Polish Journal of Pharmacology
Pol. J. Pharmacol., 2004, 56, 353–357
ISSN 1230-6002

SHORT COMMUNICATION

EFFECT OF SILDENAFIL ON ANXIETY IN THE PLUS-MAZE TEST IN MICE

Mehmet Kurt, Sirri S. Bilge[#], Elif Aksoz, Osman Kukula, Suleyman Celik, Yuksel Kesim

Department of Pharmacology, University of Ondokuz Mayis, Samsun, 55139, Turkey

Effect of sildenafil on anxiety in the plus-maze test in mice. M. KURT, S.S. BILGE, E. AKSOZ, O. KUKULA, S. CELIK, Y. KESIM. Pol. J. Pharmacol., 2004, 56, 353–357.

Several studies have shown a role of nitric oxide/cyclic guanosine monophosphate signaling pathway in the regulation of anxiety. The effects of the phosphodiesterase (PDE) 5 inhibitors on anxiety are not fully understood. The aim of present study was to investigate the possible role of sildenafil, an inhibitor of cyclic GMP-specific phosphodiesterase, on anxiety in the plus-maze test in mice. Sildenafil at a dose of 0.5 mg/kg had no significant effect on the behavior in the plus-maze test but at doses of 1 and 3 mg/kg induced an anxiogenic effect. The combination of sildenafil (1 mg/kg, *ip*) and methylene blue (1 mg/kg, *ip*) abolished the anxiogenic-like effect of sildenafil. The combination of sildenafil (1 mg/kg, *ip*) and L-arginine (50 mg/kg, *ip*) decreased the percentage of time spent in open arms compared to saline-treated group. Diazepam at a dose of 2 mg/kg significantly increased the percentage of time spent in open arms ($p < 0.05$). Sildenafil at a dose of 3 mg/kg and the combination of L-arginine (50 mg/kg, *ip*) and sildenafil (1 mg/kg, *ip*) significantly decreased the locomotor activity ($p < 0.05$). These results suggest that a nitric oxide-cGMP pathway seems to play an important role in sildenafil-induced anxiogenic-like effect.

Key words: *sildenafil, anxiety, methylene blue, cGMP*

[#] *correspondence;* e-mail: ssbilge@omu.edu.tr

INTRODUCTION

Nitric oxide (NO) is an important mediator in the central nervous system. NO is synthesized from L-arginine, in a NADPH-dependent reaction, by NO synthases (NOS) [11]. The presence of NOS in all areas of the brain indicates that NO is implicated in the control of a variety brain functions including locomotion, neurotoxicity, spinal pain processing and anxiety [4]. Some data suggest that NO plays a modulatory role in the effects of benzodiazepines [5]. NOS has also been localized in brain regions involved in anxiety especially the hypothalamus, amygdala and hypocampus [16]. There is increasing evidence that NO may underlie anxiety in the elevated plus-maze test, an animal model of anxiety. The elevated plus-maze test, one of the most popular animal test for research on anxiety, is based on the natural aversion of rodents for open spaces and uses an elevated plus-maze with two open and two closed arms [10, 14]. This test is rapid and was found to be sensitive to the effects of both anxiolytic and anxiogenic agents. The administration of L^{ω}-nitro--L-arginine methyl ester (L-NAME), a NOS inhibitor, induced an anxiolytic-like effect. In addition, pretreatment with L-arginine (L-arg) counteracted the anxiolytic effect induced by diazepam [17]. Taken together, these results suggest that NO may have anxiogenic-like effect in the elevated plus-maze test [17]. Several lines of evidence have shown a role of the NO/cyclic guanosine monophosphate (cGMP) signaling pathway in the action of NO on anxiety [3]. Intracellular cGMP concentrations are regulated by both guanylyl cyclase and phosphodiesterase (PDE) [2]. It has been suggested that NO activates guanylyl cyclase, and promotes to cGMP synthesis. PDE catalyzes the hydrolysis of cGMP to GMP. Sildenafil is a selective PDE5 type 5(PDE5) inhibitor increasing the cGMP level in the target tissues and represents a powerful therapy for male erectile dysfunction [12]. PDE5 is expressed in the brain and an inhibition of PDE5 increases the release of glutamate in the nucleus accumbens which is involved in anxiety [8]. These results indicate that the participation of the NO-cGMP pathway in anxiety deserves further investigation. It has recently been shown that sildenafil produces anxiogenic-like effect in light-dark compartment test [18], but the effects of sildenafil on anxiety has not been well investigated in elevated plus-maze test. We now examine the effects of sildenafil, methylene blue (MB), L-arg and diazepam on anxiety in the elevated plus-maze test.

The aim of present study is to evaluate the effect of sildenafil on anxiety and the role of the NO-cGMP pathway in the anxiety in mice.

MATERIALS and METHODS

Ethical guidelines

All procedures in this study are in accordance with the Guide for the Care and Use of Laboratory Animals as adopted by the National Institutes of Health.

Animals

Male ICR mice weighing 25–40 g were used in all experiments. All animals were obtained from our department breeding facility. Animals were housed in groups of eight in a temperature controlled room (20 ± 2°C) with a 12 h light/12 h dark cycle (lights on at 07.00 a.m.). The animals had a period of adaptation of three days and were allowed free access to food and water prior to the experiments. Each animal was used once.

Drugs

Diazepam, Tween 80, MB, L-arg were obtained from Sigma (USA). Sildenafil citrate was a gift from Fako Ilaclari A.S (Turkey). Diazepam was dissolved in few drops of 0.3% Tween-80 and water. MB, L-arg and sildenafil citrate were dissolved in saline. Saline was used as vehicle. All drugs were injected intraperitoneally (*ip*) 30 min before the testing in a volume of 10 ml/kg. When combinations of sildenafil and other drugs were employed, sildenafil was administered 15 min later than other drugs.

Elevated plus-maze test

Anxiety-related behavior was measured by the elevated plus-maze test. The elevated plus-maze consisted of two open arms, 50 × 10 cm, and two closed arms, 50 × 10 × 40 cm. The maze was elevated to a height of 15 cm above the floor. Each mice was placed on the central platform facing a closed arm. During 5 min test period, the following measures were taken by an observer unaware of a treatment: the time spent in open arms, percentage of time spent in open arms, percentage of open arm entries, time spent in closed arms, number of

Table 1. Effect of sildenafil, methylene blue (MB), L-arginine (L-arg) on mouse behavior in the plus-maze test

Treatment	Dose (mg/kg)	n	Percentage of open arm entries	Time spent in open arms (s)	Percentage of time spent in open arms	Time spent n closed arms (s)	Closed arm entries
Saline		8	57.5 ± 8.4	67.0 ± 14.8	27.9 ± 3.2	172.9 ± 4.6	4.8 ± 1.1
Saline + saline		8	58.8 ± 9.6	65.9 ± 12.5	25.6 ± 6.4	179.66 ± 8.4	4.4 ± 0.9
Diazepam	2	8	65.2 ± 4.2*	120.1 ± 11.6	50.7 ± 3.4	116.5 ± 8.1	4.9 ± 1.2
Sildenafil	0.5	8	50.4 ± 5.1	70.6 ± 25.7	30.4 ± 2.5	161.2 ± 6.2	5.2 ± 2.6
Sildenafil	1	8	37.5 ± 2.9*	26.4 ± 4.4*	11.4 ± 5.4*	203.5 ± 2.8	4.5 ± 1.2
Sildenafil	3	8	36.8 ± 4.8*	39.8 ± 8.9*	16.6 ± 4.9*	198.8 ± 5.4	2.8 ± 2.2*
MB	1	8	58.0 ± 6.1	74.8 ± 5.1	31.8 ± 2.4	159.7 ± 2.9	4.4 ± 1.6
L-arg	50	8	31.0 ± 5.2*	25.8 ± 3.2*	11.2 ± 2.1*	203.9 ± 4.4	5.1 ± 0.7
Sildenafil + MB	1 + 1	8	50.5 ± 4.8	69.5 ± 12.7#	29.0 ± 3.5#	169.6 ± 6.4	4.7 ± 0.5
Sildenafil + L-arg	1 + 50	8	33.8 ± 6.4$^{\beta}$	25.1 ± 10.3$^{\beta}$	10.5 ± 3.4$^{\beta}$	213.5 ± 8.2	4.1 ± 1.8

The results are expressed as means ± SEM; * $p < 0.05$ significantly different from saline-treated group, # $p < 0.05$ significantly different from sildenafil (1 mg/kg)-treated group, $^{\beta}$ $p < 0.05$ significantly different from saline + saline-treated group

closed arm entries. Entering into an arm was noted only when all paws had crossed out central area.

Locomotor activity

Spontaneous locomotor activity was measured in an activity cage (Ugo Basile, Verase, Italy) having dimensions of 39 × 28 × 26 cm. The data are presented as the number of pulses recorded by the apparatus as the stainless steel bars tilt in response to animal movements. The activity of each mice was automatically recorded for 10 min.

Table 2. Effect of sildenafil, methylene blue (MB), L-arginine (L-arg) on mouse behavior in the locomotor activity test

Treatment	Dose (mg/kg)	n	Locomotor activity counts (10 min)
Saline		8	109.5 ± 10.9
Saline + saline		8	112.9 ± 11.5
Diazepam	2	8	106.9 ± 8.2
Sildenafil	0.5	8	139.6 ± 12.3
Sildenafil	1	8	98.2 ± 10.9
Sildenafil	3	8	52.1 ± 8.9*
MB	1	8	114.3 ± 6.9
L-arg	50	8	96.2 ± 8.7
Sildenafil + MB	1 + 1	8	122.8 ± 10.8
Sildenafil + L-arg	1 + 50	8	56.0 ± 7.9$^{\beta}$

The results are expressed as means ± SEM; * $p < 0.05$ significantly different from saline-treated group, $^{\beta}$ $p < 0.05$ significantly different from saline + saline-treated group

Statistics

All results are expressed as the means ± SEM. Saline + saline, saline + sildenafil 1, MB 1 + saline, L-arg 50 + saline, MB 1 + sildenafil and L-arg + sildenafil groups were analyzed using two–way analysis of variance (ANOVA) followed by the *post-hoc* test. Data from locomotor activity testing were analyzed using one–way ANOVA for treatment as between–subject factor. Statistical analysis was performed using a software package (SPSS 10.1). The level of significance was defined as $p < 0.05$.

RESULTS

Elevated plus-maze

Diazepam at a dose of 2 mg/kg significantly increased the open arm entries and percentage of time spent in open arms ($p < 0.05$). Sildenafil at a dose of 0.5 mg/kg had no significant effect on the behavior in the plus-maze test but at a dose of 1 mg/kg induced an anxiogenic-like effect that was evidenced by significantly decreased entries into open arms and percentage of time spent in open arms. Sildenafil at a dose of 3 mg/kg significantly decreased the percentage of time spent in open arms and open arm entries ($p < 0.05$). Pretreatment with MB (1 mg/kg *ip,* 15 min before sildenafil) abolished the anxiogenic-like effect of sildenafil. Pretreatment with L-arg (50 mg/kg *ip*, 15 min before sildenafil) decreased the open arm entries and percentage of time spent in open arms compared to saline group (Tab. 1).

Locomotor activity

Sildenafil did not change the spontaneous locomotor activity except at the 3 mg/kg dose which significantly decreased this parameter. MB (1 mg/kg, *ip*) did not change the locomotor activity in activity cage. The combination of L-arg (50 mg/kg, *ip*) with sildenafil (1 mg/kg, *ip*) significantly decreased the locomotor activity ($p < 0.05$) (Tab. 2).

DISCUSSION

The effects of sildenafil, L-arg, diazepam and MB on anxiety in the elevated plus-maze test were investigated in the present study. The percentage of the time spent in the open arms was used for the evaluation of anxiety. The present study showed that sildenafil, an inhibitor of cGMP-specific PDE, (1 and 3 mg/kg, *ip*) significantly decreased the percentage of the time spent in open arms. Sildenafil-induced anxiogenic-like effect at 1 mg/kg dose in the plus-maze test was not related to motor depression, since it did not influence the locomotor activity of mice in activity cages. In contrast, sildenafil-induced anxiogenic-like effect at 3 mg/kg dose might be explained by decreased locomotor activity. Diazepam selectively elevated the percentage of time that mice spent in the two open arms of the maze, compared to the percentage of time spent in the two closed arms. L-arg diminished the percentage of time spent in the open arms.

The most important finding of present study is that sildenafil produced anxiogenic-like effect and pretreatment with MB abolished this effect. The data about the effect of sildenafil on anxiety in experimental animal models are very limited. Volke and Vasar have shown that effect of 0.2 mg/kg of sildenafil was anxiogenic-like in light-dark compartment test [18]. Sildenafil is an important inhibitor of cGMP-specific PDE [9] that crosses the blood-brain barrier and exerts various biochemical and physiological effects in the brain. The possible mechanism of sildenafil-produced anxiogenic-like effect can be explained by its influence on NO-cGMP signaling pathway and enhancing intracellular cGMP concentrations. It has been suggested that the NO/cGMP pathway in the hippocampus is responsible for alternation behavior [12]. Talarek and Fidecka suggested that the NO-cGMP pathway was involved in the mechanism of antinociceptive activity of diazepam [15]. Jain et al. have shown the sildenafil activated NO-cGMP pathway [7]. In addition, there are several reports demonstrating an interaction between intracellular cGMP concentration and behavioral effects of sildenafil [1, 6, 13].

L-arg, a NO precursor, activated NO-cGMP pathway and sildenafil also activates this pathway. An increase in intracellular cGMP results in anxiogenic-like effect. Both sildenafil and L-arg produced anxiogenic-like effect in this study. Anxiogenic effects of these drugs may be explained by an increase of intracellular cGMP level. In addition, the combination of sildenafil and L-arg also decreased the percentage the time spent in the open arms.

In the present study, anxiogenic-like effect of sildenafil disappeared with pretreatment with MB. MB is an inhibitor of guanylyl cyclase [3]. It has been known that MB produces behovioral effects due to decreasing intracellular cGMP concentration. The inhibition of sildenafil-induced anxiety by MB seems to be releated to the fact that sildenafil and MB have opposite effects on intracellular cGMP level. Similar findings have been reported previously by Mixcoatl-Zecuatl et al. for sildenafil in hot plate and tail-flick model [13]. On the other hand, both sildanafil and MB may be able to modulate NO-cGMP pathway.

Conclusion

Our results are the first demonstration that sildenafil, administrated at a dose of 1 mg/kg, produced anxiogenic-like behavior in the elevated plus-maze test in mice. The findings suggest that NO-cGMP pathway plays an important role in sildenafil-induced anxiety.

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Received: December 16, 2003; in revised form: March 31, 2004