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Interactions between mu opioid receptor agonists and cannabinoid receptor agonists in rhesus monkeys: antinociception, drug discrimination, and drug self-administration

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Running Title Page

Running title: Interactions between opioids and cannabinoids

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Abbreviations: Δ^9-THC, Δ^9-tetrahydrocannabinol; CP 55,940, 2-[(1R,2R,5R)-5-hydroxy-2-(3-hydroxypropyl) cyclohexyl]-5-(2-methyloctan-2-yl)phenol; 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

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Abstract

Cannabinoid receptor agonists enhance the antinociceptive effects of mu opioid receptor agonists suggesting that combinations of these drugs might enhance therapeutic effectiveness (e.g., analgesia). However, it is not clear whether combinations of these drugs also enhance abuse or dependence liability. This experiment examined whether combinations of cannabinoids and opioids that enhance antinociception also increase abuse-related effects by studying the effects of the cannabinoid receptor agonists CP 55,940 and WIN 55,212 on the antinociceptive, discriminative stimulus, and positive reinforcing effects of mu opioid receptor agonists in rhesus monkeys. In one group of monkeys (n=3), morphine (s.c., 0.1-5.6 mg/kg), CP 55,940 (s.c., 0.0032-0.032 mg/kg), and WIN 55,212 (s.c., 0.1-1.0 mg/kg) dose-dependently increased tail withdrawal latency from 50°C water, and pretreatment with small, otherwise ineffective, doses of CP 55,940 and WIN 55,212 shifted the morphine dose-effect curve to the left. In monkeys (n=3) discriminating 3.2 mg/kg morphine, CP 55,940 (s.c., 0.01-0.032 mg/kg) and WIN 55,212 (s.c., 0.1-1.78 mg/kg) attenuated the discriminative stimulus effects of morphine, shifting the dose-effect curve to the right. In monkeys (n=4) self-administering heroin (i.v., 0.32-32.0 µg/kg/infusion), CP 55,940 (s.c., 0.001-0.032 mg/kg) and WIN 55,212 (s.c., 0.1-1.0 mg/kg) shifted the heroin dose-effect curves rightward and downward. Cannabinoid receptor agonists CP 55,940 and WIN 55,212 enhanced the antinociceptive effects but not the discriminative stimulus or positive reinforcing effects of mu opioid receptor agonists in rhesus monkeys, supporting the view that combining cannabinoid and opioid receptor agonists might

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result in enhanced treatment effectiveness for pain without similarly enhancing abuse and dependence liability.

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Introduction

Pain remains a significant clinical problem (Gaskin and Richard 2012) and mu opioid receptor agonists (e.g., morphine and hydrocodone) continue to be the most effective treatment for moderate to severe pain. However, the use of opioids to treat pain is limited by unwanted effects (e.g., constipation, respiratory depression, and nausea), and the use of opioids for extended periods can result in the development of tolerance and physical dependence (e.g., Gutstein and Akil 2005; Ballantyne and Shin 2008; Manchikanti and Singh 2008). One strategy for enhancing therapeutic effectiveness of a drug is to administer it in combination with another drug that has a different mechanism of action and that shares a therapeutic effect.

Mu opioid receptor agonists and cannabinoid receptor agonists (e.g., Δ^9-tetrahydrocannabinol [Δ^9-THC]) share many behavioral and pharmacological effects including antinociception (see Walker and Hohmann 2005 for a review), and cannabinoid receptor agonists are increasingly used for treatment of pain (e.g., Hosking and Zajicek 2008). Medications including cannabinoid receptor agonists have been used to treat some types of pain (e.g., dronabinol; Narang et al., 2008). However, the effectiveness of cannabinoids alone can be modest, and the doses required to achieve therapeutically relevant effects can have unwanted effects, thus limiting their clinical utility (Kraft 2012). Despite these limitations, combining opioids and cannabinoids to treat pain continues to be a promising therapeutic approach. Drug combinations allow for the possibility that smaller doses of individual drugs can be combined to maintain or improve the therapeutic effects while reducing the likelihood of encountering the

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adverse effects associated with larger doses of either drug administered alone (Smith 2008).

Preclinical research supports combining mu opioid receptor agonists and cannabinoid receptor agonists to treat pain. For example, in rodents, Δ^9-THC has robust antinociceptive effects (e.g., Welch and Stevens 1992; Smith et al. 1998), and acute administration of Δ^9-THC enhances the effectiveness of mu opioid receptor agonists (e.g., morphine, codeine, or fentanyl [see Pertwee 2001; Cichewicz 2004; Welch 2009 for reviews]). Similarly, morphine enhances Δ^9-THC-induced antinociception in mice (Reche et al. 1996; Smith et al. 1998). Moreover, in rhesus monkeys, Δ^9-THC and other cannabinoid receptor agonists (e.g., WIN 55,212) have antinociceptive effects when administered alone (Vivian et al. 1998; Ko and Woods 1999; Manning et al. 2001), and acute administration of Δ^9-THC enhances the antinociceptive effects of morphine (Li et al. 2008).

Although current behavioral and pharmacological evidence indicates that combining cannabinoid receptor agonists with mu opioid receptor agonists might be an especially useful strategy to enhance antinociceptive effects, less is known about interactions between these drugs with regard to effects that are predictive of abuse. Opioid abuse continues to be a significant public health problem (e.g., Compton and Volkow 2006). Cannabinoids (e.g., marijuana) are also abused and might confer physical dependence as a consequence of repeated use (e.g., Lichtman and Martin 2005). Despite the potential for increased therapeutic effectiveness, the benefit of combining opioids and cannabinoids to treat pain could be undermined by the perceived risk of enhancing unwanted effects, especially if combinations increase abuse. Some

studies indicate that Δ^9-THC increases the reinforcing and discriminative stimulus effects of opioids in rodents (Norwood et al. 2003; Manzanedo et al. 2004; Solinas et al. 2005); however, in rhesus monkeys, Δ^9-THC fails to enhance and can attenuate the discriminative stimulus effects of morphine and heroin (Li et al. 2008). Moreover, acute administration of Δ^9-THC does not enhance and, at large doses, suppresses heroin self-administration (Li et al., 2012). Inconsistencies in the reported interactions between cannabinoids and opioids could be the consequence of numerous differences across studies related to species, drug, dose, or experimental history. Nevertheless, it appears as though in human and non-human primates Δ^9-THC enhances the antinociceptive effects of morphine across a range of doses that fail to enhance and possibly attenuate the discriminative stimulus and reinforcing effects of mu opioid receptor agonists, suggesting that combining cannabinoids and opioids might not increase abuse liability.

The current study examined interactions between mu opioid receptor agonists and cannabinoid receptor agonists by studying the effects of cannabinoid receptor agonists on the antinociceptive, discriminative stimulus, and reinforcing effects of mu opioid receptor agonists in rhesus monkeys. These studies extend previous research (Li et al. 2008; 2012) by examining interactions of opioids with two cannabinoid receptor agonists, CP 55,940 and WIN 55,212, that have overlapping but not identical (e.g., greater efficacy at cannabinoid receptors; Breivogel and Childers 2000) pharmacology as compared to Δ^9-THC. Moreover, the current experiment investigated the mechanism(s) underlying such interactions by assessing the ability of the cannabinoid receptor antagonist rimonabant to attenuate the effects of CP 55,940 and WIN 55,212 on morphine discrimination and heroin self-administration.

Materials and Methods

Animals. Ten adult rhesus monkeys (*Macaca mulatta*) served as subjects. Body weight (range: 5 to 11 kg) was maintained via post-session feeding (Harlan Teklad, High Protein Monkey Diet, Madison, WI, USA). Monkeys also received fresh fruit and peanuts daily; water was continuously available in the home cage. Subjects were housed individually in a colony room and under a 14/10-hr light/dark cycle (lights on at 0600 hr). Animals used in these studies were maintained in accordance with the Institutional Animal Care and Use Committee, The University of Texas Health Science Center at San Antonio, and 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 in the antinociception experiment 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 stop watch. Monkeys participating in the morphine discrimination and heroin self-administration experiments were seated in primate chairs (Model R001, Primate Products) and positioned in sound-attenuating operant conditioning chambers containing two horizontally aligned response levers located approximately 32 cm apart. Located above each lever were circular, translucent disks that could be trans-illuminated green or red. Extraneous sounds were masked by white noise and an exhaust fan. Experimental events were arranged and data were recorded by an interface (Med Associates, Inc., East Fairfield, VT) connected to a PC computer running

Med-PC IV software (Med Associates, Inc.). During drug discrimination sessions, feet were placed in shoes containing brass electrodes to which a brief (250 ms, 3 mA) electric stimulus could be delivered from a remote current generator (H13-15, Coulbourn Instruments, Allentown, PA). During drug self-administration sessions, infusions were delivered i.v. by connecting a s.c. vascular access port (Access Technologies, Skokie, IL) to a 185-cm extension set (Abbott Laboratories, Stone Mountain, GA) via a 20-g Huber-point needle (Access Technologies). The opposing end of the extension set was connected to a 30-ml syringe that was mounted in a syringe driver (Razel Scientific Instruments, Inc., Stamford, CT) located outside the chamber.

Warm water tail withdrawal. One male (JO) and two female (SA and ME) monkeys were studied in the warm water tail withdrawal study. The lower portion (approximately 15 cm) of the shaved tail was inserted into a mug containing 40, 50, or 55ºC 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 sec, the mug was removed and the tail withdrawal latency was recorded as 20 sec (maximum effect). Each temperature was tested once every 15 min (once per cycle) and temperatures were tested in random order across consecutive tests and across monkeys. Sessions comprised 8 cycles and began with one saline cycle followed by cumulative doses of morphine across successive cycles; the dose of morphine was increased in 0.25-log unit increments across cycles. Test sessions in which a morphine dose-effect curve was determined ended after 8 cycles or when the tail withdrawal latency reached 20 sec with 50 ºC water, whichever occurred first. When tested in

combination with morphine, WIN 55,212 was administered during the first cycle (substituted for saline) and CP 55,940 was administered 60 min prior to the first cycle.

Morphine discrimination. Three female monkeys (CI, AM, and HA) were trained previously to discriminate morphine from saline during daily sessions; each of 2 to 8 cycles comprised a 10-min timeout period followed by a 5-min response period. During the timeout period, all lights were off and responding had no programmed consequence. Injections occurred during the first minute of each timeout period. The beginning of the response period was signaled by illumination of both side key lights red, in the presence of which a brief electric stimulus was scheduled to be delivered every 45 sec. Thirty consecutive responses on the correct lever (determined by the training or testing condition as described below) extinguished the red lights and suspended the schedule of electric stimulus presentation; after 30 sec the red key lights were illuminated and the electric stimulus presentation schedule was restarted. Responses on the incorrect lever reset the response requirement on the correct lever and otherwise had no programmed consequence. Response periods lasted either until 5 min elapsed or until 4 electric stimuli were delivered whichever occurred first.

During training sessions, levers were designated as correct based on the injection given during the first minute of the cycle. For two monkeys (CI and HA), the left lever was designated as correct following saline injections; conversely, the right lever was designated correct following injections of 3.2 mg/kg (s.c.) morphine (i.e., training dose). For monkey AM the contingencies were reversed. Individual monkeys were trained under the same lever designations for the entire experiment. During saline training sessions, saline or sham (a capped needle was pressed firmly against the back)

injections were administered prior to 2 to 6 cycles. During morphine training sessions, the training dose of morphine was administered at the beginning of one cycle that was preceded by 0 to 5 saline or sham training cycles. The number of saline or sham cycles preceding the morphine training cycle varied quasi-randomly across sessions.

All monkeys in this study had previously met the initial testing criteria which were 5 consecutive or 6 of 7 sessions in which at least 80% of total responses occurred on the correct (injection-appropriate) lever and fewer than 30 responses occurred on the incorrect lever prior to the first reinforcer of the cycle. Thereafter, monkeys were tested when the training criteria were met for two consecutive sessions consisting of one drug training session and one saline training session. Test sessions were identical to training sessions except that completion of the ratio requirement on either lever was reinforced.

Morphine dose-effect curves were determined under test conditions using a cumulative dosing method. Saline was administered during the first cycle of the session; thereafter, increasing doses of morphine (in 0.25-log unit increments) were administered across successive cycles until at least 80% of total responses in a cycle occurred on the drug-associated lever, four electric stimuli were delivered, or 8 cycles were completed, whichever occurred first. When tested in combination with morphine, CP 55,940 was administered s.c. 60 min prior to determination of a morphine dose-effect curve and WIN 55,212 was administered during the first cycle of the session. After the effects of CP 55,940 and WIN 55,212 were studied alone and in combination with morphine, selected doses of each cannabinoid receptor agonist were studied in combination with morphine (dose-effect curve determination) and the cannabinoid receptor antagonist rimonabant (1.0 mg/kg). Rimonabant was always administered 45

min prior to the session. Control dose-effect curves (morphine alone) were determined immediately prior to and following a series of tests with both CP 55,940 and WIN 55,212.

Heroin self-administration. Two male (PE and NA) and two female (BE and MA) monkeys participated in the self-administration study. Monkeys were initially sedated with 10 mg/kg (s.c.) ketamine and anesthesia was maintained by isoflurane throughout surgery. After being placed in either the jugular or femoral vein, the polyurethane catheter (5-Fr, SIMS Deltec Inc., St. Paul, MN) was tunneled s.c. to the midscapular region where it was attached to a s.c. access port. During daily sessions, monkeys lever-pressed under a fixed-ratio 30 schedule for i.v. infusions of either heroin or saline. Sessions comprised four 25-min cycles. Each cycle began with a 5-min timeout period during which all lights were extinguished and responding had no programmed consequence, followed by a 20-min response period. Each response period began with a priming infusion of the unit dose of heroin that was available for self-administration in that component. During the response period, a green light located above the active lever (right lever for MA and BE, and left lever for PE and NA) was illuminated, and 30 responses on the active lever turned the lever light red for 2 sec, activated the syringe pump, and initiated a 180-sec timeout, during which all lights were extinguished. Responses on the active lever during timeouts and on the inactive lever at any time were recorded but had no programmed consequence. A maximum of 7 infusions could be obtained per cycle.

Heroin dose-effect curves were determined within single sessions; multiple unit doses of heroin (0.32-32.0 µg/kg/infusion) were available within a single session, and a

different unit dose was available in each cycle. Doses were presented in ascending order, and the range of doses of heroin was adjusted for individual monkeys to include doses comprising the ascending limb of the dose-effect curve; the unit dose of heroin was altered across cycles by varying the infusion duration which ranged from 0.6 to 55 sec. Once a range of doses was identified for each monkey, that range remained constant for a particular monkey for the remainder of the experiment. Heroin dose-response curves were considered stable when the average number of infusions received in each of the four cycles did not vary by more than ± 1.5 infusions as compared to the number of infusions obtained in the corresponding cycle for three consecutive sessions. After stable heroin dose-effect curves were established, the effects of CP 55,940 were determined first by administering the drug s.c. 60 min prior to the beginning of a heroin self-administration session. Tests occurred no more frequently than once every 4 sessions and only when responding was stable (see above). The effects of WIN 55,212 were determined in the same fashion; however, WIN 55,212 was administered s.c. immediately prior to the beginning of the self-administration session. After the effects of CP 55,940 and WIN 55,212 on heroin self-administration were studied, selected doses of the cannabinoid agonist were studied in combination with rimonabant (1.0 mg/kg) prior to a heroin self-administration session. Rimonabant was administered 45 min prior to the session.

Data Analysis. For the antinociception study, tail withdrawal latencies were expressed as a percentage of maximal possible effect (MPE) according to the following formula: % MPE = [(test latency – control latency)/(20 sec – control latency)] x 100. Control latencies were determined in the absence of drug. The mean (± SEM) MPE was

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calculated for each agonist dose administered alone and in combination and plotted as a function of dose of morphine. For the discrimination studies, the dependent measures were the distribution of responses between the two levers and response rate. For each cycle, the total number of responses on the drug lever was divided by the total number of responses on either lever and multiplied by 100 to yield the percentage of drug lever responding. Response rate was calculated by dividing the total number of responses by the duration of the response period excluding timeouts. The mean (± SEM) percent drug-lever responding and response rate were calculated for each dose of morphine alone and in combination with test compounds and plotted as a function of dose of morphine. Control dose-effect curves for morphine antinociception and discrimination represent the average of two determinations. Tests with CP 55,940, WIN 55,212, and rimonabant were determined once. For antinociception and discrimination studies, potency was estimated by calculating the dose required to produce 50% of the maximal response (ED_{50}) for individual monkeys by fitting straight lines to dose-response data. Only the linear portion of the curve was fit; this portion of the curve ranged from the largest dose that produced less than 20% to the smallest dose that produced more than 80% of the maximum effect. For self-administration studies, the mean (± SEM) number of infusions received per component was plotted as a function of unit dose of heroin. Control heroin dose-effect curves represent the average (± SEM) number of infusions obtained for the three consecutive sessions immediately prior to the first tests during which responding was deemed stable. All curve fits and analyses were conducted using GraphPad Prism (5.0, GraphPad Software, Inc., San Diego, CA).

Drugs. Morphine sulfate, heroin hydrochloride, and rimonabant were provided by the National Institute on Drug Abuse (Research Technology Branch, Rockville, MD). CP 55,940 was purchased from Sigma-Aldrich (St. Louis, MO) and WIN 55,212 was purchased from Tocris (Ellisville, MO). Morphine was dissolved in sterile water and heroin was dissolved in 0.9% saline. Rimonabant, CP 55,940, and WIN 55,212 were dissolved in a 1:1:18 mixture of absolute ethanol, Emulphor-620 (Rhone-Poulenc Inc., Princeton, NJ) and 0.9% saline. All doses are expressed as the salt, and all drugs except heroin were administered s.c. in the back in a volume of 0.2 to 0.8 ml. Heroin was administered i.v. during self-administration studies.

Results

Under control conditions the average tail withdrawal latencies (mean ± SEM) for 40, 50, and 55°C water were 20 ± 0, 2.52 ± 0.28, and 1.12 ± 0.01 sec, respectively. For all monkeys, doses of CP 55,940 up to and including 0.01 mg/kg and doses of WIN 55,212 up to an including 0.32 mg/kg failed to increase tail withdrawal latencies above control levels in 50°C water; ED_{50} values were 0.018 (SA), 0.011 (ME), 0.021 (JO) mg/kg for CP 55,940 and 0.35 (SA), 0.37 (ME), and 0.52 (JO) mg/kg for WIN 55,212 (data above “S”, Fig 1). Morphine dose-dependently increased tail withdrawal latency in 50°C water (open circles, Fig 1; ED_{50} = 0.51, 0.70, 2.57 for monkeys SA, ME, JO, respectively). Pretreatment with 0.01 mg/kg CP 55,940 (inverted triangles, top panels, Fig 1), a dose that was ineffective in all monkeys when administered alone, reduced the ED_{50} for morphine to 0.35 (SA), 0.14 (ME) and 0.19 (JO) mg/kg, resulting in a 1.6 (SA)-, 5.2 (ME)-, and 13.4 (JO)-fold leftward shift in the morphine dose-effect curve. For monkey JO pretreatment with 0.0178 mg/kg CP 55,940 (stars, top panels, Fig 1) did not

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increase tail withdrawal latency when administered alone and reduced the ED_{50} for morphine to 0.11 mg/kg, resulting in a 23.1-fold leftward shift. Likewise, pretreatment with 0.32 mg/kg WIN 55,212 (upright triangles, bottom panels, Fig 1), a dose that was ineffective when administered alone, reduced the ED_{50} for morphine to 0.14 (SA), 0.11 (ME), and 0.47 (JO) mg/kg, resulting in a 3.9 (SA)-, 7.3 (ME)-, and 5.4 (JO)-fold leftward shift in the morphine dose-effect curve. In one monkey (ME), 0.1 mg/kg WIN 55,212 (squares) also shifted the morphine dose-effect curve 6.6-fold to the left.

In monkeys trained to discriminate 3.2 mg/kg morphine, saline occasioned less than 10% responding on the drug lever (Fig 2, data above “S”). Morphine dose-dependently increased drug-lever responding (Fig 2, circles) with 3.2 (CI), 0.56 (HA), and 1.0 (AM) mg/kg occasioning greater than 80% responding on the drug lever. The ED_{50} values for morphine control dose-effect curves were 1.72 (CI), 0.46 (HA), and 0.69 (AM) mg/kg. CP 55,940 alone did not occasion drug-lever responding in any monkey (top panel, Fig 2, data above “S”) and dose-dependently shifted the morphine dose-effect curve rightward up to 6.6- (AM) and 5.7- (HA) fold and, in some cases, flattened the curve (CI; larger doses were not studied). Often rightward shifts in the discrimination dose-effect curves occurred in the absence of substantial decreases in response rate (Table 1) with the exception on 0.032 mg/kg in monkey AM. When administered alone, rimonabant (1.0 mg/kg) had no effect on the morphine dose-effect curve for any monkey (data not shown) and attenuated the effects of CP 55,940, resulting in a leftward shift in the discrimination dose-effect curve back to near control (solid symbols). Like CP 55,940, WIN 55,212 did not occasion drug-lever responding in any monkey (bottom panel, Fig 2, data above “S”) and dose-dependently shifted the morphine dose-effect

curve rightward up to 2.5-fold (AM) and downward (HA and CI). Shifts in the discrimination dose-effect curves occurred in the absence of substantial decreases in response rate (Table 2). Rimonabant (1.0 mg/kg; filled symbols) attenuated the effects of WIN 55,212 on the morphine discrimination dose-effect curves in two monkeys (see 0.32 mg/kg in HA and 1.78 mg/kg in AM), indicated by leftward shifts in the dose-effect curves, but was ineffective in preventing the effects of WIN 55,212 in monkey CI.

In all monkeys self-administering heroin, the number of infusions obtained per cycle increased as the unit dose of heroin increased (open circles, Fig 3). When saline was available (open circles above "S"), the mean (± SEM) number of infusions obtained for all four cycles of the session was 1.9 ± 0.4 (NA), 1.4 ± 0.3 (PE), 0.8 ± 0.2 (BE), and 0.8 ± 0 (MA) and mean response rates were 0.06 ± 0.02 (NA), 0.05 ± 0.01 (PE), 0.03 ±0.01 (BE), 0.03 ± 0 (MA) responses per sec (data not shown). The largest number of infusions was obtained and the highest response rates were maintained at a unit dose of 3.2 µg/kg/infusion for 2 monkeys (PE and MA) and a unit dose of 10 µg/kg/infusion for 2 other monkeys (NA and BE) with the highest average number of infusions ranging from 4.7 ± 0.3 (MA) to 6.0 ± 0 (NA and PE) infusions and response rates ranging from 0.4 ± 0.1 (MA) to 4.4 ± 0.1 (NA) responses per sec.

CP 55,940 dose-dependently decreased heroin self-administration in all four monkeys (top panels, Fig 3), shifting heroin dose-effect curves rightward in two monkeys (NA and PE) and downward in two monkeys (BE and MA). Monkey NA was most sensitive to the effects of CP 55,940; 0.0032 mg/kg (upright triangles) reduced the number of infusions obtained per cycle at the three largest unit doses (3.2, 10, and 32 µg/kg/infusion). For monkeys PE and MA, 0.01 mg/kg CP 55,940 (inverted triangles)

reduced the number of infusions obtained per cycle at intermediate unit doses of heroin (3.2 and 10 µg/kg/infusion). For monkey BE, 0.01 mg/kg CP 55,940 had no effect on the number of infusions received; however, 0.032 mg/kg (diamonds) reduced the number of infusions obtained per cycle at each of the three highest unit doses (3.2, 10, and 32 µg/kg/infusion). That same dose of CP 55,940 increased the number of infusions obtained at the lowest unit dose of heroin (1.0 µg/kg/infusion) from 0.7 on average to 3.0 infusions. Pretreatment with 1.0 mg/kg rimonabant alone did not impact heroin self-administration in any monkey (data not shown) and attenuated the effects of CP 55,940 when administered in combination with 0.0032 (NE), 0.01 (PE and MA), and 0.032 (BE) mg/kg, in each case, resulting in heroin dose-effect curves that were similar to those obtained prior to treatment (filled symbols).

WIN 55,212 also dose-dependently reduced heroin self-administration in all four monkeys, resulting in substantial rightward (NA and PE) and downward (BE and MA) shifts in the heroin dose-effect curves (bottom panels, Fig 3). For two monkeys (NA and ME), doses of 0.32 (NA and ME) and 1.0 (PE and BE) mg/kg generally reduced the number of infusions received of all unit doses of heroin and in no case increased the number of infusions received. For all four monkeys, 1.0 mg/kg rimonabant (filled symbols) attenuated the effects of WIN 55,212 on heroin self-administration when administered in combination with effective doses of WIN 55,212 (0.32 mg/kg for NE and MA and 1.0 mg/kg for PE and BE), resulting, in each case, in heroin dose-effect curves similar to those obtained prior to treatment.

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Discussion

Pain remains a significant public health problem, and despite their widespread use for treating pain, opioids are limited by unwanted effects, ineffectiveness in some patients, and the development of physical dependence following repeated administration. Consequently, there is an unmet need for more effective pain treatments. Cannabinoid and opioid systems interact at cellular, neurochemical, and behavioral levels and some interactions are likely mediated by common pathways (e.g.,Vigano et al. 2005; Bushlin et al. 2010) that might be important for developing novel therapeutics that target both systems. Cannabinoid receptor agonists have robust antinociceptive effects in nonhumans (see Pertwee 2001; Welch 2009 for reviews), but their effectiveness in humans is unclear (Kraft 2012). One strategy for enhancing therapeutic effectiveness is to combine cannabinoids with other drugs. In several species, Δ^9-THC enhances the antinociceptive effects of opioids (Welch and Stevens 1992; Finn et al. 2004; Li et al. 2008). Moreover, supplementing ongoing opioid pain treatments with cannabinoids (e.g., dronabinol) can increase treatment effectiveness (e.g., Narang et al., 2008). However, because opioids (e.g., prescription analgesics) and cannabinoids (e.g., marijuana) are abused individually, there is some concern that combining opioids and cannabinoids might increase abuse. The current study examined interactions between mu opioid receptor agonists and cannabinoid receptor agonists by studying the effects of cannabinoid receptor agonists CP 55,940 and WIN 55,212 on the antinociceptive, discriminative stimulus, and reinforcing effects of mu opioid receptor agonists morphine and heroin in rhesus monkeys.

Morphine, CP 55,940, and WIN 55,212 dose-dependently increased tail withdrawal latency from 50°C water (e.g., Dykstra et al. 1986; Vivian et al. 1998). Pretreatment with doses of CP 55,940 and WIN 55,212 that did not increase tail withdrawal latency when tested alone shifted the morphine dose-effect curve leftward, consistent with effects in rodents (e.g., Welch and Stevens 1992; Smith et al. 1998; Miller et al. 2012) and rhesus monkeys (Li et al. 2008). Given that CP 55,940 and WIN 55,212 increased the antinociceptive effects of morphine, it might be expected that they would similarly enhance other (e.g., discriminative stimulus and positive reinforcing) effects of mu opioid receptor agonists. To the contrary, CP 55,940 and WIN 55,212 shifted the morphine discriminative stimulus and heroin self-administration dose-effect curves rightward and downward. The mechanisms underlying the differential effects of CP 55,940 and WIN 55,212 in these studies (i.e., enhancing some but not other effects of opioids) remain unclear. Differences in interactions are not likely due to pharmacokinetics since the routes of administration and pretreatment times for morphine and cannabinoid receptor agonists were consistent across studies. Moreover, it is clear from the consistent rank order potency of agonists and from quantitatively consistent antagonism studies that these behavioral effects of morphine and heroin are mediated by mu opioid receptors (e.g., Bowen et al. 2002; Gerak et al. 1994).

Opioid receptor agonists and cannabinoid receptor agonists individually can have robust and pharmacologically distinct discriminative stimulus effects. Mu opioid receptor agonists (e.g., morphine and hydromorphone) fail to occasion drug-appropriate responding in rhesus monkeys (e.g., Wiley et al. 1995a; McMahon 2006; Li et al. 2008) or humans (Lile et al. 2008) discriminating Δ^9-THC. Similarly, Δ^9-THC does not occasion

drug-appropriate responding in rhesus monkeys discriminating morphine (e.g., Li et al. 2008). Consistent with these findings, neither CP 55,940 nor WIN 55,212 occasioned drug-appropriate responding in monkeys discriminating morphine. However, CP 55,940 and WIN 55,212 dose-dependently shifted the morphine discriminative stimulus dose-effect curve rightward and did so at doses that generally did not impact rates of responding. Attenuation of the discriminative stimulus effects of morphine could result from pharmacological antagonism or some other mechanism (e.g., physiological antagonism). Given that CP 55,940 and WIN 55,212 did not attenuate the antinociceptive effects of morphine and that mu receptors mediate antinociceptive and discriminative stimulus effects of morphine, pharmacological antagonism seems an unlikely mechanism. Rather, CP 55,940 and WIN 55,212 might reduce the discriminability of morphine which necessitates an increase in the dose of morphine required for drug-appropriate responding. Masking is a phenomenon whereby one stimulus interferes with the ability of the subject to detect another stimulus; CP 55,940 and WIN 55,212 might mask the discriminative stimulus effects of morphine (see Li et al. 2008). Consistent with this view, the discriminative stimulus effects of morphine can be attenuated by non-opioid drugs (e.g., amphetamine; Gauvin and Young 1989) and Δ^9-THC attenuates the discriminative stimulus effects of non-cannabinoid drugs (e.g., phencyclidine; Doty et al. 1994). Thus, CP 55,940 and WIN 55,212 might attenuate the discriminative stimulus effects of morphine through perceptual (i.e., non-pharmacologic) mechanisms. Whether this interaction between opioids and cannabinoids is due to masking and whether such attenuation of drug effects has implications for drug abuse

and drug abuse treatment (i.e., reduction in subjective effects) remains to be determined.

Cannabinoid receptor agonists have positive reinforcing effects under some conditions (e.g., Tanda et al. 2000) and can enhance the positive reinforcing effects (self-administration) of heroin in rats (e.g., Δ^9-THC and WIN 55,212; Solinas et al. 2005). In the current study, neither CP 55,940 nor WIN 55,212 enhanced heroin self administration; rather, both agonists decreased the number of heroin infusions received, shifting the heroin dose-effect curve rightward and downward. It is unclear whether reductions in heroin self-administration are due specifically to attenuation of the positive reinforcing effects since decreased self-administration could be result from several factors. For example, as in the discrimination study, it might be the case that CP 55,940 and WIN 55,212 attenuate the discriminative stimulus effects of heroin that occasion reliable self-administration. Alternatively, decreases in heroin self-administration could be due to nonselective suppression of responding. Doses of CP 55,940 (0.0032-0.032 mg/kg) and WIN 55,212 (0.32-1.0 mg/kg) that decreased heroin self-administration did not markedly reduce rates of responding maintained by shock avoidance (current discrimination study) whereas those doses decrease rates of responding maintained by other reinforcers (e.g., food) in rhesus monkeys (McMahon 2011).

The failure of CP 55,940 and WIN 55,212 to enhance the reinforcing effects of heroin might be a consequence of the manner in which drug combinations were studied. Contingency of drug administration can impact the behavioral effects of some drugs (e.g., Lecca et al. 2007) and it is possible that allowing monkeys to self-administer CP 55,940 and WIN 55,212 each in combination with heroin would have enhanced the

reinforcing effects of heroin. However, in a previous study (Li et al. 2012) in which rhesus monkeys could self-administer Δ^9-THC in combination with heroin, the combinations resulted in an overall decrease in responding and in the amount of drug self-administered with no evidence of enhancement. Although CP 55,940 and WIN 55,212 were administered response-independently in the current study, based on previous work it appears unlikely that these cannabinoid receptor agonists would enhance the reinforcing effects of heroin in rhesus monkeys under other conditions.

Rimonabant (1.0 mg/kg) completely blocked the effects of CP 55,940 and WIN 55,212 on the discriminative stimulus effects of and morphine on the reinforcing effects of heroin, confirming that these effects of CP 55,940 and WIN 55,212 are mediated by CB1 receptors (e.g., Wiley et al. 1995b; McMahon 2006). When administered alone the same dose of rimonabant had no effect on the discriminative stimulus effects of morphine or heroin self-administration. In rats, rimonabant decreases heroin self-administration that is maintained under a progressive ratio schedule (Solinas et al. 2003); conversely, the mu opioid receptor antagonist naltrexone attenuates the discriminative stimulus effects of Δ^9-THC in rats (Solinas and Goldberg 2005). Taken together, these studies demonstrate that in rodents there is a significant interaction between cannabinoid and opioid systems with regard to the discriminative stimulus and reinforcing effects of cannabinoid receptor and mu opioid receptor agonists (see Solinas et al. 2007 for review). However, the nature of interaction between cannabinoid and opioid systems in human and nonhuman primates is less clear. Naltrexone has inconsistent effects on the subjective and physiological effects of Δ^9-THC in humans (Greenwald and Stitzer 2000; Haney et al. 2003; Haney 2007). Moreover, Δ^9-THC

attenuates the discriminative stimulus effects of morphine and heroin (Li et al. 2008) and reduces heroin self-administration (Li et al. 2012) in rhesus monkeys.

In summary, acute administration of the cannabinoid receptor agonists CP 55,940 and WIN 55,212 enhanced the antinociceptive but not the discriminative stimulus or positive reinforcing effects of the mu opioid receptor agonists morphine and heroin. Taken together with previous work (e.g., Li et al. 2008; 2012), these data indicate that combinations of cannabinoids and opioids might enhance therapeutic effectiveness of opioids without similarly enhancing their abuse liability; the current study also demonstrates that these interactions are likely mediated through CB1 receptors. Moreover, in the current and in previous studies cannabinoid receptor agonists robustly attenuated the discriminative stimulus and reinforcing effects opioids. This suggests that administration of cannabinoids might attenuate the subjective effects of opioids, and thereby reduce effects related to abuse liability but not the therapeutic effects (e.g., antinociception). It is important to determine whether the nature of these drug interactions are the same or different during the course of chronic treatment.

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Footnotes

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Legends for Figures

Fig 1 Effects of CP 55,940 (top panels) and WIN 55,212 (bottom panels) on the antinociceptive effects of morphine for each of three rhesus monkeys. The mean (± SEM) tail withdrawal latency in 50°C water, expressed as a percentage of the maximum possible effect (%MPE; see Data Analysis), is plotted as a function of the dose (mg/kg) of morphine. "S" indicates data that were collected when saline was administered during the first test cycle.

Fig 2 Effects of CP 55,940 (top panels) and WIN 55,212 (bottom panels) on the discriminative stimulus effects of morphine for each of three rhesus monkeys. The mean (± SEM) percentage of responses on the drug-associated lever is plotted as a function of the dose (mg/kg) of morphine. "S" indicates data that were collected when saline was administered during the first test cycle. Filled symbols indicate when rimonabant (1.0 mg/kg) was also administered.

Fig 3 Effects of CP 55,940 (top panels) and WIN 55,212 (bottom panels) on heroin self-administration for each of four rhesus monkeys. The mean (± SEM) number of infusions received per cycle is plotted as a function of the unit dose (µg/kg/infusion) of heroin available during that cycle. "S" indicates data that were collected during sessions when saline was available for self administration. Filled symbols indicate when rimonabant (1.0 mg/kg) was also administered.

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Table 1: Effects of morphine and CP 55,940 alone and in combination on response rate (responses/sec) in monkeys trained to discriminate 3.2 mg/kg morphine from saline.

			Morphine (mg/kg)							
Monkey	CP 55,940 (mg/kg)	Rimonabant (mg/kg)	0	0.32	0.56	1	1.78	3.2	5.6	10
CI	0	0	2.8 (0)[a]	2.7[b]	2.6 (0.2)	2.8 (0.2)	3.3 (0.1)	3.2[b]		
	0.001	0	2.8	2.5	2.6	2.8	2.8	3.0	3.6	
	0.0032	0	2.9		2.9	3.1	3.0	3.1	3.0	2.8
	0.0032	1.0	2.5	2.4	2.5	2.6	2.9	3.4		
HA	0	0	3.1 (0.4)	3.3 (0.2)	3.0 (0.2)	2.8[b]				
	0.0032	0	2.4	2.9	2.6					
	0.01	0	2.4	2.8	2.4	2.8	2.4	2.4		
	0.01	1.0	3.0	3.1	2.7					
AM	0	0	3.1 (0.1)	3.1 (0.3)	3.2 (0.2)	2.8 (0.4)				
	0.01	0	2.8	2.7	3.1	3.1				
	0.032	0	3.3	2.2	2.4	1.1	0.7	1.7	0.1	
	0.032	1.0	3.1	3.1	2.9	2.6	2.6			

[a.] Indicates the mean (±SEM) of two determinations of the control dose-effect curve.

[b.] Represents single determination.

Table 2: Effects of morphine and WIN 55,212 alone and in combination on response rate (responses/sec) in monkeys trained to discriminate 3.2 mg/kg morphine from saline.

			Morphine (mg/kg)							
Monkey	WIN 55,212 (mg/kg)	Rimonabant (mg/kg)	0	0.32	0.56	1	1.78	3.2	5.6	10
CI	0	0	2.6 (0)[a]		2.7 (0.1)	2.8 (0)	3.2 (0.3)	3.0[b]	3.5[b]	
	0.1	0	2.4		2.3	2.6	2.6	3.0	3.3	
	0.32	0	2.1		2.1	2.3	2.7	2.7	2.8	
	0.32	1.0	1.8			1.6	1.9	2.1	1.9	1.8
HA	0	0	3.1 (0.3)	2.9 (0.1)	2.8 (0.1)					
	0.1	0	3.1	2.4	2.6					
	0.32	0	3.2	2.3	2.4	2.1	2.1	1.9		
	0.32	1.0	3.1	3.0	2.7	2.7	2.5			
AM	0	0	3.6 (0.5)	3.8 (0.4)	3.9 (0.4)	3.8 (0.4)				
	1.0	0	3.7	3.2	3.5					
	1.78	0	3.1	2.5	0.6	2.4	1.7	2.5		
	1.78	1.0	3.3	2.4	2.6	2.2				

[a.] Indicates the mean (±SEM) of two determinations of the control dose-effect curve.

[b.] Represents single determination.

Fig 1

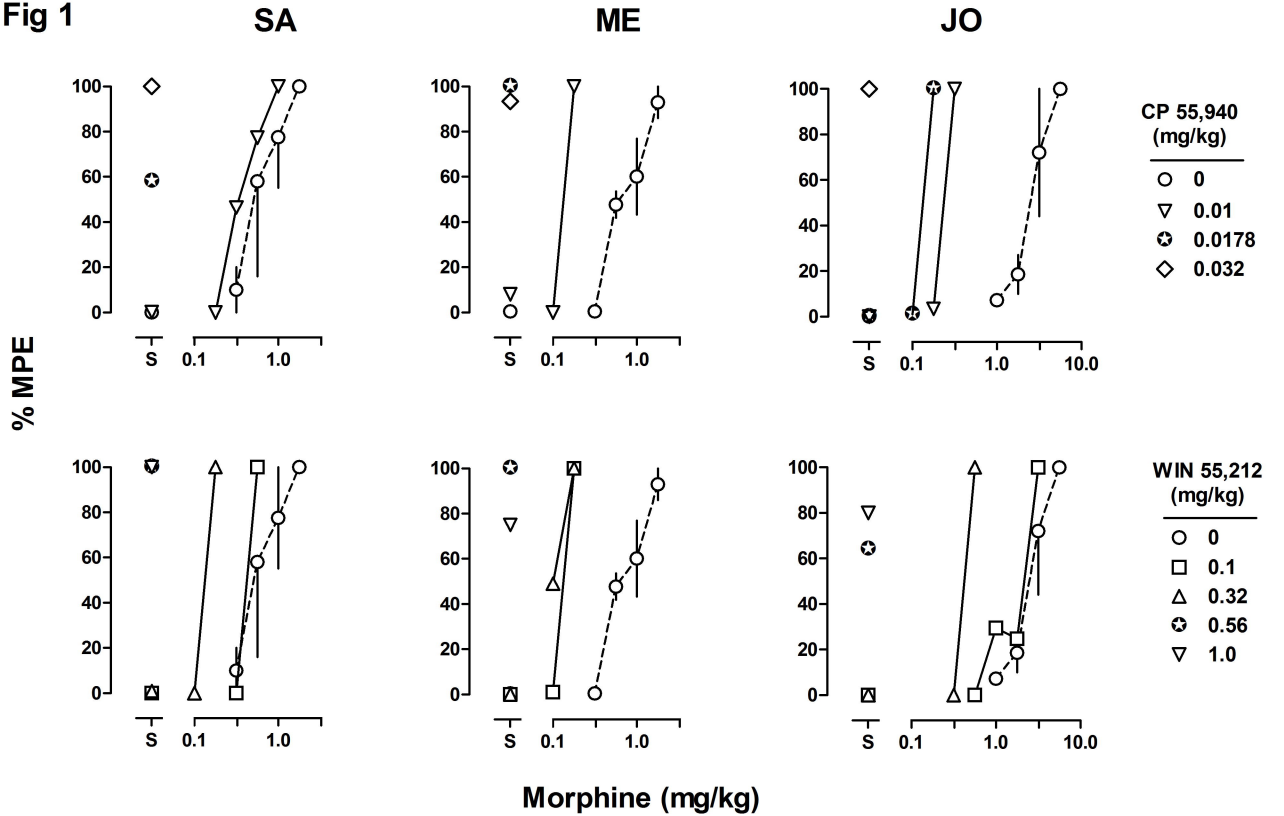
SA
ME
JO
% MPE
100
80
60
40
20
0
S
0.1
1.0
10.0
CP 55,940
(mg/kg)
0
0.01
0.0178
0.032
WIN 55,212
(mg/kg)
0
0.1
0.32
0.56
1.0
Morphine (mg/kg)

Fig 2

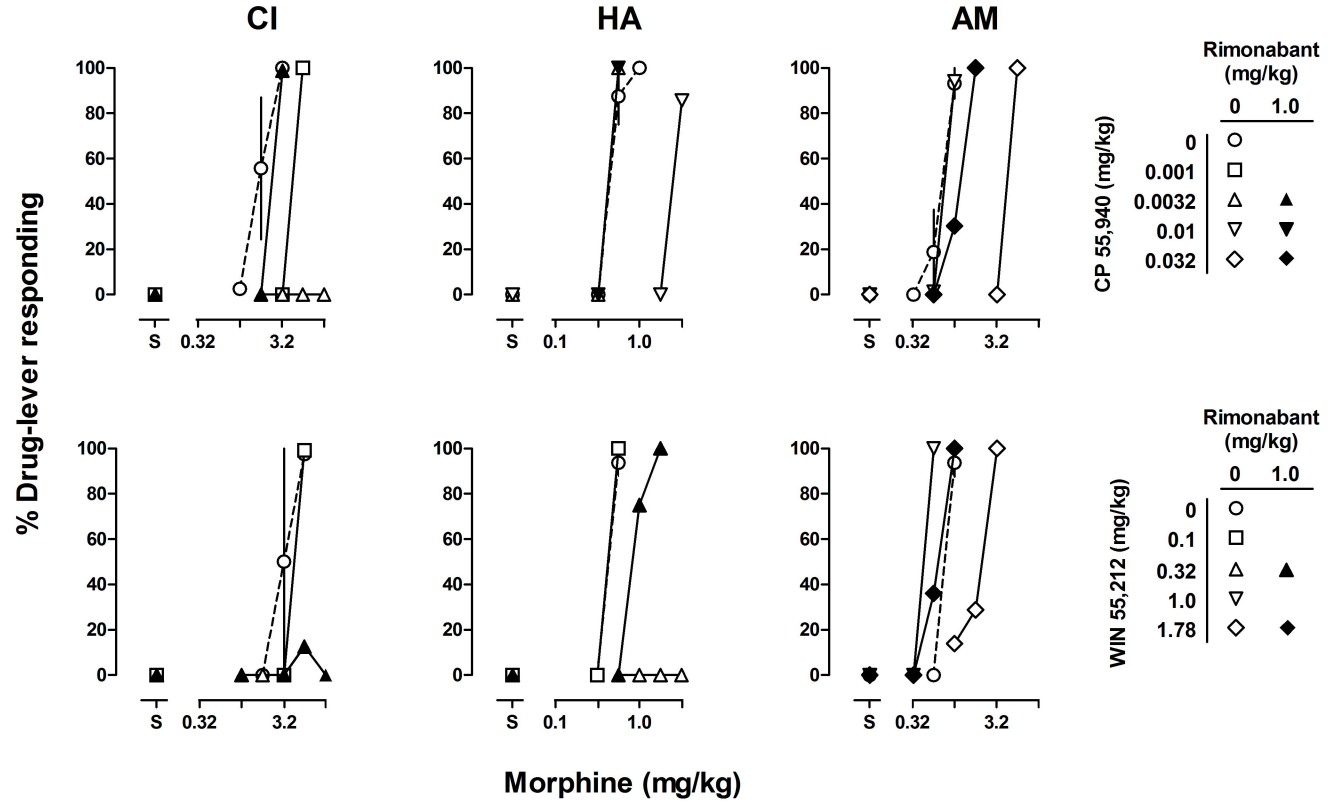
CI
HA
AM
% Drug-lever responding
100
80
60
40
20
0
S
0.32
3.2
0.1
1.0
Morphine (mg/kg)
Rimonabant (mg/kg)
0
1.0
CP 55,940 (mg/kg)
0
0.001
0.0032
0.01
0.032
WIN 55,212 (mg/kg)
0
0.1
0.32
1.0
1.78

Fig 3