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In vivo B_1 kinin-receptor upregulation. Evidence for involvement of protein kinases and nuclear factor κB pathways

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1 Intradermal (i.d.) injection of cytokines, IL-1β and TNFα (5 ng, 60 and 30 min prior) produces a rapid onset up-regulation of des-Arg^9-BK-mediated rat paw oedema. Here we analyse the mechanisms involved in des-Arg^9-BK-induced oedema in animals pre-treated with IL-1β or TNFα.

2 Co-injection of anti-IL-1β, anti-TNFα and anti-IL-8 (50 ng) significantly inhibited des-Arg^9-BK-induced oedema in animals pre-treated with IL-1β (65, 37 and 42%) or TNFα (39, 64, 25%). IL-1 receptor antagonist (IRA, 100 μg) or IL-10 (10 ng) inhibited the oedema caused by des-Arg^9-BK, in rats that had received either IL-1β (67 and 63%) or TNFα (46 and 35%).

3 Co-injection of the PKC inhibitors, staurosporine (10 nmol) or RO 318220 (30 nmol) inhibited des-Arg^9-BK-induced paw oedema (44 and 42% for IL-1β and, 53 and 30% for TNFα, respectively). Genistein (tyrosine kinase inhibitor, 2.5 mg kg^{-1}, s.c.) or PD 098059 (MAP-kinase inhibitor, 30 nmol) produced marked inhibition of des-Arg^9-BK-induced oedema (58 and 39% for IL-1β and 31 and 35% for TNFα respectively).

4 The NF-κB inhibitors TLCK (2 mg kg^{-1}, i.p.) and PDCT (100 mg kg^{-1}, i.p.) significantly inhibited the oedema of des-Arg^9-BK in IL-1β (27 and 83%) or TNFα (28 and 80%) pre-treated animals.

5 It is concluded that up-regulation of B_1 receptors modulated by IL-1β or TNFα involves the release of other cytokines, activation of PKC and tyrosine kinase pathways, co-ordinated with the activation of MAP-kinase and nuclear factor κB, reinforcing the view that B_1 receptors may exert a pivotal role in modulating chronic inflammatory processes.

Keywords: B_1 kinin receptor; cytokines; protein kinases; nuclear factor κB

Abbreviations: BCG, *Mycobacterium bovis* bacillus Calmette-Guérin; BK, bradykinin; des-Arg^9-BK, des-Arg^9-bradykinin; IL, interleukin; IRA, interleukin receptor antagonist; MAP-kinase, mitogen-activated protein kinase; NF-κB, nuclear factor κB; PBS, phosphate buffered saline; PDTC, pyrrolidine-dithiocarbamate; PKC, protein kinase C; TLCK, Nα-tosyl-L-chloromethylketone; TNFα, tumor necrosis factor α

Introduction

Kinins are endogenous peptides formed in plasma and peripheral tissues in response to trauma or infection, or during inflammatory processes, from kininogen precursors, by the action of serine protease kallikreins. Kinins are involved in many physiological processes, such as control of blood pressure, increase of blood flow and vascular permeability, oedema formation at the site of injury and induction of pain and hyperalgesia (Regoli & Barabé, 1980; Bhoola *et al*., 1992; Hall, 1992).

The actions of kinins are mediated by the activation of two receptor subtypes, B_1 and B_2. The genes that encode these receptors have been cloned in several animal species, being receptors with seven transmembrane domains, coupled to G proteins (Eggerix *et al*., 1992; Hess *et al*., 1992; 1994; McEachern *et al*., 1991; Pesquero *et al*., 1996). The B_2 receptors are constitutively expressed throughout either the central or peripheral nervous system, mediating the majority of pharmacological actions evoked by kinins and exhibiting high affinity for bradykinin (BK) and kallidin. On the other hand, the B_1 receptors show higher affinity for the kinin metabolites des-Arg^9-BK and des-Arg^{10}-kallidin, and are in some manner atypical, as their expression is often inducible. The B_1 receptors are upregulated after tissue trauma or following challenge of animals with certain agents, such as endotoxins, Freund's adjuvant, some cytokines or *Mycobacterium bovis* bacillus Calmette-Guérin (BCG) (Marceau, 1995; 1997; Campos *et al*., 1996; 1997; Hall, 1997; Hall & Morton 1997).

Recently, it has been suggested that the mechanisms mediating the expression of inducible receptors, including B_1, may involve the activation of a common signalling pathway, involving some specific transcription factors, allowing the rapid expression of the receptors, under some special circumstances (Donaldson *et al*., 1997). Nuclear factor κB (NF-κB) is a well-characterized transcription factor, activated by several stimuli, especially bacterial products and some of the cytokines. Its most usual form is a heterodimer, composed of two subunits p50 and p65, that is normally found inactive in cytoplasm and bound to an inhibitory protein 1κ-B. When cells are stimulated, specific kinases phosphorylate l-κB, causing its rapid degradation. Then, NF-κB translocates to the nucleus and activates a series of target genes, including cytokines, inflammatory enzymes and inflammatory receptors (Barnes & Adcock, 1997; Barnes & Karin, 1997; Donaldson *et al*., 1997). The mechanism(s) by which lκB is phosphorylated remains unclear, but recent data (Ghoda *et al*., 1997) suggest the possible involvement of a protein with 90 KDa, which belongs to the mitogen-activated protein-kinase (MAP-kinases) family. This class of

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proteins includes a group of serine/threonine kinases, which are activated by dual phosphorylation on the threonine and tyrosine residues, in response to many extracellular signs (Whitmarsh & Davis, 1996). It has recently been demonstrated (Larrivé *et al*., 1998) that B_1 receptor up-regulation in isolated rabbit aorta involves the activation of some MAP-kinases. Ni *et al*. (1998) reported the existence of a sequence containing a NF-κB-like binding site on the promoter of the human kinin B_1 receptor, sufficient for the B_1 receptor transcription, following exposure to some inflammatory agents such as LPS, IL-1β or TNFα in vascular smooth muscle cells. In addition, it has also been shown that in cultured human lung fibroblasts, the upregulation of kinin B_1 receptor by stimulus with IL-1β is strongly related to the activation of NF-κB (Schanstra *et al*., 1998). However, in spite of this molecular evidence, so far there are no *in vivo* studies demonstrating the role played by NF-κB and kinase phosphorylation on the upregulation of the B_1 receptor.

We have recently demonstrated that i.d. injection of the cytokines IL-1β and TNFα produces a rapid up-regulation of B_1 receptor-mediated rat paw oedema, without affecting the responses mediated by activation of B_2 receptors, in a process largely sensitive to dexamethasone and cycloheximide, requiring the activation of both cyclo-oxygenase-1 and 2 (Campos *et al*., 1998). In the present study, we have therefore investigated, in detail, some *in vivo* mechanisms involved in kinin B_1 receptor up-regulation following i.d. injection of IL-1β or TNFα. Attempts have also been made in order to investigate further the role of some specific kinases, and also, the participation of NF-κB pathways in modulating the increase of functional responses mediated by B_1 receptors *in vivo*, in animals treated with IL-1β or TNFα.

Methods

Measurement of rat paw oedema

Experiments were conducted with non-fasted male Wistar rats (140–180 g) kept in controlled room temperature ($22 \pm 2^{\circ}$C) under a 12 h : 12 h light-dark cycle (lights on 06.00 h). All animals were pre-treated with the angiotensin-converting enzyme inhibitor, captopril (5 mg kg^{-1}, s.c.) 1 h before any experiment, in order to prevent the degradation of peptides (Corrêa & Calixto, 1993; Campos & Calixto, 1995). The animals received a 0.1 ml i.d. injection in one hindpaw (right paw) of phosphate buffered saline (PBS, composition mmol l^{-1}: NaCl 137, KCL 2.7 and phosphate buffer 10) containing des-Arg^9-BK (100 nmol paw^{-1}) or BK (3 nmol paw^{-1}). The contralateral paw (left paw) received 0.1 ml of PBS and was used as 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) after injection of peptides. Oedema is expressed in ml as the difference between the right and left paws.

In most experiments, animals were treated with the cytokines IL-1β or TNFα (5 ng paw^{-1}) (60 and 30 min before injection of des-Arg^9-BK, respectively) at the same site of injection of kinins, as described previously (Campos *et al*., 1998). In all experiments, the i.d. injections were performed under slight anaesthesia with 2,2,2 tribromoethanol (0.125 g kg^{-1}). The reported experiments were carried out in accordance with current guidelines for the care of laboratory animals and ethical guidelines for investigations of experiments in conscious animals (Zimmermann, 1983).

Analysis of the mechanisms involved in B_1-receptor-mediated paw oedema in rats pre-treated with IL-1β or TNFα

In another series of experiments, to assess the possible participation of secondary cytokine synthesis on des-Arg^9-BK-induced rat paw oedema, animals received an i.d. injection of the anti-murine antibodies anti-IL-1β, anti-TNFα, anti-IL-8 or anti-IL-6 (50–100 ng paw^{-1}), co-injected with the cytokines IL-1β and TNFα (both 5 ng paw^{-1}), 60 and 30 min before the injection of des-Arg^9-BK (100 nmol paw^{-1}), respectively. Other animals received IL-1β or TNFα (5 ng paw^{-1}) in association with the recombinant interleukin receptor antagonist (IRA, 100 μg paw^{-1}) or with the anti-inflammatory cytokine, IL-10 (10–30 ng paw^{-1}).

To investigate the possible involvement of protein kinase C (PKC) activation in rat paw oedema-induced by des-Arg^8-BK animals received an i.d. injection of staurosporine (10–50 nmol paw^{-1}) or RO 318220 (30–50 nmol paw^{-1}) in combination with either IL-1β or TNFα (5 ng paw^{-1}). Other groups of rats received an i.d. injection of PD98059 (a MAP-kinase inhibitor, 30 to 50 nmol paw^{-1}) in order to evaluate the participation of MAP-kinase pathway in B_1 receptor upregulation after IL-1β or TNFα i.d. injection. In a separate series of experiments, the animals were pre-treated with the tyrosine-kinase inhibitor, genistein (2.5 to 5 mg kg^{-1}, s.c.) 30 min before injection of cytokines. Finally, to assess the possible involvement of NF-κB in development of B_1-receptor mediated paw oedema in rats that had received IL-1β or TNFα, the animals were pretreated with the NF-κ-B inhibitors, PDCT (100 mg kg^{-1}, i.p.) or TLCK (2 mg kg^{-1}, i.p.) 30 min before injection of the proinflammatory cytokines. In other groups of experiments, to assess the selectivity of these drugs, the effects of staurosporine, RO318220, PD098059, genistein, PDTC or TLCK (at the same range of doses) were tested against BK (3 nmol paw^{-1})-induced rat paw oedema.

Drugs

The following drugs were used: des-Arg^9-bradykinin, bradykinin, captopril, staurosporine, Nα-tosyl-L-chloromethylketone (TLCK), pyrrolidine-dithiocarbamate (PDCT). 2,2,2 tribromoethanol, PBS tablets (all from Sigma Chemical Company, St. Louis, U.S.A.). Genistein, PD98059 and RO31820 (Research Biochemical International, RBI, MA, U.S.A.). Recombinant murine cytokines IL-1β (Lot BN091), TNFα (Lot CS184) and IL-10 (Lot BC110); anti-murine neutralizing antibodies anti-IL-1β (Lot B01D3), anti-TNFα (Lot CT101), anti-IL-6 (Lot BF 123), anti-IL-8 (Lot BB282) and human recombinant interleukin receptor antagonist (IRA) were obtained from R&D Systems Inc., Minneapolis, U.S.A. The stock solutions for all peptides used were prepared in PBS 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^{-1} NaCl solution.

Statistical analysis

The results are presented as the mean $\pm$ s.e.mean of 4–6 animals. The percentages of inhibition are reported as mean $\pm$ s.e.mean of inhibitions obtained in each individual experiment at the peak of oedema (20 min after injection of the peptides). Statistical comparison of the data was performed by analysis of variance (ANOVA) followed by Dunnett's test or by use of Student's unpaired *t*-test. *P* values less than 0.05 were considered significant.

Results

As reported previously (Campos & Calixto, 1995; Campos *et al*., 1996; 1997), i.d. injection of the selective kinin B_1 receptor agonist des-Arg^9-BK (100 nmol paw^{-1}) in naive animals produced a very weak increase in rat paw oedema (0.07 ± 0.004 ml, $n=5$). In contrast, i.d. injection of des-Arg^9-BK in rats that had been treated i.d. with either IL-1β (5 ng paw^{-1}, 60 min before) or TNFα (5 ng paw^{-1}, 30 min before) (Campos *et al*., 1998) caused a marked increase in rat paw oedema (0.58 ± 0.02 and 0.44 ± 0.03 ml, $n=5$, respectively).

The oedemas induced by des-Arg^9-BK (100 nmol paw^{-1}) in rats pre-treated with either IL-1β (Figure 1A, C and E) or

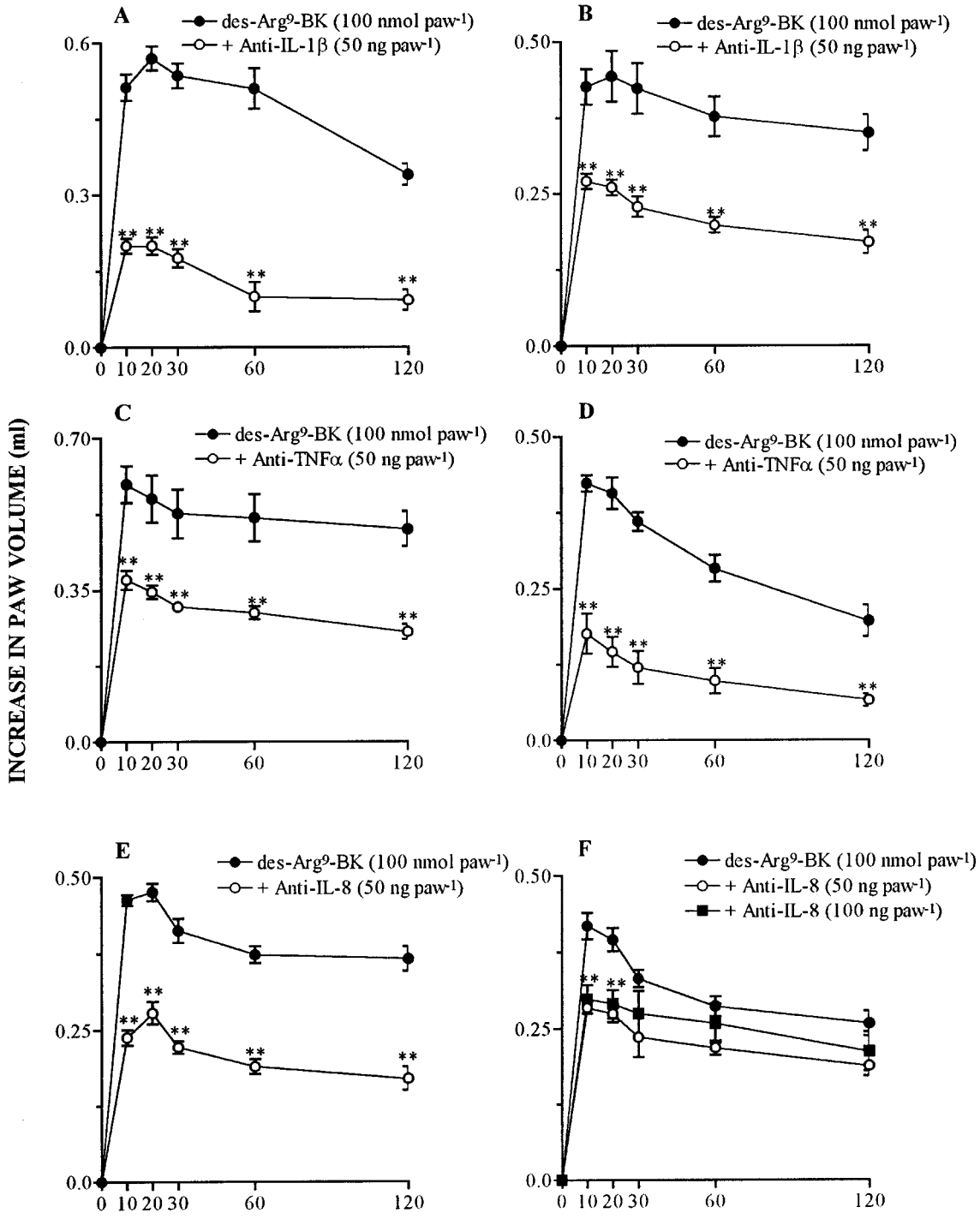

Figure 1 Effect of co-injection of anti-murine antibodies anti-IL-1β (A and B), anti-TNFα (C and D) or anti-IL-8 (E and F) on des-Arg^9-BK-induced paw oedema in rats pre-treated with IL-1β (5 ng paw^{-1}, 60 min beforehand; A, C and E) or with TNFα (5 ng paw^{-1}, 30 min beforehand; (B, D and F)). Values represent the differences between volumes (in ml) of vehicle-injected (0.1 ml of PBS solution) and drug-injected paws. Each point represents the mean $\pm$ s.e.mean of 4–6 rats. In some cases the error bars are hidden within the symbols. Significantly different from control values $^*P<0.05$; $^{**}P<0.01$ (A–E, Student's unpaired *t*-test) (F, ANOVA followed by Dunnett's test).

TNFα (Figure 1B, D and F) were significantly inhibited by the co-injection of the murine antibodies anti-IL-1β, anti-TNFα and anti-IL-8 (50 ng paw^{-1}), with inhibitions of 65±3; 37±3 and 42±4% for IL-1β, and 39±3; 64±6 and 25±7% for TNFα, respectively. On the other hand, the co-injection of the anti-murine antibody anti-IL-6 (50–100 ng paw^{-1}) had no effect on the rat paw oedema caused by des-Arg^9-BK in animals treated with either IL-1β or TNFα ($n=4$, results not shown). The results of Figure 2 show that the combination of IL-1 receptor antagonist (IRA, 100 μg paw^{-1}) or of the anti-inflammatory cytokine, IL-10 (10 ng paw^{-1}) significantly reduced the oedema formation caused by des-Arg^9-BK in rats that received IL-1β (67±4 and 63±2%; Figure 2A and C) or TNFα (46±5 and 35±5%; Figure 2B and D).

The co-injection of the PKC inhibitors staurosporine (10 nmol paw^{-1}, Figure 3A and B) or RO 318220 (30 nmol paw^{-1}, Figure 3C and D), in association with either IL-1β or TNFα, produced significant inhibition of des-Arg^9-BK-induced paw oedema. The inhibitions obtained for these drugs were: 44±3 and 42±2% for IL-1β, and 53±7 and 30±3% for TNFα, respectively.

The rat paw oedema induced by des-Arg^9-BK in rats treated with either IL-1β or TNFα was markedly inhibited by previous treatment of animals with the tyrosine kinase inhibitor, genistein (2.5 mg kg^{-1}, s.c., 30 min prior to the cytokines) (58±3 and 31±8% of inhibition for IL-1β and TNFα, respectively) (Figure 4A and B). In addition, the co-injection of the MAP-kinase inhibitor PD 098059 (30 nmol paw^{-1}) significantly inhibited des-Arg^9-BK-induced paw oedema by 39±4 and 35±7%, in rats pre-treated with IL-1β (Figure 4C) or with TNFα (Figure 4D), respectively.

The results of Figure 5 demonstrate that the pre-treatment of animals with the NF-κB inhibitors, TLCK (2 mg kg^{-1}, i.p.) or PDCT (100 mg kg^{-1}, i.p.), both 30 min before, significantly inhibited the increase in paw volume produced by des-Arg^9-BK in rats pre-treated with either IL-1β (27±4 and 83±3%) or with TNFα (28±6 and 80±2%), respectively. Additional doses of the inhibitors staurosporine, RO 318220 or PD 098059 (up to 50 nmol paw^{-1}) or genistein (up to 5 mg kg^{-1}) did not display any further inhibition of B_1 receptor-mediated rat paw oedema (results not shown, $n=4$). In the case of TLCK, it was not possible to test doses superior to 2 mg kg^{-1}, because it produced some toxic effects.

Finally, the treatment of animals with RO 318220, genistein, PD098059 or TLCK (at the same range of doses used above) did not display any significant effect on rat paw oedema induced by BK (3 nmol paw^{-1}) (Table 1). On the other hand the oedema formation mediated by BK was

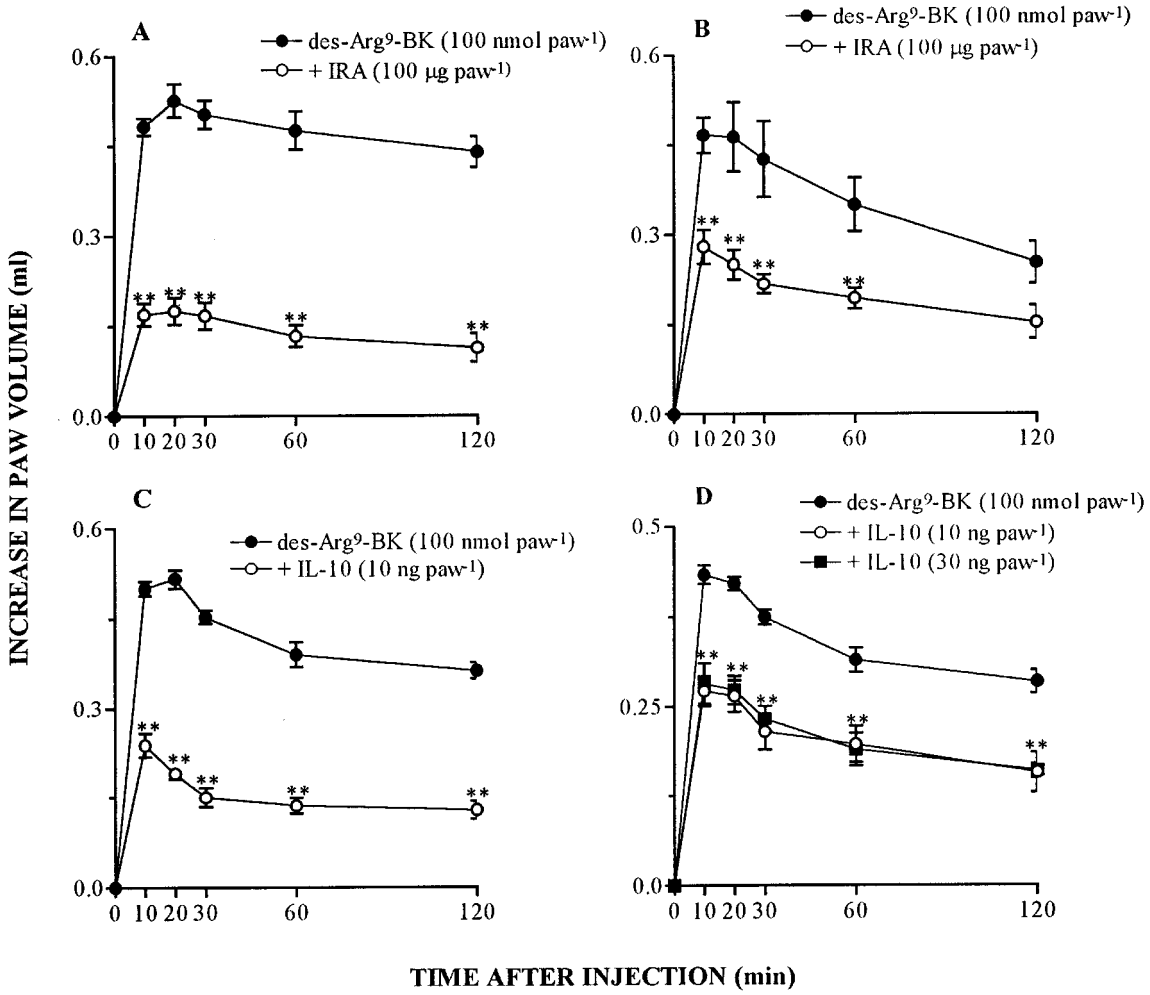

Figure 2 Effect of co-injection of the recombinant human IL-1 antagonist (IRA, A and B) or of IL-10 (C and D) on des-Arg^9-BK-induced paw oedema in rats pre-treated with IL-1β (5 ng paw^{-1}, 60 min beforehand; (A and C)) or with TNFα (5 ng paw^{-1}, 30 min beforehand; (B and D)). Values represent the differences between volumes (in ml) of vehicle-injected (0.1 ml of PBS solution) and drug-injected paws. Each point represents the mean±s.e.mean of 4–6 rats. In some cases the error bars are hidden within the symbols. Significantly different from control values *$P<0.05$; **$P<0.01$ (A–C, Student's unpaired t-test) (D, ANOVA followed by Dunnett's test).

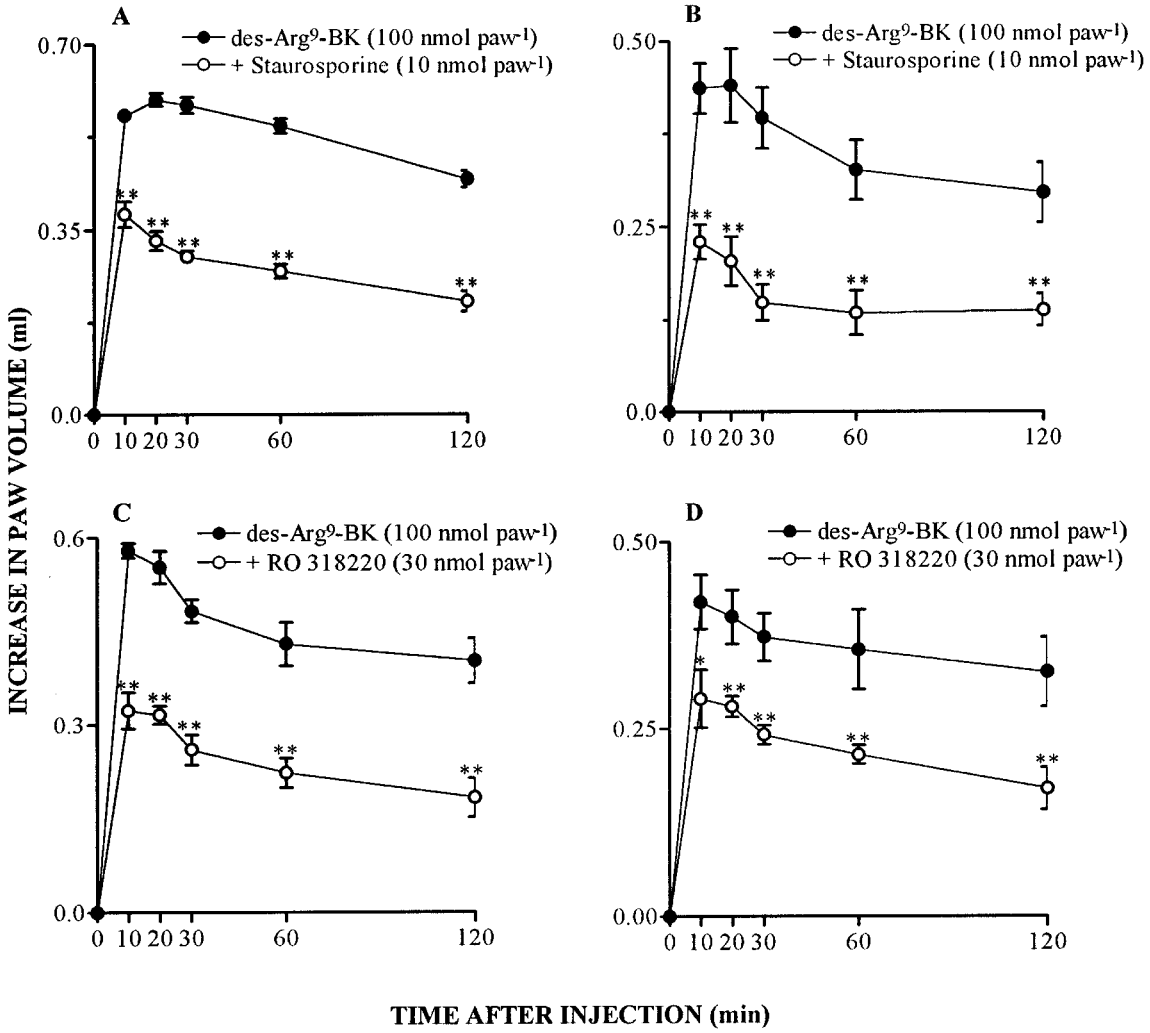

Figure 3 Effect of co-injection of staurosporine (A and B) or of RO 319220 (C and D) on des-Arg^9-BK-induced paw oedema in rats pre-treated with IL-1β (5 ng paw^{-1}, 60 min beforehand (A and C)) or with TNFα (5 ng paw^{-1}, 30 min beforehand (B and D)). Values represent the differences between volumes (in ml) of vehicle-injected (0.1 ml of PBS solution) and drug-injected paws. Each point represents the mean$\pm$s.e.mean of 4–6 rats. In some cases the error bars are hidden within the symbols. Significantly different from control values $^*P<0.05$; $^{**}P<0.01$ (Student's unpaired *t*-test).

slightly, but significantly inhibited by staurosporine (29$\pm$8%) or PDTC (24$\pm$4%).

Discussion

Despite the evidence showing the upregulation of B_1 kinin receptors following several inflammatory stimuli or after tissue trauma, the precise mechanisms involved in this effect are still not completely understood. The increase of functional responses mediated by B_1 receptors has been extensively reported for both *in vitro* and *in vivo* models. We have previously demonstrated that the complete desensitization of the rat paw oedema after repeated injection of either BK or tyrosine^8-BK results in the upregulation B_1 receptors-mediated responses (Campos & Calixto, 1995). Similar induction of B_1 receptors-mediated oedema formation, sensitive to dexamethasone and cyclohexymide treatments, has been shown after acute (24 h) treatment of animals with lypopolysaccharide of *E. coli* (Campos *et al.*, 1996), following long-term systemic treatment with BCG (Campos *et al.*, 1997) or i.d. injection of the pro-inflammatory cytokines IL-1β or TNFα (Campos *et al.*, 1998). Therefore, such data confirm the notion that induction of B_1 receptors may be relevant in either acute or chronic inflammatory processes. In the present study, besides confirming the involvement of cytokines in this process, we also investigated the possible mechanisms responsible for the *in vivo* B_1 receptor upregulation following i.d. treatment of animals with IL-1β or TNFα.

Our results suggest that the rat paw oedema induced by des-Arg^9-BK after i.d. injection of IL-1β or TNFα involves the secondary production of other cytokines, since the increase in B_1-receptor-mediated oedema formation was inhibited to a great extent by the co-injection of the antibodies anti-IL-1β, anti-TNFα or anti-IL-8, as well as by the association of the natural IL-1 receptor antagonist, IRA. These results fully agree with other evidence indicating that the upregulation of B_1 receptors depends on the local production (autocrine or paracrine) of distinct cytokines (Marceau, 1995). It has been demonstrated that the induction of B_1 receptors evoked by intra-articular injection of IL-1, IL-6 or by IL-8, in a rat model of inflammatory hyperalgesia, is consistently inhibited by co-injection of IRA, indicating that the effects of these cytokines are mediated by the release of IL-1 (Davis & Perkins, 1994; for

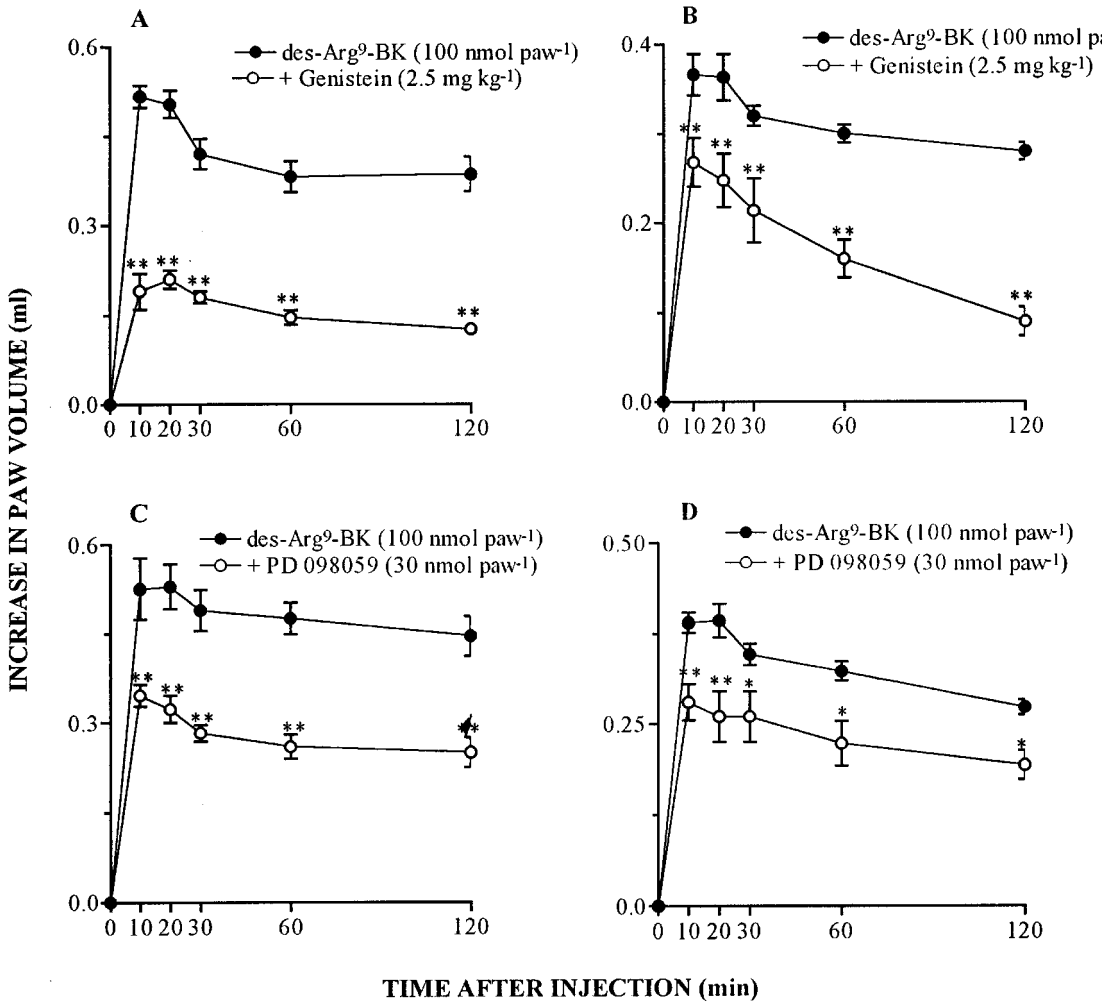

Figure 4 Effect of systemic treatment with genistein (A and B) or with PD 098059 (C and D) on des-Arg^9-BK-induced paw oedema in rats pre-treated with IL-1β (5 ng paw^{-1}, 60 min beforehand (A and C)) or with TNFα (5 ng paw^{-1}, 30 min beforehand (B and D)). Values represent the differences between volumes (in ml) of vehicle-injected (0.1 ml of PBS solution) and drug-injected paws. Each point represents the mean $\pm$ s.e.mean of 4–6 rats. In some cases the error bars are hidden within the symbols. Significantly different from control values $^*P<0.05$; $^{**}P<0.01$ (Student's unpaired *t*-test).

review see Marceau, 1997). Furthermore, our data also demonstrate that the upregulation of kinin B_1 receptor-mediated oedema formation was significantly inhibited by co-injection of IL-10, an important anti-inflammatory cytokine which induces downregulation of pro-inflammatory cytokines, mainly IL-1β and TNFα at the site of inflammation (Firestein & Zvaifler, 1997; Nikolaus *et al*., 1998). In contrast to results reported in the rabbit aorta *in vitro* (DeBlois *et al*., 1988) using human recombinat TNFα, this study, together with the results of a previous one (Campos *et al*., 1998), clearly demonstrate that i.d. treatment with murine TNFα largely mediates the upregulation of the B_1 receptor agonist des-Arg^9-BK-mediated oedema formation in the rat paw. In addition, our results are in line with those recently reported by Ni *et al*. (1998), affirming that human TNFα up-regulates B_1 receptor gene expression and increases NF-κB binding activity in vascular smooth muscle.

It has been suggested that activation of some classes of protein kinases are meaningful to the induction of B_1 kinin receptor. Recently, it has been demonstrated that both spontaneous and IL-1β-induced upregulation of B_1 receptor in rabbit aorta is very sensitive to some MAP-kinase inhibitors, e.g. PD98059, an agent which inhibits MAP kinases activated by extracellular signals or by the p38 MAP kinase inhibitor, SB203580 (Larrivé *et al*., 1998). Our data confirm and extend these findings, and demonstrate for the first time the involvement of MAP kinase pathways in B_1 receptor upregulation *in vivo*, following i.d. injection of the pro-inflammatory cytokines, IL-1β or TNFα. This conclusion arises from the fact that oedema caused by des-Arg^9-BK was significantly prevented by the co-injection of the MAP kinase inhibitor, PD 98059. The activation of MAP-kinases cascades occur in response to several stimuli including inflammatory cytokines (IL-1β and TNFα), heat and chemical shock, or bacterial products, all capable of stimulating the B_1 receptor induction (Paul *et al*., 1997). In addition, MAP-kinases seem to be important pathways responsible for the link between some transcriptional factors (e.g. NF-κB) and intracellular signals (Larrivé *et al*., 1998; Yamakawa *et al*., 1998).

Also relevant are the results demonstrating that the previous treatment of animals with the tyrosine kinase inhibitor genistein produced a marked inhibition of B_1 receptor-mediated rat paw oedema in animals that had received IL-1β or TNFα. The involvement of tyrosine kinase pathways in B_1 kinin receptor upregulation has recently been reported for rabbit aortic strips, since the treatment of

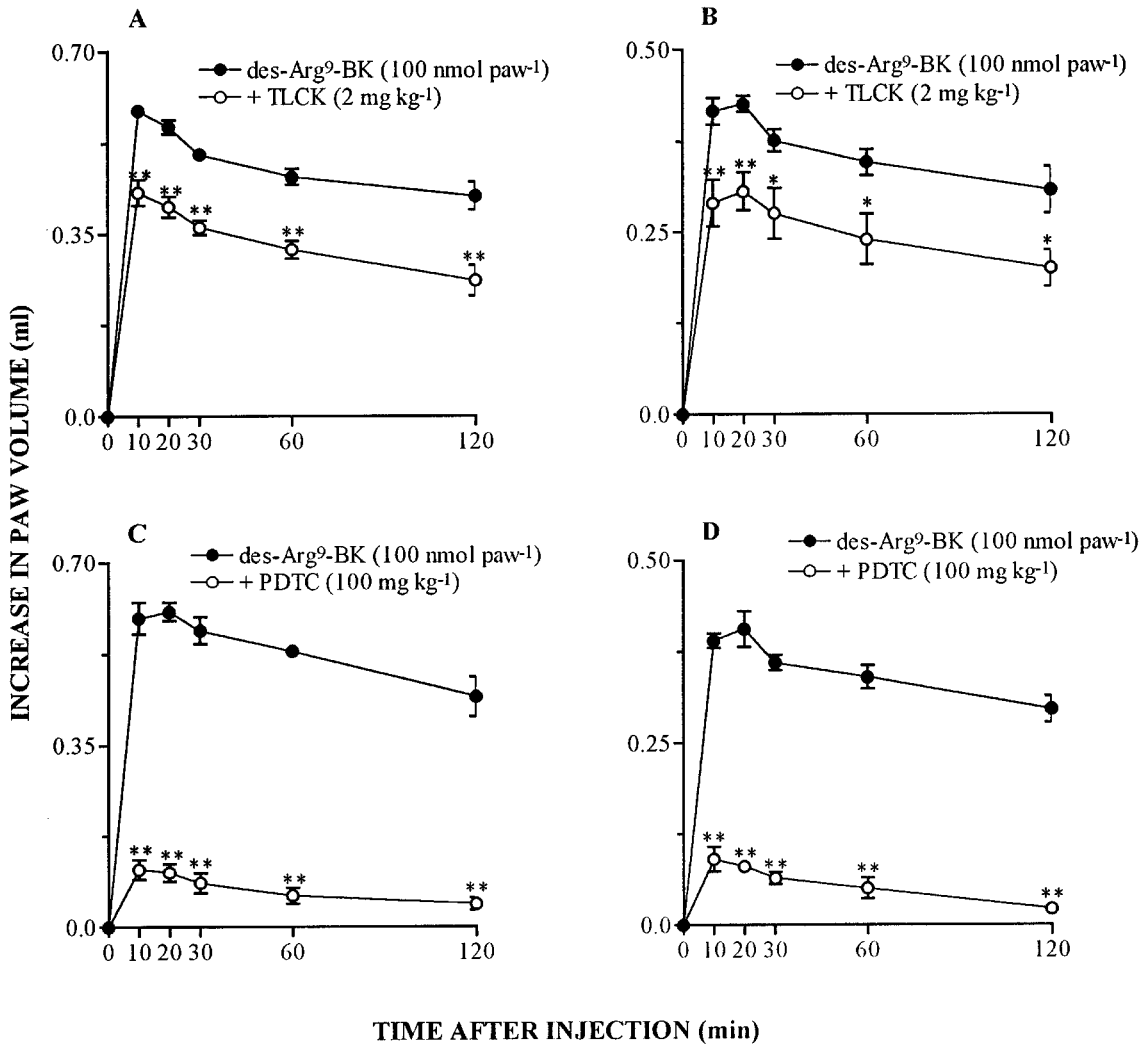

Figure 5 Effect of systemic treatment with TLCK (A and B) or with PDCT (C and D) on des-Arg^9-BK-induced paw oedema in rats pre-treated with IL-1β (5 ng paw^{-1}, 60 min beforehand (A and C)) or with TNFα (5 ng paw^{-1}, 30 min beforehand (B and D)). Values represent the differences between volumes (in ml) of vehicle-injected (0.1 ml of PBS solution) and drug-injected paws. Each point represents the mean$\pm$s.e.mean of 4–6 rats. In some cases the error bars are hidden within the symbols. Significantly different from control values $^*P<0.05$; $^{**}P<0.01$ (Student's unpaired *t*-test).

Table 1 Effect of several classes of drugs on BK-induced rat paw oedema

	Oedema formation (ml)[a]
Control (BK 3 nmol paw^{-1})	0.40$\pm$0.02
+ Staurosporine (10 nmol paw^{-1})	0.28$\pm$0.04**
+ RO 318220 (30 nmol paw^{-1})	0.38$\pm$0.04
+ PD 098059 (10 nmol paw^{-1})	0.40$\pm$0.03
+ Genistein (2.5 mg kg^{-1}, s.c., 30 min)	0.44$\pm$0.03
+ TLCK (2 mg kg^{-1}, i.p., 30 min)	0.37$\pm$0.02
+ PDTC (100 mg kg^{-1}, i.p., 30 min)	0.30$\pm$0.02**

Each group represents the mean$\pm$s.e.mean of 4–5 experiments. [a]Increase in paw volume (ml) measured 20 min after injection of peptides. Significantly different from control values. $^{**}P<0.01$ (ANOVA followed by Dunnett's test).

preparations with genistein significantly prevented the contraction produced by des-Arg^9-BK (Larrivé *et al.*, 1998).

It has been described that PKC is directly related to functional and morphological changes in endothelial cells, being responsible for some effects observed in response to important inflammatory mediators such as BK or TNFα (Ross *et al.*, 1997). Thus, PKC appears to be activated and involved in the sequential activation of MAP kinases following treatment with BK (Lal *et al.*, 1998). Our results clearly demonstrate an involvement of PKC in the upregulation of B_1 receptor after i.d. injection of IL-1β or TNFα, since the rat paw oedema produced by des-Arg^9-BK was notably inhibited by the two PKC inhibitors staurosporine and RO 318220. Also, the present results are in agreement with recent evidence showing that the pre-treatment of human lung fibroblasts with the PKC inhibitor, GF 109203, prevents in a significant manner the expression of B_1 kinin receptor RNAm, induced by the B_1 agonist des-Arg^{10}-kallidin (Schanstra *et al.*, 1998).

Another interesting aspect investigated in the present work was the participation of the transcriptional factor NF-κB in the B_1 receptor up-regulation *in vivo*. There are some *in vitro* studies showing that induction of B_1 receptors is directly related to previous activation of NF-κB. Thus, it has been demonstrated that in cultured human lung fibroblasts, the B_1 receptor upregulation induced by IL-1β is modulated at the transcriptional level, in a process mediated by the activation of

NF-κB (Schanstra *et al*., 1998). In addition, the presence of an NF-κB like sequence on B_1 receptor promoter, has recently been shown, which seems to be mainly responsible for the inducibility of the receptor in response to IL-1β, TNFα or LPS (Ni *et al*., 1998). In the present study, we have demonstrated for the first time the involvement of NF-κB pathway in B_1 kinin receptor upregulation *in vivo*. This inference is a consequence of the fact that the paw oedema caused by des-Arg^9-BK, in rats which have received the pro-inflammatory cytokines IL-1β or TNFα, was significantly prevented by the systemic pre-treatment of animals with NF-κB inhibitors, PDCT or TLCK. The inhibition of most of the tested drugs on des-Arg^9-BK, seems to be mediated by a selective mechanism, as the paw oedemas induced by BK, which involve an action on B_2 constitutive receptors, were not affected. However, part of the inhibitory action of staurosporine and PDTC on des-Arg^9-BK-mediated paw oedema may involve additional mechanisms, not related to B_1 receptor inhibition.

These results confirm and also extend the recently-reported *in vitro* evidence (Ni *et al*., 1998; Schanstra *et al*., 1998) and are consistent with the notion that the inflammatory cytokines such as IL-1β and TNFα can activate NF-κB which in turn increases their expression and jointly stimulates the upregulation of B_1 receptors. It has recently been demonstrated that the anti-inflammatory cytokines IL-10 and IL-13 are capable of suppressing activation of NF-κB in alveolar macrophages (Lentsch *et al*., 1997). As IL-10 was found to be effective in preventing the oedema formation caused by des-Arg^9-BK in animals treated with IL-1β and TNFα it is tempting to speculate that the modulation of anti-inflammatory cytokines levels and the activation of NF-κB might be one of the main cellular mechanisms responsible for the upregulation of B_1 receptors following i.d. treatment with inflammatory cytokines.

In conclusion, the current results give for the first time, strong evidence indicating that the *in vivo* up-regulation of B_1 receptor mediated rat paw oedema, following i.d. treatment of animals with IL-1β or TNFα, involves the additional release of other cytokines such as IL-1β, TNFα and IL-8. Furthermore, our results also clearly demonstrate that activation of protein kinases such as PKC, tyrosine kinase or MAP-kinases, and the transcription factor NF-κB, has a critical *in vivo* role in modulating the upregulation of des-Arg^9-BK induced paw oedema in rats treated with the pro-inflammatory cytokines IL-1β or TNFα. Together, the present results provide additional evidence supporting the role played by B_1 receptors in controlling and maintaining of inflammatory processes.

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