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The site of anti-arthritic action of the κ-opioid, U-50,488H, in adjuvant arthritis: importance of local administration

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1 Currently available pharmacological therapies treat arthritis inadequately. We have previously found that the kappa (κ)-opioid, U-50,488H (trans-(±)-3,4-dichloro-N-methyl-N-[2-(1-pyrrolidinyl) cyclohexyl]-benzene-acetamide methane sulphonate), possesses anti-arthritic effects. In light of the finding that opioid receptors in the periphery are upregulated during inflammation, κ-opioids may represent a novel therapy for arthritis. The primary aim and unique feature of the present study is to investigate whether opioids exert their anti-arthritic effects in the periphery. Thus, the dose-effect relationship of a κ-opioid agonist, U-50,488H was compared after both local and distant administration. Further, we tested whether the anti-arthritic effects of this drug are stereospecific and receptor-mediated by use of opioid antagonists.

2 Using an adjuvant model of arthritis in male Lewis rats, arthritis was judged by oedema, radiography and histological changes in the contralateral ankle of the hind limb. Treatment with (±)-U-50,488H for 3 days during disease onset and 3 days during established disease significantly attenuated arthritis, but the effects of (±)-U-50,488H on radiology and histology varied according to treatment time. Administration of (±)-U-50,488H during disease onset had a more marked effect on radiography, suggesting that treatment with that drug should be started early to prevent progressive joint destruction. Further, it was found that (±)-U-50,488H, administered for 3 days during the disease onset, either by direct subcutaneous injection into the inflamed paw or at a more distant site into the back of the neck, dose-dependently attenuated arthritic damage as measured by an index which pooled all three variables. More importantly however, (±)-U-50,488H was approximately fourfold more potent as an 'anti-arthritic' agent after local compared to distant subcutaneous injection (ED_{50}; local vs distant: 5.8 ± 1.6 vs 19.5 ± 0.8 mg kg^{-1}).

3 Equivalent doses of the (−)-enantiomer (20 mg $kg^{-1}day^{-1}$) and the racemate (±) of U-50,488H (40 mg $kg^{-1}day^{-1}$), elicited a similar attenuation of arthritic parameters while the (+)-enantiomer exacerbated arthritis, suggesting that the anti-arthritic activity lies solely with the (−)-enantiomer.

4 Both the peripherally selective antagonist, naloxone methiodide, and the κ-selective antagonist, MR2266 ((−)-5,9α-diethyl-2-(3-furylmethyl)-2′-hydroxy-6,7-benzomorphan), were able to reverse fully the peripheral anti-arthritic effects of U-50,488H, indicating that it exerts its effects through peripheral κ-opioid receptors.

5 Taken together, these results not only confirm our previous findings that demonstrate anti-arthritic effects of U-50,488H but they indicate that the opioid attenuation of experimental arthritis is mediated via peripheral κ-receptors in the arthritic joint. Peripherally acting κ-opioid agonists should lead to new therapies for arthritis.

Keywords: (±)-U-50,488H; (−)-U-50,488H; (+)-U-50,488H, naloxone methiodide; MR 2266; κ-agonist; κ-antagonist; peripheral effects; adjuvant arthritis; inflammation

Introduction

Rheumatoid arthritis is a common, chronic and potentially disabling disease associated with increased mortality. It is now recognised that aggressive drug therapy needs to be started early to prevent progressive joint destruction (Wilske & Healy, 1989; Pincus, 1994). Present therapies include nonsteroidal anti-inflammatory drugs and disease-modifying anti-rheumatic drugs (e.g. gold, sulphasalazine and methotrexate). These drugs not only inadequately treat the disease, but result in serious side effects such that 30% of patients discontinue therapy (Pincus *et al.*, 1992). More effective drugs for arthritis are clearly required. There is a long history of the use of opioids in the treatment of patients with arthritis; however, the rationale has been for the treatment of pain rather than inflammation.

The opioid system involves at least three receptor subtypes, mu (μ), delta (δ) and kappa (κ), each mediating different physiological and biochemical processes (Carmody, 1987). Previous studies from this laboratory and that of others, utilising experimental arthritis, have indicated that opioid drugs attenuate the severity of arthritis but the receptor subtype that is involved is still unclear (Walker *et al.*, 1995). At high but not low doses, the prototype opioid antagonist, naloxone, is also able to attenuate the severity of experimental arthritis (Millan & Colpaert, 1991); it has been suggested that this in fact could be an agonist action (Ferreira and Nakamura, 1979; Brown *et al.*, 1986) but in any case naloxone is known to act at all receptor classes (Jaffe & Martin, 1994).

Concerns about the central side effects of opioids, including addiction, limit the extent to which opioids are prescribed, especially for chronic diseases such as arthritis. In this regard, κ-opioid agonists possess some advantages over μ-agonists: they are devoid of side effects such as dependence liability, constipation and respiratory depression (Horwell, 1988) but possess a spectrum of other side effects including CNS disturbances and dysphoria (Pfeiffer *et al.*, 1986; Dykstra *et al.*, 1987).

It has now been shown that opioids, by local injection, have pronounced analgesic effects at peripheral sites in the presence of inflammation. They do so by interacting with peripheral opioid receptors on primary afferent neurones and/or cells of

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the immune system (for review see Stein, 1995). Together with the fact that opioid receptors in the periphery may be upregulated during inflammation, new avenues for treatment of chronic inflammatory diseases are opened up (Ferreira & Nakamura, 1979; Russell *et al.*, 1985; Sibinga & Goldstein, 1988; Basbaum & Levine, 1991; Stein *et al.*, 1989; 1990; 1991; Stein, 1991; Barber & Gottschlich, 1992). Thus, central side effects may be avoided by administering very low doses of the drug locally at the site of inflammation.

We have previously found that the prototype κ-opioid receptor agonist, U-50,488H, attenuates the progression of experimental arthritis as judged by oedema, radiology and histology (Walker *et al.*, 1995). While opioids have been previously found to have peripheral analgesic effects, the primary aim and unique feature of the present study is to investigate whether the anti-arthritic effects are also mediated via a peripheral site of action. In this study we have examined the dose-effect relationship of U-50,488H after both local and distant administration in rats with chronic arthritis. Additionally, we tested whether the anti-arthritic effects of this drug are time-dependent and stereospecific. The participation of opioid receptors was also established using the κ-opioid selective antagonist, MR2266 and the peripherally selective antagonist, naloxone methiodide.

Methods

Experimental animals

Experiments were performed in male Lewis rats (Animal Resource Centre, Perth, WA) (150–210 g). They were housed in groups of 10 in cages lined with cellulose bedding (Australian Cellulose Industries) and shredded paper in a temperature controlled room (22±1°C) with a 12 h alternating light and dark cycle (06 h 00 min to 18 h 00 min). Animals were given food (rat chow) and water *ad libitum* for one week prior to, and throughout the experiments. All experiments were approved by the Animal Care and Ethics Committee of the University of New South Wales, Australia.

Induction of arthritis

To produce adjuvant arthritis, rats were anaesthetized with a ketamine, (50 mg kg^{-1} i.p.) and xylazine (5 mg kg^{-1} i.p.) mixture and injected with 50 μl of complete Freund's adjuvant (i.e., 5 mg ml^{-1} heat killed and dried *Mycobacterium butyricum* suspension in paraffin oil and mannide monooleate, Difco Laboratories, Detroit, Michigan, USA) intracutaneously into the right hind paw on Day 1. Control rats received similar injections of incomplete Freund's adjuvant (paraffin oil and mannide monooleate, Sigma Chemicals, U.S.A.). Two groups of controls were included in each experiment. Adjuvant-treated rats received subcutaneous injections of saline (2 ml $kg^{-1}day^{-1}$) to serve as an arthritic control group ($n=10$), while non-arthritic rats received injections of U-50,488H[(±) or (−)] as a non-arthritic treatment control ($n=5$ per group).

All rats were handled every 2 to 3 days for 2 weeks prior to and throughout the study. During this time water intake and body weights were monitored. The same trained observer performed the measurements throughout the study. Three indices of polyarthritic damage were measured: oedema by plethysmometry in both the ipsilateral and contralateral ankle (Ugo Basile, Comerio, Italy), followed by radiography and histology of the contralateral ankle to assess joint damage.

Experiments in our laboratory have indicated that adjuvant arthritis produces a biphasic response (Walker *et al.*, 1995). In the first stage, (i.e., days 1 to 3), an acute local inflammatory reaction develops and then subsides by the twelfth day when a diffuse inflammatory reaction develops in the distal joints of the limbs, particularly the hind limbs. The disease then peaks between days 18 and 30 as judged by oedema, radiography and histology (unpublished data). For this reason, the experiments were terminated at day 21 post-adjuvant. The rats were killed (pentobarbitone, 60 mg i.p.) and the contralateral paw removed so that joint damage could be assessed by radiology and histology.

Assessment of arthritic damage

Radiology was performed on a mammography machine (General Electric 600T) using a fine focus of 0.3, an aluminium filter and Mammoray MR5 film (Agfa). The exposure was 27 kV and 4 mA.s for bony detail, and 24 kV and 4 mA.s for soft tissue. The specimens were radiographed at a 65 cm focus to film distance and viewed at a magnification of 1.85. The following parameters were evaluated without knowledge of treatment by a trained radiologist:

(1) soft tissue swelling: increased width in the soft tissue shadows and alterations in the normal configuration of soft tissue planes.

(2) erosion: destruction of bony tissue with increased radiolucency developing at the site of erosion.

(3) osteoporosis: decreased density of the bone recognised as increased radiolucency relative to uninvolved adjacent bone.

(4) joint space loss: narrowing of the joint space; and

(5) joint damage: destruction of the normal architecture and configuration of the joint.

A subjective rating scale was used to grade each parameter with 0 indicating normal, 1 indicating mildly, 2 moderately and 3 severely affected. Thus, a normal joint would score 0 and the maximum score in an arthritic joint would be 15 (Walker *et al.*, 1995).

Following radiography, specimens were placed in 10% formalin for 7–10 days, and then decalcified in 30% formic acid for an additional 3–5 days. Longitudinal sections were prepared such that the dorsoventral faces of the tarsal, metatarsal and phalangeal joints, bones and soft tissues were presented. The specimens were embedded in wax and blocks were cut at 7 μm on a rotary microtome. Sections were mounted on glass slides and stained with haematoxylin and eosin. Each slide was evaluated, without knowledge of treatment, by a trained observer, for character of pathological change and alteration of bone and cartilage using the following parameters: (1) periarticular inflammation; (2) cartilage erosion; (3) pannus formation; (4) osteomyelitis; (5) new bone formation and (6) overall bone loss.

A subjective rating score from 1 to 8 was assigned for each parameter. Thus, a normal joint would score 0 and the maximum score in an arthritic joint would be 48 (Ackerman *et al.*, 1979).

Drugs

The following agonist drugs were used: (±)-U-50,488H (kindly supplied by Dr M. Piercey, Upjohn, Kalamazoo, U.S.A.); (−)- and (+)-U-50,488H (Research Biochemicals, MA, USA). The peripherally selective antagonist, naloxone methiodide, was also obtained from Research Biochemicals (MA, U.S.A.), while the centrally acting κ-opioid antagonist, MR2266, was kindly donated by Boehringer, Germany. The agonist, U-50,488H, and antagonist, naloxone methiodide, were dissolved in sterile normal saline while MR2266 was dissolved in 0.1 M HCl and adjusted to pH 7 with 0.1 M NaOH.

Experimental protocols

Time course of opioid action The biphasic time-course of adjuvant arthritis in rats allows for testing the anti-arthritic effects of opioids during the different stages of the disease. Thus, either (±)-U-50,488H (40 mg $kg^{-1}day^{-1}$, $n=35$) or saline ($n=21$, 2 ml $kg^{-1}day^{-1}$), respectively, were administered subcutaneously twice daily, into a skin fold in the back of the neck, either during the primary inflammatory phase (1 to 3;

$n=21$) or once the disease was established (days 18 to 20; $n=19$). Disease parameters were assessed throughout the experiment and on day 21 as described above.

Dose-response relationships Firstly, the anti-arthritic effects of (±)-U-50,488H administered subcutaneously into a skin fold in the back of the neck, twice daily for the first three days (days 1 to 3), were examined over the dose-range: 0 mg $kg^{-1}day^{-1}$ ($n=21$), 10 mg $kg^{-1}day^{-1}$ ($n=7$); 20 mg $kg^{-1}day^{-1}$ ($n=17$); and 40 mg $kg^{-1}day^{-1}$ ($n=20$). Secondly, (±)-U-50,488H was administered by subcutaneous injection into the dorsum of the right (ipsilateral) hind foot near the ankle joint, twice daily for days 1 to 3, over the dose range 1 to 50 mg $kg^{-1}day^{-1}$ (50 μl per injection; $n=10$ rats per dose).

Stereospecificity and receptor specificity Two separate experiments were carried out. Firstly, to determine whether the anti-arthritic effects were stereospecific, equivalent doses of (±)-U-50,488H (40 mg $kg^{-1}day^{-1}$, $n=5$), (−)-U-50,488H (20 mg $kg^{-1}day^{-1}$, $n=5$) were given to rats (two groups) subcutaneously via a skin fold in the back of the neck. A pilot experiment found the effective dose for 50% effect of (±)-U-50,488H to be approximately 14 mg kg^{-1}, and therefore, a dose of 7 mg kg^{-1} of (+)-U-50,488H ($n=7$) was also evaluated. Arthritic controls ($n=10$) received saline while non-arthritic controls ($n=5$) received (−)-U-50,488H (20 mg $kg^{-1}day^{-1}$); this compound, but not (+)-U-50,488H, has been shown to have activity in anti-nociceptive tests (Rothman *et al.*, 1989).

Local anti-arthritic actions of U-50,488H were tested for by injecting the κ-opioid antagonist, MR2266 (50 μl to deliver 25 mg $kg^{-1}day^{-1}$, concomitantly with an equimolar dose of (±)-U-50,488H (50 μl to deliver 20 mg $kg^{-1}day^{-1}$, $n=11$), twice daily for 3 days, subcutaneously into the dorsum of the ipsilateral foot near the ankle joint. To evaluate whether the site of action for U-50,488H is local, the peripherally selective opioid antagonist, naloxone methiodide (NALM; 25 mg $kg^{-1}day^{-1}$), was administered simultaneously with an equimolar dose of (±)-U-50,488H (20 mg $kg^{-1}day^{-1}$, $n=11$) intracutaneously into the dorsum of the foot. Experiments in our laboratory have previously indicated that MR2266 alone had no effect on the progression of chronic arthritis ($n=10$; Walker *et al.*, 1995). Further, pilot experiments ($n=10$) found that naloxone methiodide administered subcutaneously at a dose of 25 mg kg^{-1} twice daily for 3 days alone to arthritic rats produced no effect on the progression of arthritis (data not shown).

Statistical analysis

Data are presented as mean ± standard error (s.e.) of the mean. Raw scores for both left and right paw volumes were normalised as % change from Day 0. The normalised paw swelling-time data were analysed using two factor (drug dose × time) repeated measures ANOVAs to calculate the average change from day 0 with respect to time. Paw volumes were then expressed as % of vehicle-treated arthritic control group (% change = (treated-control/control) × 100). Anti-inflammatory effect is represented by a negative change while a positive change indicates pro-inflammatory actions. The three indices of arthritic damage: contralateral paw swelling, radiography and histology were expressed as a percentage of vehicle treated arthritic rats (control), defined as 100%, and totalled to obtain a 'pooled severity index' (PSI). Animals were judged to be arthritic if this score increased two standard deviations above the mean of the non-arthritic control group.

Multiple comparisons between means were calculated using two factor repeated measures ANOVA. The factors (groups and days) were considered fixed. If a significant difference was found ($P<0.05$), *post-hoc* analysis was performed on planned comparisons using Fisher's LSD multiple comparison tests (NCSS, Utah, U.S.A.).

To obtain the ED_{50} for U-50,488H (the dose at half maximal effect), dose-response relationships were generated by fitting the percentage inhibition of PSI data to the nonlinear form of the Hill equation (Effect (PSI) = $E_{max} \times dose/ED_{50} + dose$) using nonlinear regression (PCNONLIN, Metzler *et al.*, 1974). The following parameters were obtained: the dose at half maximal effect, ED_{50} and the maximum possible response, E_{max}.

Results

In animals receiving complete Freund's adjuvant and no drug treatment the incidence of arthritis was 100%. Non-arthritic control animals injected with the incomplete adjuvant did not develop arthritis. All animals were well groomed throughout the progression of the disease. Arthritic rats were able to maintain their weight, albeit at a slower growth rate, compared to non-arthritic rats (Table 1).

Time course of opioid action

Treatment with (±)-U-50,488H, for 3 days during disease onset (1–3) and 3 days during established disease (18–20), significantly attenuated experimental arthritis as judged by a decrease in the paw volume, radiography and histology scores relative to control rats (Figure 1). Although there was no significant difference between each of the dosing-time paradigms for the paw volume measurements (disease onset vs established disease: 55 vs 64, $P>0.05$), the effects of U-50,488H on radiography and histology varied according to treatment time. For example, treatment during disease onset had a more marked effect on radiography (disease onset vs established disease: 37 vs 74, $P<0.05$). In contrast, U-50,488H administered during established disease produced lower histology scores (disease onset vs established disease: 68 vs 35, $P<0.05$). Thus, to prevent disease progression as judged by radiographic damage, subsequent studies utilised the three day, during disease onset, dosing regimen.

Dose response relationships of U-50,488H

Ipsilateral limb Distant administration of (±)-U-50,488H decreased the amount of swelling in the ipsilateral paw in a dose-dependent manner (Table 2). In contrast, local administration produced maximal anti-inflammatory effect in the ipsilateral limb at the lowest dose used in the present study; thus no dose-response relationship was evident over the range 1 to 50 mg $kg^{-1}day^{-1}$ (Table 2).

Contralateral limb On the basis that arthritis is a systemic disease, it is more important to assess the indices of arthritic damage in the contralateral paw (paw swelling, radiography and histology). The data for these three parameters were therefore combined to give the PSI. After distant administration, (±)-U-50,488H dose-dependently attenuated experimental arthritis (Figure 2). From these data, the ED_{50} for

Table 1 Effect of arthritis on body weight (% increase from day 0)

	Treatment	
Days post-adjuvant	*Vehicle*	*Freund's adjuvant*
0	0.0 ± 2.2	0.0 ± 0.7
3	6.2 ± 2.2*	−1.3 ± 0.7
7	17.6 ± 2.2*	4.4 ± 0.7
13	36.9 ± 2.2*	10.3 ± 0.7
17	42.2 ± 2.2*	4.0 ± 0.7
21	53.4 ± 2.2*	5.7 ± 0.7

*Significantly different, $P<0.05$.

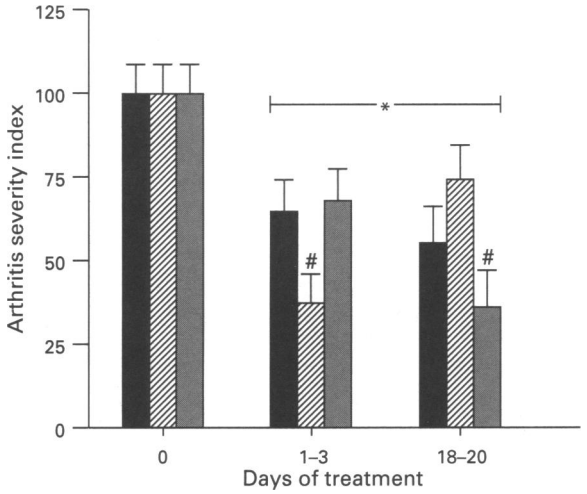

Figure 1 Effect of vehicle (0 days of treatment, $n=21$) or (±)-U-50,488H after dosing for either 3 days during the development of arthritis (1–3, $n=14$), or 3 days during established arthritis (18–21, $n=11$) on the arthritis severity indices. These are paw volume (solid columns), radiography (hatched columns) and histology (stippled columns) expressed as % of the values estimated in vehicle-treated control rats. *Denotes $P<0.05$ compared to vehicle-treated controls and # $P<0.05$ between treatment groups.

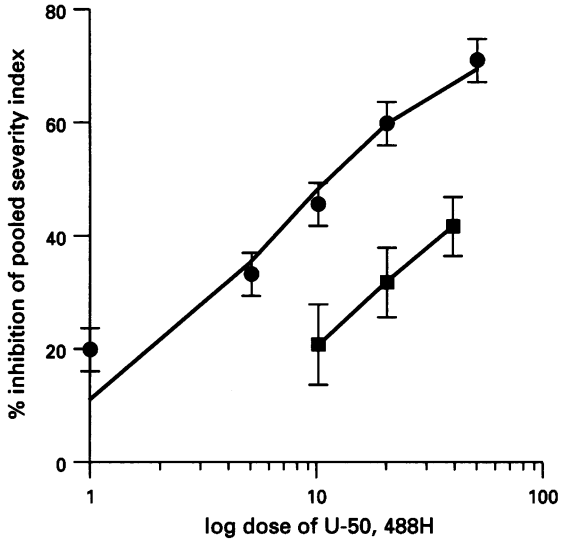

Figure 2 Effect of (±)-U-50,488H on % inhibition of pooled severity index after 10 to 50 mg kg^{-1}day^{-1} by subcutaneous bolus injection twice daily for 3 days (1 to 3) either distantly (■) or locally (●). The curves were generated by fitting the data to the E_{max} form of the Hill equation. The ED_{50}'s were 19.4±0.8 and 5.8±1.6 mg kg^{-1}day^{-1}, respectively ($n=7$ to 10 animals per dose).

Table 2 Effect of κ-agonist, U-50,488H, on the ipsilateral limb volume after either distant or local administration

Treatment/dose (mg kg^{-1} day^{-1})	*(±)-U-50,488H distant*[a]	*(±)-U-50,488H local*[b]
0	0.0±2.8 (22)	0.0±2.6 (10)
1		17.9±2.6* (10)
5		14.4±2.6* (10)
10	0 (7)	17.3±2.6* (10)
20	6.7±3.2* (17)	
40	17.8±2.5* (28)	
50		17.2±2.6* (10)

Values are mean±s.e. (number of rats is given in parentheses following value) % change from control; [a]distant subcutaneous administration into the back of the neck; [b]local administration into the ipsilateral paw; *significantly different from control ($P<0.05$).

distant administration was estimated to be 19.5±0.8 mg kg^{-1}day^{-1} with $E_{max}=63\pm1$ mg kg^{-1} ($r=0.99$). There was, however, a parallel shift in the dose-response relationship to the left when (±)-U-50,488H was administered directly to the inflamed ipsilateral paw ($ED_{50}=5.8\pm1.6$ mg kg^{-1}day^{-1} and $E_{max}=77\pm6$ mg kg^{-1}day^{-1}, $r=0.99$; Figure 2). It is noteworthy that the calculated maximal effect was comparable for both distant (63±1 mg kg^{-1}day^{-1}) and local administration (77±6 mg kg^{-1}day^{-1}). As judged by histology, in both the vehicle-treated group (Figure 3a) and distant-treated group ((±)-U-50,488H; 20 mg kg^{-1}day^{-1}; Figure 3d), there was severe periarticular inflammation, bone destruction with replacement fibrosis, joint space loss and organisation of intra-articular exudate (pannus). In contrast, local treatment with equal doses of (±)-U-50,488H (20 mg kg^{-1}day^{-1}) produced much less arthritic damage as shown by minimal joint space loss and very little pannus formation (Figure 3c), similar to a non-arthritic control (Figure 3b).

Stereospecificity and receptor selectivity

If we consider the two phases of arthritis, acute (days 1 to 7) and chronic (days 12 to 21), the different treatments produced contrasting effects on the ipsilateral limb swelling. Administration of the (−)-enantiomer of (±)-U-50,488H significantly attenuated oedema in the ipsilateral limb during the primary inflammatory phase of experimental arthritis while the (+)-enantiomer was inactive during this phase (Table 3). During the chronic phase, (−)-U-50,488H (20 mg kg^{-1}day^{-1}) markedly decreased swelling in the ipsilateral limb while the (+)-enantiomer (7 mg kg^{-1}day^{-1}) exacerbated arthritis in the ipsilateral limb (Table 3). However, (±)-U-50,488H (40 mg kg^{-1}day^{-1}) had no effect on the ipsilateral paw. In contrast, both (±)-U-50,488H and (−)-U-50,488H significantly decreased the PSI in the contralateral limb to the same extent (Figure 4) while the (+)-enantiomer (7 mg kg^{-1}day^{-1}) exacerbated arthritis in the contralateral limb (Figure 4).

Concomitant administration of both agonist, (±)-U-50,488H and either the peripheral antagonist, NALM, or the κ-selective antagonist, MR2266, into the ipsilateral limb, exacerbated the swelling in this limb during the acute phase. In contrast, during the chronic phase, (±)-U-50,488H attenuated oedema in the ipsilateral paw while NALM had no effect and MR2266 slightly exacerbated oedema (Table 3).

At equimolar doses, the peripherally selective antagonist, naloxone methiodide (25 mg kg^{-1}day^{-1}), was able to antagonize fully the anti-arthritic effects of 20 mg kg^{-1}day^{-1} (±)-U-50,488H (a dose equivalent to the ED_{50}) by local s.c. injection as judged by measurement of the PSI in the contralateral limb (Figure 5). Similarly, the κ-receptor antagonist, MR2266 (25 mg kg^{-1}day^{-1}) was able to antagonize fully the anti-arthritic effects of 20 mg kg^{-1}day^{-1} (±)-U-50,488H by local subcutaneously injection into the ipsilateral paw (Figure 5).

Discussion

In the present study, inflammatory arthritis occurred in 100% of animals treated with Freund's adjuvant. Arthritic rats maintained their weight and growth rate throughout the course of the experiment, albeit at a slower rate than non-arthritic rats. Animals experienced only moderate discomfort of the injected limb and remained well groomed. Based on these and our previous findings we propose that adjuvant arthritis is a reliable and ethically acceptable model (Walker & Kasmerski, 1988; Walker *et al.*, 1995).

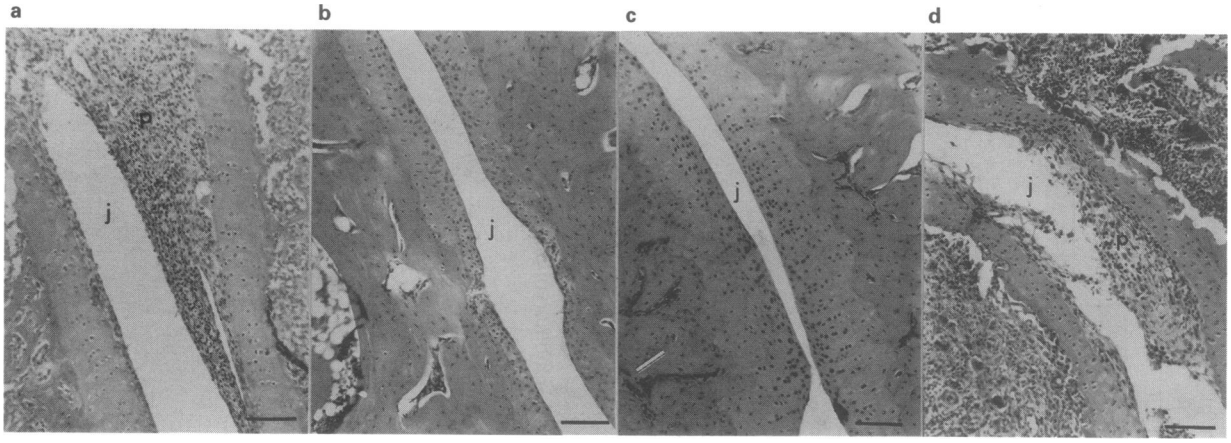

Figure 3 Histological sections from (a) a vehicle-treated arthritic rat with severe periarticular inflammation, bone destruction with replacement fibrosis, ankylosis and organisation of intra-articular exudate (pannus, p) and severe joint space loss (j); (b) a non-arthritic control rat, with a normal joint space; (c) a rat treated locally with (±)-U-50,488H, showing only mild joint space loss and (d) a rat treated systemically with (±)-U-50,488H, showing more severe joint space loss and pannus formation. Scale bar represents 100 μm.

Table 3 Stereospecificity and receptor selectivity effects of opioids on ipsilateral limb volumes during acute and polyarthritis

Treatment	*Acute-arthritis*	*Polyarthritis*
Stereospecificity[a]		
(±)-U-50,488H (40 mg kg^{-1} day^{-1})	−27 ± 1*	9 ± 1
(−)-U-50,488H (20 mg kg^{-1} day^{-1})	−34 ± 1*	−85 ± 10*
(+)-U-50,488H (7 mg kg^{-1} day^{-1})	−15 ± 3	37 ± 2*
Receptor selectivity[b]		
(±)-U-50,488H (20 mg kg^{-1} day^{-1})	84 ± 6*	−32 ± 2*
(±)-U-50,488H + NALM (25 mg kg^{-1} day^{-1})	96 ± 6*	0 ± 2
(±)-U-50,488H + MR2266 (25 mg kg^{-1} day^{-1})	92 ± 6*	25 ± 2*

Values are mean ± s.e., % change from control; [a] distant subcutaneous administration into the back of the neck; $n = 5$ to 7 per group and [b] local administration into the ipsilateral paw; $n = 11$ per group; *significantly different from control ($P < 0.05$). NALM = naloxone methiodide.

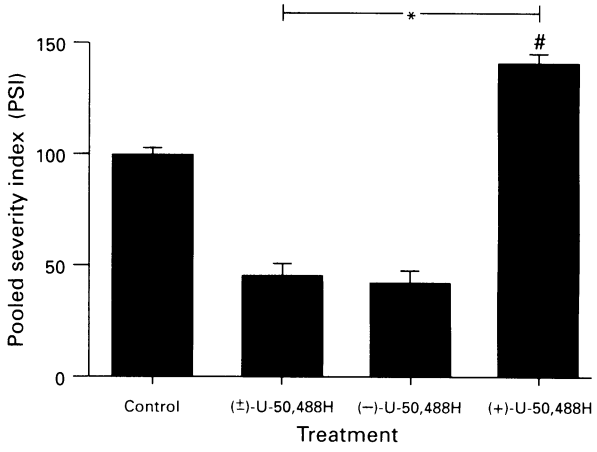

Figure 4 Anti-arthritic effect, as judged by the pooled severity index (PSI) of vehicle ($n = 5$), (±)-U-50,488H (40 mg kg^{-1}day^{-1}, $n = 5$) (−)-U-50,488H (20 mg kg^{-1}day^{-1}) or (+)-U-50,488H (7 mg kg^{-1}day^{-1}, $n = 5$) by subcutaneous injection into the back of the neck for 3 days during acute arthritis. * $P < 0.05$ compared to vehicle-treated controls; # $P < 0.05$ compared to (±) and (−)-U-50,488H.

The present study confirmed our previous findings that administration of the prototype κ-opioid agonist (±)-U-50,488H for three days during the onset of arthritis attenuated the severity of chronic arthritis in rats (Walker *et al.*, 1995), and the work of others reporting that (±)-U-50,488H inhibited inflammation in acute arthritis models (Russell *et al.*, 1985). However, we have now shown that the anti-inflammatory/anti-arthritic effects of (±)-U-50,488H are both dose-dependent and stereospecific. These data indicate that the anti-arthritic effects of (±)-U-50,488H are receptor-mediated. We also found that the anti-arthritic effects were time-dependent. Radiographic damage was lower if (±)-U-50,488H was administered during disease onset compared to established disease. This implies that the early prevention of bone damage can be sustained over almost three weeks; this would be a most advantageous effect if it could be replicated in patients. By contrast, there was less inflammation, as judged by histology, if U-50,488H was administered during established disease. The implication of this result is that the anti-inflammatory effects are more labile and that, after early drug administration, the disease process has largely reasserted itself by the end of the experimental period. In view of the fact that arthritis is a progressive disease and radiographic damage is observed early in its course, U-50,488H shows promise in halting early damage. Our data support current opinion that aggressive drug therapy needs to be started as soon as possible after disease onset to prevent progressive joint destruction (Pincus, 1994).

The unique finding of the present study is the remarkable peripheral anti-arthritic potency of (±)-U-50,488H. Arthritis severity was reduced by almost fourfold after direct peripheral administration to the site of inflammation compared to the same dose administered at a distant subcutaneous site. This indicates that the effect is local rather than systemic. Further, the anti-arthritic effects were antagonized by both a peripherally selective (NALM) and a κ-opioid selective antagonist (MR2266) suggesting the involvement of peripheral κ-opioid receptors. These findings indicate that exogenous κ-opioids can have potent anti-arthritic effects by acting at inflamed peripheral sites. Others have found that opioid anti-nociceptive effects require the presence of inflamed tissue (Stein, 1991). They have postulated that opioid agonists have easier access to neuronal opioid receptors during inflammation because inflammation disrupts the perineurium and because opioid receptors are upregulated in inflamed tissue (Antonijevic *et al.*, 1995).

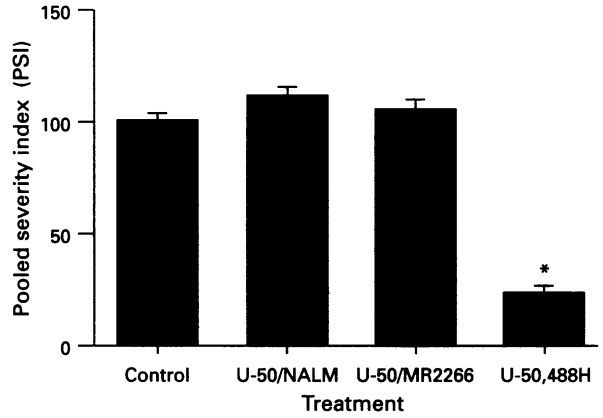

Figure 5 The effects of local injection of vehicle (control, $n=10$), U-50,488H (U50; 20 mg kg^{-1}day^{-1}, $n=10$) and concomitant administration of either naloxone methiodide (NALM; 25 mg kg^{-1}day^{-1}, $n=10$) or MR 2266 (25 mg kg^{-1}day^{-1}, $n=10$) and U-50,488H (20 mg kg^{-1}day^{-1}) on the pooled severity index (PSI).

Typical opioid receptors show a striking degree of stereospecificity for (−)-isomers of their specific ligands. For example, the (−)-isomer of U-50,488H is 100-fold more immunosuppressive than the (+)-isomer (Taub *et al.*, 1991). Further, the analgesic effects of (±)-U-50,488H reside with the (−)-enantiomer (Rothman *et al.*, 1989). We found that, compared to the racemate, administration of half the dose of the (−)-enantiomer produced a similar attenuation of arthritis. Our results are consistent with the view that the (−)-enantiomer is also the most potent anti-arthritic agent. In contrast, we found that the (+)-enantiomer, at a dose rate equivalent to the ED_{50} of the racemate (7 mg kg^{-1}day^{-1}), exacerbated polyarthritis while others have reported it to be inactive in nociceptive tests (Rothman *et al.*, 1989; Taub *et al.*, 1991). Therefore, the (−)-enantiomer has the greater therapeutic potential. Taken together with the fact that the effects of the κ-agonist are dose-dependent, the present findings suggest that U-50,488H mediates its anti-arthritic effects through κ-opioid receptors.

Our results are at variance with those of others who have postulated a centrally mediated site of action after morphine administration to arthritic rats. Morphine, administered intracerebroventricularly by continuous infusion (0.9 mg kg^{-1}day^{-1}) to block descending nociceptive circuits, was found to attenuate the severity of adjuvant arthritis in the opposite limb as judged radiographically. It was therefore concluded that opiate drugs can attenuate neurogenic inflammation via blockade of descending neural circuits (Levine *et al.*, 1985b). However, continuous infusion of morphine for two days could also produce systemic effects.

Our finding that an opioid agonist reduces the severity of arthritis raises interesting questions about the mechanisms and inflammatory mediators involved. An important conceptual advance of recent years has been the recognition that sensory nerve terminals can release inflammatory mediators (perhaps through antidromic impulses) and show chemosensitivity to such mediators in their immediate environment (Moskowitz *et al.*, 1983). Peripheral nerve endings and immune cells (e.g. macrophages and mast cells) are in close spatial and functional association in the joint (Leon *et al.*, 1994). Both neural and immune cells could thus be involved in inflammation. First, opioids may be acting to inhibit the release of neurotransmitters from either central or peripheral endings of primary afferents (Lembeck & Donnerer, 1985; Yaksh, 1988). The relatively few studies that have investigated the effects of the nervous system on arthritis are however controversial. Some have reported that depletion of neuropeptides with capsaicin or peripheral nerve section reduces joint swelling and arthritic damage in experimental arthritis (Colpaert *et al.*, 1983; Levine *et al.*, 1987; Cruwys *et al.*, 1995; Donaldson *et al.*, 1995) while others have reported no effects (Ahmed *et al.*, 1995a,b). Opioid receptors are found not only on joint afferents but also on immune cells such as lymphocytes, macrophages and mast cells (Stein *et al.*, 1990). Thus, alternatively, opioids might be exerting their anti-arthritic effects directly by occupying opioid receptors on immune cells and thereby preventing the release and/or synthesis of inflammatory mediators such as cytokines. Further, the immune and nervous system are inter-related and are both likely to be responsible for the anti-arthritic effects of opioids (Levine *et al.*, 1985a; Stein *et al.*, 1990; Czlonkowski *et al.*, 1993; Stein, 1995). For example, cytokines can induce the release of inflammatory peptides, like substance P, from peripheral nerve terminals and in turn substance P degranulates mast cells and stimulates macrophages to produce more pro-inflammatory cytokines (for review see Watkins *et al.*, 1995). Future studies will elucidate the contributions of these individual systems to the mechanisms responsible for the anti-arthritic effects of κ-opioids.

In summary, we have confirmed our previous findings that the κ-opioid agonist, (±)-U-50,488H is able to attenuate the severity of experimental arthritis in rats. In the present study, the anti-arthritic effects of U-50,488H were found to be dose-dependent and stereospecific and reversible by selective antagonists. These findings indicate that the effects are receptor-mediated. More importantly, we found U-50,488H to have approximately fourfold higher potency if administered locally into the inflamed paw, possibly due to increased access to receptors and/or receptor upregulation. Sites of action in the periphery may include the terminals of sensory neurones or receptors on the surface of immune cells. In the light of our findings with (±)-U-50,488H, peripheral administration of opioids show promise as an effective treatment for arthritis, which avoids tolerance and other compromising central side effects associated with prolonged administration. Selective κ-opioid agonists that can be given orally, and do not cross the blood brain barrier, would also simultaneously eliminate the need for multiple joint injections and avoid CNS toxicity. Our results may have important clinical applications in the treatment of rheumatoid arthritis sufferers.

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