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Bradykinin down-regulates LPS-induced eosinophil accumulation in the pleural cavity of mice through type 2-kinin receptor activation: a role for prostaglandins

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1 The role of both exogenously administered and endogenously generated bradykinin (BK) on LPS-induced eosinophil accumulation in the mice pleural cavity was investigated by means of treatment with BK selective receptor agonists/antagonists and captopril.

2 Intrathoracic (i.t.) injection of LPS (250 ng cavity^{-1}) induced eosinophil influx at 24 h as previously described (Bozza *et al*., 1993). Pretreatment with the B_1 receptor antagonist des-Arg^9-[leu-^{8}]BK (0.025 and 0.25 nmol cavity^{-1}) showed no effect on this phenomenon, whereas pretreatment with the B_2 receptor antagonists, NPC 17731 (0.025 and 0.25 nmol cavity^{-1}) or HOE 140 (2.5 nmol cavity^{-1}), increased LPS-induced eosinophil influx. Accordingly, pretreatment with captopril at 10 mg kg^{-1} i.p., inhibited eosinophil infiltration induced by LPS in the pleural cavity, suggesting that endogenous BK is down-regulating LPS-induced eosinophil accumulation.

3 BK administered at 15 and 25 nmol cavity^{-1}, i.t. or i.p. also inhibited LPS-induced eosinophil accumulation. BK alone had no effect on the basal number of leucocytes in the pleural or peritoneal cavity in doses up to 25 nmol cavity^{-1}. Nevertheless, when injected at doses of 50 and 100 nmol cavity^{-1} BK induced leucocyte influx characterized by neutrophil and eosinophil accumulation at 24 h.

4 Similarly to what was observed with BK, a specific B_2 receptor agonist, Tyr^{8}BK, administered at 0.25 nmol cavity^{-1} i.p., significantly inhibited the eosinophil influx induced by LPS.

5 The mechanism by which B_2 receptor agonists inhibit LPS-induced eosinophil accumulation was investigated by pretreating the animals with indomethacin or a selective cyclooxygenase-2 inhibitor, NS-398. Pretreatment with either indomethacin or NS-398 had no effect on eosinophil influx induced by LPS alone, but those drugs were able to restore the LPS-induced eosinophil influx in Tyr^{8}BK (0.25 nmol cavity^{-1}) injected mice.

6 In conclusion, endogenously generated bradykinin seems to modulate, through activation of B_2 receptors, eosinophil accumulation induced by LPS *via* a mechanism dependent on prostanoid synthesis.

Keywords: Bradykinin; LPS; eosinophils; bradykinin B_2 receptors; prostaglandins

Abbreviations: BK, bradykinin; COX, cyclooxygenase; IL, Interleukin; LPS, lipopolysaccharide; PAF, platelet-activating factor; TNF, tumour necrosis factor

Introduction

Endotoxin or lipopolysaccharide (LPS) is a component of the cell wall of Gram-negative bacteria that elicit a wide range of biological effects (Rietschel & Brade, 1992). We have previously demonstrated that the intrathoracic injection of LPS in rodents induces leucocyte infiltration characterized by acute neutrophil accumulation, followed by mononuclear cell and eosinophil influx (Bozza *et al*., 1993). LPS-induced neutrophil influx is related to bone marrow mobilization of these cells and involves PAF-dependent mechanisms as well as the secretion of IL-1, TNFα and IL-8 (Bozza *et al*., 1994a; Horgan *et al*., 1993; Faccioli *et al*., 1990). On the other hand, LPS-induced eosinophil accumulation is independent of PAF, arachidonic acid metabolites (Bozza *et al*., 1993) and IL-5 (Bozza *et al*., 1994b). The eosinophil accumulation induced by LPS seems to be accounted for by the generation of heat-stable protein with molecular weight ranging between 10 and 50 kDa exhibiting a selective eosinophilotactic effect (Bozza *et al*., 1993). Subsequent studies showed that both resident macrophages and $\gamma\delta^+$ T lymphocytes contribute to the eosinophil infiltration observed after LPS stimulation of the pleural cavity (Bozza *et al*., 1994c; Penido *et al*., 1997).

The nonapeptide bradykinin (BK) is an inflammatory mediator involved in several events, such as pain, smooth muscle contraction, oedema formation and cell proliferation (Bhoola *et al*., 1992). These effects depend on the interaction between BK and one of the two so far identified subtypes of BK receptors, namely B_1 and B_2 receptors (Regoli & Barabé, 1980). The interaction of BK with the receptors expressed in polymorphonuclear leukocytes (PMN), mast cells, macrophages and endothelium results in the release of several inflammatory mediators such as prostaglandins, leukotrienes,

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histamine, nitric oxide, PAF and cytokines (Bathon & Proud, 1991; Cruwys *et al*., 1994). The recent development of specific B_1 and B_2 receptor antagonists provided a suitable tool to investigate the involvement of BK on different physiological and pathological phenomena (reviewed by Regoli *et al*., 1998). Studies with specific BK receptor antagonists and agonists showed that very few normal non-traumatized tissues express B_1 receptors and that most of the *in vivo* effects of BK are mediated by B_2 receptor activation (reviewed by Stewart, 1995). Nevertheless, B_1 receptors may be relevant for BK induced effects during inflammatory reactions since its expression is highly increased in such situations (reviewed by Marceau, 1995). Interestingly, Campos *et al*. (1996) have described that LPS induces upregulation of B_1 receptor in a process dependent on protein synthesis and activation of the cyclooxygenase pathway.

We and others have previously reported that BK is able to induce neutrophil and eosinophil recruitment to the pleural cavity after its i.t. administration. Eosinophils can be detected at 24 and 48 h, while neutrophils appear at early time points (Pasquale *et al*., 1991; Martins *et al*., 1992; Ferreira *et al*., 1996). Based on the evidence presented above we hypothesized that BK could be involved in LPS-induced eosinophil accumulation. In the present work, we used specific B_1 and B_2 receptor agonists and antagonists as tools to investigate this possibility.

Methods

Animals

Swiss mice of both sexes weighing 20–25 g were obtained from the Oswaldo Cruz Foundation animal house (R.J., Brazil) and used throughout this study. The animals were maintained in a room with a constant temperature and humidity and had free access to a pelleted diet and water.

Mouse pleurisy and peritonitis model

Briefly etherized mice received intrathoracic (i.t.) injection of 0.1 ml of the stimulus or vehicle (control group) using a 1 mm long insulin needle. At stipulated time points the animals were killed in a CO_2 chamber, the thoracic cavity was opened and washed with 1 ml of heparinized saline (10 UI ml^{-1}). Pleural wash aliquots were collected and diluted in Turk solution (2% acetic acid) for total leucocyte count in Neubauer chambers. Differential leucocyte analysis was performed on cytocentrifuged smears stained by the May-Grunwald-Giemsa method.

In a different set of experiments BK, at doses of 5, 15, 25, 50 and 100 nmol $cavity^{-1}$, was injected i.p. and the peritoneal cavity was opened, rinsed and had its cellular content analysed as described above. Sterile saline (0.9%)-injected animals constituted the control group. Intraperitoneal (i.p.) injections were performed in a volume never exceeding 0.3 ml.

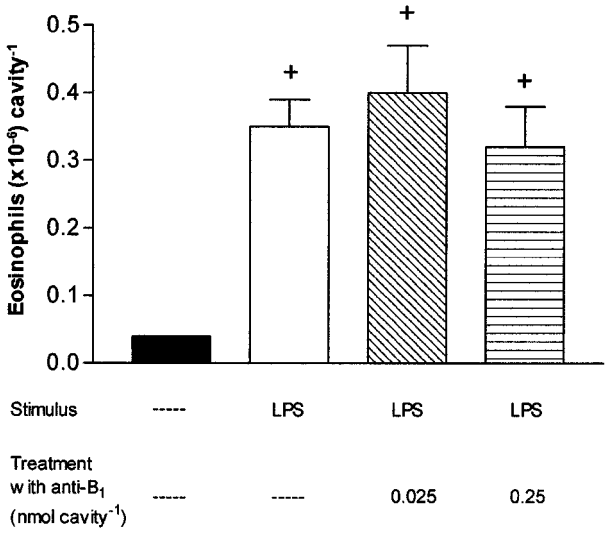

Figure 1 The effect of B_1 receptor antagonist, des-Arg^9[Leu^8]BK, on the eosinophil accumulation induced by LPS in the mice pleural cavity. des-Arg^9[Leu^8]BK was injected i.p. 1 h before the i.t. injection of 250 ng $cavity^{-1}$ of LPS. Eosinophil counts were performed 24 h after the stimulus by LPS. Each bar is the mean, with s.e.mean represented by vertical lines, from at least six animals. (+) indicates statistically significant differences as compared to non-stimulated animals.

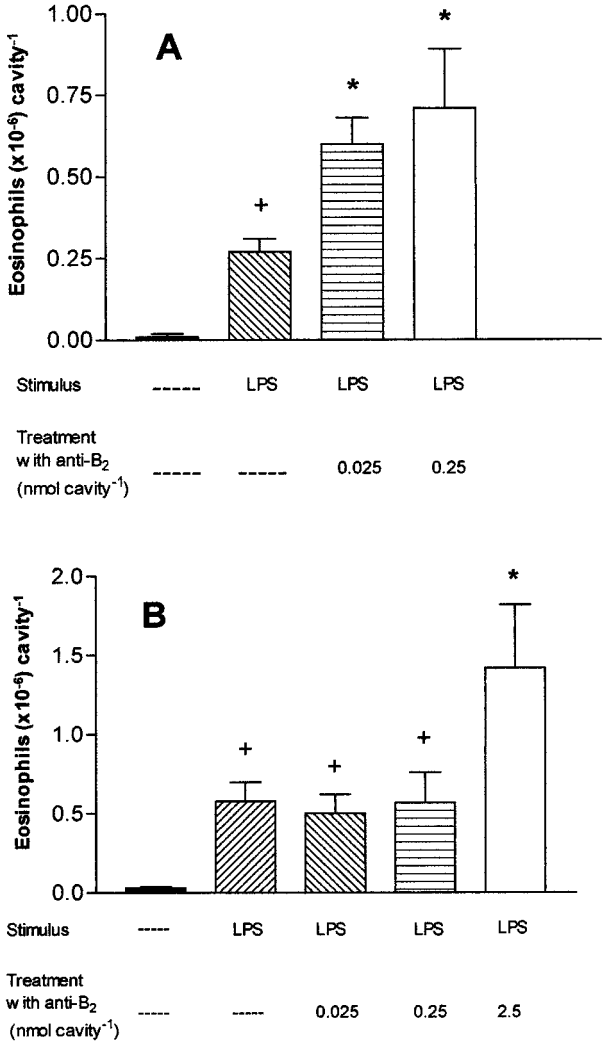

Figure 2 The effect of B_2 receptor antagonists, NPC 17731 (A) and HOE 140 (B), on the eosinophil accumulation induced by LPS in the mice pleural cavity. NPC 17731 or HOE 140 were injected i.p. 1 h before the i.t. injection of 250 ng $cavity^{-1}$ of LPS. Eosinophil counts were performed 24 h after the stimulus by LPS. Each bar is the mean, with s.e.mean represented by vertical lines, from at least six animals. (+) indicates statistically significant differences as compared to non-stimulated animals whereas (*) indicates comparison with non-treated animals stimulated by LPS.

Drugs

Lipopolysaccharide (serotype 0127:B8) from *E. coli*, bradykinin, captopril and indomethacin were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.); NS 398 was obtained from Biomol (U.S.A.); NPC 17331 (D-Arg^0-[Hyp^3 D-Hyp^E (transpropyl^7) Oic^8]bradykinin was from Scios Nova Pharmaceutical (U.S.A.); HOE 140 (D-Arg-[Hyp^3,Thi^5,D-Tic^7,Oic^8]bradykinin) was from Hoechst (Frankfurt, Germany); Tyr^{8}bradykinin and des-Arg^9[Leu^8]bradykinin were from Peninsula (U.S.A.).

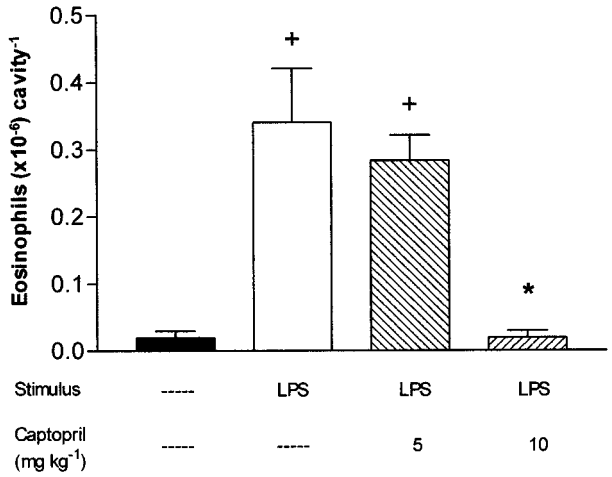

Figure 3 The effect of captopril treatment on the eosinophil accumulation induced by LPS in the mice pleural cavity. Captopril was injected i.p. at 5 or 10 mg kg^{-1} 1 h before the i.t. injection of 250 ng cavity^{-1} of LPS. Eosinophil counts were performed 24 h after the stimulus by LPS. Each bar is the mean, with s.e.mean represented by vertical lines, from at least six animals. (+) indicates statistically significant differences as compared to non-stimulated animals whereas (*) indicates comparison with non-treated animals stimulated by LPS.

Statistical analysis

Data are expressed as mean ± s.e.mean from six to eight animals per group. Statistical differences between groups were determined by analysis of variance (ANOVA) followed by Newman-Keuls-Student test with the level of significance set at $P<0.05$.

Results

Effect of bradykinin receptor antagonists on the eosinophil influx induced by LPS

Confirming previous results (Bozza *et al.*, 1993) the i.t. injection of LPS (250 ng cavity^{-1}) induced eosinophil influx into the mice pleural cavity 24 h after the challenge. In order to study the involvement of bradykinin on LPS-induced eosinophil accumulation, the animals were treated intraperitoneally with selective B_1 or B_2 receptor antagonists 1 h before the injection of LPS. Treatment with the B_1 receptor antagonist des-Arg^9[Leu^8]BK (0.025 and, 0.25 nmol cavity^{-1}) did not affect LPS-induced eosinophil influx (Figure 1),

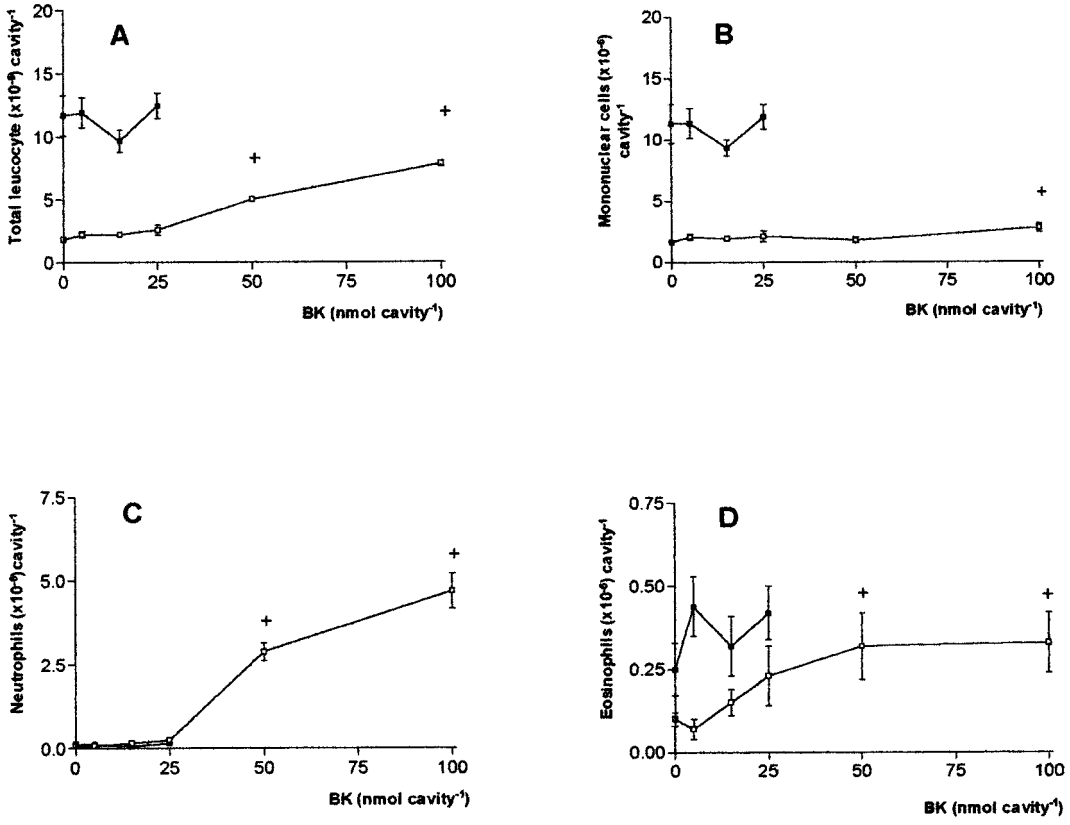

Figure 4 Dose-response curve for BK-induced leucocyte influx to the pleural (□) and peritoneal cavity (■) of mice. Total leucocyte (A), mononuclear cells (B), neutrophils (C) and eosinophils (D) counts were performed in the pleural or peritoneal wash 24 h after the i.t. or i.p. injection of BK, respectively. Each point is the mean, with s.e.mean represented by vertical lines, from at least six animals. (+) indicates statistically significant differences as compared to animals that were stimulated with saline.

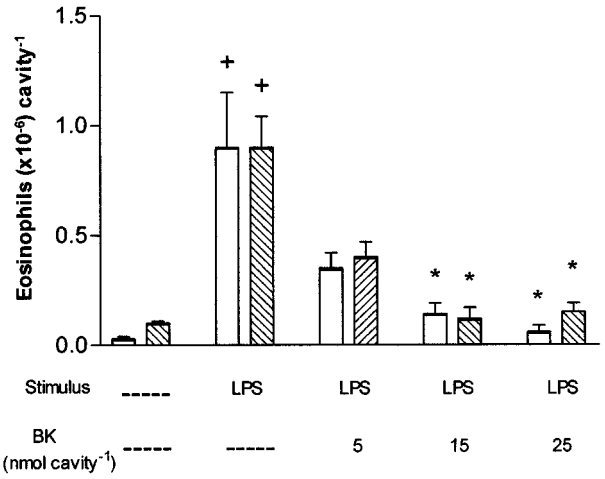

Figure 5 The effect of pretreatment with BK on LPS-induced eosinophil accumulation in the pleural cavity of mice. BK was administered i.t. (open columns) or i.p. (hatched columns) 5 min or 1 h prior to the i.t. injection of 250 ng cavity^{-1} of LPS, respectively. Eosinophil counts were performed 24 h after the stimulus by LPS. Each column is the mean, with s.e.mean represented by vertical lines, from at least six animals. (+) indicates statistically significant differences as compared to non-stimulated animals whereas (*) indicates comparison with non-treated animals stimulated by LPS.

Table 1 Inhibition of LPS-induced eosinophil accumulation by low doses of BK in captopril-treated animals

Treatment	*Stimulus*	*Eosinophils* ($\times 10^{-5}$) cavity^{-1}
Saline	Saline	0.1 ± 0.0
Saline	LPS	1.8 ± 0.3†
BK (0.025 nmol cavity^{-1})	LPS	1.7 ± 0.4
BK (0.25 nmol cavity^{-1})	LPS	1.9 ± 0.5
Captopril (5 mg kg^{-1})	LPS	1.5 ± 0.2
Captopril (5 mg kg^{-1}) + BK (0.025 nmol cavity^{-1})	LPS	0.3 ± 0.1*
Captopril (5 mg kg^{-1}) + BK (0.25 nmol cavity^{-1})	LPS	0.5 ± 0.3*

Captopril was given i.p. 1 h before the injection of BK. LPS at 250 ng cavity^{-1} was injected i.t. 1 h after the i.p. injection of BK. The evaluation of the number of eosinophils accumulating in the pleural cavity was performed 24 h after the injection of LPS. Data are represented as mean ± s.e.mean from at least six animals. (†) Indicates statistically significant differences as compared to non-stimulated animals. (*)Indicates statistically significant differences as compared to the group of animals treated with saline and stimulated with LPS.

whereas the administration of B_2 receptor antagonists NPC 17731 (0.025 and 0.25 nmol cavity^{-1} i.p.) or HOE 140 (2.5 nmol cavity^{-1} i.p.) increased the LPS response (Figure 2). The effect of higher dose of the B_1 receptor antagonist (2.5 nmol cavity^{-1}) was also analysed, but at this dose des-Arg^9[Leu^8]BK induced an inflammatory reaction that was still evident in the peritoneal cavity 24 h after its administration (data not shown), therefore complicating the analysis of its effect on the inflammatory reaction induced by LPS.

Effect of captopril on LPS-induced eosinophil accumulation

The results obtained with B_2 receptor antagonists indicated that endogenously produced BK is able to down modulate

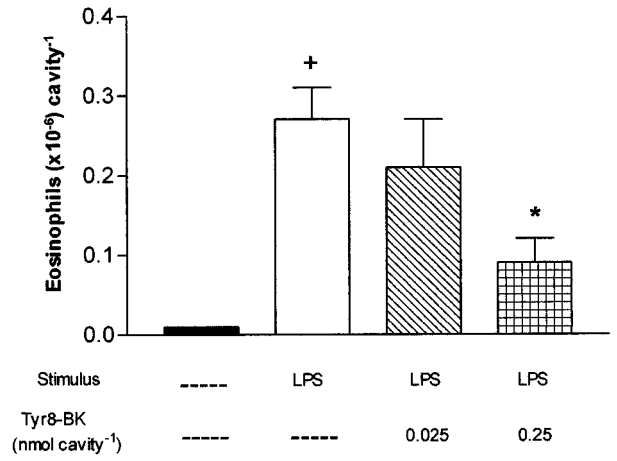

Figure 6 The effect of Tyr^{8}BK, a B_2 receptor agonist, on the eosinophil accumulation induced by LPS in the mice pleural cavity. Tyr^{8}BK was given i.p. 1 h before the i.t. injection of 250 ng cavity^{-1} of LPS. Eosinophil counts were performed 24 h after the stimulus by LPS. Each column is the mean, with s.e.mean represented by vertical lines, from at least six animals. (+) indicates statistically significant differences as compared to non-stimulated animals whereas (*) indicates comparison with non-treated animals stimulated by LPS.

LPS-induced eosinophil accumulation. To test this possibility we pretreated the animals with the kininase II inhibitor, captopril. Intraperitoneal injection of captopril at 10 mg kg^{-1}, but not at 5 mg kg^{-1}, 1 h before the challenge with LPS significantly inhibited the eosinophil influx at 24 h (Figure 3). It is noteworthy, that this same result was obtained when a small amount (50 μg cavity^{-1}) of captopril was given locally (i.t.) 5 min before LPS ($3.4 \pm 0.8 \times 10^5$ eosinophils cavity^{-1} in non-treated animals versus $0.3 \pm 0.2 \times 10^5$ eosinophils cavity^{-1} in captopril treated animals, $P < 0.05$) reinforcing the claim that endogenous BK is modulating LPS-induced eosinophil influx to the pleural cavity. In addition, the effect of captopril was reversed by treatment with 2.5 nmol of the B2 receptor antagonist Hoe140 (data not shown).

Effect of exogenous bradykinin or specific B_2 receptor agonist on LPS-induced eosinophil accumulation into the mice pleural cavity

In order to investigate if exogenously administered BK would also have a modulatory effect upon LPS-induced eosinophil accumulation, different doses of BK were administered i.p or i.t. before LPS challenge. Bradykinin alone failed to induce leucocyte influx to the peritoneal or pleural cavity in doses ranging from 5–25 nmol cavity^{-1} both at 1 h (data not shown) or at the 24 h time point. In doses of 50 and 100 nmol cavity^{-1}, BK induced leucocyte influx, characterized by neutrophil and eosinophil accumulation into the pleural cavity 24 h after its i.t. injection. BK also induced a discrete, but significant, mononuclear cell infiltration at the dose of 100 nmol cavity (Figure 4). When BK at doses of 15 and 25 nmol cavity^{-1} was administered i.p. 1 h prior, or i.t. 5 min prior to LPS, a significant inhibition of LPS-induced pleural eosinophil influx was observed (Figure 5). Lower doses of BK (0.025–0.25 nmol cavity^{-1}, i.p.) could also inhibit LPS-induced eosinophil accumulation providing the animals were pretreated with 5 mg kg^{-1} of captopril i.p. 1 h before the injection of BK (Table 1).

Confirming that the effect of BK was due to its interaction with B_2 receptor, the injection of a specific B_2 receptor agonist, Tyr^{8}BK (0.25 nmol cavity^{-1}, i.p., 1 h before LPS), signifi-

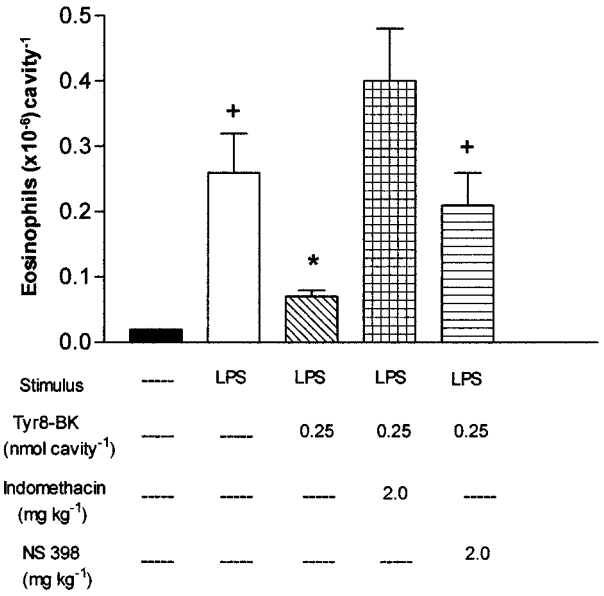

Figure 7 The effect of pretreatment with indomethacin or NS 398 on the inhibition of LPS-induced eosinophil accumulation by Tyr^8BK. Indomethacin or NS 398 were administered i.p. 1 h prior to the i.p. injection of Tyr^8BK. LPS at 250 ng cavity^{-1} was injected i.t. 1 h after the administration of Tyr^8BK. Eosinophil counts were performed 24 h after the stimulus by LPS. Each column is the mean, with s.e.mean represented by vertical lines, from at least 12 animals. (+) indicates statistically significant differences as compared to non-stimulated animals whereas (*) indicates comparison with non-treated animals stimulated by LPS.

cantly inhibited LPS-induced eosinophil influx to the pleural cavity (Figure 6).

Inhibition of LPS-induced eosinophil accumulation by B_2 receptor agonists is dependent on prostanoid synthesis

To investigate if the eosinophil accumulation induced by LPS was being down modulated as a consequence of prostanoid generation after B_2 receptor activation by BK or Tyr^8BK, animals were pretreated with indomethacin (2 mg kg^{-1}, i.p.) or with a specific cyclooxygenase-2 inhibitor, NS-398, (2 mg kg^{-1}, i.p.) 1 h before the injection of Tyr^8BK (0.25 nmol, i.p.). As shown in Figure 7, pretreatment with either indomethacin or NS-398 restored LPS-induced eosinophil accumulation in Tyr^8BK injected mice. In fact, indomethacin increased the eosinophil accumulation observed in animals treated with Tyr^8BK in about 50% above the levels observed in non-treated animals injected with LPS. This result was consistent and observed on several repetitions of the experiment although it did not reach statistical significance in any of them. It is worth mentioning, that indomethacin did not significantly affect the eosinophilia induced by LPS in non-treated animals (not shown).

Discussion

Our group has been developing a broad study about the mechanisms of LPS-induced eosinophil recruitment. Several aspects of this phenomenon have been focused on previous studies, including the partial characterization of the inflammatory mediators and cells involved in the LPS response (Bozza *et al*, 1993, 1994a,b,c; Penido *et al*., 1997). In the present study, we have investigated the effect of bradykinin on eosinophil accumulation induced by LPS.

Evidence has accumulated that BK is generated in response to LPS stimulation. For instance, there is a massive consumption of kininogen after LPS injection in rabbits suggesting that LPS is able to induce bradykinin synthesis *via* activation of the kallikrein system (Erdös & Miwa, 1968). It was reported that bradykinin is generated throughout the inflammatory processes evoked by LPS in pleural cavity, as indicated by the consumption of high molecular weight kininogen in the exudate (Uchida *et al*., 1983; Katori *et al*., 1989) and also by the presence of high levels of bradykinin-(1–5), a stable metabolite of BK (Shima *et al*., 1992; Majima *et al*., 1993). BK produced in the pleural cavity after LPS stimulation could be important in several aspects of the response. Concerning specifically the effects on eosinophils, we and others have previously demonstrated that the i.t. injection of BK is able to induce eosinophil accumulation in the pleural cavity with similar kinetics to that described for LPS (Pasquale *et al*., 1991; Ferreira *et al*., 1996). These results were confirmed here leading us to the hypothesis that BK might account for the eosinophil recruitment induced by LPS in the pleural cavity. Nevertheless, this seems not to be the case since BK receptor antagonists failed to inhibit LPS-induced eosinophil accumulation in the pleural cavity. It is important to note that exogenous BK was only able to induce cell influx at high concentrations (above 50 nmol cavity^{-1}) which may be difficult to achieve locally *in vivo*. On the other hand, low concentrations of BK, more likely to occur locally in a pathological situation, did not induce cell influx to the pleural or peritoneal cavity.

Despite the fact that i.p. injections of low concentrations of BK failed to attract leucocytes, it caused a significant inhibition of LPS-induced eosinophil accumulation. This inhibitory effect could be the result of a counter irritation phenomenon, since BK could be acting as a floggogen in the peritoneum. This possibility was ruled out based on two pieces of evidence; first the low concentrations of BK used in this experiment did not cause peritonitis; and secondly low concentrations of BK given intratoracically 5 min before LPS also inhibited the eosinophil influx, suggesting that BK may in fact down modulate the LPS reaction. These findings are in agreement with the inhibition of LPS-induced eosinophil accumulation observed after pretreatment with captopril since, in this case, the decrease in BK metabolism induced by captoril would reproduce the situation where low amounts of exogenous BK were administered to naive animals. Taken together, these results indicate a modulatory role for endogenously generated BK on LPS-induced eosinophil influx. Furthermore, the demonstration that pretreatment with two specific B_2 receptor antagonists increased eosinophil accumulation induced by LPS, together with the inhibitory effect of the B_2 agonist, Tyr^8BK, further suggests that endogenous BK, *via* interaction with its B_2 receptor, is down modulating rather than contributing to the eosinophil accumulation triggered by LPS.

In fact, B_2 receptors mediate most effects assigned to kinins, including vasodilatation, pain, increased vascular permeability, increased production of eicosanoids (Marceau *et al*., 1983; Steranka *et al*., 1988) and epithelial and mesangial cell proliferation (Wiernas *et al*., 1998; Castaño *et al*., 1998). In contrast to what we observed, Saleh *et al*. (1997) showed that activation of B_2 receptors by a low concentration of BK (10 nmol cavity^{-1}) induced leucocyte accumulation in the pleural cavity of rats. This apparent contradiction can be resolved if we consider that those authors used animals

pretreated with captopril in their experiments. In this situation, low concentrations of BK would have an effect similar to the one seen in the present study after the administration of high concentrations of BK to non-treated animals. This rationale seems to be correct since we could scale down the modulatory effect of exogenous BK in animals pretreated with captopril. In this condition, 0.025 nmol cavity^{-1} of BK was enough to impair LPS-induced eosinophil accumulation.

It has been shown that the B_1 receptor may be up-regulated by a variety of stimuli *in vivo*, including LPS, cytokines or long-term treatment with *Mycobacterium bovis* bacillus Calmette Guérin (BCG) (reviewed by Marceau, 1995; Campos *et al*., 1996; Ahluwalia & Perretti, 1996; Campos *et al*., 1997). Recently, Ahluwalia & Perretti (1996) showed that B_1 receptor antagonists are able to reduce PMN leucocyte recruitment induced by IL-1β in the mouse air pouch. In addition, desArg^{9}BK, a B_1 receptor agonist, was shown to induce neutrophil accumulation mediated by constitutive B_1 receptors present in the mouse pleural cavity (Vianna & Calixto, 1998). Surprisingly, the B_1 receptor antagonist, desArg^{9}Leu^{8}BK, failed to inhibit eosinophil influx observed after LPS stimulation suggesting that B_1 receptors are not important in this phenomenon. This result might also indicate differences in the response of eosinophils and neutrophils to substances that activate or block the B_1 receptor.

It has been reported that BK is able to induce prostaglandin E_2 (PGE_2) synthesis through stimulation of B_2 receptors in different cell types such as peritoneal macrophages (Bockmann *et al*., 1998) and cultured airway muscle cells (Pyne *et al*., 1997). BK-induced generation of PGE_2 is dependent on the activity of both cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) isozymes (Pang & Knox, 1997). Our observations that a non-selective COX inhibitor, indomethacin, and also a selective COX-2 inhibitor, NS-398, were able to restore LPS-induced eosinophil accumulation in animals treated with a B_2 agonist raise the possibility that down modulation of LPS-induced eosinophil influx by agonists of the B_2 receptor involves generation of prostanoids. Prostaglandins do exert a modulatory role in several different inflammatory conditions. For instance, prostaglandins were shown to inhibit the increase in vascular permeability of allergic pleurisy, probably acting *via* an increase in cyclic AMP levels (Bandeira-Melo *et al*., 1996). Furthermore, PGE_2 primes naive T cells for the production of anti-inflammatory cytokines IL-10 and IL-13, known to deactivate macrophages (Demeure *et al*., 1997), in addition to inhibits macrophage-induced inflammatory cytokine secretion (Williams & Shacter, 1997). PGE_2 may also affect eosinophil migration since it inhibits the shedding of L-selectin from eosinophils (Berends *et al*., 1997). Teixeira *et al*. (1997) have shown that prostanoid occupation of EP-2 receptors inhibited PAF-induced eosinophil aggregation, a CD18-dependent functional response (Teixeira *et al*., 1996). Because CD18 seems to be important for LPS-induced eosinophil recruitment (Larangeira *et al*., in preparation), these findings may be of relevance to explain the modulatory role of prostanoids on this phenomenon. Another interesting correlation has been described between prostaglandins and NO. It has been shown that NO may activate COX enzymes increasing the production of prostaglandins that are important in some inflammatory reactions (Sautebin *et al*., 1995, 1998). Since LPS is a well recognized stimulus for NO synthase induction, this cascade may be relevant for the phenomenom observed in this work. Nevertheless, in preliminary experiments we observed that inhibition of NO synthase by L-NAME failed to change both the eosinophil accumulation induced by LPS and the inhibitory effect of BK (not shown) suggesting that NO is playing a minor role in this system.

In fact, our results showed that indomethacin treatment not only restored, but also increased the eosinophil accumulation observed in animals treated with B_2 agonist, Tyr^{8}BK. This effect could be the consequence of the inhibition of the two COX isozymes by indomethacin. It has been shown that PGE_2 synthesis from COX-1 starts within minutes after BK stimulation while PGE_2 synthesis derived from COX-2 activity commences after a delay of more than 1 h and continues for at least 4 h (Pang & Knox, 1997). We can speculate that inhibition of PGE_2 production in the early and in the late phase of the LPS response in animals treated with B_2 agonist would facilitate the eosinophil accumulation. However, since indomethacin did not change the intensity of the eosinophil accumulation in animals receiving LPS alone this possibility seems less probable. Another possible explanation is that the prostaglandins synthesized from the two COX enzymes may control different functions in the cells in which they are formed, since the two isozymes may act upon with different pools of arachidonic acid (Reddy & Herschman, 1994). Additional experiments are needed to help further clarify this point.

Overall, our results demonstrated that bradykinin may have a modulatory role on LPS-induced eosinophil influx in the mice pleural cavity. This effect of BK is mediated by its interaction with the B_2 receptor subtype and seems to involve prostanoid synthesis by both COX-1 and COX-2 isozymes.

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