The Role of Central and Peripheral μ Opioid Receptors in Inflammatory Pain and Edema: A Study Using Morphine and DiPOA ([8-(3,3-Diphenyl-propyl)-4-oxo-1-phenyl-1,3,8-triaza-spiro[4.5]dec-3-yl]-acetic Acid)

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ABSTRACT
The role of opioid receptors located in the central nervous system (CNS) and peripheral nervous system in inflammatory pain is well established. In contrast, although it is has been shown that μ agonists can reduce other manifestations of inflammation, such as edema, the mechanism of action remains unclear. In this study, we have activated μ receptors located centrally, those located peripherally, and those located both centrally and peripherally and compared the effects on pain and edema using the rat carrageenan model of acute inflammation. Activation of μ receptors located only in the periphery, by administration of the peripheralized μ agonist [8-(3,3-diphenyl-propyl)-4-oxo-1-phenyl-1,3,8-triaza-spiro[4.5]dec-3-yl]-acetic acid (DiPOA) or local administration of morphine, resulted in antihyperalgesia (30 mg/kg DiPOA, 83% inhibition; 100 μg/rat morphine, 75% inhibition) without affecting edema. In contrast, activation of both central and peripheral μ receptors using systemically administered morphine resulted in antihyperalgesia (1 mg/kg, 80% inhibition) and inhibition of edema (10 mg/kg, 54% inhibition). Finally, activation of only receptors located in the CNS, by central administration of DiPOA or systemic administration of morphine after block of only the peripheral μ receptors using q-naltrexone, resulted in a significant reduction in edema. Our findings confirm the role of peripheral μ receptors in the pathology of pain associated with acute inflammation and argue against the involvement of these receptors in edema formation. Furthermore, our data demonstrate that activation of μ receptors in the brain inhibits carrageenan-induced edema and suggest that the antiedematous effect of morphine is due to action at central receptors alone.
Walker et al., 1996; Alebouyeh et al., 2002; Amann et al., 2002). Furthermore, Sacerdote et al. (1996) and Planas et al. (1995) observed a proinflammatory effect of the opioid antagonists naltrexone and naloxone, respectively. The mechanisms of the antiedematous effect of systemically administered opioids are not clearly understood (Joris et al., 1990; Perrot et al., 1999). Proposed hypotheses include activation of neuronal μ receptors present in either the CNS or the PNS. Morphine, and other centrally active opioids, reduce muscular tone in conjunction with sedation. This reduction in tone could have effects on local blood flow in peripheral tissues. Alternatively, opioids could reduce edema via interaction with receptors present on cells of the immune system such as leukocytes and macrophages either, circulating or resident at the site of inflammation. In contrast to the well-documented anti-inflammatory effect of systemically administered morphine, the effect of locally administered μ agonists on inflammation-induced edema has received much less attention.

DiPOA was administered either intraperitoneally (i.p.) or i.c.v. in 25% dextrose solution (0.76 g/rat DiPOA) or a single dose of 1, 3, or 10 mg/kg morphine s.c.; or a single dose of 30, 100, or 200 μg/rat morphine i.p. (animals were anesthetized as described above for i.pl. injections). In each experiment, a positive control (naproxen i.p., 30 mg/kg, 30 min before carrageenan) was included. Four hours after carrageenan injection, PWTs were again measured as described above (postdose PWT).

### Materials and Methods

#### Compounds and Administration Procedures.

All reagents are from Sigma-Aldrich (St. Louis, MO) unless otherwise stated. The synthetic route for DiPOA (free base) has been recently disclosed in patent application WO 2003101593 (Victory and Chen, 2003). DiPOA was used in all experiments as its free base (molecular weight 483.6; Kᵢₐ at μ = 0.76 ± 0.15 μM; solubility in 100 mM KH₂PO₄ at pH 7.4 > 50 μM). DiPOA was administered either intraperitoneally (i.p.) or i.c.v. in 25% β-cyclodextrin dissolved in distilled water in a dose volume of 2 ml/kg or 50 μl/rat, respectively. The opioid agonist morphine sulfate was administered either subcutaneously (s.c.) in 0.9% saline in a dose volume of 2 ml/kg or intraplantar (i.pl.) in 0.9% saline in a dose volume of 50 μl/rat. The peripheral opioid antagonist naltrexone was administered s.c. in 0.9% saline in a dose volume of 2 ml/kg. The nonsteroidal anti-inflammatory drug naproxen hydrochloride served as a positive control and 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 experiments, were used. Animals were group-housed and had free access to food and water at all times. 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-treated rats were identical.

### Carrageenan Model: Inflammatory Hyperalgesia.

For this assay, hind paw withdrawal thresholds (PWTs) to a noxious mechanical stimulus were determined using an analogesymeter (model 7200; Ugo Basile, Varese, Italy). Cut-off 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 (predose PWT), the rats were anesthetized with isofluorane (2% in oxygen) and received an intraplantar injection of 2% carrageenan (50 μl, diluted in 0.9% saline) to the left hind paw. Compounds were administered either 30 min before carrageenan injection (to investigate the effect on the development of inflammatory hyperalgesia) or 210 min after carrageenan injection (to investigate the effect on an established hyperalgesia). Rats received either a single dose of 3, 10, or 30 mg/kg DiPOA i.p.; a single dose of 1, 3, or 10 mg/kg morphine s.c.; or a single dose of 30, 100, or 200 μg/rat morphine i.pl. (animals were anesthetized as described above for i.pl. injections). In each experiment, a positive control (naproxen i.p., 30 mg/kg, 30 min before carrageenan) was included. Four hours after carrageenan injection, PWTs were again measured as described above (postdose PWT).

### Carrageenan Model: Inflammatory Edema.

For this assay, hind paw volume was determined using a plethysmometer (model 7140; Ugo Basile). Paw volume was determined once for each rat at each time point. Baseline paw volume was determined (predose paw volume), the rats were anesthetized with isofluorane (2% in oxygen) and received an intraplantar injection of 2% carrageenan (50 μl, diluted in 0.9% saline) to the left hind paw. Compounds were administered either 30 min before carrageenan injection (to investigate the effect on the development of inflammatory edema) or 210 min after carrageenan injection (to investigate the effect on an established edema). Rats received a single dose of either 3, 10, or 30 mg/kg DiPOA i.p.; a single dose of 10 μg/rat DiPOA i.c.v.; a single dose of 1, 3, or 10 mg/kg morphine s.c.; or a single dose of 30, 100, or 200 μg/rat morphine i.pl. (animals were anesthetized as described above for i.pl. and i.c.v. injections). q-Naltrexone (10 mg/kg s.c.) was administered 10 min before administration of 10 mg/kg morphine s.c. In each experiment, a positive control (naproxen i.p., 30 mg/kg, 30 min before carrageenan) was included. Four hours after carrageenan injection, paw volume was again measured as described above (postdose paw volume).

### Statistical Analysis.

Untransformed data were analyzed using a one-way analysis of variance. In instances were a main effect was detected, planned comparisons were made using Fisher’s PLSD test. The level of significance was set at $p < 0.05$. Data are shown as mean ± S.E.M. Percentage of inhibition of hyperalgesia and percentage of inhibition of edema were calculated according to eqs. 1 and 2, respectively.

#### % inhibition of hyperalgesia =

\[
100 - \left( \frac{\text{predose mean PWT} - \text{postdose mean PWT}}{\text{prevehicle mean PWT} - \text{postvehicle mean PWT}} \right) \times 100
\]  

#### % inhibition of edema =

\[
100 - \left( \frac{\text{predose mean paw volume} - \text{postdose mean paw volume}}{\text{prevehicle mean paw volume} - \text{postvehicle mean paw volume}} \right) \times 100
\]
Results

Carrageenan-Induced Hyperalgesia and Edema. In each experiment, injection of carrageenan produced a significant reduction of PWT 4 h later (range 60 ± 9 to 88 ± 9 g) compared with precarrageenan levels (range 149 ± 10 to 170 ± 13 g). Carrageenan injection also produced a significant increase in paw volume 4 h later (range 2.77 ± 0.17 to 3.25 ± 0.12 ml) compared with precarrageenan levels (range 1.57 ± 0.02 to 1.73 ± 0.03 ml). Intraperitoneal administration of 30 mg/kg naproxen (the positive control) produced a statistically significant inhibition of both hyperalgesia, with a maximum inhibition of 74%, and edema, with a maximum inhibition of 68% (data not shown for experiments presented in Figs. 1 and 3).

Systemic Administration of DiPOA. DiPOA (3, 10, or 30 mg/kg i.p.) administered 30 min before carrageenan injection did not result in a statistically significant inhibition of hyperalgesia (Table 1; Fig. 1A), although a trend was noted at the highest dose. Administration of DiPOA (3, 10, or 30 mg/kg i.p.) 210 min after carrageenan produced a significant inhibition of hyperalgesia at the highest dose (83%) with a trend observed at the 10 mg/kg dose (Table 1; Fig. 1A). DiPOA (3, 10, or 30 mg/kg i.p.), administered either before or after carrageenan, was not efficacious in inhibiting edema (Table 1; Fig. 1A).

Local Administration of Morphine. Morphine (30, 100, or 200 µg i.pl.) administered 30 min before carrageenan injection did not result in a statistically significant inhibition of hyperalgesia (Table 1; Fig. 1B). Administration of morphine (100 and 200 µg i.p.l.) 210 min after carrageenan produced a significant and dose-dependent inhibition of hyperalgesia (Table 1; Fig. 1B) with a maximum inhibition of 110% (200 µg). Morphine (30, 100, or 200 µg i.p.l.), administered either before or after carrageenan, did not inhibit edema (Table 1; Fig. 1B).

Systemic Administration of Morphine. Administration of morphine (3 and 10 mg/kg s.c.) 30 min before carrageenan produced a significant and dose-dependent inhibition of hyperalgesia (Table 1; Fig. 1C) with a maximum inhibition of 72% (10 mg/kg). When morphine was administered 210 min after carrageenan treatment a dose-dependent inhibition of hyperalgesia was observed with a maximum percentage of inhibition of 186% (10 mg/kg) (Fig. 1C). A percentage of inhibition of hyperalgesia that is above 100% is indicative of analgesia. The highest dose of morphine (10 mg/kg) also significantly inhibited edema when administered either pre- or post-carrageenan (Table 1; Fig. 1C) with higher efficacy when administered before carrageenan (54 versus 13%) (Fig. 1C).

Intracerebroventricular Administration of DiPOA. DiPOA (10 µg/rat i.c.v.) administered 30 min before carrageenan injection produced a statistically significant inhibition of edema with a percentage of reversal of 69%, comparable with that produced by naproxen in this experiment (58%) (Fig. 2).

q-Naltrexone Antagonism of Systemically Administered Morphine. Administration of morphine (10 mg/kg s.c.) produced a statistically significant reduction in edema when administered before carrageenan (Fig. 3), comparable with the results shown in Fig. 1. This effect was not inhibited by q-naltrexone pretreatment (10 mg/kg s.c.), with no statistically significant difference compared with the morphine alone group (Fig. 3). When administered alone in this model, q-naltrexone (10 mg/kg) had no effect on paw volume. We did not examine the effect of activation of µ receptors located only in the CNS on pain responses because this is widely accepted to cause analgesia.

Discussion

Activation of µ receptors in the brain results in analgesia, whereas activation of those in the periphery, after inflammation, results in antihyperalgesia. The µ receptors have also been implicated in the inflammatory process; systemically administered morphine reduces edema and plasma extravasation with an unknown mechanism of action (Hargreaves et al., 1988; Joris et al., 1990; Sacerdote et al., 1996; Walker et al., 1996; Alebouyeh et al., 2002; Amann et al., 2002). In the current study, we sought to investigate the role of centrally and/or peripherally located µ receptors in pain and edema caused by acute inflammation. To this end, we have activated

Table 1

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<th>0 (Vehicle)</th>
<th>30 µg/rat</th>
<th>100 µg/rat</th>
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<tr>
<td><strong>PWT (g)</strong></td>
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<tr>
<td>Pre-Carr DiPOA (i.p.)</td>
<td>65 ± 10</td>
<td>64 ± 7</td>
<td>99 ± 8</td>
<td>93 ± 8</td>
<td>89 ± 17</td>
<td>99 ± 13</td>
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<tr>
<td>Morphine (i.pl.)</td>
<td>84 ± 4</td>
<td>107 ± 13</td>
<td>116 ± 14</td>
<td>116 ± 14</td>
<td>116 ± 14</td>
<td>123 ± 12</td>
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<tr>
<td>Morphine (s.c.)</td>
<td>64 ± 7</td>
<td></td>
<td>99 ± 8</td>
<td>102 ± 8</td>
<td>118 ± 20</td>
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<td>Post-Carr DiPOA (i.p.)</td>
<td>88 ± 9</td>
<td>94 ± 9</td>
<td>144 ± 18</td>
<td>181 ± 19</td>
<td>143 ± 15</td>
<td>200 ± 0</td>
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<tr>
<td>Morphine (i.pl.)</td>
<td>73 ± 7</td>
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<td>144 ± 18</td>
<td>181 ± 19</td>
<td>195 ± 21</td>
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<td>Morphine (s.c.)</td>
<td>69 ± 9</td>
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<td>144 ± 18</td>
<td>181 ± 19</td>
<td>250 ± 0</td>
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<td><strong>PV (ml)</strong></td>
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<tr>
<td>Pre-Carr DiPOA (i.p.)</td>
<td>2.77 ± 0.07</td>
<td>3.25 ± 0.12</td>
<td>3.20 ± 0.05</td>
<td>3.38 ± 0.08</td>
<td>3.21 ± 0.08</td>
<td>2.54 ± 0.11</td>
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<td>2.75 ± 0.11</td>
<td>3.20 ± 0.05</td>
<td>3.20 ± 0.05</td>
<td>3.21 ± 0.08</td>
<td>2.73 ± 0.07</td>
<td>2.57 ± 0.16</td>
<td>2.12 ± 0.15*</td>
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<tr>
<td>Morphine (s.c.)</td>
<td>2.8 ± 0.08</td>
<td>3.17 ± 0.07</td>
<td>3.2 ± 0.08</td>
<td>3.14 ± 0.06</td>
<td>2.88 ± 0.05</td>
<td>2.87 ± 0.08</td>
<td>2.81 ± 0.06*</td>
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PWT, paw withdrawal threshold; PV, paw volume; Pre-Carr, compound administration 30 minutes prior to carrageenan injection; Post-Carr, compound administration 210 minutes following carrageenan injection (baseline PWT, 149 ± 10 g to 170 ± 13 g; baseline PV, 1.57 ± 0.02 ml to 1.73 ± 0.03 ml).
μ receptors located centrally, those located peripherally, and those located both centrally and peripherally, and compared the effects on pain and edema.

Systemically administered morphine, which activates both central and peripheral μ receptors, has been previously shown to be both antihyperalgesic and antiedematous (Hargreaves et al., 1988; Joris et al., 1990; Sacerdote et al., 1996; Walker et al., 1996; Alebouyeh et al., 2002; Amann et al., 2002). In the current study, systemic administration of morphine inhibits both pain and edema, confirming these reports.

We activated μ receptors located in the periphery first by systemic administration of the peripherally restricted μ opioid agonist DiPOA, and second by local administration of morphine. Both treatments were antihyperalgesic without affecting edema. The antihyperalgesic effects of DiPOA are in-line with our previous demonstration of naltrexone-sensitive antihyperalgesia in the FCA model of chronic inflamma-
The antihyperalgesic effect of morphine, administered to the site of inflammation has been previously well documented. In contrast, we are aware of only two studies that have investigated the action of local morphine on edema; Sacerdote et al. (1996) assessed the effects of a single dose of morphine (10 µg) administered into the inflamed paw in a yeast model of acute inflammation and observed no effect on paw edema. Similarly, Perrot et al. (1999) administered morphine locally (50–200 µg) both before and after intraplantar carrageenan. The authors concluded that neither preemptive nor curative administration of morphine affects paw circumference. Our results are consistent with these observations and support the conclusion that peripheral µ receptors, at the site of inflammation and on circulating or local immune cells, are not involved in edema formation due to acute inflammation. Furthermore, the antihyperalgesic effects of DiPOA are not secondary to a reduction of edema and may indicate an action at µ receptors on nerve terminals rather than on immune cells; however, further experimentation would be required to demonstrate this. In contrast, a number of studies investigating alternative endpoints to edema have demonstrated the involvement of peripheral µ receptors in the inflammatory process (Green and Levine, 1992; Barber, 1993; Hong and Abbott, 1995; Taylor et al., 2000; Vujic-Redzic et al., 2000; Stanojević et al., 2002; Wenk et al., 2003; McDougal et al., 2003; Romero et al., 2005). It is possible that activation of µ receptors can inhibit inflammation early in the inflammatory process, and this would not be observed in the current study. Alternatively, µ agonism may affect components of the inflammatory process, such as plasma extravasation, without affecting the resultant edema. An alternative explanation is that activation of µ receptors can affect inflammation which is predominantly neurogenic in origin. Indeed µ agonists reduce plasma extravasation in the formalin model (Hong and Abbott, 1995; Taylor et al., 2000) and after antidromic nerve stimulation (Barber, 1993), two models that induce substantial neurogenic inflammation. In contrast, the carrageenan model has been described as inducing “non-neurogenic” inflammation (Handwerker et al., 1987). Furthermore, Green and Levine (1992) demonstrated that a µ agonist inhibits capsaicin-induced extravasation but has no effect on bradykinin-induced inflammation, implicating the involvement of peripheral nerves. Finally, destruction of unmyelinated afferents in the knee attenuates the anti-inflammatory action of endomorphin-1, suggesting a neurogenic mechanism (McDougal et al., 1998). Investigation into the action of DiPOA in neurogenic pain models such as the formalin model would help to confirm this hypothesis.

Finally, we activated µ receptors located only in the CNS first by i.c.v. administration of DiPOA and second by blocking only peripheral µ receptors before systemic administration of morphine. Central administration of 10 µg of DiPOA proved antiedematous. This is consistent with the effect of 20 µg of i.c.v. morphine, which elicits a comparable, significant, and naloxone-sensitive inhibition of carrageenan induced edema (Bhattacharya et al., 1992). In the current study, pretreatment with the peripheral µ antagonist q-naltrexone did not inhibit the antiedematous effect of systemically administered morphine. The dose and route of administration of q-naltrexone used in the current study (10 mg/kg i.p.) has been previously shown to effectively block peripheral µ receptors, without affecting those in the CNS (Fecho et al., 1996). Importantly, higher doses of q-naltrexone will penetrate the brain and could therefore not be used (Fecho et al., 1996). q-Naltrexone alone did not affect edema. Although we are unaware of any previous studies that have investigated the effect of q-naltrexone on edema, our results are consistent with two studies that considered the effect of q-naltrexone on pain; Morgan et al. (1991) demonstrated that systemic q-naltrexone does not affect PWT in the rat FCA model, and Ko et al. (1998) who described no effect of q-naltrexone on capsaicin-induced hyperalgesia, upon local administration to the tail of primates.

There is a clear difference between systemically administered DiPOA and morphine in their respective effects on edema. There are four possible explanations for this observation. First, it may be due to differences in each compounds...
profile at opioid receptors. Second, off-target effects may explain the observations. Third, differences in pharmacokinetics and fourth, penetration of the blood-brain barrier may explain the data. Considering the first possibility, comparison of the in vitro profiles of DiPOA and morphine has been published previously (Valenzano et al., 2004). Briefly, the in vitro potency and selectivity data for DiPOA and morphine are comparable; both are potent μ agonists with high affinity for the receptor (Kᵢ = 0.76 and 2.06 nM, respectively), both are agonists at κ with comparable affinity (Kᵢ = 243 and 134 nM, respectively), and both are inactive at δ (Kᵢ > 10,000 nM). DiPOA and morphine do differ in their affinity for opioid receptor-like 1 receptors (Kᵢ = 286 and >10,000 nM, respectively); however, it is unlikely that this difference would explain the results; should the inhibition of edema be opioid receptor-like 1-mediated, morphine would not be expected to have an effect. We therefore feel that differing profiles at opioid receptors is unlikely to explain why systematically administered morphine is antiedematous, whereas systemically administered DiPOA is not. Addressing the second possibility, morphine is recognized to have some nonopioid activity, such as mast cell activation (Blunk et al., 2004), and this could potentially contribute to the observed differences in effects on edema. It would, be surprising, however, that two compounds of such divergent structure would exhibit the same nonopioid activity, yet both are antiedematous when administered directly into the brain. We therefore feel that this is an unlikely explanation for the current results. Furthermore, the antihyperalgesic and antiedematous effects of DiPOA and morphine, respectively, are reversible by opioid antagonists (Bhattacharya et al., 1992; Whiteside et al., 2004). The third possible explanation is differences in the compounds pharmacokinetics; our choice of route of administration and dose range attempted to achieve approximately equiefficacious plasma levels over a comparable time course. This was based on the pharmacokinetic profile of DiPOA (Valenzano et al., 2004) and morphine (unpublished observations) and on the efficacy observed in the FCA model (Whiteside et al., 2004). Morphine and DiPOA were more efficacious against hyperalgesia when administered postcarrageenan. This is likely due to the higher quantities of compound present 30 min postdosaging compared with 210 min postdosaging. In contrast, systemically administered morphine is more efficacious against edema when administered precarrageenan compared with postcarrageenan. We hypothesize that reduction of edema is not reliant on circulating levels of compound and remains when these have fallen. We therefore think that differences in plasma levels do not explain our results. Last, morphine and DiPOA differ substantially in their penetration of the CNS (brain-to-plasma ratios for morphine and DiPOA are 0.42 ± 0.13 and 0.019 ± 0.014, respectively) (Valenzano et al., 2004); this finding combined with the demonstration that both morphine and DiPOA administered directly into the brain reduce edema, supports the conclusion that systemically administered morphine is antiedematous through action at central receptors, whereas systemically administered DiPOA lacks efficacy due to the extremely low levels that penetrate the CNS.

Together, our findings confirm the role of peripheral μ receptors in the pathology of pain associated with acute inflammation and argue against the involvement of these receptors in edema formation. Furthermore, our data demonstrate that activation of μ receptors in the brain inhibits carrageenan-induced edema and suggest that the antiedematous effect of morphine is due to action at central receptors alone. The clinical implication of this work is that although peripheral μ agonists may prove useful in the treatment of pain associated with inflammation, they may not be as effective in reducing other manifestations of inflammation, such as edema.

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References


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