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Carnosine ameliorates cognitive deficits in streptozotocin-induced diabetic rats: Possible involved mechanisms

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ABSTRACT

Diabetic patients are at increased risk to develop cognitive deficit and senile dementia. This study was planned to assess the benefits of chronic carnosine administration on prevention of learning and memory deterioration in streptozotocin (STZ)-diabetic rats and to explore some of the involved mechanisms. Rats were divided into 5 groups: i.e., control, carnosine100-treated control, diabetic, and carnosine-treated diabetics (50 and 100 mg/kg). Carnosine was injected i.p. at doses of 50 or 100 mg/kg for 7 weeks, started 1 week after induction of diabetes using streptozotocin. Treatment of diabetic rats with carnosine at a dose of 100 mg/kg at the end of the study lowered serum glucose, improved spatial recognition memory in Y maze, improved retention and recall in elevated plus maze, and prevented reduction of step-through latency in passive avoidance task. Furthermore, carnosine at a dose of 100 mg/kg reduced hippocampal acetylcholinesterase (AChE) activity, lowered lipid peroxidation, and improved superoxide dismutase (SOD) activity and non-enzymatic antioxidant defense element glutathione (GSH), but not activity of catalase. Meanwhile, hippocampal level of nuclear factor-kappaB (NF-κB), tumor necrosis factor α (TNF-α), and glial fibrillary acidic protein (GFAP) decreased and level of nuclear factor (erythroid-derived 2)-like 2 (Nrf2) and heme oxygenase 1 (HO-1) increased upon treatment of diabetic group with carnosine at a dose of 100 mg/kg. Taken together, chronic carnosine treatment could ameliorate learning and memory disturbances in STZ-diabetic rats through intonation of NF-κB/Nrf2/HO-1 signaling cascade, attenuation of astrogliosis, possible improvement of cholinergic function, and amelioration of oxidative stress and neuroinflammation.

1. Introduction

Diabetes mellitus (DM) is a disease of glucose homeostasis with various chronic complications and a high incidence of morbidity and mortality and the fastest growing non-communicable disorder worldwide due to improper lifestyle habits [41]. DM has been closely linked to complications in the peripheral and central parts of the nervous system [60]. Neuropathy in diabetic patients is one of the common and unresolved complications [75] and affected individuals are at increased risk to develop several forms of cognitive dysfunction and senile dementia that is also a characteristic phenotype of Alzheimer's disease [65]. Chronic DM in rodents impairs hippocampus-dependent synaptic plasticity with ensuing deficit of learning and memory [45]. Enhanced oxidative stress with concomitant weakening of antioxidant defensive system [59,64], development of an inflammation and astrogliosis, and enhanced activity of the enzyme acetylcholinesterase (AChE) [3,58] are considered important pathogenic factors responsible for cognitive deterioration in DM. Potential protective approaches are strongly required for such problems in DM [10].

In recent years, some research trends have directed towards the possible benefits of natural antioxidants in lowering devastating complications of DM in experimental animals and patients [64,71,80,99]. Carnosine is a dipeptide compound that is mainly found in mammalian skeletal muscle and synthesized by carnosine synthase from the substrates beta-alanine and histidine [25]. Pilot intervention data has indicated its supplementation may improve

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impaired glucose tolerance and it may be of potential benefit for prevention or retarding type 2 diabetes [23]. Carnosine treatment could prevent renal damage in diabetic condition [108] and its supplementation lowers triglyceride level and plaque instability in DM-associated atherosclerosis [15]. In addition, carnosine could improve microvascular circulation by increasing erythrocyte deformability and suppressing lipid peroxidation [105]. Oral carnosine treatment is able to protect retinal microvasculature in diabetic retinopathy [77] and to inhibit pro-apoptotic cascade [79]. Furthermore, carnosine administration could reduce hypoxia-ischemic brain damage and improve brain function [109]. Neuroprotective effect of carnosine in a model of unilateral common carotid arteries occlusion has been attributed to suppression of oxidative stress, glia activation, and myelin degeneration [57]. Carnosine can also protect against NMDA-induced necrosis in PC12 cells [89] and to exert neuroprotection against 6-hydroxydopamine neurotoxicity [1]. Carnosine reduces $Ab_{1\text{-}42}$ aggregation and lowers fibril formation [2] and ameliorates cognitive deficits [19]. A previous report has suggested that carnosine released from muscles following exercise reduces blood glucose level through affecting autonomic nerves [67]. Therefore, this study was planned to judge the benefits of chronic carnosine administration on prevention of learning and memory deterioration in streptozotocin (STZ)-diabetic rats and to explore in more depth some of the involved mechanisms.

2. Material and methods

2.1. Experimental design

Male albino Wistar rats (procured from Shaheed Beheshti Univ. Med. Sci. animal facility (Tehran), 8–10 weeks old, 185–240 g) were housed in an animal house (21–23 °C, 40–55% humidity, and 12:12 h lighting period) with free access to food and water. Applied maneuvers were in line with NIH guidelines for the care and use of laboratory animals and were approved by Ethics Committee of Shahed University (Tehran, Iran) in 2014. The rats (n = 50) were randomly divided into 5 equal-sized groups, i.e. control, carnosine100-treated control, diabetic, and carnosine50- and carnosine100-treated diabetics. Diabetes was induced by a single *i.p.* injection of streptozotocin (STZ; 60 mg/kg; SigmaAldrich, USA) dissolved in cold normal saline immediately before use [30,81]. Seven days post-STZ injection, overnight fasting blood samples were collected under diethyl ether anesthesia and serum glucose concentration was measured (glucose oxidation method, ParsAzmun, Tehran). Animals with a serum glucose level over 250 mg/dl were selected for treatment. During the following weeks, symptoms of diabetes including polydipsia, polyphagia, polyuria, and weight loss were observed. L-carnosine (SigmaAldrich, USA) was injected *i.p.* (dissolved in normal saline) at doses of 50 and/or 100 mg/kg/day, started 8 days post-STZ injection for 7 weeks. Dose of carnosine at the used range (50–100 mg/kg) was selected from previous studies on its protective effect in alcohol-induced hepatic injury in rats [9] and on its effect in enhancing diabetic wound healing in the db/db mouse model of type 2 diabetes [5]. Body weight was measured every week and serum glucose level was determined two days before STZ injection and on days 28 and 56 post-STZ injection. All behavioral tests were conducted during the light on phase (09 a.m. till 03 p.m.) by a trained experimenter that was blind to treatments. Behavioral tests including Y-maze, elevated plus maze, and passive avoidance task were conducted at 8th week.

2.2. Y-maze task

Y-maze task is a valid and non-invasive behavioral test to study spatial recognition memory in rodents including rats via assessment of spontaneous alternation [45]. The maze consisted of three arms and a triangular central arena. All animals were checked once. Rats were placed at the end of one arm and allowed to move through the maze for a period of 8 min. An arm entrance was counted when the hind paws of the rat were fully in the arm. Alternation was considered as successive entrances into the three arms on overlapping triplets (i.e. A, B, C or B, A, C, etc.). The number of maximum spontaneous alternation was then the total number of arms entrances subtracted from 2 and its percentage was the ratio of actual to possible alternations. In addition, total number of arm entries was used as an index of motor activity. The maze was cleaned with 70% alcohol between sessions to diminish compounding odor cues. Y-maze task was conducted on day-36 post-STZ injection.

2.3. Elevated plus maze task

Memory acquisition and retention and recall capability was assessed using this task, as reported before [86]. The apparatus composed of two crossed arms, a closed and an open ones. Each animal was placed on the open arm, facing outwards. The time taken by the animal to enter into the closed arm in the first trial (acquisition trial) on the first day was recorded as initial transfer latency. Cut-off time was 90 s and in case a rat did not move into the closed arm within this period, it was gently guided into one of the closed arms and allowed to explore the maze for further 30 s. Second trial (retention and recall trial) was conducted 24 h after the acquisition trial and retention transfer latency was recorded. The latter was reported as a percentage of initial trial latency.

2.4. Passive avoidance task (shuttle box)

This task was performed in accordance to a previous study with some modifications [83] using the shuttle box apparatus (BPT Co., Tehran). It was made of two chambers, i.e. one light and one dark with a grid floor and interconnected through a guillotine door. Electric shock was delivered by an isolated stimulator. On the first and second days, each rat was placed into the light chamber and allowed to explore the chambers for 5 min to be adapted. During the acquisition trial (third day), an animal was placed in the light chamber and after a 5 min habituation period, the guillotine door was opened and the latency to enter the dark chamber (the initial latency or IL) was recorded. After rat entrance into the dark chamber, the door was lowered and an electric shock (1 mA, 1 s) was applied. Rats with ILs more than 60 s were excluded from the study. Twenty-four hours later, retention and recall trial was done and time needed for rat entrance into the dark chamber was recorded as step-through latency (STL up to a maximum cut-off time of 480 s).

2.5. Determination of hippocampal oxidative stress

At the end of week 8 post-STZ injection, hippocampal tissue (n = 6 from each group) was punched out and 10% homogenate was prepared in lysate buffer in the presence of protease inhibitor cocktail (SigmaAldrich, USA) and the supernatant was aliquoted and stored at −80 °C.

Malondialdehyde (MDA) level as a marker of lipid peroxidation was determined as described before [48,71]. For determination of MDA concentration (thiobarbituric acid reactive substances, TBARS), trichloroacetic acid and TBARS reagent were added to supernatant, then mixed and incubated at boiling water for 90 min. After cooling on ice, samples were centrifuged at $1000 \times g$ for 10 min and the absorbance was read at 532 nm and its final value was obtained from tetraethoxypropane standard curve.

For catalase activity assay, Claiborne's method was applied [18]. Briefly, H2O2 was added to a mixture of 50 mM potassium phosphate buffer (pH 7.0) and supernatant and the rate of H2O2