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SILDENAFIL IMPROVES ACQUISITION AND RETENTION OF MEMORY IN MICE

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Abstract : Sildenafil (Viagra) has been introduced recently in market to correct male impotency and has gained immense popularity for its dramatic effects all over the world. The present study was designed to investigate the effect of sildenafil on learning and memory in mice using elevated plus maze. A total of XV groups of animals were employed in the present study. Central cholinergic pathways play a crucial role in learning and memory processes. Physostigmine, an anticholinesterase agent (0.5 mg, 1.0 mg kg^{-1}, i.p) was employed for its memory enhancing property and alprazolam a benzodiazepine receptor agonist served as a memory-impairing agent. In the present study, alprazolam produced anterograde amnesia (at 0.5 mg kg^{-1}, i.p) and retrograde amnesia (at 0.25 mg, 0.5 mg, 0.75 mg kg^{-1}, i.p.) in separate groups of animals. Caffeine at 5 mg, 10 mg and 20 mg kg^{-1}, i.p. (an established psychostimulant) did not show any significant change in learning and memory of mice. Sildenafil (at 8 mg kg^{-1}, i.p.) administered 30 minutes prior to training on first day produced a marginal decrease in transfer latency time on first day; whereas, sildenafil (at 2 mg, 4 mg, 8 mg kg^{-1}, i.p.) administered immediately after training on first day produced a dose–dependent improvement of memory in mice. However, further studies need to be carried out to elucidate the underlying mechanism of sildenafil as a memory enhancer.

Key words: learning memory sildenafil amnesia

INTRODUCTION

Learning refers to the information that living beings acquire about their surroundings. Whereas, storage and retrieval of this information is referred to as Memory (1). Memory comprises of registration (short term memory), consolidation (long term memory) and retrieval (process of recalling). Central cholinergic pathways play a crucial role in learning and memory processes and the degree of cholinergic neurodegeneration correlates positively with severity of memory impairment (2, 3, 4, 5). Benzodiazepines (BZ) have been reported to induce memory impairment in animals as well as in humans (6, 7). Recent studies have also pointed out the involvement of nitric oxide (NO) (8, 9)

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and platelet activating factor (PAF) (8) in memory processes. NO donors like L-Arginine enhanced long-term memory in amnesic rats (10). Stimulation of NO production by L-deprenyl enhanced cognitive functions in Alzheimer's disease (11). On the other hand, inhibition of NO production resulted in decreased cortical acetylcholine (ACh) release leading to memory impairment (12, 13).

Recently, sildenafil has gained immense popularity all over the world for its dramatic effects in correcting erectile dysfunctions. Underlying mechanism of sildenafil has been attributed to its specific inhibition of enzyme phosphodiesterase-5, leading to accumulation of cGMP and eventual release of NO (14, 15). Caffeine, another phosphodiesterase inhibitor and a psycho stimulant has been observed to improve attention and alertness in human beings. Therefore, the present study was designed with an *objective* to investigate the effects of sildenafil on acquisition and retention processes of memory in mice using Elevated Plus-Maze. Physostigmine (an anticholinesterase agent) served as a memory enhancer and alprazolam (a BZ receptor agonist) served as a memory-impairing agent in this study.

METHODS

Swiss Albino mice (18–30) of either sex, exposed to alternate light and dark cycles & having free access to food and water were employed in the present study. All the animals used in the study were naive to the elevated plus-maze. The animals were acclimatized to the laboratory conditions at least 24 hours prior to the test. The experiments were conducted between 10.00 hrs to 17.30 hrs in the research laboratory, wherein access was restricted to research associates only. The experimental protocol was approved by institutional animals ethics committee (IAEC). The care of animals was taken keeping CPCSEA guidelines in mind (Reg. No.–436).

Measurement of transfer latency (TL): Transfer latency of each animal was measured by employing the elevated plus-maze test (16). The plus-maze consisted of two open (16x 5 cm^2) and two closed (16x 5x 12 cm^3) arms, connected by a central platform of 5x 5 cm^2. The apparatus was elevated to a height of 25 cm. above the floor. A fine line was drawn in the middle of the floor of each closed arm. All the animals were given a single trial on the plus-maze. Each mouse was individually placed at the end of an open arm facing away from the central platform of the maze. TL was taken as the time taken by the mouse to move from an open arm to any one of the closed arms with all its four legs crossing the middle line. In case, the animal did not enter the closed arm within 90 seconds, it was gently pushed into the closed arm and a transfer latency of 90 seconds was assigned to it.

After an interval of 24 hours each animal was again subjected to elevated plus-maze test. TL measured on plus-maze on first day served as an index of learning and acquisition, whereas TL on 2nd day served as an index of retrieval and memory. Utmost care was taken not to change the relative location of plus-maze with respect to any object serving as a visual clue in laboratory to the animals.

XV groups of animals were employed in the present study.

Control group: Group – I comprised of animals (n = 15) treated with distilled water or 0.5% w/v carboxymethylcellulose sodium (cmc).

Physostigmine treated groups: (Group II and III) : group II (n=7) and group III (n=7) comprised of mice administered (i. p.) with 0.5 and 1.0 mg kg^{-1} of physostigmine, respectively half an hour before subjecting them to elevated plus-maze training on first day.

Alprazolam treated groups: (Groups IV, V, VI, VII) : Group IV (n=7), Group V (n=7), & Group VI (n=7) were administered 0.25, 0.5 and 0.75 mg kg^{-1} alprazolam respectively, immediately after (for retrograde amnesia) the plus-maze training on first day. Group VII (n=7) animals were injected 0.5 mg/kg i.p alprazolam 30 min. prior (for anterograde amnesia) to elevated plus-maze training on first day.

Sildenafil treated groups: (Groups VIII, IX, X and XI) : Group VIII (n=7), Group IX (n=7) and Group X (n=7) comprised of animals injected i.p. with 2, 4, and 8 mg kg^{-1} of sildenafil respectively, immediately after training them on elevated plus-maze on the first day. Group XI (n=7) animals were administered with 8 mg kg of sildenafil 30 min before subjecting them to elevated plus-maze training on first day.

Caffeine treated groups: (Groups XII, XIII, XIV and XV) : Mice of groups XII (n=7), XIII (n=7) and XIV (n=7) were injected (i.p.) with 5, 10 and 20 mg kg^{-1} caffeine respectively, immediately after plus-maze training on first day. Mice of group XV (n=7) were administered with 20 mg kg^{-1} of caffeine half an hour before elevated plus-maze training on first day.

Drugs and solutions: All the drug solutions were freshly prepared before use. Physostigmine Sulfate (Sigma Aldrich, USA), Sildenafil Citrate (Ranbaxy, India) and Caffeine (Kim labs. India) were dissolved in distilled water. Aprazolam (Ranbaxy, India) was suspended in 0.5% w/v suspension of carboxymethylcelluose sodium.

Statistical analysis: All the results were expressed as mean ± S.E. Data was analyzed using paired student's t-test and one-way analysis of variance (ANOVA) followed by Dunnett test. $P<0.05$ was considered statistically significant.

RESULTS

Effect of physostigmine on transfer latency (TL)

The transfer latency in vehicle treated (control) animals decreased significantly on day two i.e. after 24 hours of training on elevated plus-maze. Physostigmine (0.5 mg kg^{-1} and 1.0 mg kg^{-1}, i.p.) administered 30 min. prior to training on plus-maze demonstrated a dose dependent decrease in TL measured on the first day but the results were not statistically significant. On the other hand, Physostigmine (in both doses) significantly ($P<0.05$) decreased TL measured on the second day as compared to the control group (Fig. 1).

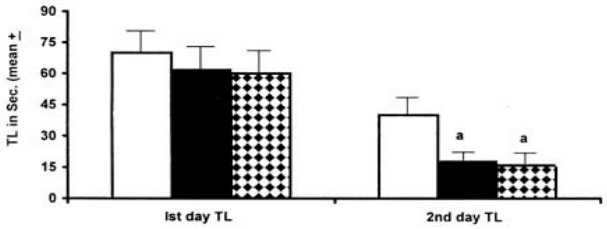

Fig. 1: Effect of Physostigmine on TL of mice using elevated plus-maze.

- Vehicle treated Control group.
- Physostigmine (0.5 mg kg-1 i.p.) injected 30 min. prior to plus-maze training on 1st day.
- Physostigmine (1mg kg-1 i.p.) injected 30 min. prior to plus-maze training on 1st day.

a denotes P<0.05 when compared to control group TL of 2nd day.

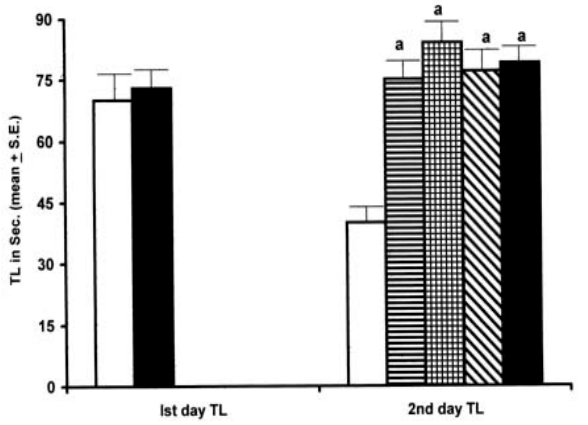

Fig. 2: Effect of Alprazolam TL of mice using elevated plus–maze.

- Vehicle treated Control group.
- Alprazolam (0.5 mg kg-1 i.p.) administered 30 min. prior to elevated plus-maze training on 1st day.
- Alprazolam (0.25 mg kg-1 i.p.) administered immediately after elevated plus-maze training on 1st day.
- Alprazolam (0.5 mg kg-1 i.p.) administered immediately after elevated plus-maze training on 1st day.
- Alprazolam (0.75 mg kg-1 i.p.) administered immediately after elevated plus-maze training on 1st day.

a denotes P<0.05 when compared to control group TL of 2nd day.

Effect of alprazolam on TL

Alprazolam administered either 30 min. prior to or immediately after training on the plus-maze produced a significant increase ($P<0.05$) in TL in mice measured on the second day at all the three dose (0.25 mg kg^{-1}, i.p. 0.5 mg kg^{-1}, i.p., 0.75 mg kg^{-1}, i.p.) levels. Moreover alprazolam 0.5 mg kg^{-1}, i.p. administered 30 min. prior to plus-maze training produced a marginal increase ($P>0.05$) in TL measured on first day, but results were not significant statistically (Fig. 2).

Effect of sildenafil on TL

Sildenafil (8 mg kg, i.p.) administered 30 min. prior to plus maze training demonstrated decrease in TL measured on first day but results were not statistically significant. On the other hand sildenafil injected either 30 min. before or immediately after (2 mg kg^{-1}, i.p., 4 mg kg^{-1}, i.p., 8 mg kg^{-1}, i.p.,) plus-maze training produced a significant decrease ($P<0.05$) in TL measured on 2nd day (Fig. 3).

Effect of caffeine on TL

Caffeine (20 mg kg^{-1}) administered 30 min. prior to plus-maze training on first day slightly decreased the TL measured on first day but the results were not statistically significant. Caffeine (5, 10 & 20 mg kg^{-1} i.p.) administered immediately after plus-maze training on first day marginally decreased

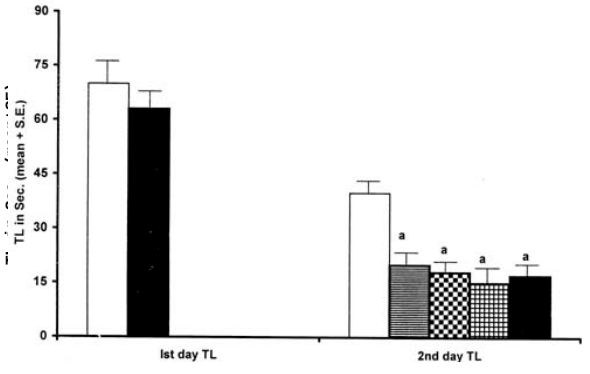

Fig. 3: Effect of sildenafil on TL of mice using elevated plus–maze.

- Vehicle treated Control group.
- Sildenafil treated group (8 mg kg-1 i.p.) administered 30 min. prior to training on plus-maze on 1st day.
- Sildenafil treated group (2 mg kg-1 i.p.) administered immediately after training on plus-maze on 1st day.
- Sildenafil treated group (4 mg kg-1 i.p.) administered immediately after training on plus-maze on 1st day.
- Sildenafil treated group (8 mg kg-1 i.p.) administered immediately after training on plus-maze on 1st day.

a denotes P<0.05 when compared to control

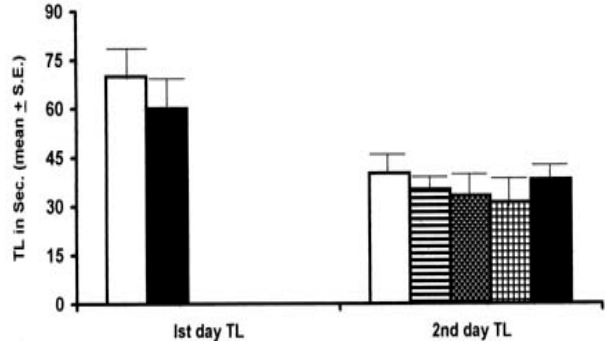

Fig. 4: Effect of caffeine on TL of mice using elevated plus-maze.

- Vehicle treated Control group.
- Caffeine (20 mg kg-1 i.p.) injected 30 min. prior to training on plus-maze on 1st day.
- Caffeine (5 mg kg-1 i.p.) injected immediately after training on 1st day.
- Caffeine (10 mg kg-1 i.p.) injected immediately after training on plus-maze on 1st day.
- Caffeine (20 mg kg-1 i.p.) injected immediately after training on plus-maze on 1st day.

(P>0.05) the TL in a dose dependent manner but the results were statistically non significant (Fig. 4).

DISCUSSION

A significant decrease in the Transfer Latency (TL) time of mice noted on second day as compared to their TL on first day in control group indicates normal memory retrieval in elevated plus-maze test (16). Physostigmine marginally reduced first day TL but significantly reduced 2nd day TL in mice as compared to control group, thereby indicating improved acquisition and retention. Physostigmine, a cholinesterase inhibitor is reported to facilitate learning and memory in animals as well as in humans (3, 4) by increasing ACh concentration in brain, which is in line with our findings.

Amnesia is inability to remember past experiences or loss of memory. Anterograde amnesia is impairment of memory for events occurring after trauma/drug treatment. In this case, new memories are not formed easily. Whereas, retrograde amnesia is impairment of memory for events occurring before trauma/drug treatment. In the present study alprazolam, when administered before training showed anterograde amnesia as indicated by significant increase in 2nd day TL. Alprazolam, when administered immediately after the training produced significant increase in 2nd day TL, reflecting retrograde amnesia as well. These findings are in conformity with earlier reports where in, benzodiazepines have been shown to produce anterograde amnesia in both, humans (6) as well as in animals (7). Alprazolam induced anterograde amnesia appears to be mediated through benzodiazepine (BZ)

receptors, since flumazenil, a selective BZ receptor antagonist attenuated alprazolam-induced anterograde amnesia (17). However, alprazolam induced retrograde amnesia may probably be mediated through its PAF receptor antagonistic property. This suggestion is in agreement with the studies showing production of retrograde amnesia by a specific PAF receptor antagonist BN 50730 (17, 18,19).

Caffeine, administered either immediately after or 30 min. prior to training slightly decreased the TL. This non-significant decrease in TL by caffeine may be attributed to its psychostimulant effect. Caffeine is reported to be a non-selective antagonist of adenosine A_1 and A_2 receptors unlike sildenafil (20, 21).

In the present study, sildenafil administered either immediately after or 30 min. prior to training, produced a significant decrease in TL measured on 2nd day and marginal decrease in TL measured on first day, thereby signaling memory improvement. Sildenafil improved penile erection by specifically inhibiting the enzyme phosphodiesterase-5, accumulating cGMP and consequently releasing NO (14, 15). Recent studies have implicated a vital role for nitric oxide in neurophysiological processes of learning and memory (8,9). Inhibition of NO synthesis impaired memory in rats (12, 13); whereas, stimulation of NO production improved cognitive functions in Alzheimer's patients (11). NO donors like molsidomine reversed scopolamine induced amnesia in rats (22). Therefore the underlying mechanism of sildenafil appears to be related to increased NO production in brain leading to cerebral vasodilatation resulting in enhanced acquisition and retention of memory. Alternatively, the enhanced NO production leading to facilitated cortical cholinergic transmission (12, 13) may possibly be responsible for improvement in memory.

Conclusion: Sildenafil, renowned for its useful effects in male impotency was found to improve learning abilities and memory capacities of mice in the present study. However, further studies need to be carried out to elucidate the underlying mechanism of sildenafil as a memory-enhancing agent.

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REFERENCES

1. Izquierdo I, Medina JH. Neuro-transmitter mechanisms in memory consolidation. *Drugs of Today* 1992; 28: 421–429.
2. Fibiger HC. Cholinergic mechanisms in learning memory and dementia: a review of recent evidence. *Trends Neurosci* 1991; 14: 220–221.
3. Smith G. Animal models for Alzheimer's disease: experimental cholinergic denervation. *Brain Res Rev* 1988; 13: 103–118.
4. Defeudis FV. Central cholinergic systems, cholinergic drugs and Alzheimer's disease an overview. *Drugs of Today* 1988; 24: 471–490.

5. Fisher A, Brandeis R, Karton I, et al. (±) cis-2-methyl-spiro (1, 3-oxathiolane-5'-3) quinoclidine, an M_1 selective chlinergic agonist, attenuates cognitive dysfunction in an animal model of Alzheimer's disease. *J Pharmacol Exp Ther* 1991; 257: 392–403.

6. Lister RG. The amnesic action of benzodiazepines in man. *Neurosci Biobehav Rev* 1985; 9: 87–94.

7. Thiebot M. Some evidence for amnesic–like effects of benzodiazepines in animals. *Neurosci Biobehav Rev* 1985; 9: 95–100.

8. Bannerman DM, Chapman PF, Kelly PAT, Butcher SP, Morris RGM. Inhibition of Nitric Oxide synthase does not impair spatial learning. *J Neurosci* 1994; 14: 7404–7414.

9. Bannerman DM, Chapman PF, Kelly PAT, Butcher SP, Morris RGM. Inhibition of Nitric Oxide synthase does not prevent the induction of longterm potentiation *In Vivo J Neurosci* 1994; 14: 7415–7425.

10. Pandhi P, Balakrishanan S. Cognitive dysfunction induced by phenytoin and valproate in rats: effect of nitric oxide. *Indian J Physiol Pharmacol* 1999; 43(3): 378–382.

11. Thomas T. Monoamine oxidase-B-inhibitors in the treatment of Alzheimer's disease. *Nuerobiol Aging* 2000; 21(2): 343–348.

12. Kopt SR, Benton RS, Kalfin R, Giovannini MG, Pepeu G. Nitric oxide synthesis inhibition decreases cortical acetylcholine release and impairs retention of a conditioned response. *Brain Res* 2001; 894(1): 141–144.

13. Reddy PL, Rajasekaran K, Paul V. Evidence for an involvement of nitric oxide in memory of shock avoidance task in rats. *Indian J Physiol Pharmacol* 2002; 46(1): 119–122.

14. Boolell M, Gepi-Attee S, Gingell JC, Allen MJ. Sildenafil a novel oral effective therapy for male erectile dysfunction. *Br J Urol* 1996; 78: 157–261.

15. Goldstein I, Lue TF, Padmanathan H, Rosen RC, Steers WD, Wicker PA. Oral sildenafil in the treatment of erectile dysfunction. *N Engl J Med* 1998; 338: 1397–1404.

16. Itoh J, Nabeshima T, Kameyama T. Utility of elevated plus–maze for dissociation of amnesic and behavioral effects of drugs in mice. *Eur J Pharmacol* 1990; 194: 71–76.

17. Singh N, Sharma A, Singh M. Possible mechanism of alprazolam induced amnesia in mice. *Pharmacology* 1998; 56: 46–50.

18. Stenzel CJ. Inhibition of PAF-induced effects by brotizolam and related compounds. *Nauyn Shmiedeberg's Arch Pharmacol* 1986; 332(Suppl): R71.

19. Singh N, Sharma A, Singh M. Effect of BN-50730 (PAF Receptor-Antagonist) and Physostigmine (AChE inhibitor) on learning and memory in mice. *Methods Findings Clin Pharmacol* 1997; 19(9): 585–588.

20. Silva RH, Frussa-Filho R. The plus-maze discriminative avoidance task: a new model to study memory-anxiety. *J Neurosci Methods* 2000; 102(2): 117–125.

21. Kopf SR, Melani A, Pedata F, Pepeu G. Adenosine and memory storage: effect of A(1) and (2) receptor antagonists. *Psychopharmacology* 1999; 146(2): 214–219.

22. Pitsikas N, Rigamonti AE, Cella SG, Locatelli V, Sala, M, Muller EE. Effects of molsidomine on scopolamine-induced amnesia and hyper motility in the rat. *Eur J Phramacol* 2001; 426(3): 193–200.