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Upregulation of B_1 receptor mediating des-Arg^9-BK-induced rat paw oedema by systemic treatment with bacterial endotoxin

Maria M. Campos, *Glória E. P. Souza & [1]João B. Calixto

Department of Pharmacology, Centre of Biological Science, Universidade Federal de Santa Catarina, 88015-420 – Florianópolis, SC, Brazil and *Laboratory of Pharmacology, Faculty of Pharmaceutical Sciences, USP, 14049-903 – Ribeirão Preto, SP, Brazil

1 The effect of pretreatment with bacterial endotoxin (LPS, 10 μg, i.v., 24 h) on the bradykinin B_1 and B_2 receptor-induced oedema in the rat paw, and the interaction of B_1-mediated responses with other inflammatory mediators, was investigated.

2 Intraplantar (i.pl.) injection of the selective B_1 agonist, des-Arg^9-BK (DABK, 100 nmol) in naive animals pretreated with the angiotensin converting enzyme inhibitor, captopril caused a small increase in paw volume (0.04 ± 0.003 ml, mean ± s.e.mean, $n=6$), while the B_2-selective agonist, tyrosine^8-bradykinin (T-BK, 3 nmol) induced marked oedema (0.36 ± 0.02 ml). However, i.pl. injection of DABK (3–300 nmol) in rats pretreated with LPS (24 h beforehand) resulted in a marked dose- and time-related increase in paw volume, with mean ED_{50} of 24.1 nmol. In contrast, oedema caused by T-BK (3 nmol) was reduced by $79 \pm 4\%$ in animals treated with LPS when compared with naive animals.

3 Oedema caused by prostaglandin E_2 (PGE_2, 10 nmol) was unaffected by LPS treatment, while oedema induced by histamine (100 nmol), 5-hydroxytryptamine (5-HT, 10 nmol) and substance P (SP, 3 nmol) was reduced ($P<0.05$).

4 The selective B_1 antagonist, des-Arg^9[Leu^8]-BK (100–300 nmol), produced dose-dependent inhibition of DABK (100 nmol)-induced paw oedema in LPS-treated animals with mean IC_{50} of 134 nmol, while the selective B_2 antagonists, Hoe 140 and NPC 17731 (each 10 nmol), had no effect.

5 Treatment of animals with dexamethasone (0.5 mg kg^{-1}, s.c.) 24 or 48 h prior to LPS injection resulted in a graded inhibition of DABK (100 nmol)-induced oedema formation (58 ± 3 and $82 \pm 2\%$, respectively), and almost reversed to control value oedema formation induced by T-BK (3 nmol) in LPS-pretreated rats. Cycloheximide (1 mg kg^{-1}, s.c.) or indomethacin (2 mg kg^{-1}, i.p.) pretreatment 24 and 1 h prior to LPS injection, respectively, markedly inhibited DABK (100 nmol)-induced paw oedema (98 ± 2 and $50 \pm 4\%$, respectively).

6 Intraplantar injection of submaximal dose of DABK (10 nmol) in LPS-treated rats produced modest paw oedema (0.09 ± 0.03 ml). However, i.pl. injections of PGE_2, prostacyclin (PGI_2), calcitonin-gene-related peptide (CGRP), SP, 5-HT, or platelet activating factor (PAF) (each 1 nmol), which alone caused little or no paw oedema, resulted in a potentiation of the DABK-induced oedema. The increases in paw volume (in ml) were: PGE_2 + DABK (0.31 ± 0.03), PGI_2 + DABK (0.39 ± 0.02), CGRP + DABK (0.35 ± 0.04), DABK + SP (0.33 ± 0.04), DABK + 5-HT (0.40 ± 0.02) and DABK + PAF (0.38 ± 0.016) ml. In contrast, histamine (1 nmol) was ineffective in potentiating the response to DABK.

7 The selective B_1 receptor antagonist, DALBK (100–300 nmol), produced dose-dependent inhibition of paw oedema potentiation induced by co-injection of DABK and other mediators with mean ID_{50}s (nmol) of: 180, 160, 139 and 135 in the presence of PGE_2, PGI_2, SP and 5-HT, respectively.

8 These results demonstrate that DABK-induced increase in paw volume in LPS-treated rats is probably mediated by induction of B_1 receptors, associated with downregulation of B_2 receptors. The induction of B_1 receptors by LPS is sensitive to dexamethasone and cycloheximide treatment and requires activation of cyclo-oxygenase pathway. In addition, B_1 receptors, when upregulated following LPS treatment, can interact in a synergistic manner with several inflammatory mediators such as PGI_2, PGE_2, CGRP, PAF and 5-HT. Such results indicate that induction of the B_1 receptor might have a significant pathophysiological role in modulating chronic inflammatory diseases.

Keywords: Paw oedema (rat); bacterial endotoxin (LPS); dexamethasone; cycloheximide; inflammatory mediators; B_1 and B_2 kinin agonists and antagonists

Introduction

Kinins are generated in plasma and peripheral tissues in response to tissue injury or infection following cleavage of low and high-molecular kininogen by the action of kallikrein enzymes. Kinins are well known mediators of several inflammatory states, and, in addition, excite nociceptors causing pain and hyperalgesia (Marceau *et al.*, 1983; Proud & Kaplan, 1988; Steranka & Burch, 1991; Dray *et al.*, 1992; Dray & Perkins, 1993). Moreover, some inflammatory diseases are associated with increased levels of bradykinin (BK) and its active carboxypeptidase metabolites des-Arg^9-BK (DABK) and des-Arg^{10}-Lys-BK (Regoli & Barabé, 1980; Hargreaves *et al.*, 1988; Damas *et al.*, 1990).

Kinin action is mediated by activation of two types of specific membrane receptors, denoted B_1 and B_2. BK preferentially acts through stimulation of constitutive B_2 receptors which are widely distributed both in the peripheral and central nervous systems. On the other hand, the active carboxypeptidase metabolites DABK and des-Arg^{10}-Lys-BK activate

[1]Author for correspondence at: Department of Pharmacology, Univesidade Federal de Santa Catarina, Rua Ferreira Lima, 82, 88015-420, Florianópolis, SC, Brazil.

B_1 receptors specifically. Both B_1 (Menke *et al.*, 1994) and B_2 (McEachern *et al.*, 1991; Hess *et al.*, 1992; 1994; Powell *et al.*, 1993) receptors have been cloned and they are members of the seven transmembrane G protein family of receptors. In contrast to B_2 receptors, B_1 receptors are rarely expressed in nontraumatized tissues, and are normally induced following *in vitro* incubation for long periods, after tissue trauma or infection or *in vivo* following treatment of animals with bacterial lipopolysaccharide endotoxin. However, it has been proposed that B_1 receptors may make an important contribution during inflammatory processes where both higher levels of the active BK metabolites (DABK and des-Arg^{10}-Lys-BK) and an increased of induction of B_1 receptors exist (Regoli & Barabé, 1980; Bathon & Proud, 1991; Marceau & Regoli, 1991; Bhoola *et al.*, 1992; Burch *et al.*, 1993).

We have recently shown that both B_1 and B_2 receptors are involved in kinin-induced oedema formation in the rat paw. The B_2 receptors are constitutive, but induction of B_1 receptors was demonstrated following complete densensitization of the paw to BK for seven consecutive days (Campos & Calixto, 1995). In addition, very low doses of inflammatory mediators such as prostaglandin E_2 (PGE_2), prostacyclin (PGI_2), calcitonin gene-related peptide (CGRP), 5-hydroxytryptamine (5-HT), platelet activating factor (PAF), but not histamine markedly potentiate oedema induced by BK, and these mediators are able to attenuate partially BK-induced paw oedema desensitization (Campos & Calixto, 1995).

In this study we have examined the modulatory role of systemic treatment of animals with bacterial endotoxin (LPS) 24 h beforehand on the selective B_1 and B_2 kinin agonist-induced oedema in the rat paw. In addition, we have also evaluated the possible synergistic interactions of oedema formation induced by the B_1-selective agonist, DABK, with several inflammatory mediators. The effects of the steroidal and non-steroidal anti-inflammatory drugs and the protein synthese inhibitor, cycloheximide, on upregulation of B_1 receptor-mediated oedema formation were also investigated.

Methods

Measurement of rat paw oedema

Experiments were conducted on non-fasted male Wistar rats (140–200 g) housed at 22±2°C with a 12 h:12 h light-dark cycle (lights on at 06 h 00 min). In experiments with BK and related kinins, animals were pretreated with the angiotensin converting enzyme inhibitor, captopril (5 mg kg^{-1}, s.c.) 1 h prior to the experiment in order to prevent kinin degradation (Corrêa & Calixto, 1993). Under ether anaesthesia, the animals received 0.1 ml intraplantar injections in one hindpaw of phosphate buffered saline (PBS; composition mmol l^{-1}: NaCl 137, KCl 2.7 and phosphate buffer 10) containing BK, DABK, tyrosine^8-BK(T-BK) (1 to 300 nmol), either alone or mixed with PGE_2, PGI_2, CGRP, SP, 5-HT, PAF or histamine (each, 1 nmol). The contralateral paw received 0.1 ml PBS and was used as a control. Oedema was measured by use of a 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 inflammatory mediators. Oedema is expressed in ml as the difference between the test and control paws. In most experiments, animals were pretreated with *E. coli* endotoxin (LPS 10 μg per animal, i.v., 24 h beforehand). Control animals received the same volume (0.1 ml per animal, i.v., 24 h prior to PBS).

Influence of drugs on des-Arg^9-BK-induced oedema formation in LPS pretreated rats

In a separate series of experiments, in order to confirm the involvement of B_1 receptors on DABK-induced rat paw oedema, animals pretreated with LPS 24 h beforehand, received the selective B_1 agonist, DABK (100 nmol)-co-injected with the selective B_1 (des-Arg^9[Leu^8]-BK, 100 to 300 nmol) or B_2 (Hoe 140 or NPC 17731, 10 nmol) receptor antagonist. To assess the possible participation of *de novo* protein synthesis on paw oedema induced by DABK, rats were pretreated with the anti-inflammatory steroid, dexamethasone (0.5 mg kg^{-1}, s.c., −24 h or −48 h) or with cycloheximide (a protein synthesis inhibitor, 1 mg kg^{-1}, −24 h) before testing. Other rats received the cyclo-oxygenase inhibitor, indomethacin (2 mg kg^{-1}, i.p., −1 h) before challenge with DABK. All animals received i.v. LPS 24 h (or control) prior to the experiments.

Drugs

The following drugs were used: BK, T-BK, PGE_2, iloprost, (a stable analogue of PGI_2), CGRP, SP, 5-HT, histamine, captopril, dexamethasone, indomethacin, cycloheximide, bacterial lipopolysaccharide (*Escherichia coli* serotype 0111, L=2630) (all from Sigma Chemical Company, St. Louis, U.S.A.), DABK and des-Arg^9[Leu^8]-BK (Peninsula Belmont, CA, U.S.A.), PAF (Bachem, Switzerland). Hoe 140 (D-Arg^0- [Hyp^3, Thi^5, D-Tic^7, Oic^8]-BK) and NPC 17731 {D-Arg^0[Hyp^3, D-HypE (transpropyl)^7], Oic^8]-BK} were kindly supplied by Hoechst (Frankfurt Main, Germany) and by Scios/Nova (Baltimore, U.S.A.), respectively. 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 NaCl.

Statistical analysis

The results are presented as the mean±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, and differences with $P<0.05$ were considered significant.

Results

Intraplantar injection of the selective B_1 agonist, DABK (100 nmol), in the naive animals pretreated with captopril caused a very small increase in paw volume (0.04±0.003 ml, mean±s.e.mean, $n=6$), while the B_2-selective agonist, T-BK (3 nmol), produced a marked and dose-related increase in paw volume with an ED_{50} value (and 95% confidence limits) of 1.3 (1.0–1.5) nmol (Figure 1 and results not shown). Intraplantar injection of DABK (100 nmol/paw), in animals that had been treated with LPS (10 μg per rat, i.v.), resulted in a marked increase in paw volume (0.46±0.08 ml). In sharp contrast, oedema formation in response to the selective B_2 agonist, T-BK (3 nmol), was reduced by 79±4% in LPS pretreated animals (Figure 1a and b). The same treatment of animals with LPS did not modify the oedema in response to PGE_2 (10 nmol), but reduced by 25±2.0; 17±2.5 and 18±4.0% oedema induced by histamine (100 nmol), SP (3 nmol) and 5-HT (10 nmol), respectively (Figure 2). Previous treatement of animals with dexamethasone (0.5 mg kg^{-1}) 24 or 48 h prior to challenge with LPS caused a dose-related inhibition of DABK (100 nmol)-induced paw oedema (58±3 and 82±2%, respectively), (Figure 1b). In marked contrast, the same treatment of animals with dexamethasone almost completely reversed to control values oedema caused by T-BK (3 nmol) in animals pretreated with LPS (Figure 1a).

Intraplantar injection of DABK (3 to 300 nmol) caused

dose-related increases in oedema with mean ED_{50} and maximal oedema value at 300 nmol of 24.1 (20.2–28.1) nmol and 0.46±0.05 ml, respectively (Figure 3a). Oedema induced by DABK (100 nmol) in LPS-treated animals was inhibited in a dose-related manner by co-injection of the selective B_1 receptor antagonist, des-Arg^9[Leu^8]-BK (100 to 300 nmol) with a mean ID_{50} value of 134.2 (129.4–143.3) nmol (Figure 3b). However, the co-injection of the selective B_2 receptor antagonists, Hoe 140 or NPC 17731 (each 10 nmol), at doses which have been shown previously to antagonize completely BK-mediated rat paw oedema (Campos & Calixto, 1995; Campos *et al.*, 1995), did not affect DABK (100 nmol)-induced paw oedema in LPS-treated animals (Figure 3c).

The intraplantar injection of a low dose of DABK (10 nmol), PGE_2, PGI_2, CGRP, SP, 5-HT or PAF (each 1 nmol) caused little or no increase in paw volume (Figures 4 and 5). However, co-administration of PGE_2, PGI_2, CGRP, SP, 5-HT or PAF (each 1 nmol) with DABK (1 nmol) resulted in significantly greater paw oedemas (Figures 4 and 5). The

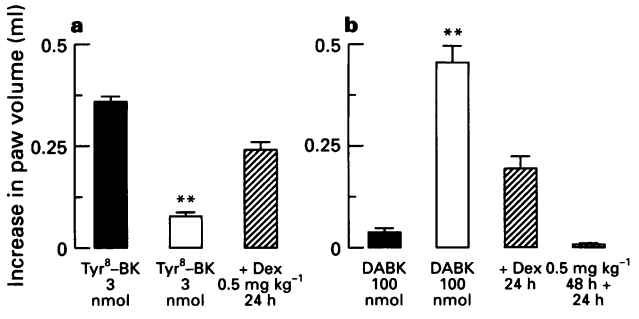

Figure 1 Rat paw oedema caused by intraplantar injection of tyrosine^8-BK (Tyr^8-BK, 3 nmol/paw, a) and by des-Arg^9-BK (DABK, 100 nmol/paw, b) in saline pretreated (solid columns) or in LPS-pretreated animals (open columns). The hatched columns represent the effect of pretreatment of rats with dexamethasone (Dex, 0.5 mg kg^{-1}, s..c.) 48 and 24 h prior to i.v. LPS. Values represent the differences between volumes (in ml) of vehicle-treated (0.1 ml of PBS solution) and drug-injected paws. Each column represents the mean±s.e.mean of 6 rats. The oedema was measured 20 min after intraplantar injection of peptides. Significantly different from control or from LPS treated animals: $^{**}P<0.01$.

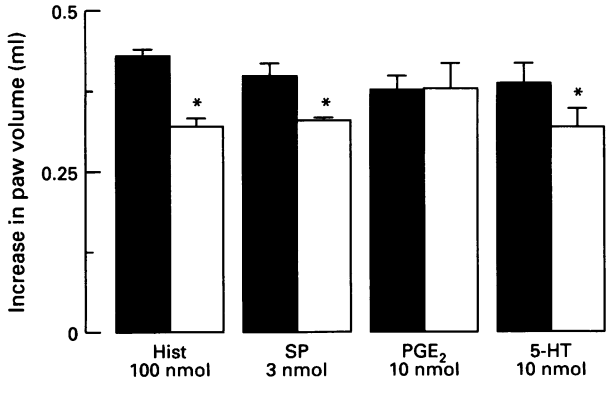

Figure 2 Effect of pretreatment of rats with LPS on oedema induced by histamine (Hist) (100 nmol/paw), substance P (SP) (3 nmol/paw), prostaglandin E_2 (PGE_2) (10 nmol/paw) or 5-hydroxytryptamine (5-HT) (10 nmol/paw). Each column (mean±s.e.mean of $n=5$) represents the oedema measured 20 min after intraplantar injection of inflammatory mediators: control, solid columns, LPS-treated animals, open columns. Significantly different from control: $^{*}P<0.05$.

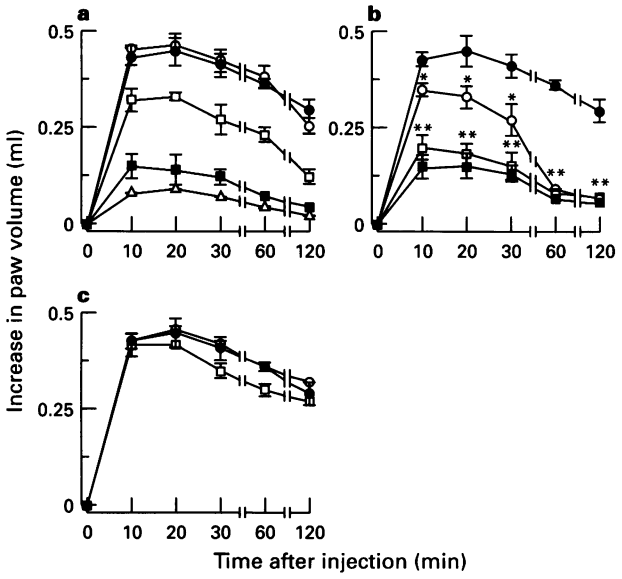

Figure 3 Dose- and time-response curves showing the increase of rat paw oedema caused by intraplantar injection of the selective B_1 receptor agonist, des-Arg^9-BK (DABK, △3; ■ 10; □ 30; ● 100 and ○ 300 nmol/paw) in LPS pretreated-animals (a). Rat hindpaw volume caused by intraplantar injection of selective B_1 receptor agonist, DABK alone (100 nmol/paw, ●) or in combination with the selective B_1 receptor antagonist des-Arg^9[Leu^8]-BK (DALBK, ○ 100; □ 200 and ■ 300 nmol/paw) (b). Effect of co-injection of Hoe 140 (○) or NPC 17731 (□) (10 nmol/paw) on des-Arg^9-BK-induced paw oedema in LPS pretreated rats. (c) Values represent the differences between volumes (in ml) of vehicle-treated (0.1 ml of PBS solution) and drug-injected paws. Each point represents the mean±s.e.mean of $n=5-6$. In some cases the error bars are hidden within the symbols. Significantly different from control values: $^{*}P<0.05$; $^{**}P<0.01$.

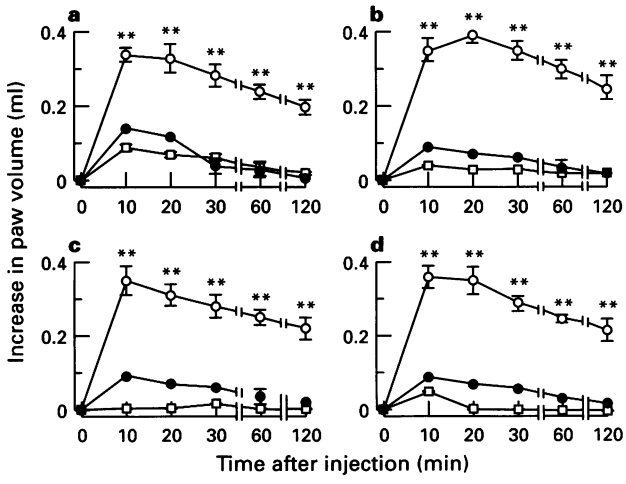

Figure 4 Effect of intraplantar injection of des-Arg^9-BK (DABK, 10 nmol/paw) in LPS pretreated animals, given alone or in combination with other inflammatory mediators, on rat hindpaw volume. Values represent differences between volumes (in ml) of vehicle-treated (0.1 ml of PBS solution) and drug-injected paws. DABK was injected either alone (● 10 nmol/paw in all panels), or in combination with SP (a), PGI_2 (b), PGE_2 (c) or CGRP (d) each 1 nmol/paw (○). The effects of each mediator alone are also shown in their respective panels (□, 1 nmol/paw). Each point represents the mean±s.e.mean of $n=5$. In some cases the error bars are hidden within the symbols. Significantly different from control values: $^{**}P<0.01$.

increases in paw volumes (in ml) induced by co-injection of DABK with each mediator (mean ± s.e.mean) were: PGE_2 (0.31 ± 0.03), PGI_2 (0.39 ± 0.02), CGRP (0.35 ± 0.04), SP (0.33 ± 0.04), 5-HT (0.40 ± 0.02) or PAF (0.38 ± 0.016) ml. In contrast, histamine (1 nmol) did not potentiate DABK-induced paw oedema (Figure 5c).

The co-injection of des-Arg^9[Leu^8]-BK (100–300 nmol) with DABK (10 nmol) plus one of the inflammatory mediators (each 1 nmol) caused a dose-dependent and significant inhibition of the paw oedema induced by DABK (1 nmol) in LPS-treated animals with the following mean ID_{50}s (nmol): 180 (143–195); 139 (130–145); 160 (139–181) and 135 (121–144), in the presence of PGE_2, PGI_2, SP and 5-HT, respectively (Figure 6).

Previous treatment of animals with indomethacin (2 mg kg^{-1}, i.p.) 1 h beforehand or with cycloheximide (1 mg kg^{-1}, s.c.) 24 h prior to LPS injection, significantly inhibited DABK (100 nmol)-induced paw oedema (50 ± 4 and 98 ± 2%, respectively), control response (mean ± s.e.mean, of 0.45 ± 0.02 versus 0.22 ± 0.01 and 0.02 ± 0.005 ml in animals pretreated with indomethacin and cycloheximide, respectively (n = 6)).

Discussion

In the present study we have demonstrated that intraplantar injection of the selective B_1 receptor agonist, DABK, was virtually inactive in naive paws. However DABK elicited significant and dose-related oedema formation in rats pretreated 24 h beforehand with LPS. DABK was about 18 fold less potent, although more efficacious, when compared with the response elicited by the selective B_2 agonist, T-BK in naive animals. By comparison, oedema caused by the selective B_2 agonist, T-BK, was greatly reduced following i.v. LPS, supporting our previous notion that, in this model, the upregulation of B_1 receptors is associated with downregulation of constitutive B_2 receptors (Campos & Calixto, 1995). The induction of B_1 receptor-mediated paw oedema following systemic LPS treatment seems to be a specific phenomenon, because the same treatment with LPS did not significantly affect oedema formation induced by PGE_2, and reduced paw oedema caused by histamine, SP and 5-HT.

Oedema formation induced by intraplantar injection of DABK in LPS-treated animals is believed to be mediated exclusively by stimulation of B_1 but not B_2 receptors, because the oedema induced by DABK was blocked in a dose-dependent manner by the selective B_1 receptor antagonist, des-Arg^9[Leu^8]-BK, and was unaffected by the selective B_2 receptor antagonists, Hoe 140 and NPC 17731. Similar induction of B_1 receptor had been described following *in vivo* administration of LPS in several models, such as the increase of responsiveness to DABK in the cardiovascular system (Regoli *et al.*, 1981; Marceau *et al.*, 1984; Deblois *et al.*, 1989), the exacerbation of formalin-induced nociception in the mouse paw (Campos *et al.*, 1995), the treatment by some cytokines (Deblois *et al.*, 1988; 1991), and following induction of arthritis by antigens (Farmer *et al.*, 1991; Cruwys *et al.*, 1994), as well as by ultraviolet and thermal-induced hyperalgesia (Perkins *et al.*, 1993; Perkins & Kelly, 1993).

A new and relevant finding of the present study are the results showing that as reported previously for BK-mediated increase in paw volume (Campos & Calixto, 1995) and BK-induced increase of vascular permeability (Brain & Williams, 1989; Buckley *et al.*, 1991; Cruwys *et al.*, 1992), the B_1 receptor agonist, DABK, can also interact synergistically with several inflammatory mediators following systemic treatment with LPS. This view is substantiated by the results showing that co-injection of very low doses of PGE_2, PGI_2, SP, CGRP, PAF or 5-HT (each 1 nmol), when injected alone caused little or no oedema formation, but induced a pronounced potentiation of DABK-induced increase in paw volume. Interestingly, histamine, when co-injected with DABK, failed to potentiate the B_1 agonist-mediated increase in paw volume. This has been reported previously for BK (Campos & Calixto, 1995). These results suggest that although histamine has an important role as an inflammatory mediator, it does not have the ability to potentiate kinin-mediated oedema formation. The potentiating effect of inflammatory mediators on the B_1-mediated response of DABK in LPS-treated animals seems to involve B_1 receptors, as revealed by the fact that the oedema elicited by co-injection of DABK together with each inflammatory mediator

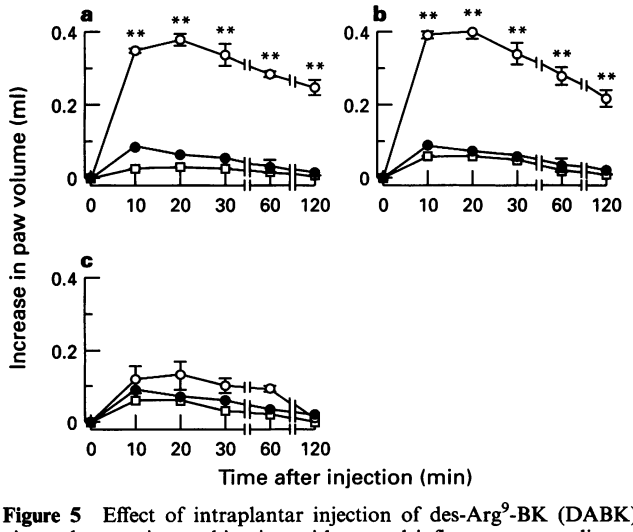

Figure 5 Effect of intraplantar injection of des-Arg^9-BK (DABK), given alone or in combination with several inflammatory mediators, on rat hindpaw volume. Values represent the differences between volumes (in ml) of vehicle-treated (0.1 ml of PBS solution) and drug-injected paws. DABK was injected either alone (●, 10 nmol/paw in all panels), or in combination with PAF (a), 5-HT (b) or histamine (c), all 1 nmol (○). The effects of each mediator alone are also shown in their respective panels (□, 1 nmol/paw). Each point represents the mean ± s.e.mean of n = 5. In some cases the bars are hidden within the symbols. Significantly different from control values: $^{**}P<0.01$.

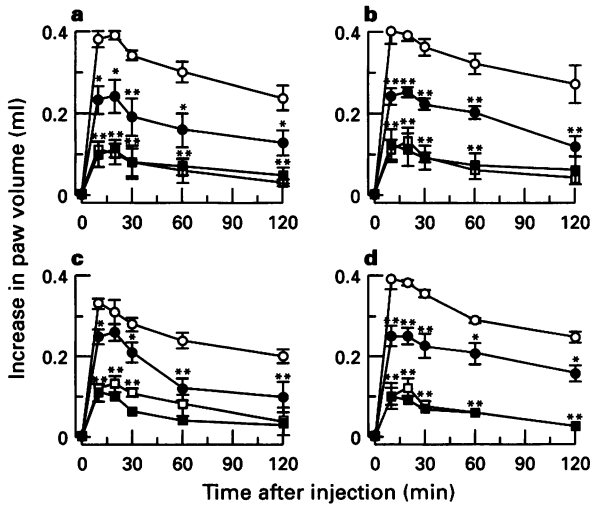

Figure 6 Effect of intraplantar injection of the selective B_1 antagonist, des-Arg^9-[Leu]^8-BK (100 nmol/paw, ●; 200 nmol/paw, □ or 300 nmol/paw, ■) given in combination with des-Arg^9-BK (10 nmol/paw) and SP (a), PGI_2 (b), PGE_2 (c) and 5-HT (d), all 1 nmol/paw (○) on rat paw volume. Each point represents the mean ± s.e.mean of n = 5. In some cases the error bars are hidden within the symbols. Significantly different from control: $^{*}P<0.05$; $^{**}P<0.01$.

was dose-dependently blocked by the B_1 receptor antagonist des-Arg^9[Leu^8]-BK, and was not affected by the selective B_2 receptor antagonists Hoe 140 and NPC 17731. It has been shown that, as reported for BK, the selective B_1 agonist, DABK, is able to stimulate the release of some inflammatory mediators, such as PGI_2 and PGE_2 (Toda *et al.*, 1987; Cahill *et al.*, 1988; Galizzi *et al.*, 1994) and interleukin-1 (IL-1) (Burch *et al.*, 1989; Tiffany & Burch, 1989; Lerner & Modeer, 1991). In addition, a synergism between DABK and IL-1 has been demonstrated to occur in increasing PGE_2 formation in human gingival and synovial fibroblasts (Lerner & Modeer, 1991; Bathon *et al.*, 1992; Lerner *et al.*, 1992). Further, IL-1 and IL-2 have been demonstrated to induce kinin B_1 receptor *in vitro* (Deblois *et al.*, 1988). As systemic treatment with LPS is capable of upregulating certain cytokine genes of macrophage, neutrophils and fibroblasts *in vivo* (Ulich *et al.*, 1992; Cockfield *et al.*, 1993; Huleihel *et al.*, 1993), through steroidal (Geiger *et al.*, 1993; Ochalski *et al.*, 1993; Pang *et al.*, 1994), and non-steroidal-sensitive mechanisms (Ochalski *et al.*, 1993; Ogle *et al.*, 1994), it seems apparent that the induction of B_1 receptor-mediated oedema formation in the rat paw after LPS treatment is mediated by cytokine release. Together, these findings are consistent with the view that the induction of B_1 receptors in animals pretreated with LPS plays an important role in maintaining the chronic inflammatory processes, as already reported in hyperalgesic and some inflammatory models (Burch *et al.*, 1989; Bhoola *et al.*, 1992; Dray & Perkins, 1993; Perkins *et al.*, 1993; Campos & Calixto, 1995; Campos *et al.*, 1995). The mechanism by which DABK interacts synergistically with various inflammatory mediators, and whether this phenomenon also occurs in other *in vivo* B_1-mediated models involving pain and cell migration, remains to be established.

As reported previously for the upregulation of B_1 receptor-mediated rat paw oedema after desensitization of B_2 receptors (Campos & Calixto, 1995), the oedema formation in response to intraplantar injection of DABK in LPS-treated animals was consistently attenuated by pretreatment of animals with dexamethasone 48 and 24 h prior to injection of LPS. Interestingly, the same treatment with dexamethasone failed to modify BK-induced paw oedema, an effect mediated by constitutive B_2 receptors (Campos & Calixto, 1995). In addition, previous treatment of animals with the protein synthesis inhibitor, cycloheximide, almost completely abolished DABK-mediated paw oedema, indicating that the oedematogenic response caused by DABK in LPS-treated animals is probably mediated by *de novo* induction of dexamethasone- and cycloheximide-sensitive B_1 kinin receptors. Evidence indicates that dexamethasone and cycloheximide are capable of preventing the induction of the B_1 receptor following tissue trauma or infection (Regoli *et al.*, 1978; Marceau *et al.*, 1980; Whalley *et al.*, 1983; Bouthillier *et al.*, 1987; Deblois *et al.*, 1988; Campos & Calixto, 1995; Campos *et al.*, 1995). The present results also show that indomethacin reduced the paw oedema in LPS-treated rats, suggesting an involvement of arachidonic acid metabolites in the oedematogenic response following B_1 receptor activation.

In summary, the current results demonstrate that DABK elicits a dose- and time-related increase in paw volume in animals treated with LPS, but not in naive animals, an effect clearly mediated through activation of B_1 receptors. The oedema caused by this B_1-selective agonist in LPS-treated animals requires activation of products from the arachidonic acid pathway and involves induction of B_1 receptors. The current results also extend and confirm our previous results that upregulation of B_1 receptors in this model is associated with downregulation of B_2 receptors (Campos & Calixto, 1995). Finally, our data show that B_1 receptors, when upregulated after systemic LPS treatment, may interact synergistically with several inflammatory mediators such as PGI_2, PGE_2, CGRP, PAF and 5-HT, but not histamine. Taken together, these results support the notion that induction of B_1 receptors largely contributes to the control of chronic inflammatory processes.

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