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The involvement of bradykinin B_1 and B_2 receptor mechanisms in cytokine-induced mechanical hyperalgesia in the rat

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1 Interleukin-1β (IL-1β), IL-2 and IL-8 induced a mechanical hyperalgesia following intra-articular (i.artic.) injection into rat knee joints, whereas IL-6 and tumour necrosis factor α (TNF-α) were without effect.

2 Co-administration of IL-1 receptor antagonist (0.1 μg) with IL-1β (1 u), IL-2 (10 u) or IL-8 (0.1 u) prevented the subsequent development of the hyperalgesia.

3 Co-administration of desArg[9]Leu[8]BK (0.5–5 nmol) with IL-1β (1 u), IL-2 (10 u) or IL-8 (0.1 u) reduced the level of hyperalgesia at 1, 4 and 6 h post administration, whereas Hoe 140 (5 pmol) antagonized the hyperalgesia only at the 1 h time point.

4 Intravenous administration of desArg[9]Leu[8]BK (10 nmol kg^{-1}) or Hoe 140 (100 pmol kg^{-1}) following IL-1β (1 u), IL-2 (10 u), or IL-8 (0.1 u) reversed the subsequent hyperalgesia.

5 Administration of desArg[9]BK into joints 24 h after pre-treatment with IL-1β (1 u) produced analgesia at low doses (50 pmol) and hyperalgesia at a higher dose (0.5 nmol). Both these effects were blocked by desArg[9]Leu[8]BK (0.5 nmol).

6 Administration of desArg[9]BK (0.5 nmol i.artic.) to animals 24 h after pre-treatment with IL-2 (1–100 u) or IL-8 (0.1–10 u) had no effect on the load tolerated by the treated joint.

7 Administration of indomethacin (1 mg kg^{-1}, s.c.) prior to IL-1β (1 u i.artic.) prevented the development of hyperalgesia. Administration of desArg[9]BK (5 pmol–0.5 nmol, i.artic.) to animals 24 h after indomethacin and IL-1β pretreatment had no effect on the load tolerated by the treated joint.

8 These data suggest that both bradykinin B_1 and B_2 receptors are involved in the induction and maintenance of cytokine-induced hyperalgesia. They also show that the induction of B_1 receptor-mediated hyperalgesia requires both cyclo-oxygenase products and IL-1 *in vivo*.

Keywords: Cytokines; hyperalgesia; interleukin-1 receptor antagonist; bradykinin; desArg[9] bradykinin; prostaglandins

Introduction

Cytokines have been implicated in the mechanisms of both inflammation and hyperalgesia. In inflammatory diseases such as rheumatoid arthritis increased levels of interleukin-1 (IL-1) tumour necrosis factor α (TNF-α), IL-6 and IL-8 have been measured in the joint (Hirano *et al*., 1988; Yocum *et al*., 1989; Brennan *et al*., 1990; Remick *et al*., 1992). These cytokines have also been shown, to varying degrees, to cause cellular infiltration and plasma extravasation in several animal models of inflammation (Colditz & Watson, 1992; Cooper *et al*., 1992; Forrest *et al*., 1992). Additionally IL-1, IL-6, IL-8 and TNF-α may contribute to the hyperalgesia accompanying inflammation since they can induce mechanical hyperalgesia following local administration in rats (Ferreira *et al*., 1988; Follenfant *et al*., 1989; Cunha *et al*., 1992).

The role of kinins in inflammation and hyperalgesia has been extensively studied. Bradykinin is known to excite nociceptors via activation of B_2 receptors (Steranka *et al*., 1988; Haley *et al*., 1989; Dray *et al*., 1992; Heapy *et al*., 1993) and can cause inflammation, extravasation and cellular migration (McFadden & Vickers, 1989; Damas & Remacle-Volon, 1992; Green *et al*., 1993). Recently we have shown that, subsequent to an inflammatory insult, the B_1 receptor plays an important role in mechanisms of mechanical (Perkins *et al*., 1992; 1993; Davis & Perkins, 1994) and thermal (Perkins & Kelly, 1993) hyperalgesia.

Interactions between cytokines and kinins have been shown in several systems. IL-1 and IL-2 have been shown to induce bradykinin B_1 receptors *in vitro* (Deblois *et al*., 1988), and synergistic interactions have been demonstrated in human fibroblasts between bradykinin, desArg[9]BK and IL-1 (Lerner & Modeer, 1991; Bathon *et al*., 1992; Lerner *et al*., 1992).

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In this study we have investigated the hyperalgesic action of inflammatory cytokines after intra-articular injection into rat knee joint and the possible involvement of B_1 and B_2 receptors.

Methods

The method used in this study for assessment of mechanical hyperalgesia has been described previously (Perkins *et al*., 1992; 1993; Davis & Perkins, 1993; 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 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 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 the dose-response relationships to test compounds could be properly assessed. All injections into the joint were made under brief enflurane anaesthesia.

Time course of cytokine-induced hyperalgesia

Animals received unilateral intra-articular injections (100 μl, phosphate buffered saline vehicle) of either IL-1β (0.1–100 u), IL-2 (1–100 u), IL-6 (1–100 u), IL-8 (0.01–10 u) or

TNF-α (1–500 u). The load tolerated by the treated joint was then assessed at 1, 4, 6 and 24 h post injection. Control animals received cytokines which had been heated to above 50°C for 60 min, the highest dose of each cytokine being used for this control. ANOVA and MANOVA followed by *post hoc* analysis of means was used to establish a reduction in tolerated load over the time course and to compare groups with their heat-treated control at each time point.

Antagonism of cytokine-induced hyperalgesia

Animals were administered a sub-maximal dose of cytokine (100 μl, i.artic.) either alone or in combination with IL-1 receptor antagonist(ra) (0.1 μg), desArg^{9}Leu^{8}BK (0.5–5 nmol), desArg^{10}[Hoe 140] (5 pmol) or Hoe 140 (5 pmol). The load tolerated by the treated leg was then assessed at 1, 4 and 6 h after administration. MANOVA followed by *post hoc* analysis of means was used to compare treatments at each time point.

Reversal of cytokine-induced hyperalgesia

Animals were injected with a sub-maximal dose of cytokine (100 μl, i.artic.) and the load tolerated by the treated joint was assessed 4 h later. Animals were injected with either desArg^{9}Leu^{8}BK (10 nmol kg^{-1}), Hoe 140 (100 pmol kg^{-1}) or saline via their tail vein, under brief enflurane anaesthesia, 30 min before assessing the level of hyperalgesia. ANOVA followed by *post hoc* analysis of means was used to compare the saline and antagonist-treated groups.

Induction of B_1 receptor

Animals received unilateral intra-articular injections (100 μl) of cytokine and then 24 h later the load tolerated by the injected leg was assessed. Control readings were taken to establish the baseline level of hyperalgesia, then 50 μl of desArg^{9}BK (5 pmol–1 nmol) was subsequently injected into the cytokine-treated joint. The level of hyperalgesia was then assessed at 30 min intervals for 2 h. Antagonists were co-administered with desArg^{9}BK. ANOVA followed by *post hoc* analysis of mean was used to study the time course of desArg^{9}BK-induced hyperalgesia for each dose administered.

Separate groups of animals were injected with indomethacin 0.1–10 mg kg^{-1}, s.c. or 2% Na_2CO_3 vehicle (buffered to pH 7 with NaH_2PO_4) 30 min before administration of IL-1β (1 u, i.artic.). The load tolerated by the treated leg was assessed at 1, 4 and 24 h after administration of IL-1β. DesArg^{9}BK (5 pmol–0.5 nmol, i.artic.) was also administered, as described above, 24 h after indomethacin and IL-1β pre-treatment.

Drugs

All cytokines used in this study were human recombinant type. IL-1β, IL-6, IL-8 (72 amino acids) and TNF-α were obtained from The National Institute for Biological Standards and Controls (NIBSC), with specific activities of 1×10^8 (IL-1β), 5×10^6 (IL-6), 1×10^6 (IL-8) and 4×10^7 u mg^{-1} (TNF-α) and code numbers 86/552, 88/514, 89/520 and 87/650 respectively. IL-2 was obtained from Sandoz Basel, with a specific activity of 2×10^6 u mg^{-1}. DesArg^{9}Leu^{8}BK and IL-1ra were obtained from Bachem A.G. and Hoe 140 (D-Arg [Hyp^3, Thi^5,D-Tic^7,Oic^8]BK) and desArg^{10}[Hoe 140] were synthesized by Dr A. Hallett, Sandoz Institute for Medical Research. Indomethacin was obtained from Sigma.

Results

IL-1β (1–100 u, i.artic.) induced a reduction in tolerated load when injected into naive joints. This hyperalgesia was significant at 1, 4, 6 and 24 h post-injection with 1–100 u, whereas 0.1 u and heat treated IL-1β (100 u) had no effect (Figure 1). The highest dose of IL-1β tested was 100 u which caused a reduction in tolerated load from 94 ± 1 g to 49 ± 5 g at 6 h ($n = 8$, $P < 0.05$). By 24 h post administration only the animals which received 10 and 100 u IL-1β showed a reduction in tolerated load when compared to the heat-treated control (60 ± 3 g and 75 ± 3 g, $n = 8$ respectively, vs 90 ± 4 g $n = 16$, $P < 0.05$); however, the animals which received 1 u IL-1β were still significantly hyperalgesic compared to their pretreatment levels (82 ± 2 g vs 94 ± 1 g, $n = 8$, $P < 0.05$).

IL-2 (1–100 u, i.artic.) produced a reduction in tolerated load when injected into naive joints. The hyperalgesia was significant at 1 and 4 h post-injection with 1–100 u, whereas heat treated IL-2 (100 u) had no effect over the time course (Figure 1). The maximum reduction in hyperalgesia occurred 1 h post-injection at all doses tested. With 100 u IL-2, the tolerated load fell from 87 ± 1 g pre-treatment level, to 44 ± 2 g at 1 h ($n = 8$, $P < 0.05$). By 6 h after all doses the level of hyperalgesia was no longer significantly different from the control group.

IL-8 (0.01–10 u) caused a reduction in tolerated load when injected into naive joints, whereas heat-treated IL-8 (10 u) had no effect (Figure 1). After 0.1–10 u IL-8 the hyperalgesia was significant at 1, 4 and 6 h post-injection. By 24 h post-injection only the group which received 10 u IL-8 were

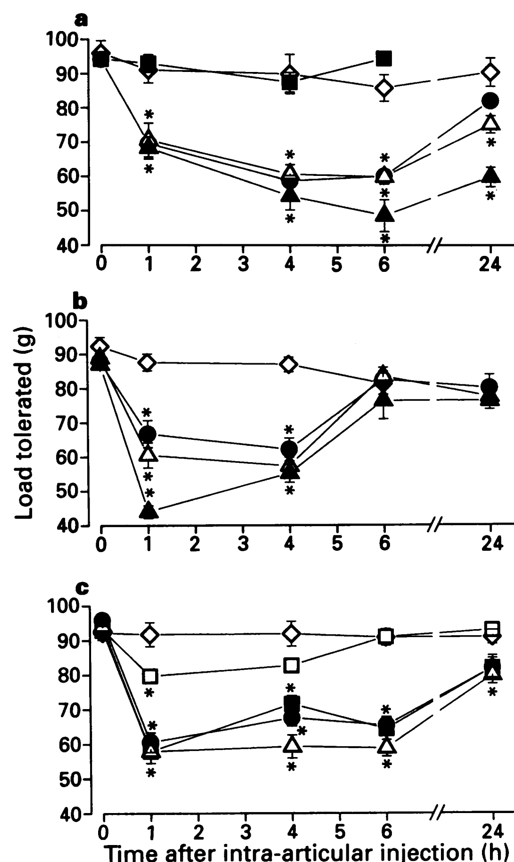

Figure 1 Load (g) tolerated by the ipsilateral leg after intra-articular administration of interleukin-1β (IL-1β) (a) IL-2 (b) or IL-8 (c) into naive joints, all injections were made after the first (0 h) reading: (a) shows the time course of hyperalgesia after IL-1β 0.1 u (■), 1 u (●), 10 u (Δ), 100 u (▲) and heat-treated 100 u (◇, $n = 16$). (b) Shows the time course of hyperalgesia after IL-2 1 u (●), 10 u (Δ), 100 u (▲) and heat-treated 100 u (◇). (c) Shows the time course of hyperalgesia after IL-8 0.01 u (□), 0.1 u (■), 1 u (●), 10 u (Δ) and heat-treated 10 u (◇). All results are expressed as mean ± s.e.mean ($n = 8$ animals per group, unless stated otherwise). *$P < 0.05$ compared to heat-treated control at each time point.

hyperalgesic, with a tolerated load of 80 ± 2 g compared to their pretreatment level of 94 ± 1 g ($n = 8$, $P<0.05$).

IL-6 (1–100 u, i.artic.) and TNF-α (1–500 u, i.artic.) did not induce hyperalgesia at 1, 4, 6 or 24 h (data not shown).

Antagonism of cytokine-induced hyperalgesia

Co-administration of desArg⁹Leu⁸BK (0.5 nmol) with IL-1β (1 u) reduced the hyperalgesia over the entire time course (Figure 2). After 1 h the load tolerated was 89 ± 2 g ($n = 16$) compared to 67 ± 2 g ($n = 24$, $P<0.05$) in animals which received IL-1β alone. The hyperalgesia was still reduced at 6 h with a tolerated load of 75 ± 3 g compared to 65 ± 2 g ($P<0.05$) with IL-1β alone. Hoe 140 (5 pmol) only blocked IL-1β-induced hyperalgesia at 1 h; animals tolerated 86 ± 5 g ($n = 8$, $P<0.05$) at this time point. By 4 h post administration the hyperalgesia was the same as in animals which received IL-1β alone (59 ± 3 g with Hoe 140 vs 60 ± 2 g with IL-1β alone).

Co-administration of desArg⁹Leu⁸BK (5 nmol) with IL-2 (10 u) prevented the development of hyperalgesia at all time points studied (Figure 2); animals tolerated 86 ± 2 g and 91 ± 2 g ($n = 8$) compared to 66 ± 2 g and 63 ± 2 g ($n = 32$, $P<0.05$) in control animals at 1 and 4 h post injection. A lower dose of desArg⁹Leu⁸BK (0.5 nmol) prevented the hyperalgesia only at the 1 h time point, however desArg¹⁰[Hoe 140] 5 pmol prevented development of hyperalgesia over the entire time course (Figure 2). Hoe 140 (5 pmol) blocked the development of IL-2-induced hyperalgesia only at the 1 h time point, when the tolerated load was 92 ± 3 g ($n = 8$) compared to 66 ± 2 g ($n = 32$, $P<0.05$) in animals which received IL-2 alone; by 4 h post injection the load tolerated fell to 60 ± 3 g in animals which received Hoe 140 compared to 63 ± 2 g in animals which received IL-2.

Co-administration of desArg⁹Leu⁸BK (0.5 nmol) with IL-8 (0.1 u) blocked the development of hyperalgesia over the entire time course (Figure 2). After 1 h the load tolerated was 94 ± 1 g ($n = 8$) compared to 63 ± 2 g ($n = 16$, $P<0.05$) in animals which received IL-8 alone. The hyperalgesia was still reduced at 6 h with a tolerated load of 94 ± 2 g compared to 70 ± 3 g ($P<0.05$) with IL-8 alone. Hoe 140 (5 pmol) blocked IL-8-induced hyperalgesia only at 1 h; animals tolerating 92 ± 2 g ($n = 8$, $P<0.05$) at this time point. By 4 h post administration the hyperalgesia was the same as in

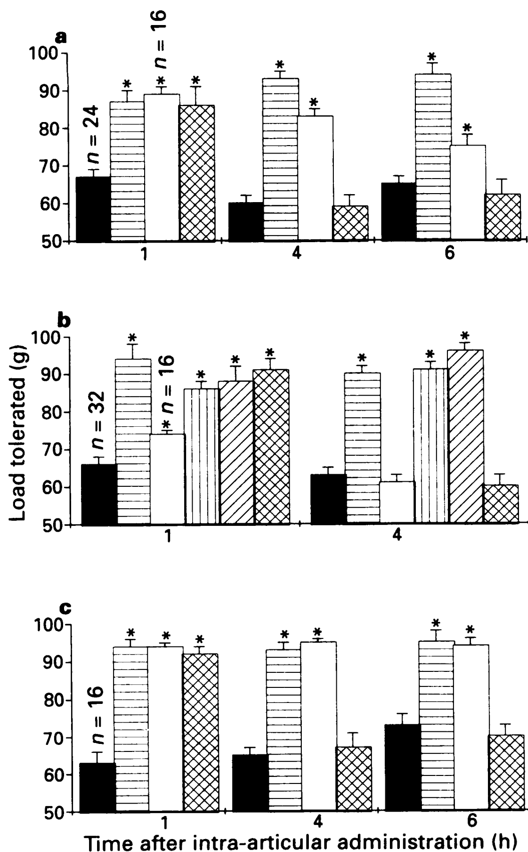

Figure 2 Load (g) tolerated by the ipsilateral leg after intra-articular administration of interleukin-1β (IL-1β) 1 u (a). IL-2 10 u (b) and IL-8 0.1 u (c). Animals either received cytokine alone (solid columns) or co-administered with IL-1 receptor antagonist (horizontally hatched columns) desArg⁹Leu⁸BK 0.5 nmol (open columns) or 5 nmol (vertically hatched columns), desArg¹⁰[Hoe 140] 5 pmol (right hatched columns) or Hoe 140 (cross hatched columns). All results are expressed as mean ± s.e.mean ($n = 8$, unless stated otherwise). $*P<0.05$ compared to control at each time point.

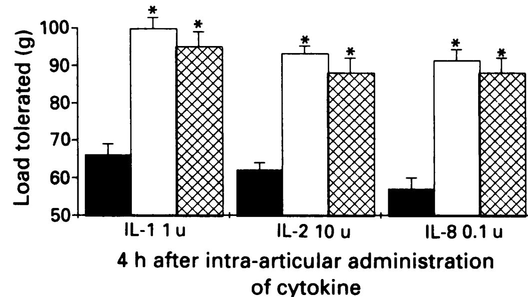

Figure 3 Load (g) tolerated by the ipsilateral leg 4 h after intra-articular administration of interleukin-1β (IL-1β) 1 u, IL-2 10 u or IL-8 0.1 u into naive joints. Saline (closed columns, $n = 16$), desArg⁹Leu⁸BK 10 nmol kg⁻¹ (open columns) or Hoe 140 100 pmol kg⁻¹ (cross hatched columns) were administered i.v. 3.5 h after each cytokine. All results are expressed as mean ± s.e.mean ($n = 8$, unless stated otherwise). $*P<0.05$ compared to saline group.

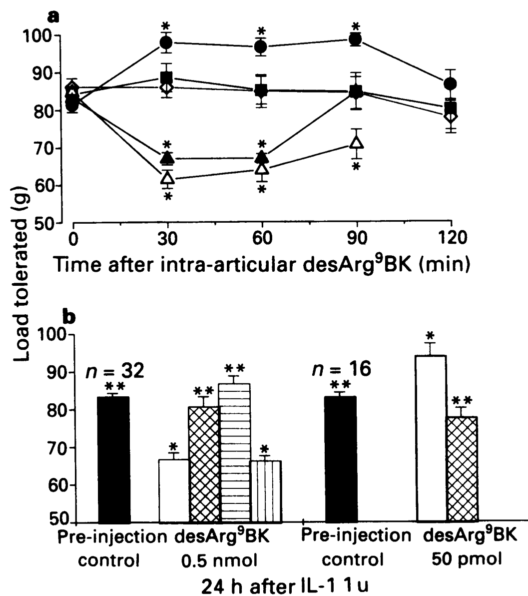

Figure 4 Load (g) tolerated by the ipsilateral leg 24 h after intra-articular administration of interleukin-1β (IL-1β) 1 u (a). desArg⁹BK was injected into the IL-1β pretreated joint after the first reading at 5 pmol (■), 50 pmol (●), 0.5 nmol (▲) and 1 nmol (△). Control joints (◇) were injected with PBS (50 μl). (b) After control readings (solid columns), desArg⁹BK was either administered alone (open columns) co-administered with desArg⁹Leu⁸BK 0.5 nmol (cross hatched columns), desArg¹⁰[Hoe 140] 5 pmol (horizontally hatched columns) or Hoe 140 (vertically hatched columns). All results are expressed as mean ± s.e.mean ($n = 8$, unless stated otherwise). $*P<0.05$ compared to pre-injection reading. $**P<0.05$ compared to desArg⁹BK-treated group.

animals which received IL-8 alone (67 ± 4 g with Hoe 140 vs 66 ± 2 g with IL-8 alone).

Co-administration of IL-1ra (0.1 μg) with IL-1β (1 u), IL-2 (10 u) or IL-8 (0.1 u) blocked the development of hyperalgesia over the entire time course (Figure 2). A lower dose of IL-1ra (0.01 μg) only partially blocked IL-1β-induced hyperalgesia (data not shown) so 0.1 μg was used to antagonize IL-2 and IL-8-induced hyperalgesia.

None of the doses of antagonists used had any effect when administered alone to the naive joint over the dose-ranges used in these experiments (data not shown).

Reversal of cytokine-induced hyperalgesia

When desArg^{9}Leu^{8}BK (10 nmol kg^{-1}) or Hoe 140 (100 pmol kg^{-1}) were administered i.v. to animals 3.5 h after intra-articular injection of IL-1β, IL-2 or IL-8 the hyperalgesia was reversed when measured 30 min later (Figure 3). The load tolerated after desArg^{9}Leu^{8}BK and Hoe 140 was 100 ± 3 g (n = 8) and 95 ± 4 g (n = 8) respectively compared to 66 ± 3 g after saline (n = 16, $P<0.05$) in IL-1β-treated animals; 93 ± 2 g (n = 8) and 88 ± 4 g (n = 8) respectively compared to 62 ± 2 g after saline (n = 16, $P<0.05$) in IL-2-treated animals; 91 ± 3 g (n = 8) and 88 ± 4 g (n = 8) compared to 57 ± 3 g after saline (n = 16, $P<0.05$) in IL-8-treated animals (Figure 3).

Induction of B_1 receptors

When desArg^{9}BK (1–100 nmol, i.artic.) was administered to naive rats there was no effect on tolerated load. After 1 and 100 nmol the loads tolerated by the treated leg at 1 h post injection were 94 ± 3 g and 92 ± 5 g respectively, compared to pretreatment values of 93 ± 2 g and 93 ± 1 g respectively (n = 8). Twenty four hours after administration of IL-1β (1 u) into the knee joint, injection of desArg^{9}BK into the same joint produced opposite effects depending on the dose used. With a low dose of desArg^{9}BK (50 pmol) the load tolerated increased from 83 ± 3 g to 96 ± 2 g (n = 8, $P<0.05$) after 1 h, whereas at higher doses (0.5 and 1 nmol) there was a reduction in load tolerated (Figure 4). After 0.5 nmol desArg^{9}BK, the mean tolerated load fell from 82 ± 2 g to 66 ± 4 g at 1 h (n = 8, $P<0.05$); this reduction was blocked when desArg^{9}BK was co-administered with desArg^{9}Leu^{8}BK (0.5 nmol) or desArg^{10}[Hoe 140] (5 pmol), but Hoe 140 (5 pmol) had no effect (Figure 4).

There was no change in load tolerated when desArg^{9}BK (0.5 nmol) was injected into joints pretreated with IL-2 (1–100 u), IL-6 (1–100 u), IL-8 (0.1–10 u) or TNF-α (1–500 u), 24 h previously (data not shown).

Following indomethacin pretreatment (1 mg kg^{-1}, s.c.) 30 min before intra-articular injection of IL-1β (1 u) there was no reduction in tolerated load when assessed 1, 4 or 24 h later (Figure 5). At 24 h, the load tolerated by the treated joint was 91 ± 1 g (n = 24) in animals which received indomethacin and IL-1β, compared to 85 ± 2 g (n = 8, $P<0.05$) in animals which received vehicle and IL-1β. Intra-articular administration of desArg^{9}BK (5 pmol–1 nmol) to animals 24 h after indomethacin and IL-1β pretreatment had no significant effect on the tolerated load (Figure 5).

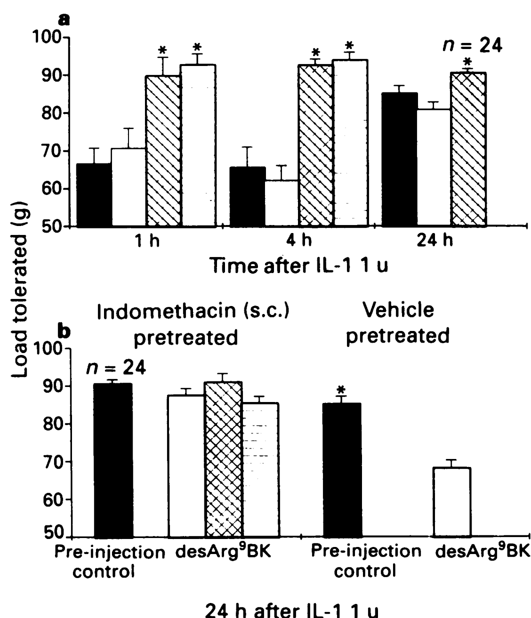

Figure 5 Load (g) tolerated by ipsilateral leg after intra-articular administration of interleukin-1β (IL-1β) 1 u. (a) Time course of hyperalgesia in animals pretreated with indomethacin 0.1 mg kg^{-1} (open columns), 1 mg kg^{-1} (cross hatched columns), 10 mg kg^{-1} (horizontally hatched columns) or vehicle (solid columns) s.c., 30 min prior to IL-1β. (b) DesArg^{9}BK was administered into the ipsilateral joint 24 h after IL1β. After pre-injection readings (solid columns), desArg^{9}BK was administered at 0.5 nmol (open columns), 50 pmol (cross hatched columns), or 5 pmol (horizontally hatched columns). All results are expressed as mean ± s.e.mean (n = 8, unless stated otherwise). *$P<0.05$ compared to pre-injection reading.

Discussion

These data show that the cytokines IL-1β, IL-2 and IL-8 all induce mechanical hyperalgesia when injected into the rat knee joint, whereas IL-6 and TNF-α were without effect. The blockade of IL-1β, IL-2 and IL-8-induced hyperalgesia by the IL-1 receptor antagonist, IL-1ra, (Eisenberg *et al.*, 1990) suggests the involvement of IL-1 in the induction of hyperalgesia by these cytokines. Furthermore, the antagonism of the cytokine-induced hyperalgesia by desArg^{9}Leu^{8}BK and Hoe 140 also implicates bradykinin B_1 and B_2 receptor systems in this hyperalgesia. These data therefore further suggests a possible role of IL-1β in hyperalgesia, and supports evidence *in vitro* (Deblois *et al.*, 1989) and *in vivo* (Perkins & Kelly, 1993) for a link between B_1 receptor expression and IL-1β.

The time course experiments did not show a clear dose-response relationship for IL-1β, IL-2 or IL-8. However, increasing the dose of IL-1β and IL-8 did prolong the duration of hyperalgesia. At the doses of cytokines used for the antagonist work, there was no significant difference in the magnitude of hyperalgesia.

When B_1 and B_2 receptor antagonists, at doses previously shown to be specific for their respective agonists (Davis & Perkins, 1994), were co-administered with IL-1β, IL-2 or IL-8, the B_1 receptor antagonist, desArg^{9}Leu^{8}BK, caused significant antagonism of hyperalgesia over the time course of study, whereas a B_2 receptor antagonist, Hoe 140, antagonized the hyperalgesia only at the 1 h time point. With IL-2, higher doses of desArg^{9}Leu^{8}BK were required to block the development of hyperalgesia, compared to the IL-1β or IL-8 experiments. The reason for this reduced potency is unclear; however, IL-2-induced hyperalgesia was blocked by IL-1ra which suggests that IL-2 causes hyperalgesia via production of IL-1. Any delay therefore in the endogenous production of IL-1 following IL-2 administration would allow time for desArg^{9}Leu^{8}BK to be metabolically degraded thereby reducing its effective concentration. This possibility is supported by the blockade of IL-2-induced hyperalgesia by desArg^{10}[Hoe 140], a metabolically protected B_1 receptor antagonist (Wirth *et al.*, 1992).

The shorter duration of Hoe 140-induced antagonism of cytokine-induced hyperalgesia, compared to desArg^{9}Leu^{8}BK seen in these experiments, was also observed in a previous study where the hyperalgesia was induced by Freund's complete adjuvant (Davis & Perkins, 1994). Since Hoe 140 has been shown to be metabolically stable in synovial fluid (Bond

et al., 1992) it is unlikely that the shorter duration of action, compared to the B_1 antagonist desArg[9]Leu[8]BK, is due to more rapid metabolism. When administered systemically, Hoe 140 has been shown to have a very long duration of action with respect to antagonism of BK (Wirth *et al.*, 1991) so it is unlikely that kinetics favour desArg[9]Leu[8]BK in this regard, particularly as desArg[9]Leu[8]BK is not metabolically stable and would be expected to have a relatively short half life. In addition, the fact that desArg[10][Hoe 140] another B_1 receptor antagonist (Wirth *et al.*, 1992) antagonized IL-2-induced hyperalgesia with a duration of action similar to desArg[9]Leu[8]BK supports the conclusion that B_1 but not B_2 receptor blockade prevents the development of hyperalgesia.

Although the data presented here suggest a more important role of B_1 receptors in the initial development of cytokine- induced hyperalgesia, both desArg[9]Leu[8]BK and Hoe 140 were equally effective in reversing the hyperalgesia after it had developed. We chose the 4 h time point to study the role of the kinin system in maintenance of hyperalgesia since IL-1β, IL-2 and IL-8 all induced a similar level of hyperalgesia at this time point. Administration of antagonists 30 min prior to testing was chosen since we have previously shown that desArg[9]Leu[8]BK and Hoe 140 are most effective at this time (Perkins *et al.*, 1993; Davis & Perkins, 1994).

Since both B_1 and B_2 receptor antagonists almost completely reverse the cytokine-induced hyperalgesia seen in this model, this suggests a complex interaction between B_1 and B_2 receptors in the development and maintenance of this type of hyperalgesia. There are several ways in which such interactions could occur. Recently it has been shown that bradykinin and desArg[9]BK can up-regulate the expression of cell specific receptors for high and low molecular weight kininogen possibly leading to an enhanced production of bradykinin (Zini *et al.*, 1993). Interestingly, this effect of bradykinin was not via a B_2 receptor but was partially blocked by desArg[9]Leu[8]BK. A B_2 receptor antagonist would, therefore, be expected to antagonize the action of BK but not affect any such increase in its production. DesArg[9]Leu[8]BK, however, would not only antagonize the action of desArg[9]BK but may also reduce an increase in production of BK (Zini *et al.*, 1993). If this up-regulation of kininogen receptors underlies to any significant extent the development of hyperalgesia in this model, then this may explain the prevention of the hyperalgesia by B_1 antagonists at the later time points. Another possibility could be that desArg[9]BK may act at B_1 receptors to produce co-factors, such as prostaglandins (Cahill *et al.*, 1988; Tiffany & Burch, 1989; Lerner & Modeer, 1991) which may be necessary to maintain the inflammatory cascade leading to further BK production and sensitization of the nociceptor to BK. In either case blockade of either B_1 or B_2 receptors would antagonize the hyperalgesia.

The initial antagonist studies suggested an involvement of B_1 receptors in both the development and maintenance of hyperalgesia; thus the latter set of experiments looked at the effect of the B_1 receptor agonist desArg[9]BK when administered to cytokine pretreated joints. These experiments were conducted 24 h after administration of IL-1β, IL-2 or IL-8 since the load tolerated by the cytokine pre-treated joint was similar with all three cytokines at this time point. Mechanical hyperalgesia was only induced by desArg[9]BK in joints pre-treated with IL-1β, whereas desArg[9]BK has no effect when administered alone into naive joints. A previous study from this laboratory has also shown the induction of thermal hyperalgesia following IL-1β administration (Perkins & Kelly, 1993). The paradoxical increase in tolerated load seen with low doses of desArg[9]BK is interesting but at present we do not have an explanation for this, although it appears to be a specific B_1 receptor-mediated effect since it was antagonized by co-administration of desArg[9]Leu[8]BK. Experiments are in progress to investigate further the pharmacology of desArg[9]BK in IL-1β pretreated joints. The lack of effect of desArg[9]BK in joints pre-treated with IL-2 or IL-8 suggests that B_1 receptors are not present 24 h after administration of these two cytokines. However, IL-2 and IL-8 showed different time courses of action and dose-response relationships from IL-1β, thus it is possible that functional expression of the B_1 receptor has a finite life, requiring the continued presence of inflammatory mediators such as IL-1β to remain functional. Since our interest was mechanisms underlying the induction of B_1 receptors, further experiments were not performed with IL-2 and IL-8.

The effect of indomethacin on the induction of B_1-mediated hyperalgesia suggests prostanoid production mediates this response. Conflicting data exist in the literature concerning the role of prostaglandins in IL-1β-induced hyperalgesia (Ferreira *et al.*, 1988; Follenfant *et al.*, 1989). Our experiments show that, at least in the knee joint, the hyperalgesia induced by IL-1β is dependent on prostaglandins, since indomethacin blocked the development of hyperalgesia at a systemic dose sufficient to inhibit prostaglandin biosynthesis completely (Salmon *et al.*, 1983; Follenfant *et al.*, 1989). Furthermore, the lack of effect of desArg[9]BK when administered to animals 24 h after indomethacin and IL-1β pretreatment, suggests that prostaglandins are also involved in the induction of the B_1 responses. This is interesting since the spontaneous induction of B_1 receptors *in vitro* is not inhibited by continuous exposure to indomethacin (Regoli *et al.*, 1978) or IL-1ra (Petitclerc *et al.*, 1992). However IL-1ra does prevent the enhanced B_1 responses induced by incubation with IL-1β, suggesting that the spontaneous induction of B_1 receptors in isolated tissues and the IL-1-induced B_1 responses could possibly occur via different mechanisms (Petitclerc *et al.*, 1992).

Although B_2 receptors are known to be present on sensory neurones (Sterenka *et al.*, 1988), the location of B_1 receptors in these studies is not known. It is possible that the cytokines induce or up-regulate B_1 receptors on the sensory neurone as seen in smooth muscle studies (Bouthillier *et al.*, 1987; Deblois *et al.*, 1988; 1989; Siebeck *et al.*, 1989) but there is, as yet, no evidence to support this. DesArg[9]BK has been shown to increase the synthesis and release of inflammatory mediators such as IL-1 and PGI_2 from other cell types (Cahill *et al.*, 1988; Tiffany & Burch, 1989; Lerner & Modeer, 1991) and such actions of desArg[9]BK côuld indirectly increase the excitability of the nociceptor. Whatever the site of the B_1 receptor, B_1 receptor antagonists are not only effective in preventing the development of hyperalgesia, but, along with B_2 receptors they can also reverse the hyperalgesia once established.

In conclusion, the present paper suggests that subsequent to a challenge of IL-1β desArg[9]BK can induce a B_1 receptor-mediated mechanical hyperalgesia. In addition the data indicate that the cytokines IL-1β, IL-2 and IL-8 can induce mechanical hyperalgesia in a rat knee joint model and this involves both B_1 and B_2 receptors. B_1 receptors, however, seem to have a greater role than B_2 receptors in the development of cytokine-induced mechanical hyperalgesia. 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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