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Effects of daily methocinnamox treatment on fentanyl self-administration in rhesus monkeys

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Running Title: Daily methocinnamox and fentanyl self-administration

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ABSTRACT

Methocinnamox (MCAM), a long-acting *mu* opioid receptor antagonist, attenuates the positive reinforcing effects of opioids such as heroin and fentanyl, suggesting it could be an effective treatment for opioid use disorder (OUD). Because treatment of OUD often involves repeated administration of a medication, this study evaluated effects of daily injections of a relatively small dose of MCAM on fentanyl self-administration and characterized the shift in the fentanyl dose-effect curve. Rhesus monkeys (3 males and 2 females) lever-pressed for i.v. infusions of fentanyl (0.032-10 µg/kg/infusion) or cocaine (32-100 µg/kg/infusion) under a fixed-ratio 30 schedule. MCAM (0.032 mg/kg) or naltrexone (0.0032-0.032 mg/kg) was administered s.c. 60 or 15 min, respectively, before sessions. When administered acutely, naltrexone and MCAM decreased fentanyl self-administration, with effects of naltrexone lasting less than 24 hr and effects of MCAM lasting for up to 3 days. Daily MCAM treatment attenuated responding for fentanyl, but not cocaine; effects were maintained for the duration of treatment with responding recovering quickly (within 2 days) following discontinuation of treatment. MCAM treatment shifted the fentanyl dose-effect curve in a parallel manner approximately 20-fold to the right. Naltrexone pretreatment decreased fentanyl intake with equal potency before and after MCAM treatment, confirming sensitivity of responding to antagonism by an opioid receptor antagonist. Although antagonist effects of treatment with a relatively small dose were surmountable, MCAM produced sustained and selective attenuation of opioid self-administration, supporting the view that it could be an effective treatment for opioid use disorder.

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SIGNIFICANCE STATEMENT

Opioid use disorder (OUD) and opioid overdose continue to be significant public health challenges despite the availability of effective treatments. Methocinnamox (MCAM) is a long-acting *mu* opioid receptor antagonist that blocks the reinforcing and ventilatory depressant effects of opioids in nonhuman subjects. This study demonstrates that daily treatment with MCAM reliably and selectively decreases fentanyl self-administration, further supporting the potential therapeutic utility of this novel antagonist.

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INTRODUCTION

Over the past two decades, drug overdose deaths have claimed hundreds of thousands of lives. In the United States, a large share of drug overdose deaths involved an opioid such as fentanyl and/or its derivatives (Hedegaard et al. 2020; Wilson et al. 2020). The opioid crisis continues largely because of high rates of opioid use disorder (OUD) and despite the availability of approved medications, including methadone, buprenorphine, and naltrexone. Although effective in many patients, currently approved medications for OUD have limitations. Methadone and buprenorphine are *mu* opioid receptor agonists that replace abused opioids; however, both drugs share many adverse effects of abused opioids, including risk for diversion, abuse, and overdose. Having antagonist properties at *mu* opioid receptors, naltrexone avoids many adverse effects of methadone and buprenorphine; however, because naltrexone binds to receptors in a competitive, reversible manner, its effects can be surmounted by taking more opioid. In light of these limitations and the ever-increasing public health challenge posed by opioids, there is a pressing need for additional safe, effective treatments for OUD.

Methocinnamox (MCAM) is an analog of buprenorphine that binds with high affinity to *mu* opioid receptors; however, unlike buprenorphine, MCAM has no intrinsic efficacy at *mu* opioid receptors and, therefore, has antagonist properties (Broadbear et al. 2000). MCAM blocks the ventilatory depressant and antinociceptive effects of *mu* opioid receptor agonists in rodents and rhesus monkeys (Broadbear et al. 2000; Gerak et al. 2019a,b; Jimenez et al. 2021; Minervini et al. 2020; Peckham et al. 2005; Zamora et al. 2021) and selectively decreases opioid self-administration (Maguire et al. 2019,

2020). Antagonist effects of MCAM far outlast those of naltrexone or naloxone, blocking effects of *mu* opioid receptor agonists for days or weeks following a single injection (Broadbear et al. 2000; Gerak et al. 2019a,b; Jimenez et al. 2021; Maguire et al. 2019, 2020; Minervini et al. 2020; Peckham et al. 2005; Zamora et al. 2021). Moreover, MCAM is effective at doses that do not decrease responding for food, disrupt cognition (delayed matching to sample), or alter physiological measures such as heart rate, blood pressure, body temperature, and body weight (Maguire et al. 2019, 2020; Minervini et al. 2020). Taken together, these results support consideration of MCAM as a safe and effective treatment for OUD and opioid overdose.

Successful treatment of OUD usually requires repeated treatment over long periods of time. In previous studies, MCAM (≥ 0.1 mg/kg) given acutely or intermittently (i.e., once every 12 days across 49 days) selectively decreased self-administration of heroin and fentanyl in rhesus monkeys (Maguire et al. 2019; 2020). Because treatment might involve frequent administration with smaller doses, this study extended characterization of the potential therapeutic utility of MCAM by evaluating effects of daily injections of a relatively small dose of MCAM (0.032 mg/kg) on fentanyl self-administration. When given acutely, this dose did not decrease heroin self-administration in an earlier study (Maguire et al. 2019). However, given the long-lasting and pseudoirreversible action of MCAM at *mu* opioid receptors (e.g. Zamora et al. 2021), it is possible that repeated treatment, even with a relatively small dose, would result in the emergence of antagonist effects (c.f., Chavkin et al. 2019), possibly due to cumulative binding of *mu* opioid receptors over successive administrations.

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Previous studies examined effects of MCAM on self-administration of relatively narrow range of unit doses of self-administered drug which offers limited information about the nature of drug-receptor interactions. Given that MCAM appears to exert long-duration antagonist activity, potentially via pseudoirreversible binding at *mu* opioid receptors, it was hypothesized that MCAM might insurmountably block the reinforcing effects of fentanyl, indicated by a flattening of the fentanyl dose-effect curve. Therefore, this study characterized effects of MCAM on a range of doses of self-administered fentanyl, with the ascending limb of the dose-effect curve determined before, during, and following discontinuation of daily MCAM treatment. Cocaine was available for self-administration intermittently to establish the pharmacological selectivity of MCAM as well as to confirm catheter patency during daily MCAM treatment when responding for fentanyl was expected to be markedly reduced. Effects of naltrexone pretreatment on responding maintained by a single unit dose of fentanyl were also determined before and after daily MCAM treatment to confirm sensitivity of fentanyl-maintained responding to opioid receptor antagonist effects. Finally, effects of a single administration of the dose of MCAM used for daily treatment were determined to compare duration of effect between a single administration with those of daily administration.

METHODS AND MATERIALS

Subjects. Five rhesus monkeys (3 males and 2 females; 11 to 24 years old) were housed individually in stainless steel cages in a colony room maintained under 14/10-hr light/dark cycle. Chow (High Protein Monkey Diet; Harlan Teklad, Madison, WI, USA), fresh fruit, peanuts, and other treats were provided daily in amounts that

maintained healthy sex- and age-appropriate weights, and water was continuously available in the home cage. Monkeys were weighed daily prior to each session; across individual monkeys, average weights were 7.8 to 11.6 kg during the study. Four monkeys (AC, PE, PR, and TA) participated most recently in studies investigating effects of MCAM on drug self-administration (e.g., Maguire et al., 2020) but had not received MCAM for at least 8 months prior to starting this study. Monkey GU had a history with drug self-administration procedures but had never received MCAM prior to this study. Experiments were conducted in accordance with guidelines set forth by the Guide for the Care and Use of Laboratory Animals (8th edition; 2011) and protocols were approved by the University of Texas Health Science Center at San Antonio Institutional Animal Care and Use Committee.

Surgery. Surgical procedures were identical to those described previously (e.g., Maguire et al. 2020). Briefly, a 5-french catheter was inserted in a vein (e.g., femoral or jugular), tunneled subcutaneously (s.c.) to the back, and connected to a stainless steel s.c. access port (Access Technologies, Skokie, IL, USA). Catheters were flushed daily after sessions with heparinized saline to maintain patency.

Drugs. Fentanyl hydrochloride, naltrexone hydrochloride, and cocaine hydrochloride were generously provided by the National Institute on Drug Abuse Drug Supply Program (Rockville, MD, USA) and dissolved in sterile saline. MCAM hydrochloride was purchased (Syncom, Groningen, NL) and dissolved in 10% w/v 2-hydroxypropyl-β-cyclodextrin (Accela ChemBio Co., Ltd., San Diego, CA, USA). Fentanyl and cocaine were administered i.v. during self-administration sessions in a volume of 1 ml per 10 kg of body weight; naltrexone and MCAM were injected s.c. prior

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to self-administration sessions in volumes of approximately 0.32 ml per 10 kg of body weight. Doses of all drugs are expressed as the salt.

Apparatus. Monkeys were seated in commercially available chairs (Primate Products, Miami, FL, USA) and positioned in light- and sound-attenuating chambers facing a stainless-steel instrument panel with two horizontally aligned levers, each located below a translucent disk that could be illuminated green or red. Infusions were delivered intravenously (i.v.) through the access port by a catheter extension connected to a plastic syringe in a pump (Med Associates, St. Albans, VT, USA) located outside of the chamber. Each chamber was equipped with an exhaust fan to provide ventilation and white noise to mask extraneous sounds. Experimental events were presented, and data were collected, using a PC-compatible computer operating Med-PC IV (Med Associates).

Drug self-administration procedure. Sessions were conducted once daily, 7 days per week, beginning at the same time each day (0800 hr). At the start of the session, the pump was activated for 15 sec to fill the catheter with saline or a fentanyl solution. After 1 min, the red light above the active lever was illuminated for 5 sec and a unit dose of drug was delivered response independently (priming infusion). Immediately after the priming infusion, the green light over one lever (active) was illuminated signaling the beginning of a response period, during which 30 consecutive responses on the active lever turned the green light off, turned the red light on for 5 sec, delivered a unit dose of drug, and initiated a 180-sec timeout period. After the timeout, the green light was illuminated once again signaling the next response period. Responses on the active lever during the timeout were recorded but had no programmed consequence.

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Responses on the other lever (inactive) at any time reset the response counter on the active lever but otherwise had no consequence.

Experimental design and timeline. The study comprised several phases; see Table 1 for the order in which various phases occurred. Fentanyl self-administration dose-effect curves were determined for individual monkeys by varying the unit dose of drug available across sessions. Each unit dose was available for 3 to 7 sessions and until one the following criteria was satisfied: 1) 8 or fewer infusions were obtained in each of 3 consecutive sessions; 2) the number of infusions obtained in each of 3 consecutive sessions did not vary by more than 20% of the mean of those sessions; or 3) a unit dose was available for 7 sessions. Once effects of a unit dose were established, a different unit dose or saline was made available for self-administration. Unit doses of fentanyl ranging from 0.032 to 10 μg/kg/infusion were generally tested in ascending order; the range of doses varied across individual monkeys to determine the ascending limb of the dose-effect curve. Fentanyl dose-effect curves were determined before, during, and following discontinuation of daily treatment with MCAM (Table 1).

Effects of naltrexone pretreatment were determined while a single unit dose of fentanyl ("maintenance dose") was available for self-administration. This was the unit dose of fentanyl that maintained the maximum number of infusions obtained for individual monkeys, either 0.32 μg/kg/infusion (monkeys AC, GU, PE, and PR) or 1.0 μg/kg/infusion (monkey TA). Monkeys received an injection s.c. 15 min prior to each session. Saline was injected prior to baseline sessions; naltrexone was injected prior to test sessions. A baseline was established initially as defined by 3 consecutive sessions in which the number of infusions obtained in each session did not vary by more than

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20% of the mean of those sessions. Thereafter, a dose of naltrexone was injected prior to the following (test) session, followed by another baseline session. At least one baseline session intervened between tests, and subsequent tests occurred once the number of infusions obtained for a single baseline session was within 20% of the initial baseline mean. Baseline and test sessions alternated in this fashion until a naltrexone dose-effect curve was established. This has proven to be an efficient and reliable method for determining effects of naltrexone on opioid self-administration (Maguire et al., 2019, 2020). A naltrexone dose-effect curve was established immediately following the first determination of the fentanyl dose-effect curve and then re-determined following discontinuation of daily MCAM treatment (Table 1).

Once effects of naltrexone were initially determined, fentanyl and cocaine self-administration sessions began alternating with fentanyl being available for 3 sessions followed by cocaine for 1 session (i.e., 1 block of sessions). When MCAM decreased self-administration of fentanyl, robust responding for cocaine confirmed i.v. catheter patency and that the monkey would work for an i.v. infusion of a nonopioid drug. This approach has been effective for examining selectivity of effects of drug pretreatments on fentanyl self-administration (Maguire et al. 2020). The maintenance dose of fentanyl for each monkey was the same dose used to determine effects of naltrexone (0.32 or 1.0 μg/kg/infusion), and all monkeys responded initially for a unit dose of 32 μg/kg/infusion cocaine.

Upon initiation of the fentanyl/cocaine alternation schedule, monkeys began receiving an injection s.c. 60 min before the start of each session. Initially, the daily injection was 0.5 ml of the MCAM vehicle (10% β-cyclodextrin in saline). After 4

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sessions (1 block) and a determination that responding was stable, the daily vehicle injection was replaced with an injection of 0.032 mg/kg MCAM starting with the first fentanyl session of a block. The maintenance doses of fentanyl and cocaine remained constant for the first 20 sessions (5 blocks) of daily MCAM treatment, at which point the fentanyl dose-effect curve was re-determined (Table 1) . The unit dose of fentanyl progressively increased in half-log unit increments across blocks (1 to 2 blocks per unit dose of fentanyl, depending on stability of responding) until the ascending limb of the fentanyl dose-effect curve was established; unit doses of fentanyl ranged from 0.32 to 10 µg/kg/infusion. After the fentanyl curve was redetermined, the initial maintenance dose of fentanyl was once again made available for 3 blocks (9 fentanyl sessions and 4 cocaine sessions), for a total of 52 daily treatments, at which point the daily MCAM injection was replaced with vehicle for at least 16 sessions. During that time, fentanyl and cocaine sessions continued to alternate. The unit dose of cocaine remained constant, with one exception; one monkey (PE) began responding at low rates for cocaine and the unit dose was increased 100 µg/kg/infusion on day 12 of daily MCAM treatment.

Once responding for fentanyl recovered (i.e., number of infusions increased to at least 80% of the baseline mean values for 3 consecutive sessions), intermittent cocaine sessions were discontinued. The naltrexone pretreatment dose-effect curve was determined a second time, followed by a third determination of the fentanyl dose-effect curve, as described above (see Table 1). Thereafter, intermittent cocaine sessions were reinstated and effects of a single administration of 0.032 mg/kg MCAM were determined using the same procedure as with daily treatment, with MCAM given 60 min prior to the

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first session of a block. Vehicle injections were given prior to at least 3 sessions that preceded and followed MCAM injection. Upon recovery of responding (after approximately 3 blocks), intermittent cocaine infusions were discontinued.

Data analyses. The number of infusions obtained each session served as the primary dependent measure. Fentanyl dose-effect curves for individual monkeys were constructed for each treatment condition by averaging the number of infusions obtained across each of the last three sessions with each unit dose of fentanyl. The ascending limb of the dose-effect curve was then fit to a straight line to quantify changes across conditions. The average number of infusions obtained with each unit dose of fentanyl was normalized to percent maximum possible effect (%MPE) using the following equation: %MPE = (test-saline) / (maximum-saline) * 100, where *test* is the number of infusions obtained with each unit dose, *saline* is the number of infusions obtained when saline was available for self-administration, and *maximum* is the maximum number of infusions obtained at any unit dose of fentanyl tested. A line was fit to the ascending limb of the dose-effect curve using linear interpolation and the logarithm of fentanyl unit dose. The slope and intercept of the fit line were used to determine the dose producing 50% of maximal number of infusions obtained (ED_{50}). Shifts in the fentanyl dose-effect curve across conditions were quantified by calculating a potency ratio; ED_{50} values during and after MCAM treatment were divided by the ED_{50} before treatment. Differences in the ED_{50} (log transformed) and slope of the fentanyl dose-effect curve across treatment conditions were analyzed using a repeated measures ANOVA with a Geisser-Greenhouse correction for potential violations of sphericity. In addition, shifts in

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the fentanyl dose-effect curve were considered significant if the 95% confidence interval for the potency ratio did not contain 1.

Effects of naltrexone were quantified by fitting a straight line to the descending portion of the naltrexone dose-effect curve and estimating the dose required to decrease the number of infusions obtained by 50% compared with vehicle pretreatment (ED_{50}); the number of infusions obtained were averaged across sessions that immediately preceded each naltrexone test to determine the value for vehicle pretreatment. The difference in potency of naltrexone before and after daily MCAM treatment was analyzed by calculating a potency ratio for each monkey (ED_{50} after treatment divided by ED_{50} before treatment), and differences were considered significant if the 95% confidence interval did not contain 1.

Effects of MCAM on fentanyl and cocaine self-administration across days were analyzed using one-way, repeated measures ANOVAs with Geisser-Greenhouse correction; data were analyzed separately for fentanyl and cocaine self-administration sessions. The number of infusions obtained during the session immediately preceding MCAM treatment served as the baseline. Dunnett's post-hoc tests were employed to determine sessions after treatment that differed significantly from baseline. Data analyses were conducted using GraphPad Prism version 9 (GraphPad Software, LLC, San Diego, CA).

RESULTS

Fentanyl dose-dependently increased the number of infusions obtained. Before MCAM treatment, monkeys received an average (± SEM) of 2.9 (0.8) infusions when

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saline was available and an average of 21.0 (2.4) infusions when a unit dose of 0.32 µg/kg/infusion of fentanyl was available (Figure 1A, open squares). The average ED_{50} for the ascending limb of the fentanyl dose-effect curve was 0.14 µg/kg/infusion (95% confidence interval [CI]: 0.08-0.26). Daily MCAM treatment shifted the fentanyl dose-effect curve approximately 21-fold to the right (Figure 1A, closed squares). Unit doses that maintained high levels of fentanyl infusions before treatment (0.32 and 1.0 µg/kg/infusion), were not different from saline during MCAM treatment. Effects of MCAM were surmounted with larger unit doses of fentanyl, as the number of infusions increased dose-dependently, with monkeys obtaining, on average, 20.3 (1.5) infusions of a unit dose of 10 µg/kg/infusion fentanyl; the average ED_{50} for fentanyl increased to 2.8 µg/kg/infusion (95% CI: 1.95-4.01). When re-determined approximately 3-4 weeks after discontinuation of daily MCAM treatment, the fentanyl dose-effect curve was shifted back to the left (Figure 1A, triangles), with an average ED_{50} of 0.11 µg/kg/infusion (95% CI: 0.05-0.23).

Prior to daily MCAM treatment, acute naltrexone pretreatment dose-dependently decreased the number of fentanyl infusions received from an average of 24.3 (0.9) following vehicle administration to an average of 2.0 (0.5) following 0.032 mg/kg naltrexone (Figure 1B, squares), with the number of fentanyl infusions received recovering fully by the next session (data not shown). The average ED_{50} for naltrexone prior to daily MCAM treatment was 0.008 mg/kg (95% CI: 0.006-0.010). When re-tested approximately 2-3 weeks (range: 15-24 days across monkeys) following discontinuation of daily MCAM treatment, naltrexone again dose-dependently decreased the number of fentanyl infusions received (Figure 1B, triangles) with an average ED_{50} of 0.009 mg/kg

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(95% CI: 0.005-0.016), which was not significantly different from effects determined prior to daily MCAM treatment.

Immediately prior to daily MCAM treatment, fentanyl and cocaine maintained an average of 24.6 (0.9) and 18.8 (1.9) infusions per session, respectively (Figure 2, data above “B”). Daily treatment with 0.032 mg/kg MCAM significantly decreased the number of fentanyl, but not cocaine, infusions beginning with the second day of treatment. Fentanyl infusions remained significantly lower than baseline, and not different from saline, for the duration of treatment. An ANOVA indicated a significant effect of treatment day on fentanyl ($F[2.6,10.3]=29.33$, $p<.0001$) but not cocaine ($p=.29$) infusions. Responding for fentanyl recovered within 2 days of daily MCAM treatment being discontinued.

An ANOVA indicated a significant effect of treatment condition on fentanyl ED_{50} ($F[1.3,5.1]=54.3$, $p=0.0005$), with the ED_{50} of fentanyl during MCAM treatment being significantly different from before treatment (Figure 3A). Potency ratio increased, on average, to 21.1 (95% CI: 13.0-29.1) during MCAM treatment and decreased following discontinuation of daily MCAM treatment to an average of 0.83 (95% CI: 0.52-1.13) (Figure 3B). Although the slope of the fentanyl dose-effect curve decreased, on average, from 154.2 (20.9) to 109.7 (18.8) during daily MCAM treatment, this decrease was not statistically significant ($p=0.24$; Figure 3C); the slope of the fentanyl dose-effect curve increased, on average, to 143.3 (18.5) following discontinuation of daily MCAM treatment.

When tested at least 10 weeks after the last daily MCAM injection, acute administration of 0.032 mg/kg MCAM significantly decreased the number of fentanyl

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infusions received 24 and 48 hr after injection. Thereafter, neither responding for fentanyl or for cocaine was decreased significantly, with full recovery of responding apparent within 5 days (Figure 4). An ANOVA indicated a significant effect of day on fentanyl ($F[2.3,9.28]=5.81$, $p=0.02$) but not cocaine ($p=.25$) infusions.

DISCUSSION

This study evaluated effects of daily injections of a relatively small dose of MCAM on fentanyl self-administration in rhesus monkeys and quantified antagonist effects by studying MCAM in combination with a range of doses of fentanyl before, during, and following discontinuation of daily treatment. Results of the study showed the following: 1) daily MCAM treatment attenuated self-administration of fentanyl, but not cocaine, and effects were maintained for the duration of treatment; 2) MCAM treatment shifted the fentanyl dose-effect curve rightward in a parallel manner by approximately 20-fold; and 3) responding recovered rapidly following discontinuation of daily treatment. These data indicate that MCAM selectively attenuates opioid self-administration, remains effective with repeated treatment, and does not alter sensitivity to opioids after treatment is discontinued, supporting the potential utility of MCAM for treating OUD.

Consistent with previous studies, the potent and high efficacy *mu* opioid receptor agonist fentanyl reliably maintained drug taking in rhesus monkeys with unit doses in the range of 0.32 to 1.0 μg/kg/infusion (e.g., Broadbear et al. 2004; Negus et al. 2008). The opioid receptor antagonist naltrexone dose-dependently decreased responding, and effects of naltrexone were relatively short-lived with responding fully recovered the

next session (e.g., Maguire et al. 2020). When administered acutely, a dose of 0.032 mg/kg MCAM produced a modest (50%) reduction in fentanyl self-administration that persisted for 2 to 3 days. The duration of action of MCAM appears to be dose-related in so far as larger doses produce longer-lasting reductions in responding for fentanyl. For example, a single injection of the dose studied in this experiment decreased responding for fentanyl for a few days, whereas a single injection of a 10-fold larger dose (0.32 mg/kg) decreased fentanyl-maintained responding for approximately 2 weeks (Maguire et al. 2020). These results parallel studies in rhesus monkeys and rats showing that the duration of antagonist action of MCAM is dose related with respect to other *mu* opioid receptor-mediated (e.g., ventilatory depressant and antinociceptive) effects of heroin and fentanyl (e.g., Gerak et al. 2019 a,b).

Daily treatment with MCAM decreased responding for fentanyl, but not cocaine, with responding remaining decreased for the duration of treatment. Thus, the antagonist effects of MCAM did not appear to wane with repeated treatment, which is an important property for sustained, long-term treatment of OUD. Moreover, MCAM treatment did not decrease responding for cocaine, in the same monkeys during the same treatment period, consistent with results from previous studies (Maguire et al. 2019, 2020) and indicating that the effects of MCAM were likely mediated by blockade of *mu* opioid receptors rather than generalized rate-suppressant effects. The involvement of antagonist effects at *mu* opioid receptors is also confirmed by the rightward shift in the fentanyl dose-effect curve and surmountability of MCAM when larger unit doses of fentanyl were made available for self-administration. In that sense, daily treatment with

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a relatively small dose of MCAM in the current study had effects comparable to what might be expected with repeated (or sustained release) treatment with naltrexone.

The long-lasting effects of larger doses of MCAM are thought to be due, at least in part, to a noncompetitive interaction whereby MCAM dissociates very slowly (if at all) from *mu* opioid receptors. Indeed, recent *in vitro* studies demonstrated that MCAM insurmountably blocked *mu* opioid receptor agonist-inhibition of cyclic adenosine monophosphate production (Zamora et al. 2021), supporting the notion that MCAM has pseudoirreversible binding properties. It was hypothesized that repeated treatment with a relatively small, modestly effective dose of MCAM would result in the emergence of antagonist effects over the course of several administrations due to an accumulation of binding to *mu* opioid receptors. A similar effect was reported for the long-acting *kappa* opioid receptor antagonist norbinatorphimine (norBNI), albeit presumably through a different mechanism (Chavkin et al. 2019). Repeated administration of otherwise ineffective doses of norBNI blocked the antinociceptive effects of the selective *kappa* opioid receptor agonist U50488 in mice, consistent with a cumulative inactivation of *kappa* opioid receptors. Indeed, MCAM produced modest, but non-significant, effects on fentanyl self-administration after the first injection, followed by a marked reduction in responding from day 2 of treatment onward. The number of infusions obtained for most daily treatment sessions was not different from saline substitution for fentanyl. Effects of daily treatment exceeded those of the same dose given acutely, which only decreased infusions by 50%, on average, one day after injection. Therefore, it appears as though an effect emerged with repeated treatment consistent with the accumulation hypothesis.

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However, there are two other predictions that were not clearly supported by current results. First, having pseudoirreversible antagonist properties, MCAM might be expected to shift the fentanyl dose-effect curve rightward and, with sufficient occupancy of *mu* opioid receptors, downward (e.g., Zernig et al. 1997). Re-determination of the fentanyl dose-effect curve began after 20 daily injections of 0.032 mg/kg MCAM, resulting in a total dose of 0.64 mg/kg (twice the dose of MCAM that decreases fentanyl self-administration for up to 2 weeks after a single injection). Although the slope of the fentanyl dose-effect curve decreased on average, it was not significantly different from the slope of the curve before treatment and the antagonist effects of MCAM were surmounted in all monkeys. Thus, there was no evidence of insurmountable antagonist effects. One possibility is that this total cumulative dose of MCAM was insufficient to produce insurmountable blockade of opioid self-administration and the resultant flattening of the fentanyl dose-effect curve, consistent with reports that opioid self-administration has a relatively low efficacy requirement and high receptor reserve (e.g., Zernig et al. 1997). It is possible that larger doses of MCAM, and higher receptor occupancy, is required to produce insurmountable blockade of opioid self-administration. Another prediction is that the time to recovery of responding following repeated MCAM administration would exceed that of a single administration of the same dose of MCAM. That is, larger cumulative doses of MCAM should bind (pseudoirreversibly) more opioid receptors resulting in diminished availability of functional receptors. Therefore, time to recovery would be dependent on the reemergence of new (i.e., functional) receptors. However, in the current study, responding for fentanyl recovered quite rapidly (within 2-3 days) following

discontinuation of MCAM treatment. The time to recovery was similar to, and possibly even shorter than, acute administration with the same dose. This is notable given the total cumulative dose of MCAM administered in this study (1.66 mg/kg) was nearly identical to the cumulative dose of MCAM administered in a previous study (1.60 mg/kg; Maguire et al. 2020), yet the time to recovery was vastly different. In the previous study, a 10-fold larger dose was administered 5 times with 12 days between injections and responding recovered very gradually over the course of 2-3 weeks. Therefore, the total cumulative dose of MCAM alone does not predict its duration of action. Taken together, these data do not clearly support the notion that effects of repeated treatment with a relatively small dose in the current study was the result of significant cumulative binding to *mu* opioid receptors.

Another possibility is that MCAM accumulated elsewhere in the body over the course of repeated treatments which resulted in sustained blood levels for the duration of treatment that decreased rapidly following discontinuation of treatment. Although not fully characterized, available pharmacokinetic data suggest that MCAM has a relatively short time to peak effect (<45 min) and short half-life (≈ 75 min) in plasma, with levels near the limit of detection 24 hr after administration of 0.32 mg/kg (10-fold larger than the dose tested in the current study; Maguire et al. 2020). Results of that study showed that MCAM remained effective even when plasma concentrations were very low. Although this does not rule out the possibility that MCAM accumulated in tissues in the current study, results to date support the view that pseudoirreversible binding to *mu* opioid receptors play a significant role in its long-lasting effects.

In summary, this study shows that daily MCAM treatment persistently and selectively decreases fentanyl self-administration in nonhuman primates, consistent with previously published studies. Results of the current study differ from those of earlier studies showing that MCAM has last long-lasting antagonist effects. Here, daily treatment with a relatively small dose of MCAM shifted the fentanyl dose-effect curve rightward in a surmountable manner with responding recovering quickly upon discontinuation of treatment. Although long-acting antagonist effects might be useful in some situations, being able to provide sustained blockade of opioid receptors with more frequent administration of smaller doses of MCAM in other situations might also have therapeutic utility. Taken together with previous studies, these data confirm that MCAM attenuates opioid self-administration in a sustained and selective manner, supporting the view that it could be an effective treatment for OUD.

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AUTHORSHIP CONTRIBUTIONS

Participated in research design: DRM, CPF

Conducted experiments: DRM

Performed data analyses: DRM

Wrote or contributed to the writing of the manuscript: DRM, CPF

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DISCLOSURES

C.P.F. is coholder of a U.S. patent for MCAM.

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FIGURE LEGENDS

Figure 1. (A) Fentanyl dose-effect curves determined before (open squares), during (filled squares), and following discontinuation of (triangles) daily treatment with 0.032 mg/kg MCAM. The number of infusions is plotted for sessions in which saline (above S) or different unit doses of fentanyl, in µg/kg/infusion, were available for self-administration. Data points show the mean (± 1 standard error of the mean) for five rhesus monkeys with the following exceptions: during daily MCAM treatment, points above 0.32 and 10 µg/kg/infusion fentanyl show the average of four monkeys; after daily MCAM treatment, the point above 0.032 µg/kg/infusion fentanyl shows the average of two monkeys. (B) Effects of naltrexone pretreatment on the number of fentanyl infusions determined before (squares) and after (triangles) daily MCAM treatment in five monkeys. The number of infusions is plotted for saline and across increasing doses of naltrexone (mg/kg). For four monkeys (AC, GU, PE, and PR) the unit dose of fentanyl available for self-administration was 0.32 µg/kg/infusion, whereas 1.0 µg/kg/infusion was available for the fifth monkey (TA). Data points show the mean (± 1 standard error of the mean) for five monkeys with the following exceptions: after daily MCAM treatment, points above 0.0032 and 0.032 mg/kg show data from four monkeys.

Figure 2. Effects of daily treatment with vehicle or MCAM on self-administration of fentanyl (squares) and cocaine (circles). The number of infusions is plotted across consecutive sessions. Symbols above B indicate data from the most recent fentanyl and cocaine sessions preceding MCAM treatment. Monkeys received a s.c. injection of vehicle or 0.032 mg/kg MCAM 60 min prior to each session. For four monkeys (AC, GU,

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PE, and PR) the unit dose of fentanyl available for self-administration was 0.32 µg/kg/infusion, whereas 1.0 µg/kg/infusion was available for the fifth monkey (TA). For all monkeys, a unit dose of 32 µg/kg/infusion cocaine was available for self-administration except for PE beginning with treatment day 12 when the unit dose of cocaine was increased to 100 µg/kg/infusion. Data points show the mean (± 1 standard error of the mean) for five monkeys, and the shaded region shows the range of for the average number of infusions obtained when saline was available for self-administration. The gap in the abscissa indicates when the fentanyl dose-effect curve was re-determined.

Figure 3. Quantification of changes in the ascending limb of the fentanyl dose-effect curve across treatment conditions. (A) Log ED_{50} is plotted for individual monkeys before, during, and after daily MCAM treatment. The horizontal line indicates the group average. Data were analyzed with a repeated measured one-way ANOVA with Dunnett's multiple comparisons tests. Brackets indicate the results of the Dunnett's test, with a significant difference between conditions denoted by an asterisk ($p<.05$; ns = not significant). (B) Potency ratio, calculated by dividing the ED_{50} during or after daily MCAM treatment by the ED_{50} before treatment, is plotted for individual monkeys during each treatment condition. The horizontal line indicates the group average. Potency ratio was considered significant if the 95% confidence interval for the group did not contain 1, denoted by an asterisk. (C) Slope is plotted for individual monkeys before, during, and after daily MCAM treatment. The horizontal line indicates the group average. Data were

analyzed with a repeated measured one-way ANOVA with Dunnett's multiple comparisons tests.

Figure 4. Effects of acute treatment with MCAM on self-administration of fentanyl (squares) and cocaine (circles). A dose of 0.032 mg/kg MCAM was administered s.c. 60 min before the session indicated by the arrow; vehicle was administered prior to the 3 sessions immediately preceding and following MCAM administration. All other details are the same as in Figure 2.

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TABLES

Table 1. Order of experimental phases

Fentanyl self-administration dose-effect curve (1st determination)
Naltrexone pretreatment dose-effect curve (1st determination)
Start daily MCAM treatment
Fentanyl dose-effect curve (2nd determination; during daily MCAM)
Daily MCAM treatment
Discontinue daily MCAM treatment
Naltrexone dose-effect curve (2nd determination; after daily MCAM)
Fentanyl dose-effect curve (3rd determination; after daily MCAM)
Acute pretreatment with MCAM

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Figure 1

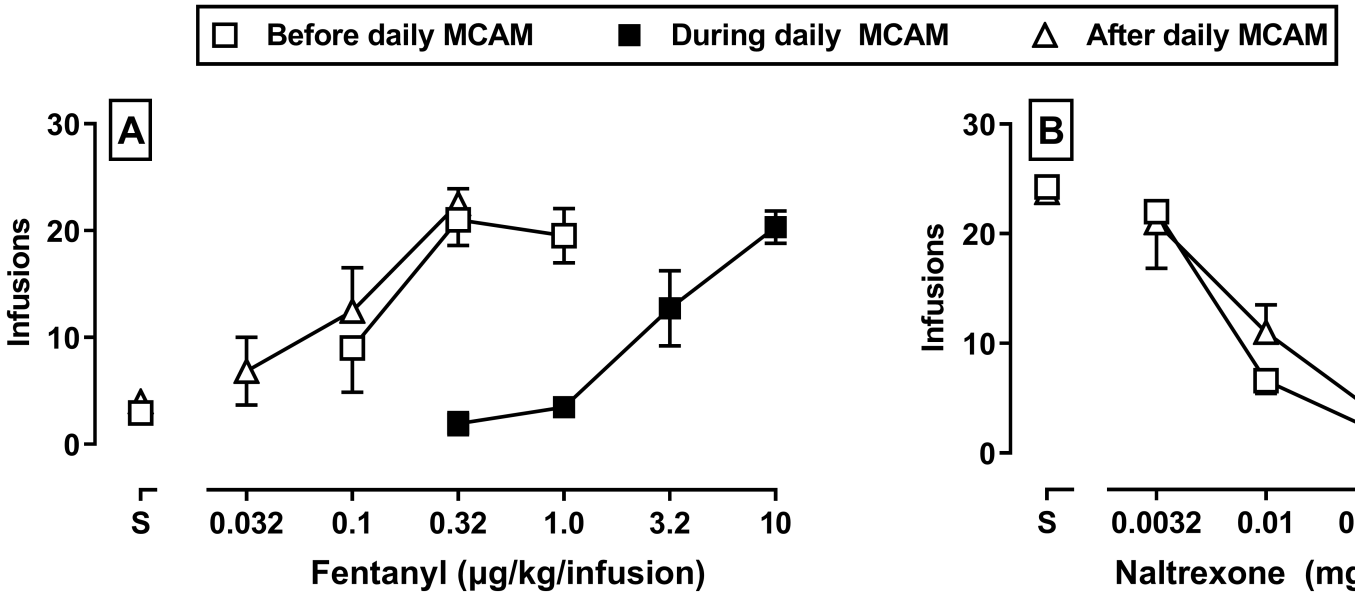

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Figure 2

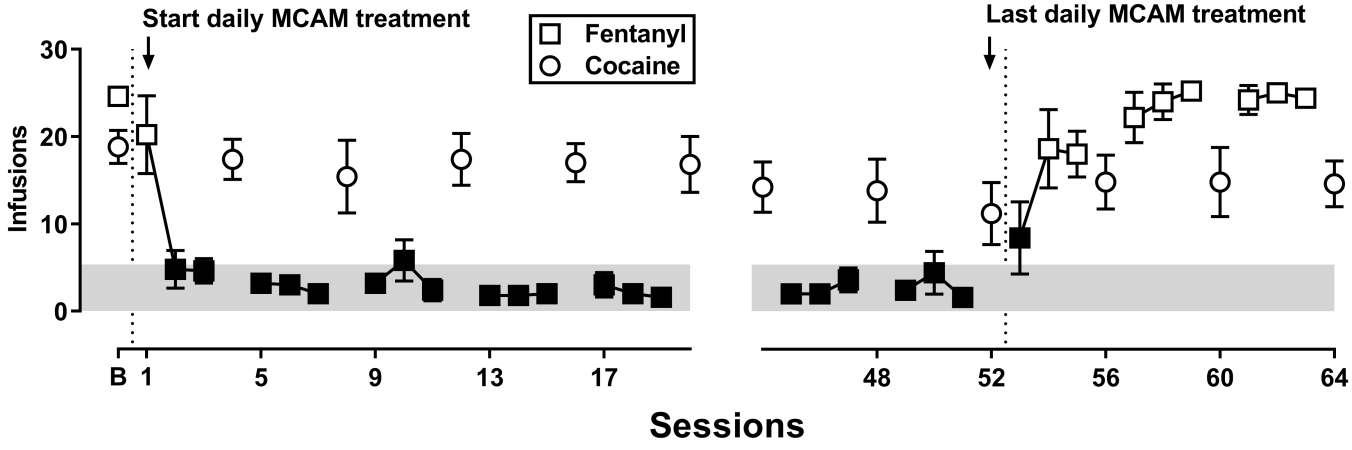

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Figure 3

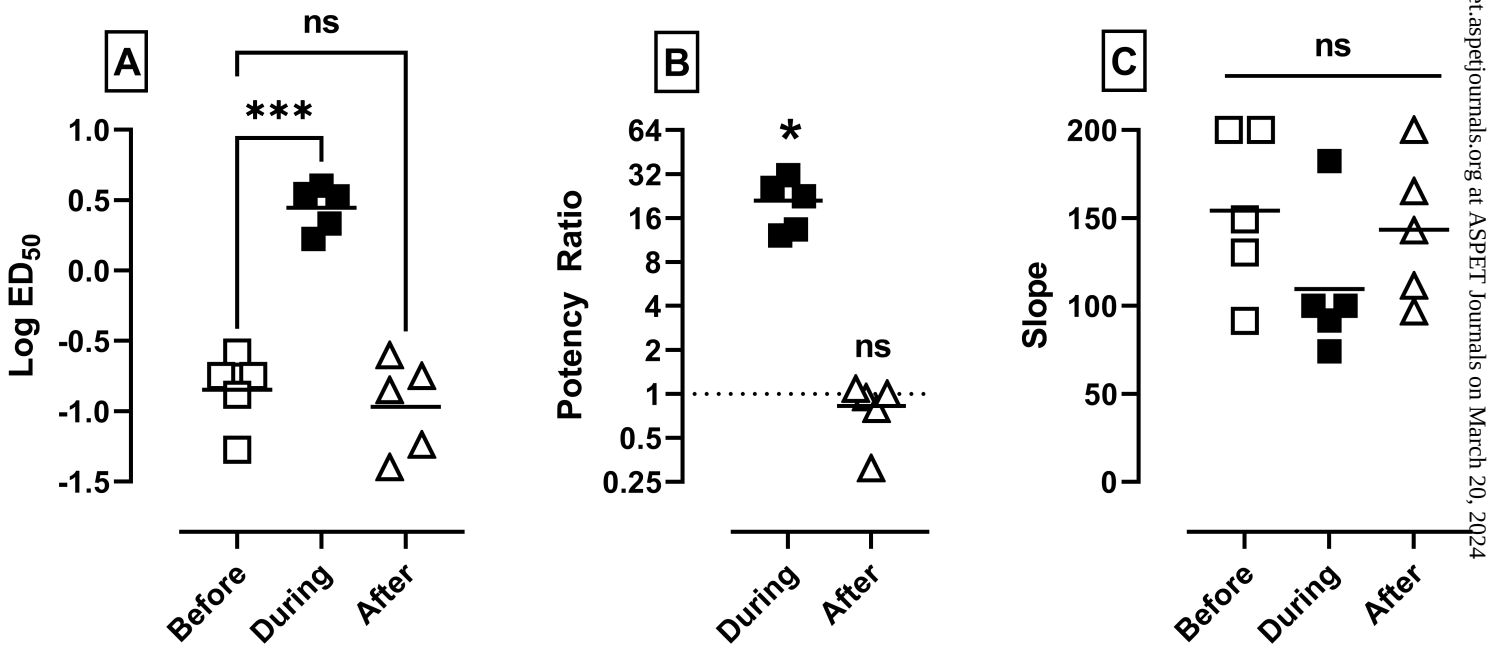

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Figure 4