1521-0103/351/2/383–389$25.00
THE JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS

http://dx.doi.org/10.1124/jpet.114.216648
J Pharmacol Exp Ther 351:383–389, November 2014

Impact of Efficacy at the μ-Opioid Receptor on Antinociceptive Effects of Combinations of μ-Opioid Receptor Agonists and Cannabinoid Receptor Agonists

David R. Maguire and Charles P. France

Department of Pharmacology (D.R.M., C.P.F.) and Department of Psychiatry (C.P.F.), University of Texas Health Science Center at San Antonio, San Antonio, Texas

Received May 15, 2014; accepted September 4, 2014

ABSTRACT

Cannabinoid receptor agonists, such as Δ^9-tetrahydrocannabinol (Δ^9-THC), enhance the antinociceptive effects of μ-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 μ-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, Δ^9-THC, and CP 55,940 (2-[(1*R*,2*R*,5*R*)-5-hydroxy-2-(3-hydroxypropyl) cyclohexyl]-5-(2-methyloctan-2-yl)phenol) each increased tail withdrawal latency. Pretreatment with doses of Δ^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. Δ^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 Δ^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 μ-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 Akil 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 Δ-9-tetrahydrocannabinol (Δ^9-THC), have antinociceptive effects (see Pertwee, 2001, and Walker and Hohmann, 2005, for reviews), are increasingly used to treat pain (Hosking and Zajicek 2008; Ware et al., 2010), 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)methanone]) 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 μ-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

This work was supported by the National Institutes of Health National Institute on Drug Abuse [Grants R01-DA005018, T32-DA031115, F32-DA035605, and K05-DA17918]. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institute on Drug Abuse or the National Institutes of Health.
dx.doi.org/10.1124/jpet.114.216648.

ABBREVIATIONS: CP 55,940, 2-[(1*R*,2*R*,5*R*)-5-hydroxy-2-(3-hydroxypropyl) cyclohexyl]-5-(2-methyloctan-2-yl)phenol; JWH018, naphthalen-1-yl-(1-pentylindol-3-yl)methanone; MPE, maximum possible effect; Δ^9-THC, Δ-9-tetrahydrocannabinol; 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.

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 Δ^9-THC and CP 55,940 (2-[(1*R*,2*R*,5*R*)-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 Δ^9-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 Δ^9-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 Δ^9-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:

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

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 Δ^9-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 ED_{50} values for each drug administered alone and for combinations using linear interpolation. Only data for 54°C were analyzed because Δ^9-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

≥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's 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 Δ^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; Δ^9-THC and CP 55940 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 listed above in milligrams per kilogram body weight. Injections were 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, μ-opioid receptor agonists etorphine, fentanyl, morphine, buprenorphine, and nalbuphine (Fig. 1) and cannabinoid receptor agonists Δ^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 Δ^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). Δ^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 Δ^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 Δ^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

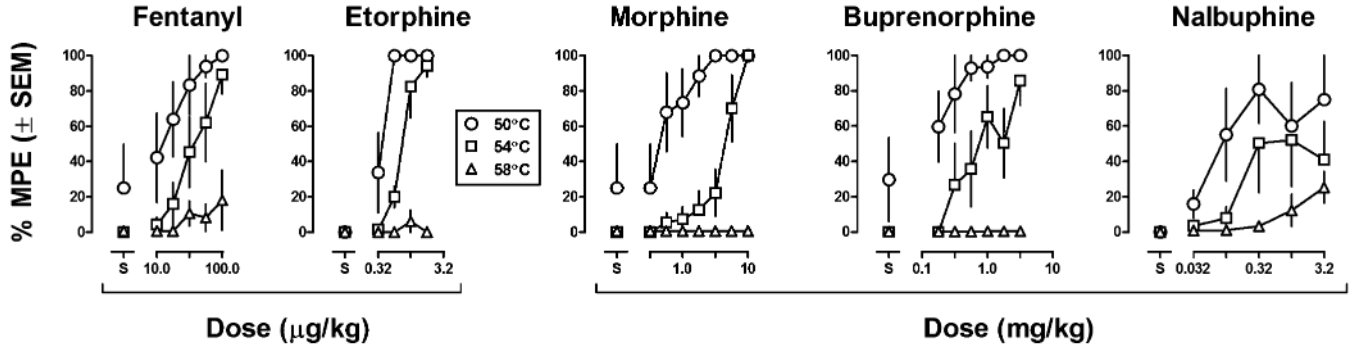

Fig. 1. Antinociceptive effects of cumulative doses of fentanyl, etorphine, morphine, buprenorphine, and nalbuphine in 50°C (s), 54°C (u), and 58°C (n) water in rhesus monkeys (n = 4 per panel). The interinjection interval was 15 minutes for all drugs. Data above "S" indicate effects after saline administration. Abscissae: 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).

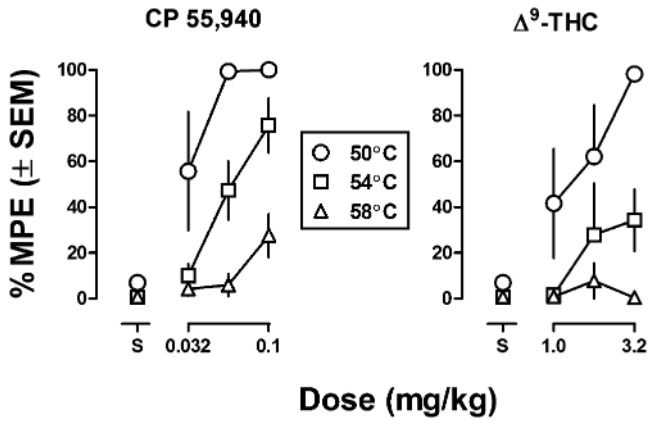

Fig. 2. Antinociceptive effects of CP 55,940 and Δ^9-THC in 50°C (s), 54°C (u), and 58°C (n) water in rhesus monkeys (n = 4 per panel). Effects were assessed during time courses studies lasting from 1 to 3 hours after acute drug injection. Data above "S" indicate effects during a time course study in 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.

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 μ-opioid receptor agonists with cannabinoid receptor agonists to treat pain. This study examined whether efficacy at the μ-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 μ-opioid receptor agonists etorphine, fentanyl, morphine, buprenorphine, and nalbuphine and the cannabinoid receptor agonists CP 55,940 and Δ^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 > 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 μ-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).

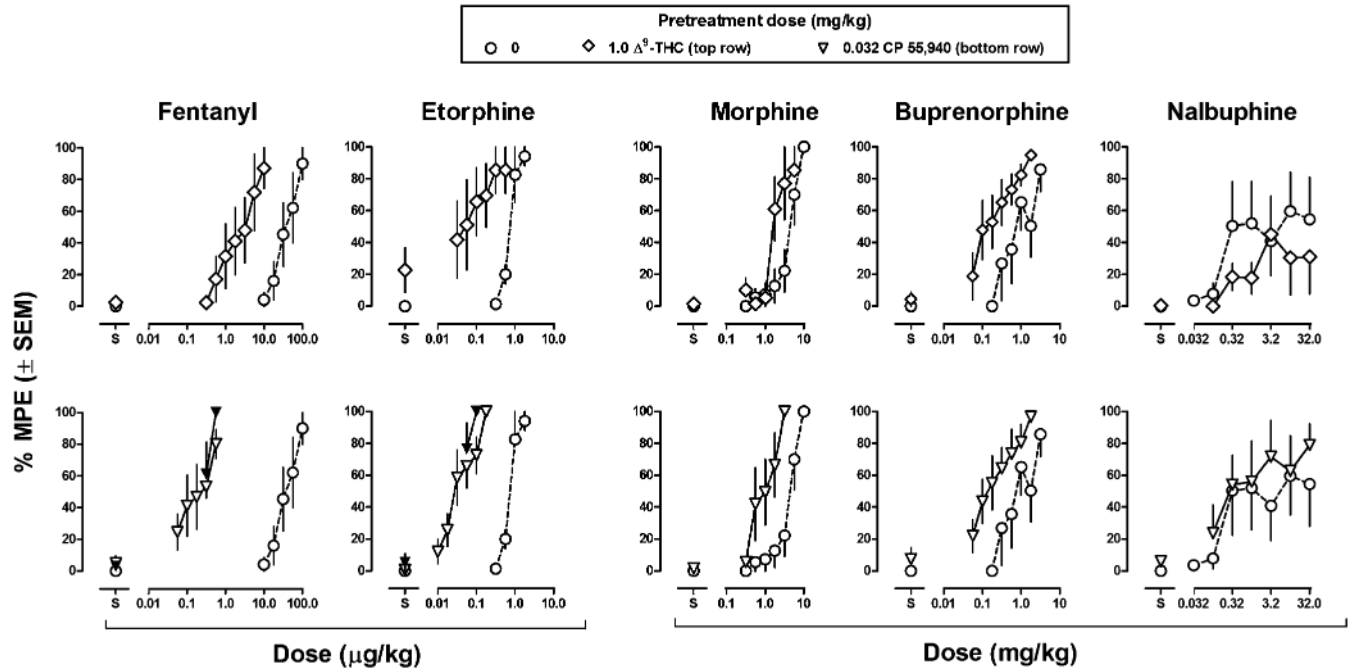

Fig. 3. Antinociceptive effects of cumulative doses of fentanyl, etorphine, morphine, buprenorphine, and nalbuphine alone (s) and after pretreatment with 1.0 mg/kg of Δ^9-THC (e , top row) or 0.032 mg/kg of CP 55,940 (, , bottom row) in 54°C water (n = 4 per drug). Filled symbols (bottom left panels) 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.

TABLE 1.
Group mean ED_{50} values for each μ-opioid receptor agonist alone or in combination with Δ^9-THC or CP 55,940 with 54°C water and group mean potency ratios for the combinations

Opioid Agonists	Control ED_{50} (95% CL)[a]	Pretreatment with a Cannabinoid Receptor Agonist			
		Δ^9-THC (1.0 mg/kg)		CP 55,940 (0.032 mg/kg)	
		Test ED_{50} (95% CL)	Potency Ratio (95% CL)[b]	Test ED_{50} (95% CL)	Potency Ratio (95% CL)[b]
Etorphine	0.00065 (0.00039–0.0011)	0.000078 (0.000020–0.00029)	12.43 (0.02–24.84)	0.000047 (0.000020–0.00010)	19.65 (2.57–36.73)[c]
Fentanyl	0.042 (0.019–0.088)	0.0024 (0.00074–0.0078)	20.60 (6.63–34.56)[c]	0.00088 (0.00036–0.0022)	52.99 (26.91–79.08)[c]
Morphine	5.24 (4.32–6.37)	1.64 (1.28–2.10)	3.37 (2.28–4.46)[c]	0.84 (0.42–1.66)	7.91 (2.33–13.48)[c]
Buprenorphine	0.93 (0.31–2.81)	0.24 (0.10–0.58)	5.42 (2.08–8.76)[c]	0.25 (0.090–0.67)	4.08 (2.17–6.00)[c]
Nalbuphine	2.40 (0.096–59.67)	7.33 (1.02–52.54)	0.55 (0.06–1.04)	1.18 (0.097–14.22)	3.58 (−1.05–8.21)

CL, confidence limit.
[a]ED_{50} expressed in milligrams per kilogram.
[b]Potency ratios were calculated for individual subjects by dividing the control ED_{50} by the test ED_{50} and then averaged for the group.
[c]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 Δ^9-THC or CP 55,940 that was ineffective when given alone enhanced the antinociceptive effects of several μ-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 μ-opioid receptor. Both cannabinoid receptor agonists were more effective when combined with agonists having higher efficacy at the μ-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 Δ^9-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 μ-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 Δ^9-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 μ-opioid receptor agonists morphine and heroin (Li et al., 2008; Maguire et al., 2013). In addition, Δ^9-THC, CP 55,940, and WIN 55,212 failed to increase, and in some cases attenuated, intravenous self-administration of the μ-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 Δ^9-THC, and this difference between CP 55,940 and Δ^9-THC was statistically significant for fentanyl. CP 55,940 alone produced a maximum of approximately 76% MPE in 54°C, whereas, Δ^9-THC was less effective, producing a maximum effect of 34% MPE. The effects of 1.78 and 3.2 mg/kg of Δ^9-THC did not differ, suggesting that increases in latency might have reached an asymptote. That CP 55,940 produced a greater maximal effect than Δ^9-THC is consistent with in vitro (Breivogel and Childers, 2000) and in vivo studies (McMahon, 2011; Hruba et al., 2012) indicating that Δ^9-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 Δ^9-THC were differentially effective in enhancing the antinociceptive effects of μ-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 μ-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 Δ^9-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, quadazocine fails to attenuate the antinociceptive effects of Δ^9-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 colocalized 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 (Rios 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 Δ^9-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 Δ^9-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.

Acknowledgments

The authors thank Christopher Cruz, Marissa McCarthy, and Jeffery Pressley for excellent technical assistance.

Authorship Contributions

Participated in research design: Maguire, France.
Conducted experiments: Maguire.
Performed data analysis: Maguire.
Wrote or contributed to the writing of the manuscript: Maguire, France.

References

Abrams DI, Couey P, Shade SB, Kelly ME, and Benowitz NL (2011) Cannabinoid-opioid interaction in chronic pain. *Clin Pharmacol Ther* **90**:844–851.
Breivogel CS and Childers SR (2000) Cannabinoid agonist signal transduction in rat brain: comparison of cannabinoid agonists in receptor binding, G-protein activation, and adenylyl cyclase inhibition. *J Pharmacol Exp Ther* **295**:328–336.
Bushlin I, Rozenfeld R, and Devi LA (2010) Cannabinoid-opioid interactions during neuropathic pain and analgesia. *Curr Opin Pharmacol* **10**:80–86.
Butelman ER, Ko M-C, Sobczyk-Kojiro K, Mosberg HI, Van Bemmel B, Zernig G, and Woods JH (1998) κ-Opioid receptor binding populations in rhesus monkey brain: relationship to an assay of thermal antinociception. *J Pharmacol Exp Ther* **285**:595–601.
Calcaterra S, Glanz J, and Binswanger IA (2013) National trends in pharmaceutical opioid related overdose deaths compared to other substance related overdose deaths: 1999–2009. *Drug Alcohol Depend* **131**:263–270.
Cichewicz DL (2004) Synergistic interactions between cannabinoid and opioid analgesics. *Life Sci* **74**:1317–1324.
Dahan A, Yassen A, Romberg R, Sarton E, Teppema L, Olofsen E, and Danhof M (2006) Buprenorphine induces ceiling in respiratory depression but not in analgesia. *Br J Anaesth* **96**:627–632.
De Souza EB, Schmidt WK, and Kuhar MJ (1988) Nalbuphine: an autoradiographic opioid receptor binding profile in the central nervous system of an agonist/antagonist analgesic. *J Pharmacol Exp Ther* **244**:391–402.
Dykstra LA and Woods JH (1986) A tail withdrawal procedure for assessing analgesic activity in rhesus monkeys. *J Pharmacol Methods* **15**:263–269.
Emmerson PJ, Clark MJ, Mansour A, Akil H, Woods JH, and Medzihradsky F (1996) Characterization of opioid agonist efficacy in a C6 glioma cell line expressing the mu opioid receptor. *J Pharmacol Exp Ther* **278**:1121–1127.
Feierman DE and Lasker JM (1996) Metabolism of fentanyl, a synthetic opioid analgesic, by human liver microsomes. Role of CYP3A4. *Drug Metab Dispos* **24**:932–939.
Finn DP, Beckett SRG, Roe CH, Madjd A, Fone KCF, Kendall DA, Marsden CA, and Chapman V (2004) Effects of coadministration of cannabinoids and morphine on nociceptive behaviour, brain monoamines and HPA axis activity in a rat model of persistent pain. *Eur J Neurosci* **19**:678–686.
Gaskin DJ and Richard P (2012) The economic costs of pain in the United States. *J Pain* **13**:715–724.
Gatch MB, Negus SS, Butelman ER, and Mello NK (1995) Antinociceptive effects of cocaine/opioid combinations in rhesus monkeys. *J Pharmacol Exp Ther* **275**:1346–1354.
Gerak LR, Butelman ER, Woods JH, and France CP (1994) Antinociceptive and respiratory effects of nalbuphine in rhesus monkeys. *J Pharmacol Exp Ther* **271**:993–999.
Gerak LR, Gauthier CR, and France CR (2003) Discriminative stimulus and antinociceptive effects of dihydroetorphine in rhesus monkeys. *Psychopharmacology (Berl)* **166**:351–359.
Gilron I, Jensen TS, and Dickenson AH (2013) Combination pharmacotherapy for management of chronic pain: from bench to bedside. *Lancet Neurol* **12**:1084–1095.

Gutstein H and Akil H (2005) Opioid analgesics, in *The Pharmacological Basis of Therapeutics* (Brunton L, Lazo J, and Parker K, eds) pp 547–590, McGraw-Hill, New York.

Hosking RD and Zajicek JP (2008) Therapeutic potential of cannabis in pain medicine. *Br J Anaesth* **101**:59–68.

Hruba L, Ginsburg BC, and McMahon LR (2012). Apparent inverse relationship between cannabinoid agonist efficacy and tolerance/cross-tolerance produced by Δ9-tetrahydrocannabinol treatment in rhesus monkeys. *J Pharmacol Exp Ther* **342**: 843–849.

Johnson JR, Lossignol D, Burnell-Nugent M, and Fallon MT (2013) An open-label extension study to investigate the long-term safety and tolerability of THC/CBD oromucosal spray and oromucosal THC spray in patients with terminal cancer-related pain refractory to strong opioid analgesics. *J Pain Symptom Manage* **46**: 207–218.

Kraft B (2012) Is there any clinically relevant cannabinoid-induced analgesia? *Pharmacology* **89**:237–246.

Li J-X, McMahon LR, Gerak LR, Becker GL, and France CP (2008) Interactions between Δ(9)-tetrahydrocannabinol and mu opioid receptor agonists in rhesus monkeys: discrimination and antinociception. *Psychopharmacology (Berl)* **199**: 199–208.

Li J-X, Koek W, and France CP (2012) Interactions between Δ(9)-tetrahydrocannabinol and heroin: self-administration in rhesus monkeys. *Behav Pharmacol* **23**: 754–761.

Lichtman AH and Martin BR (1991) Spinal and supraspinal components of cannabinoid-induced antinociception. *J Pharmacol Exp Ther* **258**:517–523.

Liguori A, Morse WH, and Bergman J (1996) Respiratory effects of opioid full and partial agonists in rhesus monkeys. *J Pharmacol Exp Ther* **277**:462–472.

Lukas SE, Sholar M, Kouri E, Fukuzako H, and Mendelson JH (1994) Marihuana smoking increases plasma cocaine levels and subjective reports of euphoria in male volunteers. *Pharmacol Biochem Behav* **48**:715–721.

Lynch ME and Clark AJ (2003) Cannabis reduces opioid dose in the treatment of chronic non-cancer pain. *J Pain Symptom Manage* **25**:496–498.

Mackie K (2005) Cannabinoid receptor homo- and heterodimerization. *Life Sci* **77**: 1667–1673.

Maguire DR, Yang W, and France CP (2013) Interactions between μ-opioid receptor agonists and cannabinoid receptor agonists in rhesus monkeys: antinociception, drug discrimination, and drug self-administration. *J Pharmacol Exp Ther* **345**: 354–362.

Manchikanti L, Helm S, 2nd, Fellows B, Janata JW, Pampati V, Grider JS, and Boswell MV (2012) Opioid epidemic in the United States. *Pain Physician* **15**(3, Suppl)ES9–ES38.

McMahon LR (2011) Chronic Δ^9-tetrahydrocannabinol treatment in rhesus monkeys: differential tolerance and cross-tolerance among cannabinoids. *Br J Pharmacol* **162**:1060–1073.

McPherson J, Rivero G, Baptist M, Llorente J, Al-Sabah S, Krasel C, Dewey WL, Bailey CP, Rosethorne EM, Charlton SJ, et al. (2010) μ-Opioid receptors: correlation of agonist efficacy for signalling with ability to activate internalization. *Mol Pharmacol* **78**:756–766.

Miller L, Shaw JS, and Whiting EM (1986) The contribution of intrinsic activity to the action of opioids in vitro. *Br J Pharmacol* **87**:595–601.

Morgan D, Cook CD, Smith MA, and Picker MJ (1999) An examination of the interactions between the antinociceptive effects of morphine and various mu-opioids: the role of intrinsic efficacy and stimulus intensity. *Anesth Analg* **88**:407–413.

Narang S, Gibson D, Wasan AD, Ross EL, Michna E, Nedeljkovic SS, and Jamison RN (2008) Efficacy of dronabinol as an adjuvant treatment for chronic pain patients on opioid therapy. *J Pain* **9**:254–264.

Nurmikko TJ, Serpell MG, Hoggart B, Toomey PJ, Morlion BJ, and Haines D (2007) Sativex successfully treats neuropathic pain characterised by allodynia: a randomised, double-blind, placebo-controlled clinical trial. *Pain* **133**:210–220.

Pertwee RG (2001) Cannabinoid receptors and pain. *Prog Neurobiol* **63**:569–611.

Preston KL and Jasinski DR (1991) Abuse liability studies of opioid agonist-antagonists in humans. *Drug Alcohol Depend* **28**:49–82.

Pugh G, Jr, Smith PB, Dombrowski DS, and Welch SP (1996) The role of endogenous opioids in enhancing the antinociception produced by the combination of delta 9-tetrahydrocannabinol and morphine in the spinal cord. *J Pharmacol Exp Ther* **279**:608–616.

Rios C, Gomes I, and Devi LA (2006) μ Opioid and CB1 cannabinoid receptor interactions: reciprocal inhibition of receptor signaling and neuritogenesis. *Br J Pharmacol* **148**:387–395.

Salio C, Fischer J, Franzoni MF, Mackie K, Kaneko T, and Conrath M (2001) CB1-cannabinoid and μ-opioid receptor co-localization on postsynaptic target in the rat dorsal horn. *Neuroreport* **12**:3689–3692.

Scavone JL, Sterling RC, and Bockstaele EJV (2013) Cannabinoid and opioid interactions: implications for opiate dependence and withdrawal. *Neuroscience* **248**: 637–654.

Schmidt WK, Tam SW, Shotzberger GS, Smith DH, Jr, Clark R, and Vernier VG (1985) Nalbuphine. *Drug Alcohol Depend* **14**:339–362.

Shapira M, Gafni M, and Sarne Y (1998) Independence of, and interactions between, cannabinoid and opioid signal transduction pathways in N18TG2 cells. *Brain Res* **806**:26–35.

Smith PB, Welch SP, and Martin BR (1994) Interactions between delta 9-tetrahydrocannabinol and kappa opioids in mice. *J Pharmacol Exp Ther* **268**:1381–1387.

Takeda S, Ishii Y, Iwanaga M, Mackenzie PI, Nagata K, Yamazoe Y, Oguri K, and Yamada H (2005) Modulation of UDP-glucuronosyltransferase function by cytochrome P450: evidence for the alteration of UGT2B7-catalyzed glucuronidation of morphine by CYP3A4. *Mol Pharmacol* **67**:665–672.

Traynor JR and Nahorski SR (1995) Modulation by mu-opioid agonists of guanosine-5′-*O*-(3-[35*S*]thio)triphosphate binding to membranes from human neuroblastoma SH-SY5Y cells. *Mol Pharmacol* **47**:848–854.

Viganò D, Rubino T, and Parolaro D (2005) Molecular and cellular basis of cannabinoid and opioid interactions. *Pharmacol Biochem Behav* **81**:360–368.

Vivian JA, Kishioka S, Butelman ER, Broadbear J, Lee KO, and Woods JH (1998) Analgesic, respiratory and heart rate effects of cannabinoid and opioid agonists in rhesus monkeys: antagonist effects of SR 141716A. *J Pharmacol Exp Ther* **286**: 697–703.

Volkow ND, Frieden TR, Hyde PS, and Cha SS (2014) Medication-assisted therapies —tackling the opioid-overdose epidemic. *N Engl J Med* **370**:2063–2066.

Walker JM and Hohmann AG (2005) Cannabinoid mechanisms of pain suppression. *Handbook Exp Pharmacol* **168**:509–554.

Walker EA, Butelman ER, DeCosta BR, and Woods JH (1993) Opioid thermal antinociception in rhesus monkeys: receptor mechanisms and temperature dependency. *J Pharmacol Exp Ther* **267**:280–286.

Walker EA, Zernig G, and Woods JH (1995) Buprenorphine antagonism of mu opioids in the rhesus monkey tail-withdrawal procedure. *J Pharmacol Exp Ther* **273**: 1345–1352.

Ware MA, Wang T, Shapiro S, Robinson A, Ducruet T, Huynh T, Gamsa A, Bennett GJ, and Collet J-P (2010) Smoked cannabis for chronic neuropathic pain: a randomized controlled trial. *CMAJ* **182**:E694–E701.

Welch SP (2009) Interaction of the cannabinoid and opioid systems in the modulation of nociception. *Int Rev Psychiatry* **21**:143–151.

Welch SP and Stevens DL (1992) Antinociceptive activity of intrathecally administered cannabinoids alone, and in combination with morphine, in mice. *J Pharmacol Exp Ther* **262**:10–18.

Woods JH and Winger G (1987) Behavioral characterization of opioid mixed agonist-antagonists. *Drug Alcohol Depend* **20**:303–315.

Zaki PA, Keith DE, Jr, Brine GA, Carroll FI, and Evans CJ (2000) Ligand-induced changes in surface μ-opioid receptor number: relationship to G protein activation? *J Pharmacol Exp Ther* **292**:1127–1134.

Zernig G, Lewis JW, and Woods JH (1997) Clocinnamox inhibits the intravenous self-administration of opioid agonists in rhesus monkeys: comparison with effects on opioid agonist-mediated antinociception. *Psychopharmacology (Berl)* **129**:233–242.

Zhu J, Luo L-Y, Li J-G, Chen C, and Liu-Chen L-Y (1997) Activation of the cloned human kappa opioid receptor by agonists enhances [35S]GTPγS binding to membranes: determination of potencies and efficacies of ligands. *J Pharmacol Exp Ther* **282**:676–684.

Address correspondence to: Dr. Charles P. France, Departments of Pharmacology and Psychiatry, University of Texas Health Science Center at San Antonio, 7703 Floyd Curl Drive, San Antonio, TX 78229-3900. E-mail: france@uthscsa.edu