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Research article

An evaluation of aversive memory and hippocampal oxidative status in streptozotocin-induced diabetic rats treated with resveratrol

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HIGHLIGHTS

- Diabetic rats exhibited normal freezing response in contextual fear conditioning.
- Hippocampal oxidative status was unaltered in diabetic rats.
- Resveratrol oral treatment had no significant effects in healthy or diabetic rats.

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ABSTRACT

The present study evaluated the effects of streptozotocin (STZ)-induced diabetes on aversive memory, free radical content and enzymatic antioxidant activity in the hippocampus of adult *Wistar* rats submitted to oral treatment with resveratrol. Animals were divided into eight groups: non-diabetic rats treated with saline (ND SAL), non-diabetic rats treated with resveratrol at a dose 5 mg/kg (ND RSV 5), non-diabetic rats treated with resveratrol at a dose 10 mg/kg (ND RSV 10), non-diabetic rats treated with resveratrol at a dose 20 mg/kg (ND RSV 20), diabetic rats treated with saline (D SAL), diabetic rats treated with resveratrol at a dose 5 mg/kg (D RSV 5), diabetic rats treated with resveratrol at a dose 10 mg/kg (D RSV 10) and diabetic rats treated with resveratrol at a dose 20 mg/kg (D RSV 20). The animals received oral gavage for 35 days. The contextual fear conditioning task was performed to evaluate aversive-based learning and memory. The oxidative status was evaluated in the hippocampus, by measuring the free radical content – using a 2′,7′-dichlorofluorescein diacetate probe – and enzymatic antioxidant activities, such as superoxide dismutase and glutathione peroxidase. Our main behavioral results demonstrated that rats from the D RSV 10 and D RSV 20 groups showed an increase in freezing behavior when compared, respectively, to the ND RSV 10 ($p < 0.01$) and ND RSV 20 ($p < 0.05$). Oxidative stress parameters remained unchanged in the hippocampus of all the experimental groups. In contrast to previous experimental findings, our study was unable to detect either cognitive impairments or oxidative stress in the hippocampus

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of the diabetic rats. We suggest additional long-term investigations be conducted into the temporal pattern of STZ-induced diabetic disruption in memory and hippocampal oxidative status, as well as the effects of resveratrol on these parameters, in a time and dose-dependent manner.

1. Introduction

Diabetes mellitus comprises a group of metabolic disorders characterized by hyperglycemia resulting from defects in insulin secretion and/or insulin insensitivity [1]. Recent clinical studies have confirmed that diabetes is associated with cortical atrophy, white matter lesions and the development of cognitive dysfunctions, which include impaired attention, intelligence, psychomotor speed and psychomotor efficiency [2,3]. However, learning and memory deficits were not observed in all clinical studies [4–6]. Therefore, it has been suggested that such clinically relevant cognitive deficits might be related to the beginning of diabetes in two specific periods in life: during brain development in childhood, and when the brain undergoes neurodegenerative changes associated with ageing [7].

Despite differences between clinical data, deficient insulin signaling in the brain and hyperglycemia are considered potential contributors to the development of neurological complications in diabetes [3,8,9]. It has been reported that insulin participates in the regulation of apoptosis and oxidative status in the central nervous system (CNS), which could explain the link between disruption in insulin activity and neuronal and oligodendroglial degeneration in the diabetic brain [8,10]. Hyperglycemia could raise free radical formation in the CNS through different mechanisms, including increased glycolysis, activation of the polyol pathway and increased formation of advanced glycation end-products (AGEs) [11,12]. Moreover, reduced antioxidant activity has been observed in diabetic rodents, which together with the above-mentioned evidence may contribute to oxidative stress and to the development of cognitive dysfunctions in diabetes [3,8,13]. Nevertheless, some studies present contrasting findings, with no significant indications of learning and memory deficits, nor significant alterations in some parameters related to oxidative stress in the diabetic rodent brain, especially in the hippocampus [14–16].

Resveratrol, a polyphenolic compound found in peanuts, some berries, grapes and wine, is known to have beneficial health effects [17]. This compound presents antioxidant activities in the CNS, demonstrated by the ability to scavenge free radicals and up-regulate antioxidant enzymes [18]. Additionally, studies have suggested resveratrol produces benefits in the CNS of diabetic rodents, protecting them from memory impairment, reducing the release of pro-inflammatory factors and reestablishing normal antioxidant enzyme levels [19,20].

The aim of this study was to verify the effects of streptozotocin (STZ)-induced diabetes on aversive memory, free radical content and enzymatic antioxidant activity in the hippocampus of adult *Wistar* rats, and evaluate the influence of oral treatment with resveratrol on these parameters.

2. Material and methods

2.1. Animals

All procedures were conducted in accordance with the University guidelines, and were previously approved by the animal ethics committee of the University. Sixty-two male *Wistar* rats obtained from the *Centro de Reprodução e Experimentação de Animais de Laboratório* (CREAL, *Universidade Federal do Rio Grande do Sul* – UFRGS), aged 12 weeks at the start of the experiment, were used. They were maintained under standard laboratory conditions, with free access to rat chow and water and a 12:12 light/dark cycle (lights on from 08:00 to 20:00 h). The animals were randomly divided into eight groups, as follows: non-diabetic rats treated with saline (ND SAL), non-diabetic rats treated with resveratrol at a dose of 5 mg/kg body weight (ND RSV 5), non-diabetic rats treated with resveratrol at a dose of 10 mg/kg body weight (ND RSV 10), non-diabetic rats treated with resveratrol at a dose of 20 mg/kg body weight (ND RSV 20), diabetic rats treated with saline (D SAL), diabetic rats treated with resveratrol at a dose of 5 mg/kg body weight (D RSV 5), diabetic rats treated with resveratrol at a dose of 10 mg/kg body weight (D RSV 10) and diabetic rats treated with resveratrol at a dose of 20 mg/kg body weight (D RSV 20). For the behavioral task, 7 to 8 rats per group were analyzed. For the biochemical analyses, 5 to 6 rats per group were used. A timeline with our experimental design can be seen in Fig. 1.

2.2. Diabetes induction

After an overnight fasting period, diabetes was induced by a single intraperitoneal (i.p.) injection of STZ (65 mg/kg of body weight) diluted in 0.1 M sodium-citrate buffer, pH 4.5. The non-diabetic rats received an equivalent amount of sodium-citrate buffer. 72 h after i.p. injections, blood glucose levels were measured in blood collected from the rat tail using a portable glucometer (On Call Plus, ACON Laboratories, USA). Only animals with blood glucose levels >300 mg/dL and symptoms of polyuria and polydipsia were considered diabetic and selected for the present study. During the experiment, the blood glucose levels of all the animals were verified at four moments: D1 (before i.p. injections of STZ and/or vehicle), D4 (72 h after i.p. injections), D30 and D64.

2.3. Oral gavage

From D30 to D64, oral treatment was provided to all groups once a day, between 10:00 and 11:00 a.m., totaling 35 days of treatment. Resveratrol was freshly dispersed in 0.9% saline solution and promptly administered via oral gavage to animals belonging to the ND RSV groups and the D RSV groups, in their respective doses, based on previous studies [14,21]. Animals from the ND SAL and D SAL groups received equal volumes of 0.9% saline solution alone. Resveratrol was stored at 5 °C in an amber flask, protected from light. The body weights of all the animals were verified on D1, D4 and D30 and, in order to better control the resveratrol dose, from D30 to D64, they were verified twice a week (on Mondays and Thursdays).

2.4. Contextual fear conditioning (CFC)

At D63, each rat was placed in a chamber (25.0 × 25.0 cm grid of parallel 0.1 cm caliber stainless steel bars, spaced 1.0 cm apart). In the training session, rats were placed in the chamber during 3 min for habituation, and after received two 2-s foot shocks of 0.7 mA, separated by an interval of 30 s. After the last foot shock, animals were kept in the conditioning chamber an additional minute, and