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ORIGINAL ARTICLE

Evaluation Effects of Verapamil as a Calcium Channel Blocker on Acquisition, Consolidation and Retrieval of Memory in Mice

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KEYWORDS

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ABSTRACT: Many factors are involved in learning and memory processes including brain nuclei, neurotransmitter systems, and the activity of ion channels. Studies showed inconsistent effects of calcium channel blockers on learning process, especially memory consolidation; however, little is known about their effect on memory acquisition and retrieval. Accordingly, the present study aimed to determine the effects of verapamil calcium channel antagonist as a representative of the phenylalkylamine group on different stages of memory and learning processes including acquisition, consolidation and retrieval in mice. In this experimental study, 150 male albino mice with a mean weight of 30 g were used. The mice were trained in a passive avoidance-learning task (1 mA shock for 2 seconds for evaluation of memory acquisition and consolidation and 3 seconds for evaluation of memory retrieval). The effect of verapamil (1, 2.5, 5, 10, and 20 mg/kg) on memory consolidation and the most effective dose of consolidation phase on memory acquisition and retrieval was assessed. For the evaluation of memory consolidation, the animals received the drug intraperitoneally immediately after training, while for evaluation of memory acquisition and retrieval, the drug was injected one hour before training. Memory retrieval test was performed 48 hours after training (the length of time it took the animal to enter the dark part of the device). The results showed that verapamil injection exerted no effect on memory acquisition and consolidation; nevertheless, it was capable to disrupt memory retrieval in 10 and 20 mg doses. These results indicate that as a phenylalkylamine calcium channel antagonist, high doses of verapamil can impair memory.

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INTRODUCTION

Learning is the process of acquiring new information, and memory is the process of encoding, storing, and remembering it. Recent evidence has shown that many factors are involved in learning and memory processes including brain nuclei, neurotransmitter systems, and the activity of ion channels. Voltage-gated $Ca_V2.1$ (P/Q-type) Ca^{2+} channels located at the presynaptic membrane are known to control a multitude of Ca^{2+}-dependent cellular processes such as neurotransmitter release and synaptic plasticity [1]. L-type Ca^{2+} channel (LTCC)-activated signaling cascades contribute significantly to psycho stimulant-induced locomotors sensitization; however, the precise contribution of the two brain-specific subunits $Ca_v1.2$ and $Ca_v1.3$ remains mostly unknown [2]. Physiological activity of the specific T-type $Ca_v3.2$ calcium channel is required for affective and cognitive behaviors suggesting potential functional implications of this calcium channel in many brain disorders [3-5]. Calcium channel antagonists are a group of various drugs, categorized according to their chemical structure, pharmacokinetics, and therapeutic use into three subgroups of dihydropyridine, benzodiazepine, and phenylalkylamine. In all the three forms, these drugs block L-type calcium channels in vessels, cardiac muscle, and nerve cells [6]. They are mostly used to treat angina, hypertension, cardiovascular disease, and migraine [7]. The dihydropyridine group is used for treatment of motor disorders and aging-induced cognitive behaviors [8, 9]. Calcium channel antagonists can strengthen cognition and increased capacity for learning and memory and their peripheral injection can improve dementia. It can also enhance learning in fear conditioning model and Morris water maze. The exact mechanism of their effects and their interpretation are very complicated [6, 10].

Considering that intracellular calcium levels serve an important role in enhancing memory, it is expected that calcium channel antagonists may lead to impairment of memory and learning, inasmuch as they inhibit the entry of calcium into cells. Some of these antagonists injected peripherally were able to reverse developed dementia, weaken the effects of brain damage on learning, and increase acquisition of spatial and non-spatial memory [6, 7, 11 and 12]. However, there are conflicting results that failed to show the effects of calcium channel antagonists on learning and memory. These studies have mainly focused on the impacts of these antagonists on acquisition and consolidation, without addressing the retrieval phase. In another study the effects of calcium channel antagonists (nimodipine, nifedipine, amlodipine, flunarizine, diltiazem, and verapamil) was investigated on passive avoidance task and linear maze and the results showed that peripheral injection of all these antagonists can improve dementia [9]. Altered calcium homeostasis has been demonstrated to serve an important role in pathogenesis of Alzheimer's disease [13, 14]. Calcium channel blockers have been studied primarily in terms of acquisition and consolidation of memory with contradictory reports, and their effects on memory retrieval have not been extensively studied.

The present study, thus, aimed to investigate the impact of different doses of verapamil as a representative of phenylalkylamine calcium-channel blockers on memory acquisition, consolidation, and retrieval in mice.

MATERIALS AND METHODS

This experimental study investigated 150 male albino mice with a mean weight of 30 g. The animals were maintained in constant room temperature (22±2 °C) under a 12-h light/dark cycle (light onset at 07:00 h) all animals were naive to tests. Each mouse was tested individually and only once. Experiments were conducted between 10:00 and 14:00 h. The animals were randomly assigned to different experimental and control groups. The mice were trained in a passive avoidance-learning task. Prior to training, the mice were firstly adapted to the avoidance apparatus (shuttle box) for 30 min. Shuttle box is common

apparatus for assessment of memory used in several investigations [15]. After entering into the dark side of the device, a shock with an intensity of 0.5 mA was given to the mice for 2 seconds. The retrieval test was performed 48 h after training. Verapamil calcium channel antagonist was purchased from Sigma Chemical Company and dissolved in saline; Verapamil was freshly prepared and administered by intraperitoneal (I.P.) in a specified dose. Control groups received the same volume of saline.

Experiment 1

The aim of this experiment was to investigate the effect of different doses of verapamil (1, 2.5, 5, 10, and 20 mg) on consolidation of information. Sixty mice were randomly divided into six groups: the control group, which received saline immediately after training (n=10) and the treatment groups, which received verapamil with doses of 1, 2.5, 5, 10, and 20 mg/kg immediately after training (n=50).

Experiment 2

This experiment investigated the most effective dose of verapamil in experiment 1 on acquisition of information. Fifty mice were randomly divided into 5 groups: the control group, which received saline immediately before training (n=10) and the verapamil groups, which received verapamil saline an hour before training (n=40).

Experiment 3

This experiment investigated the most effective dose of verapamil in experiment 1 on retrieval of information. Forty mice were randomly divided into 4 groups: the control group, which received saline an hour before retrieval (n=10) and the verapamil groups, which received verapamil saline an hour before retrieval (n=30).

Data are shown as mean ± SEM in the figures. Given the normal distribution of data, one-way ANOVA and Tukey's test were used to analyze them (P<0.05).

RESULTS

Figure 1 illustrates the effects of different doses of verapamil on learning acquisition. The evaluation criterion was the time, which lasted until the animal entered the dark side of the device. One-way ANOVA of the animals entering into the dark compartment during training showed no significant difference between the groups ($F_{4,40}$=0.57, P=0.99) (Figure 1A). These findings indicate the homogeneity of the groups. One-way ANOVA of the learners time of entry into the dark part showed no significant difference between the groups receiving verapamil and saline ($F_{4,40}$=0.228, p=0.92). Therefore, injection of verapamil had no significant effect on learning acquisition process (P>0.05) (Figure 1B).

Figure 2 shows the effects of different doses of verapamil on memory consolidation. The evaluation criterion was the time, which lasted until the animal entered the dark side of the device. One-way ANOVA of the animals entering into the dark compartment during training showed no significant difference between the groups ($F_{5,54}$=0.471, P=0.79) (Figure 2A). These findings indicate the homogeneity of the groups. One-way ANOVA of the time of entry into the dark part for evaluation of memory consolidation showed no significant difference between the groups receiving verapamil and saline ($F_{5,54}$=0.141, P=0.98). Therefore, injection of verapamil had no significant effect on memory consolidation process (P>0.05) (Figure 2B).

Figure 3 shows the effects of different doses of verapamil on memory retrieval. The evaluation criterion was the time, which lasted until the animal entered the dark side of the device. One-way ANOVA of the animals entering into the dark compartment during training showed no significant difference between the groups ($F_{3,32}$=0.16, P=0.92) (Figure 3A). These findings indicate the homogeneity of the groups. One-way ANOVA of the time of entry into the dark part for evaluation of memory retrieval showed no significant difference between the groups receiving verapamil and saline ($F_{3,32}$=5.27, P=0.004). Further analysis with Tukey's test showed that delay in entering the dark area of the device was significantly lower in the 10 and 20 mg/kg groups

than the control group (P<0.01) (Figure 3B). Therefore, verapamil injection had significantly impaired memory retrieval.

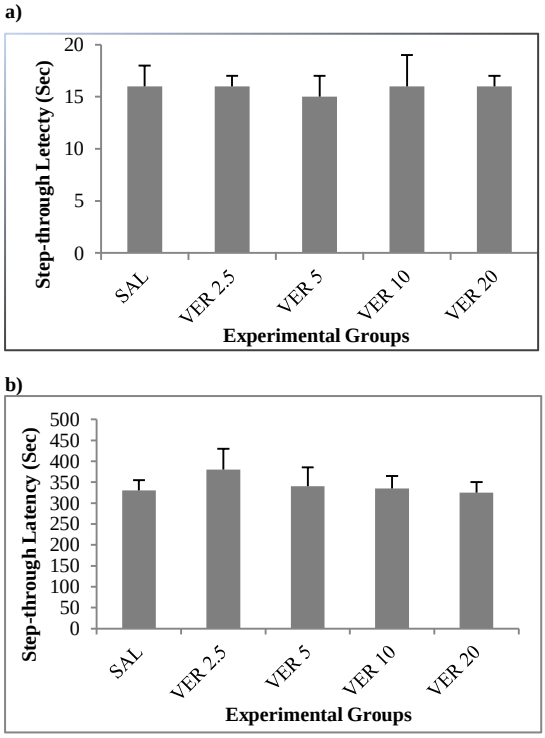

Figure 1. The effects of different doses of verapamil on learning acquisition in passive avoidance learning model .vertical axis mean ± SEM shows step through latency to dark area during training (a) and during retrieval test (b)

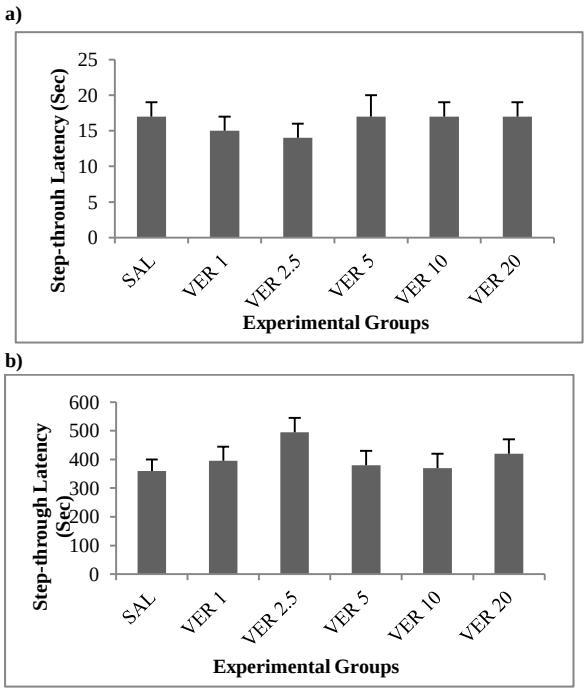

Figure 2. the effects of different doses of verapamil on learning consolidation in passive avoidance learning model .vertical axis mean ± SEM shows step through latency to dark area during training (a) and during retrieval test (b)

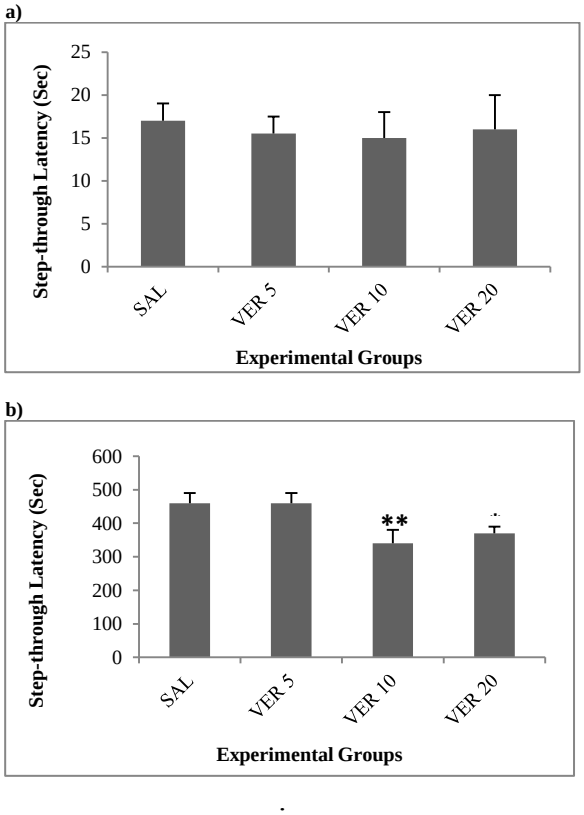

Figure 3. the effects of different doses of verapamil on learning retrieval in passive avoidance learning model .vertical axis mean ± SEM shows step through latency to dark area during training (a) and during retrieval test (b) . ** P < 0.01 with compared control group.* P < 0.05 with compared control group

DISCUSSION

We previously assessed the effect of Gabapantine on passive avoidance learning and found that it exerted no destructive effects on cognition and improved emotional cognitive performance in mice [16]. Verapamil is an inhibitor of voltage-dependent calcium channels in the family of phenylalkylamine calcium channel inhibitors [17]. The drug is a potent vasodilator, but has also a suppressive effect on heart rate [18]. Verapamil is also a strong antagonist of alpha-1 and alpha-2 adrenergic receptors. It can also inhibit the uptake of norepinephrine and dopamine in synapses [19]. The injection of different doses of verapamil had no effect on acquisition and consolidation of passive avoidance learning. This finding is consistent with a previous study regarding ineffectiveness of verapamil on acquisition and consolidation of memory [20]. However, the effects of this drug in other learning models are facilitating or

destructive, for instance, injection of verapamil could increase learning of linear maze [20]. While recent studies have shown that, its injection can damage cognitive learning acquisition [21-23]. These findings indicate that the effect of verapamil on acquisition is dependent on its type.

The mechanism of this dependence is unclear and requires further studies. On the other hand, high-dose injection of verapamil disturbed retrieval of information. This finding is in agreement with previous findings in our laboratory [24] and those of others [17, 22]. It seems that this effect of verapamil is specific and not due to impaired motor activity. Because in our previous study, it was found that verapamil had no effect on animals' motor activity [24]. As noted above, verapamil is a vasodilator that is used in treatment of hypertension, angina, and coronary artery spasm. Given that this problem occurs in older ages when age-related deficits in memory occur [17], consumption of the drug in the elderly can intensify memory impairment; this should be considered when taking the medicine.

CONCLUSIONS

As the representative of phenylalkylamine group of calcium channel blockers, high doses of verapamil can exert disruptive effect on memory retrieval in animal models, while it has no obvious impact on acquisition and consolidation of passive avoidance memory. Identification of involved mechanisms and neural location in these interactions requires further studies.

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