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Effect of the peripherally selective κ-opioid agonist, asimadoline, on adjuvant arthritis

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1 Opioids, though widely used as analgesics, have not been seriously considered as therapy for rheumatoid arthritis. The present study evaluated the dose-effect and time-dependence relationships of a new peripherally selective κ agonist, asimadoline, in rats with adjuvant arthritis.

2 The arthritis was assessed by a pooled severity index combining the comprehensive criteria of oedema, radiography and histological changes, in the hind limbs. Asimadoline was extremely effective in attenuating joint damage (by up to 80%) when administered parenterally (0.5 to 10 mg kg^{-1} day^{-1}, i.p.) throughout the disease or during its early phase; treatment was less successful if confined to the latter stages. Ten fold higher doses were effective orally.

3 Equimolar doses of a peripherally-selective antagonist, naloxone methiodide, and the κ-selective antagonist, MR2266, fully reversed the peripheral anti-arthritic effects of asimadoline (5 mg kg^{-1} day^{-1}), indicating that asimadoline acts through peripheral κ-opioid receptors. However, an equivalent dose of MR2266 did not fully reverse the anti-arthritic effects of the highest dose of asimadoline (40 mg kg^{-1} day^{-1}), suggesting a loss of κ-selectivity at this dose.

4 Asimadoline also exhibited analgesic effects (mechanical nociceptive thresholds) in arthritic but not non-arthritic rats, indicating that inflammation is necessary for asimadoline-induced analgesia.

5 These data confirm our previous findings that κ-opioids possess anti-arthritic properties and that these effects are mediated via peripheral κ-receptors. The present results are new in showing that the peripherally acting κ-opioid agonist, asimadoline, is a potent anti-arthritic agent. Such novel drugs, essentially lacking central side effects, herald new treatments for rheumatoid arthritis.

Keywords: Asimadoline; adjuvant arthritis; naloxone methiodide; MR2266; κ agonist; κ antagonist; peripheral effects; inflammation; analgesia; motor activity

Introduction

We and others have found that opioids influence the inflammatory processes of experimental adjuvant arthritis in rats. A property which, together with their analgesic actions, suggests a potential therapeutic role in this disease (for review see Walker *et al*., 1997). For example, our group showed that morphine (albeit in high doses) attenuates the inflammation and progress of this disease when administered systemically (Walker *et al*., 1996). Such high doses would preclude clinical use of opioids in arthritis so we gave specific attention to κ-agonists, partly because of the analgesic action of dynorphins and partly because their agonist-receptor relations are better understood. In addition, opioids acting at κ-receptors produce little or no constipation and exhibit dysphoric rather than eurphoric effects which limit their physical-dependence liability (Horwell, 1988; Wollemann *et al*., 1992). Thus, κ-opioid agonists offer advantages in comparison to μ-opioids and have been pursued as analgesic agents. We found the specific κ-agonist U50488H to be anti-inflammatory (Wilson *et al*., 1996). It attenuated adjuvant arthritis in a dose-dependent, stereo-selective, antagonist-reversible manner (using the comprehensive and quantitative criteria of paw swelling, radiography and histology) suggesting that it produces its anti-inflammatory effects by interacting with specific opioid receptors.

Historically, opioids have been thought to produce their antinociceptive effects exclusively on the central nervous system. However, more than a century ago, Wood demonstrated that morphine elicited analgesic effects when applied locally to 'painful areas' in the periphery (Wood, 1885). This finding has now been confirmed in clinical studies in which intra-articular morphine produced pain relief without systemic effects following knee arthroscopy (Stein, 1991). We have found that the κ-opioid agonist, PNU50488H, has analogous peripheral anti-inflammatory effects. When it is administered directly into an inflamed paw, at a dose that produces no systemic effects, it has a potent anti-inflammatory action; to achieve the same result with systemic administration requires at least four times the dosage, with the concomitant risk of systemic effects (Wilson *et al*., 1996).

In rheumatoid arthritis, a potentially successful strategy to reduce the adverse side effects of opioids, is to restrict the access of these compounds to the central nervous system (CNS). Selective opioid agonists that can be absorbed through the gut, but do not cross the blood brain barrier, would simultaneously eliminate the need for multiple injections and avoid CNS toxicity. This selectivity can, in principle, be achieved by changing the chemical structure of these drugs, but early attempts to reduce their access to the brain by increasing hydrophilicity, as in quaternary ammonium analogues, also decreased their potency (Barber & Gottschlich, 1992). Despite this initial failure, a potent κ-agonist, asimadoline, that includes a hydrophilic and hydrophobic portion in the molecule, has now been developed for review see (Barber & Gottschlich, 1997). It has very limited ability to cross the blood brain barrier and inhibits the plasma extravasation that is evoked by electrical stimulation of the saphenous nerve, an anti-inflammatory action which is reversible by administration of the κ-antagonist, nor-binathorstimm (nor-BNI), directly into the inflamed site at doses insufficient to inhibit systemic

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opioid effects (Barber *et al*., 1994). In addition, it exhibits antihyperalgesic activity in animal models of inflammatory pain at doses which do not cause signs of central action (Gottschlich *et al*., 1995).

The aim of this study was to evaluate the dose-effect relationship of the peripherally selective κ-opioid agonist, asimadoline, and the time-dependence of these effects, in rats with developing adjuvant arthritis. The participation of opioid receptors was established using the κ-opioid selective antagonist MR2266 as well as the peripherally selective opioid antagonist, naloxone methiodide.

Methods

Experimental animals

Adjuvant arthritis was induced in male Dark Agouti (DA) or Lewis rats (Animal Resource Centre, Perth, Australia) weighing approximately 200 g. The animals were housed in groups of 10 in large cages lined with cellulose bedding and shredded paper and were handled regularly for two weeks before the study to accustom them to the experimenters and the procedures. The rats were kept in a temperature-controlled room ($22 \pm 1^{\circ}C$) with a 12 h alternating light–dark cycle and were given rat chow (Gordon's Speciality Stockfeeds, Yandera, Australia) and water *ad libitum*. All experiments were approved by the Animal Care and Ethics Committee of the University of New South Wales, Australia. The same trained observer performed measurements throughout the study without knowing the treatment details of particular animals.

Induction of arthritis

To induce adjuvant arthritis, rats were anaesthetized with fentanyl (50 $\mu g\ kg^{-1}$, i.p., Hypnorm) and inoculated with 100 μl of Freund's complete adjuvant (10 mg ml^{-1}, heat-killed and dried *Mycobacterium butyricum* suspension in paraffin oil and mannide monooleate, Difco Laboratories, Detroit, Michigan, U.S.A.) intradermally into the base of the tail on day 1. Non-arthritic controls received similar injections of Freund's incomplete adjuvant (paraffin oil and mannide monooleate, Difco Laboratories, Detroit, Michigan, U.S.A.). Two groups of controls were included in each experiment: adjuvant-treated rats received intraperitoneal (i.p.) injections of saline 2 ml kg^{-1} day^{-1}, twice a day to serve as an arthritic control group, while non-arthritic control rats received either asimadoline or saline 2 ml kg^{-1} day^{-1}, twice a day by i.p. injection. Three indices of arthritic damage were used. Oedema was measured by plethysmometry in both the left and right paw on days 3, 6, 12, 18 and 21 post-adjuvant (plethysmometer from Ugo Basile, Comerio, Italy); quantitative radiographic (unfixed tissue) and histological examination of the left ankle assessed joint damage (detail below). Motor activity was monitored in two awake rats simultaneously using an activity meter (Varimex, Columbus Instruments, Ohio, U.S.A.). Rats were acclimatized to the equipment for 1 min and then measured for 1 min every 3 days between 10 h 00 min and 11 h 00 min. This protocol was adopted for pragmatic reasons. All measurements were necessarily done in daylight but the normally nocturnal rats showed reasonable levels of activity probably because it was measured last in the sequence; furthermore, they were observed in pairs to avoid the possibility that they might show less activity if left alone.

Mechanical pain thresholds were obtained by using an analgesymeter (Ugo Basile, Comerio-Varese, Italy), every three days (sequence as above), 2 h after drug administration (11 h 00 min). This device applied linearly increasing pressure (16 g s^{-1}) to the hind paw. The stimulus was applied between the third and fourth metatarsals until the rat withdrew its paw; two sequential measurements, one minute apart, were made on each paw. Rats were trained on this equipment for one week before the start of the experiment.

Assessment of arthritic damage

The disease progression was monitored from the induction of arthritis (day 1) until the disease peaked (typically this was at day 21) and the rats were killed (pentobarbitone, 60 mg, i.p.). All radiographs were performed on the left hind limb with 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 at a focus to film distance of 65 cm; the pictures were viewed at a magnification of 1.85. This work was done by a specialist clinical radiologist who was unaware of the treatment regime.

The following variables were evaluated: (I) Soft tissue swelling – increased width of the soft tissue shadows and alterations in the normal configuration indicating oedema, effusion, and possibly synovial thickening. (II) Erosion – destruction of bone architecture seen as increased radiolucency. (III) Osteoporosis – decreased density of the bone, recognised as increased radiolucency relative to uninvolved adjacent bone, and thinning of the cortex, particularly in the juxta-articular regions. (IV) Joint space reduction – narrowing of the joint spaces due to loss of cartilage. (V) Joint damage – disruption of the alignment and configuration of tarsal bones.

Each variable was evaluated as objectively and consistently as possible by a single observer using a strict rating scale: 0 indicated normality, 1 mildly, 2 moderately and 3 severely affected states. Thus the maximum obtainable score was 15.

Following radiography the specimens were skinned, placed in 10% formalin for 7–10 days and then decalcified in 30% formic acid for 5 days. The tissues were dehydrated and embedded in paraffin wax; 7 μm thick sections were cut on a rotary microtome, mounted on glass slides and stained with haematoxylin and eosin. The tissue blocks were oriented to allow longitudinal sections to be cut so as to show the dorsoventral faces of the tarsal, metatarsal and phalangeal joints, bones and soft tissue on each slide. Each section was quantitatively evaluated by a single observer (who was 'blind' to the treatment) using the following criteria: (I) periarticular inflammation i.e. density of inflammatory cells; (II) pannus formation i.e. degree of pannus intrusion into the joint space; (III) periosteal reaction i.e. extent of new bone formation.

Each variable was rated 0–8 (i.e. maximum obtainable score 24). This procedure was a simplified version of the methodology originally described by Ackerman and co-workers (1979).

Experimental protocols

Time course of drug action Asimadoline (5 mg kg^{-1} day^{-1}, $n = 10$ per group) or saline (2 ml kg^{-1} day^{-1}, $n = 10$), was administered to DA rats by i.p. injection twice daily (a) during disease onset (days 1 to 3), (b) once the disease was established (days 13–21) or (c) throughout the entire timecourse (days 1 to 21). Non-arthritic controls received asimadoline (5 mg kg^{-1} day^{-1}, $n = 5$) or saline (2 ml kg^{-1} day^{-1}, $n = 5$) by i.p. injection twice daily. In all cases, disease parameters were assessed every three days for 21 days as described above.

Dose-response relationships The anti-arthritic effects of asimadoline were examined as a function of dose. A pilot experiment using Lewis rats and a dose range of 0.1 to 40 mg kg^{-1} day^{-1} ($n=10$ per group) determined that the maximum effect was at 5 mg kg^{-1} day^{-1}, while a dose of 40 mg kg^{-1} day^{-1} was less effective. This enabled us to estimate the slope of the dose-response curve ($ED_{50} \sim 1.2$ mg kg^{-1} day^{-1}) and determine appropriate doses for a full dose-response experiment carried out in 80 DA rats. Thus, asimadoline ($n=10$ per group) was administered twice daily by i.p. injection during disease onset (days 1 to 3) using the following doses: 0, 0.5, 1, 1.5, 2, 5, 10 and 40 mg kg^{-1} day^{-1}.

Receptor specificity The κ-specificity of asimadoline was tested in arthritic rats using the κ-opioid antagonist, MR2266. Asimadoline was administered i.p. at two dose rates, 5 mg kg^{-1} day^{-1} and 40 mg kg^{-1} day^{-1} to DA rats ($n=10$ per group), with the antagonist MR2266 given concomitantly i.p. in three distinct dosages: (a) twice equimolar (agonist/antagonist: 5 mg/7.2 mg or 40 mg/58 mg), (b) equimolar (agonist/antagonist: 5 mg/3.6 mg; 40 mg/29 mg) and (c) half equimolar (agonist/antagonist: 5 ml/1.8 mg; 40 mg/14 mg). Arthritic controls ($n=10$) received vehicle 30% polyethylene glycol in sterile saline i.p. To determine if this antagonist had anti-arthritic properties of its own, MR2266 was administered to arthritic rats over the following dose range ($n=5$ per group): 0, 14, 29 and 58 mg kg^{-1} day^{-1}, these doses corresponded to those administered in the first part of this experiment (with use of asimadoline at 40 mg kg^{-1} day^{-1}).

To evaluate whether asimadoline was acting in the periphery via opioid receptors, this peripherally selective agonist was administered either alone or concomitantly with an equimolar dose of the peripherally selective opioid antagonist naloxone methiodide. Two doses of the agonist were used, 5 mg kg^{-1} day^{-1} and 40 mg kg^{-1} day^{-1} (for reasons of animal availability, Lewis rats were used for the first dose and DA rats for the higher dose, $n=10$ in each case; ED_{50} was identical in these strains. Naloxone methiodide was administered in doses of 6 mg kg^{-1} day^{-1} and 48 mg kg^{-1} day^{-1} ($n=10$ in each group). Arthritic controls ($n=10$) received sterile saline *in lieu* of drugs.

Oral activity The oral activity of asimadoline was assessed in Lewis rats at three dose rates, 10, 20 and 50 mg kg^{-1} day^{-1} ($n=10$ per group). The doses chosen correspond approximately to i.p. doses of 1, 2 and 5 mg kg^{-1} day^{-1} as asimadoline has an oral availability of 13% (Barber *et al*., 1994). The agonist was mixed in sweetened condensed milk ('No Frills', Sydney) and 0.4 ml administered by mouth, twice daily during the primary inflammatory phase, days 1 to 3. Arthritic controls received sweetened condensed milk alone ($n=10$).

Drugs

The following drugs were used: asimadoline (EMD 61753, donated by Dr Andrew Barber, Merck, KGaA, Darmstadt, Germany), naloxone methiodide (Research Biochemicals, MA, U.S.A.) and MR2266 ((−)-5,9alpha-diethyl-2-(3-furylmethyl)-2′-hydroxy-6,7-benzomorphian; donated by Boehringer KG, Ingelheim, Germany). For parental administration the asimadoline and naloxone were dissolved in 30% polyethylene glycol in sterile normal saline whilst MR2266 was dissolved in 0.1 M HCl and adjusted to pH 7 with 0.1 M NaOH; for oral administration, asimadoline was dissolved in sweetened condensed milk and administered with a syringe.

Generally opioid receptors exhibit a remarkable stereospecificity for the (**S**)-isomers of their specific ligands (Taub *et al*., 1991). Asimadoline contains two chiral centres in its molecular structure leading to the existence of four stereoisomers but only the (**S**,**S**) configuration shows high biological activity and affinity for κ opioid receptors (Gottschlich *et al*., 1994). For this reason only the pure (**S**,**S**) isomer was used here.

Data analysis

Measurements of paw volume, motor activity and nociception were made over the entire 21 day observation period and averaged (V_T); in each animal the changes in three values were normalized (V_N) relative to the measures obtained before any treatment (V_0) such that: $V_N = 100[(V_T - V_0)/V_0]$.

Estimates of drug treatment efficacy were made from the results of dividing the individual V_N values (in treated and untreated animals) by V_N for saline-treated animals, expressing each such result as a percentage (untreated animals being 100%) and, finally, pooling the accumulated results appropriately. The quantitative histological and radiographic data (day 21 values) were also normalized: the mean was calculated for the untreated animals then the individual values (drug-treated and saline-treated rats) were divided by that mean, the results being expressed as percentages. Finally, a pooled severity index (PSI) was calculated by adding these normalized paw volume, histological and radiographic results (the normative value in untreated arthritic animals being, therefore, 300). All data (except for time-course plots) are expressed in this way, as means and standard errors (s.e.mean).

The PSI was subjected to parameteric statistical analysis (one-way ANOVA) and multiple comparisons were performed by the Newman-Keuls test using the Number Cruncher Statistical System V6.06 (NCSS, Kaysville, Utah, U.S.A.). Dose-response relationships for asimadoline were analysed by fitting the PSI data (% reduction in PSI = E, as described) to the non-linear form of the Hill equation ($E = (E_{max} \times dose^S)/(ED_{50}{}^S + dose^S)$) using the non-linear regression programme PCNONLIN (Statistical Consultants Inc, Apex, NC, U.S.A.), where ED_{50} is the dose for half-maximal effect, S is the slope factor and E_{max} is the maximum possible response. A P value of 0.05 or less was deemed statistically significant.

Results

By the criterion of an increase in PSI of at least 30%, all rats treated with complete Freund's adjuvant (but no other drug) developed arthritis; none of the animals which received the incomplete adjuvant developed disease. All arthritic animals were well groomed and maintained their weight throughout the progress of the disease.

Timecourse of drug action

Anti-inflammatory action Models of arthritis in rats allow investigation of drug effects at different stages of the disease (Walker *et al*., 1995). As shown in Figure 1, treatment with asimadoline (5 mg kg^{-1} day^{-1}) significantly attenuated experimental arthritis provided it was administered during the period of disease onset, i.e. in animals treated over the first three days or over the entire period (days 1–21). When the animals received this drug only over days 13–21, no significant change occurred in averaged paw volume and radiology measurements though improvement was observed in the

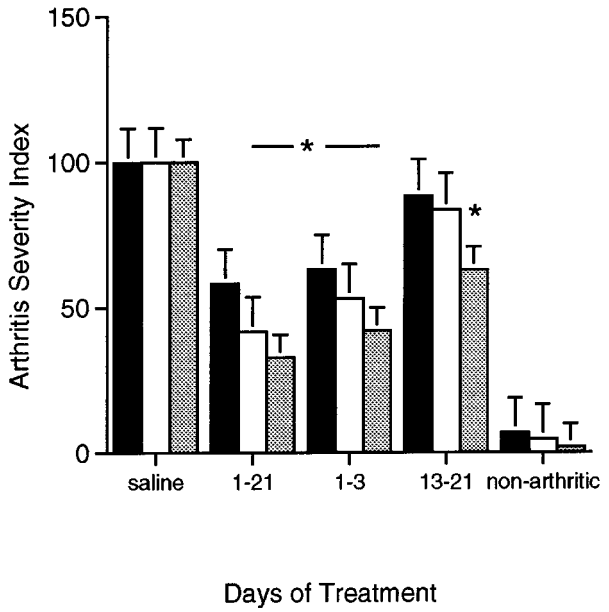

Figure 1 Effect of asimadoline or vehicle (saline) administration on indices of arthritis severity: paw volume (solid columns; in normal animals, the mean paw volume was 1.41 ± 0.004 while in untreated arthritic rats the time-averaged paw volume was 1.78 ± 0.06, i.e. there was a $26.8 \pm 2.3\%$ increase in paw volume induced by the disease); radiography (open columns; the score for normal animals was typically zero and for saline-treated arthritic rats was 6.4 ± 0.87); and histology (stippled columns; the score for normal animals is zero while for saline-treated arthritic rats in this experiment it was 14.6 ± 1.15); all as a function of time. Data are expressed as percentage of vehicle-treated control values (100%; see Methods). *Denotes a significant difference from vehicle-treated controls ($P < 0.05$).

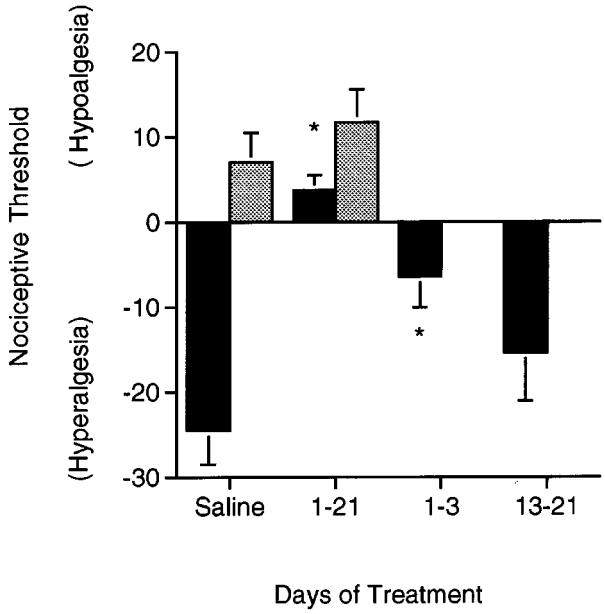

Figure 2 Nociceptive (mechanical) paw thresholds in either non-arthritic rats (stippled columns) or arthritic rats (solid columns), as a function of asimadoline treatment schedule. Time averaged data are expressed as percentages of pretreatment values (mean 69 ± 2 g). *Denotes a significant difference from saline-treated controls ($P < 0.05$).

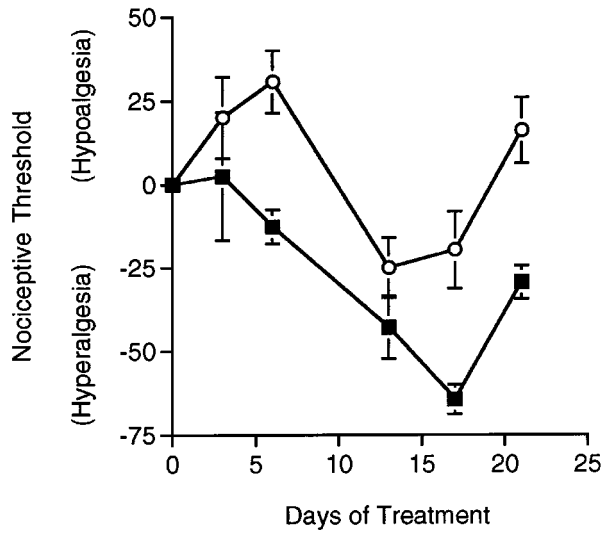

Figure 3 Time-course of changes in nociception (mechanical) paw thresholds in arthritic rats treated daily with either asimadoline (circles) or saline vehicle (squares). Note the hyperalgesia in both groups as the disease progressed. The differences in sensitivity between drug-treated and saline-treated animals were essentially consistent over the treatment period. Data are expressed as a percentage of pretreatment values (mean 69 ± 2 g).

histology assessment. For subsequent experiments, therefore, drugs were administered during disease onset (days 1–3).

Analgesic action (antinociception)

Non-arthritic rats Mechanical nociceptive thresholds were unchanged in non-arthritic rats which received saline over days 1–21, but there was a slight reduction in nociception (by 12%, $P < 0.05$) in the animals which received asimadoline (5 mg kg^{-1} day^{-1}) over this period (Figure 2).

Arthritic rats These animals (when treated with saline vehicle over days 1–21) showed a marked overall hyperalgesia in comparison with non-arthritic rats: the thresholds for their mechanosensitive responses were reduced by a mean of 24% (Figure 2). Treatment with asimadoline during the primary inflammatory phase or throughout the entire timecourse of the disease completely abolished this hyperalgesia (Figure 2). When the asimadoline treatment was confined to the period of established disease (days 13–21) the hyperalgesia persisted; this result is consistent with the data of Figure 1.

This hyperalgesia increased progressively over the course of the disease, until day 17 when the disease peaked (Figure 3); compared with saline, the effect of the drug was essentially constant over the treatment period.

Motor activity

Non-arthritic rats Non-arthritic rats treated with asimadoline (days 1 to 21) showed a marked decrease in motor activity, measured every three days and averaged over the 21 days of the experiment, in comparison to saline-treated controls (Figure 4; difference in normalized scores = 21, $P < 0.05$). Note that in Figure 4, the results are changes from normal (i.e. from pretreatment values) and upgoing columns indicate decreased activity.

Arthritic rats Arthritic rats showed a decrease in time-averaged motor activity when treated with vehicle alone (days 1–21; Figure 4). When the drug was given only for the first

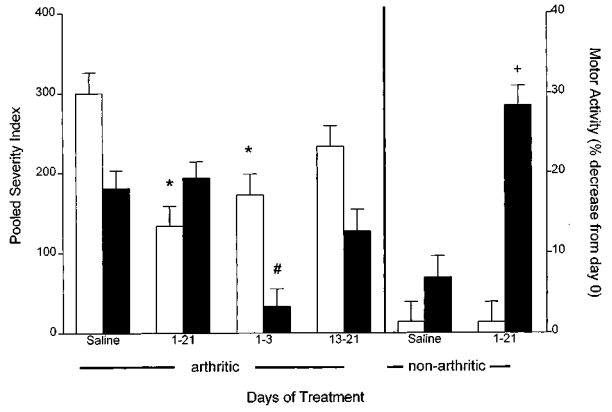

Figure 4 Effect of asimadoline treatment on motor activity (solid columns) and pooled severity index (open columns) as a function of treatment schedule. Motor activity is expressed as a % decrease from pretreatment values (42 ± 0.7 counts min^{-1} on day 0). *Denotes a significant difference in PSI from arthritic saline-treated controls; #denotes a significant difference in motor activity from arthritic saline-treated controls; +denotes a significant difference in motor activity from non-arthritic saline-treated controls ($P < 0.05$).

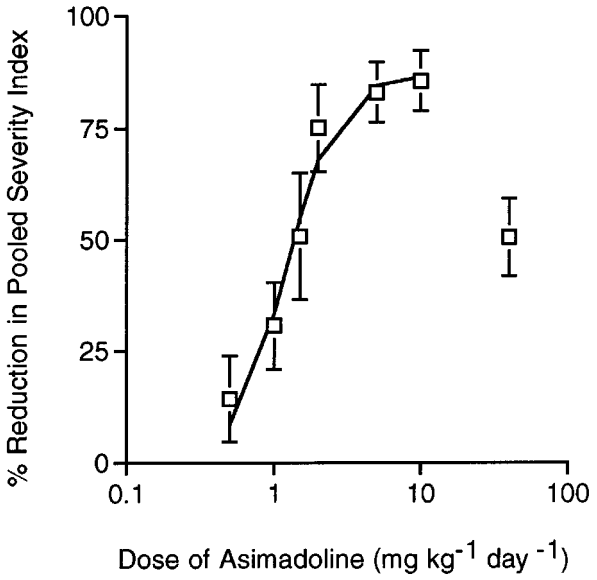

Figure 5 Effect of asimadoline administered over days 1 to 3 (during disease onset) on % reduction of pooled severity index (PSI) plotted as a function of dose (range: 0.5 to 40 mg kg^{-1} day^{-1}). The curve was generated by fitting the data (0.5–10 mg kg^{-1} day^{-1}) to the E_{max} form of the Hill equation with slope factor 2.5 ± 0.51 ($r = 0.992$; $n = 70$); ED_{50}: 1.3 ± 0.1 mg kg^{-1} day^{-1}.

three days the disease-induced reduction in activity was abolished but, paradoxically, this reduced activity persisted during the regimes of extended drug treatment (days 1–21 and 13–21; Figure 4).

Dose-response relationships

In order to quantify the dose-response relationship, asimadoline was administered in a dose range of 0.5 to 40 mg kg^{-1} day^{-1}. As judged by PSI, it dose-dependently attenuated experimental arthritis up to 10 mg kg^{-1} day^{-1} where a plateau was reached. The ED_{50} was estimated to be 1.3 ± 0.1 mg kg^{-1} day^{-1} (the same value as obtained in the pilot experiment – 1.2 ± 0.9 mg kg^{-1} day^{-1}) and E_{max} was $86.9 \pm 4.3\%$; the slope factor was 2.5 ± 0.5 ($r = 0.992$, $n = 70$; Figure 4). The highest dose (40 mg kg^{-1} day^{-1}) attenuated the experimental arthritis by 50%, but was less effective than the 5 and 10 mg kg^{-1} day^{-1} doses (Figure 5).

Receptor specificity

Effect of the κ opioid antagonist MR2266 Specific opioid antagonists were used, firstly to determine if the effects of asimadoline were opioid receptor-mediated and secondly, to determine whether the decrease in effectiveness observed at the 40 mg kg^{-1} day^{-1} dose could be due to confounding results from actions at other classes of receptors. Figure 6 shows the effect of the selective κ-opioid antagonist MR2266 when administered concomitantly with asimadoline (5 and 40 mg kg^{-1} day^{-1}). The action of asimadoline (5 mg kg^{-1} day^{-1}) was fully reversed by an equimolar dose of MR2266 (Figure 6a); a dose of the antagonist of half-molar equivalence to asimadoline was less effective and so, paradoxically, was a dose of twice molar equivalence (Figure 6a). As before (see Figure 5) the high dose of asimadoline (40 mg kg^{-1} day^{-1}) was distinctly less effective in ameliorating disease severity over the 21 days of observation and treatment; the severity index was reduced by only 43% (Figure 6b). However, this result was completely unaffected by half-molar or equimolar dosage with MR2266 while, remarkably, co-treatment with a twice-molar dosage resulted in a reduction of the severity index essentially to zero (Figure 6b).

To evaluate possible actions of MR2266 itself in this disease model, a dose-response study with the treatment levels used in the experiment of Figure 6b was performed. At a dose of 14 mg kg^{-1} day^{-1} but not at higher amounts (29 and 58 mg kg^{-1} day^{-1}), MR2266 alone exacerbated the arthritis severity (by 72%; Table 1).

Effect of peripherally-acting opioid antagonist naloxone methiodide In order to examine whether the opioid action of asimadoline was peripheral, it was co-administered with naloxone methiodide, a peripherally selective opioid antagonist. The results are summarized in Table 2. Consistent with our previous findings with an opioid antagonist (Figure 6), it is clear that, at the lower dose, asimadoline acted entirely via peripherally opioid receptors (the reduction of severity to 180 being entirely abolished). However, at the higher dose there was no suggestion of an opioid mechanism, i.e. the severity index was essentially unchanged by naloxone methiodide. On its own in equimolar (high) doses (48 mg kg^{-1} day^{-1}) naloxone methioide produced no change in the pooled severity index (Table 2; PSI saline *vs* naloxone methiodide: 303 ± 16 *vs* 292 ± 18).

Oral activity

Asimadoline when administered orally at doses of 20 and 50 mg kg^{-1} day^{-1}, but not at 10 mg kg^{-1} day^{-1}, was significantly able to attenuate adjuvant arthritis in comparison to untreated controls (Figure 7). Moreover, the two highest doses were equally effective and attenuated the disease by approximately 34%.

Discussion

Administration of asimadoline at various stages of the disease showed that early and continuous treatment were equally

effective in attenuating arthritis, whereas delayed treatment was ineffective, as judged by the PSI. When administered during disease onset, asimadoline produced a marked attenuation in the adjuvant arthritis, which was sustained well beyond the administration of the drug. This persistence of the therapeutic benefit in rats endorses the argument that early diagnosis and treatment of inflammatory joint are of great importance in patients (Pincus, 1994). The consistency of the three indices of disease, as with previous work (Walker *et al*., 1995; 1996; Wilson *et al*., 1996), supports the use of a pooled index of severity in this experimental disease, a procedure which mirrors clinical practice (Boers & Tugwell, 1993).

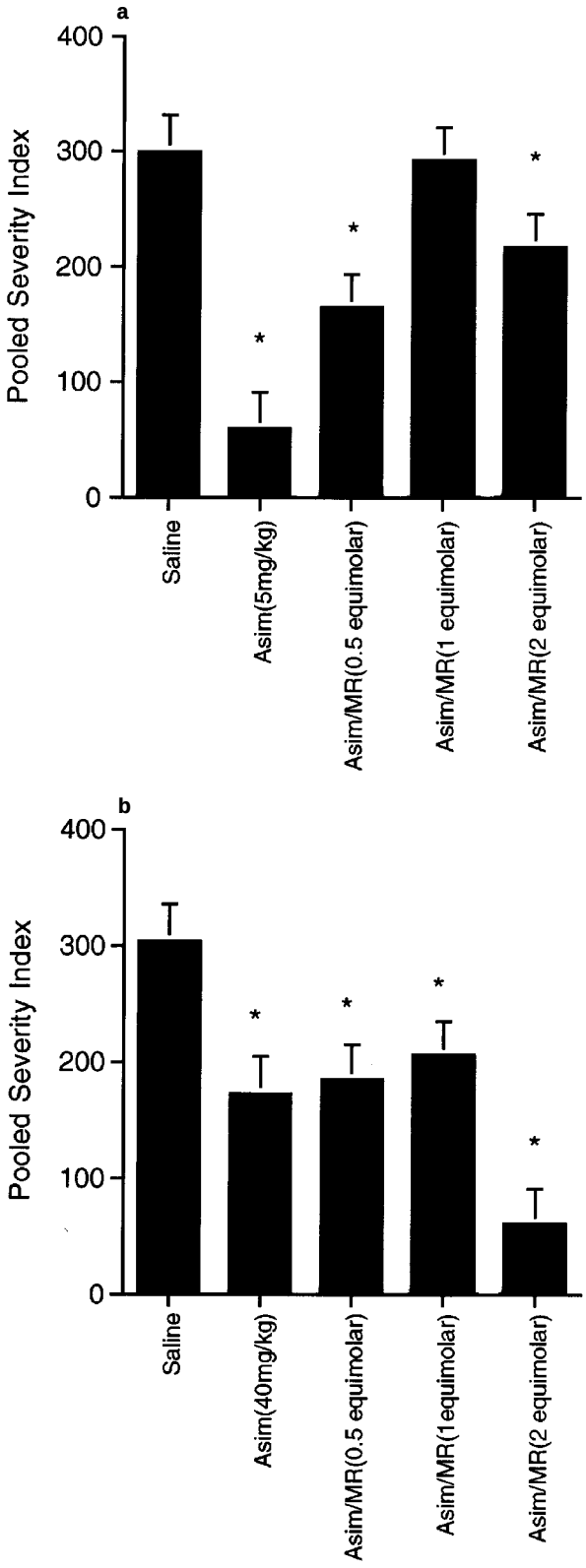

Figure 6 Effect of asimadoline on adjuvant arthritis pooled severity index (day 21 data) in animals treated with dosages of (a) 5 mg kg^{-1} day^{-1} and (b) 40 mg kg^{-1} day^{-1} with concomitant administration of MR2266 (MR) days 1 to 3. See text for details of the treatment regimens. *Denotes a significant difference from saline-treated controls, $P<0.05$.

Table 1 Effect of MR2266 on pooled severity index (PSI) as a function of dose

Dose (mg kg^{-1} day^{-1})	*PSI*[a]
0	300±70
14	517±70*
29	196±77
58	325±74

[a]Mean±s.e.mean. *Denotes significance from vehicle-treated controls, $P<0.05$.

Table 2 Effect of naloxone methiodide on action of asimadoline

Treatment	*PSI* (normalized)
Saline	301±26
ASIMD (5 mg kg^{-1} day^{-1})	187±29*
ASIMD and naloxone methiodide†	286±39
Saline	303±16
ASIMD (5 mg kg^{-1} day^{-1})	162±21*
ASIMD and naloxone methiodide†	213±33*
Naloxone methiodide (48 mg kg^{-1} day^{-1})	292±18

ASIMD = asimadoline; †equimolar doses; *significant difference from control (saline-treated) animals.

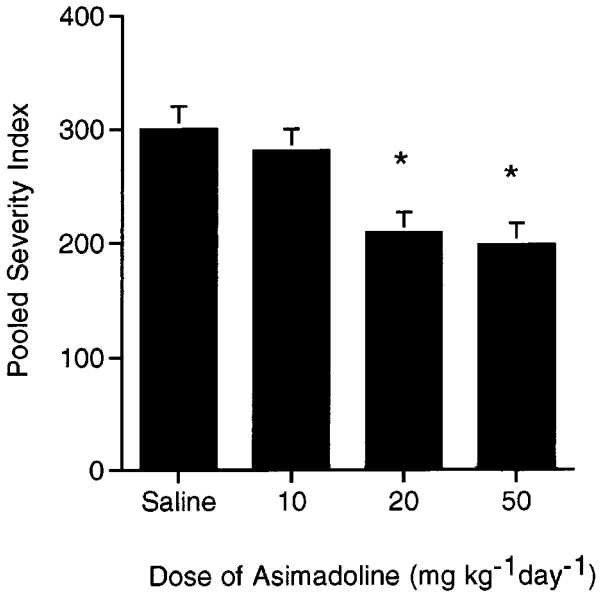

Figure 7 Effect of oral asimadoline, administered over days 1 to 3, on pooled severity index as a function of dose (in the range 10 to 50 mg kg^{-1} day^{-1}). *Denotes significant difference from saline-treated controls ($P<0.05$).

Furthermore, this attenuation by asimadoline was dose-dependent and was virtually complete at doses of 5–10 mg kg^{-1} day^{-1} (Figures 5 and 6a). The rising phase of the dose-response plot was very steep (as indicated by the slope factor of 2.5) and the ED_{50} was found to be 1.3 ± 0.1 mg kg^{-1} day^{-1} indicative of high potency compared with other opioid agonists previously used by us: U50488H (ED_{50} 19.4 ± 0.8 mg kg^{-1} day^{-1}, Wilson *et al*., 1996) and morphine (ED_{50} 58 ± 2 mg kg^{-1} day^{-1}, Walker *et al*., 1996).

If the drug were to be used clinically, oral dosage would be ideal; our results show that asimadoline is orally active (Figure 7) but that higher dosage is essential. We used an oral dosage 10 times the parenteral one but to determine whether the lesser effect which we observed indicates that even higher oral dosage was necessary or simply experimental variation would require further experiments. Lower oral activity is typical of opiates and Barber *et al*. (1994) showed the bioavailability of asimadoline to be only 13%.

Intriguingly, a bell-shaped dose-response curve was observed with asimadoline; the highest dose of 40 mg kg^{-1} day^{-1} produced only 50% attenuation. This raised the question of whether such a high dose involved non-opioid actions which possibly aggravate the disease. This theory was investigated with two opioid antagonists, the peripherally-selective naloxone methoidide and the κ-antagonist, MR2266 (Table 2; Figure 6). Whereas the attenuation achieved with the lower dose of asimadoline was completely abolished by equimolar doses of three antagonists, they had no effect on the action of the higher dose (40 mg kg^{-1} day^{-1}) of asimadoline. Such results argue cogently for a loss of κ-opioid selectivity at this high dose, which, in any case, is not absolute (Barber *et al*., 1994) being 1 : 125, $\kappa : \delta$. However, they also show that the actions of MR2266 are not straightforward either. At the highest dose (two molar equivalents), this putative antagonist, which alone had some paradoxical actions (Table 1) produced some attenuation of the disease severity itself, less marked with the smaller doses (Figure 6a; from 300 to 217). This result is consistent with the previous suggestion that MR2266 may have agonist properties (Walker *et al*., 1995). Clearly these issues require further investigation.

In addition to the anti-arthritic effects discussed here, asimadoline also exhibited effects on nociception. In Figure 3, in the early part of the plot from the drug-treated animals (open circles) there is a positive slope, i.e. the rats show hypoalgesia; interestingly, at this early stage the untreated animals already show hyperalgesia though they have not yet developed measurable oedema. Thereafter the two plots are essentially parallel, making it difficult to distinguish any possible analgesia from anti-inflammatory effects. However, others have found that asimadoline exhibited potent dose-dependent and naloxone-reversible paw pressure antinociception under hyperalgesic conditions, but was inactive or only weakly effective under normalgesic conditions (Barber *et al*., 1994); Russell *et al*. (1987) described opioid inhibition of fine afferents from inflamed joints. Similarly, Stein and co-workers have shown that opioids produce antinociception by acting at peripheral opioid receptors and that this antinociception is enhanced in inflamed tissue (Stein, 1991; 1995; Barber & Gottschlich, 1992; Stein *et al*., 1993). The most plausible explanation of this dichotomous behaviour is that without inflammation the nociceptors are, in the terminology of Schaible and Schmidt (1985) 'sleeping', in which case there would be no neural activity for the opioids to inhibit in normal tissue.

It might additionally be argued that the anti-arthritic effects of asimadoline were secondary to effects on motor activity, though we found no evidence for this. The motor activity of rats receiving asimadoline was dependent on the treatment regime. As Figure 4 shows, prolonged treatment (days 1–21) and early treatment (only days 1–3) both produced clear amelioration of the arthritis yet the motor behaviour was strikingly different in the two groups. Whether the change in motor behaviour is on account of drug or arthritis is not clear, though the drug has motor effects (a 30% reduction) in healthy rats. Furthermore, in the late-treated animals (days 13–21) there were no significant changes in disease severity or motor activity.

The precise mechanisms by which opioids act to reduce experimental arthritis remain unknown. Opioids and opioid peptides can certainly regulate the release of neurotransmitters such as acetylcholine, noradrenaline, dopamine and substance P (SP) in the central and peripheral nervous systems (for review, see Sarne *et al*., 1996). Furthermore, given the close spatial and functional association between nerve and immune cells (for review, see Walker *et al*., 1997), immune mechanisms are also likely to be involved in the anti-arthritic effects of opioids; in particular, opioid receptors are located on immune cells and have similar characteristics to those found on neurones (Carr *et al*., 1988; Bidlack *et al*., 1992). The cells of the immune system are also known to be affected by opioids: *in vivo* and *in vitro* exposure to morphine results in variable immunomodulatory actions such as increased production of the cytokine, interleukin-6, and suppression of T-lymphocyte function (Carr, 1991; Thomas *et al*., 1995). Because immunohyperactivity might be an important aspect of arthritis, opioid modulation of the immune system could therefore be an important component of the attenuation of adjuvant arthritis by these drugs. Any of these intricate mechanisms may be involved in the opioid attenuation of experimental arthritis. Experiments to understand more precisely which mechanisms are involved are currently underway in our laboratory.

In summary, therefore, we have found that the peripherally selective κ-opioid asimadoline is a very potent anti-arthritic agent especially when administered during onset of the disease. Its effects are dose-dependent, at least up to a daily dose of 10 mg kg^{-1} day^{-1} and, within this range, are reversible by specific opioid antagonists. It has the additional advantage, for possible clinical use, of being orally active. The current studies should lead not only to a better understanding of inflammatory joint disease but also offer the promise of improved therapy for human arthritis which is, universally, widespread and debilitating.

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