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Short communication

The effects of pre-treatment with vitamin B_6 on memory retrieval in rats

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ABSTRACT

The effects of pre-treatment with vitamin B_6 on memory retrieval in adult male Wistar rats were evaluated using a step-through passive avoidance task. The rats were divided into three groups of 10 each. All animals were fed standard rodent chow. Vitamin B_6 (50 or 100 mg/kg) was administered intraperitoneally (i.p.) every other day for 1 month before training was initiated. Three retention tests were performed to assess the memory of the rats. Vitamin B_6 (100 mg/kg) significantly increased the step-through latency of the passive avoidance response compared with the control in the first retention test of the passive avoidance paradigm ($p < 0.05$). In addition, vitamin B_6 at 100 mg/kg significantly increased memory retrieval in the second and third retention tests conducted 2 days and 1 week after training, respectively, compared with the control ($p < 0.05$). These results indicate that pre-treatment with vitamin B_6 potentially enhances memory retrieval.

1. Introduction

The prevalence of Alzheimer disease (AD) has increased progressively over the past 100 years, and AD is currently the most common form of dementia (Cornutiu, 2011). Higher circulating levels of total homocysteine (tHcy) have been detected in patients with neurodegenerative diseases. tHcy concentrations have been observed to be higher in patients with dementia associated with AD (Clarke et al., 1998; Duthie et al., 2002). The risk of AD has been significantly associated with high plasma levels of Hcy and low levels of folic acid and vitamin B_6 (Miller, 2003). The simultaneous supplementation of folate and vitamin B_{12} in rats attenuated Hcy-induced overproduction of β-amyloid and memory deficits (Zhang et al., 2009). Furthermore, supplementation with B vitamins, including vitamin B_6, has been shown to reduce blood Hcy levels (Malouf & Grimley Evans, 2003).

Low concentrations of vitamin B_6, vitamin B_{12}, and folate were significantly associated with a decline in spatial copying measures during a 5-year follow-up in a general population of ageing men (Tucker, Qiao, Scott, Rosenberg, & Spiro, 2005). Furthermore, it was reported that AD patients consumed significantly less dietary vitamin B_6 and folate after the age of 60 years than did the controls (Mizrahi et al., 2003).

A vitamin B-deficient diet induced hyperhomocysteinaemia in an inbred mouse strain, and this short-term dietary challenge significantly impaired cognition and caused hippocampal microvasculature deficits (Troen et al., 2008).

An increase in the intake of vitamins B_6 and B_{12} in a community-dwelling, non-pathological ageing population was associated with an increase in grey matter volume (Erickson et al., 2008). The association between vitamin consumption and cognitive function is of scientific interest. However, studies on the role of vitamin B_6 in humans are controversial owing to the use of different methods to examine cognition performance; this difference confounds and limits the scope of studying vitamin B_6 effects in humans. Thus, this study was conducted to determine the effects of pre-treatment with vitamin B_6 on cognitive performance in normal rats.

2. Materials and methods

2.1. Animals

Male Wistar rats (200–250 g) obtained from the Razi Institute (Karaj, Iran) were housed in groups of four animals per cage under standard laboratory conditions. The rats were kept at a constant room temperature (21 ± 2 °C) under a normal 12:12 h (light:dark) cycle with free access to food and water. All animal experiments were performed in accordance with the European Communities Council Directive of 24th November 1986 (86/609/EEC) to minimise the number of animals used and their suffering.

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2.2. Drugs

Vitamin B_6 was purchased in an injectable form from Tamin Pharmaceutical Co. (Tehran, Iran). The control animals were administered saline.

2.3. Experimental procedure

Animals were fed standard rodent chow (67.5% carbohydrate, 11.7% fat, 20.8% protein, and 1% supplement (0.15% vitamin B_6); Khorak-Dame Pars, Tehran, Iran), and were divided into three groups of 10 each. The first group received saline. In the remaining two groups, 50 or 100 mg/kg of vitamin B_6 were administered. All animals received the drugs intraperitoneally (i.p.) every other day for 1 month prior to starting the acquisition trials.

2.4. Passive avoidance

A learning box consisting of two compartments, one light (white compartment; 20 × 20 × 30 cm) and one dark (black compartment; 20 × 20 × 30 cm), was used. A guillotine door opening (6 × 6 cm) was constructed on the floor of the box in the centre of the partition between the two compartments. Stainless steel grids (5 mm in diameter) were placed at 1-cm intervals on the floor of the dark compartment to produce the foot shock.

All animals were allowed to habituate in the experimental room prior to the experiments. The acquisition trial was performed 30 min after the habituation trial. In each trial, an animal was placed in the light compartment and, after 5 s, the guillotine door was opened. Once the animal crossed into the dark compartment, the door was closed and a foot shock (5 s, 0.2 mA intensity) was immediately delivered to the grid floor of the dark room by an insulated stimulator. Two minutes later, the procedure was repeated. The rat received a foot shock each time it re-entered the dark compartment and placed all four paws into it. The training was terminated when the rat remained in the light compartment for 120 consecutive seconds. The number of trials (entries into the dark chamber) was recorded. All animals were trained with a maximum of three trials (Darbandi, Rezayof, & Zarrindast, 2008; Nassiri-Asl et al., 2010; Nassiri-Asl, Zamansoltani, Javadi, & Ganjvar, 2010).

The retention tests were performed 1, 2, and 7 days after training to evaluate memory. During these sessions, no electric shock was applied. The test session ended when the animal entered the dark compartment or remained in the light compartment for 300 s.

2.5. Data analysis

The data were analysed using a one-way analysis of variance followed by Dunnett's post-hoc test. A p-level <0.05 was considered statistically significant.

3. Results

There was no significant difference between the groups in terms of the number of trials, confirming the uniformity of the groups. All animals met the criteria during the training procedure.

The administration of a pre-treatment with vitamin B_6 at 100 mg/kg every other day for 1 month prior to training induced a significant increase in the memory retrieval of the rats compared with the control group in the first retention test (1 day after training) [$F_{(2,24)} = 3.37$, $p < 0.05$] (Fig. 1). Vitamin B_6 at 50 mg/kg also increased memory retrieval compared with the control group; however, this effect was not significant. Vitamin B_6 at 100 mg/kg significantly increased memory retrieval in the second and third retention tests of the passive avoidance paradigm, compared with the control (2 days and 1 week after training, respectively) [$F_{(2,26)} = 3.45$, $p < 0.05$ and $F_{(2,27)} = 3.96$, $p < 0.05$, respectively] (Fig. 1).

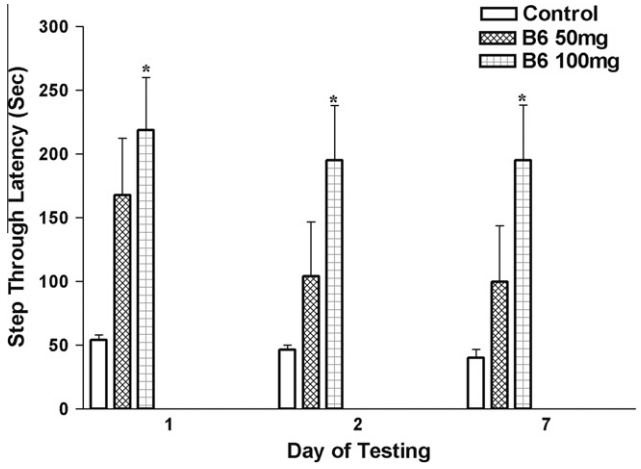

Fig. 1. The effects of pre-treatment with vitamin B_6 (50 or 100 mg/kg) on the step-through latencies in rats. Values are expressed as means ± SEM. Rats (10 per group) were i.p. injected with drugs every other day for 1 month prior to the experiments. The retention tests were performed 1, 2 and 7 days after training, $^*p < 0.05$, compared to the same day control.

4. Discussion

The present study showed that pre-administration of vitamin B_6 every other day prior to training enhanced memory retrieval in rats. Vitamin B_6 (100 mg/kg) significantly increased memory retrieval in the first, second, and third retention tests of a passive avoidance task, compared with the control group. The memory-enhancing effect of vitamin B_6 was found to be dose-dependent.

Similar to our results, the administration of nutraceutical supplements containing phosphatidylserine, *Ginkgo biloba*, vitamin E, and vitamin B_6 improved short-term memory performance in aged dogs (Araujo, Landsberg, Milgram, & Miolo, 2008). Furthermore, in human studies, supplementation of vitamin B_6 with vitamin B_{12} and folate had a significant positive effect on cognitive performance (Bryan, Calvaresi, & Hughes, 2002).

On the other hand, the results from randomised control trials with a high dose of vitamin B, including vitamin B_6, supplements did not support the treatment of individuals with mild to moderate AD (Aisen et al., 2008). In addition, it was shown that 6 months supplementation of physiological dosages of antioxidants and B vitamins, including vitamin B_6, had no effect on cognitive performance in healthy and well-nourished women (Wolters, Hickstein, Flintermann, Tewes, & Hahn, 2005). In a systematic review of randomised trials, it was suggested that there is no adequate evidence of an effect of vitamin B_6, vitamin B_{12}, or folic acid supplementation, alone or in combination, on cognitive function in people with either normal or impaired cognitive function (Balk et al., 2007). However, the limitations of the use of single nutrients and different durations of therapy in these human studies should be taken into account when evaluating their results.

Vitamin B_6 inhibits the release of glutamate from rat cortical synaptosomes by suppressing the pre-synaptic voltage-dependent entry of Ca^{2+} and protein kinase C activity. Thus, the protective effect of vitamin B_6 against excitotoxicity has received some discussion (Yang & Wang, 2009).

It has also been suggested that pre-treatment with vitamin B_6 has modulatory effects on the metabolism of neurotransmitters in the brain, and this may explain the cognition-enhancing effects of vitamin B_6 and its ability to act as a free radical scavenger. It

seems that other possibilities for the memory-enhancing effect of vitamin B_6 should also be studied, as should other behavioural methods for evaluating memory performance.

As in previous studies, an excess of vitamin B_6 in the diet did not cause any neurotoxicity depending on behavioural action (Schaeffer, Gretz, Gietzen, & Rogers, 1998; Schaeffer, Gretz, Mahuren, & Coburn, 1995). The administration of vitamin B_6 at a dose of 600 mg/kg resulted in a better recovery of behavioural function and enhanced neuroprotection, compared with a dose of 300 mg/kg in a controlled cortical impact model (Kuypers & Hoane, 2011).

However, high doses of vitamin B_6 have been shown to cause some adverse effects. In a study on mice, 350 and 700 mg/kg vitamin B_6 resulted in auditory neuropathy (Hong, Yi, Kim, & Kang, 2009). Although that study reported no ataxia or motor incoordination, a study on rats showed that vitamin B_6 at a dose of 400 mg/kg caused ataxia (Perry, Weerasuriya, Mouton, Holloway, & Greig, 2004). In another study, short-term subcutaneous administration of vitamin B_6 (150 mg/kg) in dogs did not induce any systemic toxicity, but induced sensory neuropathy (Chung, Choi, Hwang, & Youn, 2008). The doses used in this study were lower than those used in studies reporting neuroprotective effects of vitamin B_6. Thus, it can be concluded that the administration of vitamin B_6 at the selected doses did not cause any changes in the locomotor activity of the animals, compared with the controls.

In summary, these results indicate that pre-treatment with vitamin B_6 has a potential role in enhancing memory retrieval in rats. Further studies are necessary to determine whether the administration of vitamin B_6 together with other supplements will also reduce the risk of cognitive diseases and/or improve cognitive functioning.

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