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Resveratrol prevents memory deficits and the increase in acetylcholinesterase activity in streptozotocin-induced diabetic rats

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ABSTRACT

The objective of the present study was to investigate the effect of the administration of resveratrol (RV) on memory and on acetylcholinesterase (AChE) activity in the cerebral cortex, hippocampus, striatum, hypothalamus, cerebellum and blood in streptozotocin-induced diabetic rats. The animals were divided into six groups ($n=6$–13): Control/saline; Control/RV 10 mg/kg; Control/RV 20 mg/kg; Diabetic/saline; Diabetic/RV 10 mg/kg; Diabetic/RV 20 mg/kg. One day after 30 days of treatment with resveratrol the animals were submitted to behavioral tests and then submitted to euthanasia and the brain structures and blood were collected. The results showed a decrease in step-down latency in diabetic/saline group. Resveratrol (10 and 20 mg/kg) prevented the impairment of memory induced by diabetes. In the open field test, no significant differences were observed between the groups. In relation to AChE activity, a significant increase in diabetic/saline group ($P<0.05$) was observed in all brain structures compared to control/saline group. However, AChE activity decreased significantly in control/RV10 and control/RV20 ($P<0.05$) groups in cerebral cortex, hippocampus and striatum, while no significant differences were observed in diabetic/RV10 and diabetic/RV20 groups in all brain structures compared to control/saline group. Blood AChE activity increased significantly in diabetic/saline group ($P<0.05$) decreased in control/RV10, control/RV20 and diabetic/RV20 groups ($P<0.05$) compared to control/saline group. In conclusion, the present findings showed that treatment with resveratrol prevents the increase in AChE activity and consequently memory impairment in diabetic rats, demonstrating that this compound can modulate cholinergic neurotransmission and consequently improve cognition.

1. Introduction

Recent estimates indicate that around 200 million people suffer from diabetes mellitus, making it the most common serious metabolic disorder worldwide (American Diabetes Association, 2007). Diabetes is characterized by hyperglycemia due to insufficient availability of, or insensitivity to insulin and is associated with slowly progressive end-organ damage in the eyes, kidneys, blood vessels, heart and peripheral nerves, as well as in the brain (Gispen and Biessels, 2000; Northam et al., 2006).

Several studies have described the effects of diabetes in the central nervous system (CNS) as a series of neurochemical, neurophysiological and structural abnormalities, a condition referred to as diabetic encephalopathy (Biessels et al., 2002a; Sima et al., 2004). In addition to these abnormalities, impairments in cognitive function have been observed in diabetic patients and also in animal models of diabetes (Strachan et al., 2003; Brands et al., 2007). These impairments have been characterized mainly by moderate deficits in learning and memory, psychomotor slowing and reduced mental flexibility (Cukierman et al., 2005; Brands et al., 2007). Furthermore, diabetic patients also seem to double the probability of developing Alzheimer's disease and other dementias (Arvanitakis et al., 2004; Biessels et al., 2006).

In fact, the mechanism causing brain damage in diabetes mellitus has not been fully elucidated, but it appears to be a multifactorial process which involves fluctuation in the blood glucose level, as well as acute and chronic metabolic and vascular disturbances, such as a decrease in cerebral blood flow (Manschot et al., 2003) and alterations in cellular calcium homeostasis (Cameron et al., 2001; Biessels, 2002b). Moreover, it has been demonstrated that hyperglycemia induces oxidative stress (Tuzcu and Baydas, 2006) in various brain regions and also alters activities of enzymes that are considered critical for normal CNS functioning, such as Na^+-K^+-ATPase (Franzon

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et al., 2004), catalase, NTPDase and 5′-nucleotidase (Lunkes et al., 2004). In addition, some studies using experimental diabetes have found an increase in acetylcholinesterase (AChE) activity which may indicate alterations in cholinergic neurotransmission and consequently be associated to cognitive impairments observed in diabetes mellitus (Sanchez-Chavez and Salceda, 2000; Kuhad et al., 2008).

Cholinergic neurons and their projections are widely distributed throughout the CNS with an essential role in regulating many vital functions, such as learning, memory, cortical organization of movement and cerebral blood flow control (Mesulam et al., 2002). Literature data have demonstrated that one of the most important mechanisms responsible for correct cholinergic function is performed by AChE (Appleyard, 1992). This enzyme hydrolyses the neurotransmitter acetylcholine in the synaptic cleft of cholinergic synapses and neuromuscular junctions (Soreq and Seidman, 2001). In addition to its role in cholinergic neurotransmission, AChE has been implicated in several non-cholinergic actions such as cell proliferation (Appleyard, 1994) neurite outgrowth (Chacón et al., 2003) and haematopoiesis (Silman and Sussman, 2005). Interestingly, AChE responds to various insults including oxidative stress, an important event that has been related to the pathogenesis and progression of a variety of CNS disorders, such as stroke (Ozkul et al., 2007), Alzheimer's diseases (Chauhan and Chauhan, 2006) and diabetes mellitus (Kuhad et al., 2008).

Resveratrol (3,5,4-trihydroxy-trans-stilbene) is a polyphenol found mainly in grapes and red wine with diverse established biological activities, such as antioxidant, anti-inflammatory, cardioprotective and anticarcinogenic roles (Baur and Sinclair, 2006; Saiko et al., 2008). Recently, a number of studies have focused on the neuroprotective effects of resveratrol, demonstrating that this compound attenuates amyloid β peptide-induced toxicity (Han et al., 2004; Anekonda, 2006), protects against cerebral ischemic injury (Wang et al., 2002; Uguralp et al., 2005) and kainic acid-induced excitotoxicity (Wang et al., 2004). Several neuroprotective properties of resveratrol have been attributed to its potent antioxidant activity that in many studies has been shown to protect the neural tissue against a variety of neurodegenerative conditions caused by oxidative stress (Ates et al., 2005; Mokni et al., 2007; Quincozes-Santos et al., 2007).

Therefore, considering that diabetes mellitus is associated with cognitive dysfunction and that resveratrol has important neuroprotective actions, the aim of this study was to investigate the effects of this compound on learning and memory as well as on activity of AChE enzyme in brain and whole blood of streptozotocin-induced diabetic rats in order to verify the participation of resveratrol in the modulation of the cholinergic system.

2. Materials and methods

2.1. Chemicals

Acetylthiocholine iodide, 5,5′dithiobis-2-nitrobenzoic acid (DTNB), tris (hydroxymethyl)-aminomethane GR, Coomassie brilliant blue G, streptozotocin, resveratrol (3,5,4′-trihydroxy-trans-stilbene, approximately 99% purity) were obtained from Sigma Chemical Co (St. Louis, MO, USA). All other reagents used in the experiments were of analytical grade and of the highest purity.

2.2. Animals

Adult male Wistar rats (70–90 days; 250–270 g) from the Central Animal House of the University Federal of Santa Maria (UFSM) were used in this experiment. The animals were maintained at a constant temperature (23 ± 1 °C) on a 12 h light/dark cycle with free access to food and water. All animal procedures were approved by the Animal Ethics Committee from the Federal University of Santa Maria (protocol under number: 21/2007).

2.3. Experimental induction of diabetes

Diabetes was induced by a single intraperitoneal injection of 55 mg/kg streptozotocin, diluted in 0.1 M sodium–citrate buffer (pH 4.5). The age-matched control rats received an equivalent amount of sodium–citrate buffer. Streptozotocin-treated rats received 5% of glucose instead of water for 24 h after diabetes induction in order to reduce death due to hypoglycemic shock. Blood samples were taken from the tail vein 48 h after streptozotocin or vehicle injection to measure glucose levels. Glucose levels were measured with a portable glucometer (ADVANTAGE, Boehringer Mannheim, MO, USA). Only animals with fasting glycemia over 300 mg/dl were considered diabetic and used for the present study. During the experiment blood glucose levels were verified four times (2, 10, 20, and 30 days after the beginning of treatment). The animals that maintained fasting glycemia higher than 300 mg/dl were considered diabetic and selected for behavioral tests and enzymatic assays.

2.4. Treatment with resveratrol (RV)

The animals were randomly divided into six groups (six to thirteen rats per group): Control/saline; Control/RV 10 mg/kg; Control/RV 20 mg/kg; Diabetic/saline; Diabetic /RV 10 mg/kg; and Diabetic/RV 20 mg/kg. One week after diabetes induction, the animals belonging to group control/RV10 and diabetic/RV10 received 10 mg/kg of resveratrol intraperitoneally and the animals from control/RV20 and diabetic/RV20 groups received 20 mg/kg of resveratrol, while the animals from control/saline and diabetic/saline groups received saline solution intraperitoneally. Resveratrol was freshly prepared in 25% ethanol and was administered at between 10 and 11 a.m. once a day during 30 days, at a volume not exceeding 0.1 ml/100 g rat weight.

2.5. Behavioral procedure

2.5.1. Inhibitory avoidance

One day after the end of the treatment with resveratrol or saline, animals were subjected to training and test in a step-down inhibitory avoidance apparatus according to Guerra et al. (2006). Briefly, the rats were subjected to a single training session in a step-down inhibitory avoidance apparatus, which consisted of a 25 × 25 × 35-cm box with a grid floor whose left portion was covered by a 7 × 25-cm platform, 2.5 cm high. The rat was placed gently on the platform facing the rear left corner, and when the rat stepped down with all four paws on the grid, a 2-s 0.4-mA shock was applied to the grid. Retention test took place in the same apparatus 24 h later. Test step-down latency was taken as a measure of retention, and a cut-off time of 500 s was established.

2.5.2. Open field

Immediately after the inhibitory avoidance test session, the animals were transferred to an open-field measuring 56 × 40 × 30 cm, with the floor divided into 12 squares measuring 12 × 12 cm each. The open-field session lasted for 5 min and during this time, an observer, who was not aware of the pharmacological treatments, recorded the number of crossing responses and rearing responses manually. This test was carried out to identify motor disabilities, which might influence inhibitory avoidance performance at testing.

2.5.3. Foot shock sensitivity test

Reactivity to shock was evaluated in the same apparatus used for inhibitory avoidance, except that the platform was removed. The modified “up and down” method of Rubin et al. (2004) was used to determine the flinch and jump thresholds in experimentally naive animals. Animals (control/saline and diabetic/saline) were placed on the grid and allowed a 3 min habituation period before the start of a series of shocks (1s) delivered at 10 s intervals. Shock intensities

Table 1
The effect of different doses of resveratrol (RV) on body weight and fasting blood glucose levels in control and diabetic rats at the onset and the end of the experiment (30 days after resveratrol treatment).

Groups	Glucose (mM)		Body weight (g)	
	Onset	End	Onset	End
Control/saline	6.30 ± 1.10^{a}	6.20 ± 1.19^{a}	256 ± 4.50^{a}	284 ± 8.15^{a}
Control/RV 10 mg/kg	5.60 ± 0.86^{a}	6.10 ± 2.87^{a}	250 ± 5.19^{a}	269 ± 9.40^{a}
Control/RV 20 mg/kg	6.20 ± 0.90^{a}	5.80 ± 1.27^{a}	240 ± 3.10^{a}	261 ± 4.68^{a}
Diabetic/saline	6.10 ± 1.28^{a}	25.10 ± 1.26^{b}	250 ± 5.09^{a}	189 ± 15.44^{b}
Diabetic/RV10 mg/kg	5.80 ± 2.13^{a}	25.60 ± 2.24^{b}	265 ± 4.17^{a}	202 ± 7.47^{b}
Diabetic/RV20 mg/kg	5.90 ± 1.19^{a}	23.20 ± 1.52^{b}	270 ± 5.24^{a}	202 ± 11.41^{b}

Values are expressed as mean ± S.E.M. Groups with different letters are statistically different (^{a,b} $P<0.05$, $n=6$–8). ANOVA–Duncan's Test.

ranged from 0.1 to 0.5 mA in 0.1 mA increments. The adjustments in shock intensity were made in accordance with each animal's response. The intensity was raised by one unit when no response occurred and lowered by one unit when a response was made. A flinch response was defined as withdrawal of one paw from the grid floor, and a jump response was defined as withdrawal of three or four paws. Two measurements of each threshold (flinch, and jump) were made, and the mean of each score was calculated for each animal.

2.6. Brain tissue preparation

After behavioral tests, the animals were submitted to euthanasia being previously anesthetized with ethyl ether and brain structures were removed and separated into cerebral cortex, hippocampus, striatum, hypothalamus and cerebellum and placed in a solution of 10 mM Tris–HCl, pH 7.4, on ice. The brain structures were homogenized in a glass potter in Tris–HCl solution. Aliquots of resulting brain structure homogenates were stored at −8 °C until utilization. Protein was determined previously in a strip that varied for each structure: cerebral cortex (0.7 mg/ml), striatum (0.4 mg/ml), hippocampus (0.8 mg/ml), hypothalamus (0.6 mg/ml) and cerebellum (0.6 mg/ml) as determined by the Coomassie blue method according to Bradford (1976) using bovine serum albumin as standard solution.

2.7. Cerebral AChE enzymatic assay

The AChE enzymatic assay was determined by a modification of the spectrophotometric method of Ellmann et al. (1961) as previously described by Rocha et al. (1993). The reaction mixture (2 ml final volume) contained 100 mM K^{+}-phosphate buffer, pH 7.5 and 1 mM 5,5′-dithiobisnitrobenzoic acid (DTNB). The method is based on the formation of the yellow anion, 5,5′-dithio-bis-acid-nitrobenzoic, measured by absorbance at 412 nm during 2-min incubation at 25°C. The enzyme (40–50 μg of protein) was pre-incubated for 2 min. The reaction was initiated by adding 0.8 mM acetylthiocholine iodide (AcSCh). All samples were run in duplicate or triplicate and the enzyme activity was expressed in μmol AcSCh/h/mg of protein.

2.8. Blood sample collection

The blood was collected in vaccutainer tubes using EDTA as anticoagulant. The samples were hemolysed with phosphate buffer, pH 7.4 containing Triton X 100 (0.03%) and stored at −20 °C for one week.

2.9. Determination of AChE activity in whole blood

The AChE enzymatic assay was determined by the method of Ellmann et al. (1961) modified by Worek et al. (1999). The specific activity of whole blood AChE was calculated from the quotient between AChE activity and hemoglobin content and the results were expressed as mU/μmol of whole blood.

2.10. Statistical analysis

Statistical analysis of training and test step-down latencies was carried out by the Scheirer–Ray–Hare extension of the Kruskal–Wallis test (nonparametric two-way ANOVA). Foot shock sensitivity was analyzed by unpaired *t* test. AChE activity, blood glucose, body weight, crossing and rearing responses were analyzed by one-way ANOVA, followed by Duncan's multiple range tests. $P<0.05$ was considered to represent a significant difference in all experiments.

3. Results

3.1. Blood glucose and body weight

"The body weight and blood glucose levels determined at the onset and at the end of the experiment are presented in Table 1. As can be observed, the blood glucose levels at the onset of the study showed no significant differences among the groups. The blood glucose levels for the diabetic/saline group ($P<0.05$) were significantly increased when compared to the control/saline group at the end of the experiment. However, the treatment with resveratrol had no effects on glucose levels in diabetic/RV10 and diabetic/RV20 groups, which remained increased, when compared to the control/saline group. Similarly, no significant differences in glucose levels were observed when resveratrol was administered per se in control/RV10 and control/RV20 groups at the end of the study when compared to the control/saline group. In relation to body weight, no significant differences among the groups were observed at the onset of the experiment. In the diabetic/saline group a significant decrease ($P<0.05$) in body weight was observed when compared to the control/saline group at the end of the experiment. The treatment with resveratrol had no effects on body weight in diabetic/RV10 and diabetic/RV20 groups at the end of the study, which remained reduced in relation to the control/saline group. Treatment with resveratrol per se also had no effects on body weight in control/RV10 and control/RV20 groups when compared to the control/saline group at the end of the study."

3.2. Behavioral tests

3.2.1. Inhibitory avoidance

Fig. 1 shows the effect of administration of resveratrol per se (10 and 20 mg/kg), as well as its administration in streptozotocin-induced diabetic rats, on step-down latencies. Statistical analysis of testing (nonparametric two-way ANOVA) showed a significant diabetic or control vs resveratrol (10 and 20 mg/kg) or saline interaction

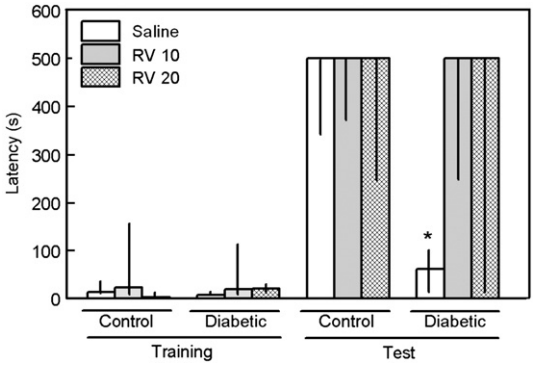

Fig. 1. Rats were made diabetic by streptozotocin. Control and diabetic rats were treated with resveratrol once daily i.p. for 30 consecutive days. After one treatment-free day animals were tested in a step-down latency test. Data are median ± interquartile of training and test. *$P<0.05$ compared with the others groups at testing by the Dunn's nonparametric multiple comparisons task. $n=6$–13.

($P<0.05$), revealing that treatment with resveratrol reversed the impairment of memory induced by diabetes. Statistical analysis of training showed no difference between groups.

Because motivational disparities in the training session may account for differences in inhibitory avoidance at testing, experiments were performed to assess whether diabetes or resveratrol affected shock threshold, or locomotor ability of the animals. Statistical analysis of open-field data (one-way ANOVA) revealed that pharmacological treatment did not alter the number of crossing ($P>0.05$) or rearing ($P>0.05$) responses in a subsequent open-field test session, suggesting that neither STZ-induced diabetes nor resveratrol caused gross motor disabilities at testing. Moreover, diabetes did not alter foot shock sensitivity, as demonstrated by the similar flinch (unpaired t test, $P=0.45$) and jump (unpaired t test, $P=1.57$) thresholds exhibited by the animals. These data suggest that neither the diabetic state nor treatment with resveratrol administered before training of inhibitory avoidance caused motor disabilities or altered foot shock sensitivity (data not shown).

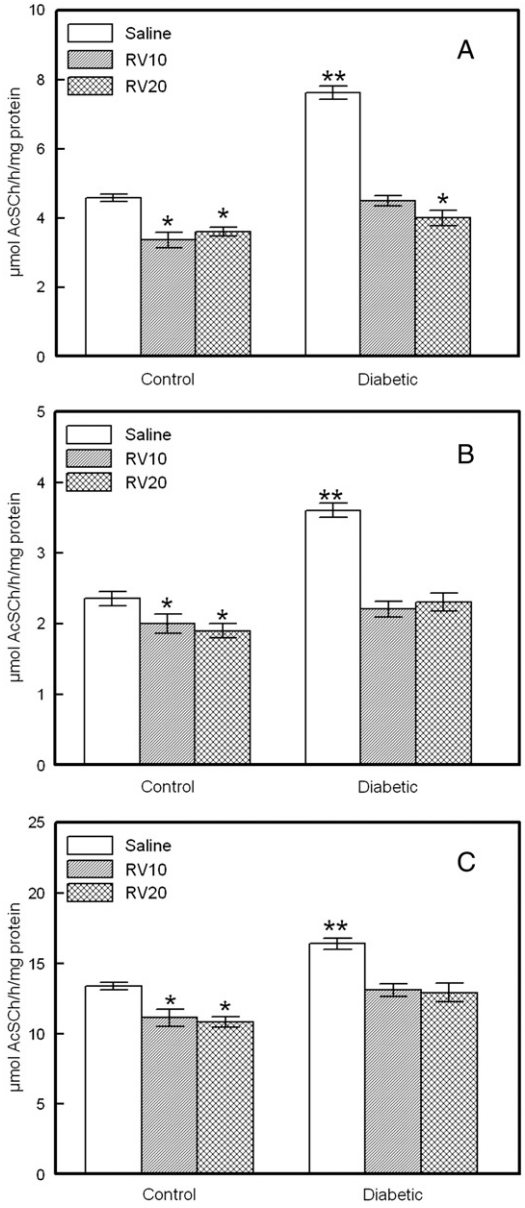

Fig. 2. AChE activity in cerebral cortex (A), hippocampus (B) and striatum (C) of streptozotocin-induced diabetic rats and those treated with resveratrol. Bars represent means ±S.E.M. (*$P<0.05$; *$P<0.01$ $n=6$–8). ANOVA–Duncan's Test.

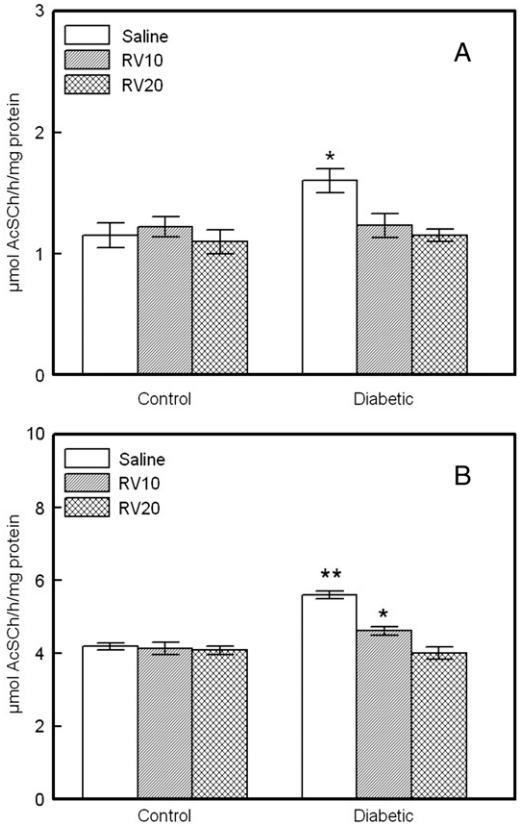

Fig. 3. AChE activity in cerebellum (A) and hypothalamus (B) of streptozotocin-induced diabetic rats and those treated with resveratrol. Bars represent means ±S.E.M. (*$P<0.05$; **$P<0.01$ $n=6$–8). ANOVA–Duncan's Test.

3.3. Activity of AChE in brain

The results obtained for AChE activity in cerebral cortex are presented in Fig. 2A. As can be observed, AChE activity was significantly increased in the diabetic/saline group ($P<0.05$) compared to the control/saline group. However, treatment with resveratrol significantly prevented the increase in AChE activity in diabetic/RV10 and diabetic/RV20 groups ($P<0.05$). On the other hand, treatment with resveratrol per se inhibited significantly AChE activity in control/RV10 and control/RV20 groups ($P<0.05$) when compared to control/saline group.

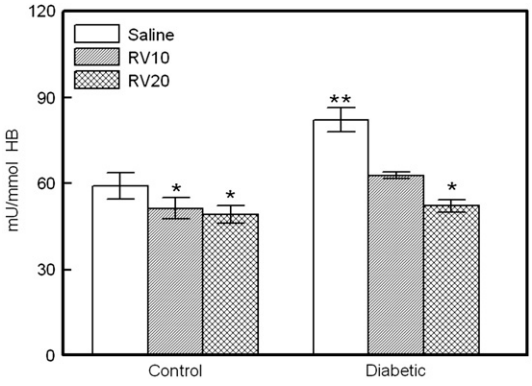

Fig. 4. AChE activity in whole blood of induced diabetic rats and those treated with resveratrol. Bars represent means ±S.E.M. (*$P<0.05$; **$P<0.01$ $n=6$–8). ANOVA–Duncan's Test.

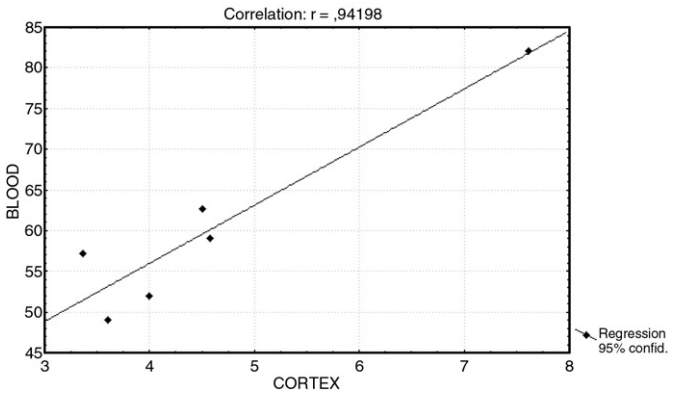

Fig. 5. Correlation between AChE activities obtained in cerebral cortex and whole blood ($r = 0.94198$, $P<0.05$). Pearson's correlation.

In relation to hippocampus, a significant increase in AChE activity in the diabetic/saline group ($P<0.05$) was observed when compared to the control/saline group (Fig. 2B). Treatment with resveratrol prevented this increase in AChE activity in diabetic/RV10 and diabetic/RV20 groups ($P<0.05$). In addition, treatment with resveratrol per se significantly inhibited AChE activity in control/RV10 and control/RV20 groups ($P<0.05$) when compared to the control/saline group.

The results obtained for striatum are presented in Fig. 2C. Similarly to cortex and hippocampus, a significant increase in AChE activity in the diabetic/saline group ($P<0.05$) was observed when compared with the control/saline group, while treatment with resveratrol prevented this rise in AChE activity in diabetic/RV10 and diabetic/RV20 groups ($P<0.05$). However, treatment with resveratrol per se significantly inhibited AChE activity in control/RV10 and control/RV20 groups ($P<0.05$) when compared to the control/saline group.

In cerebellum and hypothalamus the results were similar and can be observed in Fig. 3A and B, respectively. AChE activity in these two cerebral regions was significantly increased in diabetic/saline group ($P<0.05$) when compared to control/saline group. Treatment with resveratrol also prevented the increase in AChE activity in control/RV10 and control/RV20 groups ($P<0.05$). However, when resveratrol was given per se no significant differences in AChE activity were observed in control/RV10 and control/RV20 groups when compared to the control/saline group.

3.4. Activity of AChE in whole blood

Similarly to brain regions, AChE activity in blood was also increased in the diabetic/saline group ($P<0.05$) when compared to the control/saline group (Fig. 4). In addition, treatment with resveratrol prevented this rise in AChE activity in diabetic/RV10 and diabetic/RV20 groups ($P<0.05$). On the other hand, treatment with resveratrol per ser significantly inhibited AChE activity in control/RV10 and control/RV20 groups ($P<0.05$) when compared to control/saline group.

Analyzing only AChE activity in cerebral cortex and whole blood, there was statistically significant positive correlation ($r = 0.94189$, $P<0.05$) (Fig. 5).

4. Discussion

Diabetes mellitus is associated with cognitive dysfunctions which are paralleled by neurophysiological and structural changes in the CNS (Biessels et al., 2008). In addition, polyphenolic compounds have recently received considerable attention since they have been shown to protect neurons against a variety of experimental neurodegenerative conditions including cognitive deficits associated with diabetes (Baydas et al., 2003, 2004). Although some studies have investigated the neuroprotective actions of resveratrol in diabetes animal models (Ates et al., 2005; Kumar et al., 2007) the effects of this compound on cholinergic neurotransmission has not been reported in the literature. Thus, in the present study the effects of this polyphenol on memory and AChE activity were investigated in streptozotocin-induced diabetic rats.

The inhibitory avoidance test is a classic model behavioral test, with a strong aversive component, utilized for evaluating learning and memory in rats and mice (Cahill et al., 1986). In our study, we observed a significant decrease in step-down latency in diabetic rats in the inhibitory avoidance test (Fig. 1), suggesting learning and memory impairment in these animals. These results are in agreement with other studies that have also verified cognitive impairment in streptozotocin-induced diabetes mellitus (Biessels and Gispen, 2005; Kuhad et al., 2008). However, when the diabetic rats were treated with resveratrol (10 and 20 mg/kg) (Fig. 1) the step-down latency in the inhibitory avoidance test was similar to that found for rats from the control group. These findings indicate that treatment with resveratrol was able to prevent learning and memory impairment induced by diabetes.

A major concern in shock motivated learning tests, particularly in those that investigate the effect of drugs given before the acquisition of a given test, is whether pharmacological treatment affects locomotor activity or motivational aspects of learning, such as shock sensitivity. To rule out this possibility, we assessed locomotor behavior immediately after the inhibitory avoidance test session in order to identify any motor disability, which might influence inhibitory avoidance performance. Our results demonstrated that locomotor activity in the control, diabetic group and the diabetic group treated with resveratrol did not affect the number of crossing or rearing responses in the open-field session. We also demonstrated that streptozotocin-induced diabetic rats did not demonstrate altered shock sensitivity (data not shown). These data exclude the possibility that locomotor activity or shock sensitivity may have contributed to the change alteration in step-down latencies at testing in the inhibitory avoidance test in diabetic rats.

Although the exact mechanism through which diabetes alters cognitive functions is still not completely understood, it has been demonstrated that AChE has a fundamental role in learning and memory (Das et al., 2002, Sato et al., 2004) and alterations in its activity as well as in the acetylcholine neurotransmitter level are neurochemically associated with cognitive deficits observed in patients and in animal models of diabetes mellitus (Kuhad and Chopra, 2007; Ghareeb and Hussen, 2008).

In the present work, we observed an increase in AChE activity in diabetic rats in all brain regions evaluated (cerebral cortex, hippocampus, striatum, cerebellum and hypothalamus) (Figs. 2A,B,C,3A and B). However, this increase was less pronounced in cerebellum and hypothalamus. The lack of uniformity in the profile of AChE may be a reflection of the functional heterogeneity in the central cholinergic system. Neurons containing choline acetyltransferase are present at virtually all levels of the CNS. In any one region, all of the cholinergic neurons are usually similar in appearance, and in some cases, the neurons in different regions are similar. However, cholinergic neurons generally vary in appearance from region to region and this difference can be quite striking (Malik et al., 1998; Das et al., 2001). For example, the hippocampus and cerebral cortex receive cholinergic projections from de nucleus basalis of Meynert and the striatum, which has as intrinsic cholinergic circuit, presented similar results. On the other hand, the cerebellum and hypothalamus presented little cholinergic neuron, that can explained the low activity of AChE in relation to other structures (Das et al., 2001).

Similarly, Sanchez-Chavez and Salceda (2000) also observed a significant elevation in AChE activity in serum and cerebral cortex of streptozotocin-induced diabetic rats. Interestingly, AChE activation

leads to a fast acetylcholine degradation and a subsequent down stimulation of acetylcholine receptors causing undesirable effects on cognitive functions (Tõugu and Kesvatera, 1996; Soreq and Seidman, 2001). Based on our results we can suggest that the increase in AChE activity caused by diabetes leads to a reduction of cholinergic neurotransmission efficiency due to a decrease in acetylcholine levels in the synaptic cleft, thus contributing to progressive cognitive impairment and other neurological dysfunctions seen in diabetic patients. Furthermore, we may infer that the activator effect elicited by diabetic state on AChE activity could be one of the mechanisms involved on the memory impairment observed in the inhibitory avoidance test in this study as well as and other behavioral tests.

It is well recognized that the diabetic state results in altered membrane functions in several tissues including brain (Ghareeb and Hussen, 2008). These alterations occur due to an enhancement of free radical formation which promotes increased lipid peroxidation, having as major consequence oxidative deterioration of the cellular membranes (Halliwell and Chirico, 1993). AChE is a significant biological component of the membrane that contributes to its integrity and changes in permeability occurring during synaptic transmission and conduction. This enzyme is present in G4 (membrane bound) and G1 (cytosolic) form in different brain regions (Das et al., 2001). In the mammalian brain the G4 form represents 60–90% of the total AChE, depending on the anatomical region, the remainder is composed by G1 and G2 forms (Descarries et al., 1997). Additionally, alterations in the lipid membrane observed during the diabetic state could be a decisive factor in the modification of the conformational state of the AChE molecule and would explain changes activity this enzyme (Das et al., 2001; Aldunate et al., 2004). Based on these observations, we can suggest that AChE activation found in diabetes mellitus may be mediated by free radical production and consequent oxidative stress in the different brain regions.

In this study, treatment with resveratrol in doses of 10 and 20 mg/kg was able for preventing the increase in AChE activity in all cerebral structures evaluated in diabetic rats (Figs. 2A,B,C,3A and B). These results are similar to those found in studies with other antioxidants such as vitamin E (Tuzcu and Baydas, 2006), curcumin (Kuhad and Chopra, 2007) and lycopene (Kuhad et al., 2008) that also prevented the rise in AChE activity and consequently in cognitive deficits induced by the diabetic state. In line with this, we can suggest that the antioxidant property of resveratrol may be responsible for preventing cholinergic dysfunction in diabetic rats. In fact, several studies have shown that resveratrol protects against oxidative stress, decreasing membrane lipid peroxidation and increasing the antioxidant defensive capacity in diabetic animal brain (Ates et al., 2005; Kumar et al., 2007).

One important aspect to be discussed in this study is that resveratrol *per se* (10 and 20 mg/kg) was capable of inhibiting AChE activity in cortex cerebral, hippocampus and striatum, which are structures rich in cholinergic pathway (Fig. 2A,B and C). AChE inhibitors are an important therapeutic target for the treatment of many neurological diseases. These compounds increase the efficiency of cholinergic transmission by preventing the hydrolysis of released ACh by inhibition of AChE, thus making more ACh available at the cholinergic synapse (Benzi and Morreti, 1998; Grisaru et al., 1999; Das et al., 2002). In this line, we suggest that a decrease of AChE activity by resveratrol can contribute for increasing levels of ACh and consequently to improve the cognitive functions, such as learning and memory (Mesulam et al., 2002; Kaur et al., 2007), suggesting an interaction between resveratrol and the neurotransmission cholinergic.

In addition to its antioxidants properties, the biological role of resveratrol may also be related to several other properties (Ovesna and Horvathova-Kozics, 2005). Resveratrol has structural similarity with estrogens and this could explain its estrogenic effect as well as, at least in part, its anti-inflammatory activity, by binding to estrogens receptors (Jannin et al., 2004). In agreement with these findings, it has been reported by Nizri et al. (2005, 2006) that AChE inhibitors possess anti-inflammatory properties that promote cholinergic up-regulation by reducing lymphocyte proliferation and the secretion of pro-inflammatory cytokines. In this context, we can suggest that, resveratrol besides possessing antioxidant and anti-inflammatory properties also inhibits AChE activity as shown in our study, combining thus different functions in a single molecule.

In relation to AChE activity in the whole blood, the results were similar with those obtained in the cerebral regions. In fact, the correlation data demonstrated similar behavior between cerebral cortex and blood AChE activity (Fig. 5). Supporting these findings, it was reported by Bernhardi et al. (2005) that the concentration of AChE in whole blood is potentially a stable biomarker for the study of neurodegenerative disorders. In addition, it was reported by Thiermann et al. (2005) that whole blood could have similar functional properties as synaptic AChE and therefore may reflect the status at the synaptic site. In this context, we can infer that AChE activity in blood could be a good peripheral marker because it permits evaluation through the more accessible methods of the action of this enzyme in CNS.

In conclusion, the results from the present study demonstrated impairment in memory and learning in diabetic rats, which was coupled with a marked increase in AChE activity in all brain structures. In addition treatment with resveratrol was able to prevent the increase in AChE activity and consequently in cognitive impairment in diabetic rats, demonstrating that this compound can modulate cholinergic neurotransmission and consequently improves cognition. These results may contribute to a better understanding of the neuroprotective role of resveratrol, emphasizing the influence of this polyphenol and other antioxidants in the diet for human health, possibly preventing brain disorders associated with cognitive impairments such as diabetes mellitus.

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