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Des-Arg^9-bradykinin increases intracellular Ca^{2+} in bronchoalveolar eosinophils from ovalbumin-sensitized and -challenged mice

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Abstract

The effects of the selective bradykinin B_1 receptor agonist, des-Arg^9-bradykinin and the bradykinin B_2 receptor agonist, bradykinin were studied on the intracellular free Ca^{2+} concentration ($[Ca^{2+}]_i$) in murine bronchoalveolar lavage cells from control and ovalbumin-sensitized mice using fura-2 microfluorimetry. The bronchoalveolar lavage cells of control mice, which were predominantly alveolar macrophages, showed an increase in $[Ca^{2+}]_i$ in response to bradykinin (1 μM) but not to des-Arg^9-bradykinin (1 μM), indicating the presence of functional bradykinin B_2 receptors and the absence of B_1 receptors. Such elevation in $[Ca^{2+}]_i$ induced by bradykinin was totally inhibited by the selective bradykinin B_2 receptor antagonist, D-Arg^0-Hyp^3-Thi^5-D-Tic^7-Oic^8-bradykinin (HOE-140; 10 μM). In contrast, bronchoalveolar lavage cells from ovalbumin-sensitized and -challenged mice significantly responded to both bradykinin and des-Arg^9-bradykinin, indicating the presence of both functional bradykinin B_1 and B_2 receptors. Eosinophils exhibited higher response to des-Arg^9-bradykinin (1 μM; 485% increase in $[Ca^{2+}]_i$) compared to bradykinin (1 μM; 163% increase in $[Ca^{2+}]_i$). This des-Arg^9-bradykinin-induced $[Ca^{2+}]_i$ increase was markedly inhibited by the selective bradykinin B_1 receptor antagonist, Ac-Lys-[D-βNal^7, Ile^8]des-Arg^9-bradykinin (R-715; 10 μM). Des-Arg^9-bradykinin neither modified the basal $[Ca^{2+}]_i$ in lymphocytes nor in mononuclear cells from ovalbumin-sensitized and challenged mice, while bradykinin produced a $[Ca^{2+}]_i$ increase in both cell types. Our results further support the implication of the inducible bradykinin B_1 receptors in airway inflammatory response in ovalbumin-sensitized and challenged mice.

Keywords: Bradykinin receptor; Bradykinin; Des-Arg^9-bradykinin; HOE-140; R-715; Ovalbumin sensitization; Bronchoalveolar lavage; Eosinophil; Ca^{2+} intracellular; Fura-2

1. Introduction

Kinins are implicated in cardiovascular homeostasis, inflammation and pain. Bradykinin, an endogenous non-apeptide, is released from tissue and plasma kininogens by the proteolytic action of kallikrein. Bradykinin is transformed by kininase I to its active metabolite des-Arg^9-bradykinin (Regoli and Barabé, 1980; McFadden and Vickers, 1989; Bascands et al., 1993; Levesque et al., 1995). The effects of kinins are mediated by two subtypes of transmembrane G-protein-coupled receptors known as bradykinin B_1 and B_2 receptors. Several studies showed that the bradykinin B_1 receptor, normally absent in tissues, is up-regulated in the presence of cytokines, endotoxins or during tissue injury (Regoli et al., 1990; Marceau et al., 1998), and is expressed during inflammatory conditions or tissue damage such as septic and endotoxic shock, rheumatoid arthritis, chronic inflammatory hyperalgesia, diabetes, lung inflammation, high blood pressure and atherogenesis (Regoli et al., 1981; Marceau et al., 1983; Farmer et al., 1991; Alvarez et al., 1992; Dray and Perkins, 1993; Correa and Calixto, 1993; Chakir et al., 1995; Raidoo et al., 1997; Perron et al., 1999; Simard et al., 2002; Gabra and Sirois, 2002, 2003). The bradykinin B_1 receptor exhibits greater affinity for des-Arg^9-bradykinin and des-Arg^{10}-kallidin. On the other hand, the bradykinin B_2 receptor is optimally stimulated by bradykinin and kallidin. It is constitutively expressed in several tissues and is considered to mediate the majority of effects of bradykinin under normal conditions (Regoli and Barabé, 1980).

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Experimental evidence suggests that bradykinin and des-Arg^9-bradykinin play an important role in inflammatory reactions and asthma (Farmer et al., 1992; Perkins et al., 1993; Perron et al., 1999). Increased levels of kinins have been detected in secretions from individuals with allergic rhinitis (Naclerio et al., 1985) and in the bronchoalveolar lavage fluid of asthmatics (Christiansen et al., 1992). Symptomatic and physiological changes, which mimic naturally occurring rhinitis and asthma, are provoked by inhaled challenge with bradykinin (Fuller et al., 1987; Proud et al., 1988). Bradykinin administration causes bronchoconstriction, microvascular leakage and mucus secretion in the airways of several animal species via the bradykinin B_2 receptor (Herxheimer and Stresemann, 1961; Bhoola et al., 1962). Inhalation of bradykinin or Lys-bradykinin provoked acute bronchoconstriction in humans (Polosa and Holgate, 1990). In addition, the selective bradykinin B_2 receptor antagonist, HOE-140 improved pulmonary function in asthma subjects in a 4 week-treatment phase (Akbary et al., 1996), abolished hyperresponsiveness to histamine and reduced antigen-induced nasal eosinophilia in subjects with allergic rhinitis (Turner et al., 2001). Recent work from our laboratory demonstrated that both bradykinin B_1 and B_2 receptor subtypes play a significant role in leucocyte migration into the airways of ovalbumin-sensitized and challenged mice (Eric et al., 2003).

The stimulation of bradykinin B_1 and B_2 receptors induces an increase in intracellular Ca^{2+} in different types of smooth muscle cells (Marsh and Hill, 1994; Smith et al., 1995; Bkaily et al., 1997). It is also known that, in several cellular models such as human and murine fibroblasts, bovine endothelial cells, rat mesangial cells, rat osteoblasts and canine tracheal epithelial cells, bradykinin produces a biphasic rise in $[Ca^{2+}]_i$: a transient peak caused by the mobilization of Ca^{2+} from intracellular pool and a sustained phase caused by influx of extracellular Ca^{2+} (Hall, 1992; Bascands et al., 1993; Bkaily et al., 1997; Luo et al., 1999). In the present study, we aimed to evaluate the involvement of both bradykinin B_1 and B_2 receptors on the changes in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) in cells obtained from the bronchoalveolar lavage of normal and/or ovalbumin-sensitized and challenged mice (murine model of allergic lung inflammation) through the use of selective bradykinin B_1 and B_2 receptor agonists and antagonists.

2. Methods and materials

2.1. Animals

Male Balb/c mice weighing 20–25 g (Charles River Laboratories, St. Constant, PQ, Canada) were used. The mice were housed four/cage and maintained under conditions of standard lighting (alternating 12-h light/dark cycle), temperature (22 ± 0.5 °C) and humidity ($60 \pm 10\%$) with food and water available at libitum. All experiments were carried out in accordance with the ethical recommendations and guidelines of the Canadian Council on Animal Care (CCAC) and were approved by the Ethic Committee of the University of Sherbrooke.

2.2. Antigen sensitization

Mice were sensitized on days 0 and 5 by intraperitoneal (i.p.) injections of 8 μg ovalbumin adsorbed to 2 mg aluminium hydroxide in saline (a total volume of 0.5 ml) according to the modified method of Kung et al. (1994). Control animals received an equal amount of $Al(OH)_3$ in saline. On days 12 and 13, both control and ovalbumin-sensitized non-anaesthetised mice were placed into a plexiglass chamber ($18 \times 10 \times 10$ cm) directly connected to an ultrasonic nebulizer (Model Spag-2, Montreal, PQ, Canada) and challenged via the airways for 30 min with 0.5% (w/v) ovalbumin solution (containing 0.8% antifoam B) in saline. Bronchoalveolar lavage, cell differential analyses and intracellular Ca^{2+} measurement were performed 24 h after the second nebulization.

2.3. Bronchoalveolar lavage and differential cell counts

Bronchoalveolar cells were obtained from the bronchoalveolar lavage of animals sacrificed following an i.m. injection of 50 μl of ketamine/xylazine (87/13 mg kg^{-1}). Briefly, the trachea was cannulated and the lungs were washed with 5 ml of phosphate-buffered saline (0.1 M, pH 7.4). The bronchoalveolar lavage fluid was collected and centrifuged ($300 \times g$, 10 min, 4 °C). Total cell count was performed using a hemocytometer, and viability was assessed with the Trypan blue exclusion test. Differential cell counts were done from duplicate cytospin smears of the original total cell suspension using Wright–Giemsa staining. The bronchoalveolar lavage fluid from untreated mice contained 100% mononuclear phagocytes (macrophages and monocytes), whereas that of ovalbumin-sensitized and -challenged mice contained approximately 30% of eosinophils, 67% of monocytes/macrophages, lymphocytes and 3% of neutrophils. Differential counts of bronchoalveolar lavage fluid cells were done every time before and after the cells sorting.

2.4. Cell isolation

The sorting of lymphocytes, monocytes/macrophages and eosinophils from bronchoalveolar lavage fluid was performed on fluorescence-activated cell sorting (FACS) vantage flow cytometer equipped with Cell Quest software (Becton Dickinson, San Jose, CA, USA) using gates defined by forward and side light scatter properties.

Briefly, cells were adjusted to 4×10^6 cells ml^{-1} in phosphate-buffered saline supplemented with 2% fetal bovine serum and were further sorted with phosphate-buffered saline as sheath buffer. The forward and side scatter was determined using 488-nm argon-ion laser. Samples were gated on to exclude debris and red cells. Granulocytes had high side scatter compared to normal lymphocytes or monocytes. Cells with high side scatter were flow-sorted and their morphology was determined as eosinophilic. The purity of isolated populations was between 95% and 99%. Total intracellular free level of Ca^{2+} was determined in bronchoalveolar lavage cells using fura-2 Ca^{2+} imaging technique.

2.5. Fura-2 loading for Ca^{2+} imaging technique

The cells attached to the poly-L-lysine treated 25-mm glass coverslips, that formed the bottom of the experimental bath chamber, were loaded with fura-2 acetoxymethyl ester (fura-2 AM) as described previously (Bkaily et al., 1992). Briefly, the cells, which had adhered to the coverslips, were loaded with freshly prepared fluorescent ratiometric Ca^{2+} indicator fura-2 AM (1 μM final concentration in Tyrode (NaCl: 130, KCl: 5.4, $CaCl_2$: 2, $MgCl_2$: 1, NaH_2PO_4: 3.6, HEPES: 25, D-glucose: 5 and $NaHCO_3$: 10 mM))/bovine serum albumin solution) for 45 min at room temperature in darkness. After loading, the cells were washed twice with Tyrode containing 0.1% bovine serum albumin buffered to pH 7.4 with Tris base and three times in Tyrode buffer alone and incubated for a further 20 min at room temperature in order to hydrolyse the acetoxymethyl ester groups of the fura-2. After hydrolysis, the cells were mounted in a coverslip holder to which was added 1 ml of bovine serum albumin free Tyrode solution.

2.6. Intracellular Ca^{2+} measurements

Dye-loaded cells were examined with a Deltascan and Imagescan microfluorometer (Photon Technology International (PTI), New Jersey, USA). Changes in the intensity of fura-2 fluorescence were obtained by exciting at 340 and 380 nm and recording the ratio at an emission of 510 nm. Fluorescence ratio measurements were performed at room temperature with a dual-wavelength spectrometer. Fura-2 fluorescence, excited alternatively at 340 and 380 nm, was measured at 510/520 nm by a microscope photometer attached to a photomultiplier detection system (Ratiomaster System; PTI). Complete data acquisition, presentation and analysis were performed computer-controlled by using commercially available software (FeliX, Version 1.1; PTI). Calibration of $[Ca^{2+}]_i$ was performed according to Grynkiewicz et al. (1985): Ca^{2+}-saturated fura-2 signals (R_{max}) were determined in the presence of 20 μM ionomycin (Mg^{2+}-free buffer) and Ca^{2+}-free signals (R_{min}) with 30 mM EGTA (Ca^{2+}-free buffer). Intracellular free Ca^{2+} level was calculated according to the following equation: $[Ca^{2+}]_i = K_D(R - R_{min})/(R_{max} - R)$. K_D is the dissociation constant of fura-2 (224 nm) and R is the experimental ratio (obtained by exciting at 340 and 380 nm).

2.7. Chemicals

Bradykinin, des-Arg^9-bradykinin, Ac-Lys-[D-βNal^7, Ile^8]des-Arg^9-bradykinin (R-715) were synthesized by Dr. Witold Neugebauer in the Institute of Pharmacology of Sherbrooke, School of Medicine, University of Sherbrooke, Canada. The D-Arg^0-Hyp^3-Thi^5-DTic^7-Oic^{8}bradykinin (HOE-140), ovalbumin (Grade II), antifoam B, Tyrode solution, bovine serum albumin, poly-L-lysine, ethylene glycol-bis-(beta-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA) and ionomycin were purchased from Sigma (St. Louis, MO, USA). Aluminium hydroxide gel (Rehydragel) was obtained from Reheis (Berkley Heights, NJ, USA). Fura-2 acetoxymethyl ester (fura-2 AM) was obtained from Calbiochem (La Jolla, CA, USA). Wright–Giemsa staining and Trypan blue were purchased from Fisher Scientific (Montreal, PQ, Canada).

2.8. Statistical analysis

The ratio of intracellular free Ca^{2+} in microfluorometry and imagery was determined using software provided by Photon Technology International (PTI). Pooled data from *n* separate identical experiments were plotted as means ± S.E.M. Statistical analysis was performed using the Student's *t*-test for unpaired data or analysis of variance (ANOVA) followed by the "Student–Newman–Keuls multiple comparisons test" using the Instat 3.0 software (GraphPad Software, San Diego, CA, USA). *P* values smaller than 0.05 were considered to be statistically significant.

3. Results

3.1. Basal $[Ca^{2+}]_i$ in control and ovalbumin-sensitized and -challenged mice

The bronchoalveolar lavage cells were essentially made of macrophages in control non-sensitized mice, whereas a small number of monocytes, lymphocytes and granulocytes was measured in the sensitized non-challeged mice (data not shown). However, 24 h after the second ovalbumin challenge, the cell population was highly modified and was made of 3.4% neutrophils, 28.6% eosinophils and 68% mononuclear cells (Eric et al., 2003). The basal intracellular Ca^{2+} levels ($[Ca^{2+}]_i$) measured in mixed cell population isolated from the bronchoalveolar lavage of control mice (24 h after the second nebulisation) averaged 93.9 ± 1.0 nM, whereas the basal $[Ca^{2+}]_i$ levels of bronchoalveolar lavage cells from ovalbumin-sensitized

and -challenged animals were 40% higher (133.5 ± 1.9 nM) (Figs. 1 and 2).

3.2. Effect of kinins on $[Ca^{2+}]_i$ in total bronchoalveolar cells of normal mice

The effect of the bradykinin receptor agonists on the level of total $[Ca^{2+}]_i$ in bronchoalveolar cells of control non-sensitized mice was evaluated. As shown in Figs. 1 and 6A, stimulation of bronchoalveolar cells with the selective bradykinin B_2 receptor agonist, bradykinin (1 μM) induced a rapid-onset 3.9-fold increase in the $[Ca^{2+}]_i$ (from 93.9 ± 1.0 to 367.0 ± 10.2 nM; $P<0.001$). Such effect was almost totally blocked by the selective bradykinin B_2 receptor antagonist, HOE-140. Pretreatment of bronchoalveolar cells with HOE-140 (10 μM), reduced the total $[Ca^{2+}]_i$ from 367.0 ± 10.2 to 96.8 ± 2.3 nM ($P<0.001$). On the other hand, the selective bradykinin B_1 receptor agonist, des-Arg^9-bradykinin (1 μM) had no significant effect on the level of intracellular Ca^{2+} in cells isolated from the bronchoalveolar of normal mice. The $[Ca^{2+}]_i$ remained close to basal level (97.7 ± 1.7 nM; $P>0.05$) (Figs. 1 and 6A).

3.3. Effect of kinins on $[Ca^{2+}]_i$ in total bronchoalveolar cells of ovalbumin-sensitized and -challenged mice

When mixed cell population isolated from the bronchoalveolar lavage fluid of ovalbumin-sensitized and -challenged mice was treated with the bradykinin B_1 receptor agonist, des-Arg^9-bradykinin (1 μM), a significant rise in steady-state basal intracellular Ca^{2+} level was observed within 2–3 min after the addition of des-Arg^9-bradykinin. The $[Ca^{2+}]_i$ increased by 3.4-fold (from 133.5 ± 1.9 to 458.4 ± 26.2 nM; $P<0.001$) (Fig. 2). Pre-incubation of the bronchoalveolar cells for 7–10 min with the selective bradykinin B_1 receptor antagonist, R-715 (10 μM) completely abolished the des-Arg^9-bradykinin-induced increase in intracellular Ca^{2+} level. The $[Ca^{2+}]_i$ decreased from 458.4 ± 26.2 to 116.8 ± 0.5 nM ($P<0.001$; Fig. 2). Furthermore, when the bronchoalveolar cells from ovalbumin-sensitized and -challenged mice were treated with the bradykinin B_2 receptor agonist, bradykinin (1 μM), an increase in $[Ca^{2+}]_i$ of 2.4 times was also noted (from 133.5 ± 1.9 to 315.0 ± 10.2 nM; $P<0.001$) (Fig. 2). This increase in intracellular Ca^{2+} level provoked by bradykinin was significantly inhibited by the selective bradykinin B_2 receptor antagonist HOE-140 (10 μM). The total $[Ca^{2+}]_i$ was reduced from 315.0 ± 10.2 to 156.3 ± 11.2 nM ($P<0.001$; Fig. 2).

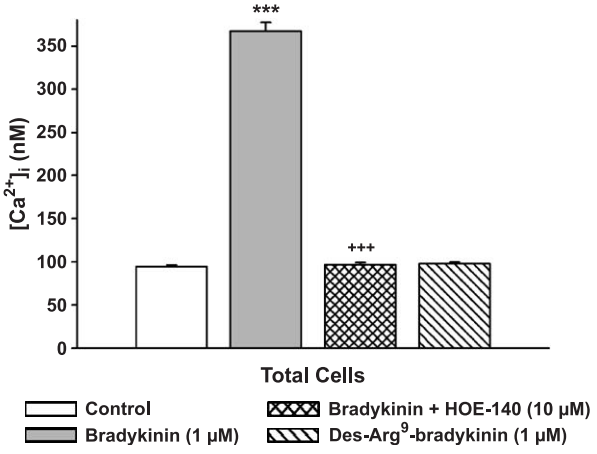


Fig. 1. Effects of kinins on $[Ca^{2+}]_i$ in total cells isolated from the bronchoalveolar lavage fluid of normal non-sensitized (control) mice. Cells were stimulated with des-Arg^9-bradykinin (1 μM), bradykinin (1 μM) or a combination of bradykinin with the selective bradykinin B_2 antagonist HOE-140 (10 μM; added 5–10 min before bradykinin). Values are means ± S.E.M. of four separate experiments. ***$P<0.001$ compared with control and $^{+++}P<0.001$ compared with the bradykinin-treated cells.

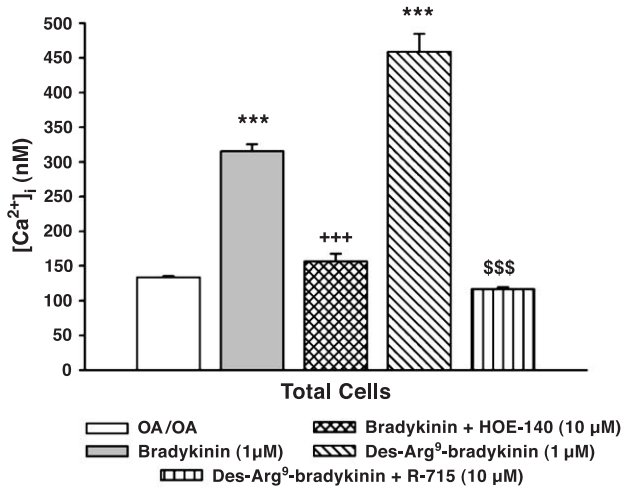


Fig. 2. Effects of kinins on $[Ca^{2+}]_i$ in the mixed cell population obtained from bronchoalveolar lavage washings of ovalbumin-sensitized and -challenged (OA/OA) mice. Cells were treated with des-Arg^9-bradykinin (1 μM), a combination of des-Arg^9-bradykinin with the selective bradykinin B_1 antagonist R-715 (10 μM; added 5–10 min before des-Arg^9-bradykinin), bradykinin (1 μM) or a combination of bradykinin with the selective bradykinin B_2 antagonist HOE-140 (10 μM; added 5–10 min before bradykinin). Bars are the means ± S.E.M. of four separate experiments. ***$P<0.001$ versus ovalbumin-sensitized and -challenged (OA/OA) mice; $^{+++}P<0.001$ versus bradykinin-treated cells and $^{\$\$\$}P<0.001$ versus des-Arg^9-bradykinin-treated cells.

3.4. Effects of kinins on $[Ca^{2+}]_i$ in eosinophils from ovalbumin-sensitized and -challenged mice

As illustrated in Figs. 3 and 6B, the treatment of eosinophils isolated from the bronchoalveolar lavage fluid of ovalbumin-sensitized and -challenged mice with des-Arg^9-bradykinin (1 μM) increased the $[Ca^{2+}]_i$ by 5.8 times (from 81.9 ± 3.7 to 474.7 ± 28.1 nM; $P<0.001$) as measured 1–2 min following the administration of the bradykinin B_1 receptor agonist and reached a steady-state level within 5 min. Eosinophil-pretreatment (5–10 min) with the selective bradykinin B_1 receptor antagonist R-715 (10 μM) totally abolished the des-Arg^9-bradykinin-mediated increase of $[Ca^{2+}]_i$ (156.3 ± 11.2 vs. 315.0 ± 10.2 nM; $P<0.001$)

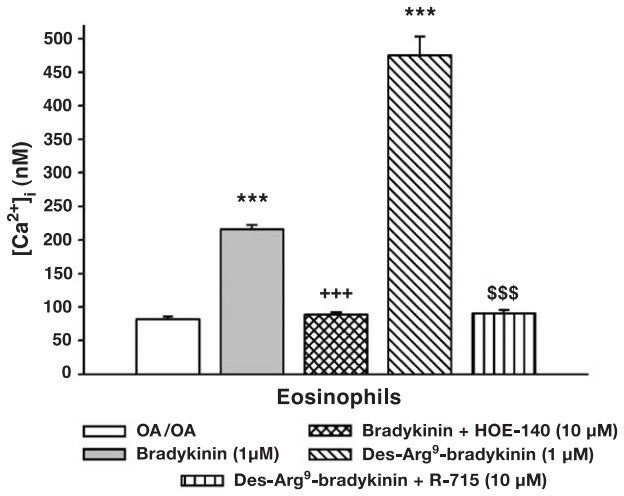


Fig. 3. Effects of kinins and their selective antagonists R-715 (10 μM) and HOE-140 (10 μM) on $[Ca^{2+}]_i$ in eosinophils isolated from the bronchoalveolar lavage fluid of ovalbumin-sensitized and -challenged (OA/OA) mice. Data are expressed as means ± S.E.M. of three to four separate experiments. ***$P<0.001$ compared to ovalbumin-sensitized and -challenged (OA/OA) mice; $^{+++}P<0.001$ compared to bradykinin-treated cells and $^{\$\$\$}P<0.001$ compared to des-Arg^9-bradykinin-treated cells.

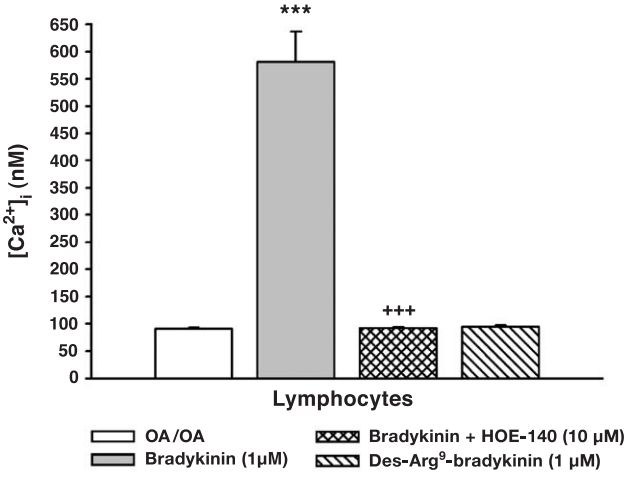


Fig. 4. Effects of kinins on $[Ca^{2+}]_i$ in lymphocytes isolated from the bronchoalveolar lavage fluid of ovalbumin-sensitized and -challenged (OA/OA) mice. Cells were stimulated with des-Arg^9-bradykinin (1 μM), bradykinin (1 μM) or a combination of bradykinin and the selective bradykinin B_2 antagonist HOE-140 (10 μM). Values are means ± S.E.M. of four separate experiments. ***$P<0.001$ compared with ovalbumin-sensitized and -challenged (OA/OA) mice and $^{+++}P<0.001$ compared with the bradykinin-treated cells.

(Figs. 3 and 6B). On the other hand, eosinophils incubated with the bradykinin B_2 receptor agonist, bradykinin (1 μM) showed an immediate but relatively smaller increase in $[Ca^{2+}]_i$ (2.6 times; from 81.9 ± 3.7 to 215.7 ± 6.3 nM; $P<0.001$) compared to that produced by des-Arg^9-bradykinin. The increase in $[Ca^{2+}]_i$ induced by bradykinin was prevented by the selective bradykinin B_2 antagonist HOE-140 (10 μM) (Figs. 3 and 6B).

3.5. Effects of kinins on $[Ca^{2+}]_i$ in lymphocytes from ovalbumin-sensitized and -challenged mice

Des-Arg^9-bradykinin (1 μM) did not modify the basal intracellular Ca^{2+} levels in lymphocytes from the bronchoalveolar lavage of ovalbumin-sensitized and -challenged mice (94.9 ± 2.1 vs. 91.01 ± 1.82 nM; $P>0.05$). Conversely, in the presence of bradykinin (1 μM), an increase of $[Ca^{2+}]_i$ by 6.4 times (from 91.0 ± 1.8 to 581.0 ± 55.9 nM; $P<0.001$) (Figs. 4 and 6B) was observed in bradykinin-treated lymphocytes compared to untreated lymphocytes. Pretreated of lymphocytes with the bradykinin B_2 receptor antagonist HOE-140 (10 μM), reversed the stimulatory effect of bradykinin on $[Ca^{2+}]_i$ increase. The Ca^{2+} level was reduced from 581.0 ± 55.9 to 92.0 ± 2.3 nM ($P<0.001$; Figs. 4 and 6B).

3.6. Effects of kinins on $[Ca^{2+}]_i$ in mononuclear cells from ovalbumin-sensitized and -challenged mice

The treatment of mononuclear phagocytes (macrophages and monocytes) isolated from the bronchoalveolar lavage fluid of ovalbumin-sensitized and -challenged mice with the bradykinin B_1 receptor agonist des-Arg^9-bradykinin (1 μM) did not induce significant change in the basal steady-state level of $[Ca^{2+}]_i$. However, stimulation with the bradykinin B_2 receptor agonist, bradykinin (1 μM), resulted in a 2.4-fold increase of $[Ca^{2+}]_i$ in macrophages/monocytes (from 87.7 ± 2.1 to 210.5 ± 5.5 nM; $P<0.001$) (Figs. 5 and 6B). Such increase was totally inhibited by the selective brady-

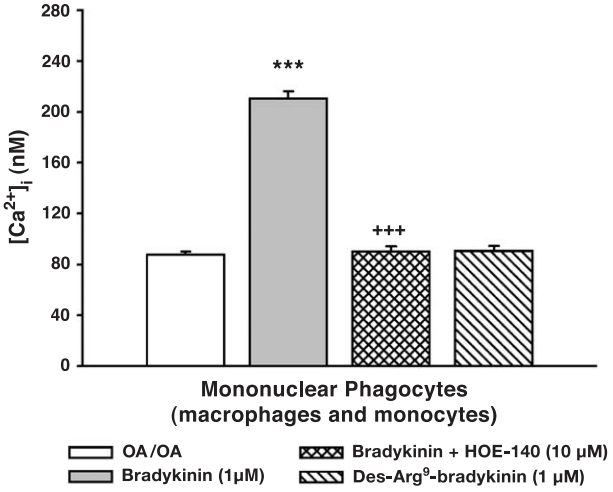
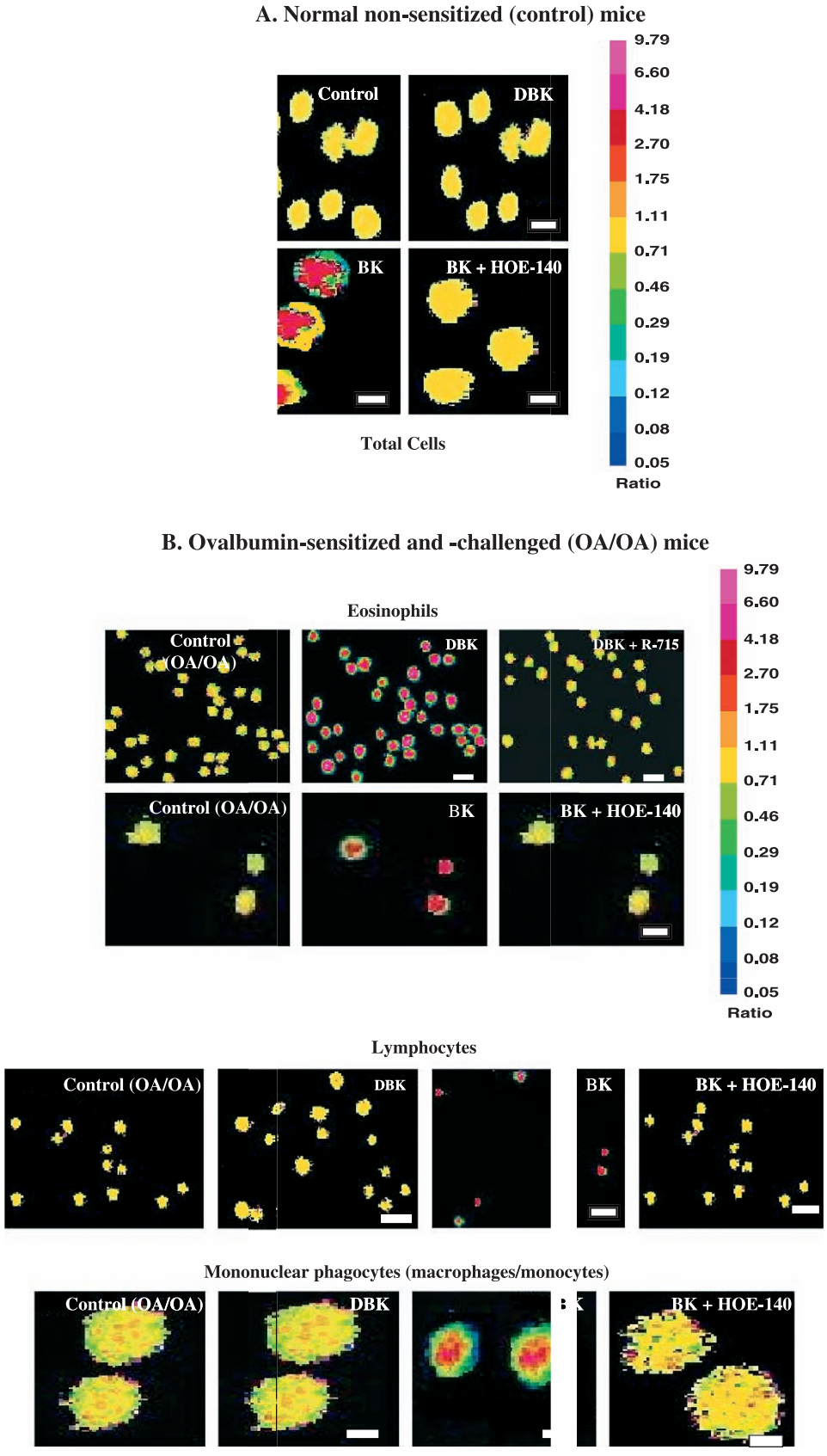


Fig. 5. Effects of kinins on $[Ca^{2+}]_i$ in mononuclear phagocytes (macrophages and monocytes) isolated from the bronchoalveolar lavage fluid of ovalbumin-sensitized and -challenged (OA/OA) mice. Cells were stimulated with des-Arg^9-bradykinin (1 μM), bradykinin (1 μM) or a combination of bradykinin and the selective bradykinin B_2 antagonist HOE-140 (10 μM). Bars are means ± S.E.M. of three to four separate observations. ***$P<0.001$ versus ovalbumin-sensitized and -challenged (OA/OA) mice and $^{+++}P<0.001$ versus bradykinin-treated cells.


A. Normal non-sensitized (control) mice
Control
DBK
BK
BK + HOE-140
Total Cells
9.79
6.60
4.18
2.70
1.75
1.11
0.71
0.46
0.29
0.19
0.12
0.08
0.05
Ratio
B. Ovalbumin-sensitized and -challenged (OA/OA) mice
Eosinophils
Control (OA/OA)
DBK
DBK + R-715
Control (OA/OA)
BK
BK + HOE-140
Lymphocytes
Control (OA/OA)
DBK
BK
BK + HOE-140
Mononuclear phagocytes (macrophages/monocytes)
Control (OA/OA)
DBK
BK + HOE-140

kinin B_2 antagonist HOE-140 (10 μM; $P<0.001$) (Figs. 5 and 6B).

4. Discussion

The present study showed that the antigenic provocation of Balb/c mice induced a significant increase in the level of basal steady-state intracellular free Ca^{2+} in bronchoalveolar lavage cells compared to the $[Ca^{2+}]_i$ levels found in cells from normal mice. The activated cells are known to secrete a number of inflammatory mediators, which interact with several intracellular signal transduction pathways and affect the basal steady-state intracellular free Ca^{2+} levels. Intracellular Ca^{2+} was shown to play a role as a second messenger in the signal transduction pathway of bradykinin B_1 and B_2 receptors in several cell and tissue types (Levesque et al., 1993; Bascands et al., 1993; Marsh and Hill, 1994; Mathis et al., 1996; Smith et al., 1995; Bkaily et al., 1997; Austin et al., 1997; MacNeil et al., 1997). We further demonstrated that the bradykinin B_2 receptor agonist bradykinin, and not the bradykinin B_1 agonist des-Arg^9-bradykinin, was able to raise the $[Ca^{2+}]_i$ in the total bronchoalveolar lavage cells from control and ovalbumin-sensitized and -challenged mice. This bradykinin-induced effect was completely blocked by the selective bradykinin B_2 receptor antagonist HOE-140 suggesting the presence of the bradykinin B_2 receptor in bronchoalveolar cells of these animals.

On the other hand, the selective bradykinin B_1 receptor agonist des-Arg^9-bradykinin caused only a sustained increase of $[Ca^{2+}]_i$ upon stimulating the total cells of ovalbumin-sensitized and -challenged mice, whereas it did not modify the $[Ca^{2+}]_i$ in the bronchoalveolar cells of non-sensitized mice. Such stimulatory effect of des-Arg^9-bradykinin was totally inhibited by the specific bradykinin B_1 receptor antagonist R-715 suggesting the presence of functional bradykinin B_1 receptors in the lavage cells of ovalbumin-sensitized and -challenged mice. This supports our previous findings that the bradykinin B_1 receptor, which is absent in control animals, is expressed in ovalbumin-sensitized and -challenged mice and is involved in the evolution of allergic inflammatory responses in pulmonary inflammation in mice (Eric et al., 2003). It is interesting to note that des-Arg^9-bradykinin induced a rapid increase of $[Ca^{2+}]_i$ in eosinophils from bronchoalveolar lavage of ovalbumin-sensitized and -challenged mice, whereas it had no influence on the basal intracellular Ca^{2+} levels of mononuclear cells and lymphocytes. Conversely, pretreatment with bradykinin B_2 receptor agonist induced a rise in $[Ca^{2+}]_i$ in various immune cells. The results obtained with isolated cell population clearly suggest that the effect of des-Arg^9-bradykinin in total cells from ovalbumin-sensitized and -challenged mice were mostly due to its effect on eosinophils, the only cell population that was clearly shown to express the bradykinin B_1 receptor. The participation of neutrophils could not be ruled out since the cell sorting technique used could not provide a sufficient number of neutrophils from mouse lungs for our Ca^{2+} fluxes analyses.

Our results suggested that the observed Ca^{2+} changes were caused by the binding of des-Arg^9-bradykinin and bradykinin to their specific receptors and that ovalbumin-sensitization and -challenge in mice promotes the induction of bradykinin B_1 receptor, the activation of which stimulates Ca^{2+} influx and increased the level of $[Ca^{2+}]_i$. The channel that seems to be activated by bradykinin B_1 and B_2 receptors in bronchoalveolar cells is not known and should be verified in the future. However, in most tissues, upon binding to its receptor, des-Arg^9-bradykinin (the metabolite resulting from the degradation of bradykinin and accumulated at sites of inflammation) stimulates a phospholipase C, which increases inositol triphosphate production and Ca^{2+} release (Regoli, 1984). In addition, Levesque et al. (1993) observed an increase of intracellular Ca^{2+} levels combined with an activation of protein kinase C following the stimulation of bradykinin B_1 receptor in rabbit aortic vascular smooth muscle cells. Recently, Bkaily et al. (1997) showed in human and rabbit aortic vascular smooth muscle cells that bradykinin B_1 receptor agonist, R-211, induced a sustained increase of $[Ca^{2+}]_c$ (cystolic) and $[Ca^{2+}]_n$ (nuclear) via activation of Ca^{2+} influx through the R-type Ca^{2+} channels which are present at the sarcolemma and the nuclear membranes. Indeed, several groups have shown, in various cellular types, that activation of bradykinin B_1 receptor induces a more persistent rise in $[Ca^{2+}]_i$ than the bradykinin B_2 receptor-induced Ca^{2+} increase. It is believed that there are at least two mechanisms involved in $[Ca^{2+}]_i$ increase provoked by the bradykinin B_1 receptor. The first is through Ca^{2+} influx across the cytoplasmic membrane and the second is through the release of Ca^{2+} from internal stores (Bascands et al., 1993; Mathis et al., 1966; Austin et al., 1997). Moreover, it was suggested by Marceau (1995) that the weak internalization of bradykinin B_1 receptor compared with the bradykinin B_2 receptor could explain this persistent Ca^{2+} signal. By integrating the present data with our earlier studies (Eric et al., 2003), it could be suggested that the absence of Ca^{2+} response in cells of normal mice stimulated with des-Arg^9-bradykinin results from the lack of expression of the bradykinin B_1 receptors. Thus, our results highly

Fig. 6. Fura-2 Ca^{2+} images showing the intracellular free Ca^{2+} level ($[Ca^{2+}]_i$) in total cells isolated from the bronchoalveolar lavage fluid of normal non-sensitized (control) (A) and in eosinophils, lymphocytes and mononuclear cells of ovalbumin-sensitized and -challenged (OA/OA) (B) mice. Cells were stimulated with the bradykinin B_1 receptor agonist des-Arg^9-bradykinin (DBK; 1 μM), a combination of DBK with the selective bradykinin B_1 antagonist R-715 (10 μM; added 5–10 min before des-Arg^9-bradykinin), bradykinin (BK; 1 μM) or a combination of BK with the selective bradykinin B_2 antagonist HOE-140 (10 μM; added 5–10 min before bradykinin). The vertical colored scale shows the fluorescence ratio (bound fura-2/unbound fura-2), while the bar corresponds to 1.25 μm.

support the concept that the bradykinin B_1 receptor can take part in the maintenance and the amplification of inflammation and "in some cases, take the relay of the function of bradykinin B_2 receptors in chronic pathologies" (Marceau, 1995). This is supported by other findings from Campos and Calixto (1995) and Campos et al. (1996) who have demonstrated that the rat paw oedema caused by the bradykinin B_2 agonist Tyr^8bradykinin was greatly reduced in animals pretreated intravenously with lipopolysaccharide compared to control groups. On the other hand, the oedematogenic response caused by the selective bradykinin B_1 agonist des-Arg^9-bradykinin was markedly increased in lipopolysaccharide-treated animals compared to normal rats. We also reported the implication of the bradykinin B_1 receptors in increasing the capillary permeability (Simard et al., 2002) and in the hyperalgesic activity (Gabra and Sirois, 2002, 2003) in diabetic mice.

In conclusion, these results are the first direct evidence that the des-Arg^9-bradykinin activates eosinophils from the bronchoalveolar lavage of ovalbumin-sensitized and -challenged mice and that eosinophils do express the bradykinin B_1 receptor during inflammatory reactions. The exact mechanisms by which the kinins increase intracellular Ca^{2+} concentration however remain to be clarified. The bradykinin B_1 receptors could play an important role in the evolution of the allergic inflammation induced by ovalbumin sensitization. Our data also indicate that the endogenous kinins, bradykinin and des-Arg^9-bradykinin, could have an important role in the initiation and development of the allergic airway inflammation in ovalbumin-sensitized mice *via* activation of bradykinin B_1 and B_2 receptors.

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