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Involvement of bradykinin B_1 and B_2 receptors in pulmonary leukocyte accumulation induced by Sephadex beads in guinea pigs

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Abstract

The effects of selected bradykinin receptor antagonists on leukocyte infiltration into the lungs were studied in a model of guinea pig lung inflammation induced by the intravenous injection of Sephadex beads. The bradykinin B_1 receptor antagonist, [Leu^8]desArg^9-BK (40 mg kg^{-1} 24 h^{-1}) and the bradykinin B_2 receptor antagonist, DArg[Hyp^3,Thi^5,DTic^7,Oic^8]BK (code name HOE 140; 4 mg kg^{-1} 24 h^{-1}), administered intravenously by osmotic pumps, significantly reduced eosinophil counts by 33% and 42% in bronchoalveolar fluid, respectively. HOE 140 decreased neutrophil counts by 35%. LysLys[Hyp^3,Igl^5,D-Igl^7,Oic^8]desArg^{9}BK (code name B 9858), a newly described bradykinin B_1 receptor antagonist, administered intraperitoneally (1 mg kg^{-1}), decreased eosinophil and neutrophil counts by 45% in bronchoalveolar fluid. D-Arg[Hyp^3,Igl^5,D-Igl^7,Oic^8]BK (code name B 9430), a non-selective bradykinin B_1/B_2 receptor antagonist, also administered intraperitoneally (1 mg kg^{-1}), decreased eosinophil and macrophage counts by 62% and 80% in bronchoalveolar fluid. These results suggest that bradykinin B_1 and B_2 receptors are involved in leukocyte recruitment in our model of lung inflammation.

Keywords: Sephadex; Leukocyte; Kinin; Bradykinin B_1 receptor; Bradykinin B_2 receptor; Antagonist

1. Introduction

Kinins were reported to induce arteriolar dilation, venoconstriction, plasma protein extravasation (Ichinose and Barnes, 1990; Rogers et al., 1990; Pedersen et al., 1991; Featherstone et al., 1996), smooth muscle contraction (Farmer, 1991), the release of inflammatory mediators such as cytokines (Koyama et al., 1995; Paegelow et al., 1995) and metabolites of arachidonic acid (Churchill et al., 1989; Farmer, 1991). In addition, kinins enhanced mucus secretion, edema and inflammatory cell infiltrate into the airways, all of which are main features of asthma. Bradykinin was shown to be a potent bronchoconstrictor in asthmatics but was essentially inactive in non-asthmatics (Fuller et al., 1987; Polosa and Holgate, 1990). Furthermore, elevated kinin levels were detected in plasma of asthmatics (Christiansen et al., 1987), and Poblete et al. (1993) demonstrated the presence of tissue kallikrein in seromucous glands of human bronchi and guinea pig respiratory tree. These data as well as their numerous effects on lower and upper airways (Farmer, 1991; Barnes, 1992) strongly suggested that kinins were involved in the pathogenesis of asthma.

Animals studies showed that the direct and indirect airway actions of kinins were mediated by two subtypes of receptors classified as B_1 and B_2 (Regoli and Barabé, 1980). Bradykinin B_2 and B_1 receptors have recently been cloned and sequenced in the human genome (Hess et al., 1992; Bachvarov et al., 1996). The bradykinin B_1 receptors have weak affinity for intact bradykinin but strong affinity for kinin metabolites without the C-terminal arginine. The bradykinin B_1 receptor has a special interest because of its apparent up-regulation following tissue injury and inflammation (Marceau, 1995). At this time, little is known about the role of bradykinin B_1 receptor subtype, if any, in human airway diseases.

In the present investigation, the effects of a potent bradykinin B_2 receptor antagonist, DArg[Hyp^3,Thi^5,DTic^7,Oic^8]BK (HOE 140; Wirth et al., 1991), two bradykinin B_1 receptor antagonists, [Leu^8]desArg^9-BK (Regoli and Barabé, 1980) and LysLys[Hyp^3,Igl^5,D-Igl^7,Oic^8]desArg^9-

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BK (code name B 9858; Stewart et al., 1996) and a non-selective bradykinin B_1/B_2 receptor antagonist, D-Arg[Hyp^3,Igl^5,D-Igl^7,Oic^8]BK (code name B 9430; Stewart et al., 1996) were studied in a model of lung inflammation induced by the intravenous injection of Sephadex beads (Maghni et al., 1991). This animal model of lung inflammation was characterized by a massive airway eosinophilia and increased bronchial hyperreactivity (Maghni et al., 1996), as seen in human asthma. By mean of these potent antagonists, the present investigation was aimed at understanding the contribution of both bradykinin B_1 and B_2 receptors in the polymorphonuclear leukocyte mobilization in inflammed guinea pig airways.

2. Materials and methods

2.1. Materials

The following compounds were used: Sephadex beads G50 (Superfine, Pharmacia); [Leu^8]desArg^9-BK (prepared in our laboratory with the procedure described by Drapeau and Regoli, 1988); DArg[Hyp^3,Thi^5,DTic^7,Oic^8]BK (HOE 140, generous gift from Dr. B.A. Schölkens, Frankfurt, Germany); LysLys[Hyp^3,Igl^5,D-Igl^7,Oic^8]desArg^{9}BK (B 9858) and D-Arg[Hyp^3,Igl^5,D-Igl^7,Oic^8]BK (B 9430), generous gifts from Dr. J.M. Stewart (Denver, CO).

2.2. Induction of leukocyte infiltration in guinea pigs lungs by Sephadex beads

Leukocytes infiltration was induced by an intravenous injection of Sephadex beads in male Dunkin Hartley guinea pigs (300–350 g), as previously described by Maghni et al. (1993). Briefly, 0.20 ml of a sterile suspension of Sephadex beads (40 mg ml^{-1}) G-50 (Superfine) was injected into the ear vein of conscious guinea pigs after local anesthesia with 2% xylocaine. Control animals received the same volume of sterile saline. In all experiments, the animals were killed by cervical dislocation 24 h after Sephadex injection and exsanguinated by cutting the inferior vena cava. We have previously demonstrated that lung eosinophilia and neutrophilia were maximal 24 h after Sephadex injection (Maghni et al., 1996). In the first series of experiments, a group of Sephadex-injected animals were treated with HOE 140 (bradykinin B_2 receptor antagonist) or with [Leu^8]desArg^9-BK (bradykinin B_1 receptor antagonist). In the second series of experiments, Sephadex-injected animals were treated with B 9858 (bradykinin B_1 receptor antagonist). In the third series of experiments, Sephadex-injected animals were treated with B 9430 (non-selective bradykinin B_1/B_2 receptor antagonist). In each series of experiments, an appropriate group of Sephadex-injected animals was done for the statistical comparison.

2.3. Administration of bradykinin B_1 and B_2 receptor antagonists

In the first series of experiments, animals were anesthetized with ketamine/xylazine to allow the dorsal implantation of osmotic pumps (ALZET Osmotic Pumps, model no 2001D, ALZA, CA) filled with the bradykinin B_2 receptor antagonist HOE 140 (4 mg kg^{-1} 24 h^{-1}) or with the bradykinin B_1 receptor antagonist [Leu^8]desArg^9-BK (40 mg kg^{-1} 24 h^{-1}). HOE 140 has been widely used in in vivo studies in guinea pigs. Wirth et al. (1993) demonstrated that HOE 140 is highly potent at inhibiting bronchoconstriction induced by intravenous bradykinin in guinea pig (ID_{50} 13.4 pmol kg^{-1}). In our study, we assumed that the intravenous infusion of 4 mg kg^{-1} 24 h^{-1} of HOE 140 was largely sufficient to block the responses to endogenous kinins. [Leu^8]desArg^9-BK, the first generation of bradykinin B_1 receptor antagonist, was shown to be rapidly degraded in vivo compared to HOE 140, and for this reason, an intravenous infusion via osmotic pump was used to effectively antagonize bradykinin B_1 receptor. Farmer et al. (1992) have previously demonstrated that [Leu^8]desArg^9-BK (400 μg kg^{-1}, i.v. plus 600 μg kg^{-1}, s.c.), inhibited leukocyte migration in antigen-induced guinea pig airway inflammation. In our study, we used an intravenous infusion of 40 mg kg^{-1} 24 h^{-1} of [Leu^8]desArg^9-BK, which is largely sufficient to block the responses. The jugular vein was canulated with a catheter connected to the implanted pumps and drugs were delivered intravenously for 24 h, at a rate of 8 μl h^{-1}. Control and Sephadex-injected animals received an intravenous infusion of saline. After the implantation of the osmotic pumps, conscious animals were injected with Sephadex beads or with sterile saline.

In second and third series of experiments, a potent and long-acting bradykinin B_1 receptor antagonist, B 9858 (1 mg kg^{-1}) and a non-selective bradykinin B_1/B_2 receptor antagonist, B 9430 (1 mg kg^{-1}) were administered intraperitonally 1 h after the injection of Sephadex, respectively. All doses and route of administration were selected according to the in vitro and in vivo antagonistic potencies (Marceau et al., 1984), as well as on the metabolic stability of these antagonists (Wirth et al., 1991; Gobeil et al., 1995, 1999; Stewart et al., 1996). At these doses, assumption was made that a full blockade of responses to endogenous kinins should be obtained.

2.4. Total and differential cell counts in bronchoalveolar lavage fluid

Immediately after killing the animals, the trachea was canulated with a catheter joined by a three-way stopcock to 50 ml syringes. A total of 50 ml of phosphate-buffered saline (pH 7.4, 37°C) was delivered into the lungs in 10 ml aliquots and reaspirated after gentle massage of the thorax.

The first 10 ml of bronchoalveolar fluid were collected and centrifuged ($360 \times g$, 10 min, 20°C), and the supernatant was divided in aliquots (1 ml) and frozen at −80°C for further use. Total cells present in the bronchoalveolar fluid were counted in a Neubauer chamber, and the cell viability was simultaneously assessed by the Trypan blue dye exclusion test. Differential cell counts were done from duplicate cytospin smears of the original total cell suspensions using Wright's stain.

2.5. Measurement of eosinophil peroxidase activity

Eosinophil peroxidase activity was determined in the bronchoalveolar lavage fluid of control and Sephadex-injected guinea pigs with a colorimetric assay. In brief, *o*-phenylenediamine dihydrochloride, a specific substrate of eosinophil peroxidase not hydrolysed by the myeloperoxydase, was used as previously described by Strath et al. (1985). Fifty microliter of each sample were mixed with 75 μl of substrate solution, containing 0.1 mM *o*-phenylenediamine dihydrochloride (Sigma, St. Louis, USA) in 0.05 M Tris–HCl with 0.1% Triton X-100 and 1 mM hydrogen peroxide (Sigma) was added. The reaction was stopped 5 min later by addition of 50 μl H_2SO_4 (4 M) and the absorbance was determined at the wavelenght of 414 nm.

2.6. Data and statistical analysis

Results were analyzed with the Student's *t*-test for unpaired data. A P value lower than 0.05 was considered to be significant. $P < 0.05$ and $P < 0.01$ were marked with one or two asterisks, respectively.

3. Results

3.1. Effects of [Leu^8]desArg^9-BK and HOE 140 on leukocyte counts of Sephadex-injected guinea pigs

A two-fold increase (from 31.45 to 60.33×10^6 cells) in total cell number recovered in bronchoalveolar fluid was observed 24 h after the injection of Sephadex beads and the sham implantation of osmotic pumps into guinea pigs. Differential counts of bronchoalveolar fluid cells after Wright's staining of cytospin smears showed that eosinophil and neutrophil numbers increased by four (from 4.36 to 19.32×10^6 cells) and 17 (from 0.64 to 11.01×10^6 cells) fold, respectively, following Sephadex injection (data not shown). Bronchoalveolar lavage fluid of control guinea pigs contained 16% polymorphonuclear cells (eosinophils and neutrophils) and 84% mononuclear cells (macrophages) compared to 49% and 51%, respectively, in bronchoalveolar lavage fluid of Sephadex-injected animals. The bradykinin B_1 receptor antagonist, [Leu^8]desArg^9-BK, administered intravenously by osmotic pump, significantly reduced eosinophil counts by 33% (from 14.96 to 10.06×10^6 cells) in bronchoalveolar lavage fluid from Sephadex-injected animals (control; $P < 0.05$, Fig. 1). The bradykinin B_2 receptor antagonist, HOE 140, also administered intravenously by osmotic pump, reduced eosinophil counts of Sephadex-injected animals by 42% (from 14.96 to 8.65×10^6 cells) (control; $P < 0.01$, Fig. 1) and neutrophil counts by 35% (from 10.37 to 6.79×10^6 cells) (control; $P < 0.05$, Fig. 1).

As shown in Fig. 3a, eosinophil peroxidase activity measured in bronchoalveolar lavage fluid as a marker of number of eosinophils, increased by three fold 24 h after the injection of Sephadex in comparison to the control levels (control; $P < 0.01$, Fig. 3a). This increase in eosinophil peroxidase activity in Sephadex-treated guinea pigs corresponded to the increase of eosinophils (from 4.36 to 19.32×10^6 cells) recovered in bronchoalveolar lavage fluid 24 h after the injection of Sephadex beads. The eosinophil peroxidase activity recovered in the bronchoalveolar lavage fluid of guinea pigs treated with [Leu^8]desArg^9-BK or HOE 140, decreased by 49% and 38%, respectively, in comparison with the levels found in bronchoalveolar lavage fluid of Sephadex-injected animals (Fig. 3a). The decreases in eosinophil peroxidase activity corresponded to the diminution in eosinophil counts recovered in bronchoalveolar lavage fluid of Sephadex-injected animals treated with bradykinin B_1 and B_2 receptor antagonists (Fig. 1).

3.2. Effects of B 9858 on leukocyte counts of Sephadex-injected guinea pigs

Cytospin smears of bronchoalveolar cells from second group of Sephadex-injected guinea pigs showed that eosinophil, neutrophil and macrophage numbers increased by seven (from 4.36 to 31.7×10^6 cells), 37 (from 0.64 to 23.91×10^6 cells) and two (from 26.45 to 54.51×10^6

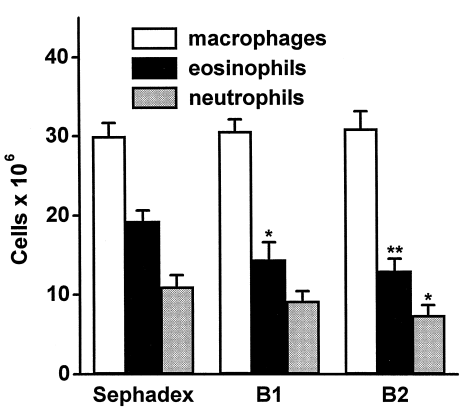

Fig. 1. Comparison of the effects of bradykinin B_1 receptor blockade using [Leu]^{8}desArg^9-BK (B1, 40 mg kg^{-1} 24 h^{-1}, i.v., $n = 12$) and bradykinin B_2 receptor blockade using HOE 140 (B2, 4 mg kg^{-1} 24 h^{-1}, i.v., $n = 10$) on Sephadex-induced leukocyte migration. Both antagonists were administered by osmotic pumps, at a rate of 8 μl per hour for 24 h. Leukocyte population ($\times 10^6$) recovered in the bronchoalveolar lavage fluid was measured 24 h after the injection of Sephadex beads. Statistical significance is shown (*$P < 0.05$; **$P < 0.01$ in comparison with the Sephadex-treated group (24 mg kg^{-1}, i.v., $n = 15$).

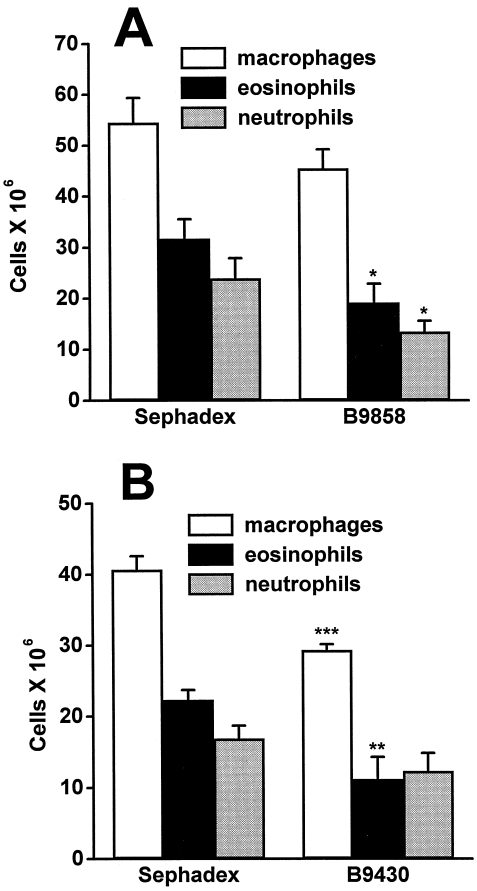

Fig. 2. Effects of bradykinin B_1 receptor blockade with B 9858 (B9858, 1 mg kg^{-1}, i.p., $n=6$) (A) and of a non-selective bradykinin B_1/B_2 receptor antagonist B 9430 (1 mg kg^{-1}, i.p., $n=5$) (B) on Sephadex-induced leukocyte migration. Both antagonists were administered 1 h after the Sephadex injection. The cell population ($\times 10^6$) recovered in the bronchoalveolar lavage fluid was measured 24 h after the injection of Sephadex beads. Statistical significance is shown ($^*P<0.05$; $^{**}P<0.01$; $^{***}P<0.001$ in comparison with their respective group of Sephadex-treated animals (24 mg kg^{-1}, i.v., $n=4$ to 6).

cells) fold, respectively, following Sephadex injection (data not shown). The bradykinin B_1 receptor antagonist, B 9858 (1 mg kg^{-1}, i.p.), reduced by 45% eosinophil (from 27.34 to 15.00×10^6 cells) and neutrophil (from 23.27 to 12.78 $\times10^6$ cells) counts of Sephadex-injected animals ($P<0.05$, Fig. 2a). B 9858 reduced macrophages counts by 32%, but this diminution is not statistically significant. As shown in Fig. 3b, eosinophil peroxidase activity measured in bronchoalveolar fluid decreased by 61% in guinea pigs treated with B 9858 ($P<0.05$). The decrement in eosinophil peroxidase activity corresponded to the decreased number of eosinophils recovered in bronchoalveolar fluid (Fig. 2a).

3.3. Effects of B 9430 on leukocyte counts in bronchoalveolar fluid of Sephadex-injected guinea pigs

Cytospin smears of bronchoalveolar lavage cells from the third group showed that eosinophil, neutrophil and macrophage numbers increased by five (from 4.36 to 22.30×10^6 cells), 26 (from 0.64 to 16.89×10^6 cells) and 1.5 (from 26.45 to 40.68×10^6 cells) fold, respectively, following Sephadex injection (data not shown). The non-selective bradykinin B_1/B_2 receptor antagonist, B 9430 (1 mg kg^{-1}, i.p.), reduced eosinophil counts by 62% (from 17.94 to 6.85×10^6 cells) ($P<0.01$, Fig. 2b) and macrophage counts by 80% (from 14.23 to 2.84×10^6 cells) ($P<0.001$, Fig. 2b) of Sephadex-injected animals. B 9430 reduced neutrophil counts by 28%, but this diminu-

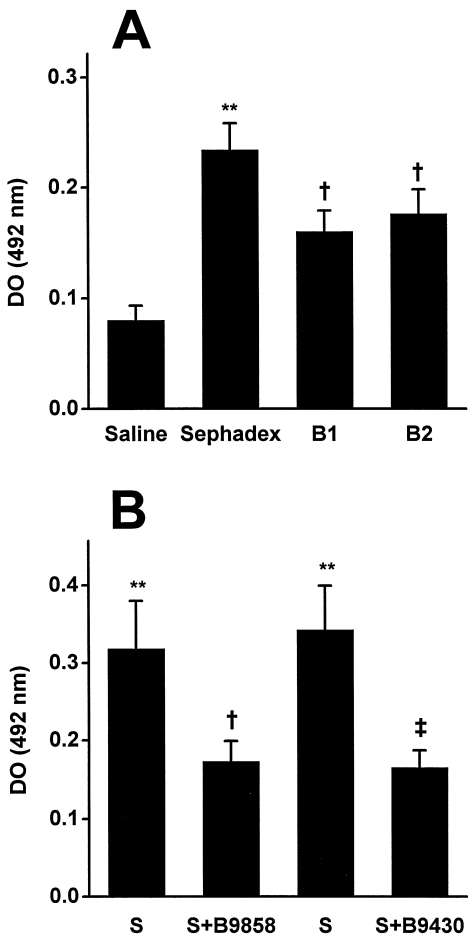

Fig. 3. Eosinophil peroxydase activity measured in bronchoalveolar lavage fluid of Sephadex-injected guinea pigs (Sephadex, 24 mg kg^{-1}, i.v., $n=15$) treated with the bradykinin B_1 receptor antagonist, (B1, [Leu]^{8}des-Arg^9-BK, 40 mg kg^{-1} 24 h^{-1}, i.v., $n=12$) or the bradykinin B_2 receptor antagonist, (B2, HOE 140, 4 mg kg^{-1} 24 h^{-1}, i.v., $n=10$). Both antagonists were administered intravenously with osmotic pumps (A). Statistical significance is shown ($^{**}P<0.01$ in comparison to controls saline (saline, 200 μl, i.v., $n=13$); $\dagger P<0.05$ in comparison with Sephadex (Sephadex, 24 mg kg^{-1}, i.v., $n=15$) treated animals). Eosinophil peroxydase activity was also measured in bronchoalveolar lavage fluid of guinea pigs treated with the potent and long-acting bradykinin B_1 receptor antagonist, B 9858 (S+B9858, 1 mg kg^{-1}, i.p., $n=6$) or with a non-selective bradykinin B_1/B_2 receptor antagonist, B 9430 (S+B9430, 1 mg kg^{-1}, i.p., $n=5$). Both antagonists were administered intraperitonally (B). Statistical significance is shown ($^{**}P<0.01$ in comparison with controls (A); $\dagger P<0.05$ in comparison with their respective group of Sephadex-treated animals (24 mg kg^{-1}, i.v., $n=4$ to 6).

tion is not statistically significant. As shown in Fig. 3b, eosinophil peroxidase activity measured in the bronchoalveolar lavage fluid decreased by 68% in guinea pigs treated with B 9430 ($P < 0.01$). The decrement again corresponded to the decreased number of eosinophils recovered in bronchoalveolar fluid 24 h after the injection of Sephadex beads (Fig. 2b).

4. Discussion

These results showed that the administration of bradykinin B_1 and B_2 receptor antagonists inhibited leukocyte recruitment into the lungs of guinea pig injected intravenously with Sephadex beads. A marked eosinophil infiltrate is indeed a key feature of this pulmonary inflammation model (Maghni et al., 1996; present study). Walls and Beeson (1972) were the first to described an eosinophilia induced by the injection of Sephadex beads in rat. The mechanism responsible for the leukocyte recruitment in this model of lung inflammation are very complex and are not well established. However, Sorden et al. (1990) demonstrated that the Sephadex beads injected intravenously in rats were trapped initially in small caliber muscular pulmonary arteries associated with terminal bronchioles and led to macrophage and multinucleated cell accumulation and to inflammatory cell responses in arteries immediately surrounding partially degraded Sephadex beads. Our previous study suggested that the induction of lung eosinophilia one day after the injection of Sephadex beads was probably not associated with an antigen–antibody type reaction and we have demonstrated that the blood complement is activated as early as 1 h after the Sephadex bead injection into guinea pigs (Blain et al., 1995).

The influx of eosinophils, neutrophils and macrophages into the airways was observed 24 h following the injection of the beads in Sephadex-injected animals (Figs. 1 and 2). Concomitant with this marked eosinophil influx, an increase in eosinophil peroxidase activity was also observed 24 h after the injection of the beads which suggested that the bronchoalveolar fluid eosinophils were activated (Fig. 3). This is in agreement with our previous findings (Maghni et al., 1996). Macrophage which constitute 84% of total cell counts in the bronchoalveolar lavage fluid of control animals were decreased to 50% of total cells after the induction of lung inflammation. It is important to note that total macrophage counts were increased in Sephadex-treated animals although the increases were less marked than those of other cell populations. Macrophages are resident cells present in large numbers in normal airways and are capable of releasing an array of pro-inflammatory products, such as MIP-1α (Macrophage Inflammatory Protein-1α), a chemotactic factor for eosinophils (Rot et al., 1992) and could be in part responsible for the recruitment of other inflammatory cells, such as eosinophils.

Using the model of lung inflammation described above, we have examined the possible roles of kinins in leukocyte migration into lungs of guinea pigs injected with Sephadex beads. In our study, selective bradykinin B_1 ([Leu^8]desArg^9-BK, B 9858) and B_2 (HOE 140) receptor antagonists were used to assess the physiological role played by endogenous kinins. The bradykinin B_2 receptor antagonist, HOE 140 (4 mg kg^{-1} 24 h^{-1}, i.v.), decreased eosinophil counts by 42% and neutrophil counts by 35% in bronchoalveolar lavage fluid of Sephadex-injected guinea pigs (Fig. 1). Concomitant with the decrease in eosinophil counts, HOE 140 decreased by 38% eosinophil peroxidase activity in bronchoalveolar lavage fluid (Fig. 3a). The inhibition of eosinophil and neutrophil migration by HOE 140 suggested that endogenous bradykinin formed in response to Sephadex injection, was involved in leukocyte migration into lungs and may play a significant role in maintaining inflammation in guinea pig airways.

B 9858 was previously shown to be a potent and long-acting bradykinin B_1 receptor antagonist in various in vivo assays (Stewart et al., 1996). In vitro functionnal studies revealed that B 9430 was a potent B_2 antagonist on both the rat uterus and guinea pig ileum and possessed a B_1 antagonist activity on the rabbit aorta (Stewart et al., 1996). In a model of dog blood pressure, B 9430 was found to have an equivalent potency to that of HOE 140 for the bradykinin B_2 receptor and to that of Lys-[Leu^8]desArg^9-BK for the bradykinin B_1 receptor. Stewart et al. (1996) demonstrated that B 9430 given at the subcutaneous dose of 30 μg kg^{-1} inhibited the hypotensive response of bradykinin within 5 min and the effect lasted for at least 24 h in the rabbit.

Our results are similar to those reported previously by Farmer et al. (1992) who have demonstrated the ability of D-Arg-[Hyp^3,Thi^{5,8},D-Phe^7]-BK (code name NPC 349), a bradykinin B_2 receptor antagonist, to inhibit antigen-induced eosinophilia and neutrophilia in bronchoalveolar lavage fluid of sensitized guinea pigs. Other bradykinin B_2 receptor antagonists were shown to have beneficial effects in selected animal models of asthma. For example, pretreatment of sheeps with a natural hypersensitivity to *Ascaris suum* with D-Arg-[Hyp^3,D-Phe^7]-BK (code name NPC 567), a bradykinin B_2 receptor antagonist, abolished the hyperresponsiveness and reduced the airway inflammation (Soler et al., 1990). Also, this antagonist was previously demonstrated to inhibit the late allergic airway response in sheep (Abraham et al., 1991). Inhalation of a bradykinin B_2 receptor antagonist prevented airway hyperresponsiveness, and dramatically reduced the number of eosinophils present in tissue sections from various airway regions of sensitized guinea pigs (Farmer et al., 1992). These results support the potential anti-inflammatory activity of the bradykinin B_2 receptor antagonist on leukocyte recruitment in guinea pig airways.

The roles of bradykinin B_2 receptor in inflammation are well documented. However, the role of desArg^9-BK, a

metabolite of bradykinin acting on bradykinin B_1 receptor, in leukocyte recruitment is unknown. To elucidate the role of bradykinin B_1 receptors in leukocyte migration in our model of lung inflammation, a classical ([Leu^8]desArg^9-BK; 40 mg kg^{-1} 24 h^{-1}, i.v.) and a newly described bradykinin B_1 receptor antagonists (B 9858; 1 mg kg^{-1}, i.p.) were used and decreased eosinophil counts by 33% and 45% and eosinophil peroxidase activity by 49% and 61%, respectively. Our results are in agreement with those of Farmer et al. (1992) who showed that [Leu^8]desArg^9-BK inhibited leukocyte migration in antigen-induced guinea pig airway inflammation. B 9858 also inhibited neutrophilia by 45% and macrophages by 32% (not significant). Compounds HOE 140 and [Leu^8]desArg^9-BK did not change macrophage counts in the first series of experiments, since the bronchoalveolar lavage fluid of the appropriate group of Sephadex-injected animals (osmotic pump) did not have an elevated macrophage population. The observation that bradykinin B_1 receptor antagonists had effects on leukocyte migration was surprising. The presence of bradykinin B_1 receptor binding sites in guinea pig trachea and lung have not been observed in healthy animals (Farmer et al., 1989) but bradykinin B_1 receptors have been shown to be induced in inflammation or following tissue injury (Marceau, 1995). The lung inflammation induced by the intravenous injection of Sephadex beads may bring about bradykinin B_1 receptor induction, but the role of these receptors in cell influx remains to be determined.

To sustain the implication of both bradykinin B_1 and B_2 receptor subtypes in leukocyte migration, a non-selective bradykinin B_1/B_2 receptor antagonist, B 9430 was used. B 9430 reduced eosinophilia by 62% and eosinophil peroxidase activity by 68% in Sephadex-injected guinea pigs. B 9430 also decreased macrophage counts by 80% and neutrophil counts by 28% (not significant). B 9430 produced a larger inhibition of eosinophilia (62%) than the inhibition produced by the same dose of the bradykinin B_1 receptor antagonist B 9858 (45%) (1 mg kg^{-1}, i.p.). The potentiation of the inhibition of eosinophil migration by a non-selective bradykinin B_1/B_2 receptor antagonist suggested that both subtypes of bradykinin receptors were involved in the inflammatory response elicited by the injection of Sephadex. Ahluwalia and Perretti (1996) demonstrated clearly for the first time a relationship between bradykinin B_1 receptor and polymorphonuclear leukocyte recruitment in a murine model of inflammation. Recently, Vianna and Calixto (1998) have demonstrated that the cell influx induced by the intratracheal injection of desArg^9-BK in a murine model of pleurisy, was mediated by stimulation of bradykinin B_1 receptor. These results support the hypothesis that the bradykinin B_1 receptor may have an important role in modulating inflammatory responses.

In conclusion, our data with bradykinin B_1 and B_2 receptor antagonists demonstrated that both subtypes of bradykinin receptors may play a role in leukocyte migration in Sephadex-injected guinea pigs airways. The ability of bradykinin B_1 and B_2 receptor antagonists to inhibit lung eosinophilia suggests a physiological role for endogenous kinins in maintaining inflammation induced by Sephadex beads in guinea pig airways and points to their possible use for the treatment of lung inflammatory conditions such as bronchial asthma.

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