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Research report

Effect of tempol on the passive avoidance and novel object recognition task in diabetic rats

Zahra Jabbarpour[a], Siamak Shahidi[a,*], Massoud Saidijam[b], Abdolrahman Sarihi[a], Taghi Hassanzadeh[c], Rasoul Esmaeili[d]

[a] Neurophysiology Research Center, Hamadan University of Medical Sciences, Hamadan, Iran
[b] Research Center for Molecular Medicine, Hamadan University of Medical Sciences, Hamadan, Iran
[c] Department of Clinical Biochemistry, School of Medicine, Hamadan University of Medical Sciences, Hamadan, Iran
[d] School of Medicine, Hamadan University of Medical Sciences, Hamadan, Iran

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ABSTRACT

Diabetes mellitus (DM) has several effects, including cognitive impairment. Oxidative stress is associated with complications from diabetes. It seems that antioxidants can reduce some complications of the diabetes induced by oxidative stress. The objective of this study was to evaluate the effect of synthetic antioxidant, tempol on the passive avoidance (PA) memory and novel object recognition (NOR) tests in the diabetic rats. Forty male Wistar rats randomly divided into the control, diabetic, diabetic receiving tempol and healthy receiving tempol groups. Diabetes was induced by injection of streptozotocin (STZ) (60 mg/kg, i.p.). Then, the rats received saline or tempol (30 mg/kg) orally by gavages for 60 days. After that, they were assessed using the PA memory and NOR tests. The results of NOR test showed that the discrimination index (DI) in the healthy receiving tempol group and diabetic control group was significantly lower than control group. Also the amount of this index in diabetic receiving tempol group was significantly higher than diabetic group. The results of PA test indicated that the number of trials to acquisition in the diabetic rats is significantly more than control and diabetic tempol treated groups. Also, the time spent in the dark compartment (TDC) in the control and diabetic receiving tempol groups was less than diabetic group. TDC in the healthy receiving tempol group was more than control group. It can be concluded that although use of tempol is restricted as a cognitive enhancer in non-diabetic subjects but long-term administration of synthetic antioxidant, tempol, is able to dramatically improve diabetes-induced learning and memory deficit in both PA and NOR tests.

1. Introduction

Diabetes mellitus (DM) is an endocrine disorder of carbohydrate metabolism resulting from impaired insulin secretion [Type I insulin-dependent diabetes mellitus], resistance to insulin action, or both [Type II non-insulin-dependent diabetes mellitus] (Balakrishnan et al., 2009). Diabetes is associated neurological complications in both the peripheral and central nervous system (Baydas et al., 2003b; Di Luca et al., 1999). Passive avoidance learning (PAL) and memory impairment also occur in streptozotocin (STZ)-induced diabetic rats (Baydas et al., 2003a,b; Kucukatay et al., 2007; Lupien et al., 2003; Patil et al., 2006). Learning deficits in diabetic rats have been partly associated with the structural and functional deficits in certain brain regions such as the hippocampus and cerebral cortex. For example, changes in hippocampal synaptic plasticity have been reported in diabetes (Baydas et al., 2003b; Biessels et al., 1994; Tuzcu and Baydas, 2006). DM has been reported to specifically impair the memory performance in experimental animals with powerful involvement of hippocampus and cerebral cortex. This finding may show impairment of acquisition and/or consolidation of memory (Flood et al., 1990). Hyperglycemia induced by diabetes is usually accompanied by increased production of free radicals and reactive oxygen species (ROS), (Baynes, 1991) or impaired antioxidant defenses (Chang et al., 1993; Halliwell and Gutteridge, 1990; Young et al., 1995). Vitamin C and vitamin E were found to reduce oxidative stress; in addition vitamin C can enhance learning and memory and prevent memory deficits in various experimental conditions (Delwing et al., 2006; Hasanein and Shahidi, 2010; Landmark, 2006; Monteiro et al., 2005; Parle and Dhingra, 2003; Reis et al., 2002; Shahidi et al., 2008a). In addition, clinical studies suggest that vitamin E may be a supplemental intervention for patients with cognitive

* Corresponding author at: Neurophysiology Research Center, Hamadan University of Medical Sciences, Hamadan, Iran. Tel.: +98 811 8380462x337; fax: +98 811 8380208.
E-mail address: siamakshahidi@yahoo.com (S. Shahidi).

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dysfunction (Chan et al., 2004; Mecocci et al., 2004). Tempol (4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl) is a member of a family of nitroxide compounds that has been studied extensively in animal models of increased ROS. Tempol is a superoxide dismutase (SOD) mimetic. It is an efficient scavenger of free radicals and improved insulin responsiveness in models of diabetes mellitus and improved the dyslipidemia (Wilcox, 2010). However, at present, no specific treatment options are available for the management of cognitive deficits induced by diabetes (Biessels et al., 2007). According to these reports so far it has not been investigated tempol effect on improves the neurological complications induced by diabetes, we examined whether treatment with tempol could protect against learning and memory deficits in diabetic rats with using PA and NOR? These tests are useful as a screen for testing new drugs and antioxidants which may alter memory processes such as diabetes (Hasanein and Shahidi, 2010; Jurdak and Kanarek, 2009; Raghavendra and Kulkarni, 2001).

2. Materials and methods

2.1. Animals

In this study, forty adult male Wistar rats with the body weight of 200–250 g were used in which they purchased from the breeding colony of Iran Pasteur Institute, Tehran. The animals were put in separate cages under 12-h light:12-h darkness periods and were fed by some standard food pellets and water ad libitum. All procedures for the treatment of animals were approved by the research committee of the Hamadan University of Medical Sciences.

2.2. Induction of diabetes and treatment

In this study, the animals were divided into the following groups as Control [C] ($n = 10$), Diabetic [D] ($n = 10$), Diabetic tempol recipients [D + T] ($n = 10$), and Control group receiving Tempol [C + T] ($n = 10$).

This experimental model of rats made diabetic with streptozotocin (STZ) injection has been validated in previous studies (Arison et al., 1967; Haider et al., 2013; Hohenegger and Rudas, 1971; Tuzcu and Baydas, 2006). Diabetes disease was induced by only a single intra peritoneal (i.p.) injection of STZ (60 mg/kg body weight) which was prepared by citrate buffer, pH 4.5 (Arison et al., 1967; Hasanein and Shahidi, 2010; Hohenegger and Rudas, 1971). The control rats received i.p. injections of physiological saline. The fasting blood glucose levels were determined three days after STZ injection by using a strip-operated blood glucose sensor (Accuchek; Roche, Mannheim, Germany). Animals were considered diabetic if plasma glucose levels exceeded 250 mg/dl.

Finally, tempol (Sigma) was fed to [D + T] and [C + T] groups by the gavage process at a dose of 30 mg/kg per day for 60 days. The control and non-treated diabetes groups received the physiological saline with the same volume during this time.

Behavioral test (PAL and NOR tests) were conducted respectively on 2 and 3 consecutive days at the same time of the day between 12:00 and 3:00 PM.

2.3. Behavioral tests

2.3.1. Novel object recognition (NOR) test

The object recognition task is a working memory task that primarily relies on cortical functioning and to a lesser extent, hippocampal functioning. The utilized set-up consists of a cubic open arena (50 cm × 45 cm × 35 cm) and a video recording system. During the first day, the rats were given one habituation session (5 min) in the arena without any object. On the second day, two identical objects were placed close to (10 cm) two adjacent corners of arena. One rat was then placed in the middle of the box and allowed to explore the two objects for 5 min. Exploration process of an object was defined as smelling the object. During the third day, one of the objects was replaced with a novel object and the rat was put back into the open field for a 5-min test session. The rat response to the novel object was assessed by subtracting the mean exploration time of the familiar object from the mean exploration time of the novel object (Akirav and Maroun, 2006; Broadbent et al., 2004; Ennaceur et al., 1997).

2.3.2. Passive avoidance learning (PAL) test

In this study, a passive avoidance apparatus and a procedure similar to our previous studies were used (Hasanein and Shahidi, 2010; Lashgari et al., 2006; Shahidi et al., 2008a,b; Shahidi et al., 2004). This step-through apparatus had a bright chamber (20 cm × 20 cm × 30 cm) made of transparent plastic and a dark chamber with walls made of dark opaque plastic (20 cm × 20 cm × 30 cm). It was also used stainless steel rods (3 mm diameter) with the distance of 1 cm from each other for the floor of the chambers. A shock generator was used for electrifying the floor of the dark chamber and a rectangular opening (6 cm × 8 cm) was located between the two chambers and could be closed by an opaque guillotine door.

2.3.2.1. Training. In order to habituate the groups to the apparatus, they were given two habituation trials. At first, the rats were entered into a lighted section of the apparatus and 5 s later the guillotine door was opened. Because of the natural tendency of the rats to the dark environment, they were trying to enter the dark compartment. The door was closed upon the rats entering the dark compartment and after 30 s they were taken from the dark compartment and placed in their cage. After 30 min this trial was repeated and then the acquisition trial was carried out after the same interval. The measurement of the entrance latency to the dark compartment (step-through latency, STLa) was carried out after the rats had completely entered the dark compartment. Then, the door was closed and an electrical shock was used (0.9 mA) for 2.5 s and the experimental rat was returned to its cage after 30 s. This procedure was repeated again after 2 min. For the next stages, the foot-shock process was applied for the rat after it reentered the dark and had placed all four paws in the dark compartment. Finally, the training method of the experimental rats was terminated when they remained continuously in the bright compartment for 120 s and the number of entries into the dark chamber to acquisition was recorded.

2.3.2.2. Retention test. Exactly 24 h after performing the PAL acquisition trial, the retention test was performed. In this step, like the PAL training trial, the rat was placed in the light chamber and after 5 s, the guillotine door was opened in order to start the recording process of the step-through latency (STLr) and the spending time in the dark compartment (TDC) for up to 300 s. If the rat does not enter the dark chamber within 300 s, the retention test is terminated and a ceiling score of 300 s was recorded.

2.4. Statistical analysis

One-way ANOVA was used to determine the statistical significance of differences between experimental groups which were followed by Tukey as post hoc test. The obtained probability values less than 0.05 were considered significant and the data were expressed as the mean ± SEM.