explain why bradykinin does not cause hyperresponsiveness in the absence of PAF.

Finally, there are many similarities between the nasal airway hyperresponsiveness induced by PAF and that caused by antigen. Both cause an eosinophilia and an increase in the production of eosinophil-derived cationic proteins in lavage fluid (Bascom *et al*., 1989; Knani *et al*., 1992), and antigen challenge itself causes the release of PAF (Shin *et al*., 1994). We have shown that icatibant and [Ad]-BK prevented the development of PAF-induced hyperresponsiveness, and we conclude that endogenous kinins are involved in the development of nasal airway hyperresponsiveness by PAF. Bradykinin alone was, however, not able to induce hyperresponsiveness.

We would like to thank the subjects who agreed to participate in these studies. P.J. Turner and J.W. Dear thank the Medical Research Council for the provision of an award for Training in Research Methods.

References

ABRAHAM, W.M., AHMED, A., CORTES, A., SOLER, M., FARMER, S.J., BAUGH, L.E. & HARBESON, S.L. (1991). Airway effects of inhaled bradykinin, substance P, and neurokinin A in sheep. *J. Allergy Clin. Immunol.,* **87,** 551 – 564.

ANDERSSON, M. & PIPKORN, U. (1988). The effect of platelet activating factor on nasal hypersensitivity. *Eur. J. Clin. Pharmacol.,* **35,** 231 – 235.

AUSTIN, C.E. & FOREMAN, J.C. (1993). The effect of platelet activating factor on the responsiveness of the human nasal mucosa. *Br. J. Pharmacol.,* **110,** 113 – 118.

AUSTIN, C.E. & FOREMAN, J.C. (1994a). A study of the action of bradykinin and bradykinin analogues in the human nasal airway. *J. Physiol.,* **478.2,** 351 – 356.

AUSTIN, C.E. & FOREMAN, J.C. (1994b). Acoustic rhinometry compared with posterior rhinomanometry in the measurement of histamine- and bradykinin-induced changes in nasal airway patency. *Br. J. Clin. Pharmacol.,* **37,** 33 – 37.

BARNES, P.J., CHUNG, K.F. & PAGE, C.P. (1988). Platelet-activating factor as a mediator of allergic disease. *J. Allergy Clin. Immunol.,* **81,** 919 – 934.

BASCOM, R., PIPKORN, U., PROUD, D., DUNNETTE, S., GLEICH, G.J., LICHTENSTEIN, L.M. & NACLERIO, R.M. (1989). Major basic protein and eosinophil-derived neurotoxin concentrations in nasal lavage fluid after antigen challenge: effect of systemic corticosteroids and relationship to eosinophil influx. *J. Allergy Clin. Immunol.,* **84,** 338 – 346.

BAUMGARTEN, C.R., NICHOLS, R.C., NACLERIO, R.M. & PROUD, D. (1986). Concentrations of glandular kallikrein in human nasal secretions increase during experimentally induced allergic rhinitis. *J. Immunol.,* **137,** 1323 – 1328.

BERTRAND, C. & GEPPETTI, P. (1996). Tachykinin and kinin receptor antagonists: therapeutic perspectives in allergic airway disease. *Trends Pharmacol. Sci.,* **17,** 255 – 259.

COYLE, A.J., ACKERMAN, S.J., BURCH, R.M., PROUD, D. & IRVIN, C.G. (1995). Human eosinophil-granule major basic protein and synthetic polycations induce airway hyperresponsiveness *in vivo* dependent on bradykinin generation. *J. Clin. Invest.,* **95,** 1735 – 1740.

COYLE, A.J., PERRETTI, F., MANZINI, S. & IRVIN, C.G. (1994). Cationic protein-induced sensory nerve activation: role of substance P in airway hyperresponsiveness and plasma extravasation. *J. Clin. Invest.,* **94,** 2301 – 2306.

DEAR, J.W., WIRTH, K., SCADDING, G.K. & FOREMAN, J.C. (1996). Characterisation of the bradykinin receptor in the human nasal airway using the binding of [^{125}I]-Hoe 140. *Br. J. Pharmacol.,* **119,** 1054 – 1062.

FARMER, S.G., WILKINS, D.E., MEEKER, S.A., SEEDS, E.A.M. & PAGE, C.P. (1992). Effects of bradykinin receptor antagonists on antigen-induced respiratory distress, airway hyperresponsiveness and eosinophilia in guinea pigs. *Br. J. Pharmacol.,* **107,** 653 – 659.

FERREIRA, S.H., LORENZETTI, B.B. & POOLE, S. (1993). Bradykinin initiates cytokine-mediated inflammatory hyperalgesia. *Br. J. Pharmacol.,* **110,** 1227 – 1231.

FOX, A.J., LALLOO, U.G., BELVISI, M.G., BERNAREGGI, M., FAN CHUNG, K. & BARNES, P.J. (1996). Bradykinin-evoked sensitisation of airway sensory nerves: a mechanism for ACE-inbitor cough. *Nature Med.,* **2,** 814 – 817.

GANBO, T. & HISAMATSU, K.-I. (1990). Mucosal dysfunction and damage induced by platelet activating factor (PAF). *Acta Otolaryngol. (Stockh.),* **110,** 427 – 436.

GUSTAFSON, E.J., SCHMAIER, A.H., WACHTFOGEL, Y.T., KAUFMAN, N., KUCICH, U. & COLMAN, R.W. (1989). Human neutrophils contain and bind high molecular weight kininogen. *J. Clin. Invest.,* **84,** 28 – 35.

HENDERSON, L.M., FIGUEROA, C.D., MULLER-ESTERL, W. & BHOOLA, K.D. (1994). Assembly of contact-phase factors on the surface of the human neutrophil membrane. *Blood,* **84,** 474 – 482.

HOWARTH, P.H. (1995). The cellular basis for allergic rhinitis. *Allergy,* **50** (Suppl.), 23, 6 – 10.

KLEMENTSSON, H., VENGE, P., ANDERSSON, M. & PIPKORN, U. (1991). Allergen-induced changes in nasal secretory responsiveness and eosinophil granulocytes. *Acta Otolaryngol. (Stockh.),* **111,** 776 – 784.

KNANI, J., CAMPBELL, A., ENANDER, I., PETERSON, C.G.B., MICHE, F.-B. & BOUSQUET, J. (1992). Indirect evidence of nasal inflammation assessed by titration of inflammatory mediators and enumeration of cells in nasal secretions of patients with chronic rhinitis. *J. Allergy Clin. Immunol.,* **90,** 880 – 889.

LADENIUS, A.R.C. & BIGGS, D.F. (1989). Capsaicin prevents the induction of airway hyperresponsiveness in a guinea pig model of asthma. *Am. Rev. Respir. Dis.,* **139,** A232.

LEGGIERI, E., TEDESCHI, A., LORINI, M., BIANCO, A. & MIADONNA, A. (1991). Study of the effects of PAF-acether on human nasal airways. *Allergy,* **46,** 446 – 471.

MATSUSE, T., THOMSON, R.J., CHEN, X.R., SALARI, H. & SCHELLENBERG, R.R. (1991). Capsaicin inhibits airway hyperresponsiveness but not lipoxygenase activity or eosinophilia after repeated aerolised antigen in guinea pigs. *Am. Rev. Respir. Dis.,* **144,** 368 – 372.

NACLERIO, R.M., MEIER, H.L., KAGEY-SOBOTKA, A., ADKINSON, N.F., MEYERS, D.A., NORMAN, P.S. & LICHTENSTEIN, L.M. (1983). Mediator release after nasal airway challenge with allergen. *Am. Rev. Respir. Dis.,* **128,** 597 – 602.

O'FLAHERTY, J.T. & WYKLE, R.L. (1983). Biology and biochemistry of platelet activating factor. *Clin. Rev. Allergy,* **1,** 353 – 367.

OHASHI, Y., NAKAI, Y., MORIMOTO, K., TANAKA, A., KAKINOKI, Y., OHNO, Y., MASAMOTO, T. & KATO, A. (1997). Platelet activating factor compromises airway epithelial defense functions. *J. Allergy Clin. Immunol.,* **100,** 520 – 526.

OMINI, C., BURNELLI, G., HERNANDEZ, A. & DAFFONCHIO, L. (1989). Bradykinin and substance P potentiate acetylcholine-induced bronchoconstriction in the guinea pig. *Eur. J. Pharmacol.,* **163,** 195 – 197.

PROUD, D., TOGIAS, A., NACLERIO, R.M., CRUSH, S.A., NORMAN, P.S. & LICHTENSTEIN, L.M. (1983). Kinins are generated in vivo following nasal airway challenge of allergic individuals with allergen. *J. Clin. Invest.,* **72,** 1678 – 1685.

RAJAKULASINGAM, K., POLOSA, R., HOLGATE, S.T. & HOWARTH, P.H. (1991). Comparative nasal effects of bradykinin, kallidin and [des-Arg^9]-bradykinin in atopic rhinitic and normal volunteers. *J. Physiol.,* **437,** 557 – 587.

SAKAMOTO, T., ELWOOD, W., BARNES, P.J. & CHUNG, K.F. (1992). Effect of Hoe 140, a new bradykinin receptor antagonist, on bradykinin- and platelet activating factor-induced bronchoconstriction and airway microvascular leakage in guinea pig. *Eur. J. Pharmacol.,* **213,** 367 – 373.

SARIA, A., MARTLING, C.R., YAN, Z., THEODORSSON-NORHEIM, E., GAMSE, R. & LUNDBERG, J.M. (1988). Release of multiple tachykinins from capsaicin-sensitive sensory nerves in the lung by bradykinin, histamine, dimethylphenyl piperazinium, and vagal nerve stimulation. *Am. Rev. Respir. Dis.,* **137,** 1330 – 1335.

SHIN, M.H., AVERILL, F.J., HUBBARD, W.C., CHILTON, F.H., BAROODY, F.M., LIU, M.C. & NACLERIO, R.M. (1994). Nasal allergen challenge generates 1-0-hexadecyl-2-lyso-sn-glycero-3-phosphocholine. *Am. J. Respir. Crit. Care Med.,* **149,** 660 – 666.

SPINA, D., MCKENNIFF, M.G., COYLE, A.J., SEEDS, E.A., TRAMONTANA, M., PERRETTI, F., MANZINI, S. & PAGE,, C.P. (1991). Effect of capsaicin on PAF-induced bronchial hyperresponsiveness and pulmonary cell accumulation in the rabbit. *Br. J. Pharmacol.,* **103,** 1268 – 1274.

TEDESCHI, A., MILAZZO, N. & MIADONNA, A. (1994a). Nasal eosinophilia induced by PAF-acether is accompanied by the release of eosinophil cationic protein. *Eur. J. Respir.* **7,** 1445 – 1451.

TEDESCHI, A., PALUMBO, G., MILAZZO, N. & MIADONNA, A. (1994b). Nasal neutrophilia and eosinophilia induced by challenge with platelet activating factor. *J. Allergy Clin. Immunol.,* **93,** 526 – 533.

TSAI, J.J., HO, C.Y. & WANG, S.R. (1995). Relationship between nasal resistance and airway hyperreactivity following nasal provocation with Dematophagoides pteronyssinus in allergic rhinitis. *Int. Arch. Allergy Immunol.,* **106,** 286 – 290.

VENGE, P., DAHL, R. & HALLGREN, R. (1979). Enhancement of factor XII reactions by eosinophil cationic protein. *Thromb. Res.,* **14,** 641 – 649.

WIHL, J.A., BAUMGARTEN, C.R. & PETERSSON, G. (1995). Contralateral differences among biomarkers determined by a modified nasal lavage technique after unilateral antigen challenge. *Allergy,* **50,** 308 – 315.

(Received October 27, 1999
Accepted November 11, 1999)