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Differential roles of neurokinin 1 and neurokinin 2 receptors in the development and maintenance of heat hyperalgesia induced by acute inflammation

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1 Following induction of acute inflammation by intraarticular injection of kaolin and carrageenan into the knee joint in rats, there was a significant decrease in the withdrawal latency to radiant heat applied to the paw (i.e. heat hyperalgesia), an increased joint circumference and increased joint temperature.

2 A neurokinin$_1$ (NK_1) receptor antagonist (CP-99,994, 10 mM) had no effect on the paw withdrawal latency when it was administered spinally through a microdialysis fibre before the induction of inflammation. Pretreatment with a NK_2 receptor antagonist (SR48968, 1 mM) administered spinally through the microdialysis fibre prevented the heat hyperalgesia from developing in the early stages of the inflammation.

3 Post-treatment through the microdialysis fibre with the NK_1 receptor antagonist (0.01–10 mM) was effective in reversing the heat hyperalgesia. In contrast, post-treatment spinally with the NK_2 receptor antagonist (0.01–1 mM) had no effect on the heat hyperalgesia. The inactive stereoisomers of the NK_1 receptor antagonist, CP100,263, or the NK_2 receptor antagonist, SR48965, administered at the same doses, had no effect on the joint inflammation or the heat hyperalgesia.

4 Pretreatment systemically with the NK_1 receptor antagonist (30 mg kg^{-1}) had no effect on the heat hyperalgesia or pain-related behaviour ratings where 0 is none and 5 is non weight bearing and complete avoidance of limb contact. Pretreatment with a NK_2 receptor antagonist (10 mg kg^{-1}) systemically prevented the heat hyperalgesia and pain-related behaviour ratings from developing in the early stages of the inflammation. The inactive stereoisomers of NK_1 receptor antagonist, CP100,263, or the NK_2 receptor antagonist, SR48965, administered at the same doses, had no effect on the joint inflammation or the heat hyperalgesia.

5 Post-treatment systemically with either the NK_1 (0.1–30 mg kg^{-1}) or the NK_2 (0.1–10 mg kg^{-1}) receptor antagonist resulted in a dose-dependent reversal of the heat hyperalgesia. Pain-related behaviour ratings were reduced by post-treatment only with the NK_1 receptor antagonist. The inactive stereoisomers of the NK_1 receptor antagonist, CP100,263, or the NK_2 receptor antagonist, SR48965, administered at the same doses, had no effect on the behavioural responses.

6 Direct pretreatment of the knee joint with either the NK_1 (30 mg) or the NK_2 (10 mg) receptor antagonist prevented the heat hyperalgesia from developing without affecting joint swelling. The inactive stereoisomers of the NK_1 receptor antagonist, CP100,263, or the NK_2 receptor antagonist, SR48965, administered at the same doses, had no effect on the joint inflammation or the heat hyperalgesia.

7 There appears to be a differential role for the spinal tachykinin receptors in the development and maintenance of the heat hyperalgesia associated with acute joint inflammation. The NK_2 receptors appear to be activated early in the development of the heat hyperalgesia and NK_1 receptors are involved in the maintenance of the heat hyperalgesia.

8 Peripherally, both NK_1 and NK_2 receptors are involved in the development of heat hyperalgesia and pain-related behaviour ratings induced by acute inflammation.

Keywords: Pain; joint; arthritis; substance P; neurokinin A; microdialysis; dorsal horn; spinal cord; inflammation; neurokinin receptors

Introduction

The tachykinins, substance P (SP) and neurokinin A (NKA), have been implicated in the processing of nociceptive information in the spinal cord. These peptides act predominantly on neurokinin$_1$ (NK_1) and neurokinin$_2$ (NK_2) receptors, respectively. Both neuropeptides are found in primary afferent fibres (Ogawa *et al*., 1985; McCarthy & Lawson, 1989) and SP in dorsal horn and spinally projecting brainstem neurones (Gibson *et al*., 1981; Bowker *et al*., 1983; Ogawa *et al*., 1985). Intrathecal administration of either SP or NKA, in awake animals, results in behavioural responses that have been interpreted as nocifensive (Yashpal *et al*., 1982; 1993; Gamse & Saria, 1986).

Involvement of these neuropeptides in peripheral inflammation is well documented. In particular, there is an increase in mRNA for preprotachykinins in the dorsal root ganglia (DRG) and the spinal dorsal horn (Minami *et al*., 1989; Noguchi & Ruda, 1992; Donaldson *et al*., 1992), as well as mRNA for the NK_1 receptor (Schafer *et al*., 1993), in chronic models of inflammation. Increased SP immunoreactivity is observed in the DRG (Smith *et al*., 1992) and in the superficial dorsal horn in both chronic and acute inflammatory pain models (Weihe *et al*., 1988; Sluka *et al*., 1992; Sluka & Westlund, 1993a). The increase in content of substance P induced by acute inflammation is blocked by spinal administration of an NK_1 receptor antagonist (Sluka & Westlund, 1993b). There is an increase in the release of substance P (Schaible *et al*., 1990) and or neurokinin A (Hope *et al*., 1990) in the spinal dorsal horn following peripheral inflammation.

There is also evidence to support a role for these neuropeptides as mediators of peripheral inflammation. For example

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SP or NKA can cause plasma extravasation in the rat knee joint (Levine *et al*., 1984; Lam & Ferrell, 1989b; Scott *et al*., 1992; Nagahisa *et al*., 1992). Conversely, plasma extravasation and vasodilatation induced by substance P or carrageenan can be prevented by NK_1 receptor antagonists (Lam & Ferrell, 1989a; 1991). Infusion of substance P was shown to cause degenerative joint changes, whereas substance P antagonists reduced the severity of inflammation (Levine *et al*., 1984).

This study was designed to address the central and peripheral roles of NK_1 and NK_2 receptors in acute inflammation and the accompanying heat hyperalgesia. Knee joint inflammation induced with intraarticular injection of kaolin and carrageenan into the knee joint is used as a model of acute arthritis. A preliminary account has appeared in abstract form (Sluka *et al*., 1995).

Methods

Placement of microdialysis fibre

All experiments were approved by the Animal Care and Use Committee at our institution. A microdialysis fibre was implanted into the spinal dorsal horn of the rat according to the protocol of Skilling *et al*. (1988), as described previously (Sluka & Westlund, 1992). Briefly, a microdialysis fibre (200 μm o.d., 45,000 MW cut-off, Hospal AN69) was passed transversely through the deep dorsal horn of the spinal cord (L5 spinal segment) and stabilized with dental cement applied to the bone while the rat was anaesthetized (sodium pentobarbitone, 50 mg kg^{-1}, i.p.). The microdialysis fibre was permeable only where it was positioned in the grey matter of the spinal cord (2 mm gap). Artificial cerebrospinal fluid (ACSF) was infused through the microdialysis fibre at a rate of 5 μl min^{-1} for delivery of receptor antagonists.

The amount of diffusion across the semipermeable membrane was estimated *in vitro*. The fibre was placed in a bath containing 1 mM solution of CP-99,994 or SR48968 (in ACSF). ACSF without drug was run through the fibre at 5 μl min^{-1} for 1 h (as in our *in vivo* experiments). The concentration collected in the dialysate following 1 h infusion of ACSF was analysed by spectrophotometry. The concentration that passed across the membrane was determined to be 8% for CP-99,994 or 0.1% for SR48968. It is thus assumed that the maximal concentration of CP-99,994 that reaches the tissue would be 800 μM and that of SR48968 would be 0.1 μM. Efficacy of the drugs was established by using a cumulative dose-response curve in animals treated with the drug after the development of hyperalgesia. The maximum effective concentration was then used to pretreat the animals with the drugs. The maximum concentration of SR48968 that would go into solution without DMSO was 1 mM. This was the limit in the concentration at which the drug was administered spinally.

Knee joint injection

Knee joint inflammation was induced while the rat was anaesthetized with a short acting barbiturate, sodium methohexitone (Brevital; 50 mg kg^{-1}). One knee joint of each of the animals was injected with a mixture of 3% kaolin and 3% carrageenan (0.1 ml; pH 7.4) in sterile saline.

Behavioural testing and assessment of inflammation

As a measure of thermal hyperalgesia, animals were tested for paw withdrawal latency (PWL) to radiant heat according to the protocol first described by Hargreaves *et al*. (1988). Briefly, animals were placed in clear plastic cages on an elevated glass plate. Radiant heat was applied to the plantar surface of the hindpaw until the rat lifted its paw. The time at which this occurred was considered the PWL. Both paws were tested independently for five trials per side with a 5 min waiting period between trials. Animals were tested before induction of arthritis, after administration of receptor antagonist, and/or induction of arthritis. Each testing period lasted 30 min.

To quantify the abnormal posture of the hindpaw induced by the arthritis, the animals were rated on a subjective pain-related behaviour scale (0–5) modified from Guilbaud and colleagues (Attal *et al*., 1990) where: 0 was normal; 1 was curling toes; 2 was eversion of the foot; 3 was partial weight bearing; 4 was non-weight bearing and guarding; and 5 was avoidance of any contact with the limb. Ratings were taken at 4 h and either (1) at 8 h and 24 h (pretreatment) or (2) 30 min after administration of each dose of receptor antagonist (post-treatment) and 24 h.

Knee joint circumference was measured in cm with a flexible tape measure around the centre of the knee joint while the joint was held in extension. The knee joints were measured before (baseline), 4 h, 8 h (or after last dose) and 24 h following injection of the knee joint with kaolin and carrageenan.

Three thermographic readings were taken after the rat was anaesthetized with sodium pentobarbitone (50 mg kg^{-1}, i.p.) before perfusion, i.e. 24 h after induction of arthritis. These readings were averaged to obtain one number per animal. Thermographic readings were obtained from a liquid crystal tablet (Thermoflex) placed over the body surface with the rat lying supine. The knee joint surface could clearly be observed (see Sluka and Westlund, 1993c). The highest reading from the joint surface on each side was then obtained and side to side differences evaluated.

Experimental groups

Male Sprague-Dawley rats ($n=69$; 250–450 g) were treated with the NK_1 receptor antagonist, CP-99,994 ((+)-(2**S**,3**S**)-3-(2-methoxybenzylamino)-2-phenylpeperidine) or the NK_2 receptor antagonist, SR48968 ((**S**)-N-methyl-N-[4-(4-acetylamino-4-phenylpiperidino)-2-(3,4-dichlorophenyl)butyl]benzamide), according to the following treatment regimens: (1) pretreatment through a microdialysis fibre in the dorsal horn for 1 h before the induction of arthritis (CP-99,994, $n=6$, 10 mM; SR48968, $n=6$, 1 mM); (2) post-treatment through a microdialysis fibre in the dorsal horn 4 h after induction of arthritis with continuous infusion of cumulative concentrations (CP-99,994, $n=7$, 0.001 mM – 10 mM, 5 doses; SR48968, $n=6$, 0.01 – 1 mM, 3 doses); (3) pretreatment systemically (i.p.) 15 min before the induction of arthritis (CP-99,994, $n=6$, 30 mg kg^{-1}, SR48968, $n=6$, 10 mg kg^{-1}); (4) post-treatment systemically (i.p.) 4 h after induction of arthritis with successively increasing doses (CP-99,994, $n=14$, 0.1 – 30 mg kg^{-1}, 5 doses; SR48968, $n=6$, 0.1 – 10 mg kg^{-1}, 3 doses); and (5) pretreatment into the knee joint 15 min before the induction of arthritis (CP-99,994, $n=6$, 30 mg; SR48968, $n=6$, 10 mg, dissolved in 20% Tween 80). Details on delivery of drugs and experimental protocol are given below.

Control groups of arthritic rats ($n=40$) included: (1) pretreatment with artificial cerebrospinal fluid through a microdialysis fibre ($n=4$) (pH 7.2 – 7.4); (2) post-treatment through a microdialysis fibre with the inactive stereoisomers of either the NK_1 receptor antagonist, CP100,263 ($n=4$, 0.001 – 10 mM, 5 doses, pH 7.2 – 7.4) or the NK_2 receptor antagonist, SR48965 ($n=4$, 0.01 – 1 mM, 3 doses, pH 7.0); (3) pretreatment systemically with sterile saline ($n=4$; pH 7.0) or with the inactive stereoisomers of either the NK_1 receptor antagonist, CP100,263 ($n=2$, 0.001 – 10 mM, 5 doses or the NK_2 receptor antagonist, SR48965 ($n=2$, 0.01 – 1 mM, 3 doses); (4) post-treatment systemically with sterile saline ($n=4$, pH 7.0, 5 injections) or with the inactive stereoisomers of CP100,263 ($n=4$, 0.1 – 30 mg kg^{-1}, 5 doses), pH 7.2 – 7.4) or SR48965 ($n=4$, 0.1 – 10 mg kg^{-1}, 3 doses, pH 7.0); and (5) pretreatment of the knee with saline and 20% Tween 80 ($n=4$) or the inactive stereoisomers of CP100,263 ($n=2$, 30 mg kg^{-1}, pH 7.2 – 7.4) or SR48965 ($n=2$, 10 mg kg^{-1}, pH. 7.0).

The inactive stereoisomers served as a control for the nonspecific effects of the tachykinin antagonists such as their

effects on calcium and sodium channels, or μ-opioid receptors (Advenier *et al*., 1992; McLean *et al*., 1993; Boyle *et al*., 1993; Lombet & Spedding, 1994; Docherty & Shah, 1995).

In experimental and control groups 1 and 2, a microdialysis fibre was implanted in the dorsal horn one day before the experiment for delivery of receptor antagonists locally in the spinal cord. On day 2, the animals were housed in small lucite cubicles which limited their movement but provided food and water. Baseline testing (see below) of the latency of paw withdrawal to radiant heat (PWL) was done for comparison with latencies after induction of knee joint inflammation.

In animals pretreated with receptor antagonists through a microdialysis fibre the drug was delivered for 1 h before baseline behavioural testing. After testing for PWL to radiant heat, the knee joint was injected with a mixture of 3% kaolin and 3% carrageenan. The injection was made after animals were anaesthetized with a short acting barbiturate (sodium methohexitone 50 mg kg^{-1}). The animals were allowed to awaken and to move about in their cages. The PWL test was then performed at 4 h, 8 h and 24 h after induction of arthritis. In animals treated with receptor antagonists through a microdialysis fibre after the development of heat hyperalgesia, the knee joint was injected following baseline behavioural testing. Receptor antagonists or their inactive stereoisomers were administered following PWL testing at 4 h. Successively increasing concentrations were administered for a total of 1.25 h per dose. Behavioural testing was repeated during the last 30 min that each concentration was given. The animals were also tested 24 h after induction of arthritis.

In animals pretreated systemically (i.p.), the receptor antagonist or saline was administered 15 min before the injection of the knee joint with kaolin and carrageenan. The PWL to radiant heat was then tested at 4 h, 8 h and 24 h after induction of arthritis. In animals post-treated systemically, the receptor antagonist, its stereoisomer or saline was injected following testing of PWL to radiant heat at 4 h. Successively increasing doses of drug were given 30 min before the PWL testing. Behavioural testing was then repeated which took approximately 30 min. Thus, there was one hour between successive increases in doses. Animals were also tested 24 h after induction of arthritis.

In animals whose knee joints were pretreated, the receptor antagonist or saline was injected 15 min before induction of arthritis. PWL to radiant heat was tested before, 4 h, 8 h and 24 h following induction of arthritis.

The concentrations of receptor antagonists used for pretreatment of the animals were based on cumulative dose-response curves that were constructed in animals post-treated with the receptor antagonists. The most effective dose was used to pretreat the animals. In the case of the NK_2 receptor antagonist, SR48968, administered through a microdialysis fibre, a 1 mM dose was ineffective with post-treatment. Since this was the highest concentration that would dissolve in ACSF it was used for pretreatment through the fibre. Pretreatment with this concentration was effective in preventing the heat hyperalgesia.

Statistical analysis

A repeated measures analysis of variance (ANOVA) was used to compare the PWL and joint circumference before, after induction of arthritis, and after administration of receptor antagonists for both the ipsilateral and the contralateral paws. If a significant difference was obtained ($P<0.05$), differences across times and between groups were compared by *t* tests. A Bonferonni correction was applied as appropriate. Since pain-related behaviour ratings did not have a normal distribution, categorical data analysis was used to assess this parameter (Freeman, 1987). *Post-hoc* testing was with a χ^2 test that compared differences between groups. All computations were performed with Statistical Analysis Software (SAS). The specific programmes were proc univariate, proc general linear modeling and proc catagorical modeling. All data are expressed as the mean $\pm$ s.e.mean.

Results

Control arthritic animals

Following induction of acute inflammation there was a significant decrease in the latency of withdrawal from radiant heat applied to the paw (i.e., heat hyperalgesia), increased pain-related behaviour ratings, an increased joint circumference and increased joint temperature. The PWL decreased from about 10 s (saline i.p., i.a., stereoisomers, ACSF) to approximately 7 s in control arthritic animals (saline i.p., i.a., stereoisomers, ACSF) (see Figures 1, 2 and 4 for specific numbers). The pain-related behaviour ratings before the induction of inflammation were zero and increased by 4 h to approximately 4 in control arthritic animals (saline, stereoisomers and ACSF) (see Figures 3 and 4 for specific numbers). This increase in pain-related behaviours remained increased through 24 h. The ipsilateral joint circumference increased by 25–30 mm in control arthritic animals by 4 h from baseline of 65–70 mm, and it remained increased 24 h after induction of inflammation (saline, stereoisomers, ACSF). The temperature change at 24 h after induction of arthritis increased on the ipsilateral side by approximately 3°C.

Microdialysis administration of receptor antagonists

Paw withdrawal latency

Pretreatment As shown in Figure 1 the latencies for withdrawal of the paw from radiant heat on the side ipsilateral to the inflammation, in control arthritic animals, significantly decreased from an average of 9.6 ± 0.41 to 7.1 ± 0.26 s by 4 h. This decrease remained nearly constant in control animals treated with ACSF for 24 h (Figure 1A,C). The NK_1 receptor antagonist (CP-99,994, 10 mM) was ineffective in preventing the decrease in PWL induced by the acute inflammation when administered spinally through a microdialysis fibre (Figure 1A). In these animals there was a significant decrease ($P<0.01$) in PWL from baseline (10.0 ± 0.2) at all time points following induction of arthritis (4 h, 7.7 ± 0.43 s; 8 h, 8.1 ± 0.25 s; 24 h, 7.8 ± 0.31 s).

In contrast, if animals were pretreated spinally with the NK_2 receptor antagonist (SR48968, 1 mM), the decrease in PWL that occurred at 4 h following induction of arthritis was significantly blocked (Figure 1A). The PWL before induction of arthritis was 8.8 ± 0.18, and 4 h following induction of arthritis it was 8.2 ± 0.1. However, the PWL to radiant heat became significantly decreased ($P<0.01$) 8 h (7.6 ± 0.33) and remained decreased 24 h (7.6 ± 0.48) following induction of arthritis. No significant changes occurred in the PWL to radiant heat on the contralateral side.

Post-treatment In all arthritic animals post-treated with the receptor antagonists there was a significant decrease in the PWL to radiant heat by 4 h after injection of kaolin and carrageenan into the knee joint (Figure 1B,C). Post-treatment of arthritic animals with the NK_1 receptor antagonist by spinal infusion through the microdialysis fibre, resulted in a concentration-dependent reversal of the decrease in PWL to radiant heat (Figure 1B). After spinal infusion of the highest concentration (10 mM) there was a significant reversal of the decrease in PWL (9.0 ± 0.12) when compared to the PWL 4 h (7.9 ± 0.12) after induction of arthritis. However, the heat hyperalgesia was only partially increased (43%).

In contrast, the NK_2 receptor antagonist, SR48968 (1 mM), had no effect on the decrease in PWL that followed induction of arthritis (Figure 1C). The PWL was significantly reduced at all times (4 h, 6.8 ± 0.33; after 1 mM SR48968, 7.0 ± 0.10; 24 h, 7.1 ± 0.15) following induction of arthritis when compared to baseline (9.6 ± 0.1) (Figure 1C). No significant changes occurred in the PWL to radiant heat on the contralateral side.

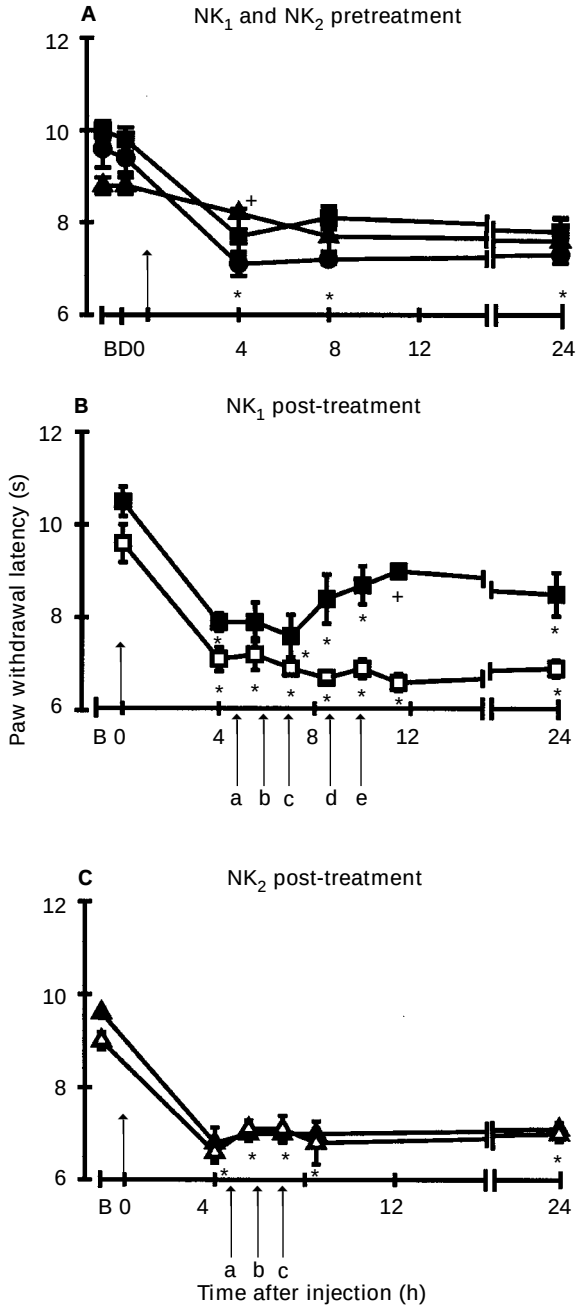

Figure 1 Paw withdrawal latencies (PWL) for arthritic animals that were pretreated (A) or post-treated (B,C) through the microdialysis fibre with a NK_1 (A,B) or NK_2 (A,C) receptor antagonist. Pretreatment: (A) animals were pretreated with CP99,994 (10 mM, $n=6$) or SR48968 (1 mM, $n=6$) for 1 h before the induction of arthritis. There was an overall significant difference for time ($F_{4,79}=14.15$, $P=0.0001$) and group ($F_{1,79}=8.57$, $P=0.02$) in the PWL of animals pretreated with ACSF, CP-99,994 or SR48968. The PWL changes were significant for time in arthritic animals pretreated with the NK_1 ($F_{4,29}=11.07$, $P=0.001$) or NK_2 receptor antagonist ($F_{4,29}=3.52$, $P=0.02$) or control arthritic animals treated with ACSF ($F_{4,19}=5.16$, $P=0.03$). Post-treatment: (B) animals were post-treated with CP99,994 ($n=11$) or CP100,263 ($n=4$) in successively increasing concentrations. An overall significant difference for time (dose) ($F_{7,76}=7.29$, $P=0.0001$) and group ($F_{1,76}=12.51$, $P=0.0008$) occurred in animals post-treated with the NK_1 receptor antagonist or its inactive stereoisomer. Doses tested: 0.001 mM (a, $n=5$), 0.01 mM (b, $n=7$), 0.1 mM (c, $n=7$), 1 mM (d, $n=6$), 10 mM (e, $n=4$). (C) There was a significant effect for time in the PWL test of arthritic animals post-treated with the NK_2 receptor antagonist ($F_{5,23}=19.04$; $P=0.0001$), with the baseline PWL significantly reduced at all times following induction of arthritis. SR48968 ($n=6$), SR48965 ($n=4$). Doses tested: 0.01 mM (a), 0.1 mM (b) and 1 mM (c). $^{*}P<0.05$, significantly different from baseline; $^{+}P<0.05$, significantly different from PWL times measured 4 h in ACSF control arthritic animals (pretreatment) or significantly different from the 4 h time point in same group of animals (post-treatment). Symbols: (●) ACSF control, (■) CP99,994, (□) CP100,263, (▲) SR48986, (△) SR48965, B = baseline, D = drug, 0 = time of injection.

Pain-related behaviour ratings

Pretreatment There was no change in the pain-related behaviour ratings of arthritic animals pretreated spinally with the NK_1 (10 mM) or the NK_2 (1 mM) receptor antagonist when compared to ACSF control animals. Pain-related behaviours 4 h after induction of arthritis were 4.0 ± 1 for ACSF controls, 4.0 ± 0.23 for animals pretreated with CP-99,994 and 3.5 ± 2 for animals pretreated with SR48968. At 8 h and 24 h after induction of arthritis, pain-related behaviour ratings remained elevated, averaging approximately 4 for each group.

Post-treatment The pain-related behaviour ratings were not significantly decreased with the highest dose of the NK_1 receptor antagonist delivered to the spinal cord. Ratings taken 4 h after induction of arthritis and before infusion of the antagonist or its isomer averaged 4.1 ± 0.26, CP-99,994 and 4.4 ± 0, CP100,263. Following infusion of the highest dose of the antagonist or the stereoisomer there was no significant change in the pain-related behaviour ratings (3.2 ± 0.18, CP-99,994, 10 mM and 4.0 ± 0.18, CP100,263, 10 mM). Post-treatment spinally with the NK_2 receptor antagonist (1 mM) had no effect on the pain-related behaviour ratings when compared to the behaviours after administration of the NK_2 stereoisomer (1 mM). Four hours after induction of inflammation, pain-related behaviour ratings averaged 4.0 ± 0.29 for the groups of animals post-treated with SR48968; and 4.0 ± 0.23 for animals treated with the inactive stereoisomer, SR48965. After the highest concentration (1 mM) of the antagonist or its inactive stereoisomer was administered, the average pain-related behaviour ratings were 4.2 ± 0.18 and 4.2 ± 0.15, respectively.

Joint swelling and temperature Pretreatment of the spinal cord with either the NK_1 or the NK_2 receptor antagonist had no effect on the swelling or increased temperature of the knee joint. Similarly, post-treatment of the spinal cord with either the NK_1 or the NK_2 receptor antagonist had no effect on the joint swelling or temperature change.

Systemic administration of receptor antagonists

Paw withdrawal latency

Pretreatment Similar to the findings in animals pretreated spinally with the NK_1 receptor antagonist, there was no effect on PWL in animals pretreated systemically with the NK_1 receptor antagonist (30 mg kg^{-1}) (Figure 2A). If animals were pretreated systemically with the NK_1 receptor antagonist, the PWL was significantly decreased from baseline (9.4 ± 0.29) at all time points tested (4 h, 6.6 ± 0.2; 8 h, 6.6 ± 0.3; 24 h, 6.7 ± 0.18). In contrast, if the animals were pretreated with the NK_2 receptor antagonist systemically (10 mg kg^{-1}), the hyperalgesia to heat did not develop (Figure 2A). Baseline PWLs averaged 9.0 ± 0.3 and remained unchanged by 4 h (9.2 ± 0.27), 8 h (8.9 ± 0.2) and 24 h (8.6 ± 0.27) following induction of arthritis. The PWLs in the NK_2 treated group of animals were significantly greater than those in ACSF control animals. The PWLs in animals pretreated with the inactive stereoisomers, CP100,263 or SR48965, after induction of inflammation decreased as in animals treated with saline.

Post-treatment There was a significant reversal of the arthritis-induced decrease in the PWL to radiant heat back toward baseline levels in this group of animals following systemic

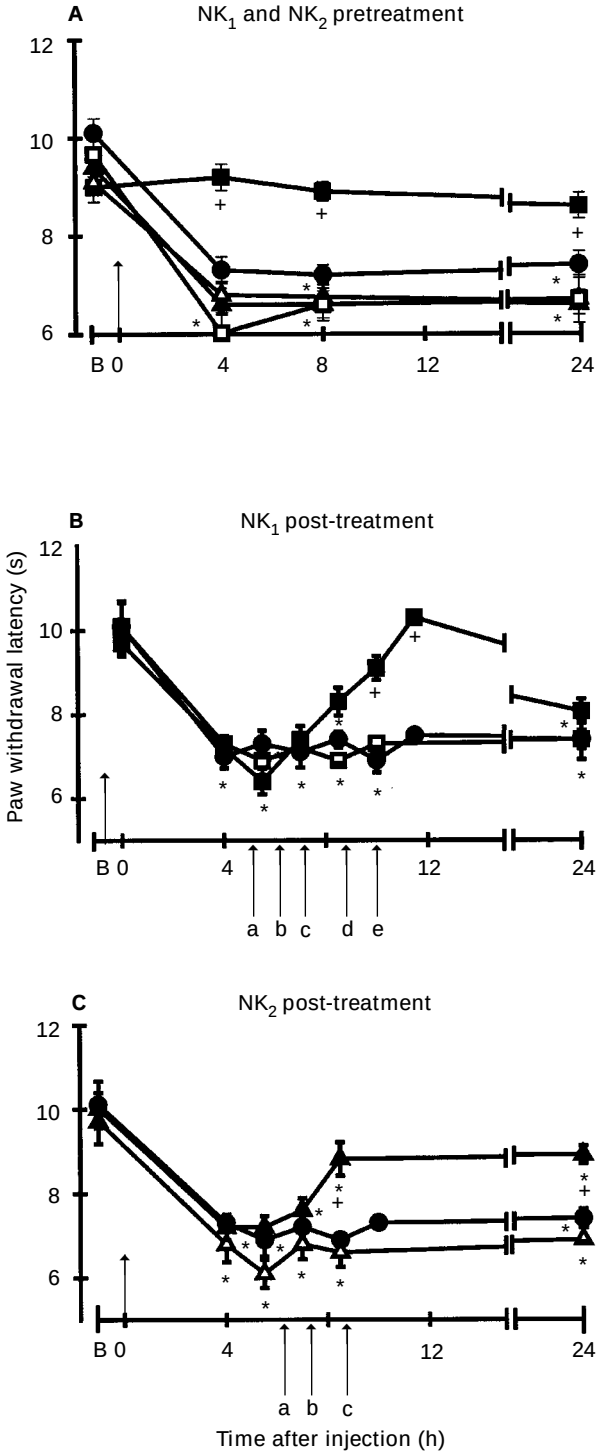

Figure 2 Paw withdrawal latencies (PWL) for arthritic animals pretreated (A) or post-treated (B,C) systemically with the NK_1 receptor antagonist, CP-99,994, or the NK_2 receptor antagonist, SR48968. Pretreatment: (A) an overall significant difference for time ($F_{3,47}=13.18$, $P=0.0001$), group ($F_{1,47}=62.96$, $P=0.0001$) and time and group ($F_{3,47}=11.19$, $P=0.0001$) occurred in animals pretreated with receptor antagonists before the induction of arthritis. CP99,994 ($n=6$, 30 mg kg^{-1}), SR48968 ($n=6$, 10 mg kg^{-1}), saline ($n=4$), CP100,263 ($n=2$, 30 mg kg^{-1}), SR48965 ($n=2$, 10 mg kg^{-1}). Post-treatment: (B) with post-treatment of animals with the NK_1 receptor antagonist, the inactive stereoisomer of the NK_1 receptor antagonist or saline, a significant change in the PWL for time (dose) ($F_{7,145}=17.63$, $P=0.0001$) and for time and group ($F_{13,145}=3.06$, $P=0.0006$) occurred. CP-99,994 ($n=14$), CP100,263 ($n=4$), saline ($n=4$). Dose tested: (a) 0.1 mg kg^{-1}, (b) 1 mg kg^{-1}, (c) 5 mg kg^{-1}, (d) 10 mg kg^{-1}, (e) 30 mg kg^{-1}, i.p.). (C) Post-treatment of arthritic animals with the NK_2 receptor antagonist, the inactive stereoisomer of the NK_2 receptor antagonist or saline, resulted in a significant effect for time ($F_{6,82}=19.26$, $P=0.0001$) and group ($F_{2,82}=12.27$, $P=0.0001$) for the PWLs ipsilaterally. SR48968 ($n=6$), SR48965, ($n=4$), saline ($n=4$). Doses tested: (a) 0.1 mg kg^{-1}, (b) 1 mg kg^{-1}, (c) 10 mg kg^{-1}. $^{*}P<0.05$, significantly different from baseline, $^{+}P<0.05$, significantly different from the 4 h time point. Symbols: saline control (●); CP99,994 (■); CP100,263 (□); SR48968 (▲); SR48965 (△); B = baseline, 0 = time of injection.

administration of the NK_1 receptor antagonist at the highest doses, 10 mg kg^{-1} or 30 mg kg^{-1} (Figure 2B). The PWL decreased from 9.7 ± 0.3 s to 7.1 ± 0.24 s at 4 h. A significant reversal of the PWL occurred following treatment with 10 mg kg^{-1} to 9.1 ± 0.28 s and 30 mg kg^{-1} to 10.3 ± 0.15 s. For control animals treated with saline or the inactive stereoisomer of CP-99,994 (CP100,263), there was a significant decrease in the PWL that remained at all time points following induction of arthritis.

A significant reversal of the arthritis-induced decrease in PWL occurred following systemic administration of the NK_2 receptor antagonist at 10 mg kg^{-1} following induction of arthritis ($P<0.01$) (Figure 2C). The PWL decreased from 10.0 ± 0.38 s to 7.2 ± 0.3 s by 4 h. Following systemic administration of the highest dose, 10 mg kg^{-1}, the PWL was increased to 8.8 ± 0.39 s. Post-treatment with repeated injections of saline or successively increasing doses of the inactive stereoisomer had no effect on the arthritis-induced decrease in PWL.

Pain-related behaviour ratings

Pretreatment Pretreatment systemically with the NK_1 receptor antagonist (30 mg kg^{-1}) had no effect on the pain-related behaviour ratings (Figure 3A). In contrast, the pain-related behaviour ratings were significantly decreased in arthritic animals pretreated systemically with a single dose of the NK_2 receptor antagonist (10 mg kg^{-1}) when compared to the animals pretreated with the NK_1 receptor antagonist or saline (Figure 3A). The greatest blockade of the pain-related behaviour ratings occurred at 4 h with a gradual increase toward that observed in the arthritic control animals and animals pretreated with the NK_1 receptor antagonist by 24 h. Pretreatment with the inactive stereoisomers, CP100,263, or SR48965, had no effect on the pain-related behaviour ratings induced by acute inflammation.

Post-treatment The pain-related behaviour ratings in the NK_1 receptor antagonist post-treated arthritic animals were significantly reduced (Figure 3B). A significant decrease in the pain-related behaviours occurred with the highest dose tested. Pain-related behaviour ratings decreased from 4.5 ± 0.17 to 2.5 ± 0.23 following administration of 30 mg kg^{-1} systemically. Post-treatment systemically with the NK_2 receptor antagonist SR48968 had no effect on the pain-related behaviours (Figure 3C).

Joint swelling and temperature Pretreatment or post-treatment systemically with either the NK_1 or NK_2 receptor antagonist had no effect on the joint swelling or increased temperature.

Local administration of receptor antagonists

Paw withdrawal latency

Pretreatment Pretreatment of the knee joint itself with either the NK_1 (30 mg) or the NK_2 receptor antagonist (10 mg) resulted in a blockade of the development of the heat hyperalgesia when compared to the arthritic animals whose knee joints were injected with saline (pH 7.0, 20% Tween 80) or the inactive stereoisomers, CP100,263 or SR48965 (Figure 4A). The control group showed a significant decrease in PWL to radiant heat at 4 h (6.9 ± 0.48), 8 h (6.7 ± 0.13) and 24 h

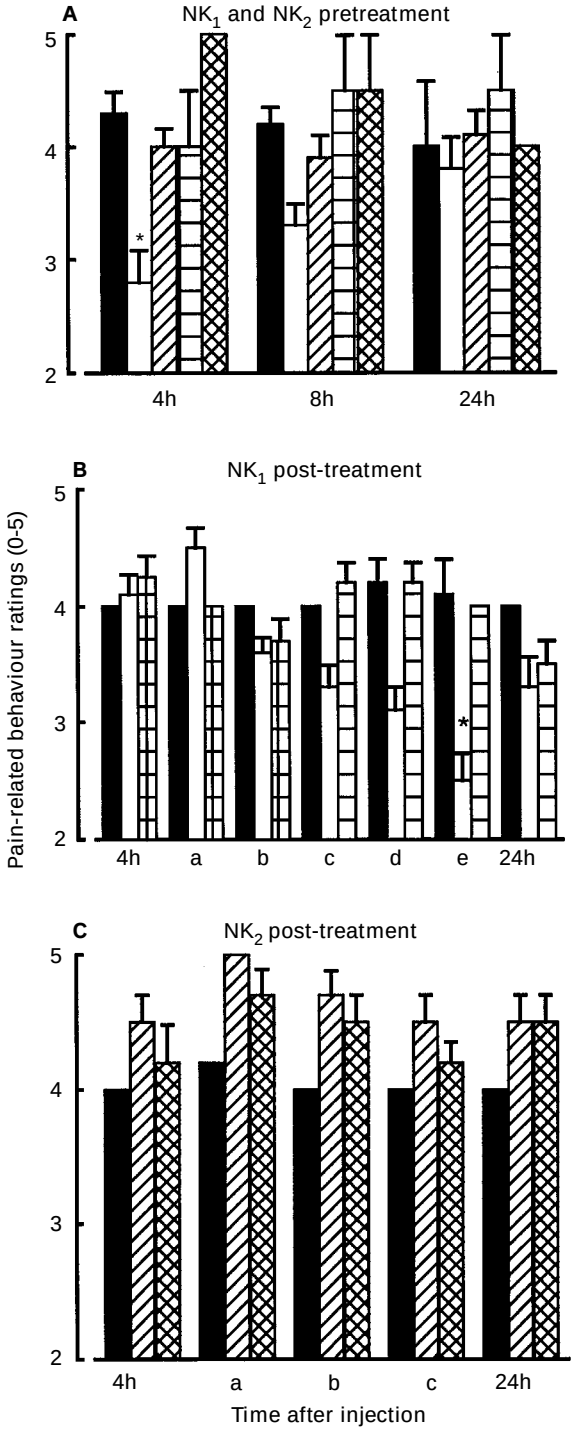

Figure 3 The pain-related behaviour ratings from arthritic animals treated systemically with the NK_1 or NK_2 receptor antagonists, their stereoisomers or saline are shown. (A) There was a significant effect for group ($\chi^2=1632.09$, $P=0.0001$) and for group and time ($\chi^2=7.13$, $P=0.03$) in the pain-related behaviour ratings of arthritic animals pretreated systemically with the tachykinin antagonists (CP99,994 or SR48968) or saline. In animals pretreated with the inactive isomers, CP100,263 or SR48965, the pain-related behaviour ratings after arthritis were similar to those seen in animals treated with saline instead of the drug. (B) There was a significant effect for group ($\chi^2=17.45$, $P=0.001$) and group and time ($\chi^2=8.65$, $P=0.04$) in the pain-related behaviour ratings of arthritic animals. Post-treatment with the NK_1 receptor antagonist, CP-99,994 (30 mg kg^{-1}), but not with its inactive stereoisomer, CP100,263 (30 mg kg^{-1}) was significantly reduced from the 4 h timepoint. Doses given: (a) 0.1 mg kg^{-1}, (b) 1 mg kg^{-1}, (c) 5 mg kg^{-1}, (d) 10 mg kg^{-1}, (e) 30 mg kg^{-1}. (C) No change in pain related behaviours occurred in animals post-treated with the NK_2 receptor antagonist, SR48968, its inactive stereoisomer, SR48965, or saline. (7.1 ± 0.23) after induction of inflammation as compared to baseline PWL before induction of inflammation (9.7 ± 0.48). Both the NK_1 and NK_2 receptor antagonists prevented the development of the heat hyperalgesia response (Figure 4A). A single pretreatment of the peripheral receptors with the NK_1 receptor antagonist, CP-99,994, prevented the heat hyperalgesia from developing for the entire 24 h testing period. On the other hand, a single dose of the NK_2 receptor antagonist was effective in preventing the heat hyperalgesia for only the first 8 h when compared to control animals treated with saline. By 24 h, the PWL was significantly decreased to times that were similar to those of the control arthritic group. No changes were observed on the side contralateral to the inflamed knee at any time point or in any group.

Pain-related behaviour ratings The increase in pain-related behaviour ratings observed in arthritic animals was unaffected by injection of saline into the knee joint, increasing to 4.0 ± 0.29 at 4 h, 4.25 ± 0.18 at 8 h and 4.0 ± 0 at 24 h. Administration of a single dose of the NK_1 (30 mg) or the NK_2 (10 mg) receptor antagonist into the knee joint before the injection of kaolin and carrageenan significantly delayed the development of the pain-related behaviour ratings when compared to control arthritic animals at the 4 h time points (Figure 4B).

Joint swelling and temperature Pretreatment with CP-99,994 or SR48968 in the knee joint had no effect on joint swelling in any of the animals. The knee joint circumferences were similar in all groups of animals at all time points. The increase in joint circumference from baseline at 4 h was not significantly different for animals injected with saline (19 ± 0.12 mm) or those injected with the NK_1 (22 ± 0.26 mm) or the NK_2 (24 ± 0.32 mm) receptor antagonist. By 24 h the joint circumference increased by 38 ± 0.20 mm in the saline injected arthritic group, 36 ± 0.32 mm in the NK_1 treated group and 38 ± 0.19 mm in the NK_2 group.

The change in knee joint temperature as measured by thermography was also similar in all animals at the 24 h time point: control arthritic animals $3.1\pm0.15^{\circ}C$; CP-99,994-treated arthritic animals $3.0\pm0.19^{\circ}C$; and SR48968-treated arthritic animals $2.5\pm0.53^{\circ}C$.

Discussion

This study provides evidence that both NK_1 and NK_2 receptors are involved in the development and maintenance of heat hyperalgesia and pain-related behaviour associated with acute joint inflammation. When heat hyperalgesia (PWL) was used for assessment of nociceptive behaviours, post-treatment spinally with the NK_1 receptor antagonist, CP-99,994, and pretreatment with the NK_2 receptor antagonist, SR48968, were effective in blocking the heat hyperalgesia. The pain-related behaviour ratings, on the other hand, were unaffected by spinal administration of tachykinin receptor antagonists. Systemic administration of the NK_1 receptor antagonist reversed both the heat hyperalgesia and the pain-related behaviour ratings if administered after the induction of arthritis. The NK_2 receptor antagonist administered systemically was effective in preventing or reducing the heat hyperalgesia with both pre- and post-treatment, while only pretreatment systemically prevented the increases in pain-related behaviour ratings. When administered peripherally directly into the knee joint, both receptor antagonists prevented the development of heat hyperalgesia and the increased pain-related behaviour ratings when delivered

Doses given: (a) 0.1 mg kg^{-1}, (b) 1 mg kg^{-1}, (c) 10 mg kg^{-1}. $^{*}P<0.01$, significant difference between groups. Solid columns, saline control; open columns, CP99,994; horizontally-hatched columns, CP100,263; diagonally-hatched columns, SR48968; cross-hatched columns, SR48965.

Table 1 Summary of the effects of pretreatment and post-treatment with either CP99,994 or SR48968 on the paw withdrawal latency (PWL) to radiant heat and the pain-related behaviours (PRB) in arthritic animals

		Microdialysis		*Systemic* (I.P.)		*Intra-articular*	
		PWL	*PRB*	*PWL*	*PRB*	*PWL*	*PRB*
CP-99,994 (NK_1)	Pretreatment	No effect	No effect	No effect	No effect	Prevented	Prevented
	Post-treatment	Reversed	No effect	Reversed	Reversed	N/A	N/A
SR48968 (NK_2)	Pretreatment	Prevented	No effect	Prevented	Prevented	Prevented	Prevented
	Post-treatment	No effect	No effect	Reversed	No effect	N/A	N/A

Abbreviations: NK_1 = neurokinin 1, NK_2 = neurokinin 2, N/A = not addressed.

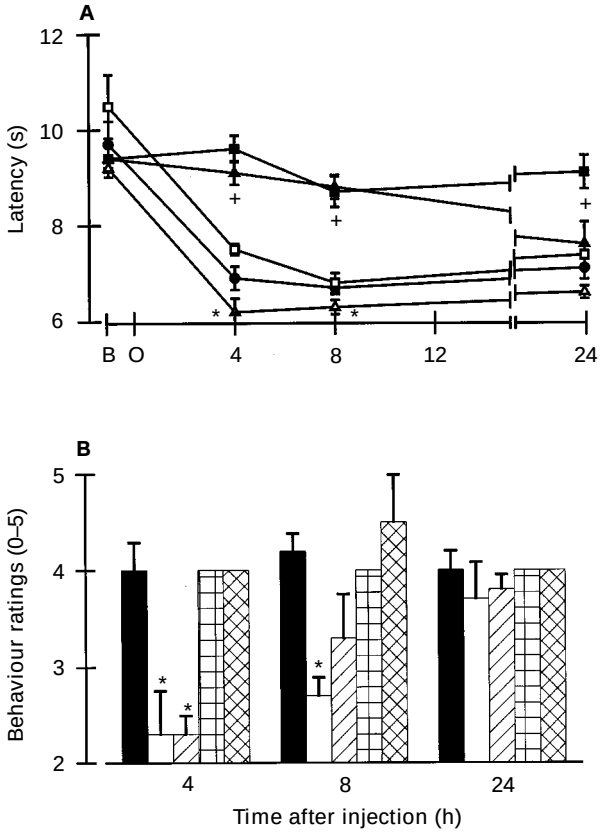

Figure 4 (A) Paw withdrawal latencies (PWL) to radiant heat in arthritic animals whose knee joints were pretreated with either 30 mg CP-99,994 (■, $n=6$), 10 mg SR48968 (▲, $n=6$) or saline (●, $n=4$). A significant change for time ($F_{3,63}=8.95$, $P=0.0001$), for group ($F_{2,63}=17.75$, $P=0.0001$) and for time and group ($F_{6,63}=4.21$, $P=0.002$) in PWL was observed. Injection of the inactive isomers, CP100,263 (□), or SR48965 (△), before the injection of kaolin and carrageenan resulted in decreases similar to those seen when saline was injected instead of the drug; thus, the inactive isomers did not prevent the heat hyperalgesia. B = baseline, 0 = time of injection. $^*P<0.01$, significantly different from the baseline. (B) Pain-related behaviour ratings from arthritic animals whose knee joints were pretreated with saline (10% Tween 80), CP-99,994 (30 mg) or SR48968 (10 mg) are shown. There was an overall significant change for time ($\chi^2=73.5$, $P=0.006$) and for group and time ($\chi^2=6.45$, $P=0.04$) in the pain-related behaviour ratings. Pretreatment with the inactive isomers, CP100,263 or SR48965, had no effect on the arthritis induced increases in pain-related behaviour ratings. $^*P<0.01$, significant difference between groups. Solid columns, saline control; open columns, CP99,994; diagonally-hatched columns, SR48968; squared columns, CP100,263; cross-hatched columns, SR48965.

before the induction of inflammation. For a summary of effects of the tachykinin antagonists see Table 1.

Tachykinin antagonists have non-selective effects although they are thought to be stereoselective. The NK_1 receptor antagonist, CP-99,994, inhibits the L-type calcium channel at a mimimum concentration of 9–100 mM (Lombet & Spedding, 1994). Taking into account the diffusion across the microdialysis fibre a minimum of 112.5 mM within the microdialysis fibre would be required to block calcium channels. In receptor binding assays CP-99,994 exhibited greater than 10,000 fold selectivity for the NK_1 relative to the NK_2 or NK_3 receptor (McLean *et al*., 1993). However, a partial reduction of the sodium current was observed with a concentration of 10 μM in guinea-pig cultured dorsal root ganglia cells (Docherty & Shah, 1995). The NK_2 receptor antagonist, SR48968, on the other hand, possesses calcium channel blocking activity at a concentration of 3 μM (Lombet & Spedding, 1994). SR48968 does not show specific blockade of the NK_2 receptor when compared to NK_1 or NK_3 receptors (Advenier *et al*., 1992; Lombet & Spedding, 1994). In addition recent evidence suggests that SR48968 also inhibits μ-opioid receptors in the guinea-pig (Docherty & Shah, 1995). However, the inactive stereoisomer was not tested and therefore there is no evidence to suggest that this is or is not a stereoselective effect. For these reasons the inactive stereoisomers, CP100,263 and SR48965, were used as a control for the nonselective effects of the receptor antagonists. In the current study, intraspinal, systemic or intraarticular administration of the inactive stereoisomers of the NK_1 and the NK_2 receptor antagonists had no effect on the behavioural responses of the animals following induction of arthritis, indicating that the effects were specific for NK_1 and NK_2 receptors but not for calcium or sodium channels, or μ-opioid receptors. Thus, this discussion is based on the assumption that the effects of CP-99,994 and SR48968 are due to their selective antagonist effects on NK_1 and NK_2 receptors, respectively.

Seguin *et al*. (1995) demonstrated very little difference in the pattern of inhibition of the nocifensive responses observed in the formalin test with several NK_1 receptor antagonists (CP99,994, RP67580, SR140,333, WIN 51,708 and WIN 62,577). In fact the doses of CP-99,994 that caused a maximal inhibition in the formalin test were quite similar to the doses used in our study (40 mg kg^{-1}). In electrophysiological recordings from dorsal horn neurones, the effects of CP-99,994 were shown to block the SP-induced depolarization (Radhakrishnan & Henry, 1995). In addition the responses of the cells to noxious heat and pinch were decreased by an NK_1 receptor antagonist (CP-99,994 or CP-96,345). The inactive stereoisomer also had no effect on the cellular responses to SP or heat (Radhakrishnan & Henry, 1995). In behavioural studies, immersion of the hindpaw in 45–55°C water increased the tail flick latency and this effect was reversed by CP-99,994 or CP-96,345 (Yashpal *et al*., 1995). These data further support a role for the specificity of CP-99,994 for NK_1 receptors.

Spinal role for tachykinin receptors

Measurement of the paw withdrawal latency in the present study, as a test of hyperalgesia to heat, most likely represents a secondary heat hyperalgesia since the area in which the heat was applied (paw) was outside the area of inflammation (knee). This secondary hyperalgesia is thought to reflect central neuronal sensitization (Woolf, 1983; Kenshalo *et al*., 1982; Simone *et al*., 1991; Törebjörk *et al*., 1992). Our studies tested whether the central neuronal changes were of spinal origin by applying tachykinin receptor antagonists directly into the spinal cord. We have clearly demonstrated a spinal site of action for the

tachykinin receptors in secondary heat hyperalgesia since the NK_1 receptor antagonist reversed the heat hyperalgesia and the NK_2 receptor antagonist prevented the heat hyperalgesia.

NK_1 and NK_2 receptors have been implicated in the processing of nociceptive information in the spinal cord. The results of the present study suggest that there is a differential role of the tachykinin receptors within the spinal cord dorsal horn with respect to the time of administration relative to the time of induction of the acute inflammation. The NK_2 receptors appear to be involved in the induction of the heat hyperalgesia while the NK_1 receptors seem to be involved in the maintenance of the heat hyperalgesia. This is in agreement with the work of several laboratories who have shown a role for NK_1 or NK_2 receptors in signalling noxious thermal (Yashpal *et al*., 1982; 1993; Yashpal & Henry, 1984; Fleetwood-Walker *et al*., 1993; Radhakrishnan & Henry, 1991; 1995; Picard *et al*., 1993) and noxious pinch (Radhakrishnan & Henry, 1995) stimuli. Sensitization of dorsal horn neurones is thought to underlie the behavioural manifestation of secondary hyperalgesia. Sensitization of dorsal horn neurones by capsaicin or kaolin and carrageenan injection is reduced by spinal or systemic administration of an NK_1 receptor antagonist (Dougherty *et al*., 1994; Neugebauer *et al*., 1995; Rees *et al*., 1995) but not by an NK_2 receptor antagonist (Dougherty *et al*., 1994; Rees *et al*., 1995). However, mustard oil-induced sensitization of dorsal horn neurones is reduced by an NK_2 receptor antagonist (Munro *et al*., 1993). Thus electrophysiological data support a role for NK_1 and NK_2 receptors in the processing of noxious stimuli in the dorsal horn, in particular with respect to sensitization of dorsal horn neurones.

Behaviourally, intrathecal administration of SP (Yashpal *et al*., 1982; 1993; Yashpal & Henry, 1984; Picard *et al*., 1993) or NKA (Picard *et al*., 1993) results in a decrease in withdrawal time to radiant heat stimuli. In fact, the activation of NK_2 receptors produces a more prolonged decrease in the withdrawal time to noxious heat stimuli than does activation of NK_1 receptors (Picard *et al*., 1993). In animals which had not received any analgesic compounds, administration of an antagonist to the NK_1 receptor did not significantly change the withdrawal latency in the tail flick, paw pinch or hot plate tests (Garces *et al*., 1994). The current study also found no effect on responses to radiant heat applied to the paw following administration of tachykinin receptor antagonists. Thus, the activation of tachykinin receptors can result in heat hyperalgesia. However, these receptors do not appear to be tonically active in normal animals since treatment with the receptor antagonists does not affect baseline parameters.

With the formalin test, pretreatment intrathecally with a NK_1 receptor antagonist reduced the typical nocifensive behaviours (Yamamoto & Yaksh, 1991; Sakurada *et al*., 1993; Seguin *et al*., 1995). Systemic administration of receptor antagonists was also effective in reducing nocifensive behaviours in the formalin test (Yamamoto *et al*., 1993b; Sakurada *et al*., 1993; Garrett *et al*., 1993; Seguin *et al*., 1995). The mechanical threshold for the flexor withdrawal reflex was significantly decreased following cutaneous injection of mustard oil or intramuscular injection of bradykinin (Ma & Woolfe, 1995). Interestingly, pretreatment with an NK_1 receptor antagonist prevented the responses to cutaneous stimulation, and an NK_2 receptor antagonist prevented the responses to muscle stimulation. However, post-treatment had no effect on responses to either of those stimuli (Ma & Woolfe, 1995). This supports our finding that hyperalgesia induced by stimulation of joint afferents was prevented by pretreatment with an NK_2 receptor antagonist and not by an NK_1 receptor antagonist. Thus, there are several reasons for the differences observed between the present and previous studies. For instance, the type of primary afferent stimulated (cutaneous vs deep) may explain some of the differences between studies with tachykinin receptor antagonists. The differences between the NK_1 and NK_2 receptor antagonists may be related to their half-lives and/or metabolic transformation. This may also be a reflection of differences between the types of stimuli used, formalin or capsaicin versus kaolin and carrageenan. Alternatively, the difference between the effects of the NK_1 and NK_2 receptor antagonists may reflect a difference in the method used to administer the antagonists, intrathecal versus microdialysis versus systemic. It has been shown that drugs administered intrathecally can redistribute to higher brain structures or into various body tissues, such as the kidney or the liver (Nässtrom *et al*., 1993). Antagonists administered by microdialysis have been shown to stay localized to the area of administration (Sluka *et al*., 1993). Systemic administration can have effects at both peripheral and central sites.

Possible sites of action for systemic administration

In the present study, the heat hyperalgesia was only partially reversed by an NK_1 receptor antagonist administered spinally. However, a full reversal of the hyperalgesia was observed when the antagonist was administered systemically. These data suggest that NK_1 receptors are involved not only spinally but also peripherally and/or at higher brain centres. The effectiveness of post-treatment with SR48968, delivered systemically, in reducing heat hyperalgesia and the lack of effect of SR48968 when delivered spinally suggests that this compound may be working at higher brain centres and not in the spinal cord at the concentration delivered systemically. Alternatively, the receptor antagonists may be effective at peripheral sites since both are effective in reducing both heat hyperalgesia and pain-related behaviour ratings when introduced directly into the knee joint before inflammation. However, both CP99,994 and SR48968 have been shown to cross the blood brain barrier (McLean *et al*., 1993; Poncelet *et al*., 1993; Steinberg *et al*., 1995). It is likely that these drugs would thus be acting not only as their peripheral receptors at the site of injury but also in the spinal cord and higher brain centres. In fact, substance P and neurokinin A have been located in brainstem nuclei known to be involved in modulation of nociception, including the raphe nuclei and periaqueductal grey (Emson, 1979; Bowker *et al*., 1983; Marcos *et al*., 1993). Thus, systemic administration of receptor antagonists has the added advantage of blocking tachykinin receptors peripherally and centrally. The effectiveness of systemic administration at reducing the hyperalgesia and pain-related behaviour ratings show that there is a potential role for these drug types in the clinical treatment of inflammatory pain.

Peripheral role for tachykinin receptors

Substance P and NKA are the endogenous ligands of the tachykinin receptors, activating cascades of cellular events associated with the inflammatory process. This study demonstrated a role for the peripheral tachykinin receptors in the development of secondary heat hyperalgesia and pain-related behaviour and thus another site for clinical intervention. The activation of the peripheral tachykinin receptors would presumably sensitize primary afferent fibres and cause primary hyperalgesia in most inflammatory models. In a study similar to ours by Yamamoto *et al*. (1993a,b), paw withdrawal latency of an inflamed paw was measured as an indicator of primary heat hyperalgesia. In this case there was no apparent spinal role of the tachykinin receptors when delivered intrathecally. Differences between the studies could be a result of differences in methods of administration (intrathecal vs microdialysis) or differences in measurement outcome (primary vs secondary hyperalgesia). However, Yamamoto *et al*. (1993a,b) demonstrated that peripheral tachykinin receptors were involved in the development of this primary hyperalgesia. Thus, primary hyperalgesia drives central neurone sensitization to peripheral mechanical stimuli and this central sensitization could then be manifested as a secondary hyperalgesia. Blockade of the peripheral tachykinin receptors could then prevent secondary hyperalgesia by eliminating sensitization of primary afferents and primary hyperalgesia. Thus, the effectiveness of antagonizing the tachykinin receptors in the periphery in reducing

heat hyperalgesia and the associated increase in pain-related behaviour ratings, in the present study, is probably the result of blockade of secondary events induced by SP and/or NKA at peripheral sites. Cohen and Perl (1990) have shown that SP does not alter the sensitivity of peripheral nociceptive terminals directly. On the other hand, SP and NKA have been shown to cause plasma extravasation (Brain & Williams, 1985; Lam & Ferrell, 1989b; Nagahisa *et al*., 1992) and the accumulation of such substances as prostaglandins and cytokines (Khalil & Helme, 1989; Barnes, 1992). Prostaglandin E_2 production and increased collagenase activity in chondrocytes have been shown to be initiated by the C-terminal fragment of SP, SP_{7-11} (Halliday *et al*., 1993). SP and NKA have been shown to increase the release of cytokines from macrophages and neutrophils, bind to leukocytes and increase neutrophilic adherence to the vascular endothelium (Bethel *et al*., 1988; McGillis *et al*., 1990). The increase in leukocyte adhesion has been shown to be mediated through NK_1 receptors (Baluk *et al*., 1995). The cytokines and prostaglandins sensitize primary afferent fibres (Schaible & Grubb, 1993; Vasko *et al*., 1994). Therefore, the peripheral effects of the tachykinins may be related to their actions on blood-borne and local inflammatory cells.

In conclusion, both spinal and peripheral tachykinin receptors appear to be involved in the development and/or maintenance of the heat hyperalgesia and pain-related behaviour ratings associated with acute joint inflammation. Systemic administration of NK_1 and NK_2 receptor antagonists effectively reduced the hyperalgesia and pain-related behaviours, suggesting a potential role of these antagonists in the treatment of inflammatory pain.

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