THE EFFECT OF HYPERGLYCEMIA ON HIPPOCAMPUS NEURONAL DENSITY IN FEMALE RATS

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Summary

The hippocampus is necessary for normal cognitive function, especially for processing recognition memory and transferring short-term memory items into long term storage. Hyperglycemia can lead to functional and structural deficits in central nervous system. The present study has been undertaken the effects of Hyperglycemia on Hippocampus neuronal density in female rats. Twenty female wistar rats were used and divided to two groups (control and hyperglycemic). Hyperglycemia was induced by stereptozotocin (55mg kg^{-1}) given by a single intraperitoneal injection to female Wistar rats. Control rats were injected with phosphate buffered saline. After four week, rats were anesthetized and brains rapidly were removed and in all sample the number of neurons in CA1, CA2, CA3 was measured via stereological method in both control and experimental groups. Statistical analysis determines that there is a meaningful reduction in number of neurons in all part of hippocampus in hyperglycemic rats ($p<o.o5$). That may be for oxidative stress in hyperglycemic rats.

Key words: hippocampus, hyperglycemia, stereological methods

Running title: Hyperglycemia on hippocampus

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Introduction

The hippocampus is an important structure for memory processing. It is a particularly vulnerable and sensitive region of the brain that is also very important for declarative and spatial learning and memory. Hippocampal neurons are vulnerable to seizures, strokes, and head trauma, as well as responding to stressful experiences. At the same time they show remarkable plasticity, involving long-term synaptic potentiation and depression, dendrite remodeling, synaptic turnover, and neurogenesis in the case of the dentate gyrus (1).The hippocampus has been implicated in certain short-term memory. Indeed hippocampal lesions often produce short-term memory deficits (2).The hippocampus is preferentially susceptible to a wide variety of toxic insults and disease processes, including hypoxia-ischemia and hypoglycemia (3).Metabolic diseases such as diabetes and obesity have been associated with increased vulnerability to stress (4)and cognitive dysfunction (5).Diabetes mellitus can lead to functional and structural deficits in both the peripheral and central nervous system. The pathogenesis of these deficits is multifactor and may involve microvascular dysfunction and oxidative stress (6).Cognitive deficits are also reported to occur in animal models of diabetes (Stroptozotocin induced)which can be prevented, but not fully reversed by insulin treatment (7). Diabetes also induced morphological changes in the presynaptic mossy fiber terminals (MFT) that form excitatory synaptic contacts with the proximal CA_3 apical dendrites (8).

Oxidative stress induced by chronic hyperglycemia contributes to cerebrovascular complication in diabetes(9).Also diabetes mellitus is associated with an increased risk for cerebrovascular disease(10).Accumulating data support the conclusion that oxidative stress induced by choronic hyperglycemia plays akey role in both microvascular and macrovascular complications of diabetes, including stroke(11).Many deleterious events contribute to oxidative damage to neurons in diabetes: because of high levels of polyunsaturated lipids in the brain, direct lipperoxidation frequently occurs causing lipid membrane disruption and consequent neurodegeneration (12).

Moreover, oxidative stress increase tissue levels of highly reactive and toxic substances and effects signal transduction pathways involved in neuronal and endothelial cell function. Primary diabetic encephalopathy is recognized as a late

complication of both type 1 and type 2 diabetes (13). Impairments in learning, memory, problem salving and mental and motor speed are more common in type1 diabetic patients than in the general population (14).A diabetic duration dependent decline in cognitive function occurs independently of hypoglycemic episodes(15) and impaired intellectual and cognitive developments in type 1 diabetic children correlate with diagnosis at young age, male sex and metabolic status. Cognitive deficits (16) and poor performances in abstract reasoning and complex psychomotor functioning occur in type 2 diabetes. Learning and memory dysfunctions are more prominent in elderly type 2 diabetic patients (17).It has not been determined whether this is because of potentiation of the normal aging process, a function of diabetes duration, or both. Notably Alzheimer disease is twice as prevalent in the diabetic population as in nondiabeti subjects (18).Several recent studies have implicated abnormal function of the insulin/IGF axis in the early pathogenesis of Alzheimer's disease.Insulin and IGF-1 are believed to regulate ß-amyloid levels and tau phosphorylation (19).

Impaired spatial learning and memory occur in animal models of both type1 and type2 diabetes.In the hippocampus of STZ-induced rats, long –term potentiation is impaired, whereas long-term depression is enhanced indicating altered hippocampal synaptic plasticity, which are corrected by insulin treatment (20).

The aim of present experimental design was to induce hyperglycemia and to assess the effects of that on Hippocampus (CA1, CA2, CA3) neuronal density.

Materials and Methods

Female Wistar rats, weigthing 200-250g, were hosed under standard laboratory conditions and kept under natural 12h light: 12h dark cycle. The animals procured from Razi Animal House, were housed 2 per cage with free access to standard food and water. Rats were acclimatized to laboratory conditions before testing. Animal were divided into two different groups for staminate the effect of hyperglycemia.

Group 1- Normal-   rats were not subjected to any procedures.

Group 2- Hyperglycemic- under STZ injection.

All the experimental protocols were conducted in faculty of science, Islamic Azad University of Mashhad, Iran (2010).All chemical used in this study were purchased from Sigma (UK).

Induction of hyperglycemia:

Hyperglycemia was induced in rats by a single injection of STZ($55mg\ kg^{-1}$) freshly dissolved in citrate buffer(PH 4.5).Age –matched control animals were injected with citrate buffer. Hyperglycemia was confirmed after 4 weeks; only the animals with blood glucose level above 400 mg/dl were included in the study.

The body weight was measured at the beginning and the end of the experiment. All animals were checked for glucose blood concentration at the beginning of the experiment. After 2 weeks from STZ-injection, as well as on the day before of experiment (21).

Tissue collection:

Animals were anesthetized with sodium pentobarbital (64mg/kg) and decapitated. The whole brain was removed and fixed in 10% paraformaldehyde. NaCl was added to the fixative to make the tissue float in order to overcome deformities during the fixation period. Paraffin embedded tissue blocks were sectioned at 7mµ thickness coronally and stained with haematoxylin-eosin (22).

Measurement of neuronal density in hippocampus:

Hemotoxylin-eosin-stained serial paraffin sections were prepared from 8 hippocampi from individual animals in each group. Regions of hippocampus (CA_1- CA_2- CA_3) were identified according to paxinos and Watson(23). Tissue blocks containing samples (brains) were serially cut throughout. Form several hundred sections per block; of each 20 section 3 serial sections were obtained. For example for the first series: 24st, 25st, 26st section and for the second series: 46st ,47st ,48st section and so on.   Therefore we mounted every 3 section on a slide. At a practical level, Stereological methods are precise tools for obtaining quantitative information about three-dimensional structures based mainly on observations made on sections (24). All experiments were performed a minimum of two times.

Statistical analyze:

The ratio of numerical density of neurons in each section of hippocampus was used as an index. Student's t test was used for comparison when only 2 groups were analyzed. Statistical significance was chosen as ($p < 0.05$).All results are reported as mean ± SEM.

Results

1-Hyperglycemia was assessed in this study by monitoring the blood glucose levels in both PBS and STZ injected rats. There was a significant increase ($p<0.001$) in blood glucose levels from 100 ± 5 mg/dL in control to 470 ± 18 mg/ml in diabetic rats. In Figur 1 was showed all part of hippocampus.
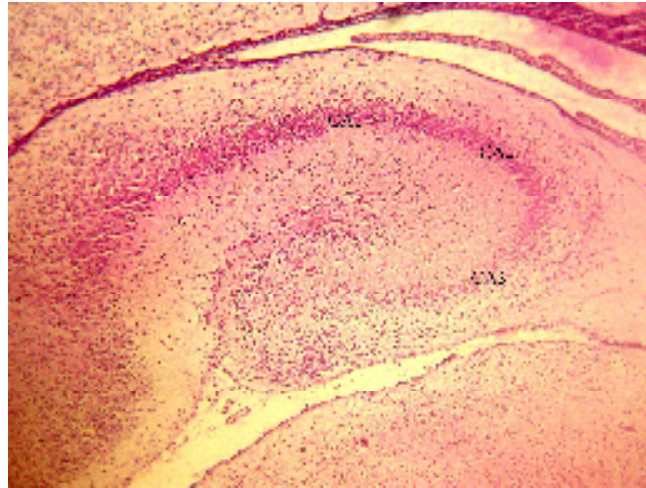

Fig.1: Photomicrograph of the brain section at the region of the midhippocampus.(X40).

2-Hyperglycemia produced evoked significant neuronal loss in CA1 (Fig.2)This index was (1479 ± 113) in control to (1182 ± 49) in hyperglycemic rats($p<o.o5$).
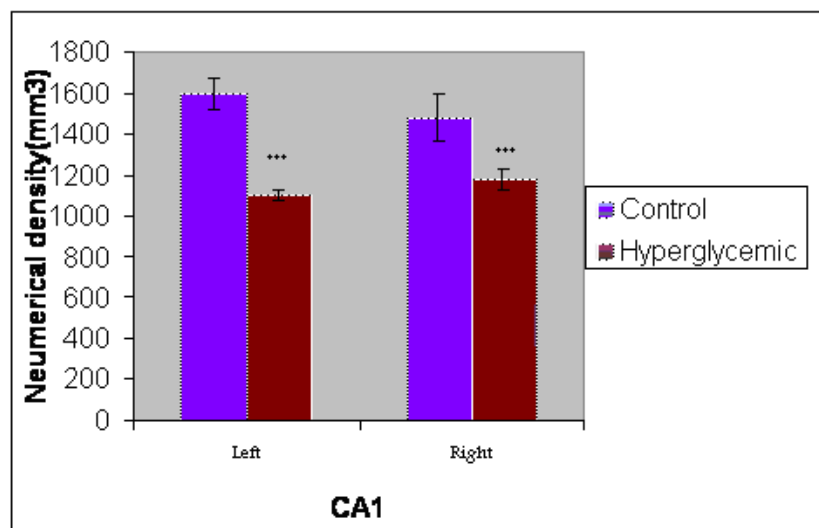

Fig.2: Neuronal density CA1 in hyperglycemic rats compare to control.The values are presented as means± SEM. n=8. *P<0.05 Student s t test compare hyperglycemic to controls.

3-In CA2 section (Fig.3) the neuronal density in control group was (1662± 124) in compare with hyperglycemic group (866±16).
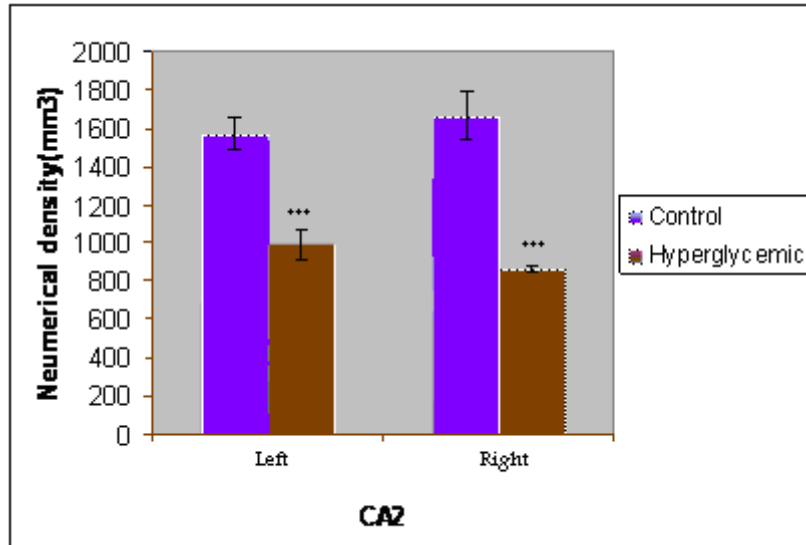

Fig.3: Neuronal density CA2in hyperglycemic rats compare to control.The values are presented as means± SEM. n=8. *P<0.05 Student s t test compare hyperglycemic to controls.

4- The average of neuronal density in CA3 section was (1836±60) in control to (1007±48) in hyperglycemic rats (p<o.o5) (Fig.4).
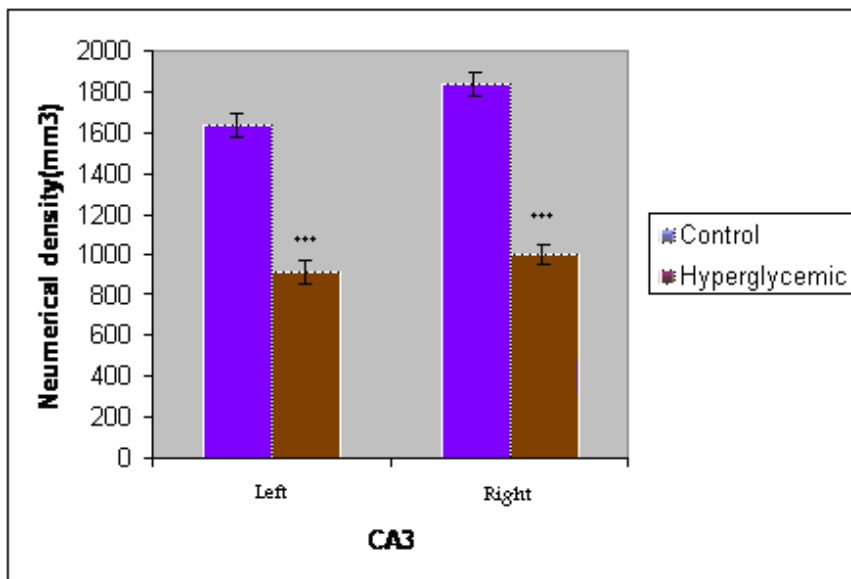

Fig.4: Neuronal density CA3 in hyperglycemic rats compare to control.The values are presented as means± SEM. n=8. *P<0.05 Student s t test compare hyperglycemic to controls.

Discussion

The hippocampus in necessary for normal cognitive function, especially for processing recognition memory and transferring short-term memory items into long-term storage (25).In our morphometric studies on pyramidal cells in CA_1, CA_2, and CA_3, it has been shown that the experimental hyperglycemia in rats results neuronal loss and damage expressed maximally in CA_3.Although in experimental groups there is a remarkable neuronal density decrease in all hippocampus sectors(Fig2,3,4),but it is abvously in CA_3(Fig1). Hyperglycemia causes morphometric neuronal changes (17). This observation has allowed us to postulate that the neuronal death in the infant from diabetic mothers proceeds on an apoptotic pathway (26).Diabetes mellitus can result in a 30-40% reduction in brain iron (9).Iron in the form of cytochoromes is a required component of cellular oxidative metabolism in the brain and is thus essential for normal neuronal function(27). Iron deficiency of the heart, liver and skeletal muscle has been shown to affect cellular energy production and organ performance. Arguably, sever iron deficiency may lead to similar deficits in cellular energy metabolism and organ performance in the brain, resulting in a reduced ability to respond to restriction of oxygen and perfusion and in greater hippocampus damage. The hippocampus, especially the DG, is one of the iron rich areas of the rat brain, Finding suggest that the integrity of the prenatal hippocampus and its cholinergic input are important for normal development of memory and learning (16)In other hand, This area of the brain(hippocampus) particularly the dentate region is also vulnerable to damage when glucocorticoids are elevated as occurs, for example, when an organism is stressed (20) .Uncontrolled experimental hyperglycemia induced by (STZ) in rats is an endogenous chronic stressor that produces retraction and simplification of apical dendrites of hippocampal CA_3 pyramidal neurons (9).

One effect, synaptic vesicle depletion and dendrite atrophy occurs in hyperglycemia as well as after repeated stress and cort treatment. These changes occurred in concert with adrenal hypertrophy and elevated basal cort release as well as hypersensitivity and defective shut off of cort secretion after stress. Thus as an endogenous stressor STZ hyperglycemia not only accelerates the effects of exogenous stress to alter hippocampal morphology: it also produce structural changes that overlap only partially with those produced by stress and cort in the nondiabetic state (8).

The hippocampal morphological changes induced by stress are mediated by interactions between Gc secretion, excitatory amino acid, and are also correlated with deficits in hippocampal dependent memory (15).These results and our results confirm that an oxidative imbalance occurs in the hippocampus of hyperglycemic rats as we have previously shown. Several studies have pointed out that NF-kB(nuclar factor) activation is inhibited by a variety of antioxidants, such as N-acetyl-cystein, butylanted hydroxyl anisole, vitamin E, and lipoic acid (10): these data suggest that antioxidants effect some steps of signaling events leading to phosphorylation, ubiquination and degradation (6).The role of oxidative stress and Nk-kB activation on hyperglycemia complications is well documented, moreover antioxidant treatment exerts a beneficial effect in experimental models of chronic injury in hyperglycemia and treatment with antioxidants can significantly reduce hyperglycemia complications (8).Reactive oxygen species activates a variety of target genes linked to the development of diabetic complications (28).

In addition, the loss of arachidonic acid content of the synaptosomal membrane, induced by hyperglycemia and by transient cerebral ischemia, making the membrane more resistant to oxidative stress. Oxidative stress induced by chronic hyperglycemia directly can damage ionic homeostasis and membrane transport systems in the brain (10) and may be this is one of the reasons for hippocampal neurons death. Apoptosis in hyperglycemia has been ascribed to oxidative stress (2).How ever, other experimental studies on streptozotocin induced rat diabetic, showed pathological changes, such as so-called dark neurons and neuronal loss, in different cerebral regions, especially in the hippocampus. A dominant opinion is that hyperglycemia aggravates ischaemic brain damage in experimental STZ-diabetes with transient cerebral ischemia in rats (3).It has been suggested that hyperglycemia and ischaemia evoke the oxidative stress following an impairment of the respiratory chain in mitochondria and an overproduction of the reactive oxygen species (ROS). ROS are considered as a main factor in the pathogenesis on neuronal death (3).The other reason for neuronal death in hyperglycemia is Iscemia. Some studies shown that there is differences in the neural death between hyperglycemia and Ischaemia (3) that differences in the neuronal death pathomechanism among hippocampal sectors in hyperglycemia and ischaemia may be related to the different duration and intensity of the oxidative stress in both applied models(acute in ischaemia and chronics in STZ-

induced diabetes).It has been postulated that in cerebral ischaemia neuronal apoptosis may be followed by necrotic phase(6).Pathomechanism of degenerative changes and neuronal loss through apoptosis or necrosis is not clear until now. It has been suggested that changes in intracellular calcium concentrations in oxidative stress may indicate the pathway of cell death .It is suggested that more sever injury with high intracellular calcium concentration (Ca^{+2}) promotes necrotic cell death, where low (Ca^{+2}) and milder injury promotes cell death through apoptosis(29). Studies on antioxidative treatment would deliver further data important in the exploration of neuronal death in diabetes and ischemia.

In total, it is concluded that hyperglycemia induces some changes in hippocampus neuronal structure and density. Statistical analysis show significant decrease in neuronal density (ND) of all part of hippocampus in hyperglycemic rats compare to control. Therefore, it is better in hyperglycemic condition the level of glucose was maintained under normal condition.

Acknowledgment

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