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The role of bradykinin B_1 receptors in the maintenance of intra-articular plasma extravasation in chronic antigen-induced arthritis

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1 The role of bradykinin B_1 and B_2 receptors in bradykinin- and des-Arg^9-bradykinin-induced plasma extravasation in normal and inflamed rat knee joints was investigated by use of an antigen-induced model of chronic arthritis. A modification of an Evans blue extraction technique allowed the unstimulated (basal) plasma extravasation to be assessed in this model. The contributions of bradykinin B_1 and B_2 receptors towards basal synovial plasma extravasation were determined.

2 In normal knees, intra-articular injection of bradykinin (BK) induced plasma extravasation in a potent, dose-dependent manner with a threshold of 0.01 nmol and an ED_{50} of 0.1 nmol. In day 5 arthritic knees, basal plasma extravasation was substantially enhanced. Lower doses of BK had no demonstrable effect and increases above basal extravasation were first observed at 0.1 nmol. Thereafter the dose-response mirrored the response in normal knees and the maximal response was unaltered.

3 The B_1 agonist, des-Arg^9-BK, induced slight but significant plasma extravasation in normal knees but was less potent than bradykinin. This response was inhibited by the B_1 receptor antagonist, des-Arg^9, [Leu^8]-BK. Lower doses of des-Arg^9-BK bradykinin did not significantly increase basal extravasation in day 5 arthritic knees but, in contrast to BK, the maximal response was significantly enhanced.

4 The B_2 antagonist, Hoe 140, inhibited BK-induced plasma extravasation in normal joints over a dose-range of 0.1–1.0 nmol but was relatively inactive in day 5 inflamed knees. The B_1 receptor antagonist, des-Arg^9, [Leu^8]-BK, was relatively inactive in normal joints but showed increased potency against BK-induced plasma extravasation in day 5 arthritic joints.

5 Hoe 140 and des-Arg^9,[Leu^8]-BK both inhibited basal extravasation in arthritic joints on days 1 and 5 post-challenge in a dose-dependent fashion. Whilst Hoe 140 was the more potent inhibitor on day 1, it was less potent than des-Arg^9,[Leu^8]-BK on day 5.

6 Although the majority of responses to BK in normal tissue are mediated via B_2 receptors, a small population of B_1 receptors may exist in normal joint tissues. The data presented in this study suggest an evolving role for B_1 receptors in the mediation of plasma extravasation in inflamed joint tissues. A role for BK antagonists in the treatment of arthritis is also suggested.

Keywords: Bradykinin; bradykinin receptors; chronic arthritis; plasma extravasation; joint inflammation

Introduction

Bradykinin (BK) is a pro-inflammatory nonapeptide which is synthesized *de novo* at sites of tissue damage producing many of the cardinal signs of inflammation (Proud & Kaplan, 1988). In both acute and chronic arthritis BK has been implicated as a potent mediator of pain and swelling and may play a role in cell proliferation, bone resorption and the secondary release of various cytokines from leucocytes (Bhoola *et al.*, 1992a; Sharma, 1992).

BK is produced in damaged tissues from kininogen substrates present in the circulation and degranulating neutrophils (Bhoola *et al.*, 1992b). Elevated levels of BK have been reported in animal models of inflammation and in patients with inflammatory joint diseases (Steranka *et al.*, 1987; Hargreaves *et al.*, 1988). It is inactivated by proteolytic enzymes, including kininase I and II, forming a variety of metabolites which include the active fragments des-Arg^9-BK and Lys-des-Arg^9-BK.

Two types of bradykinin receptor, B_1 and B_2, have been characterized to date (Regoli & Barabé, 1980). Most actions of BK have been reported to be mediated via the B_2 receptor (Steranka *et al.*, 1988; Dray *et al.*, 1992) which is optimally stimulated by BK and kallidin (Lys-BK). In normal uninflamed tissues selective B_1 agonists, which include des-Arg^9-BK and Lys-des-Arg^9-BK (Dray & Perkins, 1993), are largely inactive possibly due to lack of expression or masking of the B_1 receptors. In contrast, increased expression of B_1 receptors has recently been reported in pathological states, particularly in inflammation or after exposure to noxious stimuli (Farmer *et al.*, 1991; Bhoola *et al.*, 1992a; Perkins *et al.*, 1993).

Plasma extravasation in arthritis has important consequences leading to joint swelling and facilitating many secondary and potentially damaging intra-articular events. Although the effects of BK on acute joint swelling are relatively well established, its role in maintenance of chronic joint swelling is less clear (Bhoola *et al.*, 1992a). We have therefore compared the actions of BK on plasma extravasation in normal and chronically inflamed joints using an antigen-induced model of chronic arthritis. In light of the evidence supporting a role for B_1 receptors in chronic inflammation we have also examined the relative actions of B_1 and B_2 agonists and antagonists on plasma extravasation in the same inflammatory model.

Methods

The experiments were performed on male Wistar rats (200–250 g) which were deeply anaesthetized with sodium pentobarbitone (65 mg kg^{-1}). Evans blue (100 mg kg^{-1}) was

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injected into the penile vein. The right knee was then injected with either 0.1 ml BK (0.01–10 nmol per knee) or sterile saline (control). The contralateral knee was left uninjected and provided an internal control. Anaesthesia was maintained for 2 h after which the animals were exsanguinated. A 2 h period was chosen we have previously found bradykinin-induced plasma extravasation to plateau between 1.5 and 3 h using this technique. The synovium was dissected from each joint, weighed and the Evans blue extracted by a modification of the dye extraction technique described by Lam & Ferrell (1989).

The synovium was mixed with 2 ml of extraction medium (acetone and a 1% aqueous solution of sodium sulphate; ratio 7:3) and then left at room temperature for 24 h. The concentration of Evans blue recovered was determined by comparing the absorbance of 620 nm with a standard curve prepared with known concentrations of Evans blue. As Evans blue binds to plasma proteins, which are largely retained within the vasculature, its increased presence within the synovium is indicative of plasma extravasation into the synovial tissues. Data are presented as mean difference in plasma extravasation (± s.e.) between the test knees and the uninjected contralateral controls.

In further studies the ability of the B_1 agonist, des-Arg^9-BK, to induce plasma extravasation was assessed. The effects of B_1 and B_2 antagonists on BK-induced plasma extravasation were also investigated.

Antigen-induced arthritis

Chronic monoarticular arthritis was induced in male Wistar rats by a modification of the method described by Brackertz *et al.* (1977). The animals were sensitized on two occasions (days 0 and 7) with 5 mg methylated bovine serum albumin (mBSA; Sigma). The mBSA (10 mg ml^{-1}) was dissolved in 0.9% saline and then emulsified in an equal volume of Freund's complete adjuvant (Sigma). The emulsion (0.5 ml) was then injected into multiple intradermal sites on the animals back. On day 21 the animals were challenged by the intra-articular injection of 100 µl mBSA (5 mg ml^{-1} in sterile saline) into the right knee. The progress of the arthritis was monitored by daily measurement of joint diameter. The response to BK and des-Arg^9-BK was assessed on day 5 post-challenge by the method described above.

Basal plasma extravasation

Antigen-induced arthritis is a model of a chronic monoarthritis (Brackertz *et al.*, 1977) and allowed the investigation of basal (i.e. unstimulated) plasma extravasation into the arthritic knee whilst using the contralateral joint as a control. Basal plasma extravasation into the arthritic knees was assessed on days 1, 3, 5 and 21 post-challenge. The arthritic rats were anaesthetized and injected with Evans blue. After 2 h the rats were exsanguinated, the synovium dissected and the Evans blue content determined. The difference between the Evans blue content of the two knees was a measure of inflammation-induced plasma extravasation into the arthritic joint.

In further experiments the effects of BK antagonists on basal plasma extravasation were investigated on days 1 and 5 post-challenge. Prior to the injection of Evans blue the arthritic rats were given an intravenous injection of the test antagonist and the experimental protocol was then as stated above. Control animals were treated with an equivalent volume of sterile saline.

Statistics

Data were expressed as means ± s.e.mean and analyzed for statistical significance by Student's t test. $P<0.05$ was considered significant. Correlation analysis was by the Pearson product-moment method.

Results

Basal plasma extravasation in arthritic knees

Chronic monoarticular arthritis was induced in the right knee. Callipers were used to measure knee diameter on days 1, 3, 5 and 21 post-challenge. The diameters of the arthritic knee were greatly increased on day 1 (mean increase 4.8 ± 0.24 mm, $n=5$, $P<0.01$) and then slowly decreased during the course of the experiment (Figure 1a). A significant increase in arthritic knee diameter compared to the control knees was observed at all four time points ($P<0.05$).

Basal plasma extravasation was assessed in different groups of animals on days 1, 3, 5 and 21 post-challenge. Basal plasma extravasation into the arthritic joints was found to dramatically increase on day 1 and then fall slowly during the course of the experiment (Figure 1b). The increase in basal plasma extravasation correlated with the increase in joint diameter (Correlation coefficient $r=0.82$). No change was noted in the control knees throughout the study.

BK-induced plasma extravasation into normal and arthritic knee joints

When injected into the synovial cavity of normal knees, BK induced plasma extravasation in a dose-dependent manner (Figure 2a). The threshold for this response was 0.01 nmol BK and the ED_{50} was approximately 0.1 nmol.

BK-induced plasma extravasation in arthritic knees 5 days after the mBSA challenge was then assessed (Figure 2a). As shown above, there was significant basal plasma extravasation into the arthritic knees compared to normal control joints. Plasma extravasation in the arthritic knees injected with lower doses of BK (0.01 and 0.03 nmol) was not significantly different from basal (unstimulated) plasma extravasation. At higher doses (0.1–10 nmol) the BK-induced plasma extravasation was significantly greater than the basal

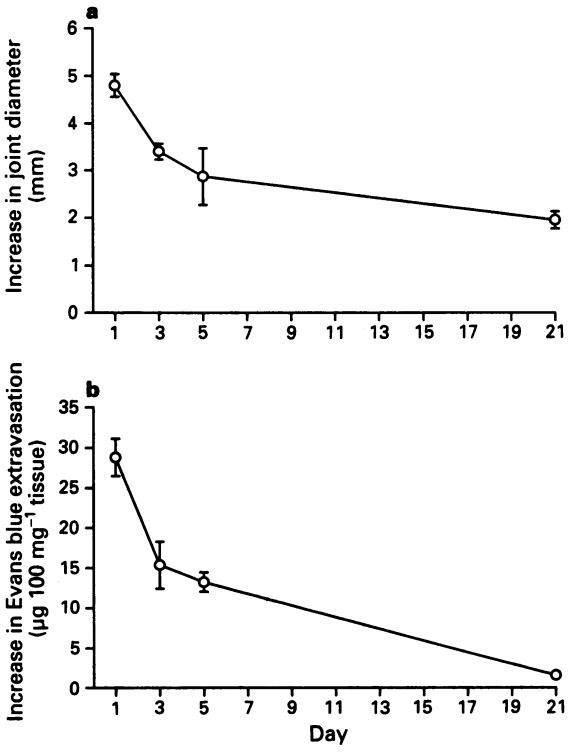

Figure 1 Increase in (a) joint diameter and (b) basal plasma extravasation following induction of antigen-induced arthritis. A significant increase was observed in both parameters at all time points ($P<0.05$). Points represent means ± s.e.mean ($n=4$ per group).

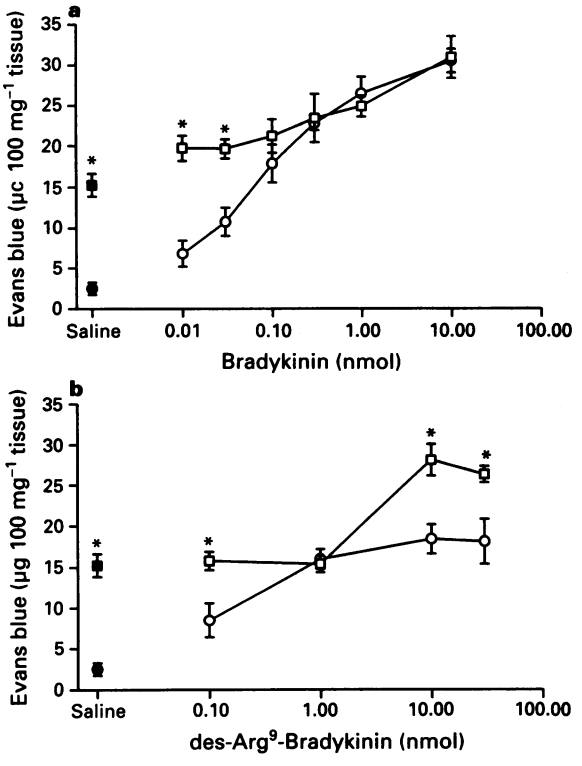

Figure 2 Dose-response curves for (a) bradykinin- and (b) des-Arg^9-Bradykinin-induced plasma extravasation into normal (○) and arthritic (□) rat knee joints. Values for unstimulated (basal) plasma extravasation in normal (●) and arthritic (■) joints are given. Points represent the means ± s.e.mean ($n = 4-6$ per group). *$P <$ 0.05 vs response in normal controls.

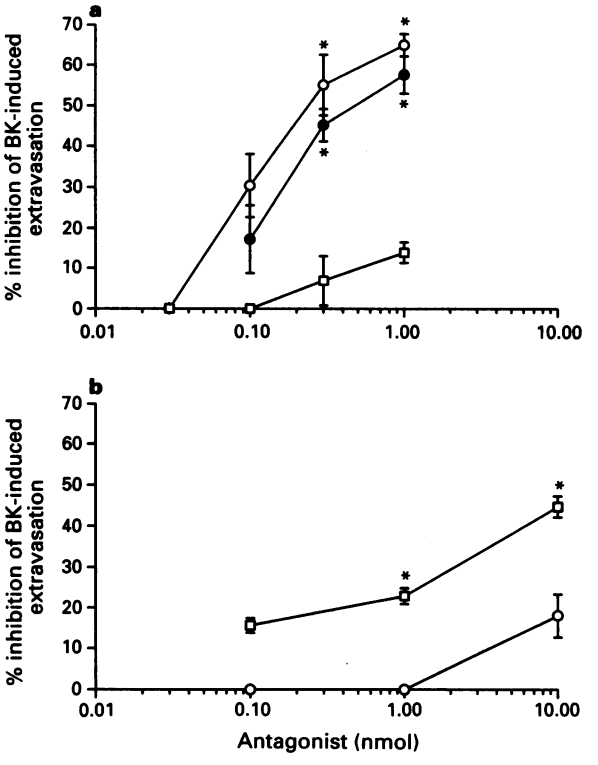

Figure 3 Inhibition of bradykinin (BK)-induced plasma extravasation by co-injection of Hoe 140 (○) and des-Arg^9,[Leu^8]-BK (□) with ED_{50} doses of BK in (a) normal and (b) arthritic knee joints. Hoe 140 was also co-injected with 1.0 nmol BK in normal joints (●). Points represent the means ± s.e.mean ($n = 3-6$ per group). *$P <$ 0.05 vs response to bradykinin alone.

extravasation. Plasma extravasation at the higher doses was similar in the arthritic and non-arthritic animals as was the maximum response (Figure 2a). The ED_{50} for plasma extravasation induced in arthritic knees by exogenous BK was 1.0 nmol and the threshold for this response was 0.1 nmol.

B_1-agonist induced plasma extravasation in normal and arthritic knees

The B_1 agonist, des-Arg^9-BK, dose-dependently induced plasma extravasation into the knee joint (Figure 2b); however, this response was less potent than that induced by BK (maximum responses 30.51 ± 1.49 BK vs 18.4 ± 1.76 des-Arg^9-BK; $P < 0.05$). In the arthritic joints, plasma extravasation in joints injected with lower dose of des-Arg^9-BK was not significantly different from the basal plasma extravasation. In contrast to BK, the responses to the higher doses of des-Arg^9-BK were greater in the arthritic joints than in the naïve controls with the maximum response in day 5 arthritic joints being 28.08 ± 1.96 μg Evans blue 100 mg^{-1} tissue compared to 18.4 ± 1.76 μg Evans blue 100 mg^{-1} tissue in the naïve controls ($P < 0.05$). The ED_{50} was raised from 0.1 nmol to 2.0 nmol (Figure 2b).

Effect of BK antagonists on BK-induced plasma extravasation in normal knees

The effects of the co-injection of BK antagonists with BK (0.1 nmol) were investigated. Hoe 140 (D-Arg[Hyp3,Thi5,D-Tic7,Oic8]-BK) inhibited BK-induced plasma extravasation over a dose range of 0.1–1.0 nmol; no inhibition was observed with 0.03 nmol (Figure 3a). BK-induced plasma extravasation was not significantly inhibited by any dose of des-Arg^9,[Leu^8]-BK (Figure 3a). This same antagonist at a dose of 1.0 nmol significantly inhibited plasma extravasation induced by the B_1 agonist des-Arg^9-BK (0.1 nmol) by 61% ($P < 0.02$), whereas Hoe 140 had no effect. With the lower doses of des-Arg^9,[Leu^8]-BK (0.03–0.3 nmol) a potentiation of the BK-induced plasma extravasation of between 5 and 13% was observed suggesting that des-Arg^9,[Leu^8]-BK may exhibit partial agonist activity.

Effect of BK antagonists on BK-induced plasma extravasation in arthritic rats

The effects of the co-injection of BK antagonists with BK (1.0 nmol) were investigated. In contrast to the antagonist effects observed in normal joints, Hoe 140 over the dose range 0.1–1.0 nmol failed to inhibit BK-induced plasma extravasation in the arthritic joints (Figure 3b). At a higher dose (10 nmol) Hoe 140 slightly inhibited the response. In normal animals, the ability of Hoe 140 to inhibit the bradykinin-induced plasma extravasation was not compromised by the higher dose of BK (1.0 nmol) used in the arthritic rats (Figure 3a). By comparison, des-Arg^9,[Leu^8]-BK, was a more potent inhibitor in the arthritic knees than in the normal joints and inhibited the response to a greater degree than Hoe 140 (Figure 3b).

Effect of BK antagonists on basal plasma extravasation in arthritic rats

On days 1 and 5 post-challenge either Hoe 140 or des-Arg^9,[Leu^8]-BK was injected (3–30 nmol kg^{-1}, i.v.) prior to the administration of Evans blue and the basal plasma extravasation was assessed over the next 2 h. There was no significant difference between basal plasma extravasation in the untreated arthritic animals and those injected with saline (data not shown). Basal plasma extravasation was significantly inhibited in a dose-dependent fashion by Hoe 140 on

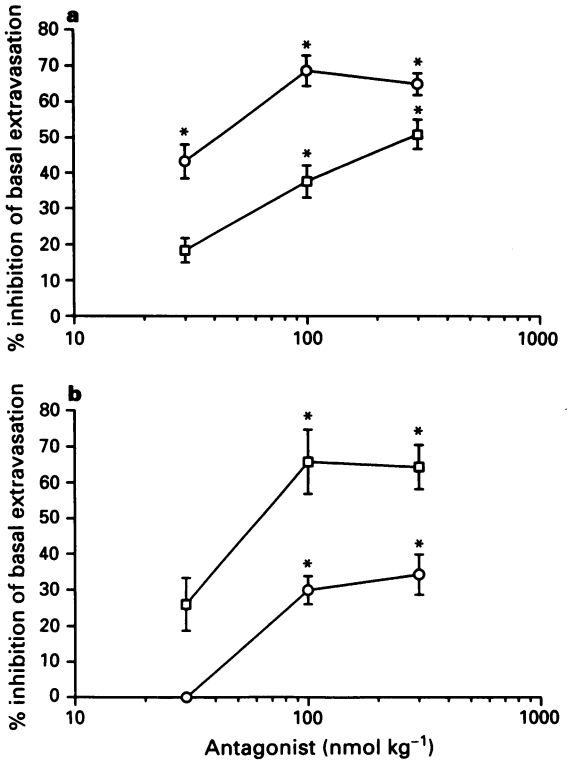

Figure 4 Effect of Hoe 140 (○) and des-Arg^9,[Leu^8]-BK (□) on basal plasma extravasation on (a) day 1 and (b) day 5 post-challenge. Points represent the means ± s.e.mean ($n = 3-6$ per group). *$P < 0.05$ vs saline treated controls.

day 1 (maximum inhibition 68.6 ± 4.2%, $n = 4$, $P < 0.002$; Figure 4a) and to a lesser extent on day 5 (maximum inhibition 34.3 ± 5.6%, $n = 6$, $P < 0.02$; Figure 4b). The results for des-Arg^9,[Leu^8]-BK showed a significant inhibition of basal plasma extravasation on day 1 (maximum 50.9 ± 4.2%, $n = 3$, $P < 0.005$; Figure 4a) and a greater inhibition on day 5 (65.9 ± 8.9%, $n = 5$, $P < 0.001$; Figure 4b).

Discussion

The present study shows that while BK and related peptides are potent mediators of intra-articular oedema in both normal and inflamed joints, the functional role of BK receptors may change with time. The relative inability of the B_1 agonist, des-Arg^9-BK, to induce plasma extravasation in the normal joint combined with the greater potency of Hoe 140 in the inhibition of BK-induced plasma extravasation suggests that, in normal joints, BK acts predominantly via B_2 receptors. These observations are in keeping with those made in other normal tissues (Steranka *et al.*, 1988; Dray *et al.*, 1992). The B_1 antagonist, des-Arg^9,[Leu^8]-BK, had only a small effect on the BK-induced plasma extravasation in normal joints. However, the observation that des-Arg^9-BK was able to induce some degree of extravasation and the ability of des-Arg^9,[Leu^8]-BK to inhibit this response, suggests that a small population of B_1 receptors may exist within the normal joint. The possibility that B_1 receptors might have been unmasked or expressed during the 2 h time-course of the experiment cannot be excluded.

Chronic joint inflammation was induced with the mBSA model of arthritis as it mirrors some of the features of chronic human arthritis, particularly rheumatoid arthritis (Brackertz *et al.*, 1977). Following prior sensitization with mBSA, a single intra-articular injection of mBSA causes an inflammatory response with a initial vascular phase (plasma extravasation and hyperaemia) followed by cellular infiltration and resultant tissue breakdown. The Evans blue extraction technique allowed for estimation of both stimulated and underlying unstimulated, basal, extravasation. The contralateral joint was not used for the saline control as recent studies have raised the possibility of contralateral sensitization following an inflammatory insult (Cruwys *et al.*, 1991; Perkins & Kelly, 1993).

In chronically inflamed joints the response to intra-articular injections of BK was unaltered although at lower doses the response appeared to be masked by increased basal plasma extravasation. In contrast, plasma extravasation in response to higher doses of des-Arg^9-BK was enhanced. This observation, together with the enhanced inhibition of BK-induced plasma extravasation by the B_1 antagonist, des-Arg^9,[Leu^8]-BK, suggested a functional role for B_1 receptors in persistant arthritis.

Further evidence of a role for B_1 receptors in chronic joint disease was evident from the basal extravasation studies. Comparison of the relative abilities of Hoe 140 and des-Arg^9,[Leu^8]-BK to inhibit basal plasma extravasation on days 1 and 5 of joint inflammation showed that whereas Hoe 140 became less potent, des-Arg^9,[Leu^8]-BK became more potent as the model progressed. Whilst it is possible that this inhibition might have been due to the antagonists having vascular effects, Wirth *et al.* (1991) demonstrated that at similar doses to those used in this study, Hoe 140 had no effect on blood pressure or heart rate.

Our results relating to changes of receptor expression in arthritis are in keeping with observations from other inflamed tissues. These show that acute oedema appears to be mediated via B_2 receptors (Burch & De Haas, 1990; Neppl *et al.*, 1991) but that the B_2 receptor antagonists, including Hoe 140, become less effective in longer duration models such as carrageenin-induced paw oedema (Beresford & Birch, 1992). Increased expression of B_1 receptors has recently been reported in aortic smooth muscle following induction of chronic inflammation (Farmer *et al.*, 1991) and B_1 receptors may be involved in the hyperalgesia accompanying inflammatory conditions (Perkins *et al.*, 1993).

BK is metabolized to des-Arg^9-BK and thereafter to a variety of other metabolites by kininases which we have recently demonstrated in inflamed but not normal joint tissues (Map *et al.*, 1992). The conversion of BK to des-Arg^9-BK has been shown to be much faster in rheumatoid arthritis than in osteoarthritis or normal individuals (Sheikh & Kaplan 1987) and levels of both BK and des-Arg^9-BK are increased in inflammatory conditions (Steranka *et al.*, 1987; Hargreaves *et al.*, 1988). In view of the probable changes to BK receptors in chronic arthritis it follows that formation of specific kinin metabolites, including des-Arg^9-BK, could result in an altered inflammatory response in these disorders (Bhoola *et al.*, 1992).

In vitro studies have demonstrated a role for inflammatory mediators known to be present in inflamed joints, particularly the cytokines interleukin-1 (IL-1) and IL-2, in the induction of B_1 receptors (De Blois *et al.*, 1988; 1989). Similarly, agents which stimulate macrophages have been shown to increase responses to des-Arg^9-BK (De Blois *et al.*, 1989). It follows that the components required for the expression and activation of B_1 receptors are present within an inflamed joint. des-Arg^9-BK is a potent stimulator of IL-1 release from macrophages (Burch *et al.*, 1989; Tiffany & Burch, 1989) and the potential exists for arthritis to be exacerbated by a positive feedback loop whereby des-Arg^9-BK stimulates the release of IL-1 from macrophages and the IL-1 in turn acts to stimulate the induction of B_1 receptors.

The clinical use of BK receptor antagonists has been proposed for the treatment of inflammatory diseases including rheumatoid arthritis (Sharma, 1992). The data presented here support this proposal and indicate that selective B_1 receptor antagonists may reduce joint swelling in inflammatory arthritis. Perkins *et al.* (1993) demonstrated that B_1 receptors are involved in the hyperalgesia which accompanies inflamma-

tion. By implication, B_1 receptors on sensory nerves may also be involved in the release of vasoactive peptides as a result of the axon reflex response. These peptides serve to modify many aspects of the inflammatory cascade (Cruwys *et al.*, 1992) and inhibition of this process may well serve to modify the outcome of chronic disease (Hakanson *et al.*, 1987; Kidd *et al.*, 1990).

In conclusion, the present study shows that BK contributes significantly to the formation and maintenance of plasma extravasation in arthritis. Whilst the majority of responses to BK in normal and acutely inflamed joints are mediated via B_2 receptors, an increasingly dominant role appears to be played by B_1 receptors in the chronic and potentially more deleterious phases of the disease. This may reflect an important role for the BK metabolite, des-Arg^9-BK, in the maintenance of arthritis and suggests that selective B_1 receptor antagonists may be of therapeutic benefit in chronic disease.

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