Modulation of Anxiety-Like Behavior in Sildenafil Citrate-Treated Mice Placed in an Elevated Plus-Maze

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ABSTRACT

Introduction: Sildenafil, a phosphodiesterase-5 (PDE5) inhibitor, may have various effects on the central nervous system via the nitric oxide (NO)-cyclic guanosine monophosphate (cGMP) pathway. Preclinical studies have shown the controversial effect of sildenafil on anxiety-like behavior in animals. This study was undertaken to investigate the acute effect of sildenafil on anxiety-like behavior in mice.

Methods: Male mice were intraperitoneally (i.p.) administrated sildenafil (1, 2, or 10 mg/kg), and the control group was given the vehicle 15 min before the plus-maze test. The number of entries into the open arms of the plus-maze, the time spent in the open arms, and the total numbers of entries into the arms were recorded.

Results: The results indicated that compared to the control group, the sildenafil-treated mice spent more time in the open arms and had a greater number of entries into open arms. The total number of entries into the arms did not significantly differ between the sildenafil-treated groups and the control group.

Discussion: Acute administration of sildenafil could have anxiolytic effects on male mice. This effect might be mediated in a time-dependent manner by the NO/cGMP/PDE5 pathway.

1. Introduction

Sildenafil is a powerful oral drug used for the treatment of erectile dysfunction (Goldstein et al., 1998; McCullough, 2002). It acts by inhibiting the action of phosphodiesterase-5 (PDE5), resulting in the accumulation of nitric oxide (NO) that in turn increases the levels of cyclic guanosine monophosphate (cGMP) (Snyder & Bredt, 1991). This leads to increased relaxation of smooth muscles, and therefore, improved erection (McCullough, 2002; Ulhayathas et al., 2007).

Evidence shows that sildenafil inhibits cGMP degradation in the brain (Hotchkiss et al., 2005; Riazi et al., 2006) and affects the central nervous system (Ulhayathas et al., 2007). In the NO-cGMP pathway, sildenafil modulates cognition, memory, reward-related stimuli, pain perception, seizure attack, neurogenesis, and drug dependency (Prickaerts et al., 2002; Boccia et al., 2011; Ulhayathas et al., 2007); it also diminishes post-stroke

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neurogenesis and neurological reactions (Zhang et al., 2002) and produces time-dependent aggressive behavioral traits in mice (Hotchkiss et al., 2005).

The results of preclinical tests on mice have shown that the use of a single injection of sildenafil 30 min before plus-maze testing does not affect anxiety and that the combination of sildenafil and L-arginine decreases the percentage of time spent in the open arms of the plus-maze (Volke et al., 1997; Volke et al., 2003; Kurt et al., 2004); in contrast, chronic administration of sildenafil has anxiolytic effects on rats (Solís et al., 2008). Sildenafil is immediately absorbed in the small intestine after oral administration, and its clinical peak efficacy has been observed within 19 min (McCullough, 2002). The relationship between the therapeutic effects of sildenafil on the central nervous system (Ulhayathas et al., 2007) and the role of sildenafil in modulating anxiety-like behavior (Kurt et al., 2004; Solís et al., 2008; Volke et al., 1997; Volke et al., 2003) leads to the hypothesis that sildenafil can modulate anxiety-like behavior in a time-dependent manner over the course of treatment.

The present study examined the effect of acute administration of sildenafil citrate 15 min before the elevated plus-maze test in mice.

2. Methods

2.1. Animals

Male, adult (age, 3–4 months), NMRI mice, weighing 20–30 g, were obtained from the Pasteur Institute (Tehran, Iran). The animals were randomly distributed to the control group and the 3 sildenafil-treated groups (3 different doses; n = 10 for each group). They were maintained at 20 °C ± 20 °C with 12 h light/12 h dark cycle. Tap water and chow pellets were available ad libitum. The mice were allowed to acclimatize to laboratory conditions for 1 week before the tests. Each mouse was used only once. All the tests were conducted between 0900 and 1500. All the experiments were performed in accordance with the Guide for the Care and Use of Laboratory Animals (National Institutes of Health Publication No. 85–23, revised 1985).

2.2. Drugs

Sildenafil citrate (Vorin, India; 1, 2, and 10 mg/kg) was used in this study. Sildenafil was already dissolved in an isotonic solution having a volume of 10 mg/kg and was injected 15 min before the test. The mice from the control group were given the vehicle. The choice of doses was based on previous studies (Prickaerts et al., 2002; Boccia et al., 2011). The 15-min interval before the test was chosen on the basis of previous experiments (Gilhotra & Dhingra, 2009; Kurt et al., 2004; Volke et al., 1997; Volke et al., 2003) and the pilot study performed by Riazi et al, (2006).

2.3. Elevated Plus-Maze

The elevated plus-maze comprised a black wooden apparatus with arms having equal dimensions. Two of its arms were enclosed by walls (30 × 15 × 5 cm) and arranged in line with 2 opposite open arms (30 × 5 cm). The maze was elevated 50 cm above the floor. The mice were placed in the center of the maze, facing the open arms. Two 100 W lamps brightly illuminated the arena. The mice were allowed to explore the maze, and their behavior was monitored by a digital camera above the maze for 10 min. After each test, the apparatus was cleaned with 10% ethanol to eliminate the remaining odors. The time spent in the open arms, the number of entries into the open arms, and the total number of entries into the arms was recorded (Gilhotra & Dhingra, 2009; Kurt et al., 2004; Volke et al., 2003).

2.4. Statistical Analysis

All the results are presented in terms of mean ± SEM. Data were analyzed by one-way ANOVA. Post hoc comparisons were performed using the Tukey-Kramer test to determine the effects of various treatments. A p value less than 0.05 was considered to be significant.

3. Results

The effects of different doses of sildenafil on the duration of time spent in the open arms are shown in Fig.1. One-way ANOVA indicated that compared to the control group, the sildenafil-treated group spent more time in the open arms ($F(3,36) = 5.68$, $P < 0.002$); in addition, Tukey-Kramer test analysis showed that sildenafil-treated groups spent a significantly longer duration of time in the open arms than the control group, when administered sildenafil at doses 1 mg/kg ($p < 0.01$) and 2 mg/kg ($p < 0.05$).

The effects of different doses of sildenafil on the number of entries into the open arms are shown in Fig.2. One-way ANOVA showed that sildenafil treatment caused an increase in the number of entries into the open arms ($F(3,36) = 5.81$, $P < 0.002$). Tukey-Kramer test analysis showed that the sildenafil-treated groups had a significantly higher number of entries into the open

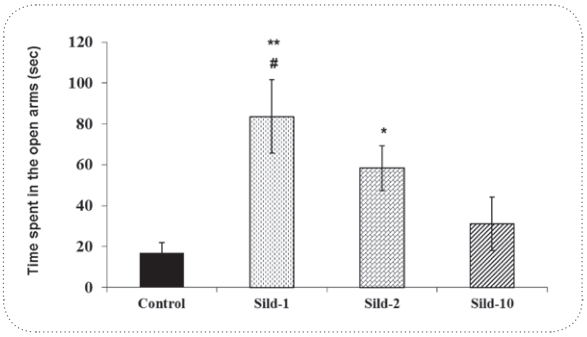

Figure 1. The effect of sildenafil treatment on the time spent in the open arms of the plus-maze within 10 min (n = 10 in each group). Data were analyzed by one-way ANOVA followed by the Tukey-Kramer post hoc test. The values are expressed in terms of mean ± SEM.
*p < 0.05, **p < 0.01 compared to the saline control group.
# P < 0.05 compared to the sildenafil-treated groups.

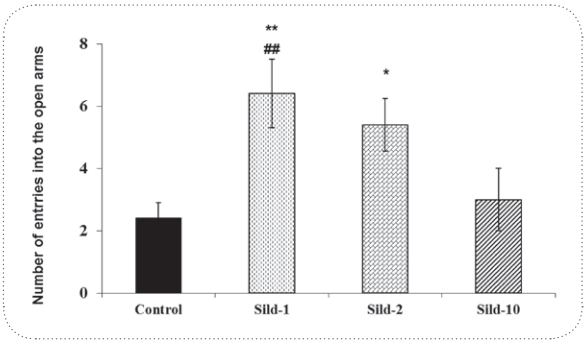

Figure 2. The effect of sildenafil treatment on the number of entries into the open arms of the plus-maze within 10 min (n = 10 in each group). Data were analyzed by one-way ANOVA followed by the Tukey-Kramer post hoc test. The values are expressed in terms of mean ± SEM.
*p < 0.05, **p < 0.01 compared to the control group.
P < 0.01 compared to the sildenafil-treated groups.

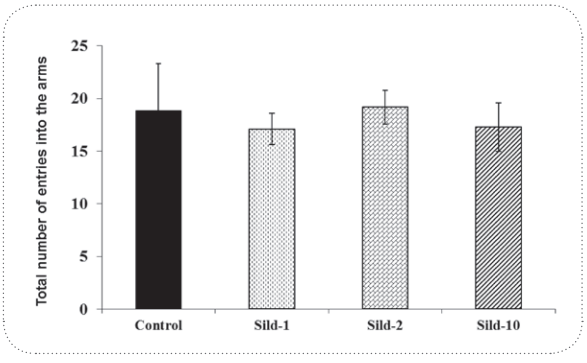

Figure 3. The effect of sildenafil treatment on the total number of entries into the arms of the plus-maze within 10 min, (n = 10 in each group). Data were analyzed by one-way ANOVA followed by the Tukey-Kramer post hoc test. The values are expressed in terms of mean ± SEM.

arms than the control group, when administered sildenafil at doses 1 mg/kg ($p < 0.01$) and 2 mg/kg ($p < 0.05$).

The effects of different doses of sildenafil on the total number of entries into the arms are shown in Fig.3. One-way ANOVA showed that sildenafil did not affect the movement of mice from the open to enclosed arms and vice versa ($F(3,36) = 0.19$, $P = 0.90$). Tukey-Kramer test analysis showed that there was no significant difference between the sildenafil-treated groups and the control group with respect to the total numbers of entries into the arms.

4. Discussion

The results show that administering sildenafil attenuates anxiety behavior—especially at low doses—and does not have any effect on the locomotion of mice in the elevated plus-maze.

Sildenafil inhibits the action of PDE5 and enhances the NO-mediated accumulation of cGMP in target tissues such as the corpus cavernsum and the brain (Gilhotra & Dhingra, 2009; Snyder & Bredt, 1991; Ulhayathas et al., 2007). Sildenafil can pass through the blood-brain barrier and has various biochemical and physiological effects (Milman & Arnold, 2002; Ulhayathas et al., 2007). In the brain, PDE5 mRNA is expressed in regions involved in anxiety behavior, such as the hippocampus (Calixto et al., 2010), and the cellular mechanism underlying this behavior involves cGMP at the levels of synthesis, neurotransmitter release, and signal transduction (Gilhotra & Dhingra, 2009; McHugh, 2004; Ulhayathas et al., 2007). It is unlikely that cGMP accumulation produces any changes in the expression of various receptors in the brain (Solís et al., 2008). The changes in levels of NO may either facilitate or inhibit anxiety, depending on the nitric oxide synthase (NOS) blocker (Li et al., 2004). The activity of the NO-cGMP pathway depends on physiological conditions.

NOS, which generates NO (Ulhayathas et al., 2007), has distinct isoforms: neuronal isoform in neuronal tissue (nNOS), inducible isoform in macrophages (iNOS), and endothelial isoform in the endothelial tissue of blood vessels (eNOS) (Volke et al., 2003; Workman et al., 2008). The different types of NOS inhibitors have varying affinities to the isoforms of the NOS enzyme (Workman et al., 2008). The nNOS and eNOS are possible principle targets for anxiolytic- and anxiogenic-like effects, respectively (Volke et al., 2003; Workman et al., 2008). Sildenafil acts as a competitive inhibitor of PDE5 (McCullough, 2002) and produces NO, caus-

ing cGMP accumulation that in turn compensates for the NO deficiency due to nNOS activity that arises via a negative feedback mechanism (Volke et al., 2003; Workman et al., 2008).

Studies have shown that administering sildenafil decreased the frequency of anxiety behavior in male rats, and after 3 weeks, the treated rats remained in the center in an open-field test for a longer duration than they did before sildenafil administration (Solís et al., 2008). Administering a single injection of sildenafil 30 min before the plus-maze test did not have any effect on anxiety-behavior (Kurt et al., 2004; Volke et al., 2003). In another study, administering sildenafil in combination with aminoguanidine, a selective inhibitor of iNOS, 30 min before the plus-maze test produced anti-anxiety effects in stressed mice 30 min prior to plus- maze test (Gilhotra & Dhingra, 2009). Sildenafil is a weak basic compound, which is therefore only partially ionized under physiological conditions and has a half-life of 15 min (McCullough, 2002). In our study, the time-dependent sildenafil treatments probably gave rise to anxiolytic effects and administering high doses of sildenafil attenuated its anxiolytic properties via the potentiated NO-cGMP pathway.

Pharmacological inhibition of NO by administering nonspecific NOS inhibitors is reported to increase the frequency of pseudo-anxiety behavior in the plus-maze and murine exploratory model tests (De Oliveira & Del Bel 1997; Vale et al., 1998; Pokk & Vali 2002; Czech et al., 2003). The selective inhibition of nNOS is responsible for anxiolytic activity (Volke et al., 2003; Workman et al., 2008). Recent studies have suggested that a microinjection of a preferential inhibitor of nNOS in the hippocampus produces an anxiolytic effect in rats in the elevated T-maze (Calixto et al., 2010). Controversial behavioral studies have been performed using different species, drug treatment protocols, and drug testing protocols in animal models of anxiety (Solís et al., 2008). Because the brain, especially the hippocampus, is enriched with NOS, GC, PDE5 (Calixto et al., 2010; Garthwaite & Boulton , 1995), NO, and probably cGMP, it possibly modulates either the release or uptake of neurotransmitters and modulates anxiety behavior (Calixto et al., 2010; Czech et al., 2003; Workman et al., 2008).

In conclusion, our results show that time-dependent acute sildenafil treatment has anxiolytic effects on male mice. Our data suggest that acute administration of sildenafil mediates the anxiolytic outcome via the NO-cGMP pathway. It is risky to consider that anxiety-like behavior is caused solely by the elevated plus-maze. Further research involving the use of different methods such as the open-field test and the light-dark box transition test for both sexes of rodents could provide a better understanding of the modification of anxiety-related behavior thorough acute administration of sildenafil in a time-dependent manner over the course of treatment.

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