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The neuroprotective effect of two statins: simvastatin and pravastatin on a streptozotocin-induced model of Alzheimer's disease in rats

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Abstract Astrocytes play a fundamental role in glutamate metabolism by regulating the extracellular levels of glutamate and intracellular levels of glutamine. They also participate in antioxidant defenses, due to the synthesis of glutathione, coupled to glutamate metabolism. Although the cause of Alzheimer's disease (AD) remains elusive, some changes in neurochemical parameters, such as glutamate uptake, glutamine synthetase activity and glutathione have been investigated in this disease. A possible neuroprotective effect of two statins, simvastatin and pravastatin (administered p.o.), was evaluated using a model of dementia, based on the intracerebroventricular (ICV) administration of streptozotocin (STZ), and astrocyte parameters were determined. We confirmed a cognitive deficit in rats submitted to ICV-STZ, and a prevention of this deficit by statin administration. Moreover, both statins were able to prevent the decrease in glutathione content and glutamine synthetase activity in this model of AD. Interestingly, simvastatin increased per se glutamate uptake activity, while both statins increased glutamine synthetase activity per se. These results support the idea that these drugs could be effective for the prevention of alterations observed in the STZ dementia model and may contribute to reduce the cognitive impairment and brain damage observed in AD patients.

Keywords Astrocyte · Glutamate · Dementia · Statins · Streptozotocin

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Introduction

Alzheimer's disease (AD) is the most common form of dementia and affects nearly 35% of the population of over 85 years (Stefani and Liguri 2009). Dementia is characterized by a decline in cognitive and social functions and severe memory loss (Weinstock 2004; Weinstock and Shoham 2004). The histopathological marks of AD are the formation of senile plaques, caused by the extracellular accumulation of amyloid fibrils in the brain, and also by the intraneuronal aggregates of neurofibrillary tangles, which lead to progressive brain dysfunction (Silvestrelli et al. 2006).

Astrocytes are closely linked to neurons and play an active role in the development of AD (Maccioni et al. 2001). In fact, activated astrocytes are found in association with senile plaques (Mrak and Griffinbc 2001). Physiologically, astrocytes play a fundamental role in glutamate metabolism by regulating the extracellular levels of glutamate and intracellular levels of glutamine (Danbolt 2001). Coupled to this function, astrocytes are also responsible for the synthesis and secretion of glutathione (GSH), the main non-enzymatic antioxidant defense in the central nervous system (Dringen and Hirrlinger 2003).

The exact cause of AD remains elusive. However, several risk factors seem to be involved in the development of this condition. Type II diabetes mellitus, the apolipoprotein ε4 allele and high levels of plasma cholesterol are some of

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these factors (Candore et al. 2010; Pasquier et al. 2006). The mechanism by which cholesterol is involved in the development of AD is still unclear, but indeed accumulation of cholesterol has been found in association with senile plaques and in transgenic models of AD (Mori et al. 2001). Taking into account the contribution of cholesterol to the development of AD, a range of studies suggest that statins, a class of lipid-lowering drugs, can confer protection against this dementia (Hoglund and Blennow 2007; Jick et al. 2000; Wolozin et al. 2007).

Statins are a class of cholesterol-lowering drugs that act by inhibiting the rate-limiting enzyme in cholesterol biosynthesis, 3-hydroxy-3-methylglutaryl coenzyme A (Zipp et al. 2007). In addition to this effect, statins also reduce the formation of isoprenoid intermediates in the cholesterol pathway (Liao and Laufs 2005), which can confer anti-inflammatory (Li et al. 2009; Yoshida 2003) and immunomodulatory properties to statins (Adamson and Greenwood 2003; Kuipers and van den Elsen 2007). The action of statins in cardiovascular disease is well known (Schonbeck and Libby 2004); however, their neuroprotective effect demands further characterization. We chose two statins, based on their different abilities to cross the blood–brain-barrier and potential neuroprotective activities (Ramirez et al. 2011; Sierra et al. 2010).

There are a few experimental models to study AD, but the intracerebroventricular (ICV) streptozotocin (STZ) model demonstrates metabolic changes that are very similar to those found in the sporadic form of AD. In this model, deficits in learning, memory and cognitive behavior are reported (Lannert and Hoyer 1998). In addition, increased oxidative damage (Sharma and Gupta 2001; Sharma and Gupta 2002), alterations in glucose utilization and neuronal damage have been described (Grunblatt et al. 2007; Weinstock and Shoham 2004).

The aim of this study was to evaluate the spatial cognitive deficit in animals exposed to ICV-STZ, and hippocampal changes in glutamate uptake, glutathione content and the activity of glutamine synthetase (GS), as well as to evaluate a possible neuroprotective effect of two statins: simvastatin and pravastatin in this model of dementia.

Materials and methods

Chemicals

Streptozotocin, simvastatin, pravastatin, albumin, standard GSH and γ-glutamylhydroxamate were purchased from Sigma, L-[H^3]-glutamate from Amersham International (UK) and HBSS from Gibco. All other chemicals were purchased from local commercial suppliers.

Animals

Forty-two male Wistar rats (90 days old, weighing 250–320 g) were obtained from our breeding colony (at the Department of Biochemistry, Universidade Federal do Rio Grande do Sul) and were maintained under controlled light and environmental conditions (12 h light/12 h dark cycle at a constant temperature of 22 ± 1°C) with free access to food and water. All animal experiments were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23), revised 1996, and following the regulations of the local animal house authorities.

Experimental groups

Rats were divided into six groups of seven rats: sham, sham–simvastatin, sham–pravastatin, STZ, STZ–simvastatin and STZ–pravastatin. (a) Sham group: the animals received ICV injection of STZ vehicle (HBSS). On day 2 after the surgery, and every 2 days, they received the statin vehicle, p.o. (b) Sham–simvastatin group: the animals received an ICV injection of STZ vehicle (HBSS, detailed further). On day 2 after the surgery, and every 2 days, they received simvastatin (5 mg/kg) p.o. (c) Sham–pravastatin group: the animals received an ICV injection of the STZ vehicle (HBSS). On day 2 after the surgery, and every 2 days, they received pravastatin (5 mg/kg) p.o. (d) STZ group: the animals received an ICV injection of STZ (3 mg/kg). On day 2 after the surgery, and every 2 days, they received the statins vehicle, p.o. (e) STZ–simvastatin group: the animals received an ICV injection of STZ (3 mg/kg). On day 2 after the surgery, and every 2 days, they received simvastatin (5 mg/kg) p.o. (f) STZ–pravastatin group: the animals received an ICV injection of STZ (3 mg/kg). On day 2 after the surgery, and every 2 days, they received pravastatin (5 mg/kg) p.o.

Surgical procedure for ICV administration of STZ

STZ was ICV infused, as previously described (Rodrigues et al. 2009; Sharma and Gupta 2001). Briefly, on the day of the surgery, animals were anesthetized with ketamine/xylazine (75 and 10 mg/kg, respectively, i.p.) and placed in a stereotaxic apparatus. A midline sagittal incision was made in the scalp. Burr holes were drilled in the skull on both sides over the lateral ventricles. The lateral ventricles were accessed using the following coordinates (Paxinos 1997): 0.9 mm posterior to the bregma; 1.5 mm lateral to the sagittal suture; 3.6 mm beneath the surface of the brain. Rats received a single bilateral infusion of 5 μL STZ (3 mg/kg) or vehicle (Hank's balanced salt solution—HBSS—containing in mM: 137 NaCl; 0.63 Na_2HPO_4; 4.17

$NaHCO_3$; 5.36 KCl; 0.44 KH_2PO_4; 1.26 $CaCl_2$; 0.41 $MgSO_4$; 0.49 $MgCl_2$ and 10 glucose, at pH 7.4) using a 10-μL Hamilton syringe. After the surgical procedure, rats were placed on a heating pad to maintain body temperature at 37.5 ± 0.5°C and kept there until recovery from anesthesia. The animals were submitted to evaluation and biochemical analysis 3 weeks after SZT injection.

Statins administration

Simvastatin and pravastatin were dissolved in 50% ethanol and administered per gavage. Rats received 0.1 mL, every 2 days, of simvastatin (5 mg/kg), pravastatin (5 mg/kg) or vehicle. The treatment lasted 4 weeks (3 weeks after the surgical procedure and 1 week during cognitive evaluation).

Cognitive evaluation

Three weeks after surgery, rats were submitted to training in the Morris water maze (Morris 1984; Silva et al. 2005). The apparatus consisted of a circular pool (180 cm in diameter, 60-cm high) filled with water (depth 30 cm; 24 ± 1°C), placed in a room with consistently located spatial cues. An escape platform (10 cm diameter) was placed in the middle of one of the quadrants, 1.5 cm below the water surface, equidistant from the sidewall and the middle of the pool. The platform provided the only escape from the water and was located in the same quadrant every trial. Four different starting positions were equally spaced around the perimeter of the pool. On each training day, all four start positions were used once in a random sequence, i.e., four training trials per day. A trial began by placing the animal in the water, facing the wall of the pool at one of the starting points. If the animal failed to escape within 60 s, it was gently conducted to the platform by the experimenter. The rat was allowed to stay there for 20 s. The inter-trial interval was 10 min. After each trial, the rats were dried and returned to their cages at the end of the session. Animals were trained for 5 days. At 24 h after the last training session, the rats were submitted to a test session (4 weeks after surgery). Before this session, the submerged platform was removed. The retention test consisted of placing the animals in the water for 1 min. The number of crossings over the original position of the platform and time spent in the target quadrant compared to the opposite quadrant were measured. After cognitive evaluation, rats were anaesthetized, as subsequently described, for obtaining CSF and preparation of brain slice.

Serum biochemical measurements

Animals were anesthetized as described above and blood was obtained by cardiac puncture. Serum contents of cholesterol, triglycerides, glucose, glutamic oxalacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) levels were analyzed using commercial kits (Human do Brasil, Itabira, Brazil).

Hippocampal tissue samples

Anesthetized animals were killed by decapitation, and the brains were removed and placed in cold saline medium with the following composition (in mM): 120 NaCl; 2 KCl; 1 $CaCl_2$; 1 $MgSO_4$; 25 HEPES; 1 KH_2PO_4 and 10 glucose, adjusted to pH 7.4 and previously aerated with O_2. The hippocampi were dissected and homogenized for measurement of glutamine synthetase activity and glutathione content or cut into transverse slices of 0.3 mm using a McIlwain tissue chopper. Slices were transferred immediately to 24-well culture plates, each well containing 0.3 mL of HBSS for measuring glutamate uptake.

Glutamine synthetase activity

The enzymatic assay was performed as previously described (dos Santos et al. 2006). Briefly, hippocampal homogenates (0.1 mL) were added to 0.1 mL of reaction mixture containing (in mM) 10 $MgCl_2$; 50 L-glutamate, 100 imidazole–HCl buffer (pH 7.4), 10 2-mercaptoethanol, 50 hydroxylamine-HCl and 10 ATP, and incubated for 15 min at 37°C. The reaction was stopped by the addition of 0.4 mL of a solution containing (in mM): 370 ferric chloride; 670 HCl; 200 trichloroacetic acid. After centrifugation, the supernatant was measured at 530 nm and compared to the absorbance generated by standard quantities of γ-glutamylhydroxamate treated with ferric chloride reagent.

Glutathione content assay

GSH levels (nmol/mg protein) were measured as described previously (Browne and Armstrong 1998). Hippocampal homogenates were diluted in 10 volumes of 100 mM sodium phosphate buffer, pH 8.0, containing 5 mM EDTA, and protein was precipitated with 1.7% meta-phosphoric acid. The supernatant was assayed with *o*-phthaldialdeyde (1 mg/mL methanol) at room temperature for 15 min. Fluorescence was measured using excitation and emission wavelengths of 350 and 420 nm, respectively. A calibration curve was performed with standard GSH solutions (0–500 μM).

Glutamate uptake assay

Glutamate uptake was performed, as previously described (Gottfried et al. 2002) with some modifications (Thomazi et al. 2004). Briefly, hippocampal slices were transferred to 24-well plates and incubated for 23 min at 37°C in a

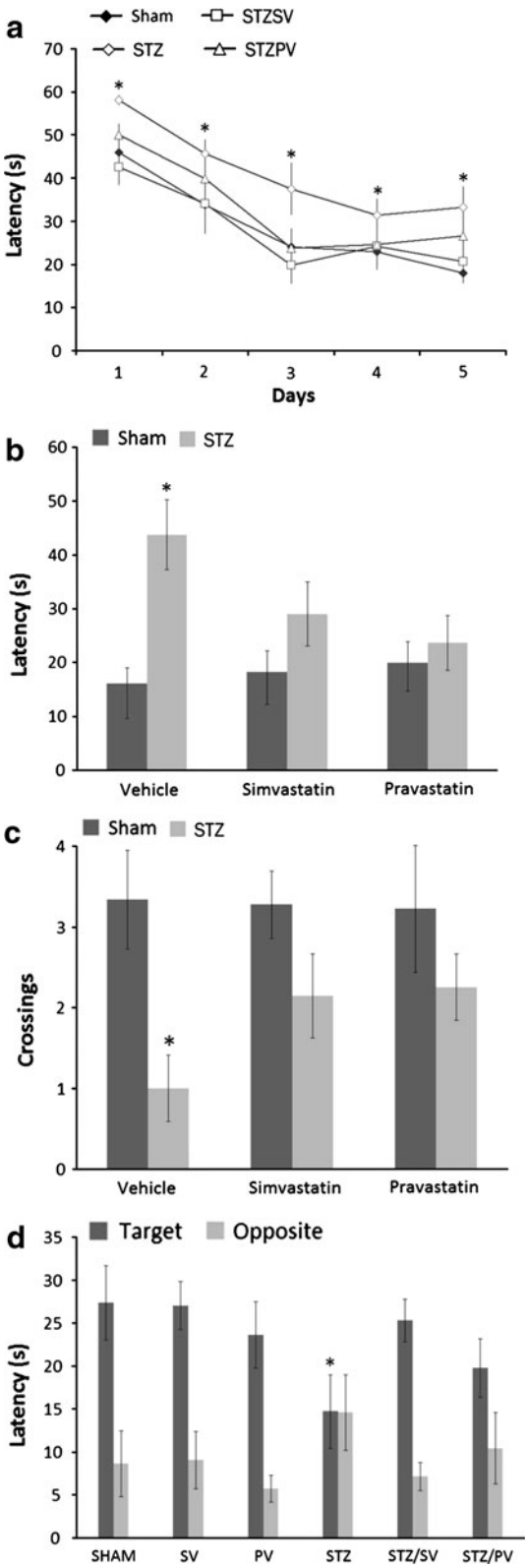

Fig. 1 Cognitive performance of rats submitted to ICV-STZ injection and oral simvastatin or pravastatin administration, evaluated by the water maze. **a** Performance in the reference memory protocol, based on escape latency. *Each line* represents the mean ± standard error. *Significant differences were detected from day 1 onward, when compared with the control group ($N = 8$, repeated measures analysis of variance, $p < 0.05$). **b** Memory in the probe trial of reference memory, based on escape latency. Values are mean ± standard error. *Significantly different from all other groups ($N = 8$, two-way ANOVA followed by Tukey's test, $p < 0.05$). **c** Number of crossings over the platform position. Values are mean ± standard error. *Significantly different from all other groups ($N = 8$, two-way ANOVA followed by Tukey's test, $p < 0.05$). **d** Memory in the probe trial of reference memory, as measured by time spent (in s) in the target and opposite quadrant. Values are mean ± standard error. *Time spent in target quadrant by STZ group was significantly different from all other groups ($N = 8$, two-way ANOVA followed by Tukey's test, $p < 0.05$)

Hank's balanced salt solution (HBSS) containing (in mM): 137 NaCl, 5.36 KCl, 1.26 $CaCl_2$, 0.41 $MgSO_4$, 0.49 $MgCl_2$, 0.63 $Na_2HPO_4.7H_2O$, 0.44 KH_2PO_4, 4.17 $NaHCO_3$ and 5.6 glucose, adjusted to pH 7.4. The assay was started by the addition of 0.1 mM L-glutamate and 0.33 μCi/mL L-[2,3-^{3}H] glutamate. Incubation was stopped after 5 min by removal of the medium and rinsing the slices twice with ice-cold HBSS. The slices were then lysed in a solution containing 0.5 M NaOH. Radioactivity was measured in a scintillation counter. Sodium-independent uptake was determined using *N*-methyl-D-glucamine instead of NaCl. Sodium-dependent glutamate uptake was obtained by subtracting the non-specific uptake from the total to obtain the specific uptake.

Protein determination

Protein content was measured by Lowry's method using bovine serum albumin as standard (Lowry et al. 1951).

Statistical analysis

Parametric data from the experiments are presented as mean ± standard error and statistically evaluated by two-way analysis of variance, followed by the Tukey's test, assuming $p < 0.05$. The escape latency parameter in the water maze task was evaluated by repeated measures analysis of variance, assuming $p < 0.05$.

Results

Cognitive evaluation

The Morris water maze was used to evaluate the reference memory in the six groups: sham, sham–simvastatin, sham–pravastatin, STZ, STZ–simvastatin and STZ–pravastatin. In the training sessions, from day 1 onward, there was a significant increase in the average time to find the platform in the STZ group (escape latency), when compared with the sham group (Fig. 1a) ($F_{(5–45)} = 1.497$, $p < 0.05$). In

the trial session, STZ rats (without statins) took more time to find the platform ($F_{(5–45)} = 3.836$, $p < 0.05$), when compared with the other groups, including the STZ + statins group (Fig. 1b). The number of crossings over the platform location was lower in the STZ group, compared to the other groups (Fig. 1c) ($F_{(5–45)} = 4.256$, $p < 0.05$). Moreover, the STZ group (without statins) also spent less time in the target quadrant than all the other groups (Fig. 1d), ($F_{(5, 45)} = 2.464$, $p < 0.05$). No differences were observed in the time spent in the opposite quadrant ($F_{(5,45)} = 1.211$, $p > 0.05$). Note that simvastatin and pravastatin "per se" had no effect on the cognitive task.

Glutamate uptake

The treatment with STZ did not affect the glutamate uptake activity in the hippocampus. However, simvastatin "per se" increased glutamate uptake (about 30%) ($F_{(5,32)} = 2.364$, $p < 0.05$) when compared with the sham group (Fig. 2). However, the simvastatin group did not differ from the STZ group. Pravastatin "per se" did not alter glutamate uptake.

Glutathione content

To evaluate a possible development of oxidative stress, a measurement of reduced GSH was performed. Glutathione content was reduced in the STZ-treated animals by about 30% when compared with the sham group (with and without statins) ($F_{(5,36)} = 2.702$, $p < 0.05$) (Fig. 3). However, administration of simvastatin and pravastatin was able to prevent the effect of STZ on glutathione content.

Glutamine synthetase activity

Hippocampal GS activity was reduced in the STZ-treated group, and both pravastatin and simvastatin were able to prevent this decrease ($F_{(5,36)} = 13.218$, $p < 0.05$) (Fig. 4).

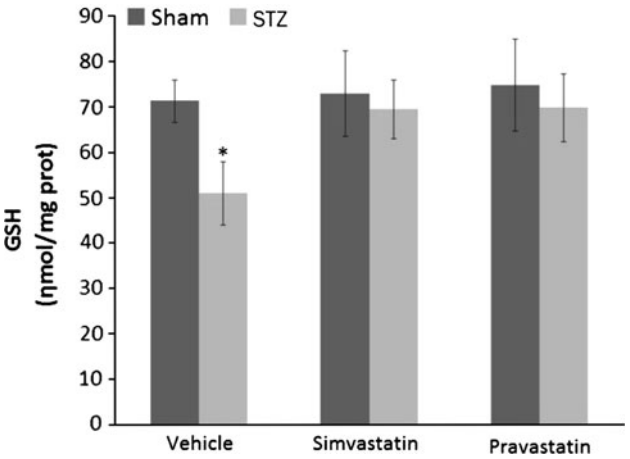

Fig. 3 Glutathione content in the hippocampus of rats submitted to ICV-STZ injection. Adult rats were submitted to ICV injection of STZ and simvastatin or pravastatin oral administration. Four weeks later, hippocampi were dissected out and homogenized for measurement of glutathione content. Values are mean ± standard error. *Significantly different from the sham groups (with or without statins). $N = 7$, two-way ANOVA followed by Tukey's test, $p < 0.05$)

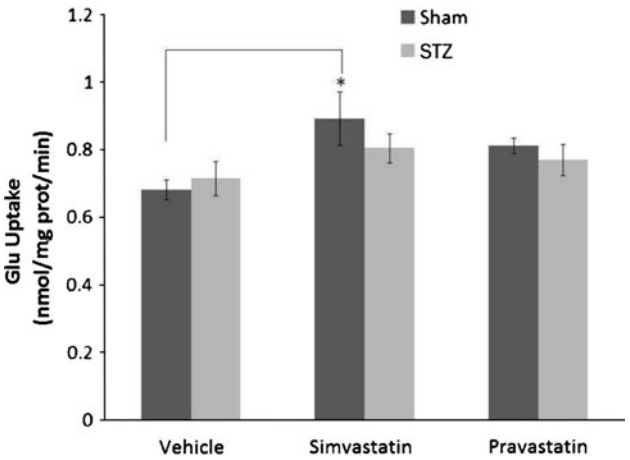

Fig. 2 Glutamate uptake in the hippocampus of rats submitted to the ICV-STZ model of dementia. Adult rats were submitted to ICV injection of STZ and oral simvastatin or pravastatin administration. Four weeks later, hippocampi were dissected out and the glutamate uptake assay performed on hippocampal slices. Values are mean ± standard error. *Significant difference between simvastatin and sham group ($N = 6$-7, two-way ANOVA followed by Tukey's test, $p < 0.05$)

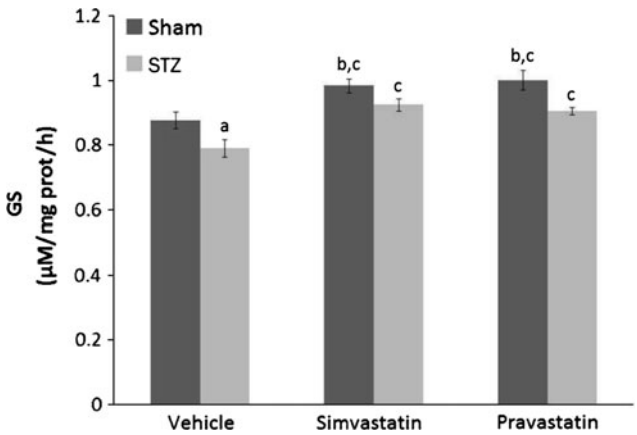

Fig. 4 Glutamine synthetase activity in the hippocampus of rats submitted to ICV-STZ injection. Adult rats were submitted to ICV injection of STZ and simvastatin or pravastatin oral administration. Four weeks later, hippocampi were dissected out and homogenized for measurement of the glutamine synthetase activity. Values are mean ± standard error. [a]Significantly different from all other groups, [b]significantly different from the sham and STZ groups, [c] significantly different from the STZ group ($N = 7$, two-way ANOVA followed by Tukey's test, $p < 0.05$)

Table 1 Serum biochemical parameters of rats submitted to ICV-STZ injection and oral simvastatin or pravastatin administration

	Sham group			STZ group		
	Vehicle	SV	PV	Vehicle	SV	PV
Glucose (mg/dl)	170.1 ± 27.0	174.0 ± 23.7	193.5 ± 19.0	166.0 ± 29.1	160.0 ± 20.1	176.0 ± 20.0
Total cholesterol (mg/dl)	84.0 ± 10.2	88.1 ± 10.1	78.1 ± 9.3	90.5 ± 20.2	87.5 ± 12.5	89.5 ± 12.5
Triglycerides (mg/dl)	125.3 ± 12.1	122.0 ± 15.0	114.5 ± 16.4	117.8 ± 24.3	121.2 ± 14.4	118.0 ± 13.4
STGO (UI)	50.1 ± 22.5	42.5 ± 13.5	56.0 ± 23.0	40.1 ± 17.8	37.2 ± 16.3	45.1 ± 20.5
STGP(UI)	20.8 ± 5.7	20.3 ± 4.5	21.1 ± 6.0	23.0 ± 7.8	20.1 ± 4.3	21.1 ± 3.9

TCholesterol total serum cholesterol, *SGPT* serum glutamic pyruvic transaminase, *SGOT* serum glutamic oxalacetic transaminase. Data are expressed as mean ± standard error ($N = 7$, two-way ANOVA)

Interestingly, simvastatin and pravastatin "per se" induced an increase in GS activity.

Serum biochemical parameters

To evaluate the effect of ICV-STZ on serum glucose levels and the effect of statins on the serum lipid profile, we carried out biochemical assays. STZ injection did not alter the serum glucose levels, triglycerides and total cholesterol levels. Moreover, statin administration had no effect on the lipid profile. We also measured serum transaminases to evaluate possible hepatic damage induced by statins. No effect was observed (Table 1).

Discussion

The STZ model of dementia has been widely used, and the injection of this drug causes increased oxidative stress and alterations in glucose metabolism, accompanied by progressive deficits in cognition and behavior (Lannert and Hoyer 1998; Terwel et al. 1995). Our data showed a reduction in glutathione content and glutamine synthetase activity with no alterations in glutamate uptake in animals that received the ICV-STZ injection, in agreement with previous results from our group (Rodrigues et al. 2009).

A variety of compounds have shown neuroprotective activities, including resveratrol (Sharma and Gupta 2002), aminoguanidine (Rodrigues et al. 2009) and tacrine and donepezil (Saxena et al. 2008), in the dementia model induced by ICV injection of STZ. Many studies have shown that statins, a class of drugs that act by the inhibition of cholesterol synthesis, can exert a neuroprotective role on the central nervous system (Sharma et al. 2008; Vaughan and Delanty 1999). In our study, we observed that simvastatin and pravastatin were able to reverse some effects of streptozotocin, without reducing the plasma cholesterol levels in STZ or control rats.

Although these statins have discrete structural differences, they exhibit different activities in diverse parameters analyzed in vitro and in vivo (Sierra et al. 2010). Simvastatin is more lipophilic and potentially crosses the blood–brain-barrier more easily than pravastatin (Saheki et al. 1994; Sierra et al. 2010). However, pravastatin inhibits HMG-CoA redutase more effectively in neural cultures (Sierra et al. 2010) and brain tissue (Kirsch et al. 2003). Importantly, despite these differences, both statins used in this study exhibited beneficial effects on cognitive and neurochemical parameters.

Despite the fact that high levels of plasma cholesterol are possibly involved in the pathogenesis of AD (Canevari and Clark 2007) and the presence of cholesterol in the amyloid plaques of the APP transgenic AD model in mice (Mori et al. 2001), no changes have been observed in total brain cholesterol in APP transgenic mice (Kurata et al. 2011). Moreover, there are no reports regarding the brain content of cholesterol in the STZ model of AD.

We did not find any changes in peripheral cholesterol in ICV-STZ treated rats that received, or did not receive, statins. In agreement with this finding, atorvastatin (30 mg/kg) and pitavastatin (3 mg/kg) improved the cognitive deficits in APP transgenic mice, but were not able to modify the serum levels of cholesterol (Kurata et al. 2011). It is important to mention that even when an elevated dose of simvastatin was administered to guinea pigs (250 mg/kg), no changes were observed in the brain content of cholesterol (Fassbender et al. 2001). It is believed that statins could act via inhibition of isoprenoid synthesis and that isoprenoids are important for the posttranslational modifications of a variety of proteins, including G proteins (Kuipers and van den Elsen 2007; Rikitake and Liao 2005). Moreover, brain cholesterol content is not dependent upon peripheral synthesis and, in AD, the release of cholesterol is altered in neurons (Kandiah and Feldman 2009). It has also been proposed that statins stabilize the cholesterol in membrane micro-

domains, preventing the cleavage of APP into beta-amyloid toxic isoforms (Kirsch et al. 2003).

There is evidence that dementia is accompanied by oxidative stress and damage to the brain tissue (Butterfield et al. 2010; Markesbery and Carney 1999). The exact mechanism of the action of STZ in not well understood, but it is well known that it causes central damage and neuronal death by induction of oxidative stress (Rodrigues et al. 2009; Sharma and Gupta 2001; Sharma and Gupta 2002). The protective effect of statins in oxidative stress is well known in cardiovascular diseases (Nagay Hernandez et al. 2008), and some recent studies demonstrate the protective effect of statins in the brains of mice exposed to beta-amyloid peptide. Previous studies also report a reduction in oxidative stress in mice (Barone et al. 2011; Piermartiri et al. 2010). In the present study, ICV-STZ injection reduced the total content of GSH in hippocampal slices, according to previous reports (Rodrigues et al. 2009), and both statins were capable of reversing this effect, in agreement with previous studies that demonstrated the antioxidant potential of statins (Bandoh et al. 2000; Piermartiri et al. 2010; Vaughan and Delanty 1999).

There is some evidence that the metabolism of glutamate is altered in AD, and neurons and astrocytes present lower levels of glutamine synthetase (Robinson 2001). The dementia model induced by ICV injection of beta-amyloid causes a reduction in glutamate uptake in hippocampal slices (Piermartiri et al. 2010); however, in our model, streptozotocin injection did not alter the hippocampal glutamate uptake, in agreement with previous results (Rodrigues et al. 2009). Interestingly, simvastatin presented an effect per se, increasing the glutamate uptake in the hippocampus. Previous reports have demonstrated the effect of statins against glutamate excitotoxicity in cortical neurons (Bosel et al. 2005; Zacco et al. 2003). Atorvastatin reversed the reduction in glutamate transporter expression in a dementia model with βA, probably mediated by PI3K and AKT pathways (Piermartiri et al. 2010). More recently, it has been suggested that simvastatin, but not pravastatin, is an effective neuroprotectant in a mouse model of neurodegeneration induced by kainate (Ramirez et al. 2011). It is possible that the effect of simvastatin (but not pravastatin) on glutamate uptake that we observed in this study could contribute to this protection against excitotoxicity.

It is important to mention that we used a low dose of simvastatin (5 mg/kg), when compared to the maximum dose approved for human use (80 mg/day) (Fassbender et al. 2001) or to the amount administered in studies aiming for neuroprotection (Li et al. 2006; Ramirez et al. 2011). However, low doses of statins also showed protection in cognitive parameters in transgenic (Kurata et al. 2011) and STZ (Sharma et al. 2008) models of dementia. Moreover, in the STZ model of dementia, as occurs in AD, we observed a decrease in glutamine synthetase activity, and both statins prevented this effect. In fact, both statins exhibited an effect "per se" on this enzyme.

Glutamatergic neurotransmission, glutamate metabolism and antioxidant defenses (particularly mediated by glutathione) are strongly coupled. Our data suggest that simvastatin and pravastatin can interfere in these parameters. Together with previous reports, our data support the neuroprotective role of statins in AD, based on changes in biochemical and cognitive parameters in the STZ model of dementia. In summary, our results confirm the cognitive deficit observed in ICV-STZ models of dementia and that this deficit was accompanied by changes in glutamate metabolic parameters. Simvastatin (and pravastatin) exhibited a neuroprotective effect on these parameters. These results support the idea that these drugs could be effective in the prevention of alterations observed in the STZ dementia model and may contribute to reduce cognitive impairment and brain damage in AD patients.

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