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Substance P and capsaicin-induced mechanical hyperalgesia in the rat knee joint; the involvement of bradykinin B_1 and B_2 receptors

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1 Substance P (SP) and capsaicin induced a mechanical hyperalgesia when injected into rat knee joints.

2 The NK_1 receptor antagonists CP 99994 (10–100 nmol) and RP 67580 (0.1–1 nmol) blocked the development of, and also reversed, SP-induced hyperalgesia. Capsaicin (10 nmol)-induced hyperalgesia was blocked by capsazepine (0.5–5 nmol).

3 Capsaicin-induced hyperalgesia was prevented and reversed by the NK_1 receptor antagonists CP 99994 (100 nmol) and RP 67580 (1 nmol).

4 The bradykinin B_2 receptor antagonist icatibant (5 pmol) blocked the development of both SP and capsaicin-induced hyperalgesia. Icatibant (100 pmol kg^{-1}, i.v.) also reversed an established SP and capsaicin-induced hyperalgesia.

5 Both low dose SP (1 nmol) and capsaicin (1 nmol)-induced hyperalgesia were potentiated by the kininase II inhibitor captopril (100 μg).

6 The B_1 receptor antagonists desArg^{9}Leu^8-bradykinin (BK) (0.5–5 nmol) and desArg^{10}[Hoe 140] (5–50 pmol) only blocked the development of SP-induced hyperalgesia for 30 min after administration. desArg^{9}Leu^8-BK (10 nmol kg^{-1} i.v.) did not reverse an established SP-induced hyperalgesia.

7 Capsaicin-induced hyperalgesia was blocked by desArg^{9}Leu^8-BK (0.5 nmol) and this antagonist also reversed an established capsaicin-induced hyperalgesia.

8 Interleukin-1 receptor antagonist (IL-1ra 0.1 μg) reduced the development of SP-induced hyperalgesia up to 4 h after administration, but did not reverse an established hyperalgesia. IL-1ra (0.1 μg) also blocked the development of and reversed an established capsaicin-induced hyperalgesia.

9 Indomethacin pretreatment (1 mg kg^{-1}, s.c.) did not reduce the development of either SP- or capsaicin-induced hyperalgesia but following indomethacin-pretreatment desArg^{9}Leu^8-BK (10 nmol kg^{-1}, i.v.) failed to reverse a capsaicin-induced hyperalgesia.

10 In conclusion, both SP and capsaicin can induce behavioural hyperalgesia when injected into the knee joint of rats. In addition, blockade of NK_1, bradykinin B_1, B_2 and IL-1β receptors can substantially modulate this hyperalgesia.

Keywords: Substance P; capsaicin; bradykinin; desArg^{9}Leu^8-bradykinin; neurogenic hyperalgesia; interleukin-1 receptor antagonist; prostaglandins

Introduction

Noxious activation of C fibres not only causes transduction of nociceptive information centrally, but also results in peripheral release of neuropeptides. Neuropeptides released into the periphery can cause inflammation, and this neurogenic inflammation has been implicated in the pathogenesis of arthritis (Levine *et al.*, 1984).

Studies into the mechanisms of neurogenic inflammation have used either electrical stimulation, application of capsaicin, or noxious heat to stimulate antidromic release of neuropeptides from primary afferent fibres (Ferrell & Russell, 1986; Yaksh 1988; Andrews *et al.*, 1989; Yonehara *et al.*, 1989). Of the neuropeptides released into the periphery, substance P (SP) has been studied most extensively. NK_1 receptor antagonists have been shown to inhibit neurogenic inflammation (Ferrell & Russell, 1986; Lembeck *et al.*, 1992; Moussaoui *et al.*, 1993), release of SP has been demonstrated after chemical, electrical and heat stimulation of primary afferent fibres (Yaksh, 1988; Yonehara *et al.*, 1989) and exogenously administered SP causes vasodilatation and plasma protein extravasation (Andrews *et al.*, 1989; Lam & Ferrell 1991; Gao *et al.*, 1993).

Although SP has little or no direct sensitizing or excitatory actions on primary nociceptive afferents (Kumazawa & Mizumura, 1979; Mizimura *et al.*, 1987; Cohen & Perl, 1990) it has a variety of cellular actions which could increase the concentration of inflammatory mediators at the nociceptive terminal. SP causes release of prostaglandins from synoviocytes (Lotz *et al.*, 1987) and induces recruitment and activation of a variety of immune cells (Lotz *et al.*, 1987; Boichot *et al.*, 1993; Kahler *et al.*, 1993; Perretti *et al.*, 1993).

Recently we have demonstrated a role of both B_1 and B_2 kinin receptor mechanisms in mechanical hyperalgesia in the knee joint after administration of Freund's complete adjuvant or the cytokines interleukin (IL)-1β, IL-2 and IL-8 (Davis & Perkins, 1994a,b). In this study we have investigated the possible involvement of the B_1, B_2 kinin receptor systems and IL-1 after administration of SP a putative mediator of neurogenic inflammation or capsaicin which causes release of endogenous SP from joint afferents.

Methods

The method used in this study for assessment of mechanical hyperalgesia has been described previously (Perkins *et al.*, 1992; 1993; Davis & Perkins, 1994) and entailed intra-articular (i.artic.) injections of inflammatory or hyperalgesic agents into one knee joint of female Sprague-Dawley rats (80–100 g Charles-Rivers, kept at 21±2°C, 12 h light/dark, food and water *ad libitum*) and subsequently measuring the load tolerated by the injected leg. For assessment of the load tolerated by

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the treated leg animals were placed with their hind paws on separate balanced force transducers and a downward force applied such that the load tolerated by the untreated joint was approximately 100 g. At this point the injected leg would tolerate less load and this reduction in load was used as a measure of hyperalgesia (Perkins *et al.*, 1992). The load of 100 g was chosen as it gave a sufficient reduction in tolerated load subsequent to intra-articular injections of inflammatory or hyperalgesic agents such that dose-response relationships to test compounds could be properly assessed.

Time course of hyperalgesia

Animals received unilateral intra-articular injections (100 μl) of either SP (0.1 – 100 nmol), capsaicin (1 – 100 nmol) or their respective vehicles. The load tolerated by the treated joint was then assessed at 1, 4, 6 and 24 h post injection.

The antagonist studies have investigated which receptors are involved in the development of the hyperalgesia. This was addressed by co-administration of specific receptor antagonists with either SP or capsaicin and the hyperalgesia assessed 1, 4 and 6 h later. In addition, the reversal of an established SP or capsaicin-induced hyperalgesia by these antagonists has been studied. In the context of these studies the established hyperalgesia was considered to be at the 4 h time point after administration of either SP or capsaicin. In these experiments the antagonist was given 30 min before the 4 h time point.

Antagonism of the development of hyperalgesia

To demonstrate that SP and capsaicin were acting via their respective receptors in this model, selective antagonists were used to block the effects of submaximal doses of SP and capsaicin. SP (10 nmol) was co-administered with the NK_1 receptor antagonists, CP 99994 (10 – 100 nmol) and RP 67580 (0.1 – 1 nmol). Whereas capsaicin (10 nmol) was co-administered with the capsaicin receptor antagonist capsazepine (0.5 – 5 nmol).

Since capsaicin acts via release of neuropeptides from sensory neurones capsaicin (10 nmol) was also co-administered with the NK_1 receptor antagonists, CP 99994 (100 nmol) and RP 67580 (1 nmol), to assess the relative contribution of endogenous SP to the capsaicin-induced hyperalgesia.

SP and capsaicin were also co-administered with IL-1 receptor antagonist (IL-1ra, 0.1 μg), the B_1-receptor antagonists desArg^{9}Leu^8-bradykinin (BK) (0.5 – 5 nmol) and desArg^{10} [Hoe 140] (5 – 50 pmol) or the B_2-receptor antagonist icatibant (5 pmol) to assess the possible contribution of kinins to SP and capsaicin-induced hyperalgesias.

Reversal of an established hyperalgesia

To assess the effect of specific receptor antagonists on the hyperalgesia once it had developed, animals were injected with a sub-maximal dose (10 nmol) of SP or capsaicin (100 μl i.artic.) and then the load tolerated by the treated joint was assessed 4 h later. For systemic administration, animals were injected with desArg^{9}Leu^8-BK (10 nmol kg^{-1}), icatibant (100 pmol kg^{-1}), CP 99994 (1 μmol kg^{-1}), RP 67580 (10 nmol kg^{-1}) or saline via the tail vein 30 min prior to measurement of the hyperalgesia. In other experiments, CP 99994 (100 nmol), RP 67580 (1 nmol), IL-1ra (0.1 μg), or PBS were injected into the treated joint, 30 min before the level of hyperalgesia was assessed. IL-1ra was only administered locally because of the availability of antagonist. CP 99994 and RP 67580 were administered both locally and i.v. at the same dose in order to demonstrate that these antagonist were acting locally to produce their effects in this model.

Potentiation of hyperalgesia

In further experiments the kininase II antagonist captopril was co-administered with a low dose (1 nmol) of either SP or capsaicin. The level of hyperalgesia was then assessed 1, 4 and 6 h later.

Indomethacin sensitivity

Indomethacin 1 mg kg^{-1} s..c. or 2% sodium carbonate vehicle (buffered to pH 7 with NaH_2PO_4) was administered 30 min prior to intra-articular SP or capsaicin. The level of hyperalgesia was then assessed at 1 and 4 h after SP or capsaicin.

Drugs

desArg^{9}Leu^8-BK and IL-1ra were obtained from Bachem A.G. CP 99994 ((+)-(2S,3S)-3-(2-methoxybenzylamino)2-phenylpiperidine), capsazepine, icatibant (D-Arg[Hyp^3,Thi^5, D-Tic^7, Oic^8]-BK) and desArg10[Hoe 140] (desArg^{10} D-Arg[Hyp^3,Thi^5, D-Tic^7, Oic^8]-BK) were synthesised at the Sandoz Institute for Medical Research. Indomethacin, SP and captopril were obtained from Sigma. RP 67580 ((3a**R**, 7a**R**)-7, 7-diphenyl-2-[1-imino-2-(2-methoxyphenyl) ethyl] perhydroisoindol-4-one) was a gift from Dr V. Fardin, Rhone Poulenc Rorer.

Statistical analysis

Appropriate statistical analysis was used throughout this study. MANOVA/ANOVA tests were used to compare time course effects and multiple comparison of a control with several test groups. Tukey's test was used for *post hoc* analysis of means.

Results

Time course of hyperalgesia

SP (1 – 100 nmol) caused a reduction in tolerated load when injected into naive joints. The hyperalgesia was significant at 1, 4 and 6 h post injection with 1 – 100 nmol SP whereas 0.1 nmol SP and PBS vehicle had no effect (Figure 1). The highest dose of SP tested was 100 nmol which caused a reduction in tolerated load from 98 ± 4 g to 53 ± 2 g by 4 h post injection ($n=8$, $P<0.01$). This reduction in tolerated load was still significant 24 h after 10 and 100 nmol SP injection.

Intra-articular capsaicin (1 – 100 nmol) also reduced the tolerated load 1, 4, 6 and 24 h after injection (Figure 1). The highest dose of capsaicin (100 nmol) reduced the tolerated load from 92 ± 2 g to 49 ± 2 g at 1 h post injection ($n=8$, $P<0.01$). Twenty four hours after administration of capsaicin the reduction in tolerated load was still significant at all doses of capsaicin. There was no evidence of distress or motor dysfunction in any of the capsaicin-treated animals during this period.

Antagonism of hyperalgesia

The NK_1 receptor antagonists CP 99994 and RP 67580 were co-administered with SP to assess the involvement of NK_1 receptors in the development of hyperalgesia. Co-administration of CP 99994 (10 and 100 nmol) or RP 67580 (0.1 and 1 nmol) with SP (10 nmol i.artic.) reduced, in a dose-dependent manner, the development of hyperalgesia (Figure 2) and this lasted for the duration of the experiment (Figure 2).

The NK_1 receptor antagonists were also administered once the hyperalgesia had developed to assess the involvement of NK_1 receptors in the maintenance of hyperalgesia. When CP 99994 (100 nmol) or RP 67580 (1 nmol) were administered intra-articularly 3.5 h after injection of 10 nmol SP, into the same joint, the hyperalgesia was reversed (Figure 2). The same doses of CP 99994 or RP 67580 administered intravenously, however, had no effect on the load tolerated by the SP-treated joint (Figure 2).

Capsaicin-induced (10 nmol) hyperalgesia was blocked by co-administration with the capsaicin receptor antagonist cap-

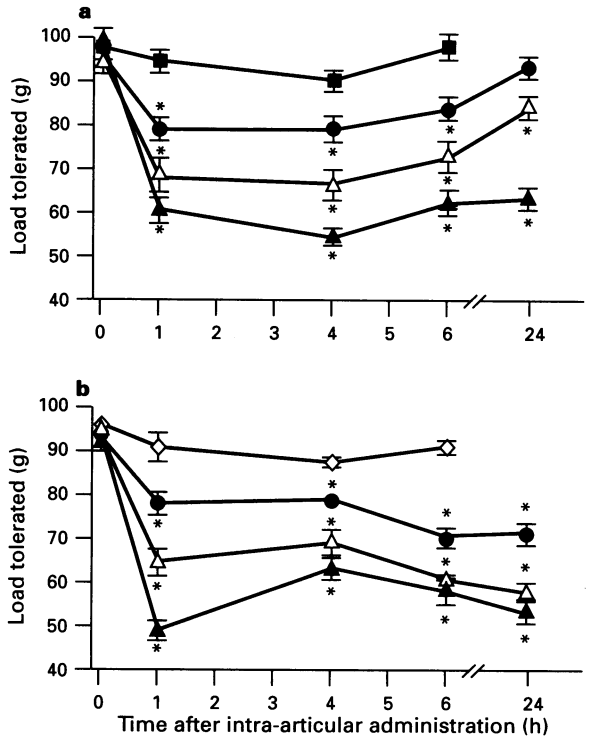

Figure 1 Load (g) tolerated by the ipsilateral leg after intra-articular administration of substance P (SP) (a) or capsaicin (b) into naive joints, all injections were made after the first (0 h) reading. (a) Shows the time course of hyperalgesia after SP 0.1 nmol (■), 1 nmol (●, $n=24$), 10 nmol (△, $n=24$), 100 nmol (▲). (b) Shows the time course of hyperalgesia after capsaicin 1 nmol (●), 10 nmol (△), 100 nmol (▲) and vehicle (◇). All results are expressed as mean ($n=8$ animals per group, unless stated otherwise); vertical lines show s.e.mean. *$P<0.05$ compared to pretreatment reading at each time point.

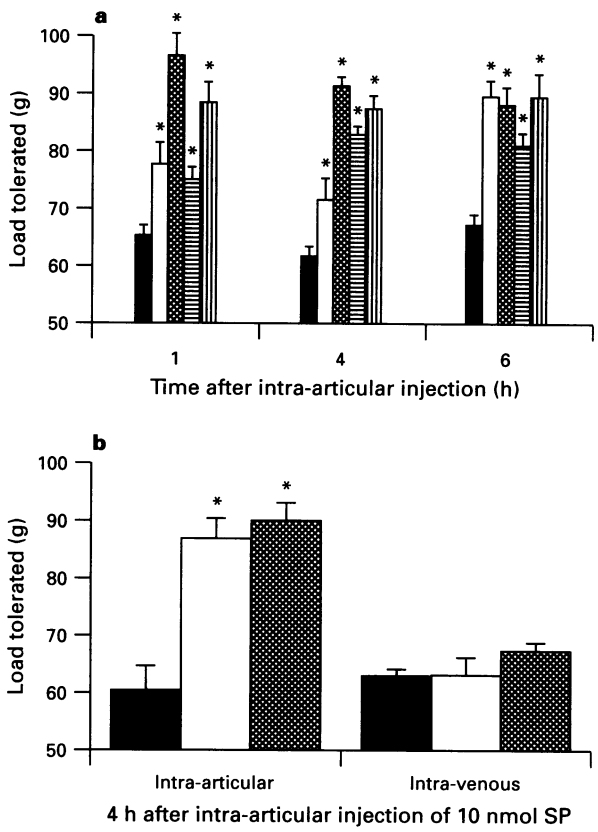

Figure 2 Load (g) tolerated by the ipsilateral leg after intra-articular administration of 10 nmol substance P (SP). In (a) animals either received SP alone (solid columns) or co-administered with CP 99994 10 nmol (open columns) or 100 nmol (cross-hatched columns), RP 67580 0.1 nmol (horizontally-hatched columns) or 1 nmol (vertically-hatched columns). In (b) animals were given an intra-articular injection of 10 nmol SP and the load tolerated by the ipsilateral leg was tested 4 h later; 30 min prior to assessment of the hyperalgesia animals received either 100 nmol CP 99994 (open columns), 1 nmol RP 67580 (cross-hatched columns) or vehicle (solid columns) either intra-articularly into the SP treated joint or intravenously. All results are expressed as mean ($n=8$, unless stated otherwise); vertical lines show s.e.mean. *$P<0.05$ compared to control group at each time point.

sazepine (0.5–5 nmol, Figure 3) with virtually complete reversal of capsaicin-induced hyperalgesia by 5 nmol of capsazepine (Figure 3). Capsazepine (5 nmol) alone had no effect on the load tolerated (data not shown).

To assess the possible contribution of NK_1 receptors in capsaicin-induced hyperalgesia, NK_1 receptor antagonists were administered at doses shown to block SP-induced hyperalgesia. Co-administration of CP 99994 (100 nmol) or RP 67580 (1 nmol) with capsaicin (10 nmol) also reduced the development of hyperalgesia over the entire time course (Figure 3). NK_1 receptor antagonists also reversed an established capsaicin-induced hyperalgesia. Intra-articular injections of CP 99994 (100 nmol) or RP 67580 (1 nmol) reversed capsaicin-induced (10 nmol) hyperalgesia when administered 3.5 h after capsaicin raising the tolerated load from 61 ± 4 g to 88 ± 3 g and 96 ± 3 g ($P<0.05$, $P<8$), respectively.

Contribution of B_2 kinin receptors

The B_2 receptor antagonist icatibant was co-administered with SP or capsaicin, at a dose previously shown to be specific for B_2 receptors in this model (Davis & Perkins, 1994a), to assess the involvement of B_2 receptors in the development of SP or capsaicin-induced hyperalgesia. Co-administration of icatibant (5 pmol) with SP (10 nmol) or capsaicin (10 nmol) blocked the development of hyperalgesia over the time course of study (Figure 4). Icatibant (5 pmol) had no effect in naive joints (data not shown).

The kininase II inhibitor captopril (100 μg) potentiated the level of hyperalgesia induced by both SP (1 nmol) and capsaicin (1 nmol) with increases in the degree of hyperalgesia between 11 and 20% for SP and capsaicin (Figure 4).

Contribution of B_1 kinin receptors

Co-administration of the specific B_1 receptor antagonists des-Arg^{9}Leu^8-BK (0.5–5 nmol) or desArg^{10} [Hoe 140] (5–50 pmol) with SP (10 nmol) only blocked the hyperalgesia for 30 min (Figure 5), by 1 h after administration there was no difference between the antagonist and control treated groups.

Unlike SP-induced hyperalgesia co-administration of des-Arg^{9}Leu^8-BK (0.5 nmol) with capsaicin (10 nmol) blocked the development of hyperalgesia over the entire time course of study (Figure 5).

When icatibant (100 pmol kg^{-1}) was injected i.v. to animals 3.5 h after intra-articular injection of SP, the hyperalgesia was reversed, 30 min later, with an increase in tolerated load from 67 ± 1 g to 94 ± 2 g ($n=8$, $P<0.01$), whereas desArg^{9}Leu^8-BK (10 nmol kg^{-1}, i.v.) had no effect on the hyperalgesia when administered 3.5 h after SP (Figure 6). In contrast, both icatibant (100 pmol kg^{-1}, i.v.) and desArg^{9}Leu^8-BK (10 nmol kg^{-1}, i.v.) reversed the capsaicin-induced hyperalgesia when administered 3.5 h after capsaicin (10 nmol, Figure 6).

Contribution of IL-1 receptors

Co-administration of IL-1ra (0.1 μg) with SP (10 nmol) reduced the development of hyperalgesia up to 4 h after ad-

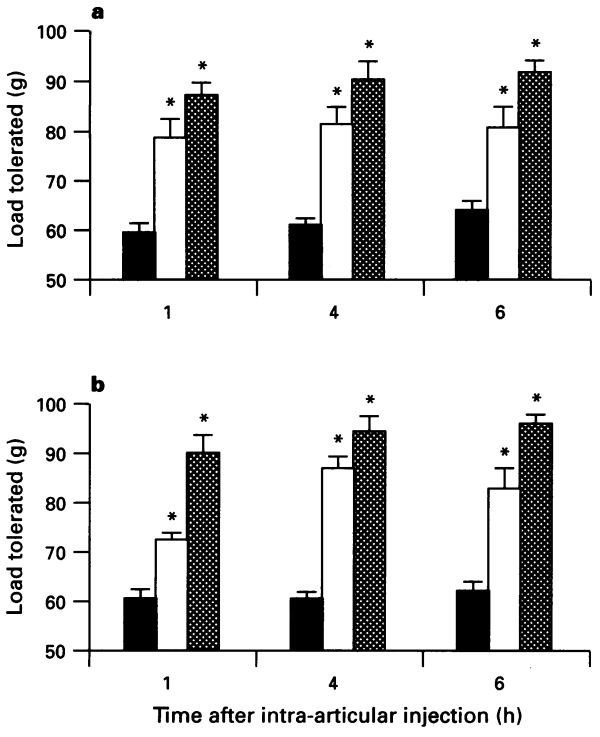

Figure 3 Load (g) tolerated by the ipsilateral leg after intra-articular administration of 10 nmol capsaicin. (a) Animals either received capsaicin alone (solid columns) or co-administered with 0.5 nmol (open columns) or 5 nmol (cross-hatched columns) capsazepine. In (b) animals either received 10 nmol capsaicin alone (solid columns) or co-administered with 100 nmol CP 99994 (open columns) or 1 nmol RP 67580 (cross-hatched columns). All results are expressed as mean ($n=8$, unless stated otherwise); vertical lines show s.e.mean. $^*P<0.05$ compared to control group at each time point.

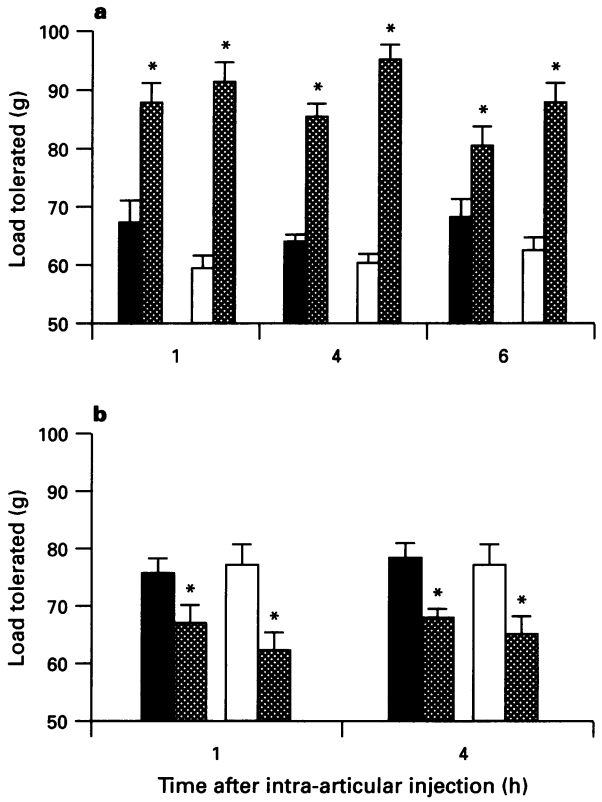

Figure 4 Load (g) tolerated by the ipsilateral leg after intra-articular administration of SP or capsaicin. In (a) animals either received 10 nmol substance P (SP, solid columns) or 10 nmol capsaicin (open columns) alone or co-administered with 5 pmol icatibant (cross-hatched columns). $^*P<0.05$ compared to control group at each time point. In (b) animals either received 1 nmol SP (solid columns, $n=16$) or 1 nmol capsaicin (open columns) alone or in combination with 100 μg captopril (cross-hatched columns). All results are expressed as mean ($n=8$, unless stated otherwise); vertical lines show s.e.mean. $^*P<0.05$ compared with relevant control group.

ministration (Figure 7). The blockade was greatest at 30 min with an increase in tolerated load from 75±3 g ($n=8$) to 97±4 g ($n=16$, $P<0.01$). By 4 h after co-administration of SP and IL-1ra the load tolerated by the treated leg fell to 74±3 g although this was still significantly greater than controls (63±2 g, $n=16$, $P<0.05$). Intra-articular administration of IL-1ra (0.1 μg) 3.5 h after intra-articular administration of SP, however, had no effect on the load tolerated (Figure 7).

When IL-1ra (0.1 μg) was co-administered with capsaicin (10 nmol) there was no significant development of hyperalgesia over the entire time course (Figure 7). In addition, intra-articular injection of IL-1ra (0.1 μg intra-artic.) 3.5 h after capsaicin (10 nmol) also reversed the hyperalgesia (Figure 7).

Indomethacin sensitivity

Indomethacin (1 mg kg^{-1}, s.c.) had no effect on the level of hyperalgesia induced by SP or capsaicin (both 10 nmol). In indomethacin-pretreated animals, desArg^{9}Leu^8-BK (10 nmol kg^{-1}, i.v.) injected 4 h after capsaicin had no effect on the level of hyperalgesia 30 min later, whereas desArg^{9}Leu^8-BK reversed the hyperalgesia of animals which had not received indomethacin (97±2 g compared to 67±2 g, $n=8$ $P<0.01$; Figure 8).

Discussion

These data show that both SP and capsaicin cause mechanical hyperalgesia when injected into the rat knee joint. The reduction in the development, and the reversal of an established SP and capsaicin-induced hyperalgesia by the NK_1 receptor antagonists CP 99994 and RP 67580 suggests that activation of NK_1 receptors can substantially modulate these hyperalgesic states. This SP and capsaicin-induced hyperalgesia is likely to be a local effect, either within the joint or in adjacent tissues, as the same dose of antagonist that was effective in blocking the response to SP and capsaicin when given intra-articularly was ineffective when given systemically. In particular it is unlikely that the NK_1 receptor antagonists were acting at the level of the spinal cord. The greater potency of RP 67580 compared to CP 99994 corresponds to their relative potencies at the rat NK_1 receptor, and the doses of RP 67580 used are much lower than those shown to produce non-specific effects on nociceptive fibres (Rupniak *et al.*, 1993).

As the capsaicin-induced hyperalgesia was blocked by the capsaicin receptor antagonist capsazepine (Bevan *et al.*, 1992) this suggests that capsaicin is acting on its receptor, probably located on sensory terminals. The actions of capsaicin on sensory nerves have been studied extensively in recent years and it has been shown that its effects are dose-related. At low doses there is excitation of small umyelinated sensory nerves with, subsequent to this excitatory action, a period of reduced sensitivity to noxious stimuli (see Bevan & Szolcsanyi, 1990; Dray, 1992). With increasing doses there is a loss of function and, eventually, destruction of the sensory neurone. In this study a hyperalgesia was only observed with no behavioural detriment subsequently, suggesting that the low doses used here did not result in irreversible effects on the joint nociceptors. There is no evidence of an anti-hyperalgesic action of capsaicin in these studies. This may be related to the very low doses employed here some 30–100 fold lower than found to cause mechanical analgesia when injected intracutaneously

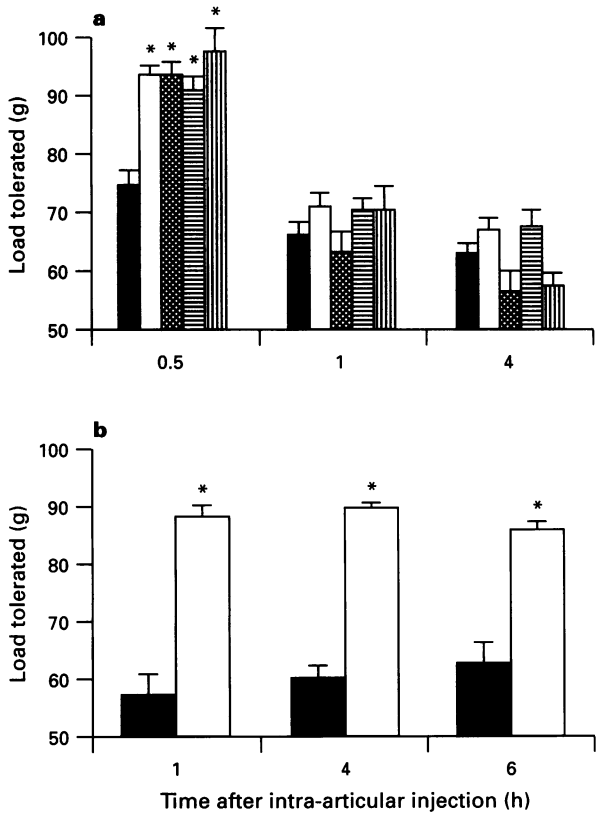

Figure 5 Load (g) tolerated by the ipsilateral leg after intra-articular administration of 10 nmol substance P (SP, a) or capsaicin (b). Animals either received agonist alone (solid columns) co-administered with desArg^{9}Leu^8-BK 0.5 nmol (open columns) or 5 nmol (cross-hatched columns), desArg^{10}[Hoe 140] 5 pmol (horizontally-hatched columns) or 50 pmol (vertically-hatched columns). All results are expressed as mean ($n=8$, unless stated otherwise); vertical lines show s.e.mean. *$P<0.05$ compared to control group at each time point.

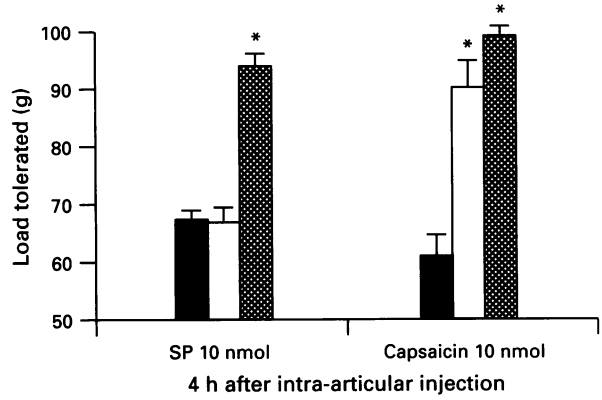

Figure 6 Load (g) tolerated by the ipsilateral leg 4 h after intra-articular administration of substance P (SP) 10 nmol or capsaicin 10 nmol into naive joints. Saline (solid columns), desArg^{9}Leu^8-BK 10 nmol kg^{-1} (open columns) or icatibant 100 pmol kg^{-1} (cross-hatched columns) were administered intravenously 3.5 h after either SP or capsaicin. All results are expressed as mean ($n=8$, unless stated otherwise); vertical lines show s.e.mean. *$P<0.05$ compared to saline group.

into a rat paw (Carter & Francis, 1991). Capsaicin has also been shown to release neuropeptides from the terminals of sensory neurones and, specifically, there are studies *in vivo* showing SP-induced plasma protein extravasation in the joint (Lam & Ferrell, 1991; Lam & Ferrell, 1993) as well as release

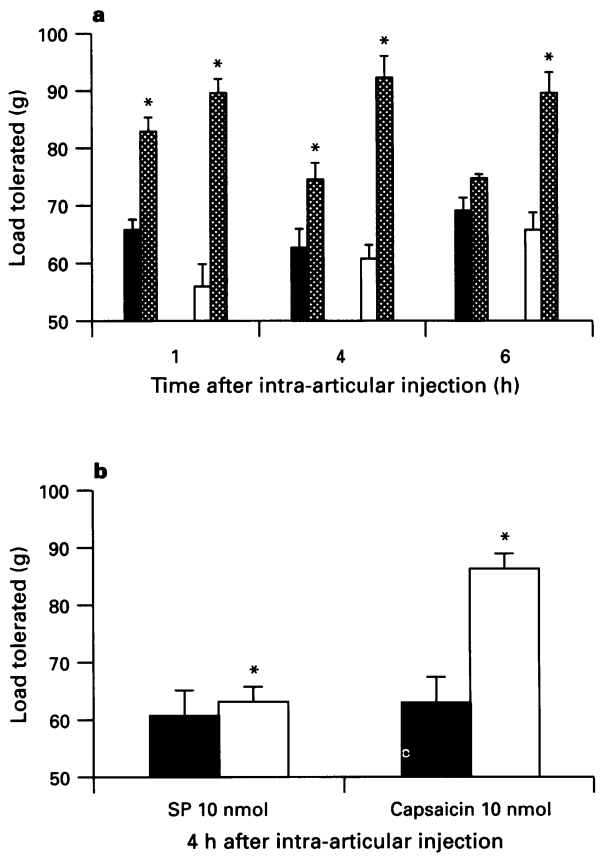

Figure 7 Load (g) tolerated by the ipsilateral leg after intra-articular administration of 10 nmol substance P (SP) or capsaicin. In (a) animals either received SP (solid columns) or capsaicin (open columns) alone or co-administered with 0.1 μg interleukin-1 (IL-1) receptor antagonist (cross-hatched columns). In (b) phosphate buffered saline (solid columns) or 0.1 μg IL-1ra (open columns) were administered intra-articularly 3.5 h after either SP or capsaicin. All results are expressed as mean ($n=8$, unless stated otherwise); vertical lines show s.e.mean. *$P<0.05$ compared to saline group.

of SP into the joint after capsaicin administration (Yaksh, 1988). The reversal of the capsaicin-induced hyperalgesia by NK_1 antagonists in the same dose range that block SP-induced hyperalgesia is consistent with a major part of the mechanism of capsaicin-induced hyperalgesia being via the release of SP.

The mechanism(s) underlying the SP-induced hyperalgesia in this model are not clear but since SP does not activate nociceptors directly (Kumazawa & Mizumura, 1979; Mizimura *et al.*, 1987; Cohen & Perl, 1990) the hyperalgesia induced by SP may be due either to a sensitization of joint afferents to mechanical stimuli, as seen *in vivo* (Nakamura-Craig & Smith, 1989) or a conditioning effect of SP on nociceptive afferents to other inflammatory mediators as has been observed *in vitro* (Kessler *et al.*, 1992). It is, however, not possible, on the basis of these results to exclude an indirect action of SP acting on cellular and vascular tissues leading to the release of other inflammatory mediators (Lotz *et al.*, 1987) which may then sensitize the nociceptor. It should also be noted that SP would be expected to increase vascular permeability within the joint resulting in oedema and a consequential rise in intra-articular pressure. This could be sufficient to activate nociceptors and lead to hyperalgesia.

The dose of SP (10 nmol) used in this study corresponds well with doses that produce plasma extravasation via NK_1 receptors in the knee joint (Lam *et al.*, 1993) and granulocyte infiltration into the mouse air pouch (Matsuda *et al.*, 1989; Iwamoto *et al.*, 1993; Perretti *et al.*, 1993). Nevertheless, there is a discrepancy in the literature between the amount of exogenous SP that needs to be administered in order to effect

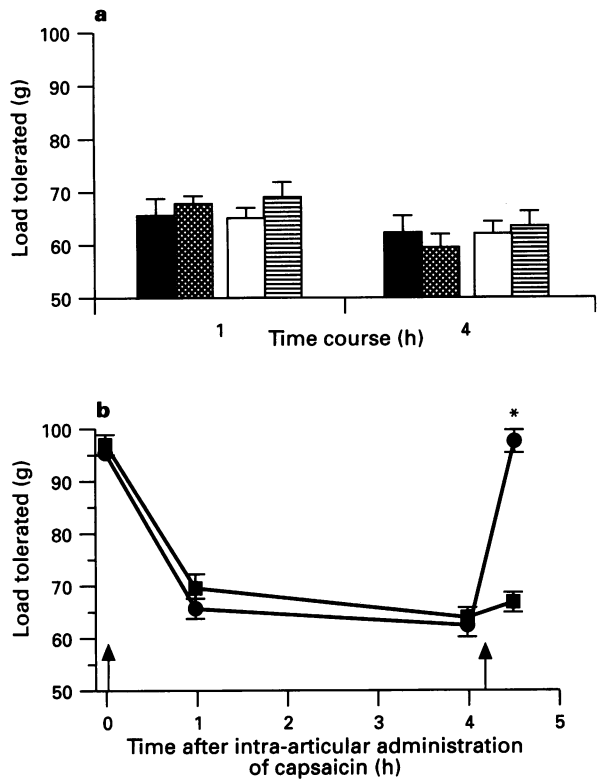

Figure 8 Load (g) tolerated by the ipsilateral leg after intra-articular administration of substance P (SP) 10 nmol or capsaicin 10 nmol. (a) Time course of hyperalgesia following intra-articular injection of SP (solid columns) or capsaicin (open columns). Indomethacin 1 mg kg^{-1} (cross-hatched columns) or vehicle (sodium carbonate) was subcutaneously administered 30 min before intra-articular injection of SP or capsaicin. (b) The time course of hyperalgesia for the animals that received vehicle 30 min prior to capsaicin (●) or indomethacin 30 min before capsaicin (■). Four h after injection with capsaicin (10 nmol, first arrow) animals were injected with desArg^9 Leu^8-BK (10 nmol kg^{-1}, i.v., second arrow) and the hyperalgesia assessed 30 min later. All results are expressed as mean ($n=8$, unless stated otherwise); vertical lines show s.e.mean. *$P<0.05$ comparing indomethacin to vehicle group at each time point.

responses, albeit inflammatory measures, and the levels of SP measured in inflammatory conditions, the latter being 2–3 orders of magnitude lower than the former (Bileviciute *et al.*, 1993, 1994; Lam *et al.*, 1993). It is difficult, however, to be certain that the concentration of SP measured in inflammatory conditions or administered exogenously is a true representation of the actual concentration at the site of action. This is particularly true with locally acting peptides, such as SP, as metabolic degradation is very rapid *in vivo*. It is probably the case that most of the exogenously applied SP is degraded before being able to act on its target cell. In addition, as discussed above, capsaicin can be regarded as being, in part, a 'releaser' of endogenous SP. As the profiles of antagonism of SP and capsaicin by the antagonists used here are remarkably similar (with the exceptions discussed below) it is suggested that the mechanisms which may underlie the action of exogenously administered SP also mediate the actions of endogenously released SP.

One major difficulty with these behavioural studies is that any inferences regarding the precise mechanisms involved in the production and maintenance of the observed hyperalgesia has to be indirect and speculative. Direct recording of the neural activity of the joint nociceptors in conscious animals would help in this context but this would be difficult, if not impossible at present, to achieve in studies such as these. The precise mechanisms involved can, therefore, only be indirectly inferred.

The results obtained following pharmacological manipulation of the actions of kinins and interleukin-1 suggest that these inflammatory mediators are either directly involved in the production and maintenance of SP and capsaicin-induced hyperalgesia or are capable of modulating the final behavioural response. The blockade of the induction of the mechanical hyperalgesia induced by SP and capsaicin by icatibant, a bradykinin B_2-receptor antagonist, at a dose previously shown to be specific for B_2-kinin receptors in this model (Davis & Perkins, 1994a) suggests that there is an early involvement of BK in the hyperalgesia induced by these agents. The potentiation of a low dose SP and capsaicin-induced hyperalgesia by captopril also suggests a role for kinins, since captopril would be expected to increase BK levels, although it should be noted that SP levels may also be influenced by captopril.

An interaction between the actions of kinins and cytokines, particularly interleukin-1 has been demonstrated in many systems (see Dray & Perkins, 1993); the data obtained here are consistent with previous studies from our laboratory and others suggesting an interdependency between kinins and cytokines with respect to behavioural hyperalgesia.

It is difficult to interpret the reversal of an established SP-induced hyperalgesia by both NK_1 and B_2 receptor antagonists. It is possible that the exogenously administered SP is still present at this 4 h time point, but, in view of metabolic degradation, this is unlikely, since administration of 0.5 nmol SP into the joint results in levels not significantly different from the levels in the contralateral knee 2 h after SP administration (Bileviciute *et al.*, 1993). One possibility is that SP induces production of BK, which subsequently acts on the nociceptor to cause sensitization as well as the further release of SP from the nociceptive terminal. Interrupting this self-reinforcing cycle with either an NK_1 or B_2 receptor antagonist would, therefore, reverse the hyperalgesia. Another possibility is that SP acts in synergy with BK to cause the hyperalgesia. It is not, however, possible to distinguish between these possibilities on the present data.

Capsaicin-induced hyperalgesia differed from SP-induced hyperalgesia in the nature of the blockade of hyperalgesia seen with B_1 and IL-1 receptor antagonists. If the capsaicin-induced hyperalgesia seen here is, indeed, due to release of neuropeptides from the sensory terminals then this difference suggests that capsaicin-induced hyperalgesia is not entirely due to SP release. It is possible that other neuropeptides are involved (for example neurokinin A (NKA) or calcitonin gene-related peptide (CGRP) which are also released by capsaicin).

The results showing antagonism of SP-induced hyperalgesia by B_1- and IL-1-receptor antagonists only when co-administered with SP suggests that there is either only a transient involvement of B_1 agonists and IL-1β in SP-induced hyperalgesia or that these agonists can only modulate this hyperalgesia in a limited way. Previously we have shown that IL-1 can induce B_1-receptor mediated hyperalgesia (Davis & Perkins, 1994b). Since B_1 receptor antagonists were effective 30 min after SP injection it is possible that there is only a transient production of IL-1 induced by SP and this is insufficient to initiate a sustained B_1-mediated hyperalgesia as has been observed after exogenous administration of IL-1β (Davis & Perkins, 1994b).

The experiments investigating the possible role of prostaglandins in capsaicin and SP-induced hyperalgesia were prompted by our previous studies demonstrating that the induction of B_1-receptor mediated hyperalgesia was indomethacin-sensitive (Davis & Perkins, 1994b). Indomethacin had no effect on the level of hyperalgesia induced by SP or capsaicin, although as the dose of indomethacin used blocked IL-1-induced hyperalgesia in this model (Davis & Perkins, 1994b) and prostaglandin production in other inflammatory models (Salmon *et al.*, 1983) we are confident that prostaglandin production was inhibited. The blockade of capsaicin-induced hyperalgesia by a B_1-receptor antagonist, but not indomethacin surprised us, since our previous studies suggest

that B_1-receptor agonists act via prostaglandin production to produce hyperalgesia (Davis & Perkins, 1994b). However, as desArg^{9}Leu^8-BK was ineffective at reversing capsaicin-induced hyperalgesia in the indomethacin pretreated animals, it appears that capsaicin can produce hyperalgesia via either a B_1-receptor-dependent or a prostaglandin- and B_1-receptor-independent route.

In conclusion, the present data suggest that subsequent to a challenge of SP or capsaicin into the knee joint, the resultant mechanical hyperalgesia either involves kinin production or that kinins can substantially modulate the hyperalgesia. In addition, we have obtained further evidence that B_1-receptor mediated hyperalgesia is an indomethacin-sensitive process. There could, therefore be potential therapeutic uses of both B_1- and B_2-receptor antagonists in conditions of inflammatory hyperalgesia.

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