Impact of Efficacy at the \( \mu \)-Opioid Receptor on Antinociceptive Effects of Combinations of \( \mu \)-Opioid Receptor Agonists and Cannabinoid Receptor Agonists

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ABSTRACT

Cannabinoid receptor agonists, such as \( \Delta^9 \)-tetrahydrocannabinol (\( \Delta^9 \)-THC), enhance the antinociceptive effects of \( \mu \)-opioid receptor agonists, which suggests that combining cannabinoids with opioids would improve pain treatment. Combinations with lower efficacy agonists might be preferred and could avoid adverse effects associated with large doses; however, it is unclear whether interactions between opioids and cannabinoids vary across drugs with different efficacy. The antinociceptive effects of \( \mu \)-opioid receptor agonists alone and in combination with cannabinoid receptor agonists were studied in rhesus monkeys \((n = 4)\) using a warm water tail withdrawal procedure. Etorphine, fentanyl, morphine, buprenorphine, nalbuphine, \( \Delta^8 \)-THC, and CP 55,940 (2-[(1R,2R,5R)-5-hydroxy-2-[3-hydroxypropyl]cyclohexyl]-5-(2-methyloctan-2-yl)phenol) each increased tail withdrawal latency. Pretreatment with doses of \( \Delta^9 \)-THC (1.0 mg/kg) or CP 55,940 (0.032 mg/kg) that were ineffective alone shifted the fentanyl dose-effect curve leftward 20.6- and 52.9-fold, respectively, and the etorphine dose-effect curve leftward 12.4- and 19.6-fold, respectively. \( \Delta^9 \)-THC and CP 55,940 shifted the morphine dose-effect curve leftward only 3.4- and 7.9-fold, respectively, and the buprenorphine curve only 5.4- and 4.1-fold, respectively. Neither \( \Delta^9 \)-THC nor CP 55,940 significantly altered the effects of nalbuphine. Cannabinoid receptor agonists increase the antinociceptive potency of higher efficacy opioid receptor agonists more than lower efficacy agonists; however, because much smaller doses of each drug can be administered in combinations while achieving adequate pain relief and that other (e.g., abuse-related) effects of opioids do not appear to be enhanced by cannabinoids, these results provide additional support for combining opioids with cannabinoids to treat pain.

Introduction

Pain remains a significant clinical problem (e.g., Gaskin and Richard 2012) and \( \mu \)-opioid receptor agonists, such as hydrocodone and morphine, continue to be the most widely used treatments for moderate to severe pain. However, the use of opioids to treat pain is limited by unwanted effects, such as constipation, respiratory depression, tolerance, and dependence (Gutstein and Aki 2005). One strategy for increasing the therapeutic window and, thus, the clinical utility of opioids is to combine them with drugs that produce analgesic effects through different mechanisms. Treating pain with drug combinations allows for the possibility that smaller doses of individual drugs can be combined to maintain or improve analgesia while reducing the likelihood of encountering the unwanted effects associated with larger doses of either drug administered alone (Gilron et al., 2013).

Cannabinoid receptor agonists, such as \( \Delta^9 \)-tetrahydrocannabinol (\( \Delta^9 \)-THC), have antinociceptive effects (see Pertwee, 2001, and Walker and Hohmann, 2005, for reviews), and medications including cannabinoid receptor agonists are approved for use in humans (e.g., Sativex; GW Pharmaceuticals, Salisbury, UK; see Nurmikko et al., 2007). However, like opioids, cannabinoids (marijuana, JWH018 [naphthalen-1-yl-(1-pentylindol-3-yl)methane] are abused, and the clinical utility of cannabinoids alone has been modest (Kraft, 2012). Combining cannabinoids with other drugs that have analgesic effects (e.g., opioids) appears to be a promising approach to treat pain. Cannabinoid receptor agonists enhance the antinociceptive effects of \( \mu \)-opioid receptor agonists in rodents (Welch and Stevens, 1992; Finn et al., 2004) and nonhuman primates (Li et al., 2008; Maguire et al., 2013). Moreover, cannabinoid receptor agonists, taken orally or through inhalation of vaporized or smoked marijuana, enhance the analgesic effects of opioids in patients (Lynch and Clark, 2003; Narang et al., 2008; Abrams et al., 2011; Johnson et al., 2013).

Though converging lines of evidence support the potential value of combining opioids with cannabinoids to treat pain, it...
is not clear what combinations of drugs and doses would be optimal. In nonhuman primates, cannabinoid receptor agonists markedly enhanced the antinociceptive effects of morphine (Li et al., 2008; Maguire et al., 2013); however, combinations with opioids that have different pharmacologic properties (e.g., different intrinsic efficacy) might offer better therapeutic outcomes by maximizing pain relief and minimizing unwanted effects. For example, some lower efficacy μ-opioid receptor agonists (e.g., nalbuphine) have fewer and less severe adverse effects, such as respiratory depression, compared with higher efficacy agonists (Liguori et al., 1996; Dahan et al., 2006). So long as adequate therapeutic effects can be achieved, combinations with lower efficacy agonists might be preferred. Moreover, drugs with lower efficacy at the μ-opioid receptor also can be less effective positive reinforcers compared with higher efficacy agonists (Zernig et al., 1997) and appear to have lower abuse liability in humans (Schmidt et al., 1985; Preston and Jasinski, 1991). Thus, in addition to providing adequate pain relief, combining small doses of lower efficacy agonists might also reduce abuse and possibly mitigate the rising abuse of prescription opioids (Manchikanti et al., 2012).

Combining lower efficacy μ-opioid receptor agonists with drugs from other classes might enhance their potency and/or maximal effect. For example, although nalbuphine has relatively weak antinociceptive effects in comparison with other μ-opioid receptor agonists (Walker et al., 1993; Gerak et al., 1994), combining nalbuphine with nonopioid drugs, such as cocaine, can enhance its antinociceptive effects (Gatch et al., 1995), raising the possibility that combining low-efficacy μ-opioid receptor agonists with cannabinoid receptor agonists might also improve analgesic effectiveness while reducing unwanted effects. However, it is unclear whether cannabinoid receptor agonists enhance the antinociceptive effects of high- and low-efficacy μ-opioid receptor agonists similarly. In the current study, the cannabinoid receptor agonists 9-THC and CP 55,940 (2-[(1R,2R,5R)-5-hydroxy-2-(3-hydroxypropyl) cyclohexyl]-5-(2-methyloctan-2-yl)phenol) were studied alone and in combination with agonists (etorphine, fentanyl, morphine, buprenorphine, and nalbuphine) varying in efficacy at the μ-opioid receptor (Schmidt et al., 1985; Woods and Winger, 1987; Walker et al., 1993; Gerak et al., 1994; Traynor and Nahorski, 1995; Emmerson et al., 1996; Zernig et al., 1997; Morgan et al., 1999; Zaki et al., 2000; McPherson et al., 2010) to determine whether efficacy at the μ-opioid receptor impacts the interaction between μ-opioid receptor agonists and cannabinoid receptor agonists with regard to antinociceptive effects. The cannabinoids Δ9-THC and CP 55,940 have been shown to enhance the antinociceptive effects of morphine using a warm water tail withdrawal procedure in rhesus monkeys (Li et al., 2008; Maguire et al., 2013).

Materials and Methods

Animals. Four adult rhesus monkeys (Macaca mulatta), three female and one male, served as subjects. Body weight (range: 6–9 kg) was maintained via postsession feeding (Harlan Teklad, High Protein Monkey Diet, Madison, WI). Monkeys received fresh fruit and peanuts daily, and water was continuously available in the home cage. Subjects were housed individually in a colony room and under a 14/10 light/dark cycle (lights on at 6:00 AM). The animals used in these studies were maintained under protocols approved by the Institutional Animal Care and Use Committee of the University of Texas Health Science Center at San Antonio, and in accordance with the 2011 Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animals Resources on Life Sciences, National Research Council, National Academy of Sciences).

Apparatus. Monkeys were tested while seated in primate chairs (Model R001-T; Primate Products, Miami, FL). Warm water baths were used to maintain water at the appropriate temperatures. Tails were dipped in plastic-insulated mugs containing water, and tail withdrawal latencies were measured using a silent handheld stopwatch.

Warm Water Tail Withdrawal. The lower portion (approximately 15 cm) of the shaved tail was inserted into a mug containing 50, 54, or 58°C water, and the time until the tail was completely removed from the mug was recorded. In the event that the monkey failed to remove the tail from the water within 20 seconds, the mug was removed, and the tail withdrawal latency was recorded as 20 seconds (i.e., maximum effect). Tests with each temperature were conducted at 15-minute intervals with temperatures presented in irregular order across cycles. Tests with different temperatures during a cycle were separated by 1 minute. Injections were given subcutaneously in the back immediately after the completion of the final test in a cycle and 15 minutes before the subsequent cycle.

Three types of tests were conducted. First, dose-effect curves for μ-opioid receptor agonists administered alone were determined using a cumulative dosing procedure with the dose increasing across cycles. Sessions ended when the tail withdrawal latency reached 20 seconds in 54°C water or after 8 cycles, whichever occurred first. Second, the effects of Δ9-THC and CP 55,940 administered alone were determined by injecting a single dose 60 minutes before a test session in which saline was administered before each cycle (time course). Time course sessions ended when tail withdrawal latency reached 20 seconds in 54°C water for a total of 4 cycles or after 8 cycles, whichever occurred first. Third, dose-effect curves for μ-opioid receptor agonists were determined as described earlier after administration of a single dose of Δ9-THC or CP 55,940 60 minutes before the start of the session. Typically, tests were separated by at least 7 days. Given that buprenorphine has long-lasting effects (Walker et al., 1995), tests after buprenorphine occurred after at least 28 days.

Data Analyses. Tail withdrawal latencies were expressed as a percentage of maximum possible effect (MPE) according to the following formula:

\[
%\text{MPE} = \left(\frac{\text{Test latency} - \text{Control latency}}{20 \text{ seconds} - \text{Control latency}}\right) \times 100.
\]

Control latencies were determined for individual subjects in the absence of drug for each temperature. The percentage of MPE was calculated for each μ-opioid receptor agonist dose administered alone and in combination with a cannabinoid receptor agonist and plotted as a function of the dose of the opioid. Dose-effect curves for Δ9-THC and CP 55,940 were constructed by averaging %MPE across all trials of a time course session after administration of each dose; the number of values comprising each average ranged from 4 to 8 depending on the effectiveness of that dose (see above).

Potency was estimated for individual monkeys by calculating ED50 values for each drug administered alone and for combinations using linear interpolation. Only data for 54°C were analyzed because Δ9-THC and CP 55,940 alone produced substantial increases in tail withdrawal latency from 50°C, precluding analysis of shifts in the dose-effect curve; data from 58°C are not shown because increases in tail withdrawal latency with combinations were comparatively small, less consistent than with 54°C, and thus less amenable to analysis. Data from the 54°C condition, comprising the linear portion of the dose-effect curve, ranging from the largest dose that produced <20% MPE to the smallest dose that produced >80% MPE, were used in the analyses. In the event that the range of doses tested did not include a dose small enough to produce >20% MPE or large enough to produce...
$\geq 80\%$ MPE, the next smaller or larger dose, respectively, that would be tested (log scale) was estimated to produce either no effect ($0\%$) or a full effect ($100\%$), respectively. This method provides a conservative estimate of shifts in dose-effect curves. Potency ratios were calculated for individual subjects by dividing the control $ED_{50}$ by the test $ED_{50}$, and shifts in dose-effect curves were considered statistically significant if the 95% confidence intervals of the potency ratios, averaged across monkeys, did not include 1.

Potency ratios were analyzed using repeated-measures analysis of variance with four observations (subjects) per cell and with opioid and cannabinoid as within-subject factors. Dunnett post-hoc analyses were used to determine statistically significant differences among potency ratios across opioids for each cannabinoid and across cannabinoids for each opioid. Statistical analyses were conducted using NCSS 8 (Kaysville, UT).

Drugs. Etorphine, fentanyl, morphine, buprenorphine, and $\Delta^9$-THC were provided by the National Institute on Drug Abuse (Research Technology Branch, Rockville, MD). Nalbuphine hydrochloride (Mallinckrodt, St. Louis, MO) and CP 55,940 (Sigma-Aldrich, St. Louis, MO) were purchased from commercial sources. Etorphine, fentanyl, morphine, buprenorphine, and nalbuphine were dissolved in sterile water; $\Delta^9$-THC and CP 55,940 were dissolved in a 1:1:18 mixture of absolute ethanol, Emulphor-620 (Rhone-Poulenc, Princeton, NJ), and 0.9% saline. Doses are expressed in terms of the forms administered subcutaneously in the back in volumes between 0.2 and 3.0 ml.

Results

Under control conditions (i.e., no drug), tail withdrawal latencies (mean ± S.E.M.) for 50, 54, and 58°C water were 4.4 ± 2.7, 1.1 ± 0.1, and 0.9 ± 0.02 seconds, respectively. When administered alone, $\mu$-opioid receptor agonists etorphine, fentanyl, morphine, buprenorphine, and nalbuphine (Fig. 1) and cannabinoid receptor agonists $\Delta^9$-THC and CP 55,940 (Fig. 2) dose-dependently increased tail withdrawal latency from 50°C (circles) and 54°C (squares) water. The range of effective doses and maximum effectiveness of each drug varied with temperature. For all drugs, smaller doses were more effective at increasing the tail withdrawal latency from 50°C compared with 54°C water. When tested alone, nalbuphine increased tail withdrawal latency from 54°C water in only two of the four monkeys tested. The $ED_{50}$ values for nalbuphine in two monkeys in which nalbuphine did not increase tail withdrawal latencies from 54°C were estimated based on the method described under "Data Analyses." When administered alone, all drugs failed to increase tail withdrawal latency more than 30% MPE from 58°C water.

Pretreatment with a dose of $\Delta^9$-THC that was ineffective when administered alone in 54°C (1.0 mg/kg) significantly shifted the dose-effect curve for fentanyl, morphine, and buprenorphine leftward 20.63-, 3.37-, and 5.42-fold, respectively (Fig. 3, top row; Table 1). $\Delta^9$-THC shifted the etorphine dose-effect curve leftward on average 12.4-fold; however, owing to large intersubject variability, the shift did not reach statistical significance (Table 1).

Pretreatment with a dose of CP 55,940 (0.032 mg/kg) that was ineffective when given alone significantly shifted the dose-effect curve of fentanyl, etorphine, morphine, and buprenorphine leftward 52.99-, 19.65-, 7.91-, and 4.08-fold, respectively (Fig. 3, bottom row; Table 1). Initially, the doses of fentanyl and etorphine studied with CP 55,940 were too large to determine a dose-effect curve (filled triangles); a redetermination of the dose-effect curve starting with smaller doses (open triangles), produced similar effects for doses that were given in both determinations. Neither $\Delta^9$-THC nor CP 55,490 significantly enhanced the effects of nalbuphine (Fig. 3, right panels).

Repeated measures analysis of variance of shifts with opioid and cannabinoid as within-subject variables indicates a significant main effect of opioid ($F_{4,40} = 11.34; P < .001$) and a significant opioid by cannabinoid interaction ($F_{4,40} = 4.05; P < .05$) but no main effect of cannabinoid. Post-hoc tests revealed that CP 55,940 produced a significantly larger leftward shift in the fentanyl dose-effect curve as compared with its effects in combination with all other opioids. Moreover, the leftward shift in the fentanyl dose-effect curve produced by CP 55,940 was significantly greater than the shift produced by $\Delta^9$-THC.

Discussion

Pain remains a significant clinical problem, and although opioids are used extensively to treat pain, their use is limited by numerous unwanted effects. Those unwanted effects are fewer or do not occur with smaller doses of opioids; thus, combining smaller doses of opioids with drugs from other classes might offer the advantage of maintaining or improving analgesic effectiveness while limiting unwanted effects.

Fig. 1. Antinociceptive effects of cumulative doses of fentanyl, etorphine, morphine, buprenorphine, and nalbuphine in 50°C (●), 54°C (□), and 58°C (△) water in rhesus monkeys ($n = 4$ per panel). The interinjection interval was 15 minutes for all drugs. Data above “S” indicate effects after saline administration. Abscissa: dose in micrograms (fentanyl and etorphine) or milligrams (morphine, buprenorphine, and nalbuphine) per kilogram body weight. Ordinate: % MPE ± 1 S.E.M. (see “Data Analyses” for details).

Fig. 3. Right panels: combination studies with CP 55,940 and opioids. Pretreatment with a dose of CP 55,940 (0.032 mg/kg) shifted the dose-effect curve for each opioid leftward on average 12.4-fold (Table 1). $\Delta^9$-THC (1.0 mg/kg) significantly shifted the dose-effect curve for each opioid leftward, 52.99-, 19.65-, 7.91-, and 4.08-fold, respectively (Fig. 3, bottom row; Table 1).
associated with larger doses of either drug alone. Preclinical (Cichewicz, 2004; Li et al., 2008; Welch, 2009; Maguire et al., 2013) and clinical (Lynch and Clark, 2003; Narang et al., 2008; Abrams et al., 2011; Johnson et al., 2013) research supports the notion of combining \(\mu\)-opioid receptor agonists with cannabinoid receptor agonists to treat pain. This study examined whether efficacy at the \(\mu\)-opioid receptor impacts the interaction between the antinociceptive effects of opioid and cannabinoid receptor agonists. Characterizing how pharmacodynamic properties, such as intrinsic efficacy, impact interactions between drugs should facilitate the development of better treatment strategies, for example, by identifying optimal drug and dose combinations.

The \(\mu\)-opioid receptor agonists etorphine, fentanyl, morphine, buprenorphine, and nalbuphine and the cannabinoid receptor agonists CP 55,940 and \(\Delta^9\)-THC increased tail withdrawal latency from warm water; for each compound, the potency and maximal effect decreased as temperature increased. The relative potency of the opioids (etorphine > fentanyl > buprenorphine > morphine) corresponds to data reported for warm water tail withdrawal procedures in rhesus monkeys (Walker et al., 1993, 1995; Gatch et al., 1995; Zernig et al., 1997; Gerak et al., 2003). Although nalbuphine increased tail withdrawal latency from 54°C water in only two of the four monkeys tested, in those two monkeys, the relative potency of nalbuphine was similar to previous reports (Walker et al., 1993; Gerak et al., 1994). The relative potency of the cannabinoids in the current study (CP 55,940 > \(\Delta^9\)-THC) was the same as reported previously for rodents (Lichtman and Martin, 1991) and nonhuman primates (Vivian et al., 1998; Maguire et al., 2013).

Although some drugs and drug combinations produced modest increases in tail withdrawal latency from 58°C water, the effects generally did not exceed 30% MPE. It is possible that cannabinoids also increase the maximal effect of opioids under conditions where the doses of opioids studied were otherwise less effective or ineffective (e.g., 58°C); the current experiment was designed to determine whether cannabinoids similarly increase the potency of opioids varying in efficacy at the \(\mu\)-opioid receptor. Testing the warmer temperature provides an important control insofar as tail withdrawal from 58°C indicates that increased latencies at lower temperatures are not likely due to sedative effects (Dykstra and Woods, 1986).

Fig. 2. Antinociceptive effects of CP 55,940 and \(\Delta^9\)-THC in 50°C (○), 54°C (□), and 58°C (△) water in rhesus monkeys (n = 4 per panel). Effects were assessed during time courses studies lasting from 1 to 3 hours after acute drug injection. Data above "S" indicate effects during a time course study in which only saline was administered (see "Warm Water Tail Withdrawal" for further details). Abscissa: cannabinoid receptor agonist dose in milligrams per kilogram body weight. Ordinate: % MPE ± 1 S.E.M.

Fig. 3. Antinociceptive effects of cumulative doses of fentanyl, etorphine, morphine, buprenorphine, and nalbuphine alone (○) and after pretreatment with 1.0 mg/kg of \(\Delta^9\)-THC (○, top row) or 0.032 mg/kg of CP 55,940 (○, bottom row) in 54°C water (n = 4 per drug). Filled symbols (bottom left panels) indicate data from the first test with CP 55,940 in combination with the opioid (see Results for further details). Abscissa: dose in micrograms (fentanyl and etorphine) or milligrams (morphine, buprenorphine, and nalbuphine) per kilogram body weight. Ordinate: % MPE ± 1 S.E.M.
Cannabinoids and Opioid Efficacy

TABLE 1.
Group mean ED$_{50}$ values for each $\mu$-opioid receptor agonist alone or in combination with $\Delta^{2}$-THC or CP 55,940 with 54°C water and group mean potency ratios for the combinations

<table>
<thead>
<tr>
<th>Opioid Agonists</th>
<th>Control ED$_{50}$ (95% CL)$^c$</th>
<th>$\Delta^{2}$-THC (1.0 mg/kg)</th>
<th>Potency Ratio (95% CL)$^c$</th>
<th>Test ED$_{50}$ (95% CL)$^c$</th>
<th>Potency Ratio (95% CL)$^c$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Etorphine</td>
<td>0.000065 (0.000039–0.0011)</td>
<td>0.000078 (0.000020–0.00029)</td>
<td>12.43 (0.02–24.84)</td>
<td>0.000047 (0.000020–0.00010)</td>
<td>19.65 (2.57–36.73)$^d$</td>
</tr>
<tr>
<td>Fentanyl</td>
<td>0.042 (0.019–0.088)</td>
<td>0.0024 (0.00074–0.0078)</td>
<td>20.60 (6.63–34.56)$^a$</td>
<td>0.000088 (0.00036–0.0022)</td>
<td>52.99 (26.91–79.08)$^a$</td>
</tr>
<tr>
<td>Morphine</td>
<td>5.24 (4.32–6.37)</td>
<td>1.64 (1.28–2.10)</td>
<td>3.37 (2.28–4.67)</td>
<td>0.84 (0.42–1.66)</td>
<td>7.91 (2.33–13.48)$^a$</td>
</tr>
<tr>
<td>Buprenorphine</td>
<td>0.93 (0.51–2.81)</td>
<td>0.24 (0.10–0.58)</td>
<td>5.42 (3.08–8.76)</td>
<td>0.25 (0.090–0.67)</td>
<td>4.08 (2.17–8.00)$^a$</td>
</tr>
<tr>
<td>Nalbuphine</td>
<td>2.40 (0.986–59.67)</td>
<td>7.33 (1.02–52.54)</td>
<td>0.55 (0.06–1.04)</td>
<td>1.18 (0.097–14.22)</td>
<td>3.38 (1.05–8.21) $^a$</td>
</tr>
</tbody>
</table>

CL, confidence limit.

$^c$ED$_{50}$ expressed in milligrams per kilogram.

$^d$Potency ratios were calculated for individual subjects by dividing the control ED$_{50}$ by the test ED$_{50}$ and then averaged for the group.

$^a$Shifts in dose-effect curves were considered statistically significant if the 95% confidence limits of the potency ratios, averaged across monkeys, did not include 1.

Pretreatment with a dose of $\Delta^{2}$-THC or CP 55,940 that was ineffective when given alone enhanced the antinociceptive effects of several $\mu$-opioid receptor agonists, replicating studies in rodents (reviewed by Cichewicz, 2004, and Welch, 2009) and nonhuman primates (Li et al., 2008; Maguire et al., 2013). This study extends this body of research by showing that enhancement by cannabinoid receptor agonists varies with efficacy at the $\mu$-opioid receptor. Both cannabinoid receptor agonists were more effective when combined with agonists having higher efficacy at the $\mu$-opioid receptor (fentanyl and etorphine), shifting the dose-effect curves leftward as much as 50-fold, compared with agonists having lower efficacy (i.e., morphine and buprenorphine; dose-effect curves shifted leftward up to 8-fold). Neither CP 55,940 nor $\Delta^{2}$-THC significantly enhanced the effects of the low-efficacy agonist nalbuphine (Woods and Winger, 1987; Walker et al., 1993; Gerak et al., 1994; Gatch et al., 1995).

One implication of these results is that the advantage of combining cannabinoids with opioids to treat pain might be greatest with drugs having high efficacy at the $\mu$-opioid receptor. Treatments including high-efficacy opioids might have the potential to produce adverse effects; however, the use of drug combinations to treat pain would require smaller doses of a high-efficacy opioid agonist, thereby reducing the risk of adverse effects. The utility of using opioid/cannabinoid combinations for treating pain depends, in large part, on the extent to which the interactions between opioids and cannabinoids are selective for therapeutic effects. For example, despite the potential for increased therapeutic potency and possible effectiveness, the benefit of combining opioids and cannabinoids to treat pain could be undermined if combinations also increase the potential for abuse and/or dependence. However, in rhesus monkeys, neither $\Delta^{2}$-THC nor CP 55,940 (the two cannabinoids used in the current study), nor the cannabinoid receptor agonist WIN 55,212 [(R)-(+)-2,3-dihydro-5-methyl-3-(4-morpholinylmethyl)pyrrolo[1,2,3-de]-1,4-benzoxazin-6-yl]-1-naphthalenylmethanone mesylate] enhanced the discriminative stimulus effects of the $\mu$-opioid receptor agonists morphine and heroin (Li et al., 2008; Maguire et al., 2013). In addition, $\Delta^{2}$-THC, CP 55,940, and WIN 55,212 failed to increase, and in some cases attenuated, intravenous self-administration of the $\mu$-opioid receptor agonist heroin (Li et al., 2012; Maguire et al., 2013), thereby demonstrating that cannabinoid receptor agonists do not increase the positive reinforcing effects of an opioid receptor agonist (and widely abused drug) under dosing conditions similar to those used in the current study.

Taken together with this previous work, results from the current study support the use of opioid/cannabinoid combinations to treat pain, particularly with drugs having higher efficacy, insofar as the potency of opioids for antinociceptive effects is markedly increased under conditions that do not increase their potency or effectiveness in procedures that are predictive of abuse. Whether other unwanted effects of opioid receptor agonists that limit their clinical utility (e.g., respiratory depression; Calcaterra et al., 2013; Volkow et al., 2014) are similarly enhanced by cannabinoid receptor agonists and whether concurrent administration of cannabinoids alters the development of tolerance to and dependence on opioids in human or nonhuman primates is not currently known.

It is unclear whether efficacy at cannabinoid receptors also impacts these drug-drug interactions. With the exception of buprenorphine, CP 55,490 shifted the dose-effect curves of opioids farther to the left than shifts obtained with the same opioid in combination with $\Delta^{2}$-THC, and this difference between CP 55,940 and $\Delta^{2}$-THC was statistically significant for fentanyl. CP 55,940 alone produced a maximum of approximately 76% MPE in 54°C, whereas, $\Delta^{2}$-THC was less effective, producing a maximum effect of 34% MPE. The effects of 1.78 and 3.2 mg/kg of $\Delta^{2}$-THC did not differ, suggesting that increases in latency might have reached an asymptote. That CP 55,940 produced a greater maximal effect than $\Delta^{2}$-THC is consistent with in vitro (Breivogel and Childers, 2000) and in vivo studies (McMahon, 2011; Hruba et al., 2012) indicating that $\Delta^{2}$-THC has lower intrinsic efficacy relative to some other cannabinoid receptor agonists (e.g., CP 55,940). Because cannabinoid receptor agonists were compared at equi-effective doses, it appears as though efficacy at the cannabinoid receptor might also impact these drug-drug interactions.

CP 55,940 and $\Delta^{2}$-THC were differentially effective in enhancing the antinociceptive effects of $\mu$-opioid receptor agonists, and neither drug enhanced the antinociceptive effects of nalbuphine. It might be expected that cannabinoids would increase the antinociceptive effects of all $\mu$-opioid receptor agonists similarly. One possibility is that cannabinoid receptor agonists differentially alter pharmacokinetic properties of opioids, perhaps by enhancing the elimination of some (e.g., lower efficacy) agonists and/or slowing the...
elimination of other (e.g., higher efficacy) agonists. Cannabinoids can modify the pharmacokinetics of other drugs; for example, smoking marijuana increases plasma levels of cocaine in healthy volunteers (Lukas et al., 1994). However, inhalation of vaporized marijuana improved the analgesic effects of morphine and oxycodone in cancer pain patients without significantly altering plasma levels or metabolism of either opioid (Abrams et al., 2011), demonstrating that opioid/cannabinoid interactions can occur in the absence of pharmacokinetic changes. Given that many opioids, including those studied in the current experiment, are metabolized through similar mechanisms (e.g., cytochrome P450 enzymes; Feierman and Lasker, 1996; Takeda et al., 2005), it is unlikely that the cannabinoids differentially impacted metabolism across opioids.

These interactions might also involve other pharmacodynamic mechanisms. For example, it might be the case that the opioid receptor agonists, while differing in efficacy at the μ-opioid receptor, also differ in activity at other (non-μ) opioid or nonopioid receptors, and that differential effects of cannabinoid receptor agonists were due to interactions at these other (non-μ-opioid) receptors. Although nalbuphine has high affinity for μ-opioid receptors, it also has affinity, albeit much lower, for κ-opioid receptors (Butelman et al., 1998) and, under some conditions, has effects that are consistent with low efficacy at κ-opioid receptors (Miller et al., 1986; De Souza et al., 1988; Zhu et al., 1997). The antinociceptive effects of cannabinoids might be mediated, in part, by release of endogenous κ-opioid receptor ligands (Smith et al., 1994; Pugh et al., 1996); therefore, one possibility is that nalbuphine, having low efficacy at κ-opioid receptors, antagonizes the effects of a higher efficacy endogenous κ-opioid receptor ligand, thereby preventing enhancement of opioid agonist–induced antinociception by a cannabinoid. Although κ-opioid receptors reportedly contribute to the antinociceptive effects of Δ⁹-THC in rodents (Smith et al., 1994; Pugh et al., 1996), their contribution to the actions of cannabinoids in nonhuman primates is less clear. For example, at doses that block μ- and κ-opioid receptors, quazacine fails to attenuate the antinociceptive effects of Δ⁹-THC or WIN 55,212 in rhesus monkeys, suggesting that endogenous κ-opioids might not mediate the antinociceptive effects of cannabinoid receptor agonists in nonhuman primates (Vivian et al., 1998).

Cannabinoid and opioid systems interact at multiple cellular and neurochemical levels (Vigano et al., 2005; Bushlin et al., 2010; Scavone et al., 2013). Opioid receptors and cannabinoid receptors are often colocized on the same neurons (Salio et al., 2001) and interactions might occur through numerous extra- and intracellular signaling mechanisms. For example, opioid and cannabinoid systems can interact though allosteric modulation (Ríos et al., 2006), possibly through the formation of heterodimers (Mackie, 2005), or through convergence on common postreceptor signaling pathways (Shapira et al., 1998). Whether those interactions contribute to interactions between the antinociceptive effects of cannabinoids and opioids in vivo has yet to be determined. Moreover, several clinical studies have been conducted using marijuana or Δ⁹-THC (a putative low-efficacy cannabinoid receptor agonist) administered in combination with various opioids; however, no systematic study with efficacy as a factor has been reported. Based on the current results in nonhuman primates, it might be useful for future studies to explore the role of efficacy in opioid/cannabinoid combinations for treating pain in humans.

In summary, this study shows that cannabinoid receptor agonists enhance the antinociceptive effects of μ-opioid receptor agonists and demonstrates that the magnitude of the interaction is determined, in part, by efficacy at the μ-opioid receptor, with the cannabinoid receptor agonists Δ⁹-THC and CP 55,940 enhancing the antinociceptive effects of high-efficacy opioid receptor agonists more than equi-effective doses of lower efficacy opioid receptor agonists. Taken together with previous work showing that cannabinoids fail to enhance other unwanted (e.g., abuse-related) effects of opioids (Li et al., 2008, 2012; Maguire et al., 2013), these data provide further support for the use of combinations of smaller doses of cannabinoids and opioids to effectively treat pain while at the same time reducing the risks associated with larger doses of either drug administered alone.

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Authorship Contributions

Participated in research design: Maguire, France.
Conducted experiments: Maguire.
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