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Chronic Methadone Treatment Shows a Better Cost/Benefit Ratio than Chronic Morphine in Mice

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ABSTRACT

Chronic treatment of pain with opiate drugs can lead to analgesic tolerance and drug dependence. Although all opiate drugs can promote tolerance and dependence in practice, the severity of those unwanted side effects differs depending on the drug used. Although each opiate drug has its own unique set of pharmacological profiles, methadone is the only clinically used opioid drug that produces substantial receptor endocytosis at analgesic doses. Here, we examined whether moderate doses of methadone carry any benefits over chronic use of equianalgesic morphine, the prototypical opioid. Our data show that chronic administration of methadone produces significantly less analgesic tolerance than morphine. Furthermore, we found significantly reduced precipitated withdrawal symptoms after chronic methadone treatment than after chronic morphine treatment. Finally, using a novel animal model with a degrading μ-opioid receptor we showed that, although endocytosis seems to protect against tolerance development, endocytosis followed by receptor degradation produces a rapid onset of analgesic tolerance to methadone. Together, these data indicated that opioid drugs that promote receptor endocytosis and recycling, such as methadone, may be a better choice for chronic pain treatment than morphine and its derivatives that do not.

Introduction

Opiate drugs are the mainstay for the treatment of severe pain, but the utility of these drugs for chronic pain conditions is curtailed by the development of tolerance to the analgesic effects of drug. It is noteworthy that the dose escalation necessary to overcome tolerance in chronic pain conditions not only puts patients at a greater risk for severe side effects, such as respiratory depression, but also increases the liability for dependence. Although there have been significant efforts designed to improve the utility of opiates, there has been little progress in identifying approaches to treatment that maintain analgesic efficacy with reduced side effects of tolerance and dependence. This is primarily because the mechanisms underlying development of tolerance and dependence as a consequence of chronic opioid use remain unresolved and are thus vigorously debated (Christie, 2008). This debate centers on the role of receptor desensitization, arrestin recruitment, and endocytosis versus the role of homeostatic adaptations in signal transduction as the primary mediators of analgesic tolerance and dependence (Raehal and Bohn, 2005; Martini and Whistler, 2007; Christie, 2008; Ingram and Traynor, 2009; Ueda and Ueda, 2009).

Two opiate drugs used for the management of chronic pain are methadone and morphine. Although these two drugs differ in their chemical structure, the primary target for their actions is the μ-opioid receptor (MOR) (Eddy and May, 1973; Raynor et al., 1994). Beyond their structural differences, these two drugs differ in a number of aspects regarding MOR pharmacology (Eddy and May, 1973). For example, methadone, but not morphine, promotes substantial arrestin recruitment (Whistler and von Zastrow, 1998; Bohn et al., 2004). In addition, whereas morphine fails to drive significant endocytosis of the MOR, methadone more closely mimics the endogenous opiates and promotes substantial endocytsois (Keith et al., 1996, 1998; Sternini et al., 1996; Borgland et al., 2003; Milan-Lobo and Whistler, 2011). Hence, these two drugs provide an opportunity to help dissect the role of receptor recruitment of arrestin and receptor endocytosis in tolerance and dependence.

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ABBREVIATIONS: MOR, μ-opiate receptor; DMOR, degrading MOR; DOR, δ-opiate receptor; WT, wild type; ANOVA, analysis of variance; NMDA, *N*-methyl-D-aspartate; WDS, wet dog shakes; PT, paw tremor; ED_{50}, effective dose at 50%.

After receptor recruitment of arrestin and receptor endocytosis, G protein-coupled receptors can either be recycled back to the plasma membrane or targeted for degradation and "down-regulated" (Hanyaloglu and von Zastrow, 2008). Although many studies have shown that the MOR is primarily recycled after endocytosis (Finn and Whistler, 2001; Whistler et al., 2002; Minnis et al., 2003; Yu et al., 2010), the hypothesis persists that receptor down-regulation also contributes to opioid tolerance (Ueda and Ueda, 2009). We have generated a knockin mouse that expresses a mutant version of the MOR, DMOR, for degrading MOR, that recruits arrestin, endocytoses, and is sorted for degradation in response to both morphine and methadone (Finn and Whistler, 2001; Whistler et al., 2002; Enquist et al., 2011). Thus, the DMOR animal model allowed us to investigate the impact of MOR down-regulation on tolerance to methadone and morphine.

The aim of this study was to use moderate, equianalgesic doses of methadone and morphine and directly compare whether these two drugs differed in their ability to promote analgesic tolerance and drug dependence. In addition, we used the DMOR knockin mice to determine whether analgesic tolerance induction is altered as a consequence of receptor down-regulation.

Materials and Methods

Materials. Morphine sulfate pentahydrate was purchased from Mallinckrodt (Hazelwood, MO). (±)-Methadone HCl and naloxone HCl were purchased from Sigma-Aldrich (St. Louis, MO), and 0.9% sodium chloride was purchased from Hospira (Lake Forest, IL).

Animals. All mice included in the study were kept on a regular light/dark cycle with ad libitum access to food and water. C57BL/6J mice were purchased from The Jackson Laboratory (Bar Harbor, ME) and allowed at least 1 week to acclimatize upon arrival. DMOR knockin mice were generated as described previously (Enquist et al., 2011) and kept with wild-type (WT) littermates. All animal experiments were performed in accordance with the guidelines of the Ernest Gallo Clinic and Research Center Institutional Animal Care and Use Committee.

Analgesic Response: Tail-Flick Reflex to Heat Irradiation. Mice were tested for antinociception by using the radiant heat tail-flick procedure. In brief, mice were transferred to the test room 1 h before tests to allow time to acclimatize. Each mouse was carefully wrapped in a small blanket, and the tail was stimulated by light heat radiation. Each mouse was tested for tail-flick reflex in response to radiant heat stimulation before test day. Mice with robust tail-flick reflexes and baseline latencies of 2.0 to 3.5 s were included in the study; a maximum latency of 10 s was set as the cutoff time to minimize damage to the tail. Maximum effect of drug on tail flick was achieved between 20 and 30 min after subcutaneous injection of drug (Fig. 1B). Dose response was measured by cumulative drug addition, and nociceptive assessment was done 20 min after each subcutaneous administered dose, three doses per animal. Data are presented as percentage of maximal possible effect = [(latency after drug − baseline)/(cutoff − baseline)] × 100.

Tolerance Induction. C57BL/6J wild-type mice were injected subcutaneously once daily at noon by using ED_{50} doses of methadone (3 mg/kg) or morphine (6 mg/kg) dissolved in sterile 0.9% saline solution. C57BL/6J:SV129 mice (knockin DMOR and WT littermates) were injected subcutaneously twice daily with 10 mg/kg methadone dissolved in sterile 0.9% saline solution. The significance of tolerance was evaluated by comparing 95% confidence intervals (Fig. 2; Table 1) or tested by repeated-measures ANOVA combined with Dunnett's multiple comparison test (see Fig. 4).

Precipitated Withdrawal: Drug Dependence. Six groups of animals were included in this study (two groups for each drug: morphine, $n = 9$; methadone, $n = 9$; saline, $n = 12$, where n is the number of animals per group). One group per drug (morphine, methadone, or saline) received a single injection of the drug (acute) followed by naloxone 30 min later, and one group per drug received 7 days of drug treatment before naloxone (chronic). Each animal was injected with the drug on the test day (3 mg/kg methadone, 6 mg/kg morphine, or equal volume of saline) followed 30 min later by a naloxone injection (0.5 mg/kg) and monitored for 15 min by three independent scorers blind to drug. Behaviors scored were paw tremor (PT; shakes of paws unrelated to grooming), wet dog shakes (WDS; full body shakes unrelated to grooming), and jumps (escape attempts, all four paws off the floor). The total (global) score for each animal was calculated by calculating the sum of each independent behavior combined. The sessions were videotaped for recordkeeping. The significance of within drug-group effects on all behaviors, and between global score of all three groups, before and after chronic drug exposure was analyzed separately by two-way ANOVA and Bonferroni post-tests.

Results

Methadone and Morphine at the ED_{50} Dose Produce Antinociception of Equal Duration in C57B6/J Mice. Each time two different drugs are used, several pharmacological parameters are altered including affinity, potency, and in vivo pharmacokinetics and dynamics. Thus, to directly compare methadone and morphine as antinociceptives, we first established the ED_{50} of each drug in the radiant heat tail-flick paradigm by a cumulative dose-response regimen

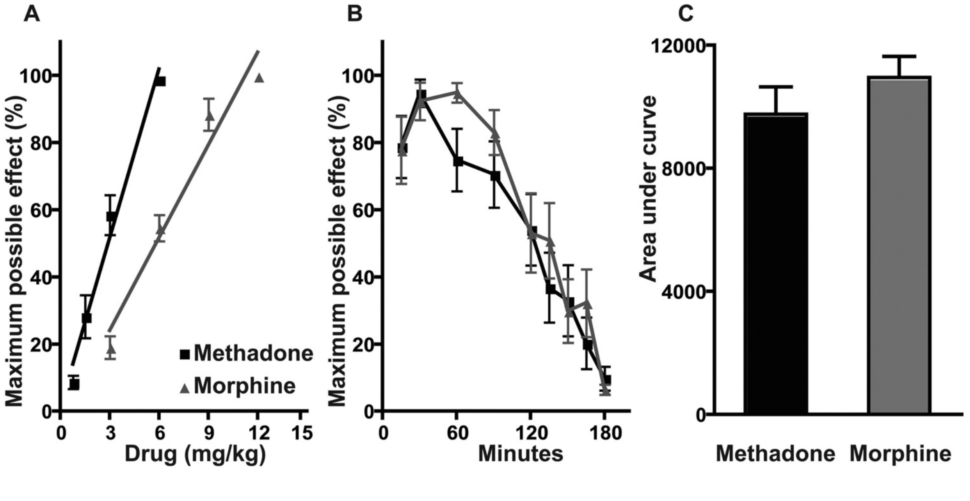

Fig. 1. ED_{50} and effect duration of methadone and morphine. Data shown are drug-induced increases in tail-flick latency described as maximum possible effect. A, antinociceptive dose response to morphine (gray triangles) and methadone (black squares) using cumulative dosing ($n = 20$ animals per drug). B, drug effect duration after a single injection of methadone (4 mg/kg) or morphine (8 mg/kg) ($n = 11$ animals per drug). C, cumulative area under the curve for each drug from B.

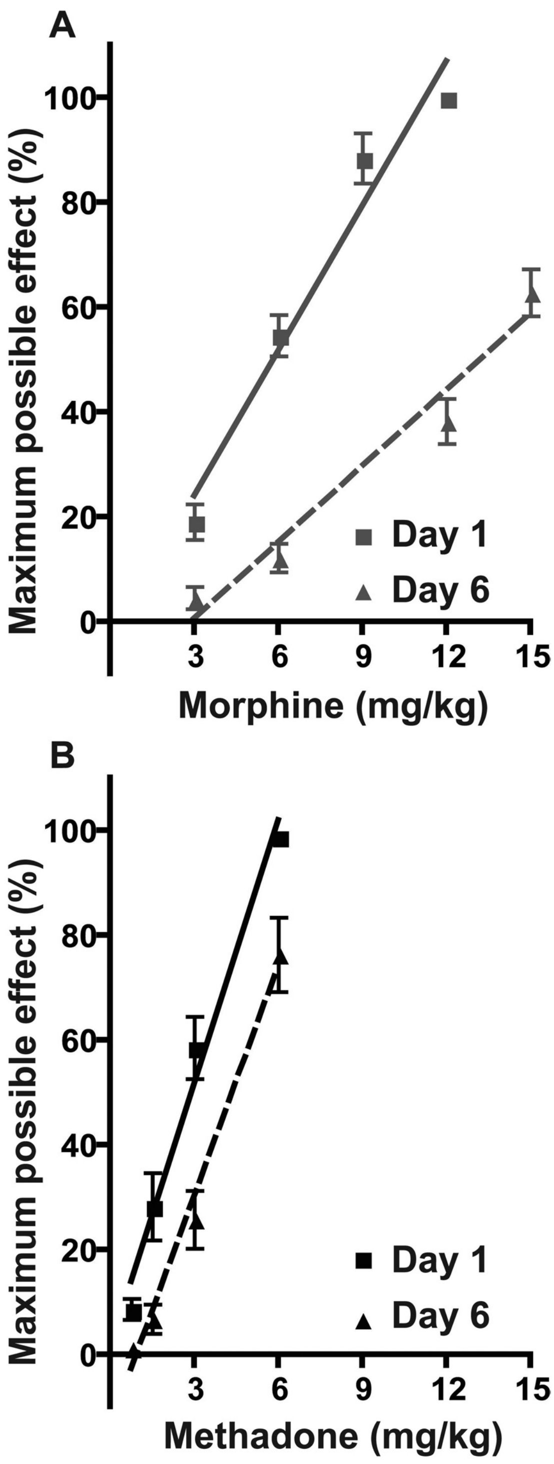

Fig. 2. Cumulative dose response of morphine and methadone on tail-flick latencies in drug-naive and drug-tolerant animals. Cumulative dose response of morphine (A) and methadone (B) on tail-flick latencies in drug naive mice (solid lines) and mice treated with repeated morphine or methadone for 6 days (hatched lines). n = 20 animals per drug. See Table 1 for confidence intervals.

(see *Materials and Methods*; n = 20 animals per drug). Methadone had an ED_{50} of 2.9 mg/kg (confidence interval 1.7–4.1 mg/kg), whereas morphine had an ED_{50} of 5.8 mg/kg (confidence interval 3.9–7.6 mg/kg) (Fig. 1A; Table 1). Because the molecular weight of morphine is twice that of methadone (for the formulations used in this study morphine pentahydrate

TABLE 1
Analgesic ED_{50} and confidence interval (in parentheses) of methadone and morphine by tail flick

Drug	Acute	Treatment Day 6	Right Shift
	mg/kg		*fold*
Methadone	2.9 (1.7–4.1)	4.3 (2.9–5.8)	1.5
Morphine	5.8 (3.9–7.6)	13.5 (9.6–16.9)	2.3

was 758 g/mol, and methadone was 346 g/mol) these data suggest that, per mole, these two drugs are equally potent.

We next examined the duration of antinociception produced by morphine (8 mg/kg) and methadone (4 mg/kg). These drug doses were carefully picked to avoid ceiling effects while eliciting a high analgesic response (90% maximum possible effect). An investigator blind to the drug administered monitored the analgesic effect (Fig. 1B; n = 11 animals per drug). The drugs showed near-identical duration of effect, producing significant antinociception for 120 min. Likewise, the areas under the curve for morphine and methadone were not significantly different (Fig. 1C).

Chronic Morphine Produces Significantly More Antinociceptive Tolerance than Chronic Equianalgesic Methadone. We next assessed the development of antinociceptive tolerance to repeated morphine and methadone exposure. On day 1, antinociception was assessed by cumulative dose response as in Fig. 1. Mice then received drug once per day for 4 days (ED_{50} dose: 3 mg/kg methadone or 6 mg/kg morphine). On day 6, antinociception was again assessed by cumulative dose response. Both treatments produced antinociceptive tolerance (Fig. 2; Table 1); however, the degree of tolerance produced by methadone was significantly less than that produced by morphine. The ED_{50} of methadone after chronic treatment was 4.3 (confidence interval 2.9–5.8 mg/kg), versus 2.9 in naive mice (a 1.5-fold shift in potency; Fig. 2A; Table 1). The ED_{50} of morphine after chronic treatment was 13.5 (confidence interval 9.6–16.9) compared with 5.8 in naive animals (a 2.5-fold shift in potency; Fig. 2B; Table 1). In addition, the effect of the maximal dose of methadone (6 mg/kg) was decreased by only 22% on day 6 compared with day 1, whereas the effect of the maximal dose of morphine was reduced by 63%.

Morphine Induces Significantly Greater Drug Dependence than Methadone. We next assessed the degree of dependence induced by chronic treatment with equiantinociceptive morphine and methadone. Mice (n = 9 animals per group) were treated once per day with an EC_{50} dose of morphine (6 mg/kg) or methadone (3 mg/kg). On day 7, mice were given their usual drug dose followed 30 min later by naloxone (0.5 mg/ml s.c.) (indicated as chronic in Fig. 3). As a control for acute withdrawal, opioid-naive mice were given a single injection of morphine (6 mg/kg) or methadone (3 mg/kg) followed 30 min later by naloxone (0.5 mg/kg) (indicated as acute in Fig. 3). As a control, we included groups that received only acute or chronic saline followed by naloxone (Fig. 3C). Naloxone-precipitated withdrawal behaviors (paw tremor, wet dog shakes, and jumps) were scored for 15 min by an investigator blind to treatment in these four groups.

Naloxone produced few signs of withdrawal, primarily paw tremors, in mice that received only a single dose of morphine (Fig. 3A, morphine acute) with a global score of 21 ± 7. In contrast, mice treated chronically with morphine showed

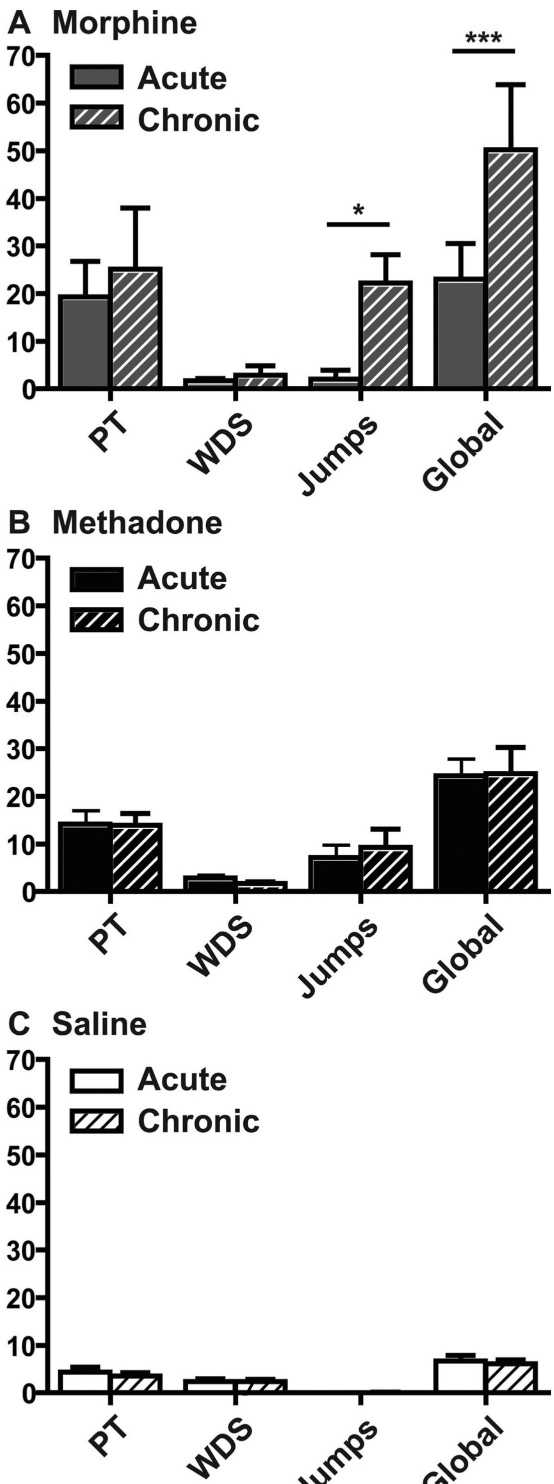

Fig. 3. Naloxone precipitated withdrawal signs after acute and chronic morphine and methadone treatment. Mice (n = 9 per group) were treated with a single dose (acute) or repeated doses (chronic) of morphine (A), methadone (B), or saline (C). Thirty minutes later mice were injected with naloxone (0.5 mg/kg), and withdrawal signs were scored for 15 min. Data are presented as number of events over the 15-min period plus a summarized global score. *, $p < 0.05$; ***, $p < 0.001$, two-way ANOVA and Bonferroni post-tests.

substantial signs of withdrawal (Fig. 3A, morphine chronic), including jumps, with a global score of 50 ± 6 [interaction significant for treatment and behavior ($F_{3,64}$ = 3.124; $p <$ 0.05); post-test revealed significant increases in jumps ($p <$ 0.05) and global withdrawal ($p < 0.001$) scores after chronic versus acute treatment with morphine]. In contrast, there was no difference in the degree of withdrawal in mice treated with acute methadone (global score 24 ± 4) versus those treated with chronic methadone (global score 25 ± 5) (Fig. 3B). Likewise, chronic saline injections did not lead to an increase in precipitated withdrawal symptoms compared with acute saline injections (chronic saline global score 6.8 ± 3.8 versus saline acute 6.1 ± 3.0) (Fig. 3C). Finally, statistical comparison of the global score after acute or chronic treatment with each drug showed a clear interaction between drug and treatment (drug × treatment, $F_{2,54}$ = 6.651, $p <$ 0.01), and post-test showed that only chronic morphine treatment led to a significant increase in withdrawal symptoms ($p < 0.001$), which was significantly higher than the chronic global score of methadone ($p < 0.001$).

MOR Down-Regulation Does Not Contribute to Analgesic Tolerance to Methadone. These data suggest that endocytosis per se is not responsible for opioid tolerance, because methadone produces greater endocytosis (Keith et al., 1998; Celver et al., 2004) but less tolerance than morphine (Fig. 1). We hypothesized that the lack of substantial tolerance to methadone, despite its ability to drive significant receptor desensitization (Arttamangkul et al., 2008; Quillinan et al., 2011), arrestin recruitment (Bohn et al., 2004), and endocytosis (Sternini et al., 1996; Keith et al., 1998; Celver et al., 2004; He and Whistler, 2005), reflects that the MOR is recycled and resensitized after endocytosis. If this were the case, we would expect methadone to produce substantial tolerance if the MOR could not be recycled. To examine this possibility we used mice expressing a mutant form of the MOR, DMOR (for degrading MOR) that is targeted for degradation after endocytosis (Finn and Whistler, 2001; Enquist et al., 2011).

The antinociceptive effect of methadone in both genotypes (WT and DMOR) was indistinguishable (Fig. 4A). To examine the rate of tolerance development mice of both genotypes were treated with methadone (10 mg/kg) twice per day, and antinociception was measured 30 min after the first dose every other day for 7 days (Fig. 4B). Unlike WT mice, DMOR mice showed significant antinociceptive tolerance to methadone by day 3 (each group analyzed separately by repeated-measures ANOVA, no significant change for WT, $F_{3,24}$ = 9186, $p < 0.0001$ for overall treatment × days in DMOR; Dunnett's multiple comparison test, $p < 0.01$ each for day 1 versus days 3, 5, and 7), which was exacerbated by additional days of treatment (Fig. 4B). Thus, endocytosis can promote tolerance under conditions where MOR is targeted for degradation (Fig. 4A), but under normal conditions where MOR is recycled endocytosis seems to protect against the development of tolerance (Fig. 2).

Discussion

Here, we show that chronic methadone treatment causes substantially reduced antinociceptive tolerance and physical dependence compared with chronic morphine treatment under conditions of equivalent pain relief. Furthermore, we show that, although receptor endocytosis protects against antinociceptive tolerance in wild-type mice, it enhances tol-

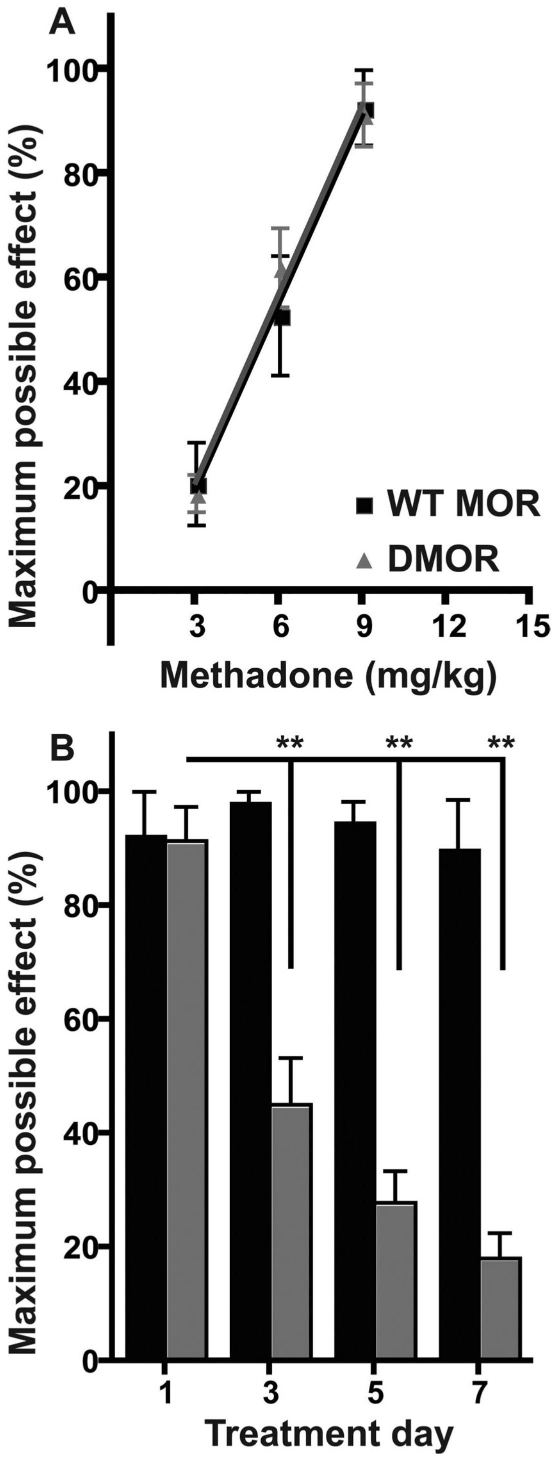

Fig. 4. Methadone antinociception and tolerance in WT and DMOR mice. Data shown are drug-induced increases in tail-flick latency described as maximum possible effect. A, cumulative dose response of methadone in WT and DMOR mice. B, WT mice (black bars) and DMOR mice (gray bars) ($n = 8$ per group) were injected with methadone (10 mg/kg) twice per day. Maximum possible effect was measured every other day starting on day 1. **, $p < 0.01$, result analyzed within each group by repeated-measures ANOVA followed by Dunnett's multiple comparison test.

erance in an animal model in which the MOR is mutated to enhance receptor degradation after endocytosis.

We carefully titrated drug levels to ensure equivalent pain relief in this study. However, a recent study that used four times the molar concentration of methadone versus morphine (and a 32× higher concentration of methadone than that used here) also showed that the potency shift was much greater with chronic morphine than with chronic methadone (Raehal and Bohn, 2011). Specifically, Raehal and Bohn showed that chronic methadone produced a 1.8× potency shift compared with the 1.5× shift we show here, whereas morphine produced a 2.9× shift compared with the 2.3× shift we show here. This would indicate that methadone could maintain its beneficial side-effect profile of reduced tolerance even across a wide concentration window.

In addition, we found no signs of dependence in mice after chronic methadone treatment compared with those produced by acute withdrawal (Fig. 3). In the human literature there is ample evidence of withdrawal signs and dependence among patients under methadone maintenance (Bakstad et al., 2009; Awgu et al., 2010; Lobmaier et al., 2010). However, it is important to keep in mind that these patients first depended on heroin, which like morphine does not promote receptor endocytosis. Thus, the methadone "dependence" in these patients probably reflects their underlying heroin dependence that cannot be reversed simply by promoting receptor endocytosis. Indeed, methadone is almost never given as a first-line analgesic in human medicine, so there is no clear data showing whether methadone would have reduced liability to produce tolerance and dependence compared with morphine (or oxycontin, Dilaudid, or fentanyl, none of which produce endocytosis at analgesic doses), when given as a first-line therapeutic. There are also reports of methadone dependence in preclinical animal models (see e.g., Raehal and Bohn, 2011). However, those reports used significantly higher doses of methadone and did not examine whether chronic treatment produced a greater degree of dependence than acute withdrawal from these single high doses of methadone. We did not examine here whether chronic administration of continuous methadone, more comparable with that used in patients, produced a different endocytic pattern than intermittent dosing. However, previously we have shown that chronic continuous administration of opioid cocktails that drive endocytosis continue to do so after at least 7 days of treatment (He et al., 2002). Furthermore, our previous studies have shown that the morphine remains unable to drive substantial endocytosis even after chronic continuous administration (He et al., 2002).

It has been suggested that methadone may produce reduced tolerance and dependence, not because it promotes receptor endocytosis, but because of its ability to antagonize NMDA receptors and thereby counteract maladaptive changes in glutamate transmission (Davis and Inturrisi, 1999; Stringer et al., 2000). Of course, these two mechanisms are not mutually exclusive. Indeed, methadone may be a better therapeutic choice than morphine because of its ability to drive endocytosis and antagonize NMDA receptors. Previously, we found that subanalgesic doses of methadone mixed with morphine reduce the development of both tolerance and dependence (He and Whistler, 2005). However, the beneficial effects of methadone in this study could not be achieved when only the methadone enantiomer with high NMDA receptor affinity but no ability to drive receptor endocytosis was used (He and Whistler, 2005). Thus, although NMDA antagonism may add to the benefits of methadone, it may be less important than the ability of this drug to promote MOR endocytosis. In support of this hypothesis, enhanced endocytosis of the MOR in response to morphine as a consequence of pharmacological cocktails that do not contain methadone (He et al., 2002) reduces the severity of morphine tolerance and dependence. Likewise, enhanced morphine induced endocytosis through the release of endogenous opioids (Zöllner et al., 2008) and in mice with mutant MORs that endocytose and recycle (Kim et al., 2008; Madhavan et al., 2010) also reduces morphine tolerance and dependence independent of any effect on NMDA receptors. Instead, we propose that the failure of morphine to induce substantial arrestin recruitment and endocytosis promotes homeostatic adaptations in MOR-expressing neurons. These adaptations are probably diverse and could include changes in channel activity, second-messenger activity, and gene expression (reviewed by Nestler, 1997, 2004; Berger and Whistler, 2010). We are also particularly interested in examining whether endocytosis of the MOR can prevent the up-regulation of the δ-opioid receptor (DOR) associated with chronic morphine (Rothman et al., 1986; Cahill et al., 2001). Indeed, up-regulation of the DOR has been implicated in morphine tolerance (Abdelhamid et al., 1991), and this redistribution depends on arrestin at least in some circuits (Hack et al., 2005). In fact, studies indicate that there is a direct interaction between DOR and MOR, these dimer interactions increase with morphine tolerance (Gupta et al., 2010), and the heteromer has a different pharmacological profile than MOR alone (Rozenfeld and Devi, 2007; Gupta et al., 2010; Milan-Lobo and Whistler, 2011).

Finally, our results from this study with the DMOR mice suggest that, although receptor endocytosis can protect against the development of tolerance, it can also produce tolerance if the receptor is not recycled. For example, etorphine at high doses has been shown to produce tolerance and accompanying down-regulation of MOR in vivo (Duttaroy and Yoburn, 1995; Yabaluri and Medzihradsky, 1997). Thus, the postendocytic fate of the MOR may depend on the agonist used. Ultimately, the combined effect of a drug's ability to induce receptor endocytosis and the postendocytic fate of the receptor will determine the physiological responsiveness to chronic drug treatment.

Authorship Contributions

Participated in research design: Enquist, Ferwerda, Milan-Lobo, and Whistler.

Conducted experiments: Enquist, Ferwerda, and Milan-Lobo.

Contributed new reagents or analytic tools: Ferwerda.

Performed data analysis: Enquist and Milan-Lobo.

Wrote or contributed to the writing of the manuscript: Enquist and Whistler.

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