A new buprenorphine analogy, thenorphine, inhibits morphine-induced behavioral sensitization in mice[1]

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KEY WORDS thenorphine; morphine; behavioral sensitization; mice

ABSTRACT

AIM: To investigate effects of thenorphine, a new compound of partial agonist of μ-opioid receptor, on the locomotor activity and the behavioral sensitization to morphine in mice. **METHODS:** Locomotor activity was observed after administration of thenorphine or co-administration of thenorphine and morphine in mice. Mice were induced behavioral sensitization to morphine by intraperitoneal injection of 20 mg/kg morphine once daily for 7 d. Thenorphine was co-administrated with morphine to observe the effects of thenorphine on the development, transfer and expression of morphine-induced behavioral sensitization. **RESULTS:** A single dose of thenorphine (0.0625, 0.25, and 1.0 mg/kg) could dose-dependently inhibit the locomotor activity in mice ($P<0.05$), repeated administrations of thenorphine, however, were not able to induce locomotor sensitization, but induced tolerance. Pretreatment with thenorphine 30 min prior to morphine effectively inhibited the psychomotor effect of morphine in mice ($P<0.01$). Co-administration of thenorphine (0.0625, 0.25, and 1.0 mg/kg) could dose-dependently inhibit the development, transfer, and expression of behavioral sensitization to morphine in mice ($P<0.05$ or $P<0.01$). **CONCLUSION:** Thenorphine inhibited morphine-induced behavioral sensitization in mice, suggesting that thenorphine may be effective against the addiction of opioids.

INTRODUCTION

Repeated intermittent administration of opioids can induce a progressive and long-lasting enhancement in behavioral responses (locomotor activity and stereotype) to the subsequent challenge of these agents[1,2]. The phenomenon is termed behavioral sensitization (reverse tolerance), which is considered to play an important role in certain aspects of addiction (eg compulsive drug-seeking and drug-taking behavior), especially in the high rate of relapse seen in drug addicts even after very long periods of abstinence[3,4]. The process of establishing behavioral sensitization can be divided into three phases: development/initiation, transfer and expression[5,6]. The stage of intermittent administration to induce sensitization is called development phase. The period following the last injection is the transfer stage, and after this, the long time during which sensitization lasts is the expression one. The three phases of sensitization are distinct events and associated with different mechanisms[5,7]. Thus, investigating drugs' effects on different phases of behavioral sensitization in animal may be helpful for better understanding the mechanisms underlying drugs

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addiction and providing new strategies for treatment.

Thenorphine is a new compound, synthesized by Beijing Institute of Pharmacology and Toxicology, China. As an analogy of buprenorphine, thenorphine is a partial agonist of μ-opioid receptor as well as buprenorphine[8] (Fig 1), which has been widely used in the therapy of opioids addiction[9,10]. However, the pharmacological characteristics of thenorphine are not very clear yet. Therefore, in this experiment, we intended to observe the effects of thenorphine on locomotor activity and morphine-induced behavioral sensitization in mice.

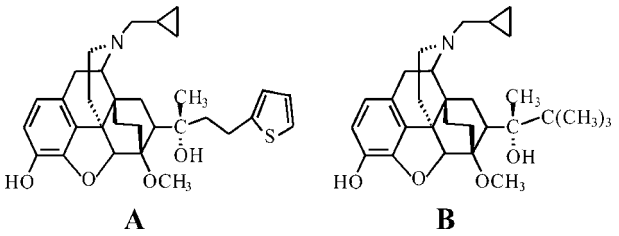

Fig 1. The chemical structures of thenorphine (A) and buprenorphine (B).

MATERIALS AND METHODS

Animals Kunming mice, weighing 18-22 g, were obtained from the Department of Laboratory Animal Science, Peking University Health Science Center. The animals were acclimated to a colony room with an ambient temperature (22±1 ºC), humidity (50 %±10 %), and a 12 h light/dark cycle for at least 3 d before experiment. Mice were randomly divided into 4 groups and each had 5 male and 5 female mice. Food and water were available *ad libitum*. All experiments were performed during daytime and were conducted according to the NIH Guide for the Care and Use of Laboratory Animals (NIH Publications No 80-23, revised 1996). The experimental procedures were approved by the local Committee on Animal Care and Use.

Drugs Thenorphine hydrochloride, which was synthesized by Beijing Institute of Pharmacology Toxicology, was generously donated by Prof Ze-hui GONG. Morphine hydrochloride was purchased from Qinghai Pharmaceutical Plant (China). Morphine was dissolved in saline (0.9 % NaCl) and thenorphine was dissolved in 5 % Tween-80 just before the experiment. Thenorphine was given sc while morphine was administered ip. All drugs were injected in a volume of 10 mL/kg (sc or ip). Control animals were given vehicle (Veh) injection in a corresponding volume.

Apparatus Locomotor activity was measured by an ambulometer with 5 activity chambers (JZZ98, Institute of Materia Medica, Chinese Academy of Medical Sciences, China). Each activity chamber of 26 cm×14 cm×15 cm consisted of white opaque perspex walls, a transparent perspex lid, and a floor. The floors were consistently composed of 25 parallel copper bars with a fixed interval of 1 cm between the adjacent bars. The odd bars were earthed and the even bars were active and connected with a few micro-amper energy source. The paws of the mice contacted or disconnected the active bars producing random configurations that were converted into pulses. The pulses, which were proportional to the locomotor activity of the mice, were recorded as the cumulative total counts of motor activity for a selected minute period.

Experimental protocols

Effects of acute administration of thenorphine on locomotor activity in mice Four groups of mice were given three doses of thenorphine (0.0625, 0.25, and 1.0 mg/kg) or Veh, and then were put into the test cages. The locomotion was recorded every 10 min for 120 min.

Effects of chronic administration of thenorphine on locomotor activity in mice Experimentally naive mice were injected with thenorphine (0.0625, 0.25, and 1.0 mg/kg) or Veh once daily for 7 consecutive days. After 7 drug free of days (on d 15), the challenge test was performed by giving animals the corresponding doses of thenorphine (0.0625, 0.25, and 1.0 mg/kg) or Veh. On d 1, 7 and 15, mice were placed individually in the test cages for 10 min immediately after the injection, and the locomotion was recorded for 30 min

Effects of thenorphine on the hyperactivity induced by acute administration of morphine in mice In this experiment, five groups of mice were given Veh or thenorphine (0.0625, 0.25, and 1.0 mg/kg). Half an hour later, the mice were injected Veh or morphine 10 mg/kg and put into the test cages for 30 min. The locomotion was recorded for 30 min

Effects of thenorphine on the development, transfer and expression of behavioral sensitization to morphine in mice Mice were treated for 7 d with one of the following drug pairs: Veh+Veh, Veh+morphine (20 mg/kg), thenorphine (0.0625, 0.25, and 1.0 mg/kg)+morphine (20 mg/kg) with a 30-min interval between the two injections. After a 7-d wash-

out period, all mice were challenged with 10 mg/kg morphine and put into the test cages for 30 min, and then the locomotion was recorded for 30 min to observe the effects of thenorphine on the development of morphine-induced behavioral sensitization.

Mice in the control group were given Veh and the other mice received 20 mg/kg morphine for 7 d to induce behavioral sensitization. From d 8 to d 14, the mice were given Veh or thenorphine (0.0625, 0.25, and 1.0 mg/kg) respectively. On d 15, all mice were challenged with 10 mg/kg morphine and put into the test cages. Half an hour later, the locomotion was recorded for 30 min to observe the effects of thenorphine on the transfer of morphine-induced behavioral sensitization.

Mice in the control group were given Veh and the other mice received 20 mg/kg morphine for 7 d to induce behavioral sensitization. On d 15 , the mice were given Veh or thenorphine (0.0625, 0.25, and 1.0 mg/kg) respectively. Half an hour later, mice were challenged with 10 mg/kg morphine and put into the test cages for 30 min. Then the locomotion was recorded for 30 min to observe the effects of thenorphine on the expression of morphine-induced behavioral sensitization.

Statistical analysis The data are expressed as mean±SD. In Fig 2, the locomotor activity was analyzed using two-factor repeated measures analysis of variance (ANOVA) for time block and treatment. For the others, statistical analysis were performed by one way ANOVA. The LSD multiple comparisons were used in all experiments at a minimum significance level of $P<0.05$.

RESULTS

Effects of acute administration of thenorphine on locomotor activity in mice Thenorphine (0.0625, 0.25, or 1.0 mg/kg) dose-dependently induced hypoactivity in mice [F(treatment)(3,36)=3.109, $P<0.05$; F(treatment×time)(33, 432)=5.158, $P<0.01$] and the dose of 1.0 mg/kg had the most effect among the three doses. The climax of thenorphine-induced hypoactivity appeared at about 30 min after thenorphine injection. Only at 20, 30, and 40 min, the hypoactivity induced by 0.25 mg/kg thenorphine reached significant. The locomotion of all thenorphine groups had no significant difference 60 min after thenorphine injection (Fig 2).

Effects of chronic administration of thenorphine on locomotor activity in mice On d 1, thenorphine (0.0625, 0.25, and 1.0 mg/kg) induced significant hypoactivity in mice [F(3,36)=3.630, $P<0.05$]. This result was corresponding with Fig 2. After chronic treatment with thenorphine for 7 d, the locomotion of 4 groups had no significant difference [F(3,36)=0.865, $P>0.05$]. On d 15, thenorphine also could not induce significant hypoactivity in the challenge test [F(3,36) =0.689, $P>0.05$] after a 7-d washout period (Fig 3).

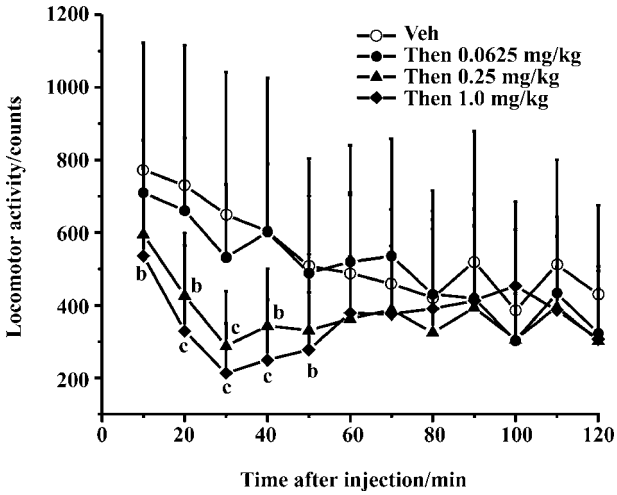

Fig 2. Effects of acute administration of thenorphine on locomotor activity in mice. *n*=10. Mean±SD. $^bP<0.05$, $^cP<0.01$ *vs* Veh group. Then: thenorphine.

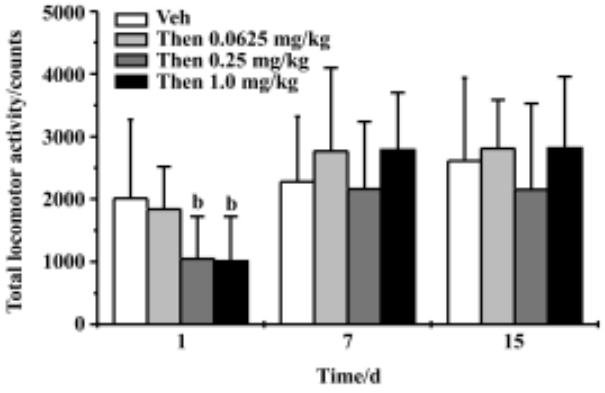

Fig 3. Effects of chronic administration of thenorphine on locomotor activity in mice. *n*=10. Mean±SD. $^bP<0.05$ *vs* Veh group of the same day. Then: thenorphine.

Effects of thenorphine on hyperactivity induced by acute administration of morphine in mice Morphine 10 mg/kg induced obvious hyperactivity in mice. Pretreatment with thenorphine 30 min prior to morphine dose-dependently decreased the hyperactivity induced by morphine [F(4,45)=6.658, $P<0.01$] (Fig 4).

Effects of thenorphine on the development, transfer, and expression of behavioral sensitization

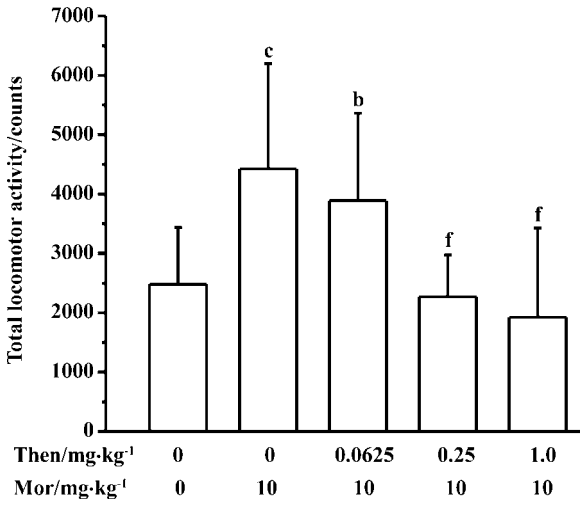

Fig 4. Effects of thenorphine on the hyperactivity induced by acute administration of morphine in mice. *n*=10. Mean±SD. $^{b}P<0.05$, $^{c}P<0.01$ *vs* Veh+Veh group; $^{f}P<0.01$ *vs* Veh+morphine 10 mg/kg group.

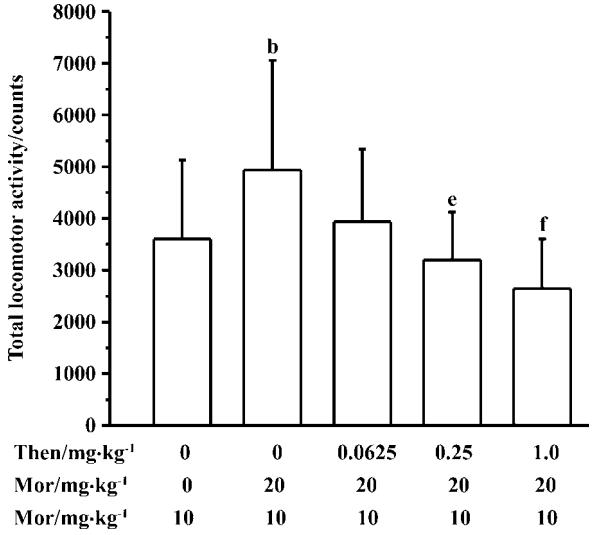

Fig 5. Effects of thenorphine on the development of morphine sensitization in mice. *n*=10. Mean±SD. $^{b}P<0.05$ *vs* Veh+Veh+morphine group. $^{e}P<0.05$, $^{f}P<0.01$ *vs* Veh+morphine+morphine group.

to morphine in mice The psychomotor effect of morphine was significantly enhanced in mice pretreated with morphine (7×20 mg/kg, ip) 7 d after cessation of treatment. Co-administration of thenorphine (0.25 and 1.0 mg/kg) 30 min prior to morphine significantly blocked the development of morphine-induced behavioral sensitization [$F(4,45)$= 3.468, $P<0.05$, $P<0.01$] (Fig 5).

Behavioral sensitization to mice was induced by 7 injections of morphine 20 mg/kg. Injections with thenorphine (0.0625, 0.25, and 1.0 mg/kg) from d 8 to d 14 dose-dependently interrupted the transfer of morphine-induced behavioral sensitization after challenged by 10 mg/kg morphine on d 15 [$F(4,45)=5.012$, $P<0.01$] (Fig 6).

Our protocol induced obvious behavioral sensitization to morphine in mice. Treatment with thenorphine (0.25 and 1.0 mg/kg) 30 min before the challenge dose of morphine significantly suppressed the expression of morphine-induced behavioral sensitization [$F(4,45)=2.823$, $P<0.05$] (Fig 7).

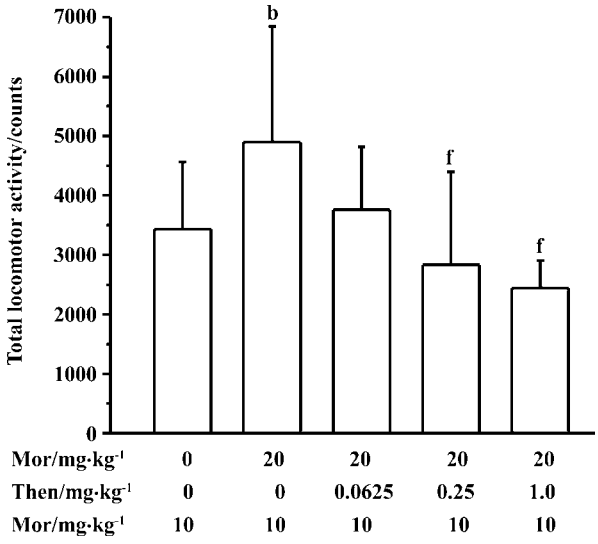

Fig 6. Effects of thenorphine on the transfer of morphine sensitization in mice. *n*=10. Mean±SD. $^{b}P<0.05$ *vs* Veh+Veh+morphine group; $^{f}P<0.01$ *vs* morphine+Veh+morphine group.

DISCUSSION

As a new compound, thenorphine is a partial agonist of μ-opioid receptor[11]. It induced hypoactivity in mice. Thenorphine's inhibition on locomotor activity significantly took effect 10 min after the thenorphine injection, peaked at 30 min and lasted only 50 min. The results in Fig 2 indicate that thenorphine could inhibit the CNS in mice. Morphine, a nonselective agonist of opiate receptors, induces hyperactivity in mice[2]. Buprenorphine, another partial agonist of μ-opioid receptor, also causes hyperactivity in mice[11]. Therefore, we might draw a conclusion that although thenorphine is a drug, which acts on opiate receptors as well as morphine and buprenorphine, its effects on locomotor activity in mice are obviously different to the two latter. The reason for the differences may be

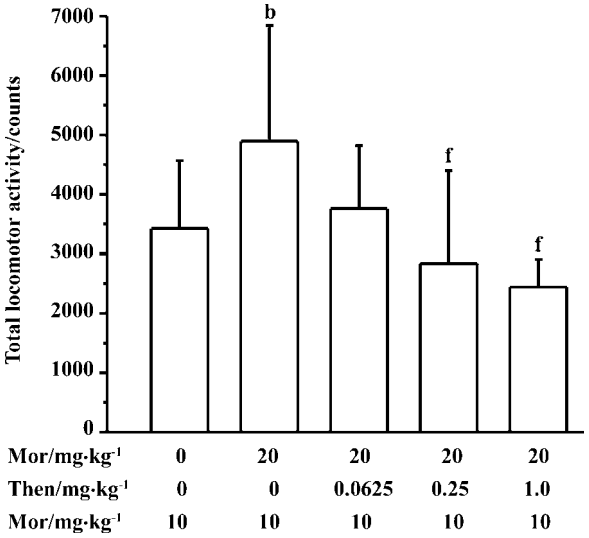

Fig 7. Effects of thenorphine on the expression of morphine sensitization in mice. n=10. Mean±SD. ^{b}P<0.05 *vs* Veh+Veh+morphine group. ^{e}P<0.05, ^{f}P<0.01 *vs* morphine+Veh+morphine group.

that the three drugs' chemical structures are different. The substitution of 23-thienyl ethyl for 23-tert-butyl possibly changes the property of drug's effects on receptors and the affinity of drug for receptors (Fig 1). Thus, thenorphine's effects on locomotor activity in mice take on opposite characteristic to buprenorphine's.

Multiple administration of morphine or buprenorphine can develop behavioral sensitization, manifested as the enhancing psychomotor effects of these drugs[2,12]. While, in this experiment, instead of behavioral sensitization, the effects of thenorphine on locomotor activity in mice developed tolerance after repeated administration, and the tolerance still existed even after a long period of free drug following the last injection (Fig 3, there were no significant differences between d 7 and d 15). This is another distinct characteristic of thenorphine, which differs from other opioids.

The locomotor hyperactivity is thought to be induced by an increase of dopamine (DA) in mesolimbic system[13,14], which arises in the ventral tegmental area (VTA) and projects mainly to the nucleus accumbens (NAC). The activation of μ-opioid receptor on the γ-aminobutyrate (GABA)-containing interneuron of VTA can bring the release of DA in mesolimbic system and it is considered that μ receptor of VTA plays a key role in modulating the hyperactivity induced by acute administration of morphine[15,16]. As a partial agonist of μ receptor, thenorphine has double properties of agonism and antagonism. In Fig 4, thenorphine was administered 30 min before morphine was given and the locomotion was recorded 30 min after morphine injection, ie 60 min after the thenorphine injection. As Fig 2 showed, thenorphine's effects on locomotor activity in mice almost completely disappeared 60 min after thenorphine was given. Thus, the inhibition of thenorphine on the hyperactivity induced by morphine is perhaps not due to the nonspecific counteraction of the effects of thenorphine and morphine on locomotor activity in mice, but due to the antagonism on the μ receptor level. This effect of thenorphine may be related to the property of antagonism, but not agonism.

Behavioral sensitization is now considered to be associated with neuroadaptations in the DA/mesolimbic system and is a useful animal model of plasticity and neuroadaptation related to repeated administration of the agents that have abuse potential[17,18]. The development of behavioral sensitization is related to the progressive augmentation in neural responses to the psychomotor effects of drugs in VTA[1]. In Fig 5, co-administration of thenorphine could dose-dependently block the development of morphine-induced behavioral sensitization, indicating that thenorphine could disrupt the process of responses sensitization in VTA. The transfer phase is the process of neurobiological changes from VTA to NAC, which is necessary for the consolidation of behavioral sensitization[5]. The interruption of thenorphine on the transfer of morphine-induced behavioral sensitization in Fig 6 suggests that thenorphine can break the consolidating process of behavioral sensitization. The enhanced responses of DA release to the challenge dose of agents in NAC underlie the expression of behavioral sensitization[19]. Thus, the suppression of thenorphine on the expression of morphine-induced behavioral sensitization in Fig 7 hints that thenorphine could inhibit the enhanced responses to drug's psychomotor effects in NAC. Although the specific pathway(s) by which thenorphine blocks the development, interrupts the transfer, and suppresses the expression of behavioral sensitization to morphine needs further study, combining previous reports, we can safely postulate that binding to opiate receptors in different regions of CNS may be the main reason for thenorphine interfering behavioral sensitization to morphine in mice.

In conclusion, as a new buprenorphine analogy, thenorphine has its own pharmacological characteristics. It induces hypoactivity in mice and its effects on locomotion develop tolerance after repeated administration, instead of behavioral sensitization. Thenorphine can

dose-dependently block the development, interrupt the transfer and suppress the expression of behavioral sensitization to morphine in mice. Although the results in animal experiment can provide references for drug's using in clinic, it needs to be further investigated whether thenorphine could effectively treat the opioids addiction.

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