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Mechanism of bradykinin-induced plasma extravasation in the rat knee joint

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1 We have investigated the mechanism of bradykinin (BK)-induced plasma extravasation into the knee joint of the anaesthetized rat. Accumulation of [^{125}I]-human serum albumin within the synovial cavity was used as a marker of increased vascular permeability.

2 Perfusion with BK (1 μM) produced significant plasma extravasation into the knee which was inhibited by co-perfusion of the selective bradykinin B_2 receptor antagonist D-Arg-[Hyp^3,Thi^5,D-Tic^7,Oic^8]-bradykinin (Hoe 140, 200 nM).

3 The bradykinin B_1 receptor agonist, [des-Arg^9]-BK (up to 100 mM), did not induce plasma extravasation into the knee joint over this time period.

4 Chemical sympathectomy by chronically administered 6-hydroxydopamine (6-OHDA) did not inhibit bradykinin-induced plasma extravasation. Acute intra-articular perfusion with 6-OHDA (to stimulate transmitter release from sympathetic nerve terminals) at concentrations up to 50 mM did not induce significant plasma extravasation. Intra-articular perfusion of 100 mM 6-OHDA induced significant plasma extravasation but produced severe systemic toxicity.

5 The selective neurokinin$_1$ (NK_1) receptor antagonist, RP67580 (230 nmol kg^{-1}), or receptor antagonists for the mast cell products histamine and 5-hydroxytryptamine did not significantly inhibit BK-induced plasma extravasation.

6 Co-perfusion of the NO synthase inhibitor, N^G-nitro-L-arginine methyl ester (L-NAME) (1 mM) did not significantly inhibit the response to BK. ^{133}Xe clearance from L-NAME (1 mM)-injected joints was significantly ($P < 0.05$) reduced compared to D-NAME injected joints, suggesting a reduction in blood flow as a result of decreased basal NO production. Systemic administration of L-NAME at doses sufficient to produce significant and sustained elevation of blood pressure (5 or 30 mg kg^{-1}, i.v. 15 min prior to BK perfusion) also failed to significantly inhibit the BK-induced response.

7 We conclude that, in normal joints, BK induces plasma extravasation by acting on bradykinin B_2 receptors and that this response is not dependent on secondary release of mediators from sympathetic nerve terminals, sensory nerves, mast cells or on generation of NO.

Keywords: Bradykinin; joint; plasma protein extravasation

Introduction

Kinins are generated from plasma and tissue precursors at sites of tissue injury and contribute to many aspects of both acute and chronic inflammation including oedema formation, vasodilatation and pain (Hall, 1992). Bradykinin (BK) has diverse actions on vascular endothelium, smooth muscle and cellular function and is a powerful algesic agent which can both sensitize and directly stimulate sensory nerve terminals. Its actions are mediated both by activation of BK receptors on target tissues and, indirectly, by release or amplification of nitric oxide or other inflammatory agents including neuropeptides and prostaglandins. In a previous study BK was found to produce a dose-dependent increase in plasma extravasation into the rat knee joint without producing systemic oedema (Cambridge & Brain, 1992). It was the most potent of the inflammatory mediators tested in this assay and, unlike histamine, was not potentiated by vasodilators. BK may act via several pathways to produce extravasation into joints. These include receptor-mediated effects on endothelial cells to increase vascular permeability and activation of sensory nerves with subsequent release of pro-inflammatory peptides including substance P, neurokinin A (NKA) and calcitonin gene-related peptide (CGRP). Recently, considerable evidence has been presented to support a role for sympathetic nerves in mediating the response to BK (Green *et al.*, 1993a,b). In addition BK may also activate synovial mast cells to release histamine (and 5-hydroxytryptamine (5-HT) in rodents). Local release of NO from endothelial cells and sensory or perivascular neurones may also contribute to the effects of BK by increasing blood flow in the synovial membrane.

In our study modulation of the response to BK was measured in the perfused knee model preparation of the rat. The release of pro-inflammatory peptides from sensory nerves was mimicked by co-perfusion of a selective NK_1 tachykinin receptor agonist, GR73632 with CGRP. GR73632 was used in preference to substance P as, in a previous study, intra-articular perfusion of substance P at sufficiently high concentrations to induce plasma extravasation, also produced systemic oedema (Cambridge & Brain, 1992). To assess the role of sympathetic nerve activation, BK-induced plasma extravasation was measured in rats which had been chemically sympathectomized by chronic treatment with 6-hydroxydopamine (6-OHDA). In addition the effects of acute sympathetic terminal activation by intra-articular perfusion of 6-OHDA on plasma extravasation were also measured. The contribution of BK-stimulated production of NO to plasma extravasation was measured indirectly by use of the NO synthase inhibitor, N^G-nitro-L-arginine methyl ester (L-NAME). Local effects of L-NAME on blood flow within the synovium were measured by use of the ^{133}Xe clearance method (Cambridge & Brain, 1992).

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Methods

Animals

Male Wistar rats (250–300 g) kept in a temperature-controlled environment and fed standard laboratory food and water *ad libitum* were used in this study. For the joint perfusion and blood flow studies and for the initial sympathectomy treatment with 6-OHDA, animals were anaesthetized with sodium pentobarbitone (50 mg kg^{-1}, i.p., additional doses as required).

Joint perfusion and quantification of plasma extravasation

Plasma extravasation was measured by accumulation of radiolabelled albumin in the synovial cavity of the rat knee after perfusion of test agents through the joint space (Cambridge & Brain, 1992). Animals were injected via the tail vein with 1.5 μCi [^{125}I]-human serum albumin, mixed with Evans Blue (0.2 ml, 25 mg ml^{-1}) as a visible marker. In each animal one knee joint was perfused with 100 μl min^{-1} Tyrode solution (composition, mM: NaCl 136.9, KCl 2.7, NaH_2PO_4 0.42, $NaHCO_3$ 11.9, $MgCl_2$ 1.0 and glucose 5.6) via 27G needles placed within the synovial cavity and connected via a polythene cannula to a roller pump (Watson-Marlow, Falmouth, U.K.). To establish a stable baseline the joint was perfused with Tyrode solution for 20 min. The solution was then changed to Tyrode (or other vehicle solution where appropriate) containing test agents and perfusion continued for 5 min. The pump was then stopped and the test agents were allowed to remain in the joint for a further 10 min after which time perfusion was recommenced with Tyrode solution alone and the perfusate (1 ml) collected and radioactivity counted in a gamma counter. Plasma extravasation was expressed as μl/joint by comparison of counts in the synovial perfusate to counts in a plasma sample.

Blood pressure measurement

Blood pressure was continuously monitored in animals which received either intravenous L-NAME (or saline) or intra-articular perfusion of 6-OHDA at both 50 mM and 100 mM. A cannula was placed in the right carotid artery and connected to a pressure transducer and chart recorder. Data are expressed as mean arterial pressure (MAP) in mmHg.

Chemical sympathectomy

Animals received either 6-OHDA or an equivalent volume of vehicle (1% ascorbic acid in saline) following the sympathectomy protocol of Green *et al.* (1993b). 6-OHDA was administered by i.p. injection on days 1, 2, and 3, (50 mg kg^{-1} daily) and on days 6 and 7 (100 mg kg^{-1} daily). Joint perfusion experiments were carried out on day 8.

To assess the functional effects of the sympathectomy regimes, the pressor response to tyramine was measured in a separate group of 6-OHDA-treated ($n=5$) and normal rats ($n=4$). Tyramine (dissolved in normal saline) was injected as a bolus via a butterfly cannula placed in the tail vein.

^{133}Xe clearance from synovial cavity

Estimation of changes in knee joint blood flow produced by L-NAME was carried out using a ^{133}Xe clearance method as previously described (Cambridge & Brain, 1992). Briefly ^{133}Xe (100 μCi) was mixed with 1 ml of L-NAME (1 mM), D-NAME (1 mM) or saline. One hundred μl of either D- or L-NAME was rapidly injected into one knee joint and 100 μl of saline injected into the contralateral joint. After a 5 min clearance period the animals were killed, the joints removed and radioactivity counted in a gamma counter. Results are expressed as percentage difference in clearance between L- or D-NAME-injected joints and saline-injected joints.

Materials

BK, [des-Arg^9]-bradykinin, 6-OHDA (hydrobromide), mepyramine, N^G-nitro-L-arginine methyl ester (L-NAME) and N^G-nitro-D-arginine methyl ester (D-NAME) were obtained from Sigma (Poole, U.K.), GR73632 (δ-Ava-Phe-Phe-Pro-MeLeu-Met-NH_2) was a gift from Dr D. Beattie, Glaxo (Ware, U.K.) and human αCGRP, a gift from Dr U. Ney, Celltech (Slough, U.K.). The BK B_2 receptor antagonist D-Arg-[Hyp^3,Thi^5,D-Tic^7,Oic^8]-BK (Hoe140) was obtained from Peninsula Laboratories, (St Helens, U.K.). [^{125}I]-human serum albumin (2.5 μCi mg^{-1}) and ^{133}Xe were obtained from Amersham International (U.K.). Agents for intra-articular perfusion were dissolved in Tyrode solution except for 6-OHDA which was dissolved in Tyrode solution containing 1% ascorbic acid (to prevent oxidation). The specific NK_1 tachykinin receptor antagonist, RP67580 ([3a**R**, 7a**R**]-7,7-diphenyl-2-[1-imino-2-(2-methoxyphenyl)-ethyl] perhydroisoindol-4-one), a gift from Dr C. Garret, Rhone-Poulenc Rorer (France) was dissolved in saline and injected (230 nmol kg^{-1}) into the tail vein 10 min before the start of the BK perfusion, a protocol which virtually abolishes neurogenic oedema induced by stimulation of the saphenous nerve (Garrett *et al.*, 1991). The 5-HT antagonist, methysergide (a gift from Sandoz, U.K.), and histamine H_1 antagonist, mepyramine were dissolved in saline and injected (both 10 mg kg^{-1}, i.p.) 15 min before the start of the BK infusion. This treatment has been shown to inhibit oedema formation in rat skin induced by the mast cell degranulating agent compound 48/80 (Brain & Williams, 1985). L-NAME was given locally (as a 1 mM solution co-perfused with BK) or systemically via a tail vein injection (5 mg kg^{-1} or 30 mg kg^{-1}) 15 min before the intra-articular perfusion with BK.

Statistical analysis

Except where indicated in the figure legend, results are expressed as mean ± s.e.mean and n refers to the number of animals in each group in all cases. One-way ANOVA, followed by Tukey's multiple comparisons test was used to assess the significance of differences between group means. Where variances were different, nonparametric analysis of variance (Kruskal-Wallis test), followed by a Dunn's multiple comparison test, was used. Blood pressure responses before and after L-NAME and 6-OHDA were compared by Student's paired t test and differences in ^{133}Xe clearance from L-NAME- and D-NAME-treated joints by Student's unpaired t test.

Results

Modulation of BK-induced plasma extravasation

As previously reported (Cambridge & Brain, 1992) perfusion of 1 μM BK induced significant ($P<0.01$, $n=10$) plasma extravasation in the knee joint when compared to joints which were perfused only with Tyrode solution ($n=8$) (Figure 1). Co-perfusion of the B_2 receptor antagonist Hoe140 (200 nM; $n=12$) significantly inhibited BK-induced plasma extravasation compared to BK alone whilst at a lower dose (20 nM; $n=13$) Hoe140 was without significant effect. The selective NK_1 receptor antagonist, RP67580 (230 nmol kg^{-1}, i.v.) did not inhibit BK-induced plasma extravasation. By contrast, RP67580 (230 nmol kg^{-1}, i.v.) significantly attenuated the response induced by co-perfusion of the specific NK_1 agonist GR73632 (1 μM) with CGRP (100 nM). Methysergide and mepyramine, the selective antagonists of the mast cell mediators 5-HT and histamine, respectively, did not significantly inhibit the response to BK (Figure 1). Co-perfusion of the NO synthase inhibitor L-NAME (1 mM; $n=6$) with BK did not significantly decrease plasma extravasation although a trend towards attenuation of the response was seen (Figure 1). The selective BK B_1 receptor agonist, [des-Arg^9]-BK, at doses up to

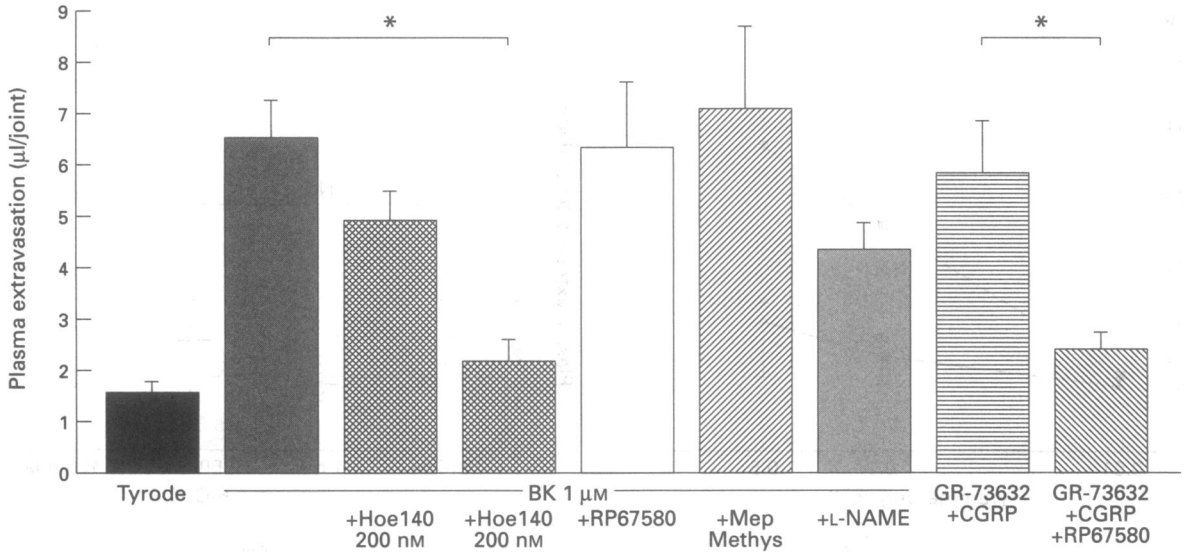

Figure 1 Modulation of BK-induced plasma extravasation into the rat knee joint. Results are shown as follows: BK (1 μM) alone (solid column); BK (1 μM) co-perfused with Hoe140 (20 nM and 200 nM), (cross-hatched columns); BK (1 μM) perfused 10 min after i.v. injection of RP67580 (230 nmol kg^{-1}), (open column); BK (1 μM) perfused 15 min after i.p. injection of methysergide and mepyramine (both 10 mg kg^{-1}), (hatched column); BK (1 μM) co-perfused with L-NAME (1 mM) (stippled column). Responses induced by co-perfusion of GR73632 (1 μM) plus CGRP (100 nM) (horizontal lined column) and 5 min after i.v. injection of RP67580 (230 nmol kg^{-1}), (hatched column) are also shown. Results are mean ± s.e.means. $n = 6-13$ animals. *$P < 0.05$, Tukey's multiple comparison test. For abbreviations, see text.

100 μM, failed to elicit significant plasma extravasation (100 μM, 2.4 ± 5.0 μl/joint, $n = 7$).

Intravenous administration of L-NAME at 5 mg kg^{-1} produced a significant ($P < 0.05$, paired t test) rise in blood pressure in all animals (mean basal, 77.5 ± 6.1 mmHg vs mean L-NAME, 101.7 ± 4.8, $n = 4$), but no significant effect on BK-induced plasma extravasation (Figure 2). At the higher dose of L-NAME (30 mg kg^{-1}) a greater increase in MAP was seen (mean basal 97.8 ± 4.0 mmHg vs L-NAME, 141.3 ± 5.0, $n = 9$, $P < 0.0001$, paired t test). However, consistent inhibition of the BK response was not evident (Figure 2). Blood pressure was unchanged over the experimental period in the control group which received i.v. saline ($n = 7$).

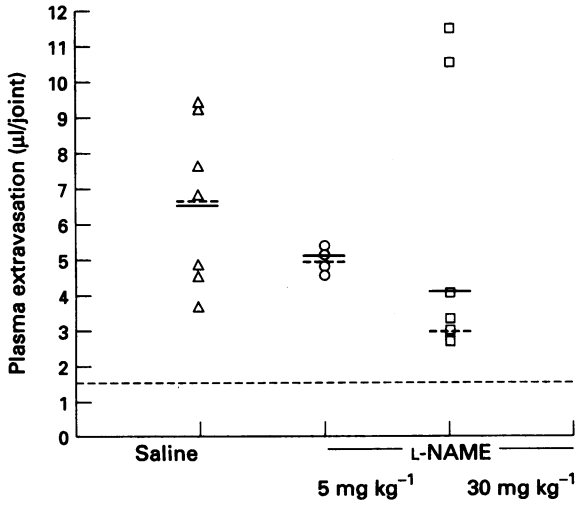

Figure 2 Effect of systemic N^G-nitro-L-arginine methylester (L-NAME) on bradykinin (BK)-induced plasma extravasation. BK-induced plasma extravasation (measured by accumulation of labelled albumin) into the knee joint of animals treated with intravenous L-NAME (5 mg kg^{-1}, $n = 4$, or 30 mg kg^{-1}, $n = 9$) and a control group ($n = 7$) which received an equivalent volume of saline. Perfusion of BK was started 15 min after L-NAME injection. Solid bars represent mean response and dashed bar represents median response in each group. No significant differences were found between groups. Dashed line represents mean response in Tyrode-perfused joints of untreated animals ($n = 8$).

Effect of L-NAME on ^{133}Xe clearance from synovial cavity

^{133}Xe clearance was reduced in L-NAME (0.1 μmol)-injected joints compared to contralateral saline-injected joints by 39.3% ± 3.6, ($n = 5$). This was significantly different ($P < 0.05$, unpaired t test) from D-NAME (0.1 μmol)-injected joints where ^{133}Xe clearance was 10.4% ± 7.0 less than in contralateral joints.

Effect of chemical sympathectomy

Pressor effect of intravenous tyramine To establish the effectiveness of the sympathectomy treatment the pressor response to i.v. tyramine, an indirect sympathomimetic (0.01 to 0.5 mg kg^{-1}), was measured in additional groups of untreated and 6-OHDA-treated animals. In untreated animals ($n = 4$) tyramine produced a dose-dependent increase in mean arterial blood pressure which was absent in 6-OHDA-treated animals ($n = 5$), demonstrating that sympathectomy was successful in this group (Figure 3a).

BK-induced plasma extravasation In animals chronically treated with 6-OHDA, BK (1 μM)-induced plasma extravasation into the knee joint was significantly ($P < 0.05$) increased compared to vehicle (1% ascorbic acid) controls (Figure 3b).

Effect of acute perfusion of 6-OHDA on plasma extravasation into the joint

Intra-articular perfusion with 6-OHDA (1 mM, $n = 6$, and 50 mM, $n = 10$), did not cause significant plasma extravasation compared to vehicle ($n = 10$) (Figure 4a). At the highest dose tested (100 mM), 6-OHDA produced a significant response ($P < 0.05$) (Figure 4a), although the results were highly variable. In the 50 mM 6-OHDA group, signs of systemic sympathetic activation including piloerection and tachycardia were observed in all rats during intra-articular perfusion. At 100 mM 6-OHDA produced obvious toxic effects, including pronounced hypersecretion in the airways, tachycardia and

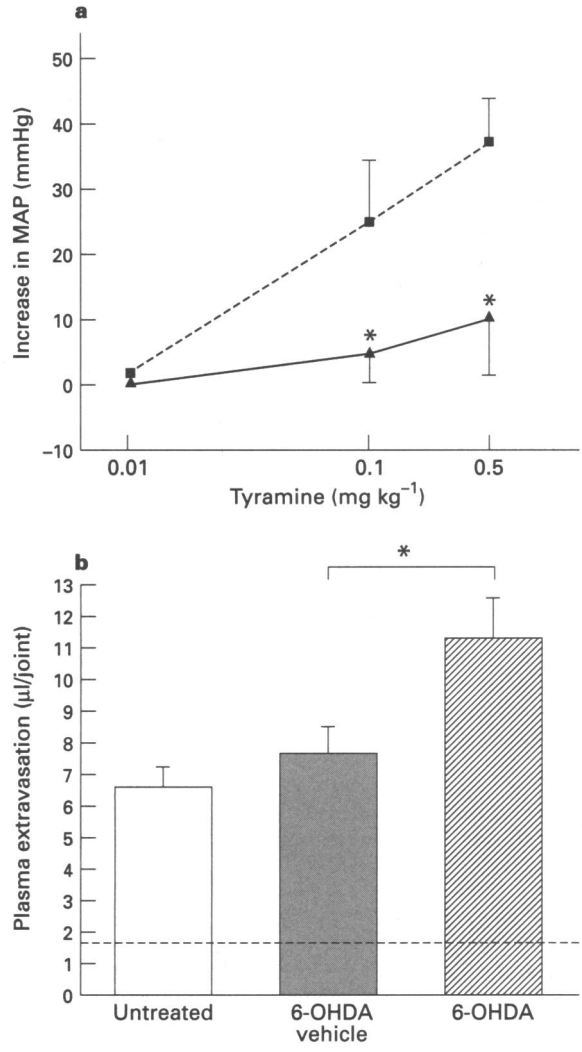

Figure 3 (a) Pressor response (mean ± s.d.) to i.v. tyramine (0.01–0.5 mg kg^{-1}) in (■) untreated rats, $n=4$; (▲) 6-hydroxydopamine (6-OHDA)-treated rats, (50 mg kg^{-1}, i.p. on days 1,2,3, 100 mg kg^{-1}, i.p. on days 6,7) $n=5$. *$P<0.05$, untreated vs 6-OHDA-treated rats, unpaired t test. (b) Effect of chemical sympathectomy on bradykinin (BK)-induced plasma extravasation. Plasma extravasation (measured by accumulation of labelled albumin in the knee joint) in response to intra-articular perfusion of BK (1 µM) in untreated rats (open columns, $n=10$); 6-OHDA vehicle (normal saline with 1% ascorbic acid) treated rats (stippled column, $n=15$); 6-OHDA-treated (as in [a] above) rats (hatched column, $n=11$). Dashed line represents the response to perfusion of Tyrode solution alone in a separate group of 8 untreated animals. Mean ± s.e.mean *$P<0.05$.

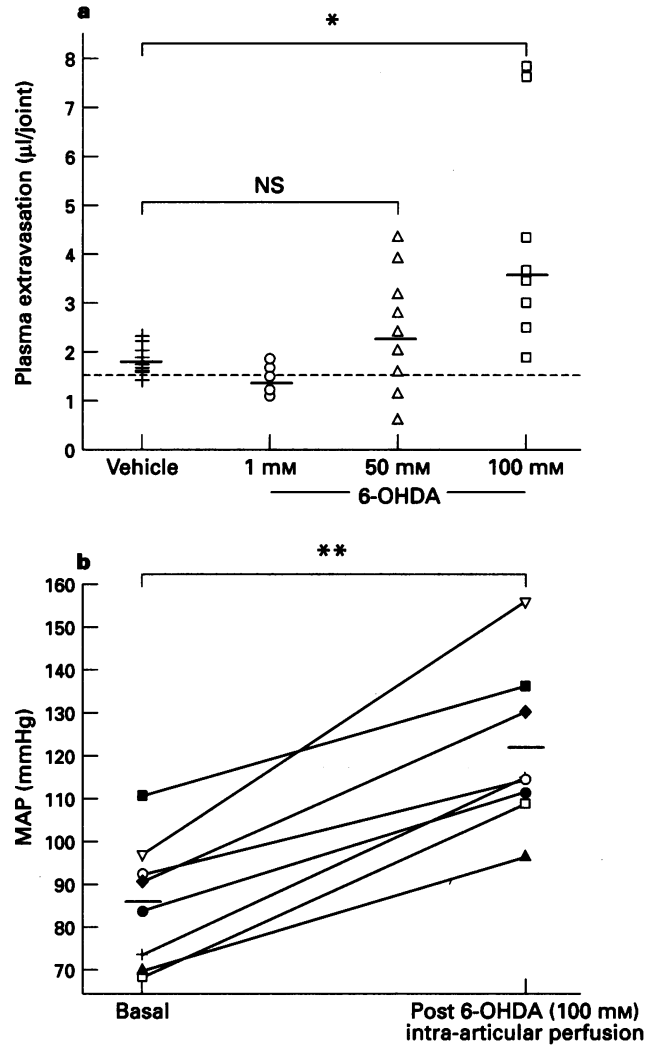

Figure 4 (a) Plasma extravasation into the knee joint induced by intra-articular perfusion of 6-hydroxydopamine (6-OHDA). Data from individual animals are shown at each dose; vehicle, $n=10$; 1 mM, $n=6$; 50 mM $n=10$; 100 mM, $n=8$. Bar represents the median response. *$P<0.05$. (b) Effect of intra-articular perfusion of 6-OHDA (100 mM) on MAP in 8 rats. Basal = MAP during perfusion of joint with vehicle (Tyrode solution with 1% ascorbic acid). Post = maximum MAP reached during perfusion of joint with 6-OHDA (100 mM for 5 min). Same animals as in (a). Bar represents mean response. **$P<0.01$.

cardiac arrhythmias. All animals in this group developed significant and prolonged hypertension with mean blood pressure increasing from a pretreatment mean of 82.4 ± 5.4 mmHg to 119.3 ± 5.9 mmHg ($P<0.0001$, $n=8$), indicating leakage of the drug into the systemic circulation. Data for individual animals are shown in Figure 4b.

Discussion

As BK-induced plasma extravasation was inhibited by co-perfusion with Hoe140, a selective B_2 antagonist (Hock *et al.*, 1991), these results show that in the normal joint, BK produces plasma extravasation via activation of the BK B_2 receptor. The selective B_1 agonist, [des-Arg^9]-bradykinin did not produce plasma extravasation at concentrations up to 100 times greater than that for BK. A recent report (Cruwys *et al.*, 1994) has described B_1 plasma extravasation in normal rat knees measured 3 h after intra-articular injection, however [des-Arg^9]-bradykinin was much less potent than BK and produced a smaller maxium response. Our findings are consistent with *in vitro* and *in vivo* studies (Boutillier *et al.*, 1987; Perkins & Dray, 1993) and *ex vivo* studies (Farmer *et al.*, 1991) where the presence of tissue damage, inflammation or the action of some cytokines (e.g. interleukin-1β) has been necessary to induce the B_1 receptor and thus allow effects of B_1 receptor activation to be measured. For example, hyperalgesia associated with adjuvant arthritis and thermal injury in rats is more effectively attenuated by B_1 than B_2-selective antagonists (Perkins *et al.*, 1993) but the effects of B_1 receptor activation on the local vascular processes of inflammation have not been extensively investigated. The significant inhibition of the response by Hoe140 provides further evidence for the importance of B_2 receptors in producing acute plasma extravasation into the joint.

A possible mode of action of BK in the joint is via activation of sensory nerves, with subsequent release of pro-inflammatory peptides and induction of neurogenic oedema. In some tissues, notably the rabbit iris, the response to BK is mediated indirectly by neuropeptides including substance P and CGRP (Ueda *et al.*, 1984; Wahlestedt *et al.*, 1985). To

mimic the release of sensory neuropeptides, the selective NK_1 agonist (GR73632), which induces oedema formation in rat skin (Richards *et al.*, 1993), and the vasodilator CGRP were co-perfused. We have previously shown that CGRP does not induce plasma extravasation at vasodilator doses (Cambridge & Brain, 1992) but it has been well established that CGRP acts synergistically with mediators of increased vascular permeability (Brain & Williams, 1985). GR73632 and CGRP produced significant plasma extravasation into the joint which could be totally inhibited by RP67580, a non-peptide NK_1 receptor antagonist. RP67580 is selective for the rodent NK_1 receptor and inhibits neurogenic oedema in many tissues (Garrett *et al.*, 1991; Beaujouan *et al.*, 1993; Shepheard *et al.*, 1993) but was without effect on BK-induced plasma extravasation in the joint. Thus, although the synovium has many nerves which show positive immunohistochemical staining for these and other peptides (Mapp *et al.*, 1990), our findings do not support a significant role for sensory neuropeptide release in BK-induced plasma extravasation. In addition, previous studies of plasma extravasation in joints showed substance P to be much less potent than BK whilst CGRP, although a potent vasodilator, could only act to potentiate oedema induced by other agents (Cambridge & Brain, 1992; Cruwys *et al.*, 1992).

NO released from endothelial cells, and possibly also from neural structures, may produce vasodilatation and thus enhance vascular leakage induced by BK. In a rat skin blister base model, an NO synthase inhibitor, N^G-nitro-L-arginine (L-NOARG), attentuated both vasodilatation and plasma extravasation to BK (Khalil & Helme, 1992). In the joint, however, co-perfusion of L-NAME, at a concentration shown to inhibit substance P-induced oedema formation by a local vasoconstrictor effect (Hughes & Brain, 1990), did not significantly depress plasma extravasation. A significant decrease in ^{133}Xe clearance produced by L-NAME (at the same concentration used for the plasma extravasation studies) suggests that perfusion of the joint is decreased by local inhibition of basal NO release and this may account for the observed trend towards attenuation of the BK response. In the rabbit knee, close arterial perfusion of L-NAME has also been shown to produce a significant drop in blood flow (Najafipour & Ferrell, 1993). Without simultaneous measurement of local blood flow or NO production it is not possible to differentiate between effects of L-NAME on basal NO release or on enhanced NO production stimulated by BK. As the effects of local L-NAME were inconclusive, the effect of intravenous administration was also measured. At a dose of 5 mg kg^{-1}, i.v., L-NAME did not significantly attenuate the BK response, although the individual data points are at the lower end of the control range. The response to BK after the higher dose of L-NAME (30 mg kg^{-1}) was highly variable but remained statistically unchanged from saline controls. Insufficient inhibition of the enzyme cannot account for these observations as a significant pressor response was observed in both treatment groups, indicative of decreased basal NO production by endothelial cells, and possibly NANC nerves (Rees *et al.*, 1989; Toda *et al.*, 1993). Thus the data obtained with intravenous L-NAME, shows clearly that NO production is not essential for BK-induced plasma extravasation in the joint. Gardiner and co-workers (1990a) demonstrated prolonged vasoconstriction in rat hindquarters and a pronounced fall in cardiac output following L-NAME (10 mg kg^{-1}, i.v.); thus it is likely that blood flow to the synovium was also decreased in treated rats and may account for the variability, and also the trend towards inhibition of plasma extravasation, seen in our study. A component of the vasodilator response induced by BK (3.2 nmol, i.v.) in rat hindquarters was found to be unaffected by L-NAME (Gardiner *et al.*, 1990b) indicating that some vascular actions of BK are also independent of NO synthesis. The lack of effect of antagonists for the important mast cell amines, histamine and 5-HT suggests that, although BK has been shown to stimulate mast cells directly, this is not an important mechanism of its action in this model.

In recent years a large body of experimental evidence has pointed to a key role for sympathetic terminals in enhancing BK-induced plasma extravasation into the joint in arthritis (Coderre *et al.*, 1991; Green *et al.*, 1993a,b). This appears to be highly specific to this property of BK as another major physiological effect of BK, nociceptor sensitization, is not dependent on sympathetic nerve activation (Koltzenberg *et al.*, 1992). This effect also seems confined to the joint as in some other sites, the pro-inflammatory effects of sympathetic nerve terminal activation have been more difficult to demonstrate (Donnerer *et al.*, 1991). Therefore sympathetic nerve activation may be of particular significance to arthritis and further investigation of this process is warranted. However, in our study chemical sympathectomy did not inhibit the response to BK and surprisingly a small increase was observed. The reason for this is unlikely to be inadequate sympathectomy as chronic 6-OHDA treatment is a well-established method for selective neurotransmitter depletion (Thoenen & Tranzer, 1968). The dosing protocol has been shown to deplete tyrosine hydroxylase containing nerves (Sulakvildze *et al.*, 1994) and has been used in previous studies in the joint (Green *et al.*, 1993a,b). In the present study the significant decrease in response to intravenous tyramine points to effective functional impairment of sympathetic nerve endings. A possible reason for the discrepancy between our results and previous findings is the use of different joint perfusion protocols. As in rat skin (Brain & Williams, 1985), the response to BK in the joint is rapid and, to minimize the possibility of systemic effects due to leakage from the joint, a relatively short (5 min) perfusion time is used. Green *et al.* (1993a,b) perfused the joints with a lower concentration of BK but at a higher flow rate and for up to 100 min, which may be sufficient time for both indirect and direct actions of BK on inflammatory cells to be of importance (Bjerknes *et al.*, 1991). In broad agreement with our results, Donnerer *et al.* (1991) found sympathectomy to have little inhibitory effect on neurogenic (saphenous nerve stimulation) or non-neurogenic inflammation (carrageenin paw oedema). The increased response to BK in sympathectomized animals may reflect loss of vasoconstrictor tone of noradrenaline and NPY, both of which inhibit plasma extravasation (Coderre *et al.*, 1989) although non-specific effects of 6-OHDA treatment cannot be ruled out.

Despite good evidence that BK can stimulate sympathetic nerves to release stored neurotransmitters (Weiss *et al.*, 1990; Green *et al.*, 1993b) and a recent report of an electrophysiological mechanism for the excitatory action of BK on cultured sympathetic ganglia (Jones *et al.*, 1995) the mechanism of the pro-inflammatory effect of sympathetic nerve stimulation is not well understood. Acute activation of sympathetic nerves by intra-articular perfusion with 6-OHDA has been reported to produce plasma extravasation in a similar fashion to BK (Coderre *et al.*, 1989); however, in our study, this was not observed. Due to drug leaking into the systemic circulation, a marked pressor response was observed in all animals in the 100 mM 6-OHDA group and severe toxic side effects of the drug were observed in several animals. From these results it is thus difficult to ascribe selective local actions of the drug on sympathetic nerves within the joint, despite significant plasma extravasation. Recently 6-OHDA has been shown to produce plasma extravasation in the rat trachea, but via a mechanism involving sensory rather than sympathetic nerves (Sulakvilidze *et al.*, 1994). Therefore further work, using alternative tools to 6-OHDA, is required to elucidate the mechanism of sympathetic nerve oedema which is clearly non-adrenergic in origin (Khalil & Helme, 1989). The effect on the BK-response of several vasoactive mediators (including ATP, adenosine, prostaglandins and NO) known to be released from sympathetic terminals has been measured (Coderre *et al.*, 1991; Green *et al.*, 1991). Each of these may modulate plasma extravasation by effects on local blood flow, however, none is a potent mediator of increased vascular permeability. The role of purines requires additional investigation as Evans Blue, used as a marker, is a purinoceptor antagonist (Bültmann & Starke, 1993).

We conclude from this study that, in the normal joint, BK mediates plasma extravasation by direct stimulation of B_2 type BK receptors which are likely to be located on vascular endothelial cells and/or smooth muscle. Although neural structures may be activated by BK there is no evidence that specific release of stored sensory neuropeptides, sympathetic neurotransmitters or activation of mast cells contribute significantly to the oedema response.

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