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The role of B_1 and B_2 kinin receptors in oedema formation after long-term treatment with *Mycobacterium bovis* bacillus Calmette-Guérin (BCG)

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1 The present study was designed to investigate the influence of long-term systemic treatment with *Mycobacterium bovis* bacillus Calmette-Guérin (BCG, 1 dose per animal, containing 6×10^4 colony-forming-units (CFu), 5 to 75 days beforehand) on oedema formation induced by intradermal injection of B_1 and B_2 selective agonists. The interaction between the B_1 agonist des-Arg^9-bradykinin and bradykinin was also investigated.

2 Intradermal injection (i.d.) of the B_2 selective agonist tyrosine^8-bradykinin (1–10 nmol) in naive (saline pretreated) animals, or in animals that had received BCG (30 days beforehand), caused dose-related and very similar oedema formation (ED_{50}; 1.1 and 1.0 nmol/paw, respectively). I.d. injection of the selective B_1 agonists des-Arg^9-bradykinin (100 nmol) or des-Arg^{10}-kallidin in naive animals caused very little paw oedema (0.04 ± 0.06 and 0.07 ± 0.02 ml, respectively, $n = 5$). However, i.d. injection of des-Arg^9-bradykinin (10–300 nmol) or des-Arg^{10}-kallidin (3–100 nmol) in animals pretreated with BCG, 30 days previously, resulted in dose-related and marked oedema formation, with mean ED_{50} values of 20.1 and 5.5 nmol/paw, respectively.

3 Oedema caused by i.d. injection of des-Arg^9-bradykinin (100 nmol/paw) in rats pretreated with BCG was evident 5 days after treatment, reaching the maximum 30 days later, remaining stable for up to 45 days, and reduced markedly at 75 days.

4 The i.d. co-injection of the selective B_1 antagonists des-Arg^9[Leu^8]-bradykinin (200 nmol), des-Arg^{10}[Leu^9]-bradykinin (30 nmol) and des-Arg^9-NPC 17731 (30 nmol) significantly (18 ± 3, 34 ± 2 and 56 ± 4%, respectively) prevented the paw oedema caused by i.d. injection of des-Arg^9-bradykinin (100 nmol) in rats treated with BCG. These effects were selective, because the i.d. injection of the B_1 selective antagonist des-Arg^{10}[Leu^9]-kallidin (30 nmol), at the same dose that consistently antagonized des-Arg^9-bradykinin (100 nmol)-mediated paw oedema, had no significant effect against tyrosine^8-bradykinin (3 nmol)-induced oedema in animals that had been treated previously with BCG. On the other hand, the i.d. co-injection of the selective B_2 antagonist, Hoe 140 (10 nmol) at a dose which markedly inhibited tyrosine^8-bradykinin (3 nmol)-induced oedema by 55 ± 4%, did not significantly affect des-Arg^9-bradykinin-induced paw oedema in animals pretreated with BCG.

5 Treatment of animals with dexamethasone (0.5 mg kg^{-1}, s.c.) every 24 h, from day 0 to day 30, inhibited significantly (67 ± 4%) the oedema caused by des-Arg^9-bradykinin (100 nmol), but did not affect the paw oedema caused by tyrosine^8-bradykinin (3 nmol) in animals pretreated with BCG.

6 Indomethacin (2 mg kg^{-1}, i.p.), administered 1 h before experiments, significantly inhibited des-Arg^9-bradykinin (100 nmol)-induced oedema formation, and, to a lesser extent, the paw oedema caused by tyrosine^8-bradykinin (3 nmol) (44 ± 4 and 20 ± 4%, respectively).

7 These findings show that the long-term systemic treatment of rats with BCG promoted a time-dependent and consistent paw oedema formation to B_1 agonists, des-Arg^9-bradykinin and des-Arg^{10}-kallidin, leaving responses to the B_2 agonist tyrosine^8-bradykinin unaffected. The upregulation of B_1 receptors after BCG treatment was inhibited by dexamethasone, suggesting the possible involvement of *de novo* protein synthesis. Finally, our results also show that in BCG-treated animals, the B_1 agonist des-Arg^9-bradykinin interacts in a synergistic manner with bradykinin. Therefore, both B_1 and B_2 kinin receptors appear to play a relevant role in modulating chronic inflammatory processes.

Keywords: Paw oedema (rat); BCG; dexamethasone; indomethacin; B_1 and B_2 agonists and antagonists

Introduction

The two naturally-occurring kinins, bradykinin and lysyl-bradykinin, are active peptides generated in plasma and peripheral tissues after trauma or infection. It has been extensively recognised that kinins are involved in many physiological processes, such as control of blood pressure, contraction or relaxation of smooth muscles, increase of vascular permeability and stimulation of sensory neurones, and they are also able to release several pro-inflammatory substances such as prostanoids, neuropeptides, cytokines and nitric oxide. Furthermore, kinins are also involved in many pathological states, including production of pain, sepsis, asthma, rheumatoid arthritis, pancreatitis and various inflammatory processes (for review see: Regoli & Barabé, 1980; Marceau *et al*., 1983; Bhoola *et al*., 1992; Farmer & Burch, 1992; Hall, 1992; Dray & Perkins, 1993).

The action of kinin involves the activation of two membrane receptors, B_1 and B_2. The B_2 receptors are present in

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peripheral and central nervous system and are believed to be responsible for maintenance of most physiological kinin actions. In addition, B_2 receptors are constitutive and exhibit higher affinity for bradykinin. The B_1 kinin receptors, in contrast, are not normally present in non-traumatized tissues in most tested species, and present greater affinity for the kinin active metabolites des-Arg^9-bradykinin and des-Arg^{10}-kallidin than for bradykinin. However, recent evidence indicates that B_1 receptors are upregulated after tissue trauma or injury and by a variety of agents administered *in vivo* or *in vitro*, such as endotoxins, Freund's adjuvant and cytokines, among others. Such results suggest that B_1 receptors may play an important role in certain pathological conditions, mainly in inflammatory processes (Farmer & Burch, 1992; Hall, 1992; Marceau, 1995). Both B_1 and B_2 kinin receptors have been cloned in several animal species and they are members of the seven transmembrane G proteins family of receptors, sharing great sequence homology at the amino acid level (McEachern *et al*., 1991; Hess *et al*., 1992; 1994; Menke *et al*., 1994; Pesquero *et al*., 1996).

In previous studies, we demonstrated that bradykinin, but not the selective B_1 agonist des-Arg^9-bradykinin, produced dose-related oedema formation when injected intradermally into the naive rat hindpaw, through stimulation of constitutive B_2 receptors. However, following complete desensitization of the paw oedema, after repeated injection of both bradykinin and tyrosine^8-bradykinin for seven consecutive days, des-Arg^9-bradykinin caused dose-related and marked oedema formation, suggesting the up-regulation of B_1 receptors after complete desensitization of constitutive B_2 receptors (Campos & Calixto, 1995; Campos *et al*., 1995). Similar induction of B_1 receptors for des-Arg^9-bradykinin, sensitive to dexamethasone and cycloheximide treatments and downregulation of B_2 receptor, have recently been described in rats which had been acutely treated with *Escherichia coli* endotoxin (Campos *et al*., 1996). Together, such results strongly suggest that induction of the B_1 receptor may play a relevant role in modulating certain inflammatory processes.

The attenuated strain of *Mycobacterium bovis* bacillus Calmette-Guérin (BCG) is commonly used in many countries as a vaccine in infants and young children to prevent disseminated tuberculosis infection and also as a therapeutic agent against neoplasic disease, such as superficial cancer of the urinary bladder (Colditz *et al*., 1994; Fine, 1995). This vaccine is administered intradermally or by the percutaneous route. Known adverse effects include regional adenitis, disseminated BCG infection and osteitis (Centers for Disease Control and Prevention. Recommendation of the Immunisation Practices Advisory Committee, 1988).

The purpose of this study was to investigate the role of B_1 and B_2 kinin receptors following long-term systemic infection of rats with BCG. Additionally, we investigated the possible synergistic interaction between the B_1 selective agonist des-Arg^9-bradykinin and bradykinin, also analysing some of the mechanisms involved in the des-Arg^9-bradykinin-mediated oedema formation in rats treated by systemic injection with BCG, 30 days previously.

Methods

Measurement of rat paw oedema

Experiments were conducted with non-fasted male Wistar rats (150–200 g) kept in a room controlled for temperature ($22 \pm 2^\circ$C) and illumination (12 h on 12 h off) and were given access to water and food *ad libitum*. Animals were pretreated with the antiotensin-converting enzyme inhibitor, captopril (5 mg kg^{-1}, s.c.) 1 h before any given experiment, in order to prevent the degradation of the peptides (Corrêa & Calixto, 1993). Under anaesthesia with 2,2,2 tribromoethanol (0.25 g kg^{-1}), the animals received 0.1 ml intradermal injections in one hindpaw of phosphate buffered saline (PBS, composition, mmol l^{-1}: NaCl 137, KCl 2.7, and phosphate buffer 10) containing des-Arg^9-bradykinin, des-Arg^{10}-kallidin, bradykinin or tyrosine^8-bradykinin. In some experiments des-Arg^9-bradykinin (3 nmol) and bradykinin (1 nmol) were co-injected in saline and BCG-treated animals. The contralateral paw received 0.1 ml of PBS and was used as a control. Oedema was measured by the use of a water plethysmometer (Ugo Basile) at several time-points (10, 20, 30, 60 and 120 min) or only at the peak (20 min) following the injection of the inflammatory mediators. Oedema is expressed as the difference between the test and the control paws.

Animals were injected by subcutaneous (s.c.) injection in dorsal region with 0.1 ml of BCG, one dose per animal (each dose contains 6.4×10^4 colony-forming-units (CFu) of *Mycobacterium bovis*) at different time intervals (5, 10, 15, 30, 45, 60 and 75 days before the experiments). Control animals received the same volume of PBS (0.1 ml per animal, s.c.) at the same time intervals.

Influence of some drugs on des-Arg^9-bradykinin or tyrosine^8-bradykinin-mediated oedema formation in BCG treated animals

In a separate series of experiments, in order to confirm the involvement of B_1 and B_2 receptors in kinin-induced oedema, animals pretreated with BCG 30 days beforehand, received an intradermal (i.d.) injection of the B_1 selective agonist des-Arg^9-bradykinin (100 nmol), or the selective B_2 agonist tyrosine^8-bradykinin (3 nmol), co-injected with the selective B_1 (des-Arg^9[Leu^8]-bradykinin, des-Arg^{10}[Leu^9]-kallidin, des-Arg^9-NPC 17731 (30–200 nmol) or B_2 (Hoe 140, 10 nmol) receptor antagonists. To assess the possible participation of *de novo* synthesis of B_1 receptors mediating paw oedema induced by des-Arg^9-bradykinin in BCG-treated animals, rats were pretreated with the anti-inflammatory steroid dexamethasone (0.5 mg kg^{-1}, s.c.) or with saline (control group), every 24 h, from day 0 to day 30. Animals were used 24 h after the last injection. The other group of rats received the cyclo-oxygenase inhibitor indomethacin (2 mg kg^{-1}, i.p., -1 h) before challenge with des-Arg^9-bradykinin.

Drugs

The following drugs were used: bradykinin, tyrosine^8-bradykinin, captopril, dexamethasone, indomethacin, 2,2,2 tribromoethanol (Sigma Chemical Company, St. Louis, U.S.A.). des-Arg^9-bradykinin, des-Arg^{10}-kallidin, des-Arg^9[Leu^8]-bradykinin and des-Arg^{10}[Leu^9]-kallidin were obtained from Peninsula Belmont Laboratories, CA, U.S.A. Hoe 140 (D-Arg^0-[Hyp^3, Thi^5, D-Tic^7, Oic^8]-bradykinin) was kindly supplied by Hoechst (Frankfurt Main, Germany). NPC 17731 (D-Arg^0[Hyp^3,D-HypE (transpropyl)^7], Oic^8]-bradykinin) and des-Arg^9-NPC 17731 (D-Arg-Arg-Pro-Hyp-Gly-Phe-Ser-D-HypE-transpropyl)-Oic) were kindly supplied by Scios/Nova (Baltimore, U.S.A.). BCG (Calmette Guérin Bacillus from *Mycobacterium bovis*, lot number 940437) was supplied by National Institute of Quality Control (FIOCRUZ, Rio de Janeiro, RJ, Brasil). Frozen ampoles were thawed, briefly sonicated, and diluted to the desired concentration in sterile 0.9% w/v of NaCl solution. The resulting suspension was dissolved in 1.0 ml in a siliconized tube and stored at -20°C until required. Plastic tube samples were sonicated before injections into animals. The stock solutions for all peptides used were prepared in PBS (1–10 mM) in siliconized plastic tubes, maintained at -18°C, and diluted to the desired concentration just before use. The other drugs were prepared daily in 0.9% w/v of NaCl solution.

Statistical analysis

The results are presented as the mean $\pm$ s.e.mean, except for the ID_{50} or ED_{50} values in individual experiments (i.e. the concentrations of antagonists that reduced oedema formation by

50% relative to control value, or concentrations of agonists needed to cause half maximal oedema increase), which are presented as geometric means accompanied by their respective 95% confidence limits. The ID_{50} or ED_{50} values were determined by use of the least squares method for individual experiments. Statistical comparison of the data was performed by the use of analysis of variance followed by Dunnett's test or by Student's unpaired t test when indicated. Differences with $P<0.05$ were considered significant.

Results

The i.d. injection of the selective B_2 agonist tyrosine^8-bradykinin (0.3 to 10 nmol) in naive (saline treated) animals, or in animals that had been treated previously with BCG (30 days beforehand) caused very similar dose-related oedema formation (Figure 1). The calculated mean ED_{50} values (and 95% confidence limit values) for these effects were 1.1. (0.8–1.9) and 1.0 (0.7–2.7) nmol, and the oedema responses induced by 10 nmol of tyrosine^8-bradykinin were 0.40 ± 0.03 and 0.41 ± 0.06 ml, respectively. Figure 2a shows that, as shown previously (Campos & Calixto, 1995; Campos *et al*., 1995; 1996), i.d. injections of the selective B_1 agonists des-Arg^9-bradykinin or des-Arg^{10}-kallidin (up to 300 nmol) in the naive animals caused a minimal increase of oedema formation (0.04 ± 0.006 and 0.07 ± 0.02 ml, respectively). However, i.d. injection of des-Arg^9-bradykinin (10 to 300 nmol) (Figure 2b) or des-Arg^{10}-kallidin (3–100 nmol) (Figure 2c) in animals that had been treated 30 days previously with BCG, resulted in dose-related oedema formation, with mean ED_{50} values (and 95% confidence limits) of 20.1 (15–22) and 5.5 (2.8–10.5) nmol, respectively. The oedema formation caused by 100 nmol of des-Arg^9-bradykinin or des-Arg^{10}-kallidin was 0.46 ± 0.04 and 0.37 ± 0.02 ml, respectively, which corresponds to 121 ± 4 and 97 ± 5%, respectively, relative to the maximal oedema induced by tyrosine^8-bradykinin (3 nmol) (0.38 ± 0.03 ml). The increase of des-Arg^9-bradykinin and des-Arg^{10}-kallidin oedema over control values (saline-treated animals) was 11 and 5 fold, respectively ($P<0.05$).

Figure 3 shows the time-course of paw oedema formation following i.d. injection of des-Arg^9-bradykinin in rats that had been previously treated with BCG. The oedema induced by des-Arg^9-bradykinin (100 nmol) was evident 5 days after BCG treatment, reaching its maximum at 30 days (0.45 ± 0.04 ml), and remaining stable for up to 45 days (0.41 ± 0.02 ml). It was markedly reduced by the 75th day after BCG treatment (0.15 ± 0.03 ml) but was still significantly greater than control values.

The i.d. injection of the selective B_1 antagonists des-Arg^9-[Leu^8]-bradykinin (200 nmol), des-Arg^{10}[Leu^9]-kallidin (30 nmol) or des-Arg^9-NPC 17731 (30 nmol) (Cabrini *et al*.,

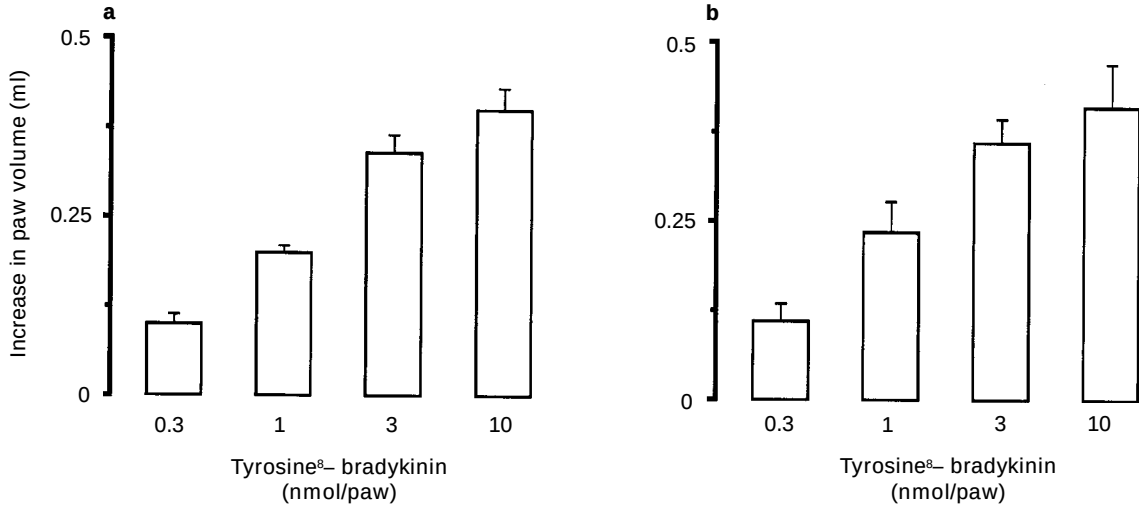

Figure 1 Dose-related rat paw oedema caused by intradermal injection of tyrosine^8-bradykinin (0.3–10 nmol/paw) in PBS pretreated (a) or in BCG (one dose per animal, 30 days before)-pretreated (b) animals. Each column represents the mean ± s.e.mean of 5–6 rats. The oedema was measured 20 min after intradermal injection of tyrosine^8-bradykinin.

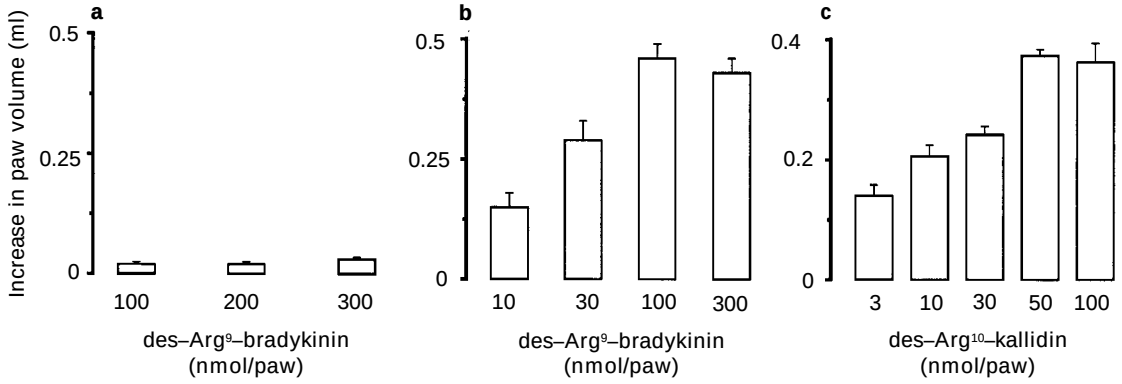

Figure 2 Rat paw oedema caused by intradermal injection of des-Arg^9-bradykinin into naive animals (100–300 nmol/paw) (a), and the dose-related rat paw oedema caused by des-Arg^9-bradykinin (10–300 nmol/paw) (b) or by des-Arg^{10}-kallidin (3–100 nmol/paw) (c) in BCG (one dose per animal, 30 days before)-pretreated animals. Each column represents the mean ± s.e.mean of 5–6 rats. The oedema was measured 20 min after intradermal injection of des-Arg^9-bradykinin or des-Arg^{10}-kallidin.

1996) alone did not produce any significant agonistic effects (results not shown), but when they were co-injected i.d. together with des-Arg^9-bradykinin (100 nmol), they significantly antagonized the paw oedema formation induced by des-Arg^9-bradykinin in rats treated 30 days before with BCG (18 ± 3; 34 ± 2 and $56\pm4\%$, respectively) (Figure 4a). In contrast, i.d. co-injection of the selective B_2 antagonist Hoe 140 (10 nmol) at a dose that markedly inhibited by $55\pm4\%$ tyrosine^8-bradykinin (3 nmol)-mediated paw oedema (Figure 4b), did not affect significantly des-Arg^9-bradykinin-mediated oedema formation in animals previously infected with BCG (Figure 4a). Consistent with a selective effect of des-Arg^{10}[Leu^9]-kallidin on B_1 receptor-mediated paw oedema, this peptide failed to inhibit tyrosine^8-bradykinin (3 nmol)-induced oedema in animals that had been treated previously with BCG (Figure 4b).

Dexamethasone (0.5 mg kg^{-1}, s.c.), administered every 24 h, from day 0 to day 30, inhibited significantly ($67\pm4\%$) the oedema elicited by des-Arg^9-bradykinin (100 nmol) in animals pretreated with BCG (30 days before the experiments), leaving the oedema caused by tyrosine^8-bradykinin (3 nmol) unaffected (Figure 5a and b). Also, the pretreatment of animals with indomethacin (2 mg kg^{-1}, i.p.) given 1 h before the challenge with des-Arg^9-bradykinin, significantly reduced the paw oedema caused by des-Arg^9-bradykinin (100 nmol) and, to a lesser extent, the paw oedema caused by tyrosine^8-bradykinin ($44\pm4\%$ and $20\pm4\%$, respectively) in animals that had been treated with BCG 30 days previously (Figure 6).

The i.d. injection of des-Arg^9-bradykinin (3 nmol) or bradykinin (1 nmol) in BCG-treated animals produced a modest paw oedema (0.08 ± 0.004 and 0.16 ± 0.05 ml, respectively). Interestingly, the i.d. co-administration of both agonists in animals treated with BCG, produced a marked potentiation of paw oedema (0.50 ± 0.019 ml) (Figure 7a). The co-injection of the selective B_1 (des-Arg^{10}[Leu^9]-kallidin, 10 to 50 nmol) or the B_2 (Hoe 140, 1 to 10 nmol) receptor antagonists, together with des-Arg^9-bradykinin and bradykinin, resulted in a dose-dependent and significant inhibition ($P<0.05$) of the paw oedema formation induced by i.d. co-administration of des-Arg^9-bradykinin and bradykinin (Figure 7b and c). The calculated mean ID_{50} values (and their 95% confidence limits) were 45.7 (38.3 – 54.4) and 3.8 (2.3 – 6.1) nmol, respectively. The inhibition of oedema caused by des-Arg^{10}[Leu^9]-kallidin (50 nmol) and Hoe 140 (3 nmol) was 54 ± 2 and $50\pm2\%$, respectively ($P<0.05$).

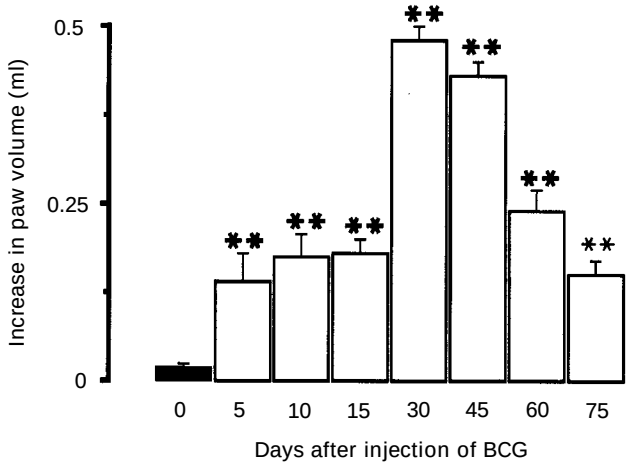

Figure 3 Time-dependent increase of rat hind paw volume in response to intradermal injection of des-Arg^9-bradykinin (100 nmol/paw) several days after treatment of animals with BCG (one dose per animal). Each column represents the mean $\pm$ s.e.mean of 4 – 5 rats. The oedema was measured 20 min after intradermal injection of des-Arg^9-bradykinin. Significantly different from control: $^{**}P<0.01$.

Discussion

In this study, we have attempted to investigate the effect of long-term treatment of rats with BCG on oedema formation induced by selective B_1 and B_2 agonists in the rat paw and also to examine the possible interaction of oedema induced by the B_1 agonist, des-Arg^9-bradykinin, with bradykinin. In earlier studies, we found that complete desensitization by daily i.d. injection of bradykinin (Campos & Calixto, 1995) or tyrosine^8-bradykinin (Campos *et al*., 1995), as well as after acute systemic treatment of rats with lypopolysaccharide (LPS) (Campos *et al*., 1996), produced an upregulation of B_1 receptor-mediated rat paw oedema followed by down-regulation of constitutive B_2 receptors. The results of the current study de-

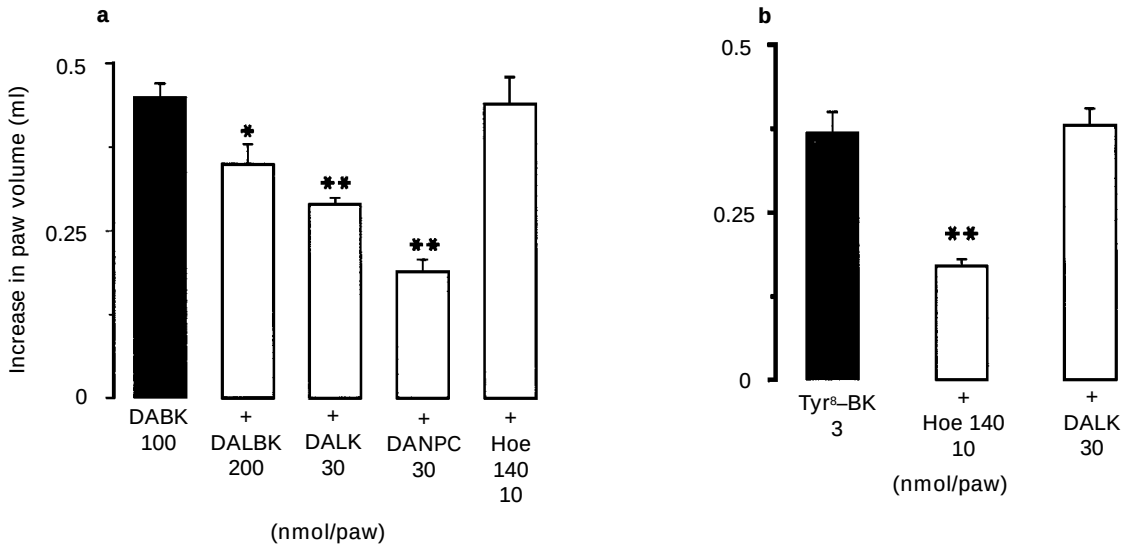

Figure 4 (a) Effect of des-Arg^9[Leu^8]-bradykinin (DALBK, 200 nmol/paw), des-Arg^{10}-[Leu^9]-kallidin (DALK, 30 nmol), des-Arg^9-NPC 17731 (DA-NPC 17731, 30 nmol/paw) and Hoe 140 (10 nmol/paw) on des-Arg^9-bradykinin (DABK, 100 nmol/paw)-induced paw oedema in animals treated with BCG 30 days previously. (b) Effect of Hoe 140 (10 nmol/paw) or des-Arg^{10}[Leu^9]-kallidin (DALK, 30 nmol/paw) on tyrosine^8-bradykinin (Tyr^8-BK, 3 nmol/paw)-induced paw oedema in rats pretreated with BCG (one dose per animal, 30 days previously). Each column represents the mean $\pm$ s.e.mean of 5 – 6 rats. The oedema was measured 20 min after the injections of the peptides. Significantly different from control: $^{*}P<0.05$; $^{**}P<0.01$.

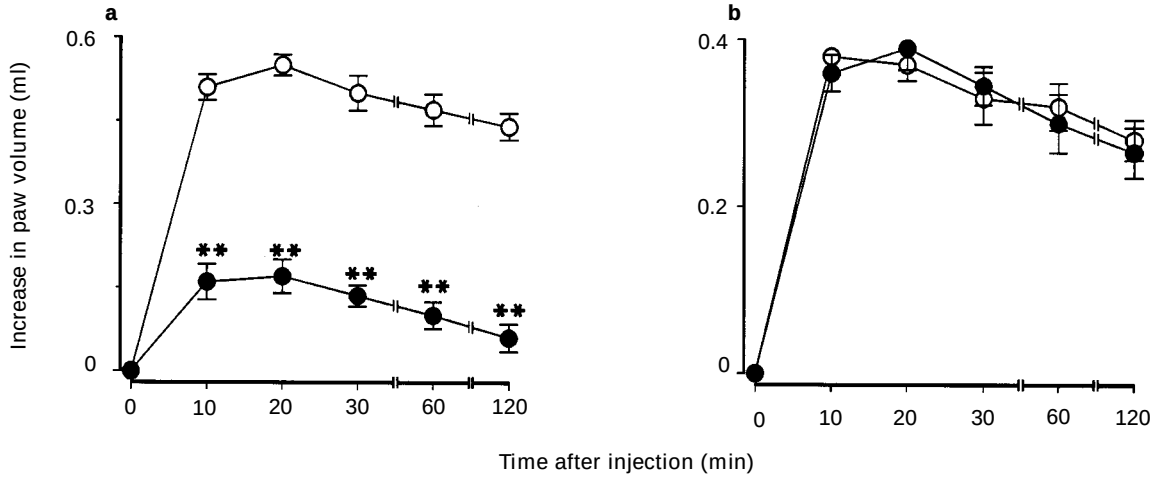

Figure 5 Effect of systemic treatment with dexamethasone (0.5 mg kg^{-1}, every 24 h, from day 0 to day 30) on oedema responses to des-Arg^9-bradykinin (100 nmol/paw) (a) or tyrosine^8-bradykinin (3 nmol/paw) (b) in rats pretreated with BCG (one dose per animal, 30 days before experiments). Control responses (○) and responses obtained in the presence of dexamethasone (●). Each point represents the mean of 6 rats; vertical lines show s.e.mean. Significantly different from control values: $^{**}P<0.01$.

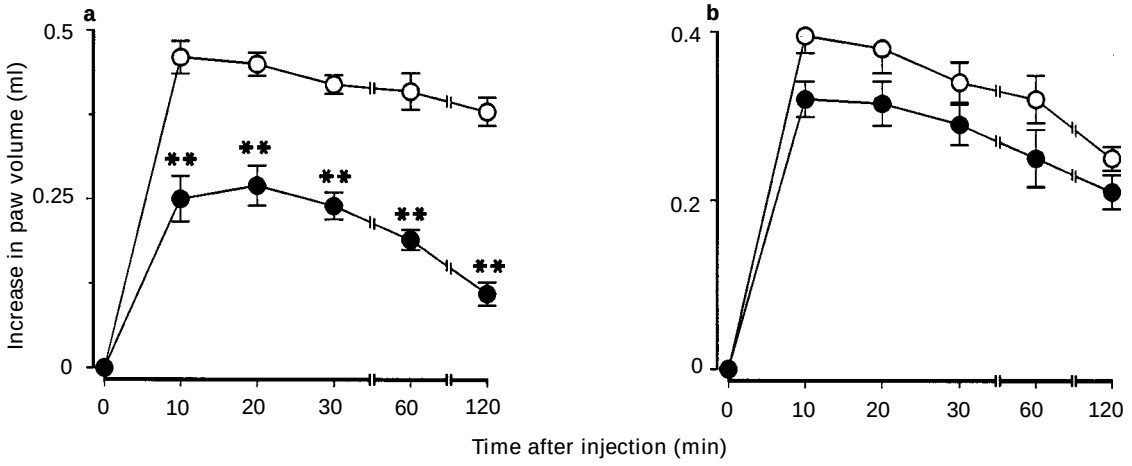

Figure 6 Effect of systemic treatment with indomethacin (2 mg kg^{-1}, i.p. 1 h before experiments) on des-Arg^9-bradykinin (100 nmol/paw) (a) or on tyrosine^8-bradykinin (3 nmol/paw) (b)-induced rat paw oedema, in animals pretreated with BCG (1 dose per animal, 30 days before experiments). Control responses (○) and responses obtained in animals treated with indomethacin (●). Each point represents the mean of 5 rats; vertical lines show s.e.mean. Significantly different from control values: $^{**}P<0.01$.

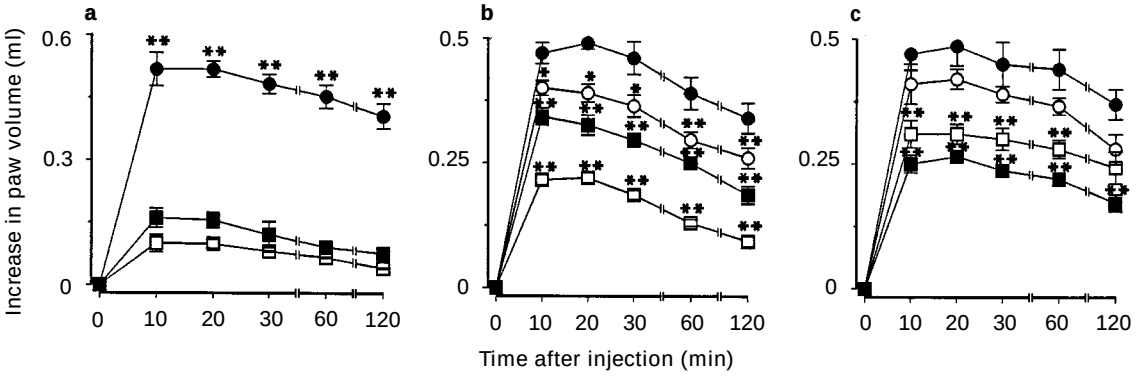

Figure 7 (a) Effect of intradermal injection of des-Arg^9-bradykinin (□, 3 nmol/paw) or of bradykinin (■, 1 nmol/paw), either alone or in combination (●). (b) Effect of intraplantar injection of the selective B_1 receptor antagonist des-Arg^{10}-[Leu^9]-kallidin (DALK) given in combination with des-Arg^9-bradykinin (3 nmol/paw) and bradykinin (1 nmol/paw), on rat hindpaw volume. Control responses (●) and responses obtained in the presence of DALK (nmol/paw): 10 (○); 30 (■) and 50 (□). (c) Effect of intraplantar injection of the selective B_2 receptor antagonist Hoe 140, given in combination with des-Arg^9-bradykinin (3 nmol/paw) and bradykinin (1 nmol/paw) on rat hindpaw volume. Control responses (●) and responses obtained in the presence of Hoe 140 (nmol/paw): 1 (○); 3 (■) and 10 (□). Values represent differences between volumes (in ml) of vehicle-treated (0.1 ml of PBS solution) and drug-injected paws. Each point represents the mean of 6 rats; vertical lines show s.e.mean. In some cases the error bars are hidden with the symbols. Significantly different from control values: $^{*}P<0.05$; $^{**}P<0.01$.

monstrate that in marked contrast with our previous data, the long-term treatment of rats with BCG did not significantly affect oedema induced by the B_2 receptor agonist tyrosine^8-bradykinin, but resulted in a marked upregulation of oedema induced by selective B_1 agonists, des-Arg^9-bradykinin and des-Arg^{10}-kallidin, which had been virtually inactive in naive animals. These observations confirm and also extend our earlier results and support the notion that induction of B_1 receptors might have a significant pathophysiological role in mediating acute inflammation, while both B_1 and B_2 kinin receptors seem to be relevant in the manifestation of chronic inflammatory processes.

Oedema formation induced by intradermal injection of kinins in BCG-treated animals seems to involve the activation of both B_1 and B_2 receptors, as indicated by the following evidence: (1) the potency of the selective B_2 agonist tyrosine^8-bradykinin to induce paw oedema was essentially the same in naive and in BCG-treated animals; (2) des-Arg^9-bradykinin and des-Arg^{10}-kallidin, two known selective B_1 agonists which caused minimal oedema formation in naive animals, produced a dose-related and significant oedema formation in animals pretreated systemically with BCG 30 days before, des-Arg^{10}-kallidin being about 3.5 fold more potent than des-Arg^9-bradykinin, and (3) oedema induced by i.d. injection of tyrosine^8-bradykinin or des-Arg^9-bradykinin in BCG-treated animals was selectively antagonised by intradermal co-injection of either of the B_2 (Hoe 140) or B_1 (des-Arg^9[Leu^8]-bradykinin, des-Arg^{10}[Leu^9]-kallidin and des-Arg^9-NPC 17731) selective antagonists, respectively.

Our results demonstrated, for the first time, that i.d. injection of a very low concentration of the selective B_1 agonist des-Arg^9-bradykinin (3 nmol), together with bradykinin (1 nmol) which caused little paw oedema when injected alone, produced a marked enhancement of the paw oedema when injected together. Such results suggest that interactions between B_1 and B_2 kinin receptors, and also the interactions of both kinin receptors with several inflammatory mediators (Campos & Calixto, 1995; Campos *et al*., 1996) could play a relevant role in maintaining the inflammatory processes. The synergistic interaction of des-Arg^9-bradykinin and bradykinin in BCG-treated animals is believed to be mediated by activation of both B_1 and B_2 kinin receptors, because the selective B_1 and B_2 antagonists, des-Arg^{10}[Leu^9]-kallidin and Hoe 140, respectively, when co-injected intradermically with these mediators, produced a graded inhibition of oedema formation. Hoe 140 was about 12 fold more potent than des-Arg^{10}[Leu^9]-kallidin at the IC_{50} level. Therefore, such results may have physiological and pathological relevance, as kinins acting through B_1 and B_2 receptors are capable of releasing many inflammatory mediators, such as PGE_2, PGI_2, cytokines and neuropeptides, including tachykinins and calcitonin gene-related peptide (Gaginella & Kachur, 1989; Bhoola *et al*., 1992; Hall, 1992; Burch *et al*., 1993).

To assess whether the upregulation of the B_1 receptor which mediates raw paw oedema following long-term systemic treatment with BCG could involve *de novo* protein synthesis, we analysed the effect of systemic treatment of animals with dexamethasone, injected every 24 h, from day 0 to day 30. As shown previously for des-Arg^9-bradykinin-induced oedema in paws desensitized to bradykinin or to tyrosine^8-bradykinin (Campos & Calixto, 1995; Campos *et al*., 1995), or after acute LPS treatment (Campos *et al*., 1996), dexamethasone consistently attenuated paw oedema formation in response to i.d. injection of des-Arg^9-bradykinin in BCG-treated animals. However, dexamethasone at the same dose failed to affect the B_2 constitutive responses mediated by tyrosine^8-bradykinin, thus confirming our previous findings (Campos & Calixto, 1995; Campos *et al*., 1996).

The mechanism by which systemic treatment of rats with BCG produced upregulation of the B_1 receptor in paw oedema has still not been completely defined. There is now considerable evidence supporting the idea that *in vitro* or *in vivo* treatment with cytokine (DeBlois *et al*., 1988; 1991; Ahluwalia & Perreti, 1996), LPS (Campos *et al*., 1996), following ultraviolet irradiation (Perkins & Kelly, 1993), Freud's adjuvant (Perkins *et al*., 1993) or after muramyl-dipeptide systemic treatment (Bouthillier *et al*., 1987), is able to induce upregulation of B_1 receptors, being prevented, in most cases, by both dexamethasone and cycloheximide treatment. It has been shown that release of cytokines (Burch & Tiffany, 1989; Tiffany & Burch, 1989; Ferreira *et al*., 1993), and the release of PGE_2 by kinins, is potentiated by interleukin-1 (IL-1) in human synovial fibroblasts (Bathon *et al*., 1992) and by IL-1 and tumour necrosis factor α (TNFα) in 3T3 fibroblasts (Burch *et al*., 1988; 1989a,b) (for review see: Burch *et al*., 1993). An enhancement of cytokine secretion has been observed namely by IL-1, IL-2, IL-6 or TNFα, *in vivo* (Bohle *et al*., 1990) or *in vitro* in the human bladder carcinoma cell line T24 (DeReijke *et al*., 1993) after treatment with BCG. Thus, most probably, the upregulation of B_1 receptors mediating rat paw oedema after long-term treatment with BCG may be secondary to the cytokine release. In addition, oedema caused by des-Arg^9-bradykinin in animals treated with BCG was significantly prevented by intraperitoneal (1 h before) treatment with indomethacin. Similar results had been obtained in animals treated with LPS (Campos & Calixto, 1995). These results reinforce our previous view, that des-Arg^9-bradykinin oedema formation involves the release of a cyclo-oxygenase product derived from arachidonic acid metabolism.

In summary, this study shows that in contrast to our previous findings (Campos & Calixto, 1995; Campos *et al*., 1995; 1996), long-term systemic treatment of rats with BCG results in a time-dependent upregulation of B_1 agonists des-Arg^9-bradykinin and des-Arg^{10}-kallidin-mediated oedema formation, leaving the response induced by the selective B_2 agonist tyrosine^8-bradykinin unaffected. The kinin-induced oedema formation in BCG-treated animals is believed to be mediated by both B_1 and B_2 receptor subtypes, as demonstrated by the ability of the selective kinin receptor antagonists to prevent such effects. Oedema induced by the B_1 agonist des-Arg^9-bradykinin, but not that caused by the selective B_2 agonist tyrosine^8-bradykinin, was consistently prevented by both dexamethasone and indomethacin, suggesting the possible *de novo* synthesis of these receptors and also the involvement of cyclo-oxygenase products derived from the arachidonic acid pathway. Finally, we have also demonstrated, for the first time, the existence of a synergistic interaction between des-Arg^9-bradykinin and bradykinin after long-term BCG treatment, but not in naive animals. Therefore, the present and also, our previous studies, are consistent with the notion that both B_1 and B_2 kinin receptors play an important role in the control of inflammatory processes.

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