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# Characterization of the bradykinin receptor in the human nasal airway using the binding of [$^{125}$I]-Hoe 140

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**1** The aim of this study was to characterize the kinin receptor in the human nasal airway using [$^{125}$I]-Hoe 140 binding to a membrane preparation from human nasal turbinates and to compare $K_i$ values from binding displacement by antagonists with the functional effects of these drugs *in vivo*. We also investigated the effect of Hoe 140 ([D-Arg$^0$, Hyp$^3$, Thi$^5$, D-Tic$^7$, Oic$^8$]-bradykinin), on bradykinin release into the nasal airway.

**2** In a membrane preparation from human nasal turbinates removed during surgery, [$^{125}$I]-Hoe 140 labelled a single, saturable binding site. The equilibrium dissociation constant (at 20°C) for [$^{125}$I]-Hoe 140 binding to the receptor was $0.46 \pm 0.08$ nM. The $B_{max}$ was $0.136 \pm 0.003$ pmol mg$^{-1}$ protein and the Hill coefficient was $1.01 \pm 0.07$.

**3** The association rate constant for [$^{125}$I]-Hoe 140 binding to the receptor was $0.20 \pm 0.06$ nM$^{-1}$ min$^{-1}$ and the dissociation rate constant was $0.14 \pm 0.01$ min$^{-1}$. These values were determined at 4°C. The equilibrium dissociation constant calculated from these rate constants was 0.70 nM.

**4** Bradykinin and the $B_2$ receptor antagonists, NPC 567, NPC 17731, NPC 17761, [1-adamantane acetyl-D-Arg$^0$, Hyp$^3$, Thi$^{5,8}$, D-Phe$^7$]-bradykinin, WIN 64338 and Hoe 140 displaced [$^{125}$I]-Hoe 140 binding: the $K_i$ values from binding displacement are consistent with values expected from a $B_2$ receptor. The $B_1$ agonist, [des-Arg$^9$]-bradykinin and the $B_1$ antagonist, [des-Arg$^9$]-Hoe 140 failed to displace [$^{125}$I]-Hoe 140 binding at concentrations up to 1 μM.

**5** The bradykinin antagonist, Hoe 140, 10 to 200 μg, given by intranasal aerosol, produced a dose-related inhibition of the reduction in minimal nasal cross-sectional area (Amin) induced by bradykinin in normal subjects and by house dust mite antigen in subjects with allergic rhinitis to house dust mite. Hoe 140, 10 to 200 μg, also caused a dose-related inhibition of the release of albumin into the nasal cavity following challenge with bradykinin.

**6** [1-Adamantane acetyl-D-Arg$^0$, Hyp$^3$, Thi$^{5,8}$, D-Phe$^7$]-bradykinin, 30 to 200 μg, caused a dose-related inhibition of the reduction in Amin and the release of albumin into the nasal cavity induced by bradykinin. NPC 567 ([D-Arg$^0$, Hyp$^3$, D-Phe$^7$]-bradykinin) failed to inhibit the reduction in Amin or the release of albumin into the nasal cavity at a dose of 10 mg.

**7** Challenge of allergic subjects with house dust mite antigen caused a significant elevation of the bradykinin concentration in nasal lavage fluid and a reduction in Amin. Hoe 140, 100 μg, prevented the antigen-induced reduction in Amin and also abolished the antigen-induced increase of bradykinin in nasal lavage fluid.

**8** We conclude that there is a $B_2$ bradykinin receptor in the human nasal airway which mediates nasal blockage and plasma extravasation induced by either bradykinin or antigen challenge. It is possible that Hoe 140 inhibits kallikrein in the human nasal airway as well as blocking the $B_2$ receptor.

**Keywords:** Hoe 140; icatibant; bradykinin; human nasal airway; $B_2$ receptor; receptor binding

## Introduction

There is evidence that bradykinin is a mediator of allergic rhinitis. First, bradykinin and kallidin are released into the nasal airway following antigen provocation of subjects with seasonal allergic rhinitis to grass pollen (Proud *et al.*, 1983). Second, in normal, non-allergic subjects, challenge of the nasal airway with bradykinin, but not with $B_1$ selective agonists, produces some of the signs of allergic rhinitis: namely, increased nasal airway resistance and increased vascular permeability of the nasal vasculature (Proud *et al.*, 1988; Rajakulasingham *et al.*, 1991; Austin & Foreman, 1994a). Third, in perennial allergic rhinitis caused by house dust mite antigen, the bradykinin receptor antagonist, Hoe 140 ([D-Arg$^0$, Hyp$^3$, Thi$^5$, D-Tic$^7$, Oic$^8$]-bradykinin; generic name, icatibant) blocks antigen-induced nasal blockage (Austin *et al.*, 1994).

Previous studies in our laboratory (Austin & Foreman, 1994a) have shown that single doses by intranasal aerosol, of the bradykinin $B_2$ receptor antagonists, Hoe 140 and [1-adamantane acetyl-D-Arg$^0$, Hyp$^3$, Thi$^{5,8}$, D-Phe$^7$]-bradykinin inhibit the effect of bradykinin in the human nasal airway. However, the bradykinin $B_2$ receptor antagonist, NPC 567 ([D-Arg$^0$, Hyp$^3$, D-Phe$^7$]-bradykinin) failed to inhibit the response to bradykinin at doses of 100 and 1000 μg. NPC 567 has a low potency as a bradykinin antagonist with estimates of the equilibrium dissociation constant in the range 9 nM to 6 μM (Lyon *et al.*, 1990; Pruneau *et al.*, 1995). Thus, although 1000 μg was used in the human nasal airway, this may have been too low a dose to antagonize the effect of bradykinin. An alternative interpretation of the functional study is that the receptor mediating the action of bradykinin in the human nasal airway is either a subtype of the $B_2$ kinin receptor or, as has been suggested in guinea-pig lung tissue (Farmer *et al.*, 1989), a $B_3$ kinin receptor might be present in the human nasal

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airway. Many of the selective antagonists at bradykinin receptors are not available for study in human subjects and hence an alternative approach to the characterization of the human nasal bradykinin receptor was needed.

The aim of this study was to use human nasal airway tissue, removed at routine surgery, to study the kinin receptor in this tissue by means of receptor binding of [$^{125}$I]-Hoe 140, and to use selective bradykinin receptor ligands to characterize the nasal kinin receptor. The aim also was to compare the $K_i$ values for antagonists, obtained from the displacement of [$^{125}$I]-Hoe 140 binding, with the effects of some of the antagonists on nasal airway function *in vivo*. The effect of Hoe 140 on bradykinin release into the nasal airway was also studied.

## Methods

### *Subjects*

All subjects gave their informed consent. The studies were approved by the local Ethics Committees at University College London and the The Royal National Throat, Nose and Ear Hospital. A total of 14 normal healthy volunteers aged 20–52 years and 14 subjects with perennial allergic rhinitis to house dust mite, aged 20–45 years, participated in the studies. Subjects suffering from a cold or reporting other nasal symptoms were excluded. No subject was taking medication at the time of, or in the four weeks prior to, the study. Experiments were conducted in a laboratory with controlled temperature and humidity.

### *Measurement of nasal patency*

Nasal patency (cross-sectional area of the nasal cavity) was determined by the method of 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.

The doses of bradykinin and antigen used to provoke blockage of the nasal airway were chosen, on the basis of previous studies (Austin & Foreman, 1994a), to cause a reduction in Amin of about 20% of the control. A reduction in Amin of this magnitude can be measured reproducibly, does not cause excessive discomfort in the subjects and is on a part of the dose-response curve which would be expected to permit the detection of an effect of an antagonist.

### *Nasal lavage and the assays of albumin and bradykinin*

Nasal lavage was performed as described by Naclerio *et al.* (1983) by the instillation of 5 ml of prewarmed (37°C) saline into each nostril for 10 s. Recovery was approximately 90%. The first two lavages at the beginning of each experiment were discarded and the third lavage was assayed for the baseline albumin or bradykinin release. The albumin content of the nasal lavage was determined with a commercially available single radial immunodiffusion assay. A protein standard serum was used to establish a calibration curve, and albumin from human sera served as a control to confirm the function of the immunodiffusion plates.

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]-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.

### *Effect of bradykinin receptor antagonists on bradykinin-induced reductions in nasal patency*

This study was a single-blind, randomized, cross-over study in which normal, non-atopic subjects were used. The minimum cross-sectional area (Amin) of the nasal cavity was determined by acoustic rhinometry. Then the nasal cavity was treated with a dose of kinin receptor antagonist or vehicle (saline) by a hand-held pump spray (Perfect-Valois) that delivered 100 $\mu$l per actuation. On separate occasions, at least 3 days apart, each subject received vehicle or one of the doses of the antagonist being tested. The order of treatments was determined randomly. Two minutes after pretreatment, bradykinin, 100 $\mu$g, was delivered into the nasal cavity, again by hand-held pump spray. The 2 min interval between antagonist and bradykinin administration was based on a previous study (Austin & Foreman, 1994a). Ten minutes after bradykinin challenge the Amin was remeasured. Amin was measured 10 min after the bradykinin application since this was found to be the time of the maximum change in Amin.

### *Effect of bradykinin receptor antagonists on the increase in albumin extravasation into the nasal cavity induced by bradykinin*

Again, in this study, normal, non-atopic subjects were used. The study of albumin release was conducted on separate occasions from the study of nasal patency. Again, a single-blind, cross-over design was used. Nasal lavage was performed, three times per nostril, prior to any treatment, and the final lavage was used for the measurement of baseline albumin release. Subjects then received either the control or one of the doses of bradykinin receptor antagonist; the order of treatments was randomly determined. On separate occasions, at least 3 days apart, each subject received vehicle or one of the doses of the antagonist being tested. Two minutes later, bradykinin, 100 $\mu$g was administered to each nostril and 10 min later another lavage was performed and the sample retained for albumin assay.

For both the studies on nasal patency and albumin release the pre-bradykinin challenge treatments administered into the nasal cavity were: saline; Hoe 140, 10, 30, 100 or 200 $\mu$g; [1-adamantane acetyl-D-Arg$^0$, Hyp$^3$, Thi$^{5,8}$, D-Phe]-bradykinin, 33, 50, 100 or 200 $\mu$g; NPC 567, 10 mg.

### *Effect of Hoe 140 on house dust mite-induced reductions in nasal patency*

For the antigen-provocation study, subjects with a history of perennial allergic rhinitis and positive skin prick tests to house dust mite antigen (HDM) were used. The protocol was similar to that for the bradykinin study except that house dust mite antigen, 500 units per nostril was used for the challenge. The dose of antigen was chosen on the basis of pilot experiments and produced approximately 50% of the maximum response to antigen. The subjects were treated with Hoe 140 or saline, each subject receiving saline or one of the doses of Hoe 140 on separate occasions at least 1 week apart. As before, there was 2 min between treatment and challenge. In addition, nasal lavage and acoustic rhinometry were performed in the same study: nasal lavage preceding the pretreatment Amin measurement and coming after the post-challenge Amin measurement. The treatments prior to house dust mite challenge were saline; Hoe 140 10, 33, 100 or 200 $\mu$g.

### *Effect of Hoe 140 on house dust mite-induced kinin generation in the nasal airway*

Using the same protocol, subjects with allergy to house-dust mite antigen were pretreated with saline or Hoe 140, 200 $\mu$g. Each subject received both treatments on separate occasions at least one week apart. The treatment given first was determined randomly. As before, nasal lavages and Amin measurements were taken, but the nasal lavages were assayed for their kinin content. The lavages were collected, EDTA was added to produce a final concentration of 40 mM, and they were stored at −70°C until assay (Proud *et al.*, 1983).

*[$^{125}$I]-Hoe 140 binding*

The tissue used was inferior turbinate bones removed at routine surgery for hypertrophy of the nasal turbinates. Soft tissue was dissected from bone and the bone discarded. The soft tissue was chopped with scissors and then homogenized in buffer A at 4°C using a Polytron blender. Five 10 s bursts of homogenization were performed in homogenization buffer A. The homogenate was centrifuged at 2500 *g* for 10 min at 4°C and the supernatant was then recentrifuged at 50,000 *g* for 10 min at 4°C. The pellet was resuspended and washed twice by alternate centrifugation and resuspension in a volume of 10 ml of buffer A. The pellet was finally resuspended in 5 ml of buffer B and the protein content was determined by the Pierce BCA protein assay. The protein concentration of the preparation was adjusted to 1 mg ml$^{-1}$.

To 100 μl of membrane preparation, [$^{125}$I]-Hoe 140 was added in 50 μl of buffer A and a further 50 μl was added containing either buffer, unlabelled Hoe 140 or displacing drug, depending on the protocol. For the competition and saturation binding studies the reaction mixture was incubated at room temperature (20°C) for 1 h, and then 2 ml of ice-cold buffer A was added and the whole mixture immediately filtered through a Whatman GF/B filter under reduced pressure. The Whatman GF/B filters had been previously treated with polyethyleneimine, 0.1% for 1 h. The filters were washed three times with 3 ml of ice-cold buffer A and then transferred to a gamma spectrometer for assay of the $^{125}$I.

For the association kinetic binding studies, the reaction mixture was membrane preparation, 100 μl; [$^{125}$I]-Hoe 140, 50 μl, (final concentration: 0.2 nM); and 50 μl of buffer or excess unlabelled Hoe 140. This was incubated at 4°C for 0–45 min. The reaction was terminated and the binding counted as described for the competition and saturation studies. The reaction mixture for the dissociation binding studies was the same as for the association studies and, again, the experiment was conducted at 4°C. The mixture was incubated for 1 h to allow equilibrium to be reached, then unlabelled Hoe 140, 1 μM, was added. Aliquots were taken at intervals between 0 and 20 min after the excess unlabelled ligand had been added, the reaction was terminated and the binding measured.

For all the binding studies, specific binding of [$^{125}$I]-Hoe 140 was defined as the binding inhibited by unlabelled Hoe 140, 1 μM.

The composition of the buffers used was based on the work of Trifilieff *et al.* (1994) and was: Buffer A: TES 25 mM, 1,10 phenanthroline 1 mM, bacitracin 140 μg ml$^{-1}$, dithiothreitol 1 mM, captopril 10 μM and bovine serum albumin 0.1%. The pH was 6.8. Buffer B: TES 25 mM and 1,10 phenanthroline 1 mM. The pH was 6.8.

*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 a percentage of the baseline control value. The absolute values for the control measurements have been given with each set of data. Means are given together with s.e.mean. The appropriate, non-parametric statistical test is given with each data set. A value of $P<0.05$ is taken as significant. The data were analysed by non-linear, least squares, curve fitting using CVFIT (courtesy of Prof. D. Colquhoun, Department of Pharmacology, University College London) and Sigma Plot (Jandel). For all the data, each separate experiment was fitted and the values calculated were used to calculate the mean and s.e.mean.

## Materials

[$^{125}$I]-Hoe 140 was synthesized by Hoechst AG, Frankfurt. Iodination of the peptide was carried out by Dr K. Wiemer, Hoechst AG. To iodinate Hoe 140, hydroxyphenyl propionic acid was coupled to the nitrogen of D-arginine in Hoe 140 prior to the iodination reaction. The specific activity of the radiolabel was 1367 mCi mg$^{-1}$ at the post-synthesis analysis. The pharmacological profile of the hydroxyphenyl propionic acid derivative of Hoe 140 is similar to that of Hoe 140 (unpublished). Unlabelled Hoe 140 was synthesized by Hoechst AG, Frankfurt. Bradykinin was obtained from Calbiochem, Nottingham. [DesArg$^9$]-bradykinin, [DesArg$^9$]-icatibant, [1-adamantane acetyl-D-Arg$^0$, Hyp$^3$, Thi$^{5,8}$, D-Phe$^7$]-bradykinin and NPC 567 were supplied by Bachem, Saffron Walden. NPC 17731 and NPC 17761 were kindly supplied by Dr D.J. Kyle, Scios Nova, Sunnyvale. WIN 64338 was kindly supplied by Dr J-P. Maffrand, Sanofi Recherche, Toulouse. House dust mite was obtained from Alleradye, Newark. The bradykinin radioimmunoassay kit was obtained from Peninsula laboratories, St. Helens. Polyethyleneimine, phenanthroline, dithiothreitol, bacitracin and TES (N-tris[hydroxymethyl]methyl-2-aminoethane-sulphonic acid) were obtained from Sigma, Poole. Captopril was obtained from Squibb, Princeton, N.J. Bovine serum albumin was obtained from Calbiochem, Nottingham. Ethylene diamine tetraacetic acid (EDTA) was obtained from BDH, Poole. Radial immunodiffusion assay plates for albumin were purchased from Behring, Marburg. The Pierce BCA protein assay kit was purchased from Rockford, IL. Whatman GF/B filters were obtained from Whatman Ltd, Maidstone.

## Results

*Effects of kinin antagonists on Amin and plasma extravasation*

Figure 1a shows that bradykinin, 100 μg introduced into the nasal airway of normal subjects resulted in a reduction of the minimal cross-sectional area (Amin) of the nasal cavity of 16±5%. Hoe 140 produced a significant dose-related reversal of this action of bradykinin, ($P<0.01$, Friedman test) (Figure 1a). The data were fitted with the Hill equation and the dose of Hoe 140 which produced a 50% reversal of the effect of the dose of bradykinin used was calculated to be 20±7 μg or 15 nmol (mean±s.e.mean). Figure 1b shows that bradykinin, 100 μg, induced release of albumin into the nasal cavity and this effect was inhibited in a dose-related manner by Hoe 140, ($P<0.02$, Friedman test). Again, fitting the data with the Hill equation produced a mean (±s.e.mean) value of 25±2 μg or 19 nmol for the dose which caused 50% reversal of the action of the dose of bradykinin used.

Figure 2 shows the dose-response relationship for the inhibition by Hoe 140 of the reduction in Amin caused by house dust mite antigen challenge in allergic subjects. House dust mite caused a significant reduction of the minimal cross-sectional area (Amin) of the nasal cavity of 22±5%. Hoe 140 significantly reversed this nasal blockage in a dose-related fashion, ($P<0.01$, Friedman test). The dose of Hoe 140 which caused a 50% reversal of the effect of this antigen dose had a mean (±s.e.mean) value of 24±3 μg.

Figure 3 shows the effect of [1-adamantane acetyl-D-Arg$^0$, Hyp$^3$, Thi$^{5,8}$, D-Phe$^7$]-bradykinin on the reduction in Amin induced by bradykinin and on bradykinin-induced albumin release in normal subjects. Figure 3a shows that this antagonist significantly inhibited bradykinin-induced nasal blockage in a dose-related manner ($P<0.01$, Friedman test). Figure 3b shows that bradykinin, 100 μg, induced release of albumin into the nasal cavity and this effect was inhibited in a dose-related manner by [1-adamantane acetyl-D-Arg$^0$, Hyp$^3$, Thi$^{5,8}$, D-Phe$^7$]-bradykinin ($P<0.02$, Friedman test). The doses of [1-adamantane acetyl-D-Arg$^0$, Hyp$^3$, Thi$^{5,8}$, D-Phe$^7$]-bradykinin which caused 50% reversal of the effects of the dose of bradykinin used on Amin or albumin release were 56±9 μg or 38 nmol and 79±7 μg or 54 nmol (mean±s.e.mean) respectively.

NPC 567, 10 mg or 8 μmol failed to inhibit the reduction in Amin induced by bradykinin and the bradykinin-induced

albumin release in normal subjects. With saline pretreatment, the Amin was reduced by bradykinin, 100 μg, to 80.56±2.33% of baseline. With NPC 567 pretreatment, the Amin was reduced by bradykinin to 74.92±1.90% of baseline. These values are not significantly different ($P>0.05$, Wilcoxon sign rank test). The baseline Amin was 0.49±0.01 cm$^2$. With saline pretreatment, the albumin content of the nasal lavage following bradykinin, 100 μg, was 29.27±4.45 mg dl$^{-1}$. With NPC 567 pretreatment, the albumin content of the nasal lavage following bradykinin, 100 μg was 27.18±6.16 mg dl$^{-1}$. These values are not significantly different ($P>0.5$, Wilcoxon sign rank test). For both the Amin and albumin data, the mean± s.e.mean from 9 subjects is given.

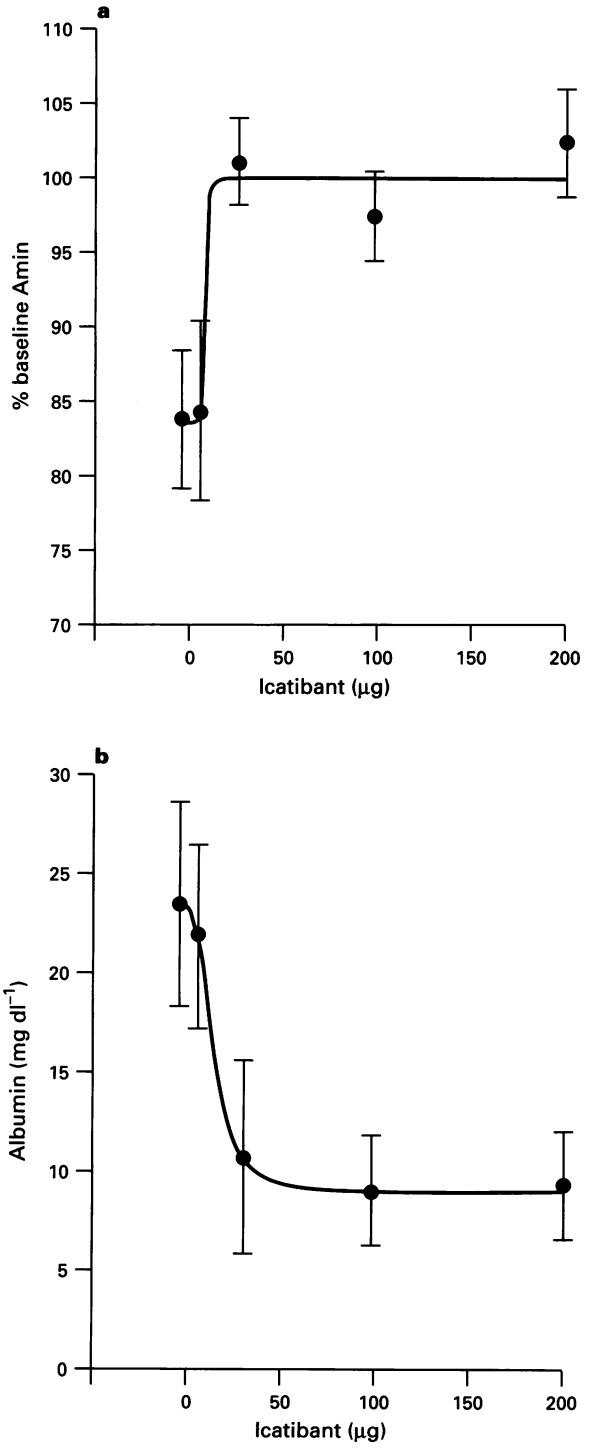


**Figure 1** Dose-response curves for: (a) the effect of Hoe 140 on the reduction of the minimal nasal cross-sectional area (Amin) and (b) albumin extravasation into the nasal cavity induced by bradykinin, 100 μg. Hoe 140 was administered 2 min before bradykinin and Amin was determined or nasal lavage was performed 10 min after bradykinin administration. Data are means±s.e.mean from 5 subjects. Changes in Amin have been normalized by expressing them as a percentage of baseline for each subject and the data have been fitted to the Hill equation. The mean±s.e.mean baseline value for Amin was 0.56±0.02 cm$^2$.

*[$^{125}$I]-Hoe 140 binding*

Figure 4a shows the total, non-specific and specific binding of [$^{125}$I]-Hoe 140 to a membrane preparation from human nasal tissue, as a function of radioligand concentration. The specific binding was found to be saturable and could be accounted for by binding to a single high affinity site over the range of ligand concentrations used (Figure 4b). From the curve fitted, using the Hill equation, to the specific binding versus ligand concentration, the estimates of the equilibrium dissociation constant ($K_d$) of Hoe 140 and of $B_{max}$ were found to be 0.46±0.08 nM and 0.136±0.003 pmol mg$^{-1}$ protein respectively ($n=6$; mean±s.e.mean). The Hill coefficient was calculated to be 1.01±0.07.

The rates of association and dissociation of [$^{125}$I]-Hoe 140 with the binding site, at 4°C, are shown in Figure 5. The dissociation of [$^{125}$I]-Hoe 140 occurred over a time of 20 min with a $t_{½}$ for dissociation of approximately 6.5 min. The mean value for the dissociation rate constant ($k_{-1}$) was 0.14±0.01 min$^{-1}$.

For the association of [$^{125}$I]-Hoe 140 with the binding site, specific binding increased over a time period of 20 min with a $t_{1/2}$ for association of approximately 3.5 min. The mean value for the association rate constant ($k_{+1}$) was 0.20±0.06 min$^{-1}$ nM$^{-1}$. From these kinetic binding studies the equilibrium dissociation constant ($k_{-1}/k_{+1}$) was calculated to be 0.70 nM.

Figure 6 shows the displacement of specific [$^{125}$I]-Hoe 140 binding by unlabelled Hoe 140 itself and by other agonists and antagonists at bradykinin receptors. For these experiments, the

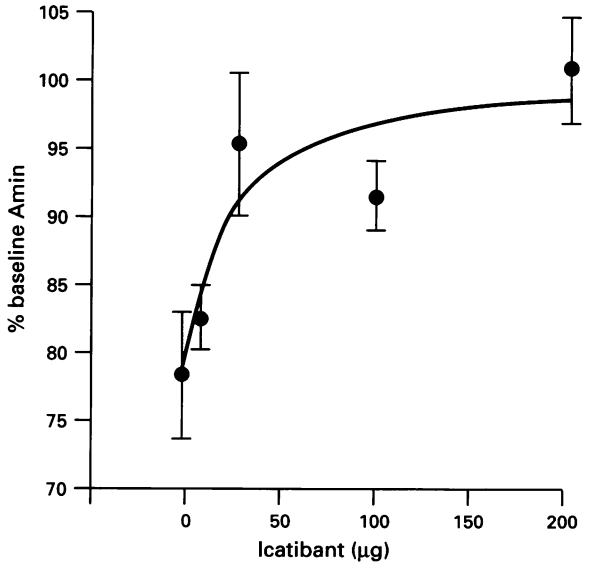


**Figure 2** Dose-response curves for the effect of Hoe 140 on the reduction of the minimal nasal cross-sectional area (Amin) induced by house dust mite, 500 u (HDM). Hoe 140 was administered 2 min before HDM and Amin was measured 10 min after HDM administration. Data are means±s.e.mean from 10 subjects. Changes in Amin have been normalized by expressing them as a percentage of baseline for each subject and the data have been fitted to the Hill equation. The mean±s.e.mean baseline value for Amin was 0.48±0.02 cm$^2$.

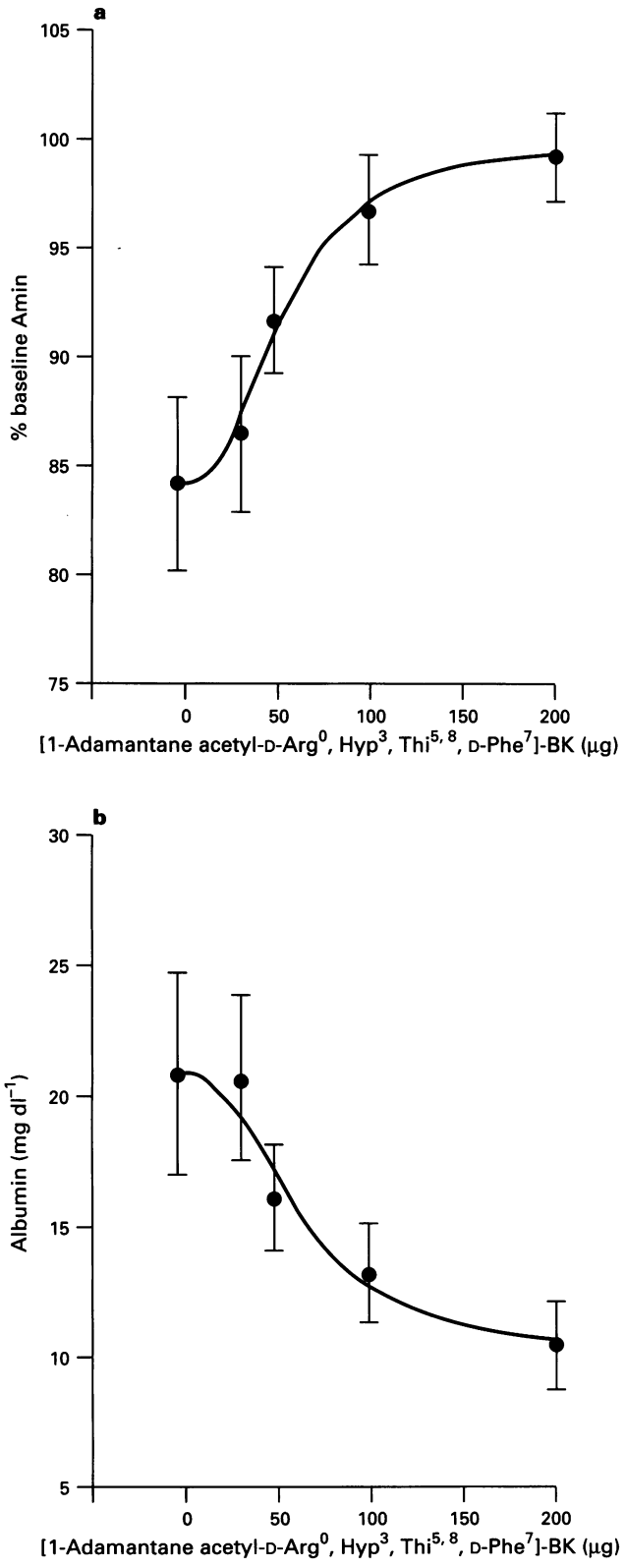


**Figure 3** Dose-response curves for the effect of [1-adamantane acetyl-D-Arg$^0$, Hyp$^3$, Thi$^{5,8}$, D-Phe$^7$]-bradykinin on: (a) the reduction of the minimal nasal cross-sectional area (Amin) and (b) albumin extravasation into the nasal cavity induced by bradykinin, 100 μg. [1-Adamantane acetyl-D-Arg$^0$, Hyp$^3$, Thi$^{5,8}$, D-Phe$^7$]-bradykinin was administered 2 min before bradykinin and Amin was determined or nasal lavage was performed 10 min after bradykinin administration. Data are means ± s.e.mean from 5 subjects. Changes in Amin have been normalized by expressing them as a percentage of baseline for each subject and the data have been fitted to the Hill equation. The mean ± s.e.mean baseline value for Amin was 0.5 ± 0.01 cm$^2$.

concentration of [$^{125}$I]-Hoe 140 was 0.2 nM. The plot shows curves fitted to the data by constraining the Hill coefficient to unity. Table 1 shows the $K_i$ values and the Hill coefficients obtained from these displacement studies by curve fitting without constraints.

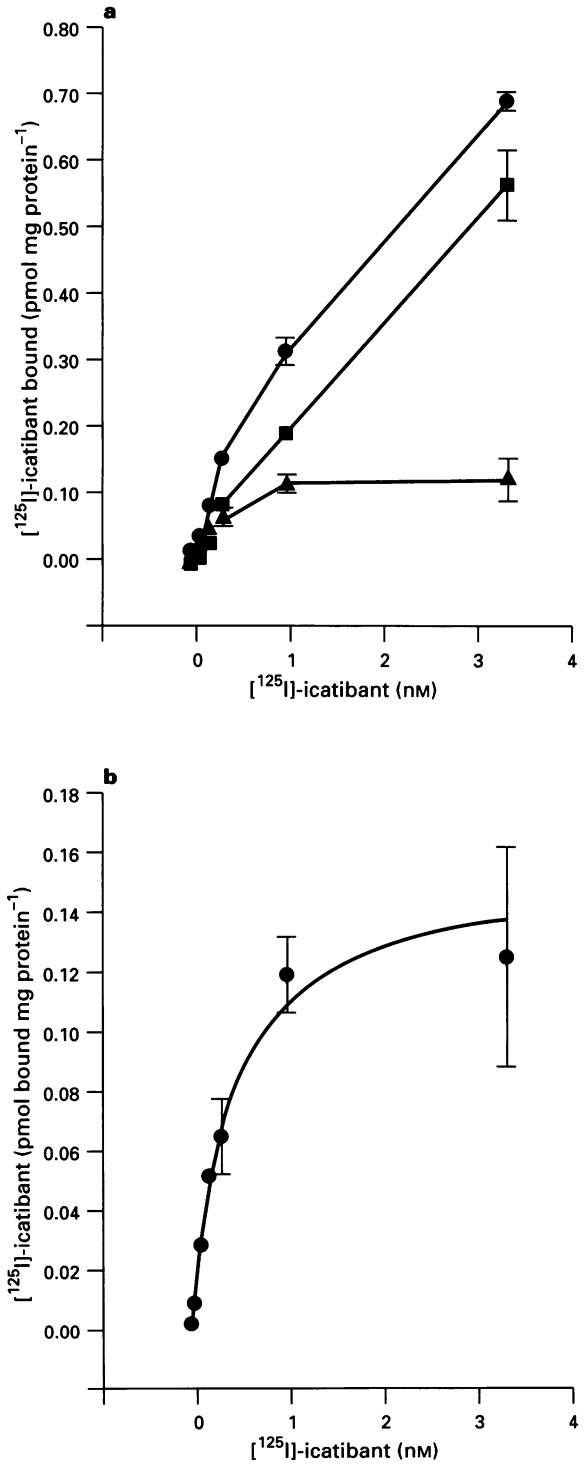


**Figure 4** (a) The total, non-specific and specific binding of [$^{125}$I]-Hoe 140 to human nasal membranes. The binding of [$^{125}$I]-Hoe 140 in the absence and presence of 1 μM unlabelled Hoe 140 corresponds to the total (●) and non-specific binding (■), respectively. Specific binding (▲) is also shown. Data are the means with s.e.mean of 6 membrane preparations. (b) Receptor specific binding of [$^{125}$I]-Hoe 140 to human nasal membranes, defined as that binding displaced by unlabelled Hoe 140, 1 μM. Data are the means with s.e.mean of six membrane preparations. The Hill coefficient has been constrained to 1 in fitting the data with the Hill equation.

To increase the certainty that only one receptor binding site is present in the tissue, binding assays were performed using ten times the labelled ligand concentration than was used in the studies so far described. Unlabelled Hoe 140 and NPC 567 also fully displaced the specific binding of [$^{125}$I]-Hoe 140, 2 nM, to human nasal membranes. The calculated equilibrium dissociation constants, $K_i$, were $0.85 \pm 0.13$ nM and $63.31 \pm 8.88$ nM for unlabelled Hoe 140 and NPC 567, respectively (mean $\pm$ s.e.mean, $n = 3$).

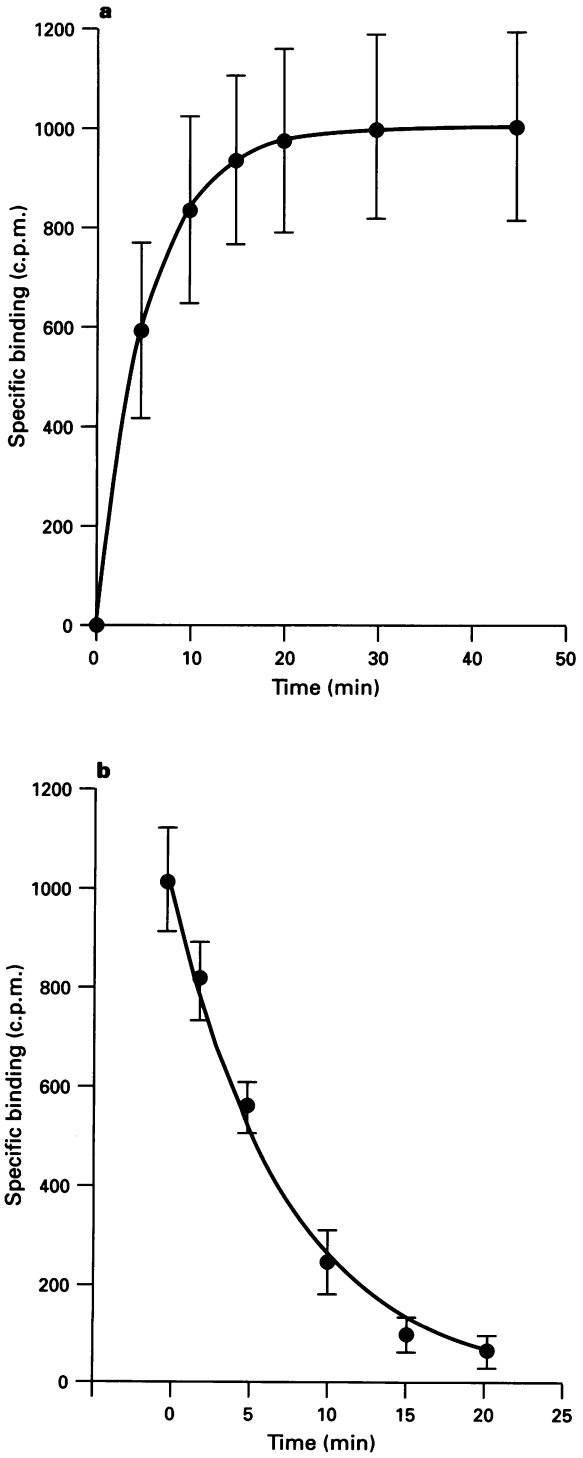


**Figure 5** Rate of association (a) and dissociation (b) of [$^{125}$I]-Hoe 140 to the binding sites on human nasal membranes. The specific binding was determined as described in the Methods section. Data are the means with s.e.mean of 3 experiments. The data have been fitted to a single exponential function.

*Effect of Hoe 140 on bradykinin release into the nasal airway*

Table 2 shows the bradykinin concentrations recovered by nasal lavage and the changes in Amin following house dust mite antigen challenge of subjects with allergic rhinitis. As previously reported, house dust mite antigen challenge significantly reduced the Amin (Austin *et al.*, 1994). It also elevated the bradykinin concentration in nasal lavage fluid. Pretreatment of subjects with Hoe 140, 100 $\mu$g, 2 min before antigen challenge significantly reduced the bradykinin level in nasal lavage fluid and reversed the effect of antigen challenge on Amin.

## Discussion

Over the range of concentrations of [$^{125}$I]-Hoe 140 that we have used, we have demonstrated the presence of a single, saturable binding site in a membrane preparation from the mucosa of human nasal turbinates. The equilibrium dissociation constants for Hoe 140 obtained from the saturation

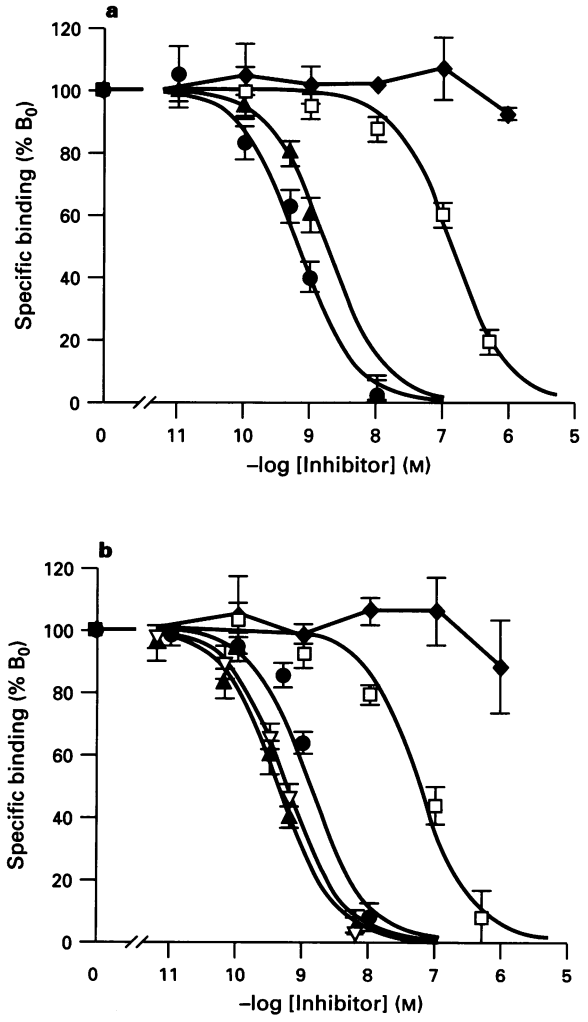


**Figure 6** Competitive inhibition of the specific binding of [$^{125}$I]-Hoe 140 to human nasal membranes. Each point represents the mean with s.e.mean from 3 experiments. The Hill coefficient has been constrained to 1 in fitting the data with the Hill equation. (a) Shows [$^{125}$I]-icatibant being displaced by unlabelled Hoe 140 (●), bradykinin (▲), NPC 567 (□) and [DesArg$^9$]-bradykinin (◆); (b) shows [$^{125}$I]-icatibant being displaced by NPC 17761 (▲), NPC 17731 (▽), [1-adamantane acetyl-D-Arg$^0$, Hyp$^3$, Thi$^{5,8}$, D-Phe$^7$]-bradykinin (●), WIN 64338 (□) and [DesArg$^9$]-Hoe 140 (◆).

**Table 1** Inhibition constants ($K_i$) and Hill ($n_H$) coefficients from the displacement of [$^{125}$I]-Hoe 140 binding to a membrane preparation from human inferior turbinates by selective agonists and antagonists at bradykinin receptors

| *Compound* | $K_i$ (nM) | $n_H$ |
|---|---|---|
| *$B_2$ antagonists* | | |
| NPC 17761 | 0.27±0.02 | 0.96±0.06 |
| Hoe 140 | 0.48±0.02 | 1.06±0.09 |
| NPC 17731 | 0.38±0.02 | 1.13±0.09 |
| [1-Adamantane acetyl-D-Arg$^0$,Hyp$^3$,Thi$^{5,8}$, D-Phe$^7$]-bradykinin | 1.20±0.04 | 1.38±0.07 |
| WIN 64338 | 41.24±8.54 | 0.86±0.03 |
| NPC 567 | 90.47±3.93 | 0.89±0.07 |
| *$B_1/B_2$ agonist* | | |
| Bradykinin | 0.99±0.13 | 1.45±0.05 |
| *$B_1$-selective agonist* | | |
| [Des-Arg$^9$]-bradykinin | >1000 | |
| *$B_1$-selective antagonist* | | |
| [Des-Arg$^9$]-Hoe 140 | >1000 | |

Each value is the mean±s.e.mean from 3 separate experiments.

**Table 2** Effect of Hoe 140 (100 μg) on the reduction of Amin and on bradykinin level in nasal lavage fluid following house dust mite antigen (HDM) challenge of subjects with allergic rhinitis

| | *Amin* (% of baseline) | *Bradykinin in nasal lavage* (pg ml$^{-1}$) |
|---|---|---|
| Baseline | 100 | 25.7±10.6 |
| HDM | 74.5±3.6* | 184.6±46.5* |
| Hoe 140+HDM | 100.9±4.3† | 23.1±9.5† |

The actual value of the baseline Amin was 0.5±0.03 cm$^2$. The values are the means±s.e.mean from 5 subjects. *Indicates a statistically significant difference from the baseline ($P<0.05$, Wilcoxon test); †indicates a statistically significant difference from HDM alone ($P<0.05$, Wilcoxon test).

binding curve at 20°C (0.48 nM) and, independently from the association and dissociation rate constants at 4°C (0.70 nM), are consistent with estimates in human lung of 0.73 nM (Trifilieff *et al.*, 1994) and in guinea-pig ileum of 0.79 nM (Hock *et al.*, 1991).

The presence of multiple [$^{125}$I]-Hoe 140 binding sites on the human nasal membranes cannot be excluded by using only a low concentration of radioligand in displacement studies as there may be high and low affinity binding sites for [$^{125}$I]-Hoe 140, as proposed for guinea-pig lung and brain (Sequin *et al.*, 1992). Under such conditions, a low concentration of [$^{125}$I]-Hoe 140 would bind predominantly to the high affinity site giving rise to a displacement curve fitted by a single-site model (Swillens *et al.*, 1995). In order to exclude the possibility of multiple [$^{125}$I]-Hoe 140 binding sites with $K_D$ values in the nanomolar range, displacement studies were conducted with a high and low concentration of radioligand. The specific binding of [$^{125}$I]-Hoe 140, 2 nM, was fully displaced by unlabelled Hoe 140 and NPC 567. The equilibrium dissociation constants calculated from this displacement study are consistent with the presence of a single kinin receptor of the $B_2$ type.

The $B_1$ selective ligands, [DesArg$^9$]-bradykinin and [DesArg$^9$]-Hoe 140 do not displace the specific binding of [$^{125}$I]-Hoe 140 over the range of concentrations used, indicating that the radioligand does not bind to bradykinin $B_1$ receptors in human nasal membranes. These data are in accord with the observations that $B_1$-selective agonists have no effect on nasal blockage or albumin extravasation in human subjects *in vivo* (Rajakulasingham *et al.*, 1991; Austin & Foreman, 1994a).

The value for the apparent equilibrium dissociation constant for bradykinin which we obtained from binding data was 1 nM and this is similar to the value of 1.1 nM reported for human lung (Trifilieff *et al.*, 1994) but rather higher than the value of 0.3 nM reported for guinea-pig ileum (Scherrer *et al.*, 1995). Multiple binding sites for bradykinin have been reported in the guinea-pig lung, ileum and brain (Trifilieff *et al.*, 1991; Sequin *et al.*, 1992). However, the displacement of [$^{125}$I]-Hoe 140 from the human nasal membranes by bradykinin is consistent with the presence of a single binding site. It should also be mentioned that, with the exception of the studies reported in this paper and the determination of the $K_i$ for [$^3$H]-NPC 17731 in the guinea-pig ileum, the $K_i$ values determined in other tissues and cited in this discussion have been obtained from binding studies with the labelled agonist, bradykinin and not with a labelled antagonist. Binding studies with agonists may involve the binding of the ligand to different activation states of the receptor and in this case only an apparent equilibrium dissociation constant is obtained.

NPC 567 is the least potent of the $B_2$ receptor antagonists that we used, giving a $K_i$ value of 90 nM. In human lung, a higher value of 270 nM was obtained (Trifilieff *et al.*, 1994). The value for guinea-pig ileum has been reported as varying from 8 to 210 nM (Farmer *et al.*, 1989; 1991; Lyon *et al.*, 1990; Sequin *et al.*, 1992). The nonpeptide kinin $B_2$ receptor antagonist, WIN 64338, exhibited a $K_i$ of 41 nM in our studies on human nasal membranes which is comparable with a value of 64 nM in human fibroblasts (Sawutz *et al.*, 1994) and 34 nM in the guinea-pig ileum (Scherrer *et al.*, 1995). In guinea-pig trachea, it is claimed that both NPC 567 and WIN 64338 fail to antagonize bradykinin-induced contractions and have equilibrium dissociation constants of more than 100 μM and 1 μM respectively, which is partly the basis for the claim that a $B_3$ receptor exists in this guinea-pig tissue (Farmer *et al.*, 1989; Farmer & DeSiato, 1994). However, the equilibrium dissociation constants of NPC 567 and WIN 64338 reported by Farmer and his colleagues in guinea-pig trachea have been challenged. It has been reported that NPC 567 antagonizes bradykinin-induced contraction of the guinea pig trachea and fully displaces, in a two binding site model, the specific binding of [$^3$H]-bradykinin to the guinea-pig airways (Trilifilieff *et al.*, 1991; Pruneau *et al.*, 1995). Scherrer *et al.* (1995) have obtained a value of 50 nM for the $K_i$ of WIN 64338 and shown that the antagonism of bradykinin-induced contraction of the guinea pig trachea was non-competitive. Farmer *et al.* (1989) reported that another kinin $B_2$ receptor antagonist, NPC 349, inhibited only weakly, bradykinin-induced bronchoconstriction in the guinea pig *in vivo* and cited this as further evidence for the presence of a $B_3$ receptor. However, another study reported that this antagonist abolishes the bronchoconstrictor effect of bradykinin in the guinea-pig lower airways (Jin *et al.*, 1989). In our studies, both NPC 567 and WIN 64338 fully displaced

icatibant binding with a $K_i$ value consistent with a $B_2$ receptor. NPC 17731 had a $K_i$ of 0.4 nM in our human nasal membranes which is, interestingly, similar to the value reported for guinea-pig trachea of 0.5 nM (Trifilieff *et al.*, 1993) where the possible existence of a $B_3$ receptor has been suggested. The $K_i$ for [$^3$H]-NPC 17731 in the guinea-pig ileum has been reported to be 0.1 nM (Burch *et al.*, 1994). Our value for the $K_i$ of NPC 17761 in human nasal membranes was 0.3 nM which is nearly an order of magnitude greater than the value of 0.05 nM reported for guinea-pig trachea (Trifilieff *et al.*, 1993). The $K_i$ values from our data are generally in good agreement with values published for other human tissues but there are some discrepancies with values in guinea-pig ileum and, more particularly, with the values from guinea-pig trachea where the existence of a $B_3$ receptor has been proposed. Our data are consistent with the receptor in the human nose being a $B_2$ receptor.

The *in vivo* functional studies on nasal blockage, as measured by changes in Amin, and on nasal plasma extravasation, as measured by albumin release, generate limited data because the experiments are time-consuming for volunteers and it is unreasonable to cause large changes in these parameters with high doses of bradykinin or antigen. Nevertheless, the data presented in this paper show that it is possible to provide estimates of the dose-response curves for the effect of two bradykinin receptor antagonists on changes in Amin or albumin leakage induced by either bradykinin or antigen. From fitted antagonist dose-response curves, estimates of the doses of antagonist required to reduce the response to a given dose of antigen or bradykinin by 50% have been determined. Clearly, these doses which produce a 50% inhibition will depend on the dose of agonist and are, therefore, only of use in calculating relative activities. For the antagonism of bradykinin-induced changes in nasal patency (Amin), Hoe 140 was 2.5 times more potent on a molar basis than [1-adamantane acetyl-D-Arg$^0$, Hyp$^3$, Thi$^{5,8}$, D-Phe$^7$]-bradykinin, and for the antagonism of bradykinin-induced albumin extravasation Hoe 140 it was 2.8 times more potent on a molar basis than [1-adamantane acetyl-D-Arg$^0$, Hyp$^3$, Thi$^{5,8}$, D-Phe$^7$]-bradykinin. From the binding data, Hoe 140 was 2.5 times more potent than [1-adamantane acetyl-D-Arg$^0$, Hyp$^3$, Thi$^{5,8}$, D-Phe$^7$]-bradykinin. Thus, there is good agreement between the functional relative activities *in vivo* and the relative activities from the binding data for these two compounds. NPC 567 has previously been shown not to block the nasal actions of bradykinin at a dose of 1 mg (Pongracic *et al.*, 1991; Austin & Foreman, 1994a) and in this study, we have failed to detect an effect at 10 mg. Taking 20 $\mu$g or 15 nmol as the dose of Hoe 140 producing 50% inhibition of the effect of bradykinin on Amin, the equivalent dose of NPC 567, based on relative potencies from the binding data, would be about 3 $\mu$mol or 4 mg. We might, therefore, have expected to see an effect with NPC 567 at a dose of 10 mg but this assumes that access of both Hoe 140 and NPC 567 to the receptor are the same. They may, of course, differ in their susceptibility to metabolism or in their ability to diffuse to the receptor. Furthermore, *in vivo* studies are unlikely to represent equilibrium conditions. Use of higher doses of NPC 567 *in vivo* is precluded by cost.

An interesting aspect of this study is the finding that Hoe 140 reduces the levels of bradykinin in the nasal airway following antigen challenge of allergic subjects. There is some evidence that Hoe 140 inhibits kallikrein in an animal model (Dr K. Wirth, unpublished) but it was less potent in this respect than as a $B_2$ receptor antagonist. Our data suggest that *in vivo*, Hoe 140 may inhibit kallikrein as well as blocking the $B_2$ receptor and that both of these effects may contribute to the reversal by Hoe 140 of the antigen-induced nasal blockage in subjects with allergic rhinitis. As previously reported (Austin *et al.*, 1994), antigen challenge of subjects with house dust mite allergic rhinitis does not result in albumin leakage into the nasal cavity, despite the fact that bradykinin does, in normal subjects, cause albumin extravasation and Hoe 140 inhibits house dust mite-induced nasal blockage. It is unlikely, therefore, that the action of Hoe 140 on bradykinin release into the nasal airway, following antigen challenge, results from blockage of a $B_2$ receptor-mediated increase in vascular permeability and a consequent fall in the supply of plasma kininogen or kallikrein.

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