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Atorvastatin evokes a serotonergic system-dependent antidepressant-like effect in mice

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ABSTRACT

Atorvastatin is a statin largely used in the treatment of hypercholesterolemia and recently revealed as a neuroprotective agent. The antidepressant-like effect of acute atorvastatin treatment in mice has been previously demonstrated by our laboratory. The purpose of this study was to explore the contribution of the serotonergic system in the antidepressant-like effect of atorvastatin in mice. Data demonstrate that the serotonin (5-HT) depleting agent p-chlorophenylalanine methyl ester (PCPA, 100 mg/kg, i.p.) completely abolished atorvastatin (0.1 mg/kg, p.o.) antidepressant-like effect. Besides atorvastatin, fluoxetine (10 mg/kg, p.o.), a serotonin selective reuptake inhibitor (SSRI) was able to exert an antidepressant-like effect, but any of them changed 5-HT content in the hippocampus or frontal cortex. The 5H-T1A (WAY100635, 0.1 mg/kg, s.c) or the 5-HT2A/2C (ketanserin, 5 mg/kg, s.c.) receptor antagonists prevented atorvastatin antidepressant-like effect. In addition, a combinatory antidepressant-like effect was observed when mice received the co-administration of sub-effective doses of atorvastatin (0.01 mg/kg, p.o.) and the SSRI fluoxetine (5 mg/kg, p.o.), paroxetine (0.1 mg/kg, p.o.) or sertraline (1 mg/kg, p.o.). Taken together, these results indicate that the antidepressant-like effect of atorvastatin depends on the serotonergic system modulation.

1. Introduction

Atorvastatin is a lipophilic and synthetic statin, largely used for the treatment of hypercholesterolemia. The mechanism of action is based on the inhibition of the 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, the rate-limiting enzyme in the cholesterol synthesis (Wang et al., 2008). Beyond this primary effect, lipid-lowering independent or pleiotropic effects have been attributed to statin treatment (Liao and Laufs, 2005). Clinical trials have shown that the use of statins reduces the incidence of stroke (Di Napoli et al., 2002; Heeschen et al., 2002) and dementia (Stêpieñ et al., 2002). Animal models (Ouk et al., 2013) and cell culture studies (Posada-Duque et al., 2013; Xu et al., 2013) have also been used to demonstrate the neuroprotective effect of statins. In our laboratory, the protective effect of atorvastatin have been already established against seizures induced by quinolinic acid, an N-methyl-D-aspartate (NMDA) receptor agonist (Piermartiri et al., 2009), amyloid-beta-induced toxicity (Piermartiri et al., 2010) and cognitive and motor impairments induced by an experimental model of Parkinson's disease (Castro et al., 2013).

An antidepressant-like effect evoked by atorvastatin in mice has recently been demonstrated. In that study, we showed that atorvastatin antidepressant-like effect is at least partially dependent on the modulation of the glutamatergic system (Ludka et al., 2013). The observation that ketamine, a selective NMDA receptor antagonist, exhibits a rapid and sustained relief of depressive symptoms has brought to the fore the glutamatergic hypothesis of depression (Irwin and Iglewicz, 2010; Li et al., 2010). Although findings support that excitatory transmission plays a central role in mediating the emotional and cognitive changes associated with depression (Pralong et al., 2002; Robbins and Arnsten, 2009), it is well established that drugs that act by modulating monoamines in the brain are largely used in the treatment of depression (Nutt, 2006).

Abnormalities in the serotonergic neurotransmission have been specifically pointed out as a common factor in mental illness, particularly in depression (Woolley and Shaw, 1954). It can be highlighted by studies that showed a reduced cerebrospinal fluid concentration of 5-HT and its main metabolite 5-hydroxyindoleacetic acid (5-HIAA) in postmortem brain tissue of depressed or suicidal patients (Asberg et al., 1976; Roy et al., 1989). 5-HT can interact with multiple receptors, and to date seven families have been characterized ($5HT_1R$–$5HT_7R$), and with exception to $5HT_3R$ that is a ligand-gated ion channel, they are all

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G-protein-coupled receptors (Barnes and Sharp, 1999; Hoyer, 1988; Kriegebaum et al., 2010).

The 5-HT_{1A} receptor subtype is the best studied (Glennon and Dukat, 1991; Pessoa-Mahana et al., 2003), and it is generally accepted to be involved in psychiatric disorders such as anxiety and depression (Blier and Abbott, 2001). 5-HT_{1A} receptors are localized dendritically as inhibitory autoreceptors on serotonergic cells or in postsynaptic sites that are enriched in corticolimbic structures as the hippocampus and frontal cortex (Millan, 2000). Postmortem and neuroimaging studies suggest an increased density of 5-HT_{1A} autoreceptors in major depressive patients compared with control subjects (Boldrini et al., 2008; Parsey et al., 2002; Stockmeier et al., 1998).

5HT_{1A} and 5-$HT_{2A/2C}$ receptors are of remarkable importance in the control of mood, motor behavior and appetite (Millan, 2005). 5-HT_{2A}R is a postsynaptic receptor localized on cortical GABAergic interneurons as well as on glutamatergic projections in humans and rodents (De Almeida and Mengod, 2007; Santana et al., 2004). Cumulative evidence indicates that the 5-HT_{2A} receptor plays a role in depression, based on the fact that atypical antipsychotic drugs (Carvalho et al., 2008) and the antidepressant mirtazapine have the ability to specifically block 5-HT_{2A} receptor-mediated responses (Marek et al., 2003).

5-HT_{2C} receptors are predominantly located in the choroid plexus, cerebral cortex, hippocampus, substantia nigra and cerebellum (Abramowski et al., 1995). Alterations in their functional status have been detected in anxiodepressive states (Niswender et al., 2001), and 5-HT_{2C} receptors are known to be involved in the action of several classes of antidepressant drugs (Artigas, 2012).

It is also important to highlight that serotonergic neurons are controlled by glutamatergic inputs at forebrain areas in physiological conditions (Celada et al., 2001; Fink et al., 1995; Martin-Ruiz, et al., 2001).

Considering that atorvastatin antidepressant-like effect involves glutamatergic system modulation (Ludka et al., 2013), and the known correlation between glutamatergic and serotonergic systems, this study investigated the participation of serotonergic system in the antidepressant-like effect of atorvastatin in mice submitted to the tail suspension test (TST). Moreover 5-HT contents in the hippocampi and frontal cortices of mice acutely treated with atorvastatin or fluoxetine were evaluated.

2. Experimental procedure

2.1. Animals

Male Swiss mice (35–45 g) were maintained at 21–23 °C with free access to water and food, under a 12:12 h light/dark cycle (lights on at 07:00 h). All manipulations were carried out between 9:00 and 16:00 h. All procedures in this study were performed in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23). The experiments were performed after approval of the protocol (PP559) by the Institutional Ethics Committee (CEUA/UFSC) and all efforts were made to minimize animals suffering and to reduce the number of animals used in the experiments.

2.2. Drugs

The following drugs were used: atorvastatin, fluoxetine hydrochloride, sertraline hydrochloride, paroxetine hydrochloride (Pfizer, Brazil), ketanserin tartarate, p-chlorophenylalanine methyl ester (PCPA), N-N-(2-pyridynyl) cyclohexanecarboxamide (WAY100635) (Sigma Chemical Co, USA). All drugs were dissolved in saline. Atorvastatin, fluoxetine, sertraline and paroxetine were administered by oral route (p.o.) in a volume of 10 mL/kg body weight using the gavage technique (Brocardo et al., 2008; Cunha et al., 2013). WAY100635, ketanserin and PCPA were administered by intraperitoneal (i.p.) route in a volume of 10 mL/kg body weight (Brocardo et al., 2008; Capra et al., 2010).

2.3. Behavioral tests

2.3.1. Tail suspension test (TST)

The total duration of immobility induced by tail suspension was measured according to the method described by Steru et al. (1985). Briefly, mice both acoustically and visually isolated were suspended 50 cm above the floor by adhesive tape and placed approximately 1 cm from the tip of the tail. Immobility time was recorded during a 6 min period (Zeni et al., 2011).

2.3.2. Open-field behavior

The ambulatory behavior was assessed in an open-field test as described previously (Zomkowski et al., 2010). The apparatus consisted of a wooden box measuring 40 × 60 × 50 cm high. The floor of the arena was divided into 12 equal squares. The number of squares crossed with all paws (crossings) was counted in a 6-min session. The light was maintained at minimum to avoid anxiety behavior.

2.4. Pharmacological treatments

The participation of serotonergic system was investigated by using the 5-HT depleting agent, PCPA (100 mg/kg, i.p.) as shown in Fig. 1A. It was given during four days (Brocardo et al., 2008; Machado et al., 2008) preceding the atorvastatin (0.1 mg/kg, p.o.) or saline administration. Twenty four hours after the last administration of PCPA (Machado et al., 2008), atorvastatin or saline was given. The TST was performed 1 h after atorvastatin or saline administration (Ludka et al., 2013).

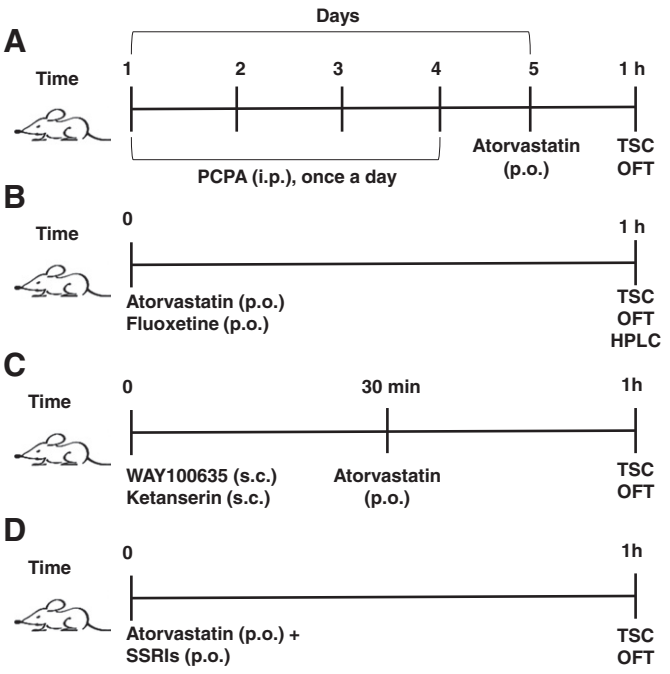

Fig. 1. Time table of treatments and schedule of behavioral and biochemical tests. (A) Adult male Swiss mice received PCPA (100 mg/kg, i.p., once a day during four consecutive days). 24 h after the last administration of PCPA animals received atorvastatin (0.1 mg/kg, p.o.) or vehicle, and 1 hour interval before tail suspension test (TST) and open field test (OFT). (B) Adult male Swiss mice received atorvastatin (0.1 mg/kg, p.o.) or fluoxetine (10 mg/kg, p.o.), and 1 h after the treatment animals were subjected to TST/OFT. Another group of animals subjected to the same treatment schedule were killed and hippocampus and cortex were dissected to measure serotonin content by HPLC. (C) Adult male Swiss mice received WAY100635 (0.1 mg/kg, s.c.; 5HT_{1A} receptor antagonist) or ketanserin (5 mg/kg, s.c.; a preferential 5HT_{2A} receptor antagonist), and after 30 min, atorvastin (0.1 mg/kg, p.o.) or vehicle was administered. Animals were subjected to TST and OFT after 1 h. (D) The effect of SSRIs and atorvastatin was assessed by co-administration of fluoxetine (5 mg/kg, p.o.), paroxetine (0.1 mg/kg, p.o.) or sertraline (1 mg/kg, p.o.) with atorvastatin (0.01 mg/kg, p.o.) and 1 h after the administration TST and OFT was carried out.