

Morphine-Induced Receptor Endocytosis in a Novel Knockin Mouse Reduces Tolerance and Dependence

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Summary

Opioid drugs, such as morphine, are among the most effective analgesics available. However, their utility for the treatment of chronic pain is limited by side effects including tolerance and dependence. Morphine acts primarily through the mu-opioid receptor (MOP-R) [1], which is also a target of endogenous opioids. However, unlike endogenous ligands, morphine fails to promote substantial receptor endocytosis both in vitro [2–5] and in vivo [6–11]. Receptor endocytosis serves at least two important functions in signal transduction. First, desensitization and endocytosis act as an “OFF” switch by uncoupling receptors from G protein. Second, endocytosis functions as an “ON” switch, resensitizing receptors by recycling them to the plasma membrane. Thus, both the OFF and ON function of the MOP-R are altered in response to morphine compared to endogenous ligands. To examine whether the low degree of endocytosis induced by morphine contributes to tolerance and dependence, we generated a knockin mouse that expresses a mutant MOP-R that undergoes morphine-induced endocytosis. Morphine remains an excellent antinociceptive agent in these mice. Importantly, these mice display substantially reduced antinociceptive tolerance and physical dependence. These data suggest that opioid drugs with a pharmacological profile similar to morphine but the ability to promote endocytosis could provide analgesia while having a reduced liability for promoting tolerance and dependence.

Results

Generation of a Novel MOP-R Knockin Mouse with Altered Trafficking Properties

To directly examine whether endocytosis of the MOP-R in response to morphine alters the development of tolerance and dependence in vivo, we generated a knockin mouse expressing

the rMOP-R mutant receptor that internalizes in response to morphine [12]. In this rMOP-R, a portion of the cytoplasmic tail of the MOP-R, encoded entirely within exon 3, has been replaced with sequence from the delta opioid peptide receptor (see Figure 1A). Mice expressing the rMOP-R were identified by Southern (DNA) blot analysis (Figures 1A and 1B). The specific mutation introduced to the MOP-R gene [12] was contained entirely within exon 3, which is common to all splice variants that have been described. Endocytic trafficking of the wild-type MOP-R and the rMOP-R mutant receptor examined in striatal neurons cultured from wild-type and mutant mice demonstrated that morphine promotes rMOP-R but not MOP-R endocytosis in response to morphine (Figure 1C and Figure S1 available online).

The mutant mice were viable, had no gross phenotypic abnormalities, and showed normal baseline pain responses (hot-plate latency, 56°C: wild-type, 4.88 ± 0.33 s; mutant, 4.55 ± 0.32 s). Consistent with their equivalent baseline pain responses, there were no genotypic differences in MOP-R distribution in the spinal cord or multiple brain regions important for the antinociceptive and reinforcing properties of opiates (Figure S2 and data not shown). In addition, ligand affinity, receptor number, and receptor G protein coupling were unaltered in the rMOP-R mice (Figures 2A–2C).

Antinociception in rMOP-R Knockin Mice versus Wild-Type MOP-R Mice

Morphine-induced antinociception was evaluated by measuring response latencies in the hot-plate test. We tested a dose of morphine (10 mg/kg) known to induce robust antinociception in mice. The acute antinociceptive effect of this dose of morphine was significantly enhanced and prolonged in knockin mice relative to their wild-type littermates (Figure 3A). A dose of 3 mg/kg in the mutant mouse was equi-antinociceptive to 10 mg/kg in the wild-type mouse (Figure 3B). Both genotypes reached a ceiling effect at the highest dose tested, 50 mg/kg. The opioid antagonist naloxone completely reversed the antinociceptive effects of morphine in both wild-type and mutant mice (Figure 3B).

We propose that the enhanced antinociception in the mutant mice reflects the restoration of the ON function provided by receptor endocytosis and recycling. Specifically, we propose that morphine-occupied MOP receptors become partially desensitized in wild-type mice and fail to resensitize due to poor endocytosis; whereas in the rMOP-R knockin mice, receptors are also desensitized but are rapidly resensitized by endocytosis and recycling. Consistent with this hypothesis, MOP receptors in wild-type mice given a single 10 mg/kg dose of morphine showed significant receptor-G protein uncoupling (Figure 3C, left). Clearly not all MOP receptors in these mice were desensitized because morphine is still an excellent acute antinociceptive agent in wild-type mice. Nevertheless, MOP-Rs in the brainstem of wild-type mice treated with morphine showed a 200-fold shift in the EC₅₀ of DAMGO (Figure 3C, left) compared to wild-type mice treated with vehicle. In contrast, receptors in rMOP-R mice given the same dose of morphine, showed no desensitization (Figure 3C, right). These data suggest that the reduced morphine antinociception

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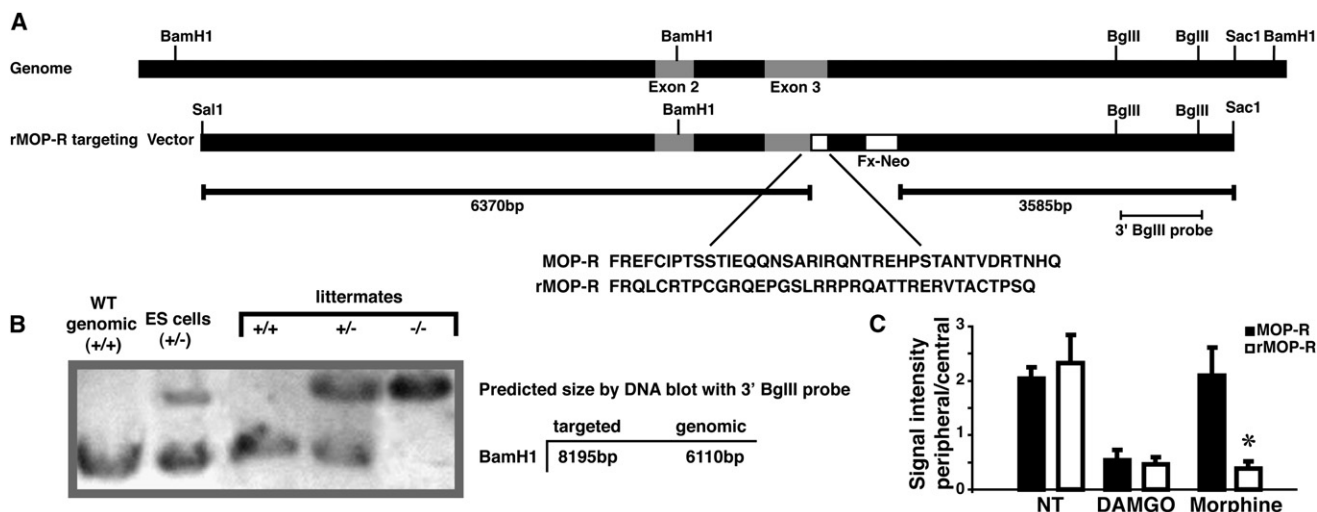


Figure 1. Generation of rMOP-R Knockin Mice

(A) Schematic of targeting strategy. A SalI-SacI genomic fragment containing the MOP-R sequence, including exons 2 and 3, was modified to contain the rMOP-R sequence (inset). A cassette containing resistance to G418 (Fx-Neo) and flanked by Lox P sites was inserted in the intron downstream of exon 3 for selection of ES clones.

(B) Detection of homologous recombinants. Genomic DNA was digested with BamHI and subjected to DNA hybridization with an ~1.1 kb BglIII fragment (see [A]). Targeted loci were confirmed by the presence of a band at ~8 kb. The intact locus gave a band at ~6 kb.

(C) Quantification of endocytosis. MetaMorph software was used to quantify the intensity of receptor signal at the plasma membrane versus the cytosol for each treatment condition and each genotype (MOP-R in black, rMOP-R in white). Data is plotted as the ratio of signal located within 0.3 μ m of the surface (peripheral) versus the amount in the cytosol (central). See Figure S1 for representative neurons and schematic. Histogram shows the mean of this ratio \pm the SD for each treatment (10–25 measurements per condition; see the Supplemental Experimental Procedures.)

in the wild-type compared to the rMOP-R mice reflects partial desensitization of MOP-Rs that is not reversed by endocytosis and recycling.

If this were the case, we would expect mice of both genotypes to show equivalent antinociception to an agonist that promotes endocytosis of the receptor in both genotypes. Indeed, there were no significant genotypic differences in antinociception induced by methadone (1–10 mg/kg; Figure 3D), a MOP-R agonist that promotes rapid internalization of both the wild-type MOP-R and mutant rMOP-R. Thus, the enhanced opioid antinociception observed in the rMOP-R knockin mice is specific to morphine. Together with our immunohistochemical and pharmacological data (Figure S2 and Figure 2), these data suggest that the enhanced morphine antinociception in the rMOP-R knockin mice cannot be accounted for by differences in MOP-R distribution, ligand affinity, receptor number, or receptor-G protein coupling. Rather, these data suggest that facilitating MOP-R endocytosis enhances morphine antinociception by reversing rapid desensitization.

Acute Antinociceptive Tolerance in rMOP-R Knockin Mice versus Wild-Type MOP-R Mice

It has been hypothesized that MOP-R desensitization contributes to acute morphine tolerance. If this were the case, rMOP-R mice would be expected to develop reduced acute tolerance compared to wild-type mice. To examine this, we evaluated the acute antinociceptive effect of equi-antinociceptive doses of morphine (3 mg/kg in rMOP-R and 10 mg/kg in MOP-R, see Figure 3B) 24 hr after pretreatment with a high dose of morphine (100 mg/kg) or saline. The day following pretreatment, baseline response latencies between genotypes were similar (rMOP-R, 5.76 ± 0.47 s; MOP-R, 5.86 ± 0.51 s). Indicative of the acute tolerance that is typically observed in this paradigm

[13], wild-type MOP-R mice that had been pretreated with 100 mg/kg of morphine showed a 43% reduction in antinociception compared to mice that had received saline the day before (Figure 4A). In contrast, the rMOP-R knockin mice maintained similar levels of morphine antinociception regardless of whether they had received morphine or saline pretreatment the day before (Figure 4A). Thus, the rMOP-R knockin mice did not develop acute antinociceptive tolerance to morphine.

Chronic Antinociceptive Tolerance in rMOP-R Knockin Mice versus Wild-Type MOP-R Mice

Although acute tolerance to high doses of opioids is most relevant to acute pain, during the treatment of chronic pain, analgesic tolerance typically develops over the course of repeated administrations of moderate levels of drug. Thus, we evaluated the development of tolerance after twice daily administrations of morphine (10 mg/kg) over 5 days. Wild-type mice in this paradigm developed antinociceptive tolerance (Figure 4B, closed squares). In contrast, their rMOP-R littermates, treated with the same dose of morphine (10 mg/kg) at the same intervals, showed no evidence of tolerance, exhibiting as much antinociception on the last day of drug treatment as they did on the first day (Figure 4B, closed circles).

To rule out the possibility that the lack of tolerance in the mutant mice was an artifact of enhanced morphine antinociception (Figures 3A and 3B), we treated a separate group of knockin mice with an equi-antinociceptive dose of morphine (3 mg/kg, see Figure 3B) given at the same intervals. These rMOP-R knockin mice still showed reduced tolerance, maintaining similar levels of antinociception over the course of treatment (Figure 4B, triangles). Thus, reduced morphine tolerance in the knockin relative to wild-type mice cannot be attributed to enhanced morphine antinociception.

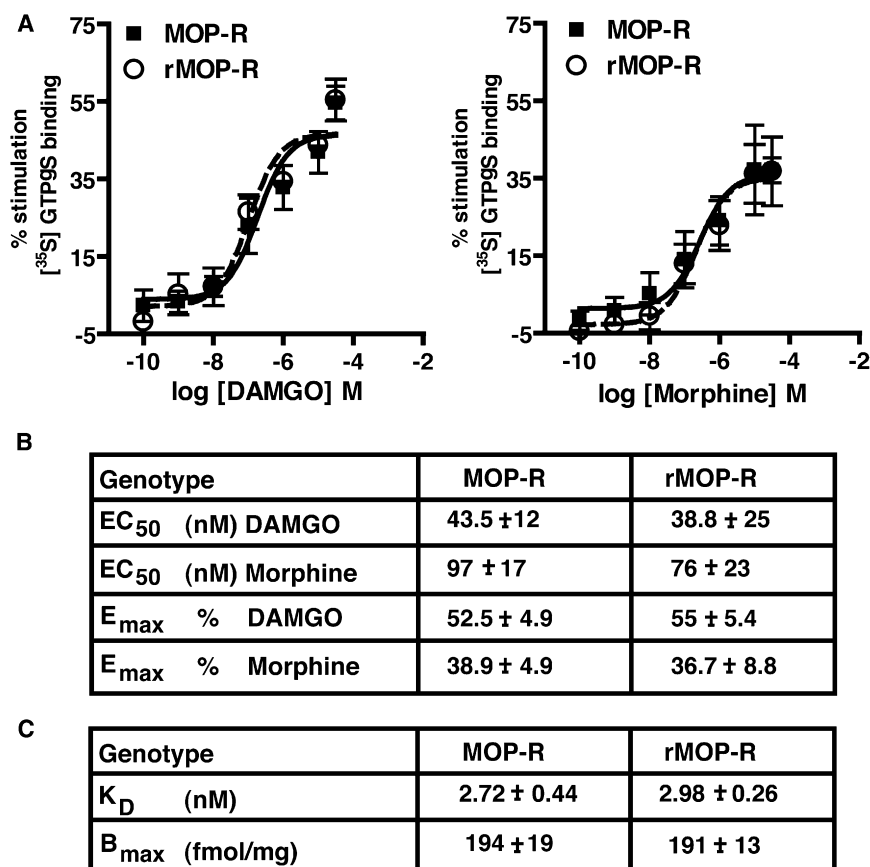


Figure 2. Pharmacological Characterization of Wild-Type MOP-R and rMOP-R Knockin Mice

(A and B) Receptor-G protein coupling. Agonist-mediated GTPγS binding was measured in brain membranes of wild-type MOP-R (squares) and rMOP-R knockin mice (circles) with increasing concentrations of DAMGO or morphine. Data were analyzed by nonlinear regression by using GraphPad Prism software and are presented as means ± SEM of at least three experiments performed in triplicate. There were no significant genotypic differences in either EC₅₀ or E_{max}. (C) Ligand affinity and receptor number. [³H] Naloxone binding in whole-brain membranes from MOP-R and rMOP-R mice. Saturation binding assays were performed on membranes (50–100 μg per well) with increasing concentrations of [³H] naloxone (0–15 nM, 55.9Ci/mmol). Nonspecific binding was measured in the presence of 10 μM naloxone. Binding parameters were determined by Scatchard analysis of specific binding. Data are means ± SEM of three experiments performed in duplicate. There were no statistically significant differences between the genotypes (One way ANOVA with Tukey's post-hoc test). B_{max}, maximum binding capacity; K_D, dissociation constant.

These results suggest that endocytosis of the receptor reduces the development of antinociceptive tolerance. If this were the case, one would expect that opiate agonists, such as methadone, that promote endocytosis of the MOP-R would have reduced liability for promoting tolerance in wild-type mice. In addition, wild-type and rMOP-R mice should show equivalent responsiveness to chronic methadone. To examine this hypothesis, we evaluated the development of tolerance to methadone in MOP-R and rMOP-R mice. In order to directly compare tolerance to morphine versus methadone, we administered a dose of methadone (4 mg/kg, see Figure 3D) that was equi-antinociceptive to the morphine dose we administered in Figure 4B. At this dose, neither genotype showed evidence of tolerance across treatment days (Figure 4C). In addition, responsiveness to methadone during all treatment days was equivalent in MOP-R (Figure 4C, squares) and rMOP-R mice (Figure 4C circles). Thus, reduced chronic opioid tolerance in rMOP-R mice relative to MOP-R mice is specific to morphine.

As was the case for reduced acute tolerance, reduced chronic tolerance to morphine in the mutant mice may reflect, at least in part, that MOP-Rs in the wild-type mice are desensitized (Figure 3C) but are unable to resensitize due to poor endocytosis of the receptor. Facilitating receptor internalization and recycling (i.e., restoring the on function of endocytosis) may protect against the development of both acute (Figure 4A) and chronic tolerance (Figure 4B).

However, receptor desensitization alone cannot explain antinociceptive tolerance to morphine. Specifically, if all MOP-Rs were desensitized in morphine tolerant mice, then displacement of morphine from these nonsignaling receptors with

antagonist should have no behavioral effect. However, morphine tolerant animals show substantial naloxone-precipitated withdrawal signs (for example, see [14]), indicating that receptors continue to signal actively in morphine tolerant

animals despite the lack of antinociception. Hence, mechanisms other than receptor desensitization are likely contributing to tolerance.

Morphine Withdrawal in rMOP-R Knockin Mice versus Wild-Type MOP-R Mice

We next examined whether facilitating endocytosis in the rMOP-R mice affected the development of morphine dependence. After chronic treatment with morphine, mice were challenged with the opioid antagonist, naloxone (2 mg/kg), 30 min after the final morphine injection. Global withdrawal responses were scored by an observer who was blind to genotype (Figure 4D). Wild-type mice expressed robust withdrawal responses compared to mutant mice, which were chronically treated with the same amount of morphine (10 mg/kg) but at a functionally higher dose (see Figures 3A and 3B). Consistent with the hypothesis that enhanced receptor endocytosis decreases withdrawal, chronic methadone treatment (4 mg/kg given at the same intervals as morphine) promoted substantially less withdrawal than did morphine in wild-type mice (Figure 4D). In fact, the moderate level of methadone withdrawal in wild-type mice was equivalent to that produced by either morphine or methadone in the rMOP-R mice (Figure 4D). Hence, we have generated a mouse line that retains morphine antinociceptive potency with markedly reduced morphine tolerance and dependence.

Discussion

In summary, we report that mice expressing a mutant rMOP-R with altered receptor trafficking properties in response to

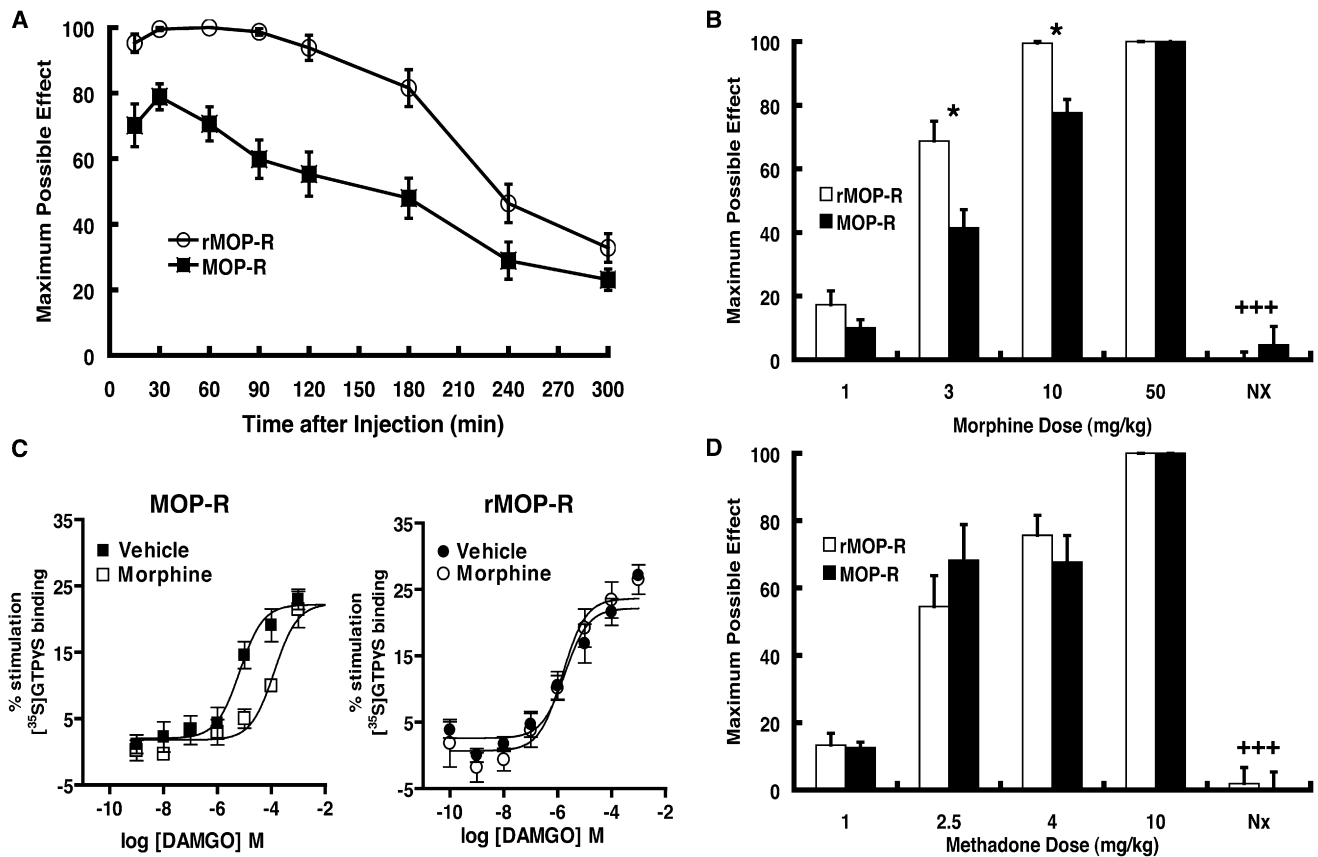


Figure 3. Antinociception in Wild-Type MOP-R and rMOP-R Knockin Mice

(A) Enhanced and prolonged morphine-induced antinociception in rMOP-R knockin mice. Antinociceptive responses were measured with the hot-plate response latency test (56°C) after morphine treatment (10 mg/kg, s.c.). A response endpoint was defined as latency to either lick the fore- or hindpaws or flick the hindpaws. To avoid tissue damage, we exposed mice to the hot-plate for a maximum of 20 s. Data are reported as the mean \pm SEM of percent maximum possible effect (MPE) by using the following formula: $100\% \times [(drug\ response\ time - basal\ response\ time) / (20\ s - basal\ response\ time)]$. A two-way analysis of variance revealed that the MPE curve for rMOP-R mice ($n = 17$) mice was significantly greater and prolonged relative to the MOP-R mice ($n = 17$), as indicated by a significant genotype [$p < 0.001$, $F(1,7) = 28.05$] and genotype \times time interaction effect [$p < 0.001$, $F(1,7) = 4.97$].

(B) Dose-dependent morphine-antinociception. Antinociceptive responses were determined with the hot-plate test, and data are reported as mean \pm SEM of MPE (see [A]). Separate groups of mice for both genotypes ($n = 7-9$) were injected with the doses of morphine indicated and assessed for antinociception 30 min later. To test whether the antinociceptive responses were mediated by opioid receptors, we injected a final grouping with morphine (10 mg/kg) followed by naloxone (2 mg/kg). rMOP-R knockin mice showed enhanced antinociception at 3 and 10 mg/kg doses (rMOP-R versus MOP-R scores for MPE at respective morphine doses, Student's t test, $*p < 0.03$) with the latter dose inducing the maximum possible response (100%) in the mutant mice. At the highest dose tested (50 mg/kg), both genotypes exhibited the maximum possible response (100%). For both genotypes, antinociception induced by 10 mg/kg of morphine was reversed by treatment with 2 mg/kg of the opioid antagonist naloxone (morphine 10 mg/kg with and without naloxone 2 mg/kg treatment for each genotype respectively, student's t test $+++p < 0.001$).

(C) MOP-R desensitization in the brainstem following acute morphine treatment. Agonist-mediated [35 S]GTP γ S binding was measured in brainstem membranes of MOP-R and rMOP-R mice with increasing concentrations of DAMGO. Left: Binding in MOP-R mice was significantly reduced ($p < 0.01$) after acute morphine-treatment (10 mg/kg s.c. 30 min; $EC_{50} = 428 \pm 141 \mu M$; open squares) compared to vehicle-treated mice ($EC_{50} = 2.35 \pm 0.9 \mu M$; closed squares). Right: Binding in rMOP-R mice was not significantly changed ($p > 0.05$) after acute morphine treatment ($EC_{50} = 3.29 \pm 1.4 \mu M$; open circles) compared to vehicle-treated mice ($EC_{50} = 1.16 \pm 0.6 \mu M$; closed circles). Data were analyzed by nonlinear regression using GraphPad Prism software and are presented as means \pm SEM of at least three experiments performed in triplicate.

(D) Enhanced antinociception in rMOP-R knockin mice is morphine specific. Separate groups of mice for both genotypes ($n = 8-10$) were injected with the doses of methadone indicated (1-10 mg/kg,) and assessed for antinociception. Methadone induced a dose-dependent increase in antinociceptive response with no genotypic differences. For both genotypes, antinociception induced by 4 mg/kg of methadone was reversed by treatment with 2 mg/kg of the opioid antagonist naloxone (methadone 4 mg/kg with and without naloxone 2 mg/kg treatment for each genotype respectively, Student's t test, $+++p < 0.001$). Thus, enhanced opioid-induced antinociception observed in the rMOP-R knockin mice is agonist specific and naloxone reversible.

morphine show enhanced morphine-induced antinociception, reduced morphine tolerance, and reduced naloxone-precipitated withdrawal compared to their wild-type littermates. These knockin mice otherwise show normal ligand affinity, receptor number, receptor-G protein coupling, and receptor distribution, consistent with the fact that both their basal pain responses as well as methadone antinociception are equivalent to that of their wild-type littermates.

These data are consistent with the hypothesis that enhanced endocytosis of the MOP-R, in response to morphine, can reduce antinociceptive tolerance and dependence while retaining the antinociceptive efficacy of morphine. It is important to note that endocytosis is only one step in a cascade of highly conserved events that occurs after G-protein-coupled receptor activation. When receptors are activated by endogenous ligand, they are rapidly desensitized by

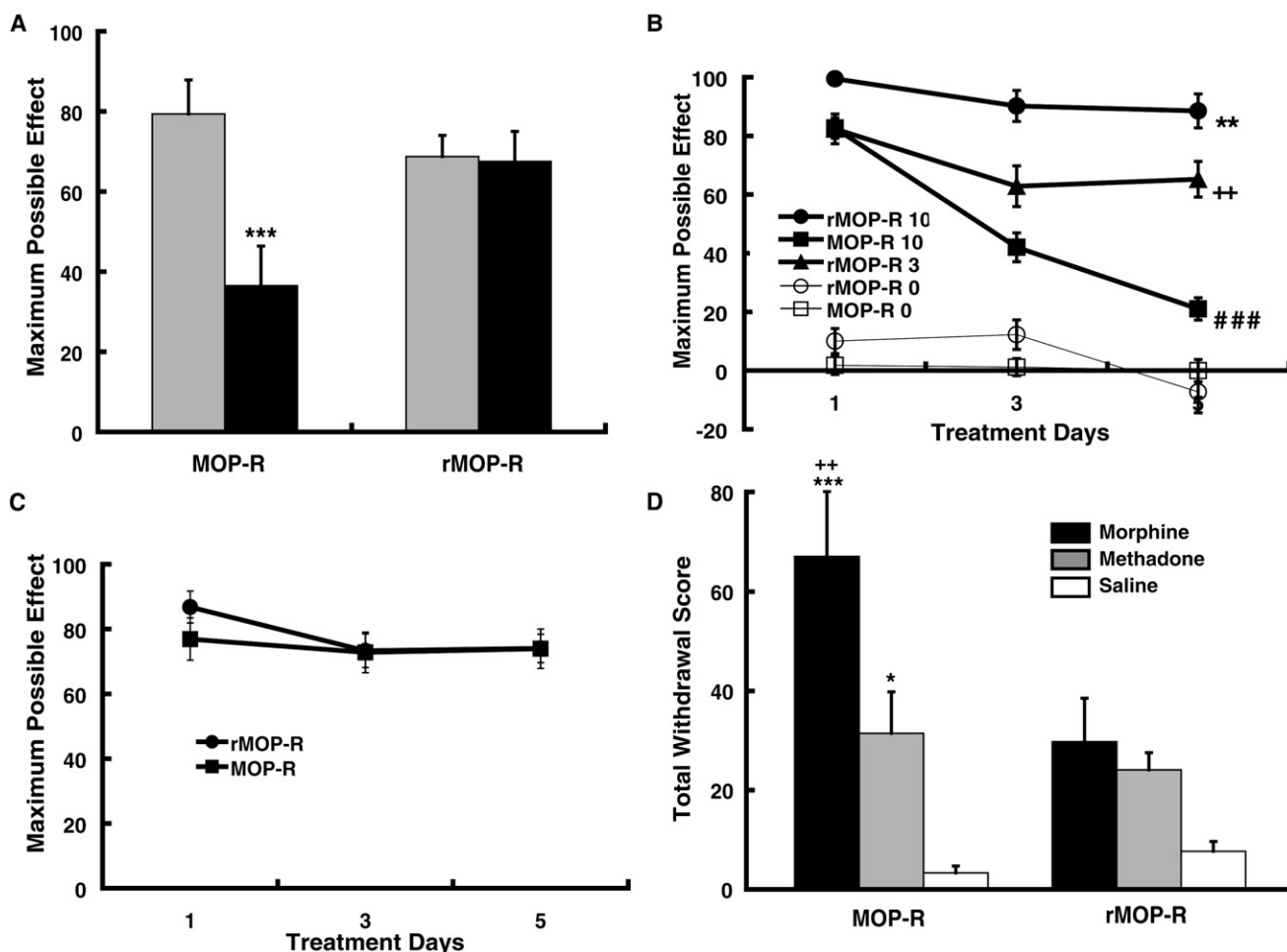


Figure 4. Opioid Tolerance and Dependence in MOP-R Wild-Type and rMOP-R Knockin Mice

(A) Acute morphine tolerance. Mice ($n = 9$) were initially treated with either saline (gray bars) or a high dose of morphine (100 mg/kg s.c., black bars). 24 hr later, mice were challenged with an acute equi-antinociceptive dose of morphine (3 mg/kg for rMOP-R and 10 mg/kg for MOP-R, see Figure 4B). Data are presented as mean \pm SEM of MPE. Wild-type MOP-R mice exhibited significant acute tolerance, showing a 43% reduction in MPE when pretreated with 100 mg/kg of morphine compared to saline 24 hr before (MOP-R comparing saline versus morphine pretreatment, Student's *t* test, *** $p < 0.001$). In contrast, rMOP-R knockin mice showed no evidence of tolerance and maintained the same level of morphine-induced antinociception whether they were pretreated with 100 mg/kg of morphine or saline 24 hr before.

(B) Chronic morphine tolerance. Mice were treated twice daily with morphine (10 mg/kg, sc) for 5 days and antinociception was assessed after the first injection of morphine each day. Mean \pm SEM of MPE across days are presented. A two-way ANOVA revealed that mice treated chronically with morphine ($n = 17$) behaved differently corresponding to genotype, as indicated by a significant group effect [$F(2,42) = 27.95, p < 0.001$] and group \times treatment days effect [$F(4,84) = 12.09, p < 0.001$]. Posthoc comparisons (Tukey's) revealed the source of the interaction. rMOP-R knockin mice treated with 10 mg/kg of morphine had significantly longer response latencies across days compared to wild-type mice treated with the same dose of morphine (10 mg/kg) and rMOP-R knockin mice chronically treated with a lower (equi-antinociceptive) dose of morphine (3 mg/kg) (rMOP-R 10 significantly different from MOP-R 10 and rMOP-R 3, ** $p < 0.01$). Additionally, rMOP-R knockin mice chronically treated with an equi-antinociceptive dose of morphine (3 mg/kg) showed significantly greater antinociception than did wild-type mice chronically treated with a higher dose of morphine (10 mg/kg) across the tolerance development days (rMOP-R 3 versus MOP-R 10, ++ $p < 0.01$). Only wild-type mice chronically treated with morphine (10 mg/kg) showed a significant decrease in antinociception from Day 1 to Day 5 (MOP-R 10 Day 1 versus Day 5, ### $p < 0.001$). Thus, the development of antinociceptive tolerance to morphine was evident in wild-type mice but attenuated in the knock in mice, whether they were chronically treated with the same (10 mg/kg) or equi-antinociceptive (3 mg/kg) dose of morphine.

(C) Methadone tolerance. Mice were injected twice daily with methadone (4 mg/kg, sc) for 5 days. For both genotypes, there was no evidence of tolerance development with both groups expressing comparable levels of methadone-antinociception following the first and last injection. Methadone antinociception was equivalent in both genotypes across all days.

(D) Naloxone precipitated withdrawal. Groups of mice were chronically treated with 10 mg/kg of morphine (black bars, $n = 10-12$), 4 mg/kg of methadone (gray bars, $n = 9$), or saline (white bars, $n = 6$) at the same intervals described for Figures 4B and 4C. Mice were challenged with naloxone (2 mg/kg, sc) 30 min after the final treatment injection. Notably, the chronic dose of morphine used (10 mg/kg) corresponded to a functionally higher dose in the rMOP-R knockin mice relative to wild-type mice (see Figures 3A and 3B). Standard withdrawal behaviors including jumping, "wet-dog" shakes, paw licks, and paw tremors, were scored by an observer blind to genotype. Total withdrawal scores (the sum of all individual withdrawal behaviors) \pm SEM are presented and group differences were analyzed with the LSD test. Compared to saline-treated mice, MOP-R wild-type mice displayed a significantly higher incidence of withdrawal when chronically treated with morphine or methadone (MOP-R MOR versus MOP-R SAL, *** $p < 0.001$, MOP-R METH versus MOP-R SAL, * $p < 0.05$), with a higher degree of withdrawal associated with chronic morphine treatment (MOP-R MOR versus MOP-R METH, ++ $p < 0.01$). In contrast, rMOP-R knockin mice displayed similar levels of withdrawal regardless of pretreatment drug. For rMOP-R knockin mice, both morphine and methadone pretreatment resulted in similar levels of moderate withdrawal, comparable to methadone pretreatment in MOP-R wild-type mice.

phosphorylation and interaction with arrestin and then endocytosed. After endocytosis, MOP-Rs are functionally resensitized by recycling to the plasma membrane. Many groups have demonstrated *in vitro* that morphine-activated receptors elude this natural cycle of receptor desensitization, endocytosis, and resensitization that is induced by endogenous MOP-R ligands [15–18]. Similarly, morphine has been found to be a poor inducer of receptor endocytosis *in vivo* [6–10]. However, *in vivo*, subtleties also emerge because in some cases desensitization has not been detected [19], whereas in other cases desensitization of the morphine-activated receptor by GRK/arrestin and/or PKC does appear to occur [20–27]. Thus, receptor desensitization may be either brain region specific, incomplete, or both.

In the context of regionally-specific or incomplete receptor desensitization, the failure to endocytose the morphine-bound receptor has the potential to affect signal transduction in at least two ways. First, in cells or brain regions where morphine does not cause substantial receptor desensitization, prolonged receptor activation may trigger downstream adaptive responses that contribute to morphine tolerance and dependence. In rMOP-R mice, this prolonged receptor activation is replaced by pulsatile receptor activation due to restoration of the OFF/ON switch of endocytosis. Second, in cells or brain regions where receptors do become desensitized after morphine activation, failure to endocytose would prevent functional resensitization of the receptor. In rMOP-R mice, resensitization would be restored. Notably, even in regions where desensitization appears to occur (Figure 3C), a significant number of receptors remain coupled. These remaining receptors would exhibit prolonged activation in wild-type mice and pulsatile activation in knockin mice.

Disruption of arrestin enhances morphine antinociception [21] and delay tolerance [21], presumably by decreasing the degree of morphine-induced desensitization. However, arrestin knockout mice show levels of withdrawal equivalent to their wild-type littermates [28], indicating that there are still a substantial number of functionally coupled MOP-Rs even in animals with intact arrestin. Promoting morphine-induced endocytosis would be expected to both facilitate receptor resensitization and alleviate the compensatory adaptive changes associated with dependence. Thus, while both preventing receptor desensitization and facilitating receptor endocytosis/resensitization are effective strategies to enhance morphine antinociception and prevent tolerance, the latter has the added benefits of reducing morphine dependence and specificity to the MOP-R.

All opioids, when given at high enough concentration for a long enough period of time, including methadone, can induce tolerance and dependence. However, when given at equi-antinociceptive doses, opioids induce different degrees of tolerance and dependence [29–31] (see Figure 4C), and some ligands even appear to cause these effects by different mechanisms [32, 33]. Hence, given the complex pharmacology of the various opioid ligands, it has been difficult to isolate the effect of endocytosis on tolerance and dependence.

The present results provide a genetic “proof of concept” that endocytosis is an important mechanism that can delay tolerance and dependence. Notably, the use of the rMOP-R knockin mice allowed the same opioid drug to be compared in mice that appear to differ only in their MOP-R trafficking properties. Importantly, even if mechanisms other than endocytosis are contributing to the behavioral differences in the MOP-R and rMOP-R mice, these mice will provide a powerful

tool for delineating which of the adaptive changes that have been observed in wild-type animals after chronic morphine treatment are relevant to behavioral tolerance and dependence.

Supplemental Data

Supplemental results, experimental procedures, and two figures are available at <http://www.current-biology.com/cgi/content/full/18/2/129/DC1>.

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