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Statins enhance cognitive performance in object location test in albino Swiss mice: Involvement of beta-adrenoceptors

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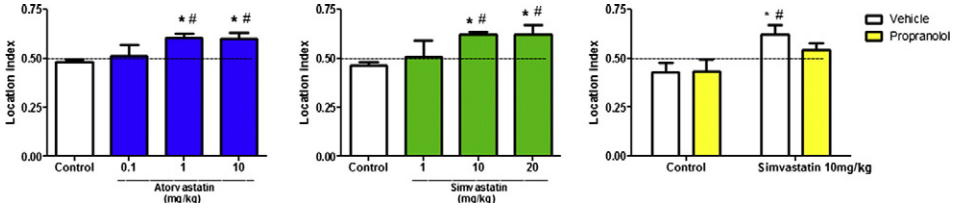
HIGHLIGHTS

- Statins improve performance of Swiss albino mice in the OLT.
- Beta-adrenergic receptor abolishes the beneficial effects of simvastatin.
- Statins do not change the exploratory parameters in the OF.
- Statins do not change the anxiety-like parameters in the EPM.

GRAPHICAL ABSTRACT



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ABSTRACT

Statins are inhibitors of the 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, thereby inhibiting cell synthesis of cholesterol and isoprenoids. Moreover, several studies have been evaluating pleiotropic effects of statins, mainly because they present neuroprotective effects in various pathological conditions. However, knowledge about behavioral effects of statins *per se* is relatively scarce. Considering these facts, we aimed to analyze behavioral responses of atorvastatin or simvastatin-treated mice in the open field test, elevated plus maze and object location test. Atorvastatin treatment for 7 consecutive days at 1 mg/kg or 10 mg/kg (v.o.) or simvastatin 10 mg/kg or 20 mg/kg enhanced cognitive performance in object location test when compared to control group (saline-treated mice). Simvastatin effects on mice performance in the object location test was abolished by post-training infusion of the beta-adrenoceptor antagonist propranolol. Atorvastatin and simvastatin did not change the behavioral response in open field and elevated plus-maze (EPM) tests in any of the used doses. These data demonstrate the positive effects of both statins in cognitive processes in mice, without any alteration in locomotor parameters in the open field test or anxiolytic-like behavior in EPM. In conclusion, we demonstrate that atorvastatin and simvastatin *per se* improve the cognitive performance in a rodent model of spatial memory and this effect is related to beta-adrenergic receptors modulation.

1. Introduction

Statins are inhibitors of the enzyme 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, thereby inhibiting cell synthesis of

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cholesterol and isoprenoids [1]. The HMG-CoA reductase is the pacemaker enzyme of cholesterol synthesis by reducing HMG-CoA to mevalonate [2]. Retrospective studies suggest that the prevalence of Alzheimer Disease (AD) and vascular dementia is lower among patients taking statins, even reducing the levels of Aβ peptides induced by cerebral trauma [3–5]. Additionally, several studies have evaluated pleiotropic effects of statins, mainly because they present neuroprotective effects on pathological conditions [6–8]. Furthermore, the safety of high doses of atorvastatin and simvastatin has been demonstrated in adult humans [9,10].

Several studies in rodent animals are performed to evaluate the neuroprotective effects of statins [11–14]. Statins promote reduction of neurological deficits and increase in synaptogenesis, angiogenesis and neuronal survival in animals exposed to a model of traumatic brain injury [15]. Atorvastatin reduced the seizure activity and the neuronal death in rat hippocampus after seizures induced by kainate [1]. Atorvastatin also reduced the number of convulsing animals and promoted neuroprotection against hippocampal cell death after seizures induced by quinolinic acid, an NMDA receptor agonist [8]. Other members of the statin family, as fluvastatin, simvastatin and pravastatin, have shown differential effects regarding on the intervention schedule against cognitive impairment induced by amyloid-beta (Aβ) peptide infusion, due to a prevention of cholinergic neuronal loss or modulation of glutamatergic system [16,17]. Moreover, clinical data indicates that statin therapy is linked to a reduction in the incidence of depression and anxiety [18], although the mechanisms of action are not yet established.

Despite the evidence of important roles for statins on neurological diseases, the knowledge about behavioral effects of statins *per se* is scarce [19]. It has been demonstrated that treatment with statins prevented spatial memory deficit induced by traumatic brain injury or scopolamine infusion in rodents [15,20,21]. Statin treatment has also been shown to prevent neuronal cell death as well to prevent cognitive deficits induced by Aβ infusion [22,23]. Besides, it has been shown that simvastatin treatment improved the performance of control rats in the object location and passive avoidance tasks [14]. A wide range of mechanisms has been proposed to explain these pleiotropic effects of statins including antioxidant, anti-inflammatory, immunomodulatory, modulation of nitric oxide production and increased expression of brain-derived neurotrophic factor (BDNF) [24–26]. However, the precise molecular mechanisms involved in the memory enhancing effects of the statins remain unknown.

In this study, mice were subjected to three different behavioral tasks. The open field task (OF) represents a new environment and it is used to analyze the locomotor behavior in mice and rats [27]. The elevated plus-maze is the classical approach to evaluate anxiolytic-like behavior in rats and currently is also used in mice [28]. The object location test (OLT) is based on rodents' natural behavior (novelty preference), an innate instinct that drives animals to learn about their environment (discrimination ratio). Additionally, it has been reported that the performance of animals in this task is dependent of the hippocampal function [29].

Considering the necessity for elucidation of the behavioral effects of statins, this study investigated the behavioral effects of atorvastatin and simvastatin treatments in mice submitted to the OF, EPM and OPR tests. Additionally, we evaluated the involvement of beta-adrenergic receptor on the cognitive effects of statin treatment.

2. Materials and methods

2.1. Animals

Male adult Swiss albino mice (3 months old/45 ± 5 g) were kept on a 12-h light/dark cycle (light on at 07.00 a.m.) at a constant temperature of 22 ± 1 °C. They were housed in plastic cages with tap water and commercial food *ad libitum*. All procedures were carried out according to the institutional policies on animal experimental handling, designed to minimize suffering and limit the number of animals used and were approved by the local Ethical Committee for Animal Research. All experiments were performed during the light phase (between 14:00 and 17:00 h) to avoid circadian variations.

2.2. Pharmacological treatments

Total of 203 male Swiss albino mice were employed to study the putative role of statins on behavioral changes, animals were treated orally with atorvastatin (Lipitor Atorvastatin calcium, Pfizer) 0.1, 1 or 10 mg/kg/day, or simvastatin 1, 10 or 20 mg/kg/day once a day during seven consecutive days [7,8]. Control animals were treated with vehicle (NaCl 0.9%) orally for the same period. One day after the last atorvastatin or saline administration animals were submitted to the specifically behavioral task. Object location test, or open field, or elevated plus maze were analyzed in this specific time.

After the initial results, we performed additional experimental procedure to evaluate the involvement of beta-adrenergic receptors in the effects of statins in the OLT (49 animals were employed in these experiments). Mice received 10 mg/kg/day of simvastatin or vehicle (NaCl 0.9%) for seven days. One day after the last simvastatin or vehicle (NaCl 0.9%) animals were submitted to OLT. The animals of two initial groups were treated with the beta-adrenergic receptor antagonist propranolol (2 mg/kg, i.p.; Sigma Chemical Co., St. Louis, U.S.A.) or vehicle (NaCl 0.9%) immediately after the training session. All treatments were done by the administration of 10 μl/g weight of the animal.

2.3. Behavioral tasks

Mice were randomly assigned for treatments. Animals were housed in the communal plastic cages (10 animals per cage). The behavioral task was performed and analyzed by a blinded observer. Every experimental procedure presents animals of each group to comparative analyses. A control experiment procedure was used by two groups to evaluate the effects of propranolol in short term memory of Swiss albino mice in OLT. All experiments were performed during the light phase (between 14:00 and 17:00 h) to avoid circadian variations. In every experiment, the animals are exposed to apparatus in randomized order to minimize the circadian effects in the behavioral analyses.

2.4. Object location test

The OLT was performed in an apparatus consisting of a wooden box chamber (40 cm × 60 cm × 50 cm). Before the experimental sessions, animals (total of 112, distributed in 4 experiments) were habituated to the experimental room for 90 min in dim light conditions. A light bulb was switched on during the experimental sessions. The light intensity was equal in different parts of the apparatus. In the adaptation sessions, mice explored the apparatus for 10 min, with no object. The objects were placed equidistant from two corners, 10 cm apart from the wall. Mice were placed individually into the chamber and performed the task for 10 min. In training sessions, 2 similar objects were utilized. In test sessions, performed 90 min (12 animals, to control the effects of propranolol in short term memory) or 24 h later (total of 100 animals), one object was replaced to the other corner of the chamber. The objects employed were two LEGO® pieces presenting the same texture, size, shape and color. The objects were not known to have any ethological significance for mice [30]. Discrimination ratio was expressed by the ratio TN/(TN + TF), (TN, time spent exploring the novel place; TF, time spent exploring familiar place), both in the training and test sessions. During the inter-trial interval objects were cleaned with 10% ethanol solution to avoid odor cues. Exploration was defined by directing the nose to the object at a distance less than 2 cm and/or touching the object with the nose or forepaws. The time of exploration was measured by two blinded observers, with the use of chronometers.