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Lack of reward and locomotor stimulation induced by heroin in μ-opioid receptor-deficient mice

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Abstract

The μ-opioid receptor is the main substrate mediating opiate reward. Multiple μ-opioid receptor subtypes have been postulated to underlie opiate actions. Animals treated with antisense oligonucleotides targeting specific μ-opioid receptor exons show differential sensitivity to morphine versus heroin. The present work examined the rewarding and locomotor activating effects of heroin in mutant mice with a disrupted exon 2 of the μ-opioid receptor. Heroin (1–3 mg/kg) produced significant place preferences and stimulated locomotor activity in wild-type mice, whereas it had no effect in μ-opioid receptor-deficient mice. In contrast, treatment with cocaine (10–30 mg/kg) produced comparable place preferences and locomotor activation in both wild-type and μ-opioid receptor-deficient mice, thus providing evidence that the mutant mice are able to show drug-induced effects in the two behavioral paradigms used here. These results support an essential role for the μ-opioid receptor in the rewarding and locomotor activating effects of heroin.

Keywords: μ-Opioid receptor; Heroin; Knockout mice; Place conditioning; Locomotor activity

1. Introduction

Opiate addiction is a major health problem of our society. Heroin is the most abused opiate drug, and recent studies show an increase in the number of addicts to this substance (Mark et al., 2001). Rewarding properties of opiate drugs play an important role in driving their abuse, and research focused on the study of the relative contribution for each of the three known opioid receptors (μ, δ and κ) to opiate reward indicates a major role for the μ-opioid receptor in this opiate effect (Negus et al., 1993; Matthes et al., 1996; Simonin et al., 1998).

In addition to opiate reward, the μ-opioid receptor plays a crucial role in mediating opiate analgesia. In this regard, several studies have suggested that distinct μ-opioid receptors might underlie the analgesic actions of different opiate drugs. In support of this, the μ-opioid receptor-deficient CXBK (C57xBalb/c) mice did not display the analgesic properties of morphine but retained the antinociceptive effects of heroin (Reith et al., 1981; Rossi et al., 1996). Moreover, mice made tolerant to the analgesic effects of morphine still showed the full analgesic properties of heroin and codeine (Rossi et al., 1996). Genetic studies have also provided evidence in favor of a role for different μ-opioid receptors in the analgesic effects of morphine and heroin. Treatment with antisense oligonucleotides to exon 1 of the μ-opioid receptor inhibited both morphine and heroin antinociceptive effects, whereas antisense oligonucleotides to exon 2 of the μ-opioid receptor blocked heroin analgesia without affecting morphine antinociception (Rossi et al., 1996). In addition, null mutant mice with a targeted disruption of exon 1 of the μ-opioid receptor showed no analgesic response to morphine but retained the analgesic effects of heroin (Schuller et al., 1999).

Evidence in favor of distinct μ-opioid receptor sites has also been provided by pharmacological studies comparing motor behaviors induced by morphine and morphine-6-glucuronide. In particular, the two opiate drugs were shown to produce different patterns of ambulatory stimulation in

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mice (Grung et al., 1998). Moreover, while treatment of mice with morphine produced no tolerance to the motor-activating effects of morphine-6-glucuronide, pretreatment with morphine-6-glucuronide caused tolerance to both morphine and morphine-6-glucuronide (Grung et al., 2000). Based on these and previous findings (Rossi et al., 1996), it has been hypothesized that heroin and morphine-6-glucuronide actions might involve a common μ-opioid receptor site that is different from that mediating morphine effects.

The studies described above strongly support the hypothesis that separate μ-opioid receptors may exist to mediate the analgesic and motor-stimulating properties of different opiate drugs. However, to date, relatively few studies have investigated the specific role for the μ-opioid receptor in the rewarding properties of opiate drugs other than morphine. Null mutant mice lacking a functional μ-opioid receptor have been generated and represent a unique tool to examine the specific function for this receptor in opiate actions. μ-Opioid receptor-deficient mice have been used to elucidate the specific role for this receptor in morphine effects such as analgesia, reward, motor activation, withdrawal symptoms and lethality (Matthes et al., 1996; Sora et al., 2001; Loh et al., 1998; Becker et al., 2000; Tian et al., 1997; Sora et al., 1997). In particular, most of these studies have demonstrated a crucial role for the μ-opioid receptor in the rewarding and motor-stimulating properties of morphine. However, to our knowledge, the rewarding and motor-activating properties of heroin have not been investigated in μ-opioid receptor-deficient mice.

Using μ-opioid receptor-deficient mice with a null mutation of exon 2 of the μ-opioid receptor gene (Matthes et al., 1996), the present study examined the specific involvement of this receptor in the rewarding and motor-stimulating properties of heroin. Rewarding effects of heroin were investigated using the conditioned place preference behavioral paradigm, and heroin-induced motor behaviors were evaluated using a fully automated computerized system.

2. Materials and methods

2.1. Subjects

Naïve male and female mice were used for conditioned place preference and locomotor activity studies, respectively. Wild-type and μ-opioid receptor-deficient (MOR −/−) mice were derived from mating of hybrid 129SV/C57BL/6 F1 wild-type or MOR −/− breeding pairs, respectively. Breeders were derived from mating of μ-opioid receptor-deficient heterozygous (MOR+/−) mice. Mice were 4–10 months old at the time of testing (age 4–6 months at first conditioned place preference test, 8–10 months second conditioned place preference test; 6–10 months at motor activity test). One week prior to the experiments, they were handled on alternate days. Mice were group-housed (three to four per cage) and kept on a 12:12 h light–dark cycle in a colony room maintained under standard laboratory conditions (relative humidity 50–60%, temperature 22±1 °C). Food and water were available ad libitum. All studies were conducted in accordance with the National Institute of Health *Guide for the Care and Use of Laboratory Animals* and were approved by the Institutional Animal Care and Use Committee of The Scripps Research Institute. Protocols used here comply with the European Community guidelines for the use of experimental animals.

2.2. Conditioned place preference apparatus

The apparatus consisted of a rectangular Plexiglas box (40 × 20 × 20 cm) divided by a central partition into two chambers of equal size (20 × 20 × 20 cm). During the test sessions, an aperture (4 × 4 cm) in the central partition allowed the mice to enter both sides of the apparatus. The apparatus used for heroin conditioned place preference consisted of two compartments. One side had black walls with a smooth floor (black side), and the other side had vertical black and white striped (2 cm) walls with a slightly rough floor (striped side). The walls of each side of the apparatus used for cocaine conditioned place preference were painted half black and half white. One compartment had the lower part of the walls painted white with a textured floor (textured floor side); the other compartment had the lower part of the walls painted black with a rough floor (rough floor side). Transparent Plexiglas lids allowed observation of the animal's behavior on a video monitor connected to a camera placed above the apparatus. Mouse behavior was recorded on videotape and later scored by an experimenter blind to treatment condition and genotype.

2.3. Locomotor activity apparatus

Mouse locomotor behavior was measured in Plexiglas cages (42 × 22 × 20 cm) placed into frames (47 × 25.5 cm) mounted with photocell beams (San Diego Instruments, San Diego, CA). Zone entries, defined as movement into one of eight equal-sized squares, reflected horizontal locomotion rather than the repeated breaking of a single beam. Rearing episodes were recorded by a set of photocell beams located above those for horizontal locomotion.

2.4. Experimental design

2.4.1. Conditioned place preference

Conditioned place preference experiments were carried out during the dark phase of the 12:12 h light–dark cycle (lights off: 10:00 a.m.). Throughout the experiment, the mice were brought into the testing room 1 h before being tested. Prior to the beginning of conditioning trials, each mouse was allowed to become familiar with the entire apparatus for 20 min. On the following 3 days, two conditioning sessions were given daily. At 11:00 a.m., the drug-treated animals were injected with vehicle and immediately

confined to one side of the apparatus (AM session). Four hours later, the same animals were injected with heroin (1 mg/kg) and confined to the opposite side of the apparatus (PM session). Control animals received vehicle prior to both AM and PM sessions. During each conditioning session, half of the mice within a group were confined to one side of the conditioned place preference apparatus whereas the other half was confined to the opposite side. Conditioning trials lasted 30 min. The day after the last conditioning trial, test sessions were performed in the undrugged state by placing each mouse in the ''AM session'' side. Following the first entry into the opposite side, the mice were allowed to explore the entire apparatus for 20 min and the time spent in each of the two compartments of the conditioned place preference apparatus was recorded. Approximately 4 months later, the same mice were tested again, this time for cocaine conditioned place preference. Conditioning and testing procedures were carried out similarly to those described above. During conditioning, mice previously exposed to heroin were treated with vehicle before being confined to both sides of the conditioned place preference apparatus (control group). In contrast, mice treated only with vehicle during the heroin conditioned place preference experiment received cocaine (10 mg/kg) during the PM sessions and vehicle during the AM sessions.

2.4.2. Locomotor activity

Locomotor activity experiments were carried out during the light phase of the light–dark cycle (lights on: 6:00 a.m.). Throughout the experiment, the mice were brought into the testing room 1 h prior to being tested. To examine baseline horizontal and vertical motor activity, wild-type and μ-opioid receptor-deficient mice were first exposed to a 3-h test without any prior drug or vehicle treatment. Subsequently, the same mice were repeatedly tested 1 week apart for their ambulatory responses to vehicle or increasing doses of heroin (0.5, 1, 2 and 3 mg/kg) in a within-subject design. In particular, during each test, the mice were first allowed to habituate to the activity chambers for 2 h prior to vehicle or heroin treatment. Mice were then injected with vehicle or drug and immediately placed in their respective test cages for an additional 2 h. To control for sensitization to the motor-activating properties of heroin, 1 week after the last heroin dosing (3 mg/kg), both wild-type and μ-opioid receptor-deficient mice were tested again with the 2 mg/kg dose of the opiate (2s). One week later, following a 2-h habituation exposure to the activity cages, wild-type and μ-opioid receptor-deficient mice were treated with cocaine (30 mg/kg, i.p.) and immediately placed in their respective activity cages for an additional 2 h. Locomotor responses to cocaine displayed during the first hour of testing were compared to those previously obtained following vehicle treatment. Throughout motor activity testing, the mice were observed through one-way glass from a room adjacent to the test room and episodes of rearing, grooming, stereotyped behaviors and Straub tail were recorded.

2.5. Drugs

Heroin HCl (0.5, 1, 2, 3 mg/kg) and cocaine HCl (10, 30 mg/kg) were dissolved in physiological saline (vehicle) and injected intraperitoneally (i.p.) in a volume of 10 ml/kg. Control mice received the same volume of vehicle.

2.6. Statistical analysis

Time spent by each genotype (wild-type or μ-opioid receptor-deficient mice) in the two compartments of the heroin or cocaine conditioned place preference apparatus during the initial 20-min exposure to the entire apparatus was analyzed using the nonparametric Mann–Whitney *U*-test. Time spent in the PM conditioning sessions-paired side of the conditioned place preference apparatus by wild-type and μ-opioid receptor-deficient mice during postconditioning tests was subjected to a two-way analysis of variance (ANOVA) with genotype and drug treatment (vehicle, heroin or cocaine) as independent factors. Total number of zone entries and rears performed by wild-type and μ-opioid receptor-deficient mice during the initial 3-h exposure to the activity test cages were analyzed using the Student's *t*-test. Total zone entries performed by wild-type and μ-opioid receptor-deficient mice during the 2-h test following each heroin dosing (0, 0.5, 1, 2, 3 and 2s mg/kg) were analyzed by a two-way ANOVA with genotype as the between-subjects factor and zone entries collected during repeated testing as the within-subject factor. The same statistical analysis was used to examine total zone entries performed by wild-type and μ-opioid receptor-deficient mice during the 2-h test preceding each heroin dosing. A two-way ANOVA with genotype and drug treatment as independent factors was used to examine zone entries performed by wild-type or μ-opioid receptor-deficient mice 1 h after treatment with vehicle or cocaine (30 mg/kg). Newman–Keuls post hoc tests were used for individual group and drug dose comparisons. The accepted value for significance was $P < 0.05$.

3. Results

3.1. Heroin conditioned place preference

Wild-type and μ-opioid receptor-deficient mice showed no unconditioned preference for either of the two compart-

Table 1
Time (s) spent by wild-type and μ-opioid receptor-deficient (MOR −/−) mice in the black and striped compartments of the heroin conditioned place preference apparatus during the initial 20-min exposure to the entire apparatus

Genotype	Black	Striped
Wild type	594.8±38.1	595.1±38.1
MOR −/−	577.9±29.8	612.0±29.8

Values represent mean±S.E.M.; $n = 16$/genotype.

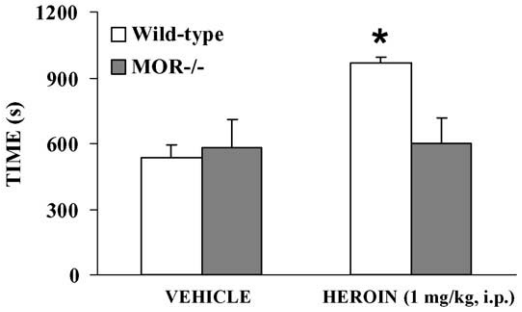

Fig. 1. Time (s) spent by wild-type ($n=8$/group) and μ-opioid receptor-deficient (MOR −/−; $n=8$/group) mice in the drug-paired side ("PM session" side for vehicle-treated mice) during the postconditioning test. *$P<0.05$ versus vehicle-treated mice and heroin-treated MOR −/− mice.

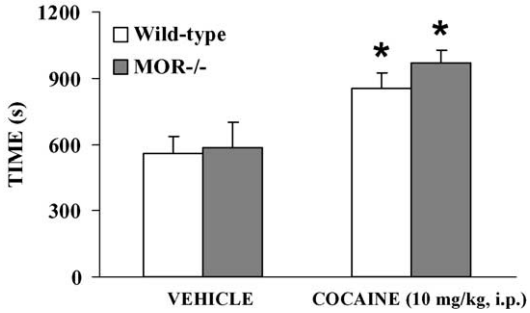

Fig. 2. Time (s) spent by wild-type ($n=8$/group) and μ-opioid receptor-deficient (MOR −/−; $n=8$/group) mice in the drug-paired side ("PM session" side for vehicle-treated mice) during postconditioning test. *$P<0.0005$ versus vehicle-treated mice.

ments of the apparatus used for heroin conditioned place preference. As shown in Table 1, during the initial 20-min exposure to the entire apparatus, both genotypes spent a similar amount of time in the two compartments of the conditioned place preference apparatus (wild-type mice: $U=128$, $P=1.00$; μ-opioid receptor-deficient mice: $U=111$, $P=0.52$). Evaluation of time spent in the drug-paired side by vehicle- or heroin-treated wild-type and μ-opioid receptor-deficient mice during the postconditioning test revealed no genotype effect [$F(1,28)=2.91$, $P=0.09$), a drug treatment effect [$F(1,28)=6.01$, $P<0.05$] and a genotype × drug treatment interaction effect [$F(1,28)=4.86$, $P<0.05$]. Post hoc comparisons showed that heroin-treated wild-type mice spent more time in the drug-paired side than heroin-treated μ-opioid receptor-deficient mice and vehicle-treated wild-type and μ-opioid receptor-deficient mice ($P<0.05$). Heroin-treated μ-opioid receptor-deficient mice, vehicle-treated wild-type and μ-opioid receptor-deficient mice spent about 50% of the total test time in each compartment of the conditioned place preference apparatus (Fig. 1).

3.2. Cocaine conditioned place preference

Wild-type and μ-opioid receptor-deficient mice showed no unconditioned preference for either of the two compartments of the apparatus used for cocaine conditioned place preference. As shown in Table 2, during the initial 20-min exposure to the entire apparatus, both genotypes spent a similar amount of time in the two compartments of the conditioned place preference apparatus (wild-type mice: $U=91$, $P=0.16$; μ-opioid receptor-deficient mice: $U=115$, $P=0.62$). Examination of time spent in the drug-paired side by vehicle- or cocaine-treated wild-type and μ-opioid receptor-deficient mice during the postconditioning test yielded no genotype effect [$F(1,28)=0.69$, $P=0.41$], a drug treatment effect [$F(1,28)=17.06$, $P<0.0005$] but no genotype × drug treatment interaction effect [$F(1,28)=0.33$, $P=0.56$]. Wild-type and μ-opioid receptor-deficient mice treated with cocaine spent more time in the drug-paired side compared to vehicle-treated wild-type and μ-opioid receptor-deficient mice ($P<0.0005$). No group difference was observed between drug-treated wild-type and μ-opioid receptor-deficient mice. Vehicle-treated wild-type and μ-opioid receptor-deficient mice spent about 50% of the total test time in each compartment of the conditioned place preference apparatus, indicating the unbiased nature of the experimental apparatus and procedures used (Fig. 2).

Table 2
Time (s) spent by wild-type and μ-opioid receptor-deficient (MOR −/−) mice in the textured and rough floor compartments of the cocaine conditioned place preference apparatus during the initial 20-min exposure to the entire apparatus

Genotype	Textured floor	Rough floor
Wild type	628.3±29.4	561.7±29.4
MOR −/−	606.8±43.5	583.1±43.5

Values represent mean ± S.E.M.; $n=16$/genotype.

3.3. Locomotor activity

No genotype difference was observed in baseline ambulatory activity displayed during the initial 3-h exposure to the activity cages [total zone entries: $t(14)=0.46$, $P=0.64$; total rears: $t(14)=0.16$, $P=0.87$; Figs. 3 and 4]. Examination of total zone entries performed by wild-type and μ-opioid receptor-deficient mice during the 2-h test preceding each

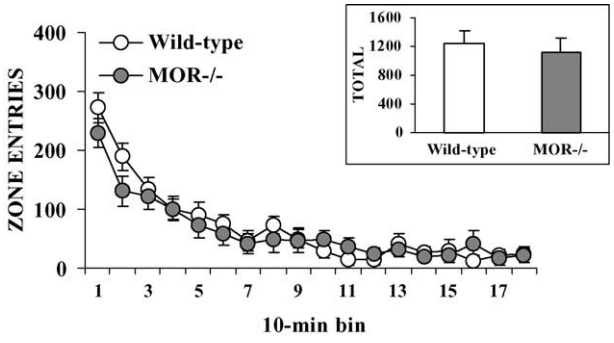

Fig. 3. Zone entries performed by wild-type ($n=8$) and μ-opioid receptor-deficient (MOR −/−; $n=8$) mice during the initial 3-h exposure to the motor activity chambers. Inset: total number of zone entries during the entire test session.

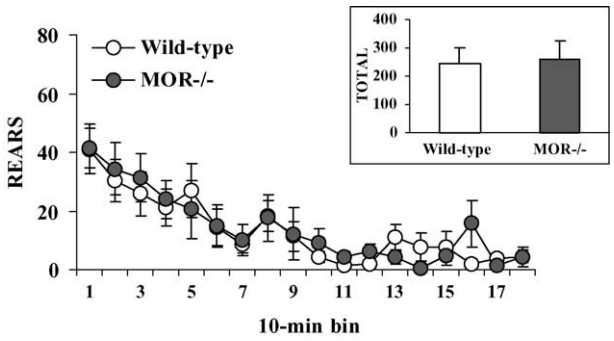

Fig. 4. Rears performed by wild-type ($n=8$) and μ-opioid receptor-deficient (MOR −/−; $n=8$) mice during the initial 3-h exposure to the motor activity chambers. Inset: total number of rears during the entire test session.

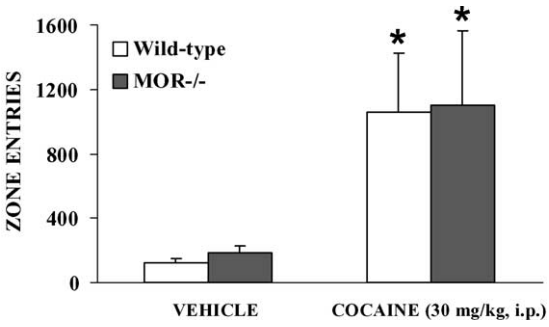

Fig. 6. Zone entries performed by wild-type ($n=8$) and μ-opioid receptor-deficient (MOR −/−; $n=8$) mice during 1-h test following treatment with vehicle or cocaine. * $P<0.005$ versus vehicle treatment.

heroin dosing revealed no genotype effect [$F(1,14)=1.42$, $P=0.25$], a repeated test effect [$F(5,70)=4.46$, $P<0.005$] but no genotype × repeated test interaction effect [$F(5,70)=1.07$, $P=0.38$]. Higher levels of zone entries were observed during the 2-h test session preceding vehicle treatment than during the test sessions preceding drug dosing, indicating the occurrence of genotype-independent habituation to the activity test cages (data not shown). Analysis of zone entries recorded during repeated tests following treatment with different doses of heroin revealed no genotype effect [$F(1,14)=3.90$, $P=0.06$], a drug dose effect [$F(5,70)=4.97$, $P<0.001$] and a genotype × drug dose interaction effect [$F(5,70)=6.28$, $P<0.0001$]. Post hoc comparisons showed that wild-type mice treated with 3 mg/kg heroin made more zone entries than μ-opioid receptor-deficient mice treated with the same opiate dose ($P<0.0005$). No heroin dose produced motor stimulation in the μ-opioid receptor-deficient mice and only the 3 mg/kg dose significantly increased ambulation levels of wild-type mice ($P<0.0005$; Fig. 5). Moreover, final challenge with the second 2 mg/kg heroin dose (2s) revealed no sensitization of the wild-type mice to the motor-activating effects of heroin (data not shown). Straub tail was detected in 5/8 and 7/8 of wild-type mice following treatment with 2 or 3 mg/kg heroin, respectively. This sign was not observed in μ-opioid receptor-deficient mice. Neither genotype-nor drug treatment-dependent changes were observed in other behavioral signs recorded by direct observation (data not shown). Finally, examination of zone entries recorded during the 1-h time period following treatment with vehicle or cocaine (30 mg/kg) revealed no genotype effect [$F(1,28)=0.03$, $P=0.86$], a drug treatment effect [$F(1,28)=9.70$, $P<0.005$] but no genotype × drug treatment interaction effect [$F(1,28)=0.01$, $P=0.96$]. Cocaine treatment produced higher levels of zone entries than vehicle treatment in both wild-type and μ-opioid receptor-deficient mice (Fig. 6).

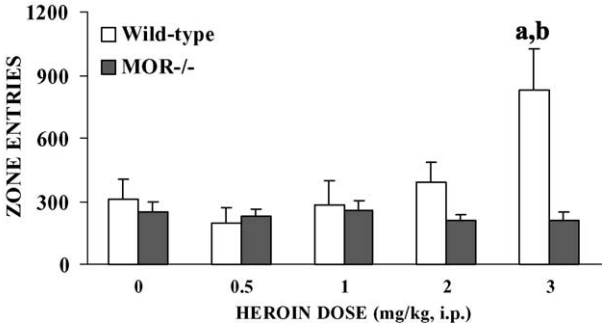

Fig. 5. Zone entries performed by wild-type ($n=8$) and μ-opioid receptor-deficient (MOR −/−; $n=8$) mice during 2-h tests following repeated dosing with heroin. Animals were exposed to increasing doses of heroin 1 week apart. [a] $P<0.0005$ versus MOR −/− mice treated with the same opiate dose. [b] $P<0.0005$ versus same genotype treated with the other drug doses.

4. Discussion

The present study shows that μ-opioid receptor-deficient mice do not display the rewarding and motor-stimulating properties of heroin. Treatment with the opiate produced a robust place conditioning and increased ambulatory behavior in wild-type mice, whereas heroin was devoid of effects in μ-opioid receptor-deficient mice. In contrast, cocaine produced comparable levels of place conditioning and locomotor stimulation in both wild-type and μ-opioid receptor-deficient mice.

Evidence indicating that distinct μ-opioid receptor systems might mediate the analgesic effects of different opiate drugs prompts the hypothesis that separate receptor sites may underlie the rewarding properties of these drugs as well. However, to date, relatively few studies have investigated the role of the μ-opioid receptor in the conditioned reinforcing effects of opiate drugs other than morphine. The present results clearly indicate the complete absence of rewarding effects of heroin in μ-opioid receptor-deficient mice as measured by the conditioned place preference paradigm. The dose of heroin used here (i.e., 1 mg/kg) produced a strong place conditioning in wild-type mice with these mice spending about 80% of the total postconditioning test time in the drug-paired compartment of the conditioned place preference apparatus. We are not aware of other studies in which the conditioned place preference paradigm has been used to examine the rewarding effects of heroin in

mice. Levels of heroin-induced conditioned place preference observed in the current study were higher than those detected in mice conditioned with morphine under the same experimental conditions (Contarino et al., unpublished observations). Thus, the dose of heroin used in this study most probably produced levels of place conditioning very close to the maximal conditioning effect that can be obtained under our experimental conditions. However, despite the strong conditioned place preference effect observed in the wild-type mice, no heroin conditioning was detected in the μ-opioid receptor-deficient mice, indicating an essential role for the μ-opioid receptor in the rewarding effects of this opiate. These results agree with the absence of morphine-induced conditioned place preference and self-administration behavior previously shown in μ-opioid receptor null mutant mice (Matthes et al., 1996; Becker et al., 2000; Sora et al., 2001). Further support for a crucial role for the μ-opioid receptor in the reinforcing properties of heroin has also been provided by pharmacological studies showing that blockade of the μ-opioid receptor with beta-funaltrexamine antagonized heroin reward to the point of producing extinction-like effects (Negus et al., 1993; Martin et al., 1998).

Despite the lack of heroin place conditioning, μ-opioid receptor-deficient mice displayed robust preferences for cocaine-paired places. Wild-type and μ-opioid receptor null mutant mice showed similar levels of cocaine conditioned place preference. This result raises two important issues. First, the presence of cocaine-induced place conditioning in μ-opioid receptor-deficient mice indicates a minor role for the μ-opioid receptor in cocaine reward, although the present experiments do not rule out a more subtle involvement of the μ-opioid receptor system in the reinforcing effects of cocaine. Accordingly, other studies have shown no change in cocaine self-administration behavior in rats treated with the μ-opioid receptor-selective antagonist beta-funaltrexamine (Martin et al., 1998). Second, findings of cocaine conditioned place preference in μ-opioid receptor-deficient mice indicate that despite the μ-opioid receptor mutation, these mice were able to acquire a drug-induced conditioned place preference. The latter point rules out the possibility that absence of heroin conditioned place preference in the μ-opioid receptor mutant mice was due to a deficit in those processes required for the acquisition and expression of this conditioned behavior.

In this study, basal ambulatory activity of the μ-opioid receptor-deficient mice was similar to that observed in wild-type mice. No genotype difference was detected in the level of horizontal and vertical locomotion displayed during the initial 3-h exposure to the activity cages. Wild-type and μ-opioid receptor-deficient mice also showed similar patterns of motor activity decline, indicating unaltered habituation processes upon exposure to the novel environment of the activity cages in the mutant mice. Accordingly, other studies have shown no change in baseline ambulatory activity displayed by μ-opioid receptor-deficient mice (Becker et al., 2000; Sora et al., 2001); however, decreased levels of ambulatory behavior in μ-opioid receptor-deficient mice could be detected by others under different experimental conditions (Tian et al., 1997) or following repeated testing (Matthes et al. 1996).

In line with the conditioned place preference findings, μ-opioid receptor-deficient mice did not show the motor stimulatory properties of heroin. Heroin (3 mg/kg) produced motor stimulation in wild-type mice, whereas the opiate did not induce any activation in the μ-opioid receptor-deficient mice. In agreement with these results, other studies have shown absence of morphine-induced motor stimulation in μ-opioid receptor-deficient mice (Tian et al., 1997; Sora et al., 2001). Higher doses of heroin were not tested since 3 mg/kg appeared to be the maximally tolerated dose. Moreover, 63% and 88% of wild-type mice showed Straub tail following treatment with 2 or 3 mg/kg of heroin, respectively. The Straub tail is a dose-dependent sign usually observed at relatively high doses of opiate drugs (Houshyar et al., 2000). Unlike the wild-type mice, the Straub tail sign was never observed in the μ-opioid receptor-deficient mice. Noteworthy, final challenge with the 2 mg/kg dose of heroin did not produce increased levels of motor activity in the wild-type mice compared to vehicle treatment, indicating that the drug treatment protocol used here produced no sensitization to the motor-activating properties of heroin. Lastly, consistent with the findings obtained in the conditioned place preference experiments, cocaine treatment produced comparable levels of locomotor stimulation in both wild-type and μ-opioid receptor-deficient mice.

Several reports have proposed the hypothesis that activation of distinct μ-opioid receptor subtypes may be responsible for the actions of different opiate drugs. Evidence in favor of this differentiation has emerged mainly from studies examining the analgesic properties of closely related opiate drugs. Results shown here demonstrate that heroin produced neither place conditioning nor motor stimulation in μ-opioid receptor-deficient mice. Thus, whereas distinct μ-opioid receptor systems might underlie the analgesic effects of different opiate drugs, the present results suggest that opiate reward and motor stimulation is mediated by a common μ-opioid receptor system. In support of this, it has been shown that 3-*O*-methylnaltrexone inhibited heroin but not morphine analgesia; however, treatment with this opiate antagonist similarly antagonized heroin and morphine intravenous self-administration behavior (Walker et al., 1999). In addition, although μ-opioid receptor-deficient CXBK mice failed to show morphine-induced analgesia, they were able to acquire consistent place preferences for morphine-associated cues (Rossi et al., 1996; Suzuki et al. 1993).

In conclusion, the use of μ-opioid receptor null mutant mouse models has revealed the essential role for this opioid receptor subtype in the biological actions of morphine and heroin. In particular, μ-opioid receptor-deficient mice did not show morphine-induced place conditioning, motor activation and self-administration behavior, indicating the absence of morphine reinforcing effects in these mice (Tian

et al., 1997; Sora et al., 2001; Becker et al., 2000). Thus, together with the present results demonstrating a lack of heroin-induced place conditioning and motor activation, these findings suggest that unlike opiate analgesia, a common μ-opioid receptor system may underlie the rewarding and motor stimulating properties of heroin and morphine.

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