Neuroscience 160 (2009) 149–155

BACOPA MONNIERA ALLEVIATES N_ω-NITRO-L-ARGININE-INDUCED BUT NOT MK-801-INDUCED AMNESIA: A MOUSE MORRIS WATER MAZE STUDY

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Abstract—*N*-methyl-D-aspartate (NMDA) receptor and nitric oxide syntheses are the emerging target sites for development of novel drug molecules because their modulation affects the long term potentiation (LTP) process. NMDA receptor antagonists and nitric oxide synthase inhibitors induce amnesia in animals and therefore have been employed for evaluation of efficacy of several novel antiamnesic agents. *Bacopa monniera* Linn (syn. Brahmi) is commonly used in the ancient Indian medical system for improvement of memory deficit. We have earlier described the involvement of GABAergic and cholinergic system to account for the antiamnesic effects of *B. monniera* on diazepam- and scopolamine-induced amnesia. In extension to our previous study this study was designed to investigate the downstream mechanism of *B. monniera* by evaluation of its effect on MK-801 (an NMDA receptor antagonist) and N_ω-nitro-L-arginine (L-NNA) (a nitric oxide inhibitor) induced memory deficit. We used a Morris water maze scale and compared the degree of reversal of amnesia induced by the two agents. Male Swiss albino mice were subjected to a Rotarod muscle incoordination test followed by water maze tasks. Our data revealed that L-NNA and MK-801 produced anterograde and retrograde amnesia and *B. monniera* significantly attenuated the L-NNA-induced anterograde amnesia, partially reversing L-NNA-induced retrograde amnesia. On the other hand, *B. monniera* neither attenuated the MK-801-induced anterograde amnesia nor improved retrograde amnesia caused by it.

Key words: amnesia, *Bacopa monniera*, L-NNA, MK-801, Morris water maze.

The interference in long term potentiation (LTP) (Bliss and Lomo, 1973; Kang and Lee, 2001) is documented to affect the synaptic plasticity. The blockade of postsynaptic receptors such as AMPA receptors, *N*-methyl-D-aspartate (NMDA) receptors and metabotropic receptors thus impairs the LTP and synaptic plasticity. Both competitive (i.e. AP5, NPC and 12,626) and non-competitive NMDA receptor antagonists (i.e. phencyclidine, ketamine and MK-801) block LTP induction (Bliss and Collingridge, 1993; Gruart et al., 2006; Harney et al., 2006) and produce amnesia (Bannerman et al., 1995; Han et al., 2000). MK-801, a prominent NMDA receptor blocker, is reported to impair both acquisition and retrieval in different paradigms of learning and memory (Filliat and Blanchet, 1995; Kant et al., 1996). It has, therefore, been used as an experimental drug to induce amnesia in animals for evaluation of various antiamnesic agents such as cannabinoid-rich extracts (Fadda et al., 2006), SB-399885 (Perez-Garcia and Meneses, 2005) and thioperamide (Bernaerts et al., 2004). Similarly, 7-nitro-imidazole (a nitric oxide synthase (NOS) inhibitor) has been reported to block hippocampal LTP and induce impairment of learning and memory in rats (Holscher, 1994; Sinz et al., 1999; Tong and Hamel, 1999).

In the absence of tangible results with single molecule approach, extracts comprising several molecules are now being tested for antiamnesic effects. Galantamine (a cholinesterase inhibitor), isolated from *Galanthus nivalis* and *Lycoris radiate* (Howes and Houghton, 2003; Howes et al., 2003) recently became licensed in the United Kingdom for treatment of mild to moderate Alzheimer's disease (Clegg et al., 2002; Tully et al., 2003). *Bacopa monniera* Linn (syn. Brahmi) is another such herbal drug which has been used for several centuries for treatment of neurological diseases in the Indian Ayurvedic system of medicine. *B. monniera* extract was later studied and found to reduce the level of amyloid, especially Abeta 1–40 and 1–42 in doubly transgenic mouse model of rapid amyloid deposition and brought favorable Y-maze performance and open field hyperlocomotion behavioral changes (Holcomb et al., 2006). The animal studies show that the *B. monniera* standard extract improves the cognition deficit as evaluated by various animal models. It has been shown to improve acquisition and retention in a brightness discrimination reaction task (Singh, 1997) and reverses amnesic effects of neurotoxin, scopolamine, electric-shock and immobilization stress (Bhattacharya et al., 2001) and attenuates motor learning deficit (Prakash and Sirsi, 1962). Like animal studies, the clinical studies also provide equally robust evidence of *B. monniera's* action on cognitive function (Stough et al., 2001). In a clinical study Sharma et al. reported *B. monniera's* effect on revitalizing the intellectual functions of children (Sharma, 1987). We have earlier reported that *B. monniera* attenuates both scopolamine-induced (Saraf et al., 2007) and diazepam-induced memory deficit (Prabhakar et al., 2008) which suggests the involvement of cholinergic and GABAergic system in modulation of learning and memory (Saraf et al., 2008). Since NMDA receptor and NOS are the important components of LTP, this study was designed to test the downstream effects of *B. monniera* on MK-801 (NMDA antagonist) and N_ω-nitro-L-arginine (L-NNA; NOS inhibitor)-induced amnesia.

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Abbreviations: ELT, escape latency time; L-NAME, N^G-nitro-L-arginine methyl ester; L-NNA, N_ω-nitro-L-arginine; LTP, long term potentiation; NMDA, *N*-methyl-D-aspartate; NOS, nitric oxide synthase; Q (with number), quadrant.

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doi:10.1016/j.neuroscience.2009.02.005

EXPERIMENTAL PROCEDURES

Male Swiss albino mice were used in the present study. They were housed in an animal house with free access to water and standard diet, and exposed to a 12-h light/dark cycle. The experiments were conducted between 09:00 and 18:00 h in a semi-soundproof laboratory. L-NNA and MK-801 were dissolved in normal saline. Standardized extract of *Bacopa monniera*, containing 55.35% bacosides, was suspended in Tween 80 (5% v/v in normal saline).

Behavioral evaluation

Morris water maze was employed to evaluate learning and memory (Morris, 1984). This model has several advantages over other models including the radial arm maze. These include (1) absence of motivational stimuli such as food and water deprivation, electrical stimulations and buzzer sounds which are required in active/passive avoidance tasks and may interfere the normal process of memory; these are not required in this setup; (2) animals can be trained in a shorter time (1 week) while the arm maze studies require several weeks of training; (3) intra-maze cues like odor trains are eliminated in the pool; (4) larger dose response studies can be conducted in a week's time; and (5) from a theoretical point of view, the water maze is an aversively motivated task, while the arm maze is appetitive motivated task. This test allows studies of spatial working memory and reference memory (Vogel, 2002). Olton and coworkers had suggested the role of the hippocampus in spatial memory using these behavioral tests (Olton and Samuelson, 1976; Olton et al., 1979). It has, therefore, been extensively used for evaluation of spatial memory deficit (Bermudez-Rattoni et al., 1991; McNamara and Skelton, 1991, 1992; Anand et al., 2007) induced by benzodiazepine (McNamara and Skelton, 1992; Shimizu et al., 1998; Saraf et al., 2003; Anand et al., 2007). This model suitably combines evaluation of both spatial memory along with locomotor activity. Therefore, it can be used for evaluation of drugs which not only affect spatial memory but also impair locomotor activity.

It consists of a circular water tank filled with water that is made opaque with a white-colored dye. The tank was divided into four equal quadrants with the help of two threads, running at right angle to each other over the rim of the pool. A tiny platform (10 cm^2) was placed 1 cm below the level of water in the center of one of these four quadrants designated as target quadrant. The position of platform was kept unaltered throughout all the training sessions. In the present study, quadrant 2 (Q2) was considered as the target quadrant. Each animal was subjected to four consecutive trials on each day with a gap of 5 min, during which they were allowed to escape on to the hidden platform. In case the animal was unable to locate the hidden platform within 120 s period, it was gently guided to the platform and allowed to remain there for 20 s. Escape latency time (ELT), as a quantitative measure to locate the hidden platform in water maze, was noted as the index of acquisition. Each animal was subjected to the four acquisition trials per day for six consecutive days. On seventh day, platform was removed and time spent by the animal in each of four quadrants (Q1, Q2, Q3, Q4) was noted. The time spent by the animal in target quadrant searching for the hidden platform was noted as an index of retrieval.

In order to study the effect of drug on acquisition, drug solution was administered before acquisition trials for six consecutive days and the diluent was administered before retrieval trial on day 7. Drug solution was administered before retrieval trial on day 7 to study its effect on retrieval of memory. The experimental mice were screened with a Rota-rod to test the muscle coordination activity of mice before conducting the maze experiments (Ghelardini et al., 2002). Mice, showing abnormal swimming pattern in water maze, and low muscle coordination activity in Rota-rod test were excluded from study. Each group comprised seven mice.

Mice in group I were administered normal saline (10 ml kg^{-1}) intraperitoneally 30 min before acquisition (day 1–6) and retrieval trial (day 7). Five percent Tween 80 was used as a diluent for preparation of *B. monniera* suspension. Group II mice were administered 5% Tween 80 (10 ml kg^{-1}) orally 60 min before acquisition and retrieval trial to record the per se effect of 10% Tween 80 on normal acquisition and retrieval of memory. Group III mice were treated with L-NNA (30 mg kg^{-1} i.p.) 30 min before acquisition trials and normal saline (10 ml kg^{-1} i.p.) 30 min before retrieval trial. Group IV mice were administered normal saline (10 ml kg^{-1} i.p.) 30 min before acquisition trials and L-NNA (30 mg kg^{-1} i.p.) 30 min before retrieval trial in order to evaluate the effect of L-NNA on retrieval process. Group V mice were administered standardized extract of *B. monniera* (80 mg kg^{-1} oral) 60 min before and L-NNA (30 mg kg^{-1} i.p.) 30 min before acquisition trials. group VI mice were treated with 5% Tween 80 (10 ml/kg i.p.) 60 min before and normal saline (10 ml/kg i.p.) 30 min before acquisition trials, and standardized extract of *B. monniera* (80 mg kg^{-1} oral) and L-NNA (30 mg kg^{-1} i.p.) were administered 60 and 30 min before retrieval trial respectively. group VII, VIII and IX mice were treated with varying doses of MK-801, i.e. 0.25, 0.17 and 0.1 mg/kg^{-1} i.p. 30 min before acquisition trials and normal saline (10 ml kg^{-1} i.p.) 30 min before retrieval trial. Group X mice were administered normal saline (10 ml kg^{-1} i.p.) 30 min before acquisition trials and MK-801 (0.25 mg kg^{-1} i.p.) 30 min before retrieval trial in order to evaluate its effect on retrieval process. Group XI mice were administered standardized extract of *B. monniera* (120 mg kg^{-1} oral) 60 min before and MK-801 (0.17 mg kg^{-1} i.p.) 30 min before acquisition trials. Group XII mice were treated with 5% Tween 80 (10 ml/kg i.p.) 60 min before and normal saline (10 ml/kg i.p.) 30 min before acquisition trials, and standardized extracts of *B. monniera* (120 mg kg^{-1} oral) and MK-801 (0.25 mg kg^{-1} i.p.) were administered 60 and 30 min before retrieval trial respectively. We also studied per se the effect of *B. monniera* on acquisition and retrieval of memory in group XIII and XIV mice with *B. monniera* suspension 80 and 120 mg kg^{-1} orally respectively 60 min before and normal saline (10 ml kg^{-1} i.p.) 30 min before trials.

Statistical analysis

We have analyzed the behavioral results of the Morris water maze using SPSS statistical software and applied one-way ANOVA followed by Tukey's post hoc analysis at $P<0.05$ for multiple comparisons among the groups. During retrieval trials, each value represents mean±SEM.

RESULTS

B. monniera reverses L-NNA induced amnesia

L-NNA (30 mg kg^{-1} i.p.) did not lead to a progressive decrease in ELT during successive acquisition trials ($F=0.46$ and P-values were 0.96, 0.88, 0.76, 0.86. 0.8 on day 2 to day 6 respectively) as compared to day 1 ELT. ELT of the L-NNA-treated group was significantly different from the ELT of the control group on day 3 ($F=3.0$), day 4 ($F=4.5$) and day 5 ($F=6.9$) at $P<0.05$ (Fig. 1a). L-NNA (30 mg kg^{-1} i.p.)-treated mice did not spend more time in the target quadrant ($F=1.81$ and P-values were 0.99, 0.5, 0.25 versus Q1, Q3 and Q4 respectively). L-NNA reduced the time an animal spent in the target quadrant ($F=2.74$ at $P<0.05$) as compared to control (Fig. 1b). These observations suggest that L-NNA impairs the process of acquisition of new memory by producing anterograde amnesia and it also produces retrograde amnesia by impairing retrieval process of the trained mice. *B. monniera* (80 mg kg^{-1} oral) administration progressively decreased ELT in L-NNA (30 mg kg^{-1} i.p.)-treated mice with ongoing acquisition trials