Original Article

Volumetric Study of Dentate Gyrus and CA3 Region in Hippocampus of Streptozotocin-Induced Diabetic Rats: Effect of Insulin and Ascorbic Acid

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ABSTRACT

Background and Objectives: **Hippocampal volume reduction has been reported in diabetes mellitus type 1. It is believed that hyperglycemia and oxidative stress mediate neuropathological changes in hippocampal neurons. In this study we aimed to study the effect of insulin and an antioxidant like ascorbic acid on preventing volume changes of dentate gyrus and CA3 region of hippocampus.**

Materials and Methods: **This study was carried out on male Wistar rats. Experimental diabetes was induced by intraperitoneal injection of streptozotocin (80 mg/kg). Control animals (C) received only saline. Six weeks later diabetic rats were divided into four groups as follows: diabetic (D), diabetic/insulin (D/Ins), diabetic/insulin + ascorbic acid (D/Ins+AA), and diabetic/ascorbic acid (D/AA). Treatments were continued for two weeks. At the end of treatment course, the hippocampi were removed and dentate gyrus and CA3 region volumes were measured using Cavalieri principle.**

Results: **STZ diabetic rats showed a reduction in DG and CA3 volumes. The volume of DG and CA3 in D and D/AA groups showed a reduction in comparison with control group ($p<0.01$). However, the volumes of DG and CA3 in groups D/Ins and D/Ins+AA showed no significant difference related to control group ($p>0.05$).**

Conclusion: **Our findings showed that insulin administration reverse volume reduction of dentate and CA3 region.**

Key words: Dentate gyrus, Diabetes mellitus, Streptozotocin, Rat

Introduction

Reduced hippocampal volumes were reported in psychiatric disorders including dementia, major depression and diabetes mellitus type1 (1,2). Recent studies have reported morphological changes in apical dendrites of CA3 pyramidal neurons, necrosis in mossy fibres, and suppressed cell proliferation in dentate gyrus (DG) and electrophysiological abnormalities in hippocampus of streptozotocin (STZ)-diabetic rats (3-5). It is suggested that

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these neuropathologic changes may contribute to hippocampal volume reduction and memory and learning impairments in diabetic subjects (6,7). These effects of diabetes on hippocampus are mediated in part by hyperglycaemia in insulin deficiency in addition to elevated glucocorticoid (GC) levels (8).

Hyperglycaemia mediates oxidative stress and free radicals generation (9).

Oxidative stress is implicated in pathological changes observed in CA3 region (10). Increased oxidative stress is associated with impairment in plasma antioxidants like ascorbic acid. It is believed that ascorbic acid protects neuron cell membrane from lipid peroxidation and damage (11,12).

It has also been shown that insulin can reverse electrophysiological abnormalities of hippocampus in STZ-diabetic rats (6). In spite of studies on hippocampus in diabetic state, there is little information about the preventive effects of insulin and ascorbic acid on the DG and CA3 volume changes in STZ-induced diabetes. Due to the importance of DG and CA3 in learning, we decided to assess the effects of insulin and ascorbic acid alone or in combination on volume changes of these regions in STZ-induced diabetic rats.

Materials and Methods

The study was carried out on male Wistar rats (aged 8 weeks and a body weight of 240-260 g). All rats were maintained in an animal house and were allowed free access to drinking water and standard rodent chow. Experiments were performed during the light period of cycle and were conducted in accordance with guidelines of animal ethics committee of Mashhad University of Medical Sciences (MUMS).

Induction of experimental diabetes

Diabetes was induced by a single intraperitoneal (IP) injection of STZ (Sigma Chemical, St. louis, MO) at a dose of 60 mg/kg dissolved in saline (control animals were injected with saline only). Four days after STZ injection, fasting blood glucose was determined in blood samples obtained by tail prick by a strip operated glucometer (Bionime, Swiss). Rats were considered diabetic and included in the study if they had fasting plasma glucose levels>250 mg/dl and non-fasting blood glucose levels >350 mg/dl in all STZ injected animals. Six weeks after diabetes induction, animals were divided into five groups as follows (each group contained 12 animals): group 1: control, non-diabetic (CON); group 2: diabetic, receiving no treatment (D); group 3: diabetic treated with insulin (D/Ins); group4: diabetic treated with insulin and ascorbic acid (D/Ins+AA); group5: diabetic treated with ascorbic acid (D/AA). Insulin (NPH, Exir Pharmacoceutical Co., Iran) was administered through subcutaneous injection at a dose of 4 U/day to maintain blood glucose levels at approximately normoglycemic range. Ascorbic acid (Daroopakhsh Co., Iran) was administered through IP injection at a dose of 80 mg/kg. Insulin dose was obtained by a preliminary study. To insure that this dose did not accompany periods of hyperglycaemia during the 24 h cycle, blood glucose (BG) levels were measured every 4 h in 5 diabetic rats over 3 consecutive 24 h cycles. Treatments were conducted for 2 weeks. Before the treatment course the animals' BG and weights were monitored. During the treatment course the BG was also checked. At the end of study, the animals were anaesthetized by chloroform. Then, the animals were transcardially perfused with 100 ml of saline followed by 200 ml of fixative containing 2% glutaraldehyde and 2% parafomaldehyde in 0.1 phosphate buffer (pH 7.4). Adrenal glands were removed, cleaned and weighed.

The brains were removed and post fixed in the same fixative for two weeks. Serial coronal sections (a thickness of 10 micron) were cut through the entire rostrocaudal extent of hippocampi in left and right hemispheres using a vibratome.

The total number and order was noted. The volumes of the right dorsal hippocampi in different groups were estimated using Cavalieri principle (13). From the complete rostrocaudal set of sections the hippocampus in each animals every 40 sections (on average) in the series was mounted in series onto the glass slides, air dried and subsequently stained with a solution of 0.1 toluidine blue in 0.1 M PB (pH 7.4) for 2 minutes. Sections were washed, dehydrated in ascending series of alcohol, passed through xylene and finally embedded in DPX. Each of these sections from any given animals was studied at a magnification of x200 with an Olympus digital microscope (Bxl, Japan) connected to a monitor. Each image was superimposed at random with a test lattice having a regular array of test points. Each point represented an area (u) of 0.00173 mm^2 in the section plane. The total number (p) of test point falling on each of tow regions for each section was counted. These counts for any region are related to the volume (v) of region by the following relationship:

V=putN/n where N= total number of serial sections through the hippocampus= the number of section

sampled and t= thickness of the section. The values were expressed as mm^3.

Statistical analysis

All data are expressed as mean ± SD. Statistical comparisons for volume data between groups were made using one way ANOVA and Tukey post hoc test. A p value less than 0.05 is considered as significant.

Results

Four days after STZ injection, rats were severely diabetic as indicated by their plasma glucose levels which were greatly elevated over those of control. Diabetic rats also exhibited obvious signs of diabetes, namely: polyurea and polydipsea. These rats were also lethargic and hypoactive, exhibited a mean weight of 216.93 ± 9.63 g (a mean weight loss of 34.35 g, $p<0.05$).

After 4 weeks the blood glucose (BG) levels of the D and D/AA groups were higher than those of CON ($p<0.05$), while the D/Ins and D/Ins and AA groups showed no significant level of difference compared with control ($p>0.05$).

STZ diabetic rats also showed a reduction in DG and CA3 volumes. The volume of DG and CA3 in D and D/AA groups showed a reduction ($p<0.01$). But the volumes of DG and CA3 in groups D/Ins and D/AA showed no significant difference relative to CON ($p>0.05$).

Discussion

Our results showed that uncontrolled STZ-induced diabetes for 8 weeks causes a volume reduction in DG and CA3 regions of hippocampus. Previous studies have also reported that STZ-induced diabetes produces a significant reduction in number of proliferating cells in the DG and morphological changes in CA3 pyramidal neurons as well as neuronal death (14-16). Musen et al reported that type 1 diabetes is associated with hippocampal grey matter reduction (2). These neuropathological changes may result from elevated circulating glucocorticoids, increased oxidative stress and an imbalance between plasma antioxidants like ascorbic acid and free radicals generation (17-19). STZ diabetes is now recognized as a hypercorticostrenomia model, associated with an increase in extracellular glutamate levels in the hippocampus that lead to glutamate excitotoxicity and neuronal death (8,20).

Stewart et al showed that increased circulating glucocorticids (GCs) lead to reduction in whole hippocampus volume (14). On the other hand, hyperglycemia and increased oxidative stress activate some neuronal death pathways (19). Ascorbic acid is a powerful antioxidant that inhibits these pathways and its administration improves antioxidant markers in vivo (13), but treated animals with ascorbic acid showed DG and CA3 volume reduction. In comparison between insulin and insulin/AA groups, it seems that treatment with ascorbic acid and insulin had no additional benefit on DG and CA3 volumes. Although ascorbic acid is of beneficial effects on lowering free radicals levels ,but adverse effects of STZ diabetes on hippocampal neurons are mediated through lack of insulin and hyperglycaemia that is indicative of special role(s) of insulin on hippocampal neurons (8). It has been shown that insulin deficiency plays a key modulator in neurodegenration. It probably acts on neurons via complex pathways (21).

It has also been reported that insulin reverses the morphological changes of STZ diabetes and GCs on hippocampus (5,8). According to our results, insulin administration even alone prevents volume changes of DG and CA3 regions of STZ-diabetic rats. Insulin at clinical dose prevents increase in extracellular levels of glucose and its neurotoxic effects on hippocampal neurons. Insulin mediates this effect on hippocampus through the regulation of specific glucose transporters (Gluts) like GLUTx1 in non-neuronal cells of hippocampus that is responsible for modulating of cytoskeleton proteins and promotion of neuronal survival (22-24).

Conclusion

Our findings clearly suggest that volume changes of DG and CA3 in diabetic state are reversible by insulin therapy.

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