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# Involvement of $B_1$ and $B_2$ receptors in bradykinin-induced rat paw oedema

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**1** The mechanisms involved in bradykinin (BK)-induced oedema in the rat paw as well as the interactions between BK and several inflammatory mediators, have been investigated.

**2** Intraplantar injection of BK (1 nmol/paw) in rats pretreated with captopril (5 mg $kg^{-1}$, s.c.) caused a small amount of oedema formation ($0.17 \pm 0.05$ ml). Des-Arg$^9$-BK (DABK, a selective $B_1$ receptor agonist) up to 300 nmol/paw caused minimal oedema ($0.03 \pm 0.01$ ml).

**3** Co-administration of prostaglandin $E_2$ ($PGE_2$), prostaglandin $I_2$ ($PGI_2$), calcitonin gene-related peptide (CGRP), 5-hydroxytryptamine (5-HT), substance P (SP) or platelet activating factor (PAF) (1 pmol–1 nmol/paw) with BK (1 nmol/paw) dose-dependently potentiated BK-induced paw oedema. The rank order of potency (mean $ED_{50}$, pmol/paw) for this effect was: SP (8.1) > PAF (13.7) > $PGI_2$ (20.5) > 5-HT (23.8) > CGRP (25.7) > $PGE_2$ (52.0). Co-administration of BK with the various inflammatory mediators resulted in maximal paw oedemas (ml) of: $PGE_2$ ($0.71 \pm 0.02$); $PGI_2$ ($0.66 \pm 0.02$); 5-HT ($0.65 \pm 0.01$); SP ($0.63 \pm 0.05$); CGRP ($0.60 \pm 0.05$) and PAF ($0.47 \pm 0.02$) ml. Histamine (up to 1 nmol/paw) was ineffective in potentiating the response to BK.

**4** Hoe 140 or NPC 17731 (two selective $B_2$ receptor antagonists, 0.1–3 nmol/paw) produced dose-dependent inhibition of paw oedema potentiation induced by co-injection of BK with other mediators with the following mean $ID_{50}$s (nmol/paw): Hoe 140–1.4; 1.3; 1.5 and 1.1 and NPC 17731–1.0; 1.0; 0.9 and 0.7; in the presence of $PGE_2$, $PGI_2$, CGRP and SP, respectively. The selective $B_1$ receptor antagonist des-Arg$^9$ [Leu$^8$]-BK (DALBK, up to 300 nmol/paw) had no effect.

**5** Daily intraplantar injections of BK (10 nmol/paw) once a day for 7 consecutive days caused a progressive and complete desensitization of the paw oedema, which was specific for BK, since paw oedema induced by PAF, $PGE_2$, SP or histamine was not affected. In addition, the oedema caused by BK in the paw desensitized to the peptide was almost completely reversed if BK was co-injected with $PGE_2$, $PGI_2$ or SP (1 nmol/paw). Injection of $PGE_2$ or SP (10 nmol/paw) together with the first BK injection (10 nmol/paw), partially prevented BK-induced desensitization.

**6** When animals were completely desensitized to BK, DABK (100 nmol/paw) caused paw oedema ($0.25 \pm 0.03$ ml) which was consistently blocked by the $B_1$ receptor antagonist, DALBK (100 nmol/paw).

**7** Treatment of animals with dexamethasone (0.5 mg $kg^{-1}$, s.c., 24 h previously) antagonized paw oedema induced by DABK (100 nmol/paw) in desensitized paws, but not that induced by BK (3 nmol/paw) in naive paws. The steroid also prevented the recovery of oedema seen after co-injection of BK with $PGE_2$ or $PGI_2$ (1 nmol/paw) in desensitized paws.

**8** These results suggest that both $B_1$ and $B_2$ receptors are involved in BK-induced rat paw oedema. The $B_2$ receptors are constitutive, but induction of expression of $B_1$ receptors seems to occur only after complete desensitization of the paw to BK. In addition, very low doses of inflammatory mediators markedly potentiate BK-induced paw oedema and can attenuate BK-induced paw oedema desensitization. Such mechanisms may be relevant for the manifestation of acute and chronic inflammatory processes.

**Keywords:** Paw oedema (rat); bradykinin; des-Arg$^9$-bradykinin; inflammatory mediators; synergism; desensitization; $B_1$ and $B_2$ kinin antagonists

## Introduction

Kinins, including bradykinin (BK) and kallidin (lys-BK) are generated in plasma and in a variety of peripheral tissues in response to tissue injury or infection by the action of kallikreins on low molecular weight kininogen precursors. Kinins are recognized as potent vasoactive peptides which promote venular dilatation, increased vascular permeability, enhanced fluid secretion from epithelia and produced pain and hyperalgesia (Lewis, 1970; Garcia Leme, 1978; Marceau *et al.*, 1983; Proud & Kaplan, 1988; Steranka & Burch, 1991; Dray & Perkins, 1993). In addition, inflammation is associated with increased levels of BK and its metabolites des-Arg$^9$-BK and des-Arg$^{10}$-Lys-BK (Regoli & Barabé, 1980; Hargreaves *et al.* 1988; Damas *et al.*, 1990).

The actions of kinins are mediated by activation of two types of specific membrane receptors, denoted $B_1$ and $B_2$. The expression of $B_1$ kinin receptors is usually restricted to some rabbit blood vessels and nonvascular smooth muscle tissues. Importantly, this type of receptor exhibits higher affinity for the kinin metabolites, des-Arg$^9$-BK and des-Arg$^{10}$-Lys-BK and can be selectively and competitively antagonized by the $B_1$ receptor antagonists, des-Arg$^9$[Leu$^8$]-BK or des-Arg$^{10}$[Leu$^8$]-Lys-BK. In contrast, $B_2$ kinin receptors are widely distributed both in the peripheral and central nervous systems and show high affinity for BK and Lys-BK, being selectively and competitively antagonized by several potent and selective $B_2$ receptor antagonists, including among others, Hoe 140 and NPC 17731. While $B_2$ receptors are constitutive and mediate the majority of kinin actions, $B_1$ receptors are expressed following *in vitro* incubation for long

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periods or after tissue trauma or infection (Regoli & Barabé, 1980; Burch & DeHaas, 1990; Bathon & Proud, 1991; Marceau & Regoli, 1991; Bhoola *et al.*, 1992; Burch *et al.*, 1993).

It has been demonstrated that most of the actions of kinins, including their pro-inflammatory and algesic properties, are indirectly mediated by production and release of pro-inflammatory mediators derived from the arachidonic acid pathway, especially prostaglandin $E_2$ ($PGE_2$) and prostaglandin $I_2$ ($PGI_2$), as well as histamine and 5-hydroxytryptamine (5-HT) following mast cell degranulation (Gaginella & Kachur, 1989; Hall, 1992; Burch *et al.*, 1993). In addition, kinins may also stimulate visceral sensory neurones to release pro-inflammatory neuropeptides such as substance P (SP), neurokinin A or calcitonin gene-related-peptide (CGRP) (Bhoola *et al.*, 1992; for review see: Farmer & Burch, 1992; Geppetti, 1993). Therefore, a large part of the pro-inflammatory and algesic actions of kinins is most likely to be due to synergistic actions between these mediators (Brain & Williams, 1989; Buckley *et al.*, 1991; Cruwys *et al.*, 1992; Warren *et al.*, 1993).

The purpose of the present study was to analyse, by use of selective BK receptor agonists and antagonists, the mechanisms involved in BK-induced oedema in the rat paw. In addition, we have also investigated the synergism between BK and co-administration of low doses of several inflammatory mediators.

## Methods

### *Measurement of rat paw oedema*

Experiments were conducted on non-fasted male Wistar rats (140–200 g) kept in a room controlled for temperature (22 ± 2°C) and illumination (12 h on and 12 h off). All animals were pretreated with captopril (5 mg $kg^{-1}$, s.c.) 1 h prior to any given experiment to prevent BK degradation (Corrêa & Calixto, 1993). Under ether anaesthesia 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 (1 nmol/paw) either alone or mixed with $PGE_2$, $PGI_2$, CGRP, SP, 5-HT, PAF or histamine (1 pmol/paw to 1 nmol/paw). 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) after injection of inflammatory mediators. Oedema has been expressed in ml as the difference between the test and control paws.

### *Influence of bradykinin receptor antagonists*

In a separate series of experiments in order to assess whether the oedema resulting from co-injection of BK and other inflammatory mediators involved activation of $B_1$ or $B_2$

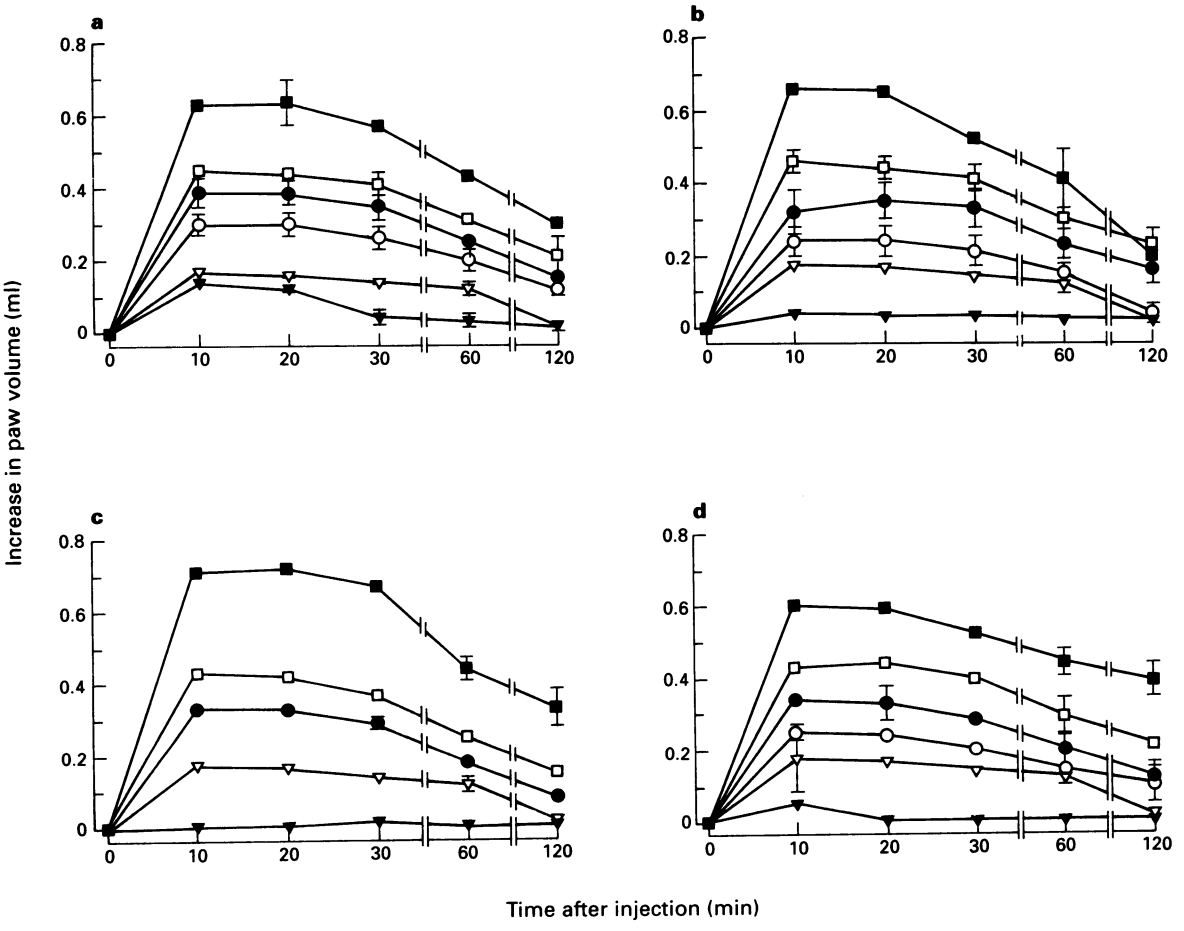


**Figure 1** Effect of intraplantar injection of bradykinin (BK), given alone or in combination with other inflammatory mediators, on rat hindpaw volume. Values shown represent the differences between volumes (in ml) or vehicle-treated (0.1 ml of PBS solution) and drug-injected paws. BK was injected either alone (▽, 1 nmol/paw in all panels), or in combination with 1(○), 10 (●), 100 (□), and 1000 (■) pmol/paw of substance P (a), prostaglandin $I_2$ ($PGI_2$) (b), $PGE_2$ (c) or calcitonin gene-related peptide (d). 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 5 to 6 animals pretreated with captopril (5 mg $kg^{-1}$, s.c.). In some cases the error bars are hidden within the symbols.

receptors, animals received intraplantar injections of BK (1 nmol/paw) together with the inflammatory mediators in the absence (control group) or in the presence of the selective $B_1$ receptor antagonist, des-Arg$^9$[Leu$^8$]-BK (DALBK) or the $B_2$ selective receptor antagonists, Hoe 140 or NPC 17731 (0.1 to 3 nmol/paw).

### *Desensitization to bradykinin*

In a further series of experiments, in order to assess the specificity of the synergism between BK and the inflammatory mediators and the possible mechanisms underlying this effect, animals were desensitized with single daily intraplantar injections of BK (10 nmol/paw) for 7 consecutive days. Paw oedema was measured as described above. The contralateral paw received only PBS and was used as a control. In another group of experiments, animals which were completely desensitized to BK (by day 7), received an intraplantar injection of BK (1 nmol/paw) together with $PGE_2$, $PGI_2$ or SP (1 nmol/paw).

The possible occurrence of cross-desensitization was also evaluated by intraplantar injection of $PGE_2$ (10 nmol/paw), SP (3 nmol/paw), PAF (10 nmol/paw) or histamine (100 nmol/paw) in naive or in BK desensitized paws. The doses of mediators were chosen because they were equieffective in increasing the response to BK alone. In other experiments, in order to evaluate the effect of inflammatory mediators on desensitization of BK-induced oedema, animals received an intraplantar injection of BK (10 nmol/paw) together with SP (10 nmol/paw) or $PGE_2$ (10 nmol/paw) on the first day, but BK (10 nmol/paw) alone was injected once daily throughout days 2 to 7. Other groups of animals which were completely desensitized with BK were treated with dexamethasone (0.5 mg kg$^{-1}$) 24 h previously or DABK (100 nmol/paw). Control animals received only BK (10 nmol/paw) from day 1 to 7 or PBS solution.

### *Drugs*

The following drugs were used: BK, $PGE_2$, iloprost, (a stable analogue of $PGI_2$), CGRP, SP, 5-HT, histamine, captopril, dexamethasone (all from Sigma Chemical Company, St. Louis, U.S.A.), des-Arg$^9$[Leu$^8$]-BK (Peninsula Belmont, CA, U.S.A.), PAF (Bachem, Switzerland), Hoe 140 (D-Arg-[Hyp$^3$, Thi$^5$, D-Tic$^7$, Oic$^8$]-BK) and NPC 17731 (D-Arg$^0$-Arg$^1$-Pro$^2$-Hyp$^3$-Gly$^4$-Phe$^5$-Ser$^6$-[D-Hype$^3$ (transpropyl)$^7$]-Oic$^8$-Arg$^9$)h, were kindly supplied by the Department of Pharma Synthesis, 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 and were kept 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 (i.e. the concentrations of drugs that reduced oedema by 50% relative to control value or concentrations which produced 50% of the maximal oedema increase), which are presented as means accompanied by their respective 95% confidence limits. The $ID_{50}$ or $ED_{50}$ values were determined by the use of the least squares method. Statistical analysis of the data was performed by analysis of variance followed by Dunnett's test or by Student's unpaired $t$ test, as indicated, and differences with $P \leq 0.05$ were considered significant.

## Results

The subplantar injection of BK (1 nmol/paw) in captopril-pretreated rats (5 mg kg$^{-1}$, s.c., 1 h previously) produced modest paw oedema (0.17 ± 0.05 ml). Intraplantar injections of $PGE_2$, $PGI_2$, CGRP, SP, 5-HT, PAF or histamine (all 1 nmol/paw) caused even smaller or no increase in paw volume (Figures 1 and 2). However, co-administration of $PGE_2$, $PGI_2$, CGRP, SP, 5-HT or PAF (1 pmol to 1 nmol/paw) with BK (1 nmol/paw) resulted in significantly greater paw oedemas (Figures 1 and 2) ($P<0.05$). These effects were dose-dependent, yielding a rank order of potency (mean $ED_{50}$ pmol/paw and 95% confidence limits) for the potentiating effects of these mediators of: SP (8.1; 6.7–9.1) > PAF (13.7; 10.2–16.8) > $PGI_2$ (20.5; 19.2–22.1) > 5-HT (23.8; 22.9–25.3) > CGRP (25.7; 23.4–26.3) > $PGE_2$ (52.0; 50.4–57.6). The maximal increases in paw volume (in ml) induced by

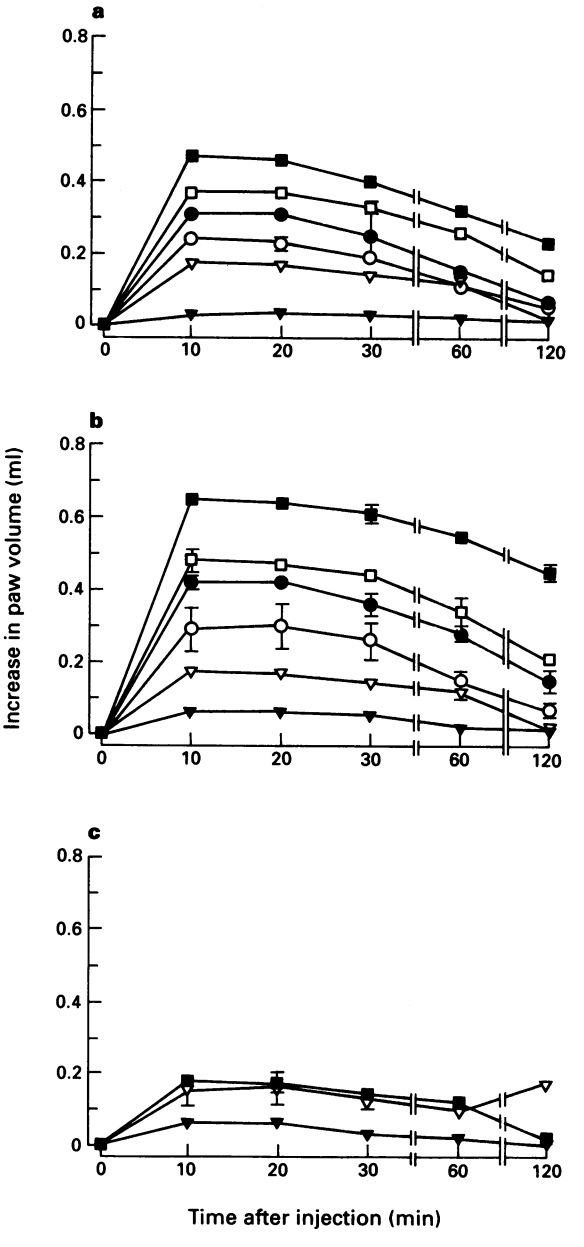


**Figure 2** Effect of intraplantar injection of bradykinin (BK), given alone or in combination with other inflammatory mediators, on rat hindpaw volume. Values shown represent the differences between volumes (in ml) of vehicle-treated (0.1 ml of PBS solution) and drug-injected paws. BK was injected either alone (▽, 1 nmol/paw in all panels), or in combination with 1(○), 10 (●), 100 (□), and 1000 (■) pmol/paw of PAF (a), 5-HT (b) or histamine (c). 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 5 to 6 animals pretreated with captopril (5 mg kg$^{-1}$, s.c.). In some cases the error bars are hidden within the symbols.

co-injection of BK together with each mediator were (mean ± s.e.mean): $PGE_2$ (0.71 ± 0.02), $PGI_2$ (0.66 ± 0.02), 5-HT (0.65 ± 0.01), SP (0.63 ± 0.05), CGRP (0.60 ± 0.05), PAF (0.47 ± 0.02) ml. In contrast, histamine (1 nmol/paw) did not potentiate BK-induced rat paw oedema (Figure 2).

On the other hand, co-injections into the paw of different combinations of $PGE_2$, $PGI_2$, SP, CGRP, and 5-HT (e.g. $PGE_2$ and $PGI_2$, SP and $PGE_2$, CGRP and SP, $PGI_2$ and 5-HT) resulted, at best, in 50% of the oedema produced by combination of any of these mediators with BK. All combinations, except those with BK, caused oedema which appeared to reflect only the summation of the individual effects of each mediator (each given at 1 nmol/paw; $n = 6-7$; results not shown), i.e. their effects were merely additive.

The intraplantar injection of the selective $B_2$ receptor antagonists, Hoe 140 or NPC 17731 (0.1, 1 and 3 nmol/paw) alone had no effect (results not shown). However, when they were co-injected with BK (1 nmol/paw) plus one of the inflammatory mediators (1 nmol/paw), both caused a dose-dependent and significant inhibition of the size of the response to BK given together with inflammatory mediators ($P<0.05$), with the followings mean $ID_{50s}$ (nmol/paw) ( and 95% confidence limits): 1.4 (0.9–2.0), 1.3 (0.8–2.2), 1.5 (1.1–2.0) and 1.1 (0.9–2.2) for Hoe 140 and 1.0 (0.9–1.1), 1.0 (0.8–1.2), 0.9 (0.9–1.5) and 0.7 (0.6–0.7) for NPC 17731 in the presence of $PGE_2$, $PGI_2$, CGRP and SP, respectively (Figures 3 and 4). In contrast, the selective $B_1$ receptor antagonist des-Arg$^9$[Leu$^8$]-BK (DALBK) (up to 100 nmol/paw) did not affect the potentiation of paw oedema (control response to BK (3 nmol/paw) (mean ± s.e.mean) of 0.41 ± 0.03 ml; treated with DALBK (100 nmol/paw) 0.44 ± 0.02 ml ($P>0.05$)).

Successive daily intraplantar injections of BK (10 nmol/paw) for 7 days caused progressive desensitization of the oedema response reaching a maximal inhibition of 93 ± 3% on day 7 (Figure 5a). This desensitization of oedema was specific for BK, since intraplantar injections of $PGE_2$ (10 nmol/paw), SP (3 nmol/paw), PAF (10 nmol/paw) or histamine (100 nmol/paw) into the BK desensitized paws produced oedema of the same magnitude observed in the naive paw, indicating the absence of cross desensitization between BK and these inflammatory mediators (Figure 5b). Co-administration of BK (1 nmol/paw) with $PGE_2$ (1 nmol/paw), SP (1 nmol/paw) or $PGI_2$ (1 nmol/paw) in the BK-desensitized paws almost completely restored to control level BK-induced paw oedema (Figure 6). Moreover, the intraplantar co-injection of $PGE_2$ or SP (10 nmol/paw) together with the first BK (10 nmol/paw) injection (day 1) partially, but significantly, prevented BK-induced desensitization (Figure 7). The maximal reductions in oedema (mean ± s.e. mean) (at the end of 7 days) were: 55 ± 3% and 78 ± 2%, in animals that received $PGE_2$ or SP respectively, compared with 93 ± 3% inhibition when desensitization was carried out in the absence of these mediators ($P<0.01$). The results in Figure 8 show that prior treatment of animals with dexamethasone (0.5 mg kg$^{-1}$, s.c., 24 h previously) markedly reduced the size of the response to BK given together with either $PGE_2$ (1 nmol/paw) or $PGI_2$ (1 nmol/paw) in the BK desensitized paws ($P<0.01$).

Intraplantar injection of the selective $B_1$ agonist, DABK (up to 100 nmol/paw) caused modest paw oedema in control

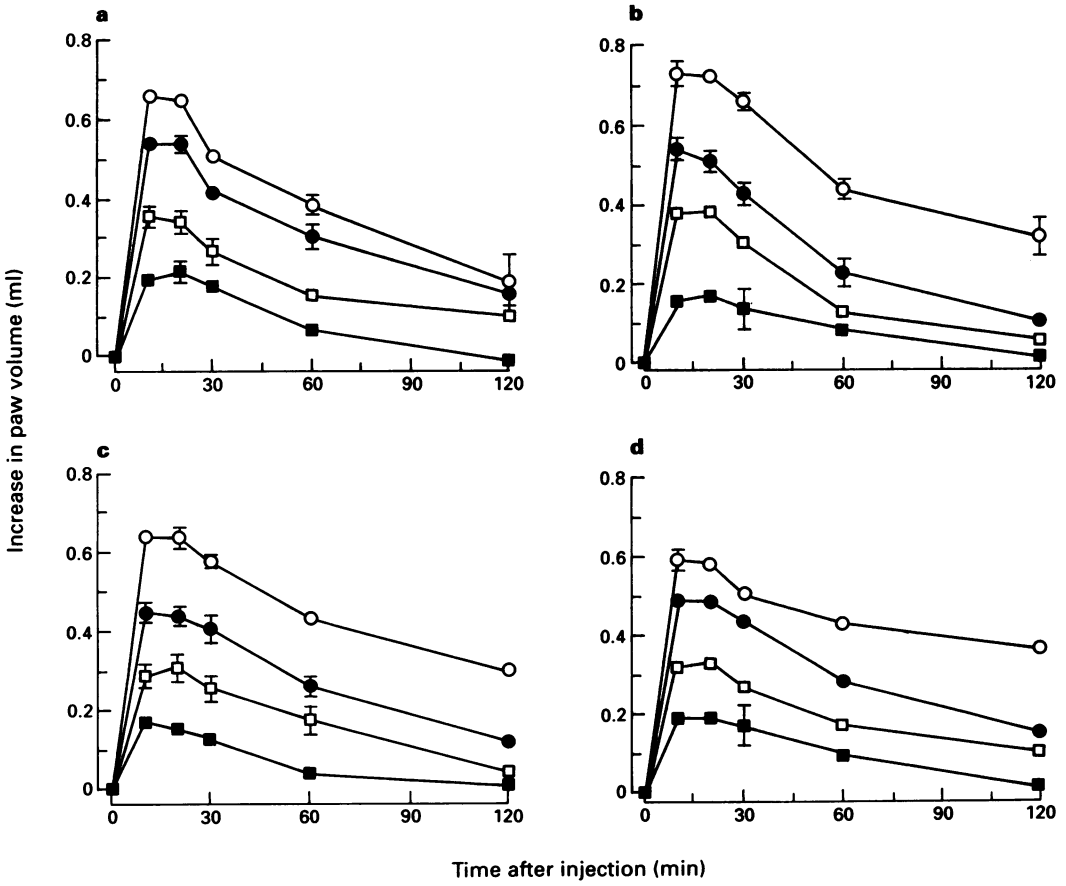


**Figure 3** Effect of intraplantar injection of the selective $B_2$ receptor antagonists Hoe 140 given in combination with BK (1 nmol/paw) and 1 nmol/paw $PGI_2$ (prostaglandin $I_2$) (a), $PGE_2$ (b), substance P (c) or calcitonin gene-related peptide (d), on rat hindpaw volume. Values shown represent the differences between volumes (in ml) of vehicle-treated (0.1 ml of PBS solution) and drug-injected paws. Control responses (○) and responses obtained in the presence of Hoe 140 (nmol/paw): 0.1 (●); 1 (□) and 3 (■). Each point represents the mean ± s.e.mean of 5 to 6 animals pretreated with captopril (5 mg kg$^{-1}$, s.c.). In some cases the error bars are hidden within the symbols.

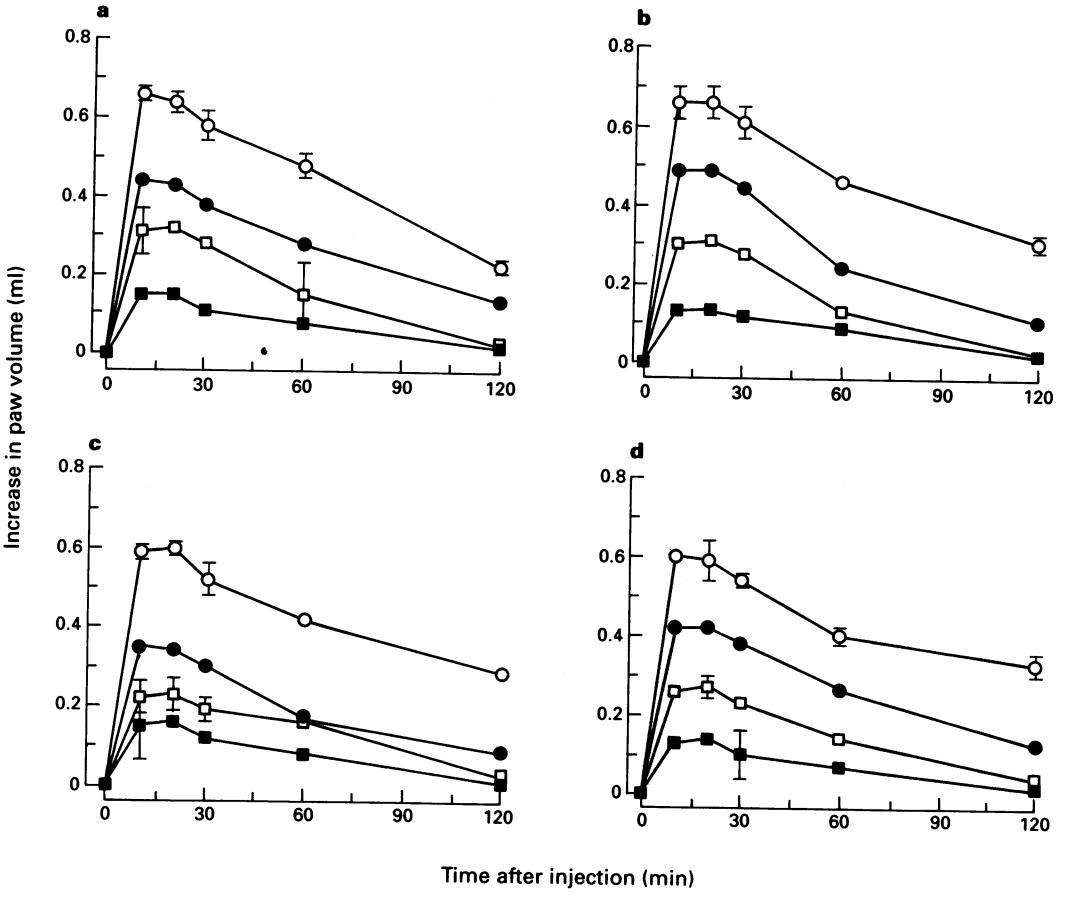


**Figure 4** Effect of intraplantar injection of the selective $B_2$ receptor antagonist, NPC 17731 given in combination with bradykinin (BK) (1 nmol/paw) and 1 nmol/paw prostaglandin $I_2$ ($PGI_2$) (a), $PGE_2$, (b), substance P (c) or calcitonin gene-related peptide (d), on rat hindpaw volume. Values shown represent the differences between volumes (in ml) of vehicle-treated (0.1 ml of PBS solution) and drug-injected paws. Control responses (○) and responses obtained in the presence of NPC 17731 (nmol/paw): 0.1 (●); 1 (□) and 3 (■). Each point represents the mean ± s.e.mean of 5 to 6 animals pretreated with captopril (5 mg $kg^{-1}$, s.c.). In some cases the error bars are hidden within the symbols.

animals (0.3 ± 0.01 ml). However, when animals were completely desensitized to BK (10 nmol/paw for 7 days), intraplantar injection of the selective $B_1$ agonist DABK (100 nmol/paw) caused marked paw oedema (0.25 ± 0.03 ml), which correspond to about 60% of the maximal oedema induced by BK (Figure 9a). Paw oedema produced by DABK (100 nmol/paw) was significantly attenuated by co-injection of the selective $B_1$ receptor antagonist DALBK (100 nmol/paw) (36 ± 6% inhibition) ($P<0.01$) (Figure 9a). In addition, prior treatment of animals with dexamethasone (0.5 mg $kg^{-1}$, 24 h previously) also consistently antagonized DABK (100 nmol/paw)-induced oedema in desensitized paws (53 ± 4% inhibition) ($P<0.05$) (Figure 9a). In contrast, the same treatment with dexamethasone had no effect on oedema induced by BK (3 nmol/paw) in naive paws (Figure 9b).

## Discussion

The present study demonstrated that BK-induced paw oedema in the rat can be markedly potentiated by several mediators of inflammation. Thus, co-injection of very low doses of $PGE_2$, $PGI_2$, SP, CGRP, PAF or 5-HT, which alone caused little or no oedema, induced marked dose-dependent potentiation of BK-induced paw oedema, whereas histamine was ineffective. The potentiating actions of all mediators seems to involve an amplification of responses to BK mediated by $B_2$ receptors, as the oedema caused by co-injection of BK together with each mediator was dose-dependently and similarly blocked by two selective and potent $B_2$ BK receptor antagonists, Hoe 140 and NPC 17731. Similar inhibition of kinin responses *in vivo* by these $B_2$ antagonists has been reported (Wirth *et al.*, 1991; Dray *et al.*, 1992; Corrêa & Calixto, 1993; Heapy *et al.*, 1993; Kyle & Burch, 1993).

It is important to mention that co-injections of different combinations of $PGE_2$, $PGI_2$, SP, CGRP or histamine always resulted in oedema that was smaller than that caused by any co-injections with BK. These results strongly suggest that BK plays a key role in this process. The mechanisms underlying the potentiating effects of these agents on $B_2$ receptor-mediated BK-induced paw oedema have yet to be characterized. Nevertheless, it is possible that the influences of prostaglandins, SP and CGRP on BK-induced paw oedema may involve their ability to enhance blood flow, as has been shown for BK-induced increases in vascular permeability (Brain & Williams, 1989; Buckley *et al.*, 1991; Cruwys *et al.*, 1992). Alternatively, there may well be interactions between the distinct second messenger systems activated by BK and these mediators. It has been shown that BK-stimulated $PGE_2$ synthesis is potentiated by interleukin$_1$ (IL-1) in human synovial fibroblasts (Bathon *et al.*, 1992) and by IL-1 and tumour necrosis factor ($TNF_\alpha$) in 3T3 fibroblasts (Burch *et al.*, 1988; 1989a,b; Burch & Tiffany, 1989; for review see Burch *et al.*, 1993). Moreover, both IL-1 and $TNF_\alpha$ have been reported to increase BK-induced membrane GTP binding and GTP activity (Burch *et al.*, 1988; Imamura *et al.*, 1988).

Both paw oedema and pleural exudation triggered by BK can be progressively inhibited by repeated daily injections of the peptide (Martins *et al.*, 1992). Confirming this report, we have found that daily intraplantar injections of BK (10 nmol/

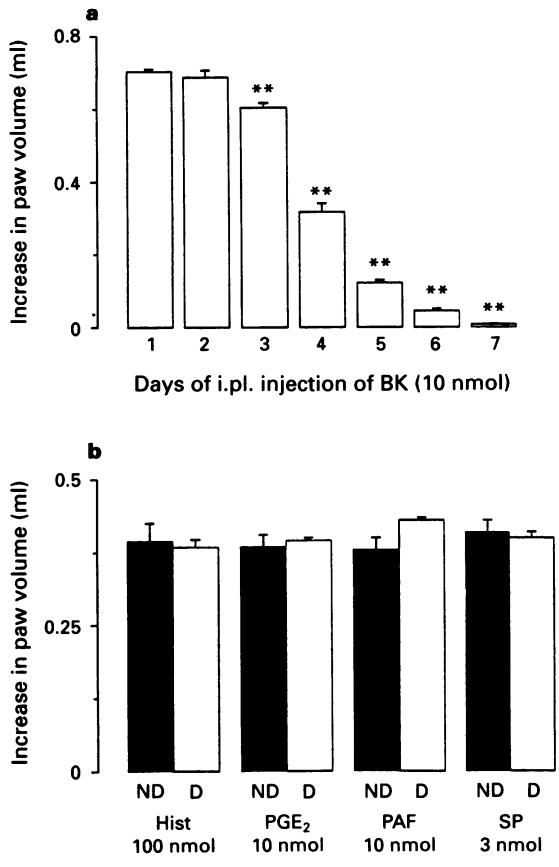


**Figure 5** (a) Desensitization of bradykinin (BK)-induced rat paw oedema caused by intraplantar (i. pl.) daily injections of BK (10 nmol/paw) once a day for 7 days. (b) Absence of cross-desensitization between BK and inflammatory mediators. Response of desensitized (D, open columns) or non-desensitized paws (ND, solid columns). Values shown 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 5 to 6 animals pretreated with captopril (5 mg $kg^{-1}$, s.c.). Significantly different from control: $^{**}P<0.01$. The oedema was measured 20 min after intraplantar injection of inflammatory mediators.

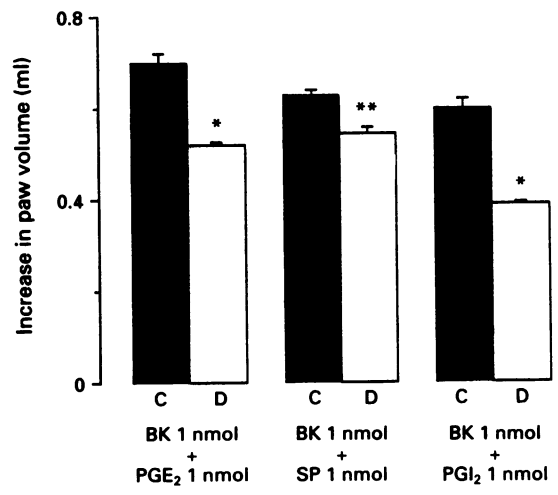


**Figure 6** Effect of intraplantar injection of bradykinin (BK) (1 nmol/paw), given in combination with prostaglandin $E_2$ ($PGE_2$) (1 nmol/paw), substance P (1 nmol/paw) or $PGI_2$ (1 nmol/paw) on rat hindpaw volume in naive (solid columns) or in BK-desensitized (seven days) paws (open columns). Values shown 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 5 to 6 animals pretreated with captopril (5 mg $kg^{-1}$, s.c.). Significantly different from control: $^{*}P<0.05$; $^{**}P<0.01$. The oedema was measured 20 min after intraplantar injection of inflammatory mediators.

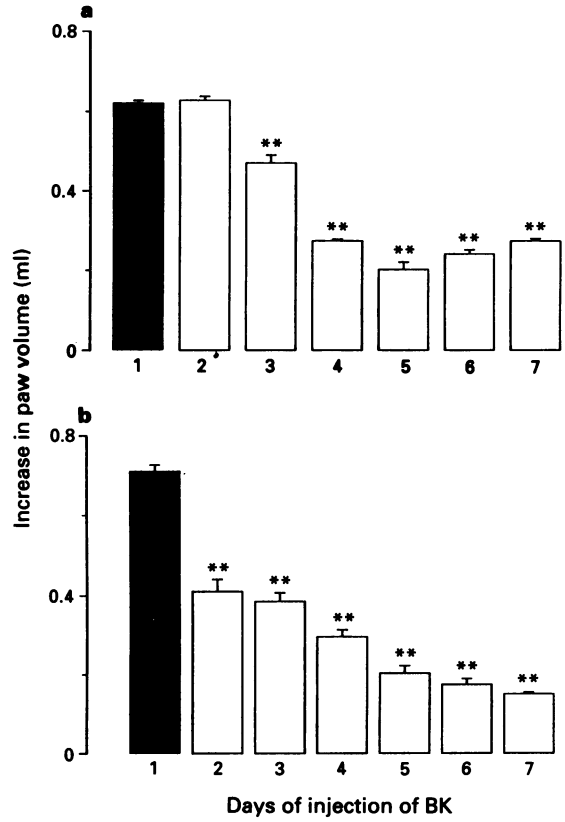


**Figure 7** Effect of intraplantar administration of substance P (SP) (a, 10 nmol/paw) or prostaglandin $E_2$ ($PGE_2$) (b, 10 nmol/paw) together with the first bradykinin (BK) injection (10 nmol/paw) (solid columns) on BK (10 nmol/paw)-induced rat paw oedema desensitization by repeated injection of the peptide once a day for 7 days (open column). Values shown 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 5 to 6 animals pretreated with captopril (5 mg $kg^{-1}$, s.c.). Significantly different from control: $^{**}P<0.01$. The oedema was measured 20 min after intraplantar injection of inflammatory mediators.

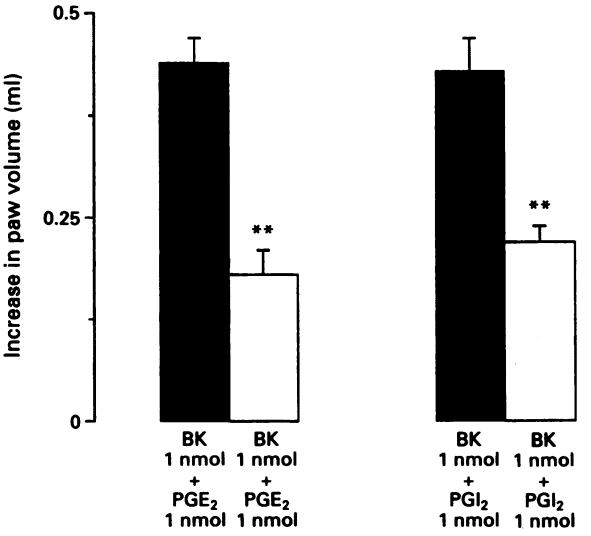


**Figure 8** Effect of pretreatment of animals with dexamethasone (0.5 mg $kg^{-1}$, s.c., 24 h previously, open columns) on the rat paw potentiation caused by intraplantar co-administration of bradykinin (BK) (1 nmol/paw) with prostaglandin $E_2$ ($PGE_2$) (1 nmol/paw) or $PGI_2$ (1 nmol/paw) in desensitized paws (solid columns). Values shown 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 4 animals pretreated with captopril (5 mg $kg^{-1}$, s.c.). Significantly different from control: $^{**}P<0.01$. The oedema was measured 20 min after intraplantar injection of inflammatory mediators.

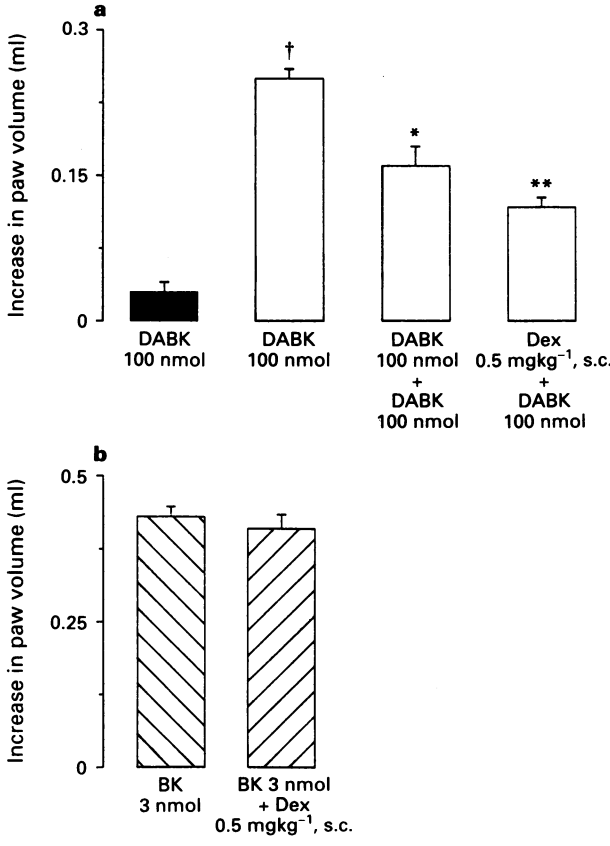


**Figure 9** (a) Effect of intraplantar injection of des-Arg$^9$-BK (DABK, 100 nmol/paw) in naive control paws (solid column) or in bradykinin (BK)-densensitized paws (open columns) in rats pretreated with captopril (5 mg kg$^{-1}$, s.c.). Values shown represent the differences between volumes (in ml) of vehicle-treated (0.1 ml of PBS solution) and drug-injected paws. Panel (a) also shows the inhibition of DABK-induced paw oedema in BK-desensitized paws caused by co-injection of des-Arg$^9$[Leu$^8$]-BK (DALBK; 100 nmol/paw) or by the treatment of animals with dexamethasone (Dex, 0.5 mg kg$^{-1}$, s.c., 24 h previously). (b) Effect of treatment of animals with Dex (0.5 mg kg$^{-1}$, s.c., 24 h previously) on BK-induced paw oedema. Each column represents the mean ± s.e.mean of 4 to 5 animals. Significantly different from control (†$P<0.01$) or from DABK-induced oedema (*$P<0.05$; **$P<0.01$). The oedema was measured 20 min after intraplantar injection of the peptides.

paw) for seven days caused a progressive and complete desensitization of paw oedema. This phenomenon was specific for BK, as BK-desensitized paws were equally responsive to intraplantar injections of PAF, $PGE_2$, SP or histamine. Interestingly, however, intraplantar co-injection of $PGE_2$ or SP (10 nmol/paw), together with the first BK (10 nmol/paw) injection, significantly attenuated the desensitization caused by daily intraplantar injections of BK. In addition, the oedema response of BK-desensitized paws to the peptide could be almost completely restored to initial levels if BK was co-injected on day 7 together with $PGE_2$, $PGI_2$ or SP (1 nmol/paw). These results are consistent with the view that these mediators are important modulators of BK action.

Recent biochemical studies have suggested that BK-induced desensitization involves changes at the receptor level, as well as of second messenger transducer mechanisms. Thus, BK-induced desensitization in cultured sensory neurones is mediated via release of nitric oxide, which, in turn, activates guanylate cyclase to increase cyclic GMP (Burgess *et al.*, 1989; Lang *et al.*, 1990; McGehee *et al.*, 1992). Furthermore, BK-induced desensitization is correlated with a down regulation and internalization of kinin receptors, allied to a decreased coupling of activated receptors to G-proteins (Roscher *et al.*, 1984; 1990; Munoz & Leeb-Lundberg, 1992; Wolsing & Rosenbauum, 1993). Such observations suggest that BK-induced desensitization may be relevant as a mechanism for regulation of its pro-inflammatory properties.

In this regard, the current study shows that the selective $B_1$ receptor agonist, DABK, which caused a very weak effect in naive paws, produced marked oedema in BK-desensitized paws. It is well known that DABK does not produce any inflammatory or algesic responses in non-traumatized tissues. This is likely to be because the $B_1$ receptors are not expressed in normal tissues (Regoli & Barabé, 1980; Marceau *et al.*, 1983; Steranka & Burch, 1991). Nevertheless, expression of $B_1$ receptors can be induced under a variety of conditions, and thus exert important roles in several pathological states, including inflammation and hyperalgesia (Marceau *et al.*, 1983; Farmer *et al.*, 1991; Dray & Perkins, 1993; Perkins & Kelly, 1993). We have attempted to provide more direct evidence that $B_2$ receptor desensitization induces the expression of $B_1$ receptors, by pretreating animals with dexamethasone 24 h before intraplantar injection of DABK. Dexamethasone consistently attenuated DABK-mediated oedema in paws desensitized to BK. Interestingly, the same treatment with dexamethasone failed to modify BK-induced paw oedema, which is mediated by activation of constitutive $B_2$ receptors. Thus, the paw oedema caused by intraplantar injection of DABK in BK-desensitized paws is likely to be associated with a dexamethasone-sensitive induction of $B_1$ kinin receptor expression. Indeed, dexamethasone has been found to inhibit the induction of $B_1$ receptor expression *in vitro* (Deblois *et al.*, 1988). Recent findings suggest that BK triggers the release of several cytokines such as IL-1, IL-2 and $TNF_\alpha$ (Tiffany & Burch, 1989; Ferreira *et al.*, 1993). Cytokines can also mediate the expression of $B_1$ receptors (Deblois *et al.*, 1988; 1991), and their synthesis is blocked by corticosteroids (Roscher & Manganiello, 1984; Deblois *et al.*, 1988). These data suggest that cytokines may be involved in the upregulation of expression of $B_1$ receptors in BK-desensitized paws. Our results strongly suggest that the desensitization of $B_2$ receptors may play an important role in expression of $B_1$ receptors following tissue damage or in inflammatory states. However, it remains to be confirmed whether $B_2$ receptor desensitization and induction of expression of $B_1$ receptors may occur in inflamed tissues.

In conclusion, we have shown that both $B_1$ and $B_2$ kinin receptors can trigger oedema in the rat paw under different conditions. The $B_2$ receptors are constitutive and can interact in a synergistic manner with several inflammatory mediators. On the other hand, $B_2$ receptor desensitization induces the expression of $B_1$ receptors through a dexamethasone-sensitive mechanism which may involve cytokine production. As BK can stimulate prostanoid synthesis in most tissues and activate sensory neurones to release the pro-inflammatory neuropeptides SP and CGRP, the current findings may have important implications for the manifestation of acute and chronic inflammatory process.

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