DiPOA ([8-(3,3-Diphenyl-propyl)-4-oxo-1-phenyl-1,3,8-triaza-spiro[4.5]dec-3-yl]-acetic Acid), a Novel, Systemically Available, and Peripherally Restricted Mu Opioid Agonist with Antihyperalgesic Activity: II. In Vivo Pharmacological Characterization in the Rat

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ABSTRACT
Mu opioid receptors are expressed throughout the central and peripheral nervous systems. Peripheral inflammation leads to an increase in mu receptor present on the peripheral terminals of primary sensory neurons. Activation of peripheral mu receptors produces potent antihyperalgesic effects in both humans and animals. Here, we describe the in vivo pharmacological properties of the structurally novel, highly potent, systemically available yet peripherally restricted mu opioid agonist, [8-(3,3-diphenyl-propyl)-4-oxo-1-phenyl-1,3,8-triaza-spiro[4.5]dec-3-yl]-acetic acid (DiPOA). DiPOA administered i.p. produced naltrexone-sensitive, dose-dependent reversal of Freund's complete adjuvant-induced inflammatory mechanical hyperalgesia (1–10 mg/kg). Maximum percent reversal (67%) was seen 1 h postadministration at 10 mg/kg (the highest dose studied). DiPOA also proved antihyperalgesic in a model of postsurgical pain with a maximum percent reversal of 85% 1 h postadministration at 30 mg/kg i.p. (the highest dose studied). DiPOA administered i.p. had no effect in the tail flick assay of acute pain (0.1–10 mg/kg), produced no ataxia as measured by latency to fall from an accelerating rotarod (3–30 mg/kg), and was not antihyperalgesic in the Seltzer model of neuropathic pain (1–10 mg/kg). This is the first report of a peripherally restricted, small-molecule mu opioid agonist that is nonsedating, antihyperalgesic, and effective against inflammatory and postsurgical pain when administered systemically.

To date, four members of the opioid receptor family have been cloned and characterized, and include the mu, kappa, delta, and opioid receptor-like 1 (ORL-1) receptors (Pleuvry, 2003). All are G-protein-coupled receptors and mediate inhibition of adenylate cyclase through activation of GTP-binding proteins. In addition, opioid receptor agonism results in the opening of receptor-operated potassium channels and suppression of voltage-gated calcium currents (Duggan and North, 1983). Several classes of endogenous peptidic ligands have been identified for the opioid receptors, including the enkephalins, dynorphins, endorphins, and nociceptin (Terenius, 2000). These ligands are distributed throughout the central and peripheral nervous system (CNS and PNS) as well as in peripheral tissues. Opioid receptors are also expressed throughout the CNS, and their activation results in potent analgesia via inhibition of ascending excitatory nociceptive transmissions and activation of descending inhibitory systems (Fields and Basbaum, 1999; Yaksh, 1999). Activation of CNS opioid receptors also results in diminished responsiveness of the brainstem respiratory centers to carbon dioxide (Gutstein and Akil, 2001) and stimulation of dopaminergic pathways, particularly the

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ABBREVIATIONS: ORL-1, opioid receptor-like 1; CNS, central nervous system; PNS, peripheral nervous system; DiPOA, [8-(3,3-diphenyl-propyl)-4-oxo-1-phenyl-1,3,8-triaza-spiro[4.5]dec-3-yl]-acetic acid; FCA, Freund's complete adjuvant; PWT, paw withdrawal threshold; PSN, partial sciatic nerve ligation; PLSD, protected least significant difference; DAMGO, [D-Ala²,MePhe⁴,Gly(ol)⁵]enkephalin; CCI, chronic constriction injury; SNL, spinal nerve ligation.
nucleus accumbens (Kieffer and Gaveriaus-Ruff, 2002); these effects lead to respiratory depression and dependence, respectively. It is important to note that for exogenous compounds to elicit these effects, penetration into the CNS is required. In situ hybridization and immunohistochemistry has localized opioid receptor mRNA and protein to the PNS, specifically to the cell bodies of primary afferent sensory neurons, located in the dorsal root ganglia (Wang and Wessendorf, 2001). Furthermore, the presence of functional opioid receptors has been demonstrated on both the peripheral terminals, located in the skin and end organs, and the presynaptic terminals, located in the spinal cord (Stein, 1993; Wenk et al., 2003).

Inflammation can play multiple roles in modulating peripheral opioid receptor function. Inflammatory conditions may sensitize peripheral opioid receptors. It has been demonstrated that increased cAMP and reduced pH, two events that occur at the site of inflammation, can increase the efficacy of opioid agonists (Selley et al., 1993) and enhance opioid-induced attenuation of neuronal excitability (Ingram and Williams, 1994). Immune cells, such as macrophages, that express opioid receptors and contain enkephalin and endorphin are commonly found at sites of inflammation (Mousa, 2003). Importantly, inflammatory mediators, such as interleukin-1, can stimulate the release of opioid peptides from macrophages, the result of which may be an endogenous attempt to attenuate pain and inflammation. Furthermore, inflammatory conditions increase the axonal transport of mu opioid receptors, resulting in increased receptor density at peripheral sites (Zöllner et al., 2003). Finally, inflammation can disrupt the perineurium, which results in a more efficient binding of opioids to opioid receptors (Antonijevic et al., 1995).

Indeed, local administration of exogenous opioids at the site of inflammation has shown therapeutic utility in animals and humans (for review, see Stein et al., 2003). In humans, intra-articular administration of morphine reverses the hyperalgesia associated with osteoarthritis (Stein et al., 1999) or resulting from arthroscopic knee surgery (Kalso et al., 1997). Preclinically, the inflammation and hyperalgesia caused by acute chemical injury to the rat cornea were reversed by direct application of morphine to the surface of the eye (Wenk et al., 2003). Additionally, local administration of the mu agonists loperamide, morphine, or fentanyl is antihyperalgesic in rodent models of inflammatory pain (Stein et al., 1988; Zhou et al., 1998; DeHaven-Hudkins et al., 2002). These data suggest that an exogenous, peripherally restricted mu opioid agonist when systemically administered would provide effective pain relief against hyperalgesia associated with inflammation without eliciting centrally mediated adverse effects.

In the accompanying manuscript (Valenzano et al., 2004), [8-(3,3-diphenyl-propyl)-4-oxo-1-phenyl-1,3,8-triaza-spiro[4.5]dec-3-yl]-acetic acid (DiPOA) is described as a high-affinity, high-efficacy, and systemically available mu receptor agonist (systemically available is defined as significant concentrations of compound in the plasma following systemic administration). Therefore, DiPOA provides us with the opportunity of testing the hypothesis that a systemically administered, peripherally restricted mu opioid agonist would be antihyperalgesic in rat pain models of inflammatory hyperalgesia without eliciting centrally mediated side effects. As such, we would expect DiPOA to be antihyperalgesic in the Freund's complete adjuvant (FCA) model of inflammatory pain without demonstrating analgesia in the rat tail flick assay of nociception or causing sedation as measured by the rotarod assay. Here, we describe the effects of DiPOA in models of acute, inflammatory, neuropathic, and incision-induced pain. We have compared the effects of DiPOA with those of the classic and clinically relevant opioid, morphine, in the acute and inflammatory models. Additionally, we have assessed the effects of the compound on motor function and investigated the ability of an opioid receptor antagonist to reverse the effects on inflammatory pain.

**Materials and Methods**

**Compounds and Administration Procedures.** The structure for DiPOA is disclosed in the accompanying manuscript (Valenzano et al., 2004), and the synthetic route for DiPOA (free base) has been recently disclosed in patent application WO 2003101953 (Victory and Chen, 2003). DiPOA was used in all experiments as it free base (molecular weight 483.6, K$_M$ at mu = 0.76 < 0.15 mM, solubility in 100 mM K$_2$HPO$_4$ at pH = 7.4 > 50 µM). DiPOA was administered i.p. in 25% β-cyclodextrin (Sigma-Aldrich, St. Louis, MO) dissolved in distilled water in a dose volume of 2 ml/kg (the dosage formulations were sonicated for 2.5 h prior to administration). The opioid agonist morphine (Sigma-Aldrich) was administered s.c. or i.p. in 0.9% saline in a dose volume of 2 ml/kg. The anticonvulsant gabapentin (Kemprotec, Middlesborough, UK) was administered i.p. in 0.9% saline in a dose volume of 2 ml/kg. The nonsteroidal anti-inflammatory drug indomethacin (Sigma-Aldrich) and the cyclooxygenase-2 inhibitor celecoxib (Toronto Research Chemicals, Toronto, Canada) were administered p.o. in 0.5% methyl-cellulose (Sigma-Aldrich) dissolved in distilled water. The mu antagonist naltrindole (Sigma-Aldrich) was administered i.p. in 0.9% saline in a dose volume of 2 ml/kg.

**Animals.** The Purdue Institutional Animal Care and Use Committee approved all animal procedures according to the guidelines of the Office of Laboratory Animal Welfare. Male Sprague-Dawley rats (Taconic Farms, Germantown, NY) weighing 180 to 200 g at the start of acute and inflammatory experiments or 90 to 110 g at the start of nerve ligation experiments were used. Animals were group housed and had free access to food and water at all times, except prior to oral administration of drugs when food was removed 12 h before dosing. For comparison with compound-treated groups, animals treated with appropriate drug vehicle were included in each experiment. The volume of administration and all other experimental procedures and conditions for vehicle and compound administration were identical.

**Acute Analgesia.** The effect of DiPOA on acute analgesia was investigated using the tail flick assay (D’Amour and Smith, 1941). Animals were placed on the apparatus (Ugo Basile, Comerio, Italy), and an infrared beam was focused onto the tail, 5 cm from the tip. Under conditions for vehicle and compound-treated rats were identical. The latency to tail flick was assessed. Cutoff was set at 30 s, and the intensity was set to 35%. Latency was determined once for each rat at each time point. Baseline latency was determined, and 1 h later the rats received a single dose of 0.1, 0.3, 1, 3, or 10 mg/kg DiPOA, 10 mg/kg morphine (the positive control), or vehicle (i.p. volume = 2 ml/kg). Latency to tail flick was again determined 1, 3, and 5 h postdrug administration.

**Inflammatory Hyperalgesia.** The efficacy of DiPOA against hyperalgesia associated with inflammation was investigated using the FCA model. For this assay, hind paw withdrawal thresholds (PWTs) to a noxious mechanical stimulus were determined using an analgesimeter (model 7200; Ugo Basile). Cutoff was set at 250 g, and the endpoint was taken as complete paw withdrawal. PWT was determined once for each rat at each time point. Baseline PWT was determined, and the rats were anesthetized with isoflurane (2% in oxygen) and received an intraplantar injection of 50% FCA (50 µl, diluted in saline) to the left hind paw. Twenty-four hours after FCA injection, predrug PWTs were measured, and the rats received a single dose of 1, 3, or 10 mg/kg DiPOA, 30 mg/kg morphine (the
positive control), or vehicle (i.p. volume = 2 ml/kg) (in addition, the effect of DiPOA (10 mg/kg, i.p.) was assessed on PWTs of animals that did not receive an intraplantar injection of FCA). PWT was again determined 1, 3, and 24 h postdrug administration. For naltrexone inhibition, rats received a single dose of 10 mg/kg naltrexone 10 min prior to 10 mg/kg DiPOA. Percent reversal of hyperalgesia for each rat was calculated according to the following equation:

\[
\text{%reversal} = \frac{\text{postdose threshold} - \text{predose threshold}}{\text{baseline threshold} - \text{predose threshold}} \times 100
\]

**Neuropathic Hyperalgesia.** The partial sciatic nerve ligation (PSN) model was used as a model of nerve injury-related pain in rats, as described previously by Seltzer et al. (1990). Baseline PWT was determined, and partial ligation of the left sciatic nerve was performed under isofluorane (2% in oxygen) inhalation anesthesia. After induction of anesthesia, the left thigh was shaved and prepared in a sterile manner. The sciatic nerve was exposed at high thigh level through small incision and was carefully cleared of surrounding connective tissue at a site near the trochanter just distal to the point at which the posterior biceps semitendinosus nerve branches off the common sciatic nerve. A 7-0 silk suture was inserted into the nerve with a three-eighths curved, reversed-cutting minineedle and tightly ligated so that the dorsal one-third to one-half the nerve thickness was held within the ligature and the wound was closed. Sham-operated control rats underwent an identical procedure on the left hind limb; however, the sciatic was not manipulated or ligated. After surgery, animals were weighed and allowed to recover before being returned to their home cages. Twenty-one days following nerve ligation, predrug PWTs were measured, and the rats received a single dose of 1, 3, or 10 mg/kg DiPOA, 100 mg/kg gabapentin (the positive control), or vehicle (i.p. volume = 2 ml/kg). PWT was again determined 1, 3, 5, and 24 h postdrug administration.

**Postsurgical Hyperalgesia.** The effect of DiPOA on postsurgical pain was assessed using an incisional pain model, as described previously by Brennan et al. (1996). Baseline PWT was determined, and the plantar surface of the left hind paw was prepared in a sterile manner. Under isofluorane (2% in oxygen) inhalation anesthesia, a 1-cm longitudinal incision was made with a number 10 scalpel, through skin and fascia of the plantar aspect of the paw starting 0.5 cm from the proximal edge of the heel and extending toward the toes. The plantaris muscle was elevated and incised longitudinally. Following hemostasis with gentle pressure, the skin was opposed with two single interrupted sutures using 5-0 nylon. The wound site was covered with povidone-iodine antibiotic powder (PRN Pharmacal, Pensacola, FL), and the animals were allowed to recover in their home cages. Unoperated rats served as controls. Twenty-four hours following plantar incision, predrug PWT were measured, and the rats received a single dose of 3, 10, or 30 mg/kg DiPOA, vehicle (i.p. volume = 2 ml/kg), or 30 mg/kg indomethacin (the positive control) orally (p.o. volume = 10 ml/kg). PWT was again determined 1, 3, 5, and 24 h post drug administration. Percent reversal of hyperalgesia for each rat was calculated according to the following equation:

\[
\text{%reversal} = \frac{\text{postdose threshold} - \text{predose threshold}}{\text{baseline threshold} - \text{predose threshold}} \times 100
\]

**Results**

**DiPOA Does Not Affect Acute Nociception.** Opioids that penetrate the blood-brain barrier, such as morphine, are analgesic in models of acute nociception. We tested the effects of DiPOA on acute nociception using the tail flick assay (D’Amour and Smith, 1941). DiPOA did not affect latency to tail flick at 1, 3, or 5 h following administration of doses up to 10 mg/kg, whereas morphine (10 mg/kg s.c.) produced a significant increase in latency to tail flick 1 h postadministration (p < 0.05; Fig. 1).

**DiPOA Reduces Mechanical Hyperalgesia Associated with Inflammation.** Intraplantar injection of 50 μl of FCA into the hind paw resulted in the development of mechanical hyperalgesia as indicated by a decreased PWT to a noxious mechanical stimulus (Fig. 2). Intraperitoneal administration of DiPOA produced a dose-dependent reduction in mechanical hyperalgesia 1 h postadministration (F<sub>5,49</sub> = 20.621; p < 0.0001; Fig. 2). DiPOA (1, 3, and 10 mg/kg i.p.) produced statistically significant antihyperalgesia 1 h following administration (p < 0.05). A dose-dependent trend was observed 3 and 5 h following administration; however, statistical significance was not reached. The maximum percent reversal (67%) was achieved 1 h following the 10 mg/kg dose. Sedation was not noted at any dose of DiPOA at any of the time points tested. Intraperitoneal administration of morphine (30 mg/kg) also produced a statistically significant reversal of hyperalgesia 1 and 3 h postadministration (Fig. 1).
2); however, sedation was noted in all animals treated with this dose. When administered to animals that did not receive an intraplantar injection of FCA, DiPOA (10 mg/kg i.p.) did not produce a statistically significant change in PWT 1, 3, 5, or 24 h following administration (data not shown).

**Naltrexone Inhibits the Antihyperalgesic Effect of DiPOA against Inflammatory Pain.** As described above, intraplantar injection of 50 μl of FCA resulted in the development of mechanical hyperalgesia as indicated by a decreased PWT to a noxious mechanical stimulus. Intraperitoneal administration of DiPOA (10 mg/kg) produced a statistically significant reduction in mechanical hyperalgesia 1 h postadministration (p < 0.05; Fig. 3), which was completely inhibited by intraperitoneal pretreatment with naltrexone (10 mg/kg); no statistical significance was observed as compared with vehicle-treated controls (p > 0.15; Fig. 3). Oral administration of celecoxib (30 mg/kg) also produced a statistically significant reversal of hyperalgesia 1 h postadministration (Fig. 3). When administered alone in this model, naltrexone (10 mg/kg) had no effect on PWT (Fig. 3).

**DiPOA Does Not Reduce Mechanical Hyperalgesia Associated with Nerve Injury.** Partial ligation of the sciatic nerve resulted in the development of mechanical hyperalgesia within 3 weeks of surgery. Intraperitoneal administration of DiPOA 21 days after partial ligation of the sciatic nerve did not result in statistically significant reversal of mechanical hyperalgesia (data not shown). Intraperitoneal administration of gabapentin (100 mg/kg) produced a statistically significant reversal of hyperalgesia 1 and 3 h postadministration.

**DiPOA Reduces Mechanical Hyperalgesia Associated with Surgical Incision.** Incision of the plantar surface of the hind paw resulted in the development of mechanical hyperalgesia as indicated by a decreased PWT to a noxious mechanical stimulus 24 h postincision (Fig. 4). Intraperitoneal administration of DiPOA produced a dose-dependent reduction in mechanical hyperalgesia 1 (F<sub>5,54</sub> = 23.131; p < 0.0001), 3 (F<sub>5,54</sub> = 14.907; p < 0.0001), and 5 (F<sub>5,54</sub> = 31.926; p < 0.0001) h postadministration. DiPOA (3, 10, and 30 mg/kg i.p.) produced statistically significant anti-hyperalgesia 1 and 3 h following administration (p < 0.05; Fig. 4). Thirty milligrams per kilogram produced significant antihyperalgesia 5 h postdosing (p < 0.05; Fig. 4). The maximum percent reversal (85%) was achieved 1 h following 30 mg/kg. Oral administration of indomethacin (30 mg/kg) also produced a statistically significant reversal of hyperalgesia 1 and 3 h postadministration.

**DiPOA Does Not Cause Ataxia or a Loss of Motor Coordination.** A common centrally mediated side effect of opioids is ataxia, which can confound the interpretation of behavioral assays. We tested rats for motor function using the rotarod assay. DiPOA did not affect rotarod performance at 0.5, 1, or 2 h following administration of doses up to 30 mg/kg, whereas morphine (10 mg/kg s.c.) produced a significant decrease in rotarod performance 0.5 and 1 h postadministration (p < 0.05; Fig. 5).

**Discussion**

A link between opioid receptors and pain is well established. The mu opioid receptor is present on neurons of both the CNS and PNS, in addition to inflammatory cells such as macrophages (for review, see Mousa, 2003). Systemic administration of mu opioid agonists that penetrate the blood-brain barrier, such as morphine, produces analgesia (Fields and Basbaum, 1999); however, this also causes unwanted and dose-limiting side effects such as sedation, respiratory depression, and dependence (Kieffer and Gaveriaux-Ruff, 2002). Activation of opioid receptors in the PNS, by local administration of morphine for example, has shown efficacy in the treatment of inflammatory pain. DiPOA dose-dependently reverses inflammatory mechanical hyperalgesia. Rats received an intraplantar injection of 50 μl of saline (open squares) or 50% FCA (all other groups) into the hind paw, followed by intraperitoneal administration of DiPOA, morphine, or vehicle 24 h later. Asterisks denote significance (p < 0.05) from FCA/vehicle-treated group according to Fisher’s PLSD post hoc test. B, baseline latency; Pre, pre-drug reading. Data shown are mean ± S.E.M.; error bars represent S.E.M. (n = 8–10 rats/group).
in both animals and humans following an inflammatory insult (for review, see Stein et al., 2003). We hypothesized that an exogenous, systemically available, and peripherally restricted opioid agonist would provide effective pain relief in an inflammatory setting without centrally mediated adverse effects; the accompanying paper (Valenzano et al., 2004) describes such a molecule. Here, we report that systemic administration of DiPOA produced significant and naltrexone-sensitive reversal of mechanical hyperalgesia associated with FCA-induced inflammation. In addition, DiPOA was also effective against hyperalgesia induced by plantar incision, yet had no effect in the partial ligation model of neuropathic pain at the doses tested. These effects were observed in the absence of ataxia or effects on acute nociception, two outcomes commonly associated with opioid analogics.

The results presented here regarding reversal of inflammatory hyperalgesia are in line with those of DeHaven-Hudkins et al. (2002), who demonstrated that local administration of the potent mu agonist loperamide is antihyperalgesic in the FCA model. Local administration of alternative mu agonists such as fentanyl (Stein et al., 1988) and the peptide [d-Ala²,MePhe⁴,Gly(ol)⁵]enkephalin (DAMGO) (Stein et al., 1989; Zhou et al., 1998) have also proven antihyperalgesic efficacy of locally administered mu opioid agonists in both animals and humans following an inflammatory insult (for review, see Stein et al., 2003). We hypothesized that an exogenous, systemically available, and peripherally restricted opioid agonist would provide effective pain relief in an inflammatory setting without centrally mediated adverse effects; the accompanying paper (Valenzano et al., 2004) describes such a molecule. Here, we report that systemic administration of DiPOA produced significant and naltrexone-sensitive reversal of mechanical hyperalgesia associated with FCA-induced inflammation. In addition, DiPOA was also effective against hyperalgesia induced by plantar incision, yet had no effect in the partial ligation model of neuropathic pain at the doses tested. These effects were observed in the absence of ataxia or effects on acute nociception, two outcomes commonly associated with opioid analogics. In Vivo Characterization of DiPOA 797

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In the present study, intraperitoneal administration of DiPOA has no effect on acute nociception as measured by the tail flick assay. This result is in direct contrast to those obtained with morphine, which leads to profound effects on acute nociception. We believe this difference is due to the extremely low levels of DiPOA that penetrate the blood-brain barrier (Valenzano et al., 2004). Therefore, these data provide strong evidence that effects on acute nociception are mediated by central opioid receptors. The literature regarding the involvement of peripheral mu receptors in acute nociception is controversial. Clinical studies indicate that local application of opioid agonists along uninjured nerve axons does not reliably produce analgesic effects (Picard et al., 1997; Likar et al., 2001). This result may indicate that peripheral opioid analgesia is not achievable in the absence of an injury or inflammation. Alternatively, it may indicate that binding of opioids to opioid receptors does not occur on nerve axons. Preclinally, peri-neural application of either morphine or fentanyl is also not analgesic (Grant et al., 2001). Intraplantar injection of DAMGO (Stein et al., 1989) or loperamide (DeHaven-Hudkins et al., 1999) into noninflamed rat paws or loperamide administered intravenously (Hurwitz et al., 1994) has no effect on acute nociception. These studies argue that peripheral mu receptors are not involved in acute nociception and are therefore in agreement with the conclusions of the current study. This is in disagreement with the results of Kolesnikov and Pasternak (1999), who demonstrated that when the tail of a mouse is immersed in a dimethyl sulfoxide solution containing either morphine or DAMGO, potent dose-dependent analgesia results only in the treated area. These seemingly opposing results may be explained by differences in administration procedures; the use of dimethyl sulfoxide (Kolesnikov and Pasternak, 1999) may disrupt the perineurium, causing an enhancement of opioid coupling, analogous to the situation following inflammation. Indeed, following intraplantar injection of mannitol, a compound that increases perineurial permeability without causing inflammation, local administration of DAMGO or fentanyl inhibits acute nociception with a similar potency to that observed after local administration to FCA-treated animals (Antonijevic et al., 1995).

Although at least three studies have demonstrated antihyperalgesic efficacy of locally administered mu opioid agonists in either the chronic constriction injury (CCI) or spinal nerve
ligation (SNL) rat models of neuropathic pain (Pertovaara and Wei, 2001; Martinez et al., 2002; Truong et al., 2003), we did not find that DiPOA was effective in the PSN model of neuropathic pain at doses that were effective against inflammatory pain. There are three potential contributing factors that may explain this difference. Firstly, the action of morphine in the CCI model of neuropathic pain has been attributed, at least partially, to action at the kappa opioid receptor (Catheline et al., 1996). This may also hold true for the effect of morphine in the SNL model. Because DiPOA has a higher selectivity for mu over kappa, as compared with morphine, this may contribute to the lack of efficacy in the PSN model. Secondly, both the CCI and SNL models involve a more substantial inflammatory component as compared with the PSN model (Lindenlaub and Sommer, 2000; Eaton, 2003), which may account for the efficacy of peripherally applied opioid agonists observed using these models. Finally, the time point in these studies was 14 days postsurgery or less; at this time, there may be a significant inflammatory component due to the surgery. In the present study, a 21-day time point postsurgery was chosen to minimize the effect of surgery-induced inflammation. In this study, DiPOA was ineffective over the same range of doses that produced antihyperalgesia in the rat FCA model of inflammatory hyperalgesia.

In addition to reversing inflammation-induced pain, systemically administered DiPOA also reversed hyperalgesia induced by plantar incision. To our knowledge, this is the first demonstration that activation of peripheral mu receptors can inhibit pain elicited by an incision in the rat and suggests that this model of post-operation pain has an inflammatory component.

Our demonstration of a complete inhibition of the antihyperalgesic effect of DiPOA against inflammatory hyperalgesia by preadministration of the opioid receptor antagonist, naltrrexone, demonstrates that DiPOA is acting through opioid receptors in vivo. Furthermore, we believe that the mechanism of action of DiPOA is predominantly through mu opioid receptors. Firstly, as compared with morphine, DiPOA has higher affinity, potency, and efficacy at the mu receptor in vitro; it also demonstrates selectivity for mu over kappa, ORL-1, and delta receptors (Valenzano et al., 2004). Secondly, if the mechanism of action of DiPOA is predominantly through the stimulation of kappa or ORL-1 receptors, then we would expect to see effects on acute nociception (Machelska et al., 1999a) and/or on neuropathic pain (Catheline et al., 1996; Abdulla and Smith, 1998).

As previously discussed, both peripheral mu and peripheral kappa receptors have been implicated in pain modulation. Our data suggests that development of a peripheralized, orally available, small-molecule mu agonist would have clinical utility in the treatment of inflammatory and postsurgical pain. Clinical proof of concept exists for peripheral mu-mediated antihyperalgesia in studies where morphine was applied locally. We anticipate the major side effect of a peripheralized, orally available, small-molecule mu agonist to be constipation. In contrast, a peripheral kappa agonist, asimadoline, has been shown to be analgesic (Machelska et al., 1999b); therefore, it may have a wider therapeutic utility, including neuropathic pain. However, in the same preclinical study, a low dose of asimadoline was hyperalgesic. Furthermore, administration of asimadoline to patients following knee surgery resulted in no beneficial effect (Machelska et al., 1999b). This lack of efficacy may have been compound specific, and data from further clinical trials with alternative peripheral kappa agonists are needed before the clinical validity of peripheral kappa receptors can be concluded.

The in vivo characterization of DiPOA in acute and chronic pain models presented here, together with the in vitro and pharmacokinetic profile presented in the accompanying manuscript, suggest a pharmacological profile unique from other opioids commonly used to study peripheral mechanisms of pain modulation. Our findings provide strong support for a role of peripheral mu opioid receptors in the pathology of pain associated with inflammation and argue against the involvement of these receptors in acute nociception, neuropathic pain, and motor coordination. In conclusion, DiPOA represents the first description of a peripherally restricted, small-molecule mu opioid agonist that is nonselective, antihyperalgesic, and effective against inflammatory and postsurgical pain when administered systemically.

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References


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