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Exenatide reduces TNF-α expression and improves hippocampal neuron numbers and memory in streptozotocin treated rats

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ABSTRACT

Recent studies suggest a possible link between type 2 diabetes and Alzheimer's disease (AD). Glucogan-like peptide 1 (GLP-1) facilitates insulin release from pancreas under hyperglycemic conditions. In addition to its metabolic effects, GLP-1 and its long-lasting analogs, including exenatide can stimulate neurogenesis and improve cognition in rodent AD model. The aim of the present study was to investigate the effects of exenatide on hippocampal cellularity, cognitive performance and inflammation response in a rat model of AD. Fourteen rats were used to create AD model using intracerebroventricular (ICV) streptozotocin (STZ) infusion while 7 rats were administered 0.9% NaCl only (sham-operated group). Following stereotaxic surgery, STZ received rats were randomly distributed into two groups, and treated with either saline or exenatide 20 μgr/kg/day through intraperitoneally for two weeks. Then, cognitive performance (passive avoidance learning), brain tumor necrosis factor alpha (TNF-α) levels, choline acetyltransferase (ChAT) activity and hippocampal neuronal count were determined. While the brain TNF-α levels were significantly high in the saline-treated STZ group, exenatide treatment suppressed the increase in TNF-α levels. Saline-treated STZ group showed reduced ChAT activity compared to sham group. However, exenatide significantly preserved brain ChAT activity. The cognitive performance was also impaired in saline group while exenatide improved memory in rats. Moreover, exenatide treatment significantly prevented the decrease in hippocampal neurons. Overall, the results of the present study clearly indicated exenatide might have beneficial effects on impaired cognitive performance and hippocampal neuronal viability in AD by suppressing the inflammation response and increasing cholinergic activity.

1. Introduction

In recent years, a growing body of evidence suggests a link between type 2 diabetes and Alzheimer's disease (AD) (Schrijvers et al., 2010). It has been demonstrated that insulin receptors in the brain are desensitized, and the insulin signal to stimulate cell metabolism and cell repair seems to be impaired in AD patients. This condition has been referred 'type 3 diabetes' by some authors (Kroner, 2009). The loss of insulin signaling in the brain can play a role in the pathogenesis of neurodegenerative disorders such as AD and Parkinson's disease (PD). The impairment of neuronal insulin signaling may trigger the intracellular accumulation of Aβ and hyperphosphorylation of tauprotein, which are both main pathological features of AD (Hoyer, 2004). Neuronal aggregates of tau molecules, as well as Aβ oligomers, are neurotoxic and can significantly impair cognitive performance (Querfurth and LaFerla, 2010).

Peripheral injection of the streptozotocin (STZ), a glucosamine–nitrosurea compound, selectively damages pancreatic β-cells, and thus, it is generally used to establish the animal model of diabetes mellitus (Plaschke and Hoyer, 1993; Lester-Coll et al., 2006; Kraska et al., 2012; Nazem et al., 2015). Also, intracerebroventricular (ICV) injection of streptozotocin (STZ), to rodents has been reported as an suitable model for AD and, characterized by a impaired neuronal plasticity and learning deficits, which has been widely confirmed in the literature. While peripherally injected STZ does not cross the blood–brain barrier, ICV injection leads to impaired brain insulin signaling in rodents (Plaschke and Hoyer, 1993; Lester-Coll

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et al., 2006; Kraska et al., 2012; Nazem et al., 2015).

The incretine hormone glucogan-like peptide 1 (GLP-1) is secreted from intestinal L cells and facilitates insulin release from pancreas under hyperglycemic conditions. Also, GLP-1 appears to be an important regulator of appetite and food intake. Because of these actions, GLP-1 and long-lasting GLP-1 analogs, including exenatide and liraglutide, are currently being evaluated for the treatment of type 2 diabetes (Kern et al., 2001). Exenatide, a synthetic form of exendin-4, is much more effective than GLP-1 in regulating the serum glucose level (Eng et al., 1992; Young et al., 1999). Exenatide and exendine-4 can increase the insulin secretion dependent on glucose and suppress glucagon secretion, regulate appetite, increase of insulin sensitivity (Szayna et al. 2000; Parkes et al., 2001). In addition to its metabolic effects, GLP-1 readily enters the brain through blood–brain barrier (BBB) and binds its receptors extensively expressed in the brain, including hippocampus, cortex and cerebellum (Hamilton and Hölscher, 2009; Hamilton et al., 2011). Recently, it has been demonstrated that GLP-1 could stimulate neurogenesis in the substantia nigra in experimental Parkinson's disease models (Rampersaud et al., 2012). Moreover, direct injection of GLP-1or long-lasting GLP-1 analogs into the brain has been shown to improve long-term potentiation (LTP) in the hippocampus (Gault and Hölscher, 2008). Therefore, GLP-1 and its analogs represent a novel treatment modality for various neurodegenerative diseases such as AD and PD (Hölscher, 2010).

In the present study, we used ICV-STZ procedure to develop in vivo experimental AD model in rats. Our purpose was to investigate whether exogenous exenatide supplementation has beneficial effect on cognitive performance and hippocampal cellularity of AD rats by using behavioral, histological and biochemical parameters.

2. Materials and methods

2.1. Animals

Twenty-one Sprague Dawley albino male mature rats, weighing between 200–220 g, were used in the study. The animals were fed ad libitum and housed in pairs in temperature controlled steel cages (22 ± 2 °C) with 12-h light and dark cycles. The Ethics Committee for Animal Research of Cumhuriyet University approved the experimental procedures. All the animal studies strictly adhered to the guidelines of the Committee.

2.2. Experimental protocol

Animals were randomly divided into 2 groups: stereotaxic infusion of STZ group ($n=14$) and stereotaxic infusion of 0.9% NaCl (sham, $n=7$). Rats were deeply anesthetized by the mixture of ketamine hydrochloride (80 mg/kg, Alfamine®, Ege Vet, Alfasan International B.V. Holland) and xylazine hydrochloride (4 mg/kg, Alfazyne®, Ege Vet, Alfasan International B.V. Holland), intraperitoneally (i.p.) and placed in a stereotaxic frame. STZ (3 mg/kg STZ in % 0.9 NaCl, $n=14$) was infused into the left (2.5 μl) and right lateral ventricle (2.5 μl) (ICV) (AP= −0.8 mm, $L=+1.6$ mm, DV= −4.2 mm) with a 28-gauge Hamilton syringe (Paxinos, 1986). Sham-operated rats received vehicle (% 0.9 NaCl) into the left (2.5 μl) and right lateral ventricle (2.5 μl). The needle was left in place for an additional 2 min for complete diffusion of the drug. After surgery, rats were monitored daily for behavior and health conditions.

2.3. Exenatide treatment

The ICV-STZ rats were randomly divided into two groups: Group 1 ($n=7$) was administered saline (1 ml/kg/day, i.p.) and Group 2 ($n=7$) was administered exenatide (Byetta, Lilly) 20 μgr/kg/day through i.p. for two-weeks. After the two-weeks treatment period, passive avoidance tasks evaluating the memory were performed in the study and control groups.

The passive avoidance learning (PAL) is a trial of fear-motivated avoidance tasks in which a rat learns to refrain from stepping through a door that would seem apparently safer but actually led to a dark compartment. The PAL box, which is equal to the size (20 cm × 20 cm × 20 cm) of the dark and light sections, was used. This PAL box has a grid system that performs electric shocks within the dark compartment. Normally when rats are placed in the light compartment, they prefer to enter the dark compartment. After a 10 s habituation period in the light section area, the guillotine door separating the light and dark chambers was opened. When a rat passed to the dark compartment, the door between the light and dark compartments was closed. A 1.5 mA electric shock was applied for 3 s, and the rat was removed from the dark chamber and returned to its home cage. After 24 h, the rats were placed in the same mechanism. The time (latency) to switch from the light compartment to the dark compartment of the rats was recorded, but at this time a shock was not delivered. The latency time was recorded up to a maximum of 300 s. The latency to refrain from crossing into the punishing compartment serves as an index of the ability to avoid and allows memory to be assessed (Erbas et al., 2014). After testing, the animals were weighed and euthanized. The brains were removed for histopathological and biochemical examinations. The brain tissues were cut in half; one half was used for the measurement of TNF-α levels whereas the second half was used for the measurement of ChAT activity. All tissues were stored at 80 °C until analysis.

2.4. Histopathological evaluation

Formalin-fixed brain sections (5 μm) were stained with cresyl violet stain to quantify the number of surviving neurons. All sections were examined and photographed with Olympus C-5050 digital camera at Olympus BX51 microscope. Cresyl violet stained neurons were counted in six sections per studied animal ($n=2$ for each group) by an image analysis system (Image- Pro Express 1.4.5, Media Cybernetics, Inc. USA). The observers blinded to the study groups accomplished all histological assessments.

2.5. Detection of brain TNF-α levels

The frozen brain tissue was homogenized with a glass homogenizer in 1 ml of buffer containing 1 mmol/l of PMSF, 1 mg/l of pepstatin A, 1 mg/l of aprotinin, and 1 mg/l of leupeptin in PBS solution (pH 7.2). The mixture was centrifuged at 16,000 g for 20 min at 4 °C. The supernatant was collected, and the total protein was determined using the Bradford method. The levels of TNF-α in the tissue supernatants were measured using an ELISA kit (eBioscience, Inc, San Diego, CA) specific for rat TNF-α. The measurement of TNF-α was performed according to the specifications given by the manufacturer. The minimum amount of TNF-α detected for this assay was 30 pg/ml. The TNF-α content in the brain tissue was expressed as ng/g protein.

2.6. Determination of brain choline acetyl transferase (ChAT) activity

The obtained frozen brain tissue was homogenized with a glass homogenizer. Ten percent cerebral homogenate (1:10, w/v) in cold saline was centrifuged with 3500g for 10 min at 4 °C. Supernatant