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Original Article

The effects of sildenafil on the hippocampal long-term potentiation in male rats

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ABSTRACT

Introduction: The hippocampal nitric oxide/cGMP signaling pathway plays a crucial role in memory processing. Phosphodiesterase interacts with this signaling pathway. There are controversial reports regarding the effect of sildenafil, a phosphodiesterase inhibitor, on learning and memory. Therefore, the effects of acute administration (intrahippocampal/intra-dentate gyrus injection) of sildenafil on long-term potentiation (LTP) of rats were investigated.

Methods: The rats were anesthetized with urethane and placed in a stereotaxic device for field potential recording. After ensuring a steady-state baseline response, a single intraperitoneal injection of saline or sildenafil (2 and10 µg/kg) was done. The population spike amplitude, the excitatory postsynaptic potentials (EPSPs) slope and paired-pulse stimuli (as an inhibitory interneuron) were compared between groups.

Results: The results showed that population spike amplitude and EPSP slope significantly increased after sildenafil administration (10µg/kg) following titanic stimulation compared with the saline group. However, the sildenafil (2µg/kg) and control groups showed no difference regarding population spike amplitude and EPSP slope. Sildenafil had no significant effects on recurrent inhibition.

Conclusion: The obtained results indicated that acute administration of sildenafil improved LTP via direct effects on the hippocampus of intact rats. Thus, sildenafil may enhance learning and memory processing by modulating the hippocampal synapse.

Keywords:
Sildenafil
Phosphodiesterase inhibitor
Long term potentiation
Hippocampus

Introduction

Long-term potentiation (LTP) is a form of synaptic plasticity which occurs in the hippocampus and cerebral cortex structures that play important roles in the formation of new memories (Ostrovskaya et al., 2020). One class of signaling that is important for LTP, the activity induced consolidation of synapses, is cyclic nucleotide signaling. This form of signaling is mediated by adenosine and guanosine cyclic monophosphate (cAMP and cGMP, respectively) as second messenger-regulating signal transduction and an expanding area of research focuses on the role of enzymes that degrade these signaling molecules, the phosphodiesterase (PDEs) (Sanderson and Sher, 2013). In recent years, PDEs have gained increased attention as potential new targets for cognition enhancement (Wu et al., 2018). PDEs are enzymes

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that hydrolyze cAMP and cGMP to the corresponding nucleotides AMP and GMP (Bender and Beavo, 2006). This enzyme was detected in the brain, PDE5 mRNA is expressed in several regions associated with cognitive functions such as the hippocampus and cortex in rat and human brain (García-Osta et al., 2012). Behavioral studies suggest that each component of the NO/cGMP/PKG pathway may be involved in learning and memory (Ota et al., 2008).

The chemical structure of sildenafil is very similar to cGMP. Sildenafil binds to PDE5 and competitively prevents cGMP degradation following increased glutamate release. There are paradoxical reports about the effects of sildenafil on memory. So, sildenafil significantly attenuated scopolamine impairment in avoiding mild foot shock and T-maze training (Devan et al., 2004). However, post-training injection of sildenafil did not facilitate retention performance of passive avoidance in young (2-month-old) and middle aged (12-month-old) rats compared to the control group (Shafiei et al., 2006). Furthermore, sildenafil impaired acquisition and consolidation of spatial learning and memory but improved retrieval of spatial memory in radial maze (Shahidi et al., 2014). Moreover, neuroprotective effects of sildenafil against oxidative stress and memory dysfunction exposed to noise stress were also demonstrated in mice (Sikandaner et al., 2017).

The beneficial effects of sildenafil on the some kind of short-term memory, such as passive avoidance memory, previously investigated by the mentioned studies and given that sildenafil crosses the blood–brain barrier (Jackson et al., 2005). Nevertheless, its direct microinjection in hippocampus (dentate gyrus) has not studied yet on long-term memory. So, the present study investigated the effect of this drug on the activity of the dentate gyrus cells in response to LTP in intact rats.

Materials and methods

Animals and groups

Eighteen adult male Wistar rats (8 weeks old, 250±50g) were purchased from the animal house of the Hamadan University of Medical Science. Animals were maintained in a 12h light/dark cycle (light on between 7:00 a.m. and 7:00 p.m.). Animal care, treatment and surgical procedures were approved by the scientific and ethics committees of the Hamadan University of Medical Sciences (IR.Umsha.REC.1394.200) and performed according to the Guide for Care and Use of laboratory animals published by the National Institute of Health, United States (NIH Publication No. 85-23, revised 1985). The rats were divided into three groups as follows: group 1 that received saline; group 2 that received 2μg/kg sildenafil and group 3 that received 10μg/kg sildenafil.

Electrophysiological recordings and LTP induction

Rats anesthetized with urethane (1.5g/kg, IP; Sigma) and the skulls were put in a stereotaxic apparatus. The sildenafil was microinjected into the rat's *brain in the intra-dentate gyrus (intra-DG) route (Mohammadi, 2020)*. The scalp was removed and two holes were made in the skull by drilling it. The recording and stimulating electrodes were positioned in the granular cells of dentate gyrus (AP=−3.8; ML=2.3; DV=3.2 mm from the skull surface) and perforant pathway (AP=−8.1; ML=4.3; DV=3.2 mm from the skull surface), respectively (Shahidi et al., 2018a). Following baseline response recording, High frequency stimulation (HFS) (stimulus intensity: 400KHz, 10 bursts of 20 stimuli, inter-stimulus interval: 40ms) was done in the perforant pathway (Shahidi et al., 2019a). Then, evoked responses were noted at 5, 30, 60 and 120min after the HFS. The excitatory postsynaptic potentials (EPSP) slope, population spike (PS) amplitude and paired stimulation of recurrent interneurons were measured. PS amplitude was measured as the distance between initial peaks of the positive wave and second negative. The EPSP slope was considered as the slope of the rising part of the first positive peak (Shahidi et al., 2018b). The recurrent interneurons of inhibitory hippocampal from second stimulation to PS amplitudes from the first stimulation (Shahidi. et al., 2021). Then saline or sildenafil (2 and 10μg/kg) dissolved in saline was injected 15min before a single excitation and subsequent response from paired stimulation were recorded in three groups (Shahidi et al., 2019b). Representative response traces are given before and after HFS for each group (Figure 1).

Statistical analysis

The data was analyzed by SPSS version 16.0. PS amplitude and EPSP slope were evaluated using two or one-way ANOVA with post-hoc tukey's test and recurrent inhibition of hippocampal by paired t-test. The significance level was set at $P<0.05$. Results are expressed

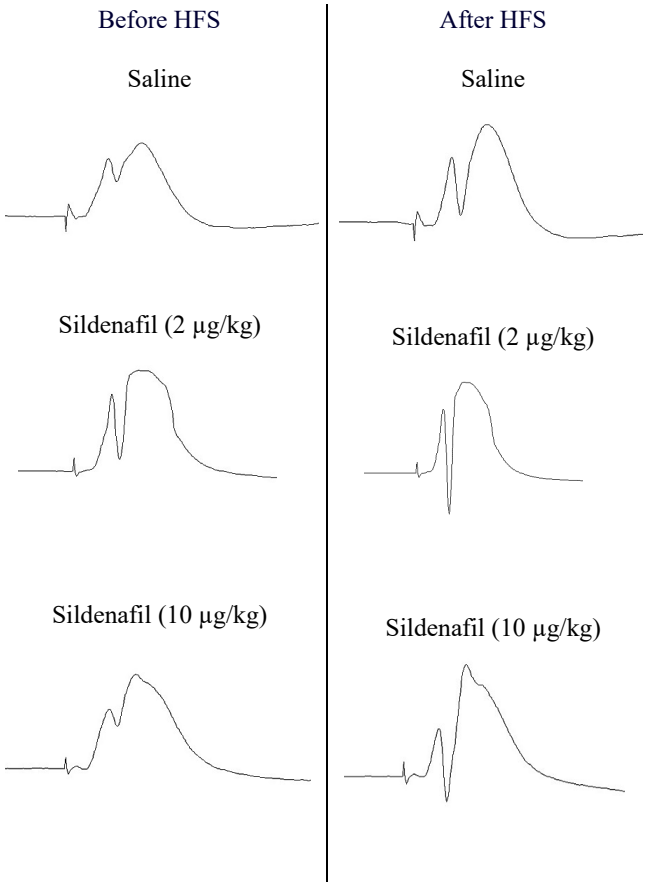

FIGURE 1. Stimulated field potential sample traces in the dentate gyrus region in the sildenafil groups before and after high-frequency stimulation.

as %mean±SEM.

Results

Effect of acute sildenafil administration on PS amplitude of DG neurons

The effect of sildenafil administration on PS amplitude in DG granular cell synapses was presented (Figs. 1 and 2). A two-way ANOVA test revealed significant effects of treatment [(F(4,20)=11.59, P<0.001], time [F(2,10)=1.181, P=0.3463] and interaction between treatment and time point [F(8,40)=2.207; P=0.0.01].

Also, the one ANOVA test indicated significant differences in the PS amplitude among groups at the 5-min [F(2,15)=16.920; P<0.001], 30-min [F(2,15)=10.969; P<0.001], 60-min [F(2,15)=7.678; P<0.001] and 120-min [F(2,15)=10.969; P<0.001] recording times. In addition, the results of tukey's post hoc analysis showed there was a remarkable increase in PS amplitude of 10µg/kg sildenafil treatment than 2µg/kg sildenafil at the 5-min (P<0.001), 30-min (P<0.001), 60-min (P<0.01) and 120-min(P<0.01) recording times. Moreover, it was seen that during all recording periods, the PS amplitude of the administration of 10µg/kg sildenafil was notably higher than the control group at the 5-min (P<0.001), 30-min (P<0.05), 60-min (P<0.01) and 120-min (P<0.01) recording times. Data analysis also revealed that there was no significant difference between the control and 2µg/kg sildenafil groups in the PS amplitude on all recordings.

Effect of acute sildenafil administration on EPSP slope of DG neurons

The effect of sildenafil administration on EPSP slope in DG granular cell synapses was presented (Figures 1 and 3). A two-way ANOVA test revealed significant effects of treatment [F(4,20)=12.88, P<0.001], time [F(2,10)=1.672, P=0.2364] and interaction between treatment and times point [F(8,40)=6.192, P<0.0.001]. In addition, there was a remarkable increase in EPSP slope of sildenafil (10 µg/kg) treatment than control at the 30-min (P<0.05) and 60-min (P<0.05).

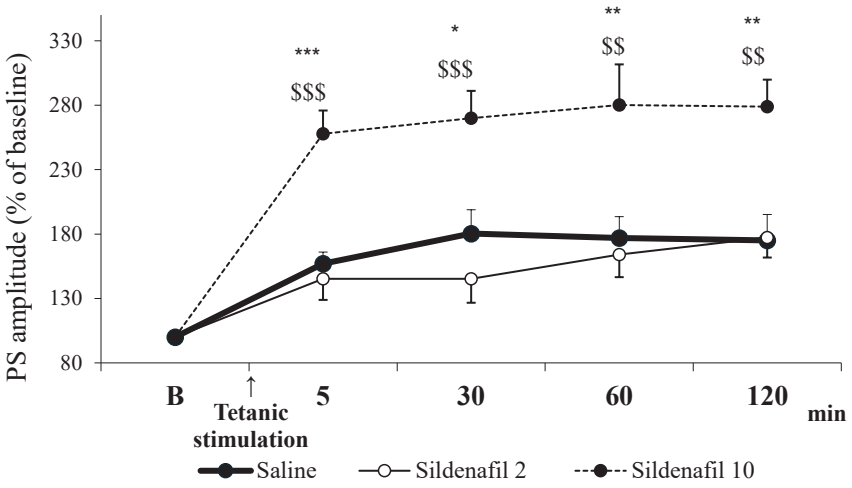

FIGURE 2. Comparison of population spike (PS) amplitude before and after15min treatment between sham, sildenafil (2 and 10 µg/kg) groups in the dentate gyrus at 5, 30, 60 and 120 minutes in response to tetanic stimulation. Data are expressed as %mean±SEM of baseline (n=6). $^{*}P$<0.05, $^{**}P$<0.01 and $^{***}P$<0.001 vs control. $^{\$\$}$P<0.01 and $^{\$\$\$}P$<0.001 vs 2µg/kg sildenafil.

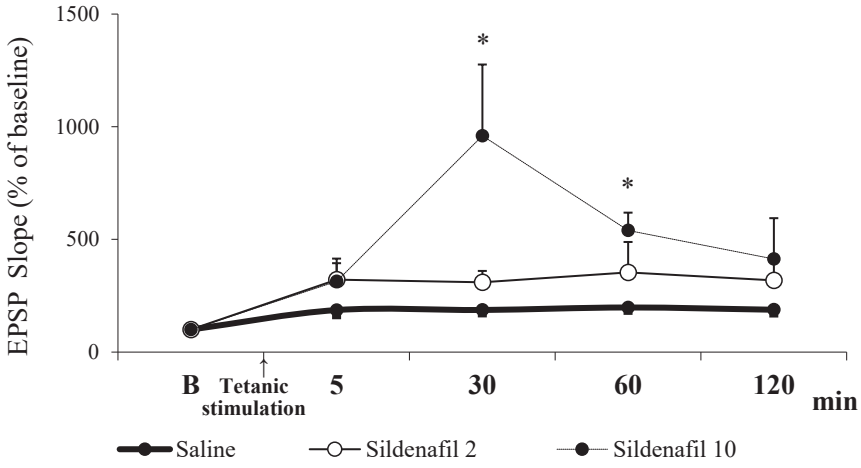

FIGURE 3. Comparison of excitatory postsynaptic potential (EPSP) slope in the dentate gyrus after administration of sildenafil (2, 10 µg/kg) or saline. At 5, 30, 60 and 120 minutes, the recording was done in response to stimulation following an HFS. $^{*}P$<0.05 vs control. Data are expressed %mean±SEM (n= 6).

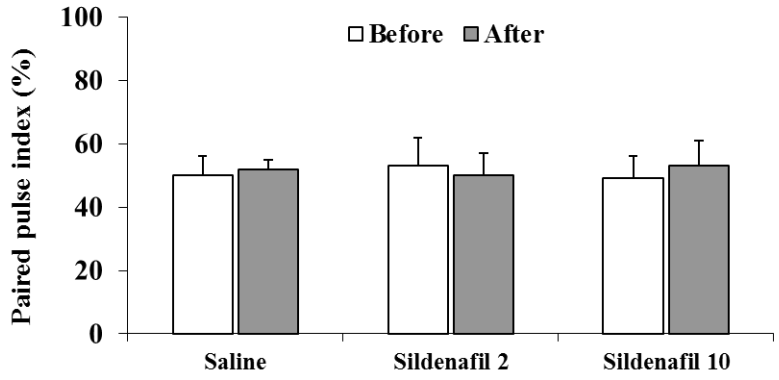

FIGURE 4. Effect of sildenafil on recurrent inhibition of interneurons were obtained from population spike (PS) amplitude divided by the second stimulation to PS amplitudes from the first stimulation. The amount recurrent inhibition in the times before and 15min after injection was no significant difference between groups (n= 6).

Administration of sildenafil on the recurrent inhibition of hippocampal interneurons

Recurrent inhibition of hippocampal interneurons was obtained by dividing the PS amplitude of second stimulation to the PS amplitude of first stimulation. There was no significant difference in the measurement of recurrence inhibition before and 15min after injection (Figure 4).

Discussion

In the present study, the effects of acute administration of sildenafil as one of the inhibitor PDE5 on LTP induction in the dentate gyrus of intact rat were evaluated. The key results showed that (1) central administration of 10μg/kg sildenafil increased long-term potentiation in the dentate gyrus-perforant pathway compared to control group; (2) Lower dose of sildenafil (2μg/kg) had no effect on the EPSP slope compared to the control group; (3) LTP induction in 10μg/kg sildenafil group was higher than 2μg/kg sildenafil group; (4) There was no significant difference before and after injection of treatment between groups in recurrent inhibition. Overall, the results of this study showed that sildenafil affects the parameters associated with long-term synaptic amplification, namely PS amplitude and EPSP slope, and strengthens these parameters as much as possible. This booster effect is dose dependent and at higher doses this boost occurs more and faster.

Also, the results of conjugate stimulation and measurement of recurrent inhibition, which indicates the inhibition of inhibitory interneurons in the dentate gyrus region, showed that none of the doses used sildenafil had an effect on the inhibition of interneurons. This suggests that the dose-enhancing effects of sildenafil doses on inhibitory interneurons in the gyrus region were not toothed but resulted from a direct effect on the dentate-perforant pathway. To date, few behavioral studies have been performed to evaluate the effect of sildenafil on learning and memory. Most of these studies have shown the effects of improving memory or preventing memory disorders for sildenafil (Erceg et al., 2005; Patil et al., 2006) which may be due directly to PDE inhibition and enhancement of the NO-cGMP pathway and better and longer-term reinforcement of synaptic plasticity in the hippocampus (Puzzo et al., 2008).

In the same study, the PDE5 inhibitor tadalafil and a highly selective PDE1 inhibitor IC354 were tested. Tadalafil did not improve either contextual fear conditioning or spatial working memory in mice, and IC354 had no effect on the LTP amplitude in hippocampal slices (Wu et al., 2018). In contrast to this study, our results showed that microinjection of sildenafil not only increased EPSP but also attenuated the PS amplitude in rats and finally improved the LTP. Furthermore, the previous study demonstrated that the selective PDE5 inhibitor, sildenafil, improves object memory in Swiss mice and increases cGMP levels in hippocampal slices. This investigation shown that sildenafil through regulation of cGMP levels could play a role in the memory process (Rutten et al., 2005).

Recently, stimulation of the cAMP pathway has been described as a new role of the NO/cGMP pathway, to induce long-term memory (LTM). This was based on the observation that injection of inhibitors of NOS, cGMP or cAMP into the hemolymph prior to multiple-trial conditioning blocked LTM, whereas injection of an NO donor, cGMP analog, or cAMP analog prior to single-trial conditioning induced LTM. Therefore, it has been suggested that the cAMP pathway is a down-stream target of the NO-cGMP pathway for the formation of LTM, and that the cyclic nucleotide-gated channel and calcium–calmodulin intervene between the NO/cGMP pathway and the cAMP pathway (Ahmadimoghaddam et al., 2021; Delhaye and Bardoni, 2021)

Hippocampal proteins are also affected by NO. Modification of hippocampal synaptic proteins by nitric oxide-stimulated ADP ribosylation and association of impaired cognitive performance in nNOS knockout mice with hippocampal protein derangements were reported (Feil and Kleppisch, 2008). According to the results obtained by previous researchers (Hofmann, 2020), the basal activity of NOS is increased while the level of cGMP is decreased in the aged brain comparing to brain of adult rats. However, in the presence of isobutylmethylxanthine, a non-specific inhibitor of PDEs, the level of cGMP is higher in the aged brain than in the adult one. These results suggest that enhanced cGMP hydrolysis occurred during brain aging. Other studies have provided evidence that the basal activity of NOS is decreased in the senescent brain compared to the mature one (Mollace et al., 1995).

Moreover, the previous study also shown the effects of inhibition of PDE2 and PDE5 on cognitive performance in 3-, 12- and 24-month-old rats and on NOS activity in

the hippocampus in animals brains (Domek-Łopacińska and Strosznajder, 2010). These results indicate that administration of the selective PDE2 inhibitor Bay 60-7550 could improve object memory in 3-, 12- and 24-month-old rats and increase NOS activity in the hippocampus. On the other hand, administration of a selective nNOS inhibitor together with Bay 60-7550 eliminated the memory improvement evoked by the PDE2 inhibitor. Wirtz-Brugger and Giovanni (2000) have shown that cGMP also protects cells from death induced by nerve growth factor withdrawal and amyloid beta. The previous data (Puzzo et al., 2005) presented that amyloid beta peptides inhibit activation of NO/cGMP/cAMP-responsive element–binding protein pathway during hippocampal synaptic plasticity (Zarei et al., 2021). They suggest that enhancement of NO/cGMP signaling provides a novel approach to the treatment of Alzheimer's.

During the damaging of the memory process, constitutive NOS activity is increased, which leads to increase NO production. This pool of NO could interact with the SH group of NMDA receptors leading to lower receptor response and NOS activation. At the same time, the activity of cGMP hydrolyzing PDEs is increased, cGMP levels are decreased and the LTP process is less efficient in the senescent compared with the adult brain, resulting in worse cognitive performance. By using a selective PDE inhibitor, we can restore cGMP levels, which induces a calcium influx through cGMP-gated ion channels, activation of nNOS, and synthesis of NO pool engaged in improvement of cognitive performance.

Conclusion

In summary, The induction of long-term synaptic reinforcement in the hippocampus is currently one of the most accepted theories of learning and memory. The results of the present study indicate that the enhancing effects on memory induced by sildenafil may be directly due to inhibition of phosphodiesterase and longer lasting synaptic plasticity in the hippocampus. This drug can be considered as a contributing factor in memory in rats with a direct impact on the hippocampus.

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Conflict of interest

The authors declare no conflicts of interest.

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