

Effects of phosphodiesterase type 5 inhibitions on morphine withdrawal symptoms in mice

Minoo Mahmoodi¹, Siamak Shahidi² and Nasrin Hashemi-Firouzi²

¹ Islamic Azad University, Hamadan Branch (Irán) and ² Hamadan University of Medical Sciences (Irán)

Abstract

Background: Chronic morphine exposure creates dependence and, upon cessation, withdrawal symptoms. Studies indicate the phosphodiesterase type 5 (PDE5) inhibitor sildenafil may provide centrally mediated benefits against withdrawal, and therefore, this study evaluated morphine withdrawal signs in dependent mice with and without sildenafil treatment. **Method:** Dependence was induced by repeated treatments with morphine over 5 consecutive days. The morphine-dependent mice received sildenafil (1, 5, 10, or 20 mg/kg, i.p.) 15 min prior to the precipitation of morphine withdrawal. On the last day, naloxone was injected 2 hours after the last morphine injection, and withdrawal signs were evaluated for 30 min after naloxone injection. **Results:** The administration of sildenafil reduced all of the morphine withdrawal symptoms. **Conclusions:** The administration of sildenafil diminished morphine withdrawal signs in morphine-dependent mice. We hypothesize that the mechanism involves enhanced cyclic guanosine monophosphate (cGMP) activity, but further studies are recommended for a better understanding.

Keywords: Sildenafil, morphine withdrawal, mice.

Resumen

Efectos comportamentales por inhibición de la fosfodiesterasa tipo 5 sobre los síntomas de abstinencia de morfina en ratones. Antecedentes: se sabe que la exposición crónica a la morfina conduce a la dependencia. El cese en el consumo de morfina conlleva al desarrollo del síndrome de abstinencia. Numerosos estudios indican los efectos beneficiosos del sildenafil en el sistema nervioso central. Es por ello que el presente estudio evaluó el efecto del sildenafil sobre el síndrome de abstinencia a la morfina. **Método:** los ratones dependientes de morfina recibieron dosis diferentes de sildenafil (1, 5, 10 o 20 mg/kg i.p.) 15 minutos antes de producir el síndrome de abstinencia a la morfina. En el último día, la naloxona se inyectó dos horas después de la última inyección de morfina. Los signos de abstinencia fueron evaluados durante 30 minutos después de la inyección de naloxona. **Resultados:** la administración de sildenafil redujo todos los síntomas de abstinencia relacionados con la morfina. **Conclusiones:** se puede concluir que la administración de sildenafil disminuyó los signos de abstinencia a la morfina. Estos resultados muestran cómo los incrementos en el nivel del GMP cíclico mediante la vía PDE5 reduce los síntomas de abstinencia a la morfina.

Palabras clave: sildenafil, abstinencia a la morfina, ratones.

Prolonged exposure to opioids such as morphine leads to dependence on the drug both in humans and animals. Opiate dependence is characterized by a withdrawal syndrome, which includes both autonomic and somatic symptoms, as well as severe physiological disturbances (Koob, Maldonado, & Stinus, 1992; Koob, Stinus, Moal, & Bloom, 1989; Kreek & Koob, 1998). Although various therapeutic methods are now used to attenuate opioid withdrawal (Rehni, Jaggi, & Singh, 2013), none of these has led to a complete, subjective elimination of withdrawal signs (Rahimi-Movaghar, Amin-Esmaili, Hefazi, & Yousefi-Nooraie, 2013). One of the suggested protocols with high hopes for more successful addiction treatment is the use of drugs without opioid properties.

Sildenafil, an inhibitor of phosphodiesterase type 5 (PDE5), is an approved treatment for human erectile dysfunction (Goldstein et al., 1998; McCullough, 2002). However, there is little information about the effects of sildenafil on opioid withdrawal syndrome. Sildenafil induces accumulation of cyclic guanosine monophosphate (cGMP) peripherally, in the human corpus cavernosum (Snyder & Bredt, 1991; Uthayathas et al., 2007), but has also been localized to brain, lung, muscle, and blood platelets (Lin, 2004). Based on experimental studies, sildenafil has a range of effects on the central nervous system (CNS), altering behaviors such as cognition, reward-related stimuli, pain perception, anxiety (Kurt et al., 2004; Shahidi, Hashemi-Firouzi, & Mahmoodi, 2011, 2013; Solis, Bethancourt, & Britton, 2008), and seizures. Sildenafil also modulates neurogenesis and drug dependency via the cGMP pathway (Boccia, Blake, Krawczyk, & Baratti, 2011; Prickaerts et al., 2002; Uthayathas et al., 2007) and diminishes post-stroke neurogenesis and neurological sequelae (Zhang et al., 2002).

Evidence indicates that cGMP plays a role in regulating dependency and opioid withdrawal (Mo, Leung, & Yung, 2005), and, in fact, cGMP activity (and content) was reduced in opioid-dependent and withdrawal brains (Askew & Charalampous,

1976; Bhargava & Cao, 1997; Javadi et al., 2013; Mo et al., 2005; Peregud, Iakovleva, Stepanichev, Panchenko, & Guliaeva, 2013). However, sildenafil is therapeutically beneficial for disorders related to the CNS and cGMP, including those related to opioid dependency (Askew & Charalampous, 1976; Bhargava & Cao, 1997; Javadi et al., 2013; Mo et al., 2005; Peregud et al., 2013). There is, however, no direct evidence linking sildenafil and opioid withdrawal. Therefore, the present study evaluates the effect of sildenafil on morphine withdrawal signs in mice.

Method

Animals

Adult male NMRI mice weighing 20-30 g were used. They were obtained from a colony from the Pasteur Institute of Iran. Animals were divided into 7 groups ($n = 7$ per group) and kept under controlled environmental conditions (temperature: 21 ± 2 °C, 12:12h light/dark cycle) with ad libitum food and water. All behavioral tests were conducted from 12 to 3 P.M. All experiments were performed in accordance to the Guide for the Care and Use of Laboratory Animals (National Institutes of Health Publication No. 85-23, revised 1985).

Instruments

Scoring of morphine withdrawal symptoms were done in a Plexiglas. Sildenafil citrate (Vorin, India, 1, 5, 10, and 20 mg/kg, i.p.), morphine sulfate (DarouPakhsh, Iran, s.c.), and naloxone hydrochloride (Tolid-Daru, 3 mg/kg, s.c.) were used. All the drugs were dissolved in physiological saline. Dosages were chosen according to previous experiments (Tahsili-Fahadan et al., 2006).

Procedure

Induction of morphine dependence: The control group received saline, while the other groups - morphine, naloxone, and sildenafil groups - received subcutaneous injections of morphine for dependency. Morphine dependence was induced by two daily subcutaneous injections (first injection at 8 a.m. and second injection at 7 p.m.) of increasing doses of morphine sulfate for five consecutive days: 1st day (8 and 15 mg/kg), 2nd day (20 and 25 mg/kg), 3rd day (30 and 35 mg/kg), 4th day (40 and 45 mg/kg), and 5th day (45 mg/kg at 8 a.m.) (Maldonado et al., 1989; Shahidi & Hashemi-Firouzi, 2014; Yamaguchi et al., 2001). Two hours after the last morphine injection on the fifth day of the experiment, the controls received saline (saline group), while all of the other morphine-dependent groups received naloxone (3 mg/kg) to precipitate morphine withdrawal symptoms. Sildenafil-treated groups received intraperitoneal injections of sildenafil (1, 5, 10, or 20 mg/kg) 30 min before administration of naloxone, which was injected to precipitate withdrawal. An equal volume of saline was intraperitoneally injected into the control and morphine groups.

The animals were placed in a Plexiglas chamber (50 cm × 25 cm × 15 cm) and the following signs were scored for 30 min after naloxone injection: Number of jumps, teeth chattering, wet dog shakes, writhing, face grooming, body grooming, standing, and percentage of weight loss (Navarro-Zaragoza et al., 2011).

Data analysis

Statistical comparisons among the experimental groups were made by one-way analysis of variance (ANOVA), followed by Tukey's post hoc test for multiple comparisons. All results are presented as the mean ± S.E.M, with a p value of less than 0.05 being considered significant.

Results

Repeated administration of morphine produced physical dependence, such that naloxone administration to the mice after the repeated morphine generated a specific set of behavioral responses including weight loss, teeth chattering, jumping, writhing, wet dog shakes, face grooming, body grooming and standing. Administration of saline did not significantly affect withdrawal in the chronically treated morphine groups. In the naloxone group that was chronically treated with morphine, significant behavioral responses (as detailed above) were induced after naloxone administration when compared to control mice receiving chronic saline.

Figure 1A illustrates the percentage of weight loss before and after naloxone injection in the sildenafil treated morphine-dependent and control groups. Naloxone injection in the morphine-dependent group induced notable weight loss in the animals. Sildenafil (1, 5, 10 and 20 mg/kg) significantly decreased

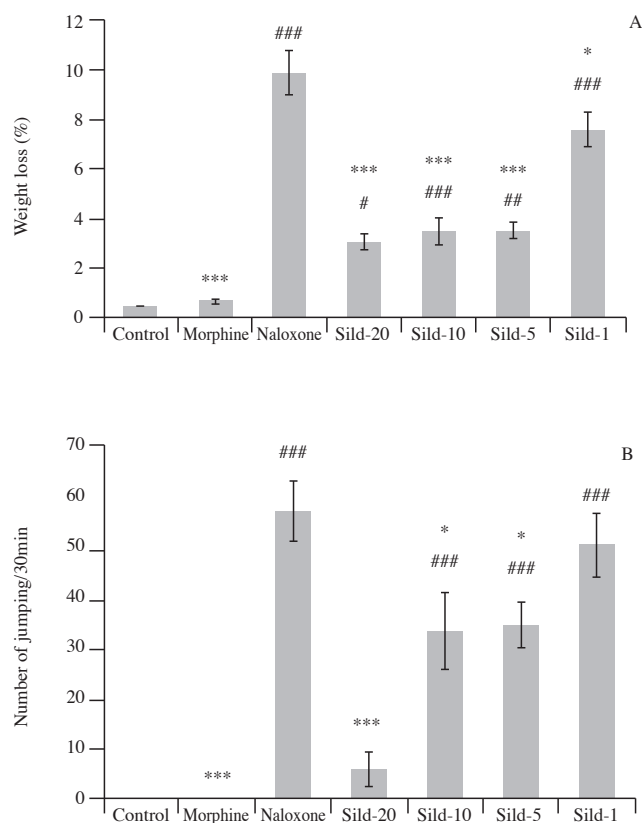


Fig. 1. The effects of sildenafil on naloxone-induced withdrawal signs: Weight loss and jumping. The percentage of weight loss (A) and number of jumps in 30 min (B) between the experimental groups.

*, $p < 0.05$ and ***, $p < 0.001$ compared to the naloxone group.

#, $p < 0.05$, ##, $p < 0.01$, and ###, $p < 0.001$ compared to the control group. Values are mean ± S.E.M.

the percent weight loss in the treated morphine-dependent mice compared to the naloxone group ($p < 0.05$, and $p < 0.001$).

Figure 1B illustrates the effect of sildenafil on the number of jumps by morphine-dependent mice. Administration of naloxone precipitated jumping in the morphine-dependent group. Treatment with sildenafil at 5, 10, and 20 mg/kg attenuated the number of jumps in morphine-dependent mice compared to the naloxone group ($p < 0.05$ and $p < 0.001$, respectively). However, administration of the lowest dose of sildenafil (1 mg/kg) to the morphine-dependent group did not significantly reduce the number of naloxone-precipitated withdrawal jumps compared to the naloxone-only (received i.p. saline instead of sildenafil) treatment group.

Figure 2A illustrates the effect of sildenafil (1, 5, 10, and 20 mg/kg) on teeth chattering in the dependent animal groups. The highest doses of sildenafil (10 and 20 mg/kg) attenuated teeth chattering in morphine-dependent mice as compared to the naloxone-only mice ($p < 0.01$ and $p < 0.001$, respectively).

The effects of different doses of sildenafil on the number of writhing movements in morphine-dependent mice are depicted in Fig. 2B. All doses of sildenafil (1, 5, 10 and 20 mg/kg) significantly attenuated the amount of writhing in morphine-dependent groups versus naloxone-only mice (all p -values < 0.001).

Figure 3A demonstrates the effects of different doses of sildenafil on the number of standing movements (rearing) in

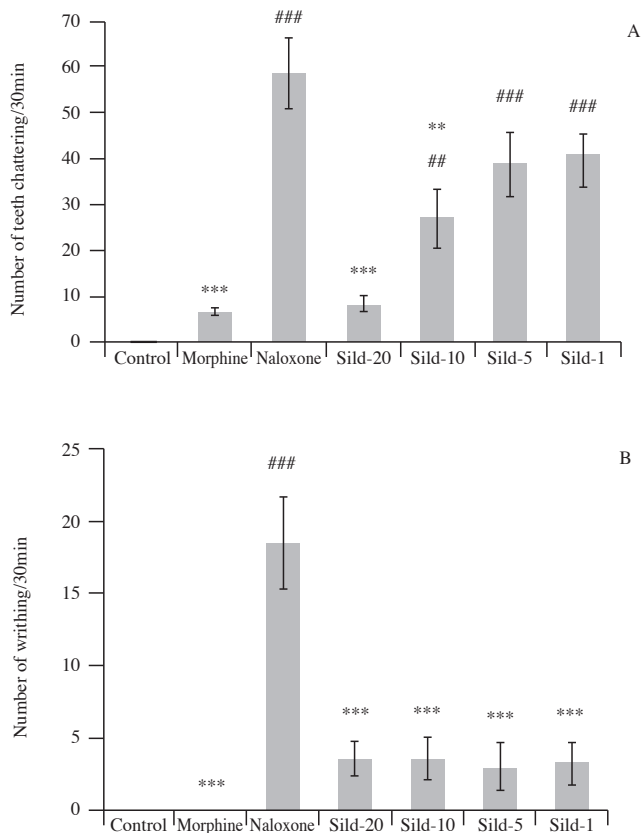


Fig. 2. The effects of sildenafil on naloxone-induced withdrawal signs: Teeth chattering and writhing. The amount of teeth chattering (A) and writhing movements (B) in 30 min between the experimental groups. **: $p < 0.01$ and ***: $p < 0.001$ compared to the naloxone group. ###: $p < 0.001$ compared to the control group. Values are mean \pm S.E.M.

morphine-dependent mice. All doses of sildenafil (1, 5, 10, and 20 mg/kg) significantly attenuated the rearing in morphine-dependent mice versus naloxone-only mice ($p < 0.05$ and $p < 0.01$ respectively). Figure 3B demonstrated that only the highest doses of sildenafil (10 and 20 mg/kg) significantly attenuated the number of wet dog shakes in morphine-dependent mice when compared to naloxone-only mice ($p < 0.001$).

Administration of naloxone precipitated both face and body grooming in morphine-dependent mice. Sildenafil significantly reduced face grooming at doses of 5, 10, and 20 mg/kg (Fig. 4A; $p < 0.001$ for all doses). Sildenafil administration significantly decreased body grooming at all administered doses (Fig. 4B; $p < 0.001$ for all doses).

Discussion

The effect of different doses of sildenafil (1, 5, 10 and 20 mg/kg) on morphine withdrawal signs was evaluated in the present study. We used the opioid antagonist naloxone to induce morphine withdrawal syndrome in the dependent mice (Koob et al., 1992; Koob et al., 1989; Kreek & Koob, 1998). Withdrawal symptoms such as the percentage of weight loss, writhing, standing, face grooming, and body grooming were attenuated using sildenafil treatment. Additionally, sildenafil in higher doses (5, 10 and 20

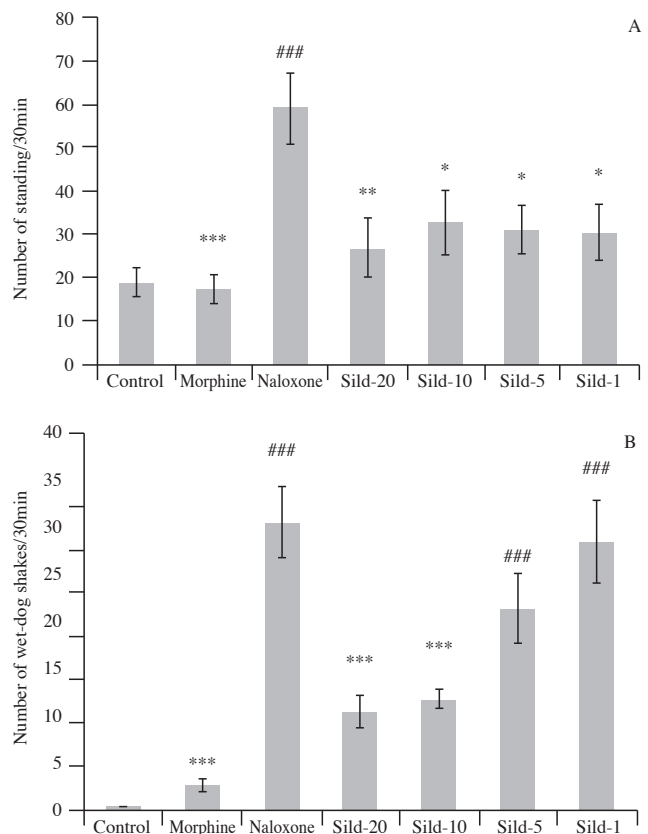


Fig. 3. The effects of sildenafil on naloxone-induced withdrawal signs: Standing (rearing) and wet dog shakes. The amount of standing (A) and wet dog shaking movements (B) in 30 min between the experimental groups. *: $p < 0.05$, **: $p < 0.01$ and ***: $p < 0.001$ compared to the naloxone group. ###: $p < 0.001$ compared to the control group. Values are mean \pm S.E.M.

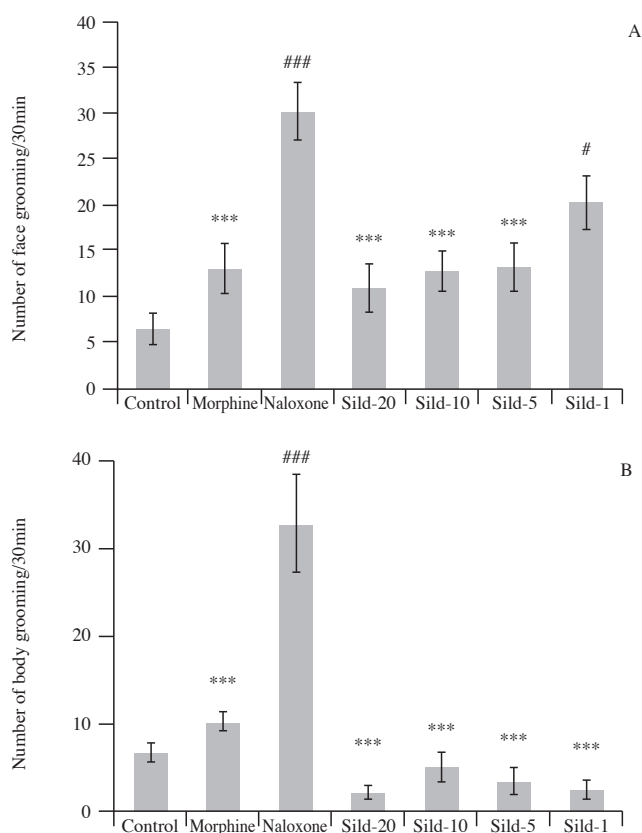


Fig. 4. The effects of sildenafil on naloxone-induced withdrawal signs: Face grooming and body grooming. The amount of face grooming (A) and body grooming (B) in 30 min between the experimental groups. ***: $p < 0.001$ compared to the naloxone group. #: $p < 0.05$ and ###: $p < 0.001$ compared to the control group. Values are mean \pm S.E.M.

mg/kg) also decreased jumping, teeth chattering, and wet-dog shakes.

Sildenafil acts by inhibiting the action of PDE5, resulting in the accumulation of nitric oxide (NO). In turn, NO elevates the levels of cGMP (Snyder & Brecht, 1991). Sildenafil has anxiolytic (Kurt et al., 2004; Shahidi et al., 2011, 2013; Solís et al., 2008), analgesic (Yoon et al., 2008) and reward-promoting activities (Tahsili-Fahadan et al., 2006) which may be involved in the relief of withdrawal signs. Sildenafil inhibited the morphine withdrawal symptoms resulting from naloxone administration, most likely by passing through the blood-brain barrier, inhibiting the action of PDE5, and thus augmenting cGMP levels in the brain (Snyder & Brecht, 1991; Uthayathas et al., 2007).

Our result confirmed the rewarding effect of sildenafil in mice on conditional place preference (Tahsili-Fahadan et al., 2006). In

contrast, it has been reported that chronic co-treatment of sildenafil with the peroxisome proliferator-activated receptor gamma (PPAR γ) agonist pioglitazone potentiated naloxone-induced withdrawal syndrome (Javadi et al., 2013). Hamdy et al. have shown that chronic treatment with rolipram, a selective phosphodiesterase type 4 inhibitor, abolishes behavioral withdrawal in rats (Hamdy et al., 2001). In addition, tolerance to morphine has been associated with increased cGMP levels in various brain regions (Bhargava & Cao, 1997). However, Cedemex attenuated behavioral withdrawal via cGMP modulation in the hippocampus, cortex, and tegmental areas (Xie et al., 2008).

Opioids are significant modulators of cGMP in neurons. For example, long-term morphine exposure alters the balance of cGMP production in cultured neurons (Mo, Leung, & Yung, 2005). In live animals, morphine, as well as mu-, kappa-, and delta-opioid receptor agonists, increases cGMP levels in several brain regions (Bhargava & Cao, 1997), though chronic exposure actually decreases cGMP (Askew & Charalampous, 1976; Javadi et al., 2013; Peregud, Iakovleva, Stepanichev, Panchenko, & Guliaeva, 2013). During development of opioid dependency, cGMP signaling activity decreased (Peregud, Iakovleva, Stepanichev, Panchenko, & Guliaeva, 2013) upon antagonism of opioid receptors (Xie et al., 2008).

The effects of sildenafil are not only mediated by PDE5. Some of the effect of sildenafil is mediated through the opioid receptors themselves (Yoon et al., 2008). Sildenafil is known to increase morphine-mediated anti-nociception through the inhibition of cGMP degradation (Jain, Patil, Singh, & Kulkarni, 2003; Mixcoatl-Zecuatl, Aguirre-Banuelos, & Granados-Soto, 2000). In fact, the mechanism of action of sildenafil can be explained through its enhancement of intracellular cGMP in brain regions related to withdrawal behaviors. Sildenafil crosses the blood brain barrier, where it can inhibit PDE5 (Snyder & Brecht, 1991), and elevate the amount of cGMP in neuronal cells, particularly in the hippocampus, cerebral cortex, and basal ganglia (Prado, Pifarre, Giral, Hidalgo, & García, 2013).

In conclusion, the acute administration of the PDE5 inhibitor sildenafil during naloxone-precipitated morphine withdrawal syndrome significantly reduced withdrawal symptoms. This finding suggests that the sildenafil may be beneficial as an adjunct treatment of morphine withdrawal syndrome.

Conflict of interest

The authors declare that there is no conflict of interest.

Acknowledgments

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