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Rewarding properties of sildenafil citrate in mice: role of the nitric oxide-cyclic GMP pathway

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Abstract *Rationale:* Sildenafil citrate is widely prescribed for erectile dysfunction and acts by inhibiting phosphodiesterase type-5, resulting in accumulation of cyclic-guanosine monophosphate (cGMP) via activation of nitric oxide synthase (NOS). The nitric oxide (NO) system is relevant to the rewarding effects of various drugs of abuse. Several epidemiologic studies indicate that sildenafil is abused in a recreational fashion. *Objectives:* In the present study, the rewarding properties of sildenafil and probable involvement of the NO-cGMP pathway were investigated in adult male NMRI mice. *Methods:* The ability of sildenafil citrate (1–40 mg/kg) to produce conditioned place preference (CPP) was studied in an unbiased CPP paradigm. The effects of NO precursor L-arginine, nonselective NOS inhibitor N^G-nitro-L-arginine methyl ester (L-NAME), and the inhibitor of guanylyl cyclase methylene blue (MB) on sildenafil-induced CPP were assessed. *Results:* Mice that received sildenafil (20 and 40 mg/kg) in one environment during conditioning phase displayed a preference for this environment. Both L-NAME (5 mg/kg) and MB (1 mg/kg) in combination with sildenafil (20 mg/kg) suppressed the acquisition of sildenafil-induced place preference. Lower and per se noneffective dose of sildenafil (10 mg/kg) and L-arginine

(60 mg/kg), when coadministered, exerted a significant place conditioning. *Conclusions:* Sildenafil shows rewarding properties that may involve the NO-cGMP pathway.

Keywords Sildenafil · Conditioned place preference · Nitric oxide · Cyclic GMP · Mice

Introduction

The Food and Drug Administration approved sildenafil (Viagra, Pfizer Inc., NY, USA) as the first oral pill to treat erectile dysfunction on 27 March, 1998. The drug quickly gained popularity and it is estimated that since its approval, more than 16 million men worldwide have used the drug (Swearingen and Klausner 2005). Recently, two additional drugs for the treatment of erectile dysfunction, tadalafil and vardenafil were approved. All three of these drugs act by inhibiting phosphodiesterase type-5 (PDE-5), resulting in the accumulation of cyclic-guanosine monophosphate (cGMP) in the glans penis, corpus cavernosum, and corpus spongiosum. This leads to an increase in smooth-muscle relaxation and better erection. Sildenafil is shown to have no effect on the penis in the absence of sexual stimulation when the concentration of nitric oxide (NO) and cGMP are low (Moreira et al. 2000; Boyce and Umland 2001).

Several epidemiologic studies indicate that sildenafil is abused in a recreational fashion. It is reported to be used as a recreational drug by 3% of nightclub customers in England in 1998. Most of them reported to have taken sildenafil simultaneously with illegal drugs (methylenedioxymethamphetamine, cocaine, and cannabis), illicit drugs (amyl nitrite, -hydroxybutyric acid), and alcohol (Aldridge and Measham 1999). A recent comprehensive study of sildenafil use among commercially insured adults in the United States from 1998 to 2002 found an 84% increase in usage rates of the drug by men and women (from 0.8% in 1998 to 1.4% in 2002). The largest increase in sildenafil use was found among younger males aged 18–45 years, who had an increase in use of 312% from 1998 to 2002. The study con-

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cluded that this large increase in use among a younger population suggested increased use of sildenafil for sexual enhancement or recreational use (Delate et al. 2004). Several studies have found increased levels of methamphetamine and sildenafil use among populations of homosexual men (see Swearingen and Klausner 2005 for review). Numerous studies established an association between sildenafil use and increased sexual risk behavior, including unprotected anal sex with a partner of unknown or serodiscordant HIV status, a major risk factor for HIV transmission (Romanelli and Smith 2004). Greater risk of sexually transmitted diseases and HIV infection, unsafe sex behavior, and concomitant abuse of other drugs raise many questions including rewarding properties of sildenafil.

Conditioned place preference (CPP) has been widely used to assess the rewarding effect of various drug and nondrug treatments. The test is based upon the principle that when a primary reinforcer is paired with a contextual stimulus, the contextual stimulus can acquire secondary reinforcing properties. These secondary reinforcing properties, which are presumably established due to a Pavlovian contingency, are thought to be capable of eliciting an operant approach response or place preference, which results in a significant increase in the time spent in the drug-paired place.

Nitric oxide is a gas produced by many mammalian cells that is synthesized from L-arginine and is a potent stimulator of guanylyl cyclase, resulting in increased levels of cGMP (Snyder and Brecht 1991). The NO system is relevant to the rewarding effects of various drugs of abuse. It is suggested that NO may play an important role in nicotine-, morphine-, methamphetamine-, alcohol-, phencyclidine-, and cocaine-induced place preference (Martin and Itzhak 2000; Manzanedo et al. 2004; Kivastik et al. 1996; Langroudi et al. 2005; Li et al. 2002; Itzhak and Martin 2000; Miyamoto et al. 2000; Kim and Park 1995; Itzhak et al. 1998).

The link between sildenafil and the NO-cGMP system raises the hypothesis that sildenafil may exert rewarding effects. This hypothesis is powered by mentioned epidemiologic studies reporting that sildenafil is abused in a recreational fashion. Sildenafil crosses well the blood brain barrier (Marmor and Kessler 1999; Milman and Arnold 2002), exerts various biochemical and physiologic effects in the brain, and affects information processing. Pretreatment with sildenafil is shown to modify several behavioral signs centrally evoked by the D2/D3 dopamine agonists in experimentally naive male rats (Ferrari et al. 2002). The potential for abuse also exists for the two more recently approved drugs in this class, vardenafil and tadalafil (Smith and Romanelli 2005).

This study was performed to investigate the effect of sildenafil citrate on the induction of CPP in mice. These experiments revealed a rewarding effect for sildenafil. Therefore, we also tested the involvement of the NO-cGMP pathway in this effect by using nitric oxide synthase (NOS) inhibitor, *N*^G-nitro-L-arginine methyl ester (L-NAME), inhibitor of guanylyl cyclase, methylene blue (MB), and NOS substrate L-arginine.

Materials and methods

Animals

A total of 191 male NMRI mice (Pasteur Institute of Iran), each weighing 20–30 g, were used. The animals were housed and grouped by 6–7 per cage in a temperature-controlled ($22 \pm 3^\circ\text{C}$) colony room. They were maintained in a 12-h on and 12-h off light/dark schedule with ad libitum food and water except during experimental procedures. All trials were carried out in the light phase. Subjects were experimentally naïve. Animals were allowed at least 3 days to acclimatize to the laboratory environment before testing began. Each mouse was used only once and each treatment groups consisted of six to seven animals. Animals housed in the same cage were subjected to the same treatment. All procedures were carried out in accordance with institutional guidelines for animal care and use and possible measures were undertaken to minimize the number of animals used and also to minimize animals' discomfort. The protocol was approved by the committee of ethics of the faculty of Sciences of Tehran University (357; 8 November 2000).

CPP

The place preference apparatus were made of wood and consisted of two square-based compartments ($15 \times 15 \times 30$ H cm each). To distinguish the two compartments, visual and sensory texture cues were used; the inner surface of one compartment was painted in black with a smooth floor; the other was white with a textured floor to create equally preferred compartments. Place conditioning was conducted using an unbiased procedure. The CPP paradigm took place in nine consecutive days, which consisted of three phases: familiarization and preconditioning, conditioning, and post-conditioning. All trials were done between Zeitgeber Time (ZT) 4 and ZT 6 (light onset is defined as ZT 0).

Familiarization and preconditioning On the first day of the trials (i.e., familiarization) and the second day (i.e., preconditioning), each mouse was placed separately into the apparatus for 10 min while they could freely access both compartments. The time spent in each compartment was recorded on the preconditioning day to determine any individual innate preference for either of the two compartments. Placement in each compartment was considered as placement of the front paws and the head. Animals showing strong unconditioned preference or aversion for any compartment were excluded from the experiments (11 mice).

Conditioning This phase consisted of six 40-min conditioning sessions held in six consecutive days. Animals were confined to the considered compartment by isolating the compartment using a removable partition. The mice received the considered drugs on days 1, 3, and 5 and vehicle injections on days 2, 4, and 6 of the conditioning

phase according to the experimental design. Treatment compartment and order of presentation of drugs and saline were counterbalanced for either group.

Postconditioning This phase was carried out in the ninth day of the trials (24 h after the last conditioning session with no preceding injections) in a drug-free state. As in the preconditioning phase, the partition was raised and the animals were placed in the apparatus for 10 min with free access to both compartments and the time spent in each compartment was recorded in real time. An observer who was unaware of mice and treatments recorded the time spent in drug-paired compartment for each animal. Change in preference (CIP) was calculated as the difference (in seconds) between the time spent in the drug-paired compartment on the postconditioning day and the time spent in this compartment in the preconditioning session.

Measurement of locomotor activity

Locomotion was measured based on a method used previously by Tzschentke and Schmidt (1997) during the test sessions (Belzung and Barreau 2000) in the drug-paired compartment. To measure the locomotor activity, the ground areas of the drug-paired compartments were divided into four equal-sized squares by two transverse lines and the number of squares mice entered during the 10-min test of CPP was measured by another observer and used as an index of locomotor activity.

Experimental design

Experiment 1: induction and assessment of place conditioning by sildenafil citrate

In this experiment, the effect of different doses of sildenafil citrate (1, 5, 10, 20, and 40 mg/kg, i.p.) on producing place preference was tested. In the first, third, and fifth day of the conditioning phase, animals received sildenafil 15 min before placement in the apparatus under the schedule described above. A control group that received saline (10 ml/kg, i.p.) in all sessions was included to confirm that the injection and conditioning schedule did not affect the time spent in the compartments.

Experiments 2 and 3: measurement of the effects of NOS and guanylyl cyclase inhibitors, L-NAME and MB, and NOS substrate L-arginine on the acquisition of CPP induced by sildenafil citrate in mice

This part of study was carried out to examine the possible involvement of the NO-cGMP pathway in rewarding properties of sildenafil citrate.

In experiment 2, the effect of nonselective NOS inhibitor L-NAME, and guanylyl cyclase inhibitor MB on sildenafil-induced place preference conditioning was assessed. In the first part of the experiment, animals received saline (10 ml/kg, i.p.), MB (0.5, 1, and 2 mg/kg, i.p.), or L-NAME (1, 5, and 10 mg/kg, i.p.) 30 min before confinement to the conditioning apparatus according to the schedule described above. The ability of these treatments to induce either place preference or place aversion was assessed in the post-conditioning day. Based on the results of this experiment, in the next part, six groups of animals received saline (10 ml/kg, i.p.), MB (1 mg/kg, i.p.), or L-NAME (5 mg/kg, i.p.) 15 min before the administration of sildenafil (20 mg/kg, i.p.) or saline (10 mg/kg, i.p.). Animals were placed in the conditioning apparatus 15 min after the second injection under the schedule.

Experiment 3 was conducted to test the probable additive effect of NO precursors and sildenafil on producing place conditioning. In the first part of this experiment, mice received saline (10 ml/kg, i.p.) or L-arginine (30, 60, 120, and 240 mg/kg, i.p.) 30 min before placement of animals in the apparatus. In the next part, L-arginine (60 mg/kg, i.p.) was injected 15 min before administration of two different and per se noneffective doses of sildenafil (5 and 10 mg/kg, i.p.) according to the results of experiment 1. Control animals received saline solution instead of sildenafil.

Drugs

Sildenafil citrate (Vorin, India) was a generously given by Poursina (Tehran, Iran). Other Drugs used in the present study were L-NAME (Sigma, UK), MB (Hoechst, Germany), and L-arginine (Fluka, Switzerland). All drugs were dissolved in sterile physiological saline solution to such concentrations that requisite doses were administered in a volume of 10 ml/kg. All drugs were injected intraperitoneally in all experiments. Appropriate vehicle controls (saline) were performed for each experiment.

Data analysis

All results are presented as mean±SEM. Data for CIP (difference between the time spent in the drug-paired compartment on the pre- and postconditioning sessions) and locomotion were assessed by two-way analysis of variance (ANOVA) or one-way ANOVA. If a significant *F* value was obtained, post hoc analyses (Tukey–Kramer's multiple comparison tests) were performed to determine the effects of various treatments on induction of place preference and changes in locomotion. *P* values less than 0.05 were considered as significant. Calculations were performed using the SPSS statistical package (version 11.5).

Results

Experiment 1: dose–response curve for place preference conditioning induced by sildenafil in mice

Figure 1 shows the effect of intraperitoneal administration of different doses of sildenafil (1, 5, 10, 20, and 40 mg/kg) on induction of CPP. One-way ANOVA revealed a significant effect ($F_{5,32}=6.530$, $P<0.001$). Post hoc analysis showed that sildenafil at doses of 20 and 40 mg/kg produces a significant place preference compared with saline-treated control animals. Sildenafil administration had no significant effect on locomotor activity (one-way ANOVA $F_{5,32}=0.188$, $P=0.965$).

Experiment 2: effect of NO synthesis and guanylyl cyclase inhibitors on the acquisition of sildenafil-induced CPP in mice

Figure 2 shows the effect of L-NAME (1, 5, and 10 mg/kg, i.p.) and MB (0.5, 1, and 2 mg/kg, i.p.) on producing place conditioning. One-way ANOVA revealed that neither L-NAME ($F_{3,22}=1.349$, $P=0.284$) nor MB ($F_{3,22}=0.625$, $P=0.607$) in doses used in this experiment are able to induce CPP or conditioned place aversion. Administration of L-NAME or MB had no significant effect on locomotor activity (one-way ANOVA $F_{3,22}=0.095$, $P=0.962$ and $F_{3,22}=0.088$, $P=0.966$ for L-NAME and MB, respectively).

The effect of L-NAME (5 mg/kg, i.p.) or MB (1 mg/kg, i.p.) on sildenafil-induced CPP is shown in Fig. 3. Two-way ANOVA indicated a significant interaction between L-NAME plus sildenafil (factor L-NAME $F_{1,21}=13.789$, $P=0.001$; factor sildenafil $F_{1,21}=11.656$, $P=0.003$; and factor L-NAME \times sildenafil $F_{1,21}=7.756$, $P=0.011$) and MB plus

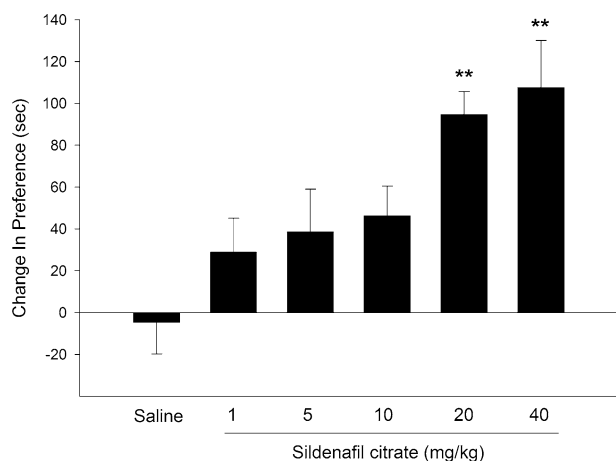


Fig. 1 The effect of different doses of sildenafil (1, 5, 10, 20, and 40 mg/kg) on producing CPP in mice. Sildenafil was injected intraperitoneally 15 min before placement of animals in the apparatus in the first, third, and fifth day of the conditioning phase. Data are expressed as mean \pm SEM. ** $P<0.005$ compared to saline control group (Tukey–Kramer’s multiple comparison tests).

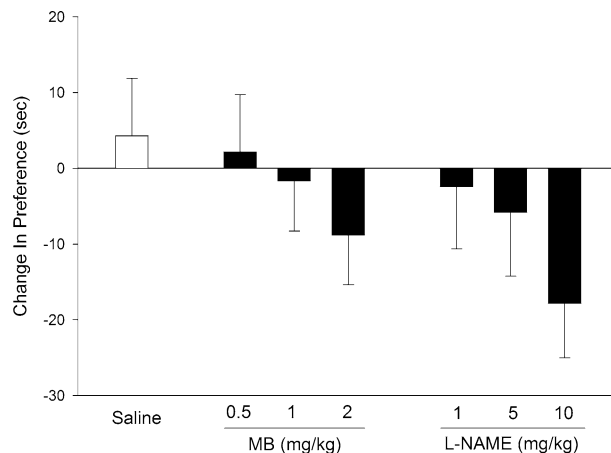


Fig. 2 The effect of NOS inhibitor L-NAME (1, 5, and 10 mg/kg) and guanylyl cyclase inhibitor MB (0.5, 1, and 2 mg/kg) on induction of place conditioning in mice. Drugs were injected 30 min before confinement of mice in the apparatus in the first, third, and fifth day of conditioning phase. A control group receiving saline in all conditioning sessions was included. Data presented as mean \pm SEM

sildenafil (factor MB $F_{1,21}=7.339$, $P=0.013$; factor sildenafil $F_{1,21}=4.797$, $P=0.040$; and factor MB \times sildenafil $F_{1,21}=6.750$, $P=0.011$, $F_{3,21}=6.195$, $P=0.017$) on induction of place conditioning. Post hoc comparisons showed a significant difference between sildenafil- and saline-treated animals in control condition (87.83 s vs 1.5 s, $P<0.001$). Both pretreatments with L-NAME and MB inhibited the effect of sildenafil (20 mg/kg) on producing CPP. Co-administration of L-NAME (factor L-NAME $F_{1,21}=0.897$, $P=0.354$; factor sildenafil $F_{1,21}=0.248$, $P=0.624$; and factor L-NAME \times sildenafil $F_{1,21}=0.392$, $P=0.538$) or MB (two-way ANOVA: factor MB $F_{1,21}=1.086$, $P=0.309$; factor sildenafil $F_{1,21}=0.651$, $P=0.429$; and factor MB \times sildenafil

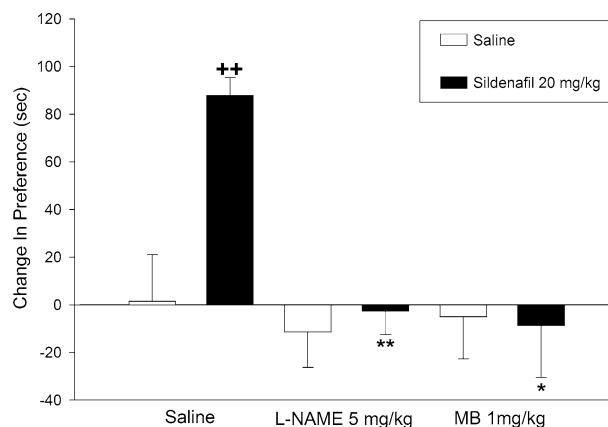


Fig. 3 The effect of pretreatment with L-NAME (5 mg/kg) and methylene blue (MB; 1 mg/kg) on the rewarding effect of sildenafil in mice. In the first, third, and fifth day of the conditioning phase, L-NAME or MB was injected 30 min before placement of animals in the apparatus and 15 min before sildenafil. Controls animals received saline injections. Data presented as mean \pm SEM. ++ $P<0.005$ compared with saline/saline group; * $P<0.05$ and ** $P<0.005$ compared with the group treated with saline/sildenafil 20 mg/kg (Tukey–Kramer’s multiple comparison tests)

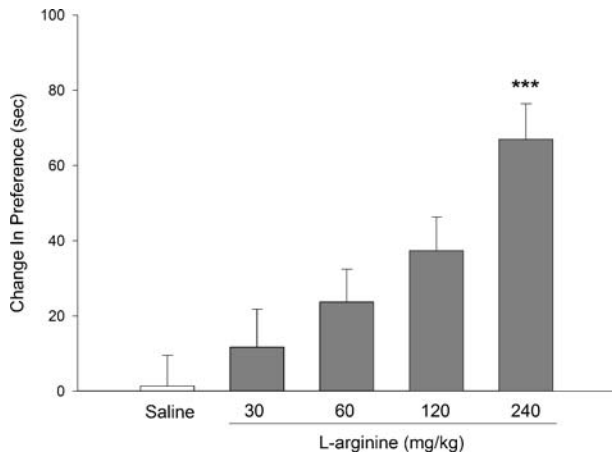


Fig. 4 The effect of L-arginine (30, 60, 120, and 240 mg/kg) on induction of CPP in mice. L-arginine or its vehicle (saline) were injected 30 min before confinement of mice in the apparatus in the first, third, and fifth day of conditioning phase. Data presented as mean \pm SEM. *** $P < 0.001$ compared to saline control group (Tukey–Kramer’s multiple comparison tests)

$F_{1,21}=3.935$, $P=0.061$) with sildenafil had no significant effect on locomotion of animals.

Experiment 3: effect of NO synthesis precursor on the acquisition of sildenafil-induced CPP in mice

Figure 4 shows the effect of L-arginine (30, 60, 120, and 240 mg/kg, i.p.) on induction of place conditioning. A significant effect was seen (one-way ANOVA $F_{4,26}=7.638$, $P < 0.001$). Further analysis showed that L-arginine at the dose of 240 mg/kg induced a significant CPP compared to saline control group while L-arginine (30, 60, and 120 mg/kg) failed to produce a significant place preference. Administration of L-arginine did not alter the locomotor

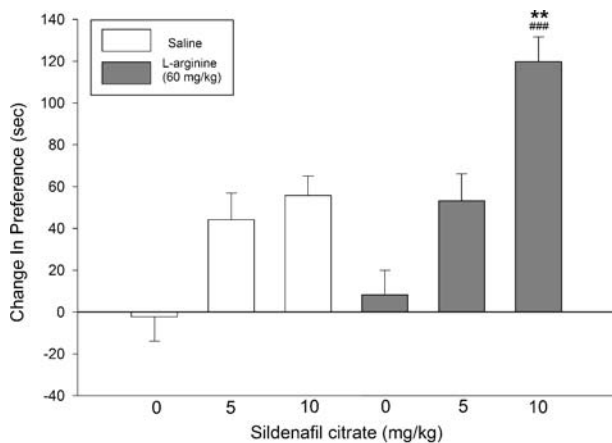


Fig. 5 Effect of pretreatment with L-arginine (60 mg/kg) on the place preference induced by sildenafil in mice. L-arginine was injected 15 min before sildenafil and 30 min before placement in the apparatus in first, third, and fifth day of the conditioning phase. Control animals received saline injections. Data presented as mean \pm SEM. ** $P < 0.005$ compared with the group treated with saline/sildenafil 10 mg/kg and ### $P < 0.001$ compared with saline/saline group (Tukey–Kramer’s multiple comparison tests)

activity of animals (one-way ANOVA $F_{4,26}=0.560$, $P=0.693$).

Figure 5 shows that coadministration of lower and per se noneffective doses of L-arginine and sildenafil is able to produce a significant place preference. Two-way ANOVA showed a significant effect (factor L-arginine $F_{1,33}=8.615$, $P=0.006$; factor sildenafil $F_{2,33}=27.614$, $P < 0.001$; and factor L-arginine \times sildenafil $F_{2,33}=3.763$, $P=0.034$). Further analysis showed that combination of per se noneffective doses of L-arginine (60 mg/kg) and sildenafil (10 mg/kg) induced place conditioning. However, this effect of L-arginine was not observed on the lower dose of sildenafil (5 mg/kg). Combination of L-arginine and sildenafil did not alter the locomotion of animals (two-way ANOVA: factor L-arginine $F_{1,33}=0.622$, $P=0.436$; factor sildenafil $F_{2,33}=0.486$, $P=0.620$; and factor L-arginine \times sildenafil $F_{2,33}=0.831$, $P=0.444$).

Discussion

In the present study, the rewarding effect of sildenafil was assessed using place-conditioning paradigm in mice. The findings indicated that sildenafil with doses of 20 and 40 mg/kg induced a significant place preference. We also showed that the rewarding effect of sildenafil is inhibited by both NOS inhibitor L-NAME, and guanylyl cyclase inhibitor MB. Pretreatment with L-arginine potentiated the effect of a subthreshold and per se noneffective dose of sildenafil.

Sildenafil citrate, widely prescribed for treatment of erectile dysfunction, acts by inhibiting PDE-5, an enzyme found in the corpus cavernosum, resulting in accumulation of cGMP and causing relaxation of the smooth muscle with concomitant increased blood flow to the penis, leading to an erection (Marmor and Kessler 1999). The localization of PDE-5 to areas of the brain provides for the possibility of central nervous system (CNS) effects of sildenafil administration. Several direct effects of sildenafil administration on the CNS were reported in both rodents and humans. These effects in human include dizziness, depression, insomnia, abnormal dreams, and nervousness (Moreira et al. 2000; Milman and Arnold 2002). Also, recreational sildenafil use and its association with men’s substance abuse behaviors are reported (Crosby and Diclemente 2004). Various adverse effects (i.e., headache, dyspepsia) were attributed to the inhibition of PDE-5 by sildenafil in smooth muscle of cerebral or other vessels, esophageal sphincter, and nasal mucosa, and by inhibition of PDE-6 (another isoenzyme form of PDE) in smooth muscle of the retina (Osterloh et al. 1999; Young 1999; Goldstein 1999).

The data about the effects of sildenafil on the CNS in experimental animal models are limited. It was shown that sildenafil interacts with other systems in which the role of the NO-cGMP pathway was demonstrated. Sildenafil produces a local peripheral antinociceptive effect via activation of the cGMP-PKG- K^+ channel pathway (Ambriz-Tututi et al. 2005) and increases brain levels of cGMP, evokes neurogenesis, and reduces neurological deficits when given to rats 2 or 24 h after stroke (Zhang et al. 2002). Kurt et al. (2004)

have recently reported that sildenafil exerts an NO-cGMP pathway-mediated anxiogenic effect in rats. Hotchkiss et al. (2005) have reported that the administration of sildenafil in mice does not induce aggressive behavior, but mice treated chronically (4 weeks) with 10 mg/kg sildenafil were generally more aggressive 1 week postcessation of drug treatment compared to vehicle-treated mice. It was shown that sildenafil produces a dose-dependent analgesic effect and significantly increased the morphine-induced antinociception via the inhibition of cGMP degradation through the NO-cGMP pathway (Mixcoatl-Zecuatl et al. 2000; Jain et al. 2001).

The possible mechanism of sildenafil-induced CPP can be explained by its influence on the NO-cGMP signaling pathway and increasing intracellular cGMP concentrations. Nitric oxide, a short-lived molecule synthesized from L-arginine by activation of NOS, functions as a neuronal messenger or neurotransmitter in the central and peripheral nervous system (Bredt et al. 1990). NO acts as an endogenous activator of guanylyl cyclase and thereby increases the level of cGMP (Snyder and Bredt 1991) that constitutes a common pathway in signal transduction in the central and peripheral nervous system (Moncada et al. 1991; Moncada and Higgs 1993). Nitric oxide is involved in the dopaminergic pathway in mesocorticolimbic tract. This may be one mechanism whereby dopamine release is enhanced after repeated administration of psychomotor stimulatory drugs (Pudiak and Bozarth 1997). Ventral tegmental area is one of the regions in which NOS was detected (Rodrigo et al. 1994) and NO release from neurons in this area is implicated in the control of dopamine release in the shell of the nucleus accumbens (Gracy and Pickel 1997). Sodium nitroprusside, an NO generator, induces a dose-dependent increase in endogenous dopamine release from rat striatum slices (Zhu and Luo 1992) and decreases dopamine uptake in synaptosomes from the nucleus accumbens (Pogun et al. 1994). In addition, it was shown that constitutive NO levels play a role in the modulation of dopamine outflow in the nigrostriatal movement and the mesolimbic and mesocortical reward and motivation circuitries (Fricchione and Stefano 2005). The intraperitoneal and intra-accumbal administrations of the NOS substrate L-arginine are shown to produce a significant place conditioning in rats (Sahraei et al. 2004). Moreover, pretreatment with L-NAME suppresses the maintenance of intravenous cocaine self-administration and the absolute reward magnitude of cocaine in rats (Pulvirenti et al. 1996). In addition, different NOS inhibitors are shown to attenuate place conditioning induced by nicotine, morphine, methamphetamine, alcohol, and cocaine (Martin and Itzhak 2000; Manzanedo et al. 2004; Kivastik et al. 1996; Langroudi et al. 2005; Li et al. 2002; Itzhak and Martin 2000; Kim and Park 1995; Itzhak et al. 1998). It was reported that in the alcoholic brain, neuronal NOS protein content is increased in the frontal cortex and the nucleus accumbens brain regions, which were suggested to be involved in the dopaminergic mesolimbic reward system (Gerlach et al. 2001). It was shown that NOS, guanylyl cyclase, and PDE-5 are present in high concentrations in regions of the brain involved in

reward circuits (Garthwaite and Boulton 1995). Also self-stimulation induces significant changes in striatum and hippocampus concentration of cyclic nucleotides (Miliaressis and Merali 1979).

The per se noneffective dose of L-arginine that we used in this study (60 mg/kg) showed an additive effect when administered with the subthreshold dose of sildenafil. These findings can suggest that the rewarding effect of sildenafil might be mediated through its capability of activating NO-cGMP signaling pathway. This implication is powered by our findings that the NO production inhibitor, L-NAME, and cGMP production inhibitor, MB, with doses ineffective on place preference inhibited the effect of sildenafil on producing CPP.

In conclusion, our results are the first demonstration that sildenafil with doses as high as 20 mg/kg, exerts rewarding properties assessed by CPP paradigm in mice. The findings suggest that the NO-cGMP pathway might play a role in the rewarding effect of sildenafil. Although this was an animal study and cannot be generalized to human, these findings warrant further investigations on possible addictive effects of sildenafil use.

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