Comparison between the effects of type 1 and type 2 diabetes on hippocampal neuronal density in Wistar rats

Zeinab Momeni¹, Morteza Behnam-Rasouli†*, Massoud Fereidouni¹, Sareh Rostami¹

¹- Biology Department, Faculty of Science, Ferdowsi University of Mashhad, Mashhad, Iran

Abstract

Background: Diabetes is one of metabolic disorders characterized by hyperglycemia due to defects in insulin secretion, insulin function, or both. Diabetes induces neuronal death in different regions of the brain especially hippocampus. In this regard, the present study has been conducted to compare the effects of type I and type II diabetes on hippocampal neuronal density in Wistar rat.

Methods: Male Wistar rats were divided into three groups including control, type 1 and type 2 diabetes. Type I diabetes was induced by a single subcutaneous injection of Alloxan (135 mg/kg body weight), and for type 2 diabetes, drinking water containing 10% fructose was administered for eight weeks. Two months after diabetes confirmation, hippocampal neuronal density was investigated using dissector technique.

Results: Neuronal density in CA1 region showed significant decrease in both experimental groups compared to control, whereas CA3 neuronal density demonstrated a remarkable reduction only in type 1 diabetic group in comparison with control (P<0.05). Likewise, in the whole hippocampus, there was a statistically meaningful difference between control and type 1 diabetic group (P<0.05).

Conclusion: Significant reduction in CA1 neuronal density may be possibly due to more vulnerability of this region to pathological conditions. In the whole hippocampus, decrement in neuronal density was also more significant in type 1 diabetes, while in type 2, neuronal density could be associated with age and diabetes duration.

Keywords: Type1 diabetes, Type2 diabetes, Neuronal density, Hippocampus, Rat

*Corresponding Author: Department of Biology, Faculty of Science, Ferdowsi University of Mashhad, Vakil Abad Boulevard, Mashhad, Iran. Tel: +98 (511) 8796416, Fax: +98 (511) 8796416, email: behnam@ferdowsi.um.ac.ir
Introduction
Diabetes is one of metabolic disorders characterized by hyperglycemia due to defects in insulin secretion, insulin function, or both. Chronic hyperglycemia is accompanied by dysfunction and sustained injuries in various organs such as eyes (retinopathy), kidneys (nephropathy), heart, blood vessels and especially the neuronal system (neuropathy) [1]. Neuropathy is considered as the most common neurological complication of diabetes, leading to some functional impairment in central nervous system, particularly brain, in addition to affecting peripheral nervous system [2]. Among different brain regions, hippocampus is one of the most sensitive areas which is highly vulnerable to harmful insults such as ischemia, stress and diabetes, and reveals neurophysiological, structural and molecular changes such as neurogenesis [3], hippocampal atrophy [4], dendritic remodeling [5], astroglial alterations [6], changes in glutamate receptors [7], insulin [8] and insulin-like growth factors receptor [9], dopamine receptors [10], advanced glycosylation end products receptors [11], as well as gene expression including nitric oxide synthase, NF-kB transcription factor and nerve growth factor [12]. Hippocampal neuronal death can be also mentioned as the other significant change caused by diabetes [13]. Hyperglycemia plays a major role in diabetes-induced neuronal degeneration through alteration in calcium homeostasis and protein kinase activity and increase in ROS production and oxidative stress [14]. It seems that CA1 is the most sensitive region of hippocampus and the first place influenced by hazardous conditions such as diabetes [15]. On the other hand, cognitive impairment, as one the most prevalent CNS complications of diabetes can be attributed to neuronal death in CA3 region of the hippocampus (which plays an important role in memory encoding) [16]. Although many researches have been conducted on the effects of type 1 and type 2 diabetes on neuronal density in different brain regions particularly hippocampus, there is not yet any study which comparatively and simultaneously investigated the effects of both types of diabetes on this region. Therefore, the present study has been carried out to simultaneously compare the effects of type 1 and type 2 diabetes on neuronal density in the rat hippocampus.

Methods
This experimental study was performed in Department of Biology, Faculty of Science, Ferdowsi University of Mashhad in 2009. Two months old male Wistar rats, fostered in the animal room of faculty of science, were used in the study. The animals were kept under standard condition at 20±2 °C and light: dark hours of 12:12 with free access to water and standard food prepared by Javaneh Khorasan Company. They were kept based on Local Animal Ethics Committee protocols. Rats were randomly divided into three groups (n=6) including control and two experimental groups. At the age of two months, one of the experimental groups was treated with regular food and drinking water containing 10% w/v fructose (Merk, Germany) for eight weeks to induce type 2 diabetes [17]. To confirm the induction of type 2 diabetes, four months old rats underwent glucose tolerance test at the end of 8th week. This group was continuously treated by regular food and drinking water containing 10% (w/v) fructose for another two months. At the end of 16th week, blood biochemical factors such as glucose, insulin, triglyceride, cholesterol, cholesterol-LDL and cholesterol-HDL were measured for six months old rats. For another experimental group, type 1 diabetes was induced by a single subcutaneous injection of 135 mg/kg of body weight Alloxan (Merck, Germany). Three days after Alloxan injection, the rats’ blood fasting glucose was measured to confirm the induction of type 1 diabetes. After diabetes confirmation, these animals were kept for another two months with free access to food and water.

Tissue sample preparation and implementation of dissector technique
Following perfusion, removing the animals' brain and additional fixation, left hemisphere of each brain was separated and underwent tissue passages procedures. Then, paraffin embedded tissues were coronally sectioned at 10 microns thick, from frontal to occipital lobe. At first, pre-hippocampal sections were excluded, and hippocampal sections were serially selected 3 per each 25 sections. Samples were randomly selected, as sampling was initiated by a number between 1 and 25 e.g. 15; thus, first series included sections number 15, 16, and 17, second series 45, 46, 47 and third series 65, 66, 67 and
so on. The aim of this approach was to possess all parts of the total samples related to each group. After conventional Hematoxylin-Eosin staining, samples were photographed using a digital camera (Olympus Dp71). Images were prepared by 40x objective lens from sections 1 and 3 per slide. For this purpose, the whole hippocampal section was divided into 20 microscopic fields, and a systematic random method was used to select the fields for photography, so as the image was taken from one microscopic field in each 7 fields; for example, in the first series, fields 2, 9 and 16 were selected and captured, and the same areas in section 3 were also photographed following being found. In the second series, fields 4, 11 and 18 and in the third series, fields 6, 13 and 20 were selected and captured and so on. In the next step, prepared images were used for neuronal counting by dissector technique [18]. In addition to calculating neuronal density in the whole hippocampus, neuronal density of the CA1 and CA3 regions were also studied.

**Results**

FIRI and the area under the curve are among the important variables used to confirm the induction of type 2 diabetes. These variables, calculated in both control and type 2 diabetic groups, are presented in Table 1. Animals’ body weight was evaluated in three levels in control and experimental groups; baseline level (two months of age), one month after diabetes confirmation (five months of age), and at the end of the study (six months of age). Serum glucose of all groups was also measured at the beginning of the study (two months of age), after induction of both types of diabetes (four months of age) and at the end of the experiment (six months of age) (Table 2). Serum changes of other biochemical factors are presented in Table 3. Estimation of neuronal density in CA1, CA3 and the whole hippocampus in control and experimental groups indicate that neuronal density is significantly low in CA1 region compared to the control (Figure 1). Furthermore, neuronal density in CA3 region of type 1 diabetic group is remarkably lower than control and type 2 diabetic group (Figure 2), and similarly, neuronal density of the whole hippocampus is significantly low in comparison with control and type 2 experimental group (Figure 3).

**Table 1. Comparison of FIRI factor and the area under the curve between control and type II diabetic group**

<table>
<thead>
<tr>
<th>T2DM</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Area under the curve (min× (mg/dl))</strong></td>
<td>19349.55 ± 6.3*</td>
</tr>
<tr>
<td><strong>FIRI (miu/ml)×(mg/dl)</strong></td>
<td>10.11 ± 1.26**</td>
</tr>
</tbody>
</table>

Comparison (Mean ± SEM) with the control group *p<0.05, **p<0.01, Sample size in each group: 6 male rats

Statistical analysis: Student t-test, Type of the study: Experimental

**Table 2. Comparison of changes in body weight and blood glucose between all the study groups during the experiment**

<table>
<thead>
<tr>
<th>6-month of age</th>
<th>Blood Glucose (mg/dl)</th>
<th>g) (Weight Body)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4-month of age</td>
<td>2-month of age</td>
</tr>
<tr>
<td>Control</td>
<td>95.5±10.2</td>
<td>108±4.4</td>
</tr>
<tr>
<td>T1DM</td>
<td>495±47***</td>
<td>474±25.6***</td>
</tr>
<tr>
<td>T2DM</td>
<td>177.5±32.6*</td>
<td>121.7±4</td>
</tr>
</tbody>
</table>

Comparison (Mean ± SEM) of body weight and serum glucose level between the study groups,
*p<0.05, **p<0.01, ***p< 0.001
Sample size: 6 male rats
Statistical methods: ANOVA and student t-test
Type of experiment: Experimental

**Table 3. Glucose, insulin, triglyceride, cholesterol, cholesterol LDL, cholesterol HDL in control and experimental groups at the end of study**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Type 1 diabetes</th>
<th>Type 2 diabetes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mg/dl)</td>
<td>95.5 ± 10.2</td>
<td>330 ± 82.55*</td>
<td>177.5 ± 32.6</td>
</tr>
<tr>
<td>Insulin (miu/ml)</td>
<td>1.07 ± 0.11</td>
<td>0.97 ± 0.08</td>
<td>1.52 ± 0.17*</td>
</tr>
<tr>
<td>Triglyceride (mg/dl)</td>
<td>50.75 ± 4.09</td>
<td>62 ± 10.64*</td>
<td>68.25 ± 16.02</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>61.25 ± 2.29</td>
<td>69.5 ± 4.15</td>
<td>61.25 ± 1.25</td>
</tr>
<tr>
<td>LDL (mg/dl)</td>
<td>36.25 ± 1.8</td>
<td>30.7 ± 3.61</td>
<td>35± ± 1.82</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>16.33 ± 1.2</td>
<td>15.5±1.41</td>
<td>15.33 ± 0.49</td>
</tr>
</tbody>
</table>

Comparison (Mean ± SEM) with the control group *p<0.05, Sample size: 6 male rats,
Statistical methods: ANOVA and student t-test , Type of study: Experimental
Figure 1. Comparison of neuronal density in CA1 region of the hippocampus (NV/mm³) between control, type 1 and type 2 diabetic groups

* p<0.05 Comparison between control and diabetic groups, Sample size: 6 male rats, Statistical methods: ANOVA and student t-test, Type of study: Experimental

Figure 2. Comparison of neuronal density in CA3 regions (NV/mm³) between control, type I and type II diabetic groups

* p<0.05 Comparison between control and diabetic groups, Sample size: 6 male rats, Statistical methods: ANOVA and student t-test, Type of study: Experimental
Discussion
As regards the main purpose of the study, simultaneous comparison between the effects of type 1 and type 2 diabetes on hippocampus neuronal density, neuronal counting was performed using dissector technique per volume unit in control and diabetic groups. Following eight weeks fructose treatment in type 2 experimental group, a significant increase in FIRI factor and the area under the curve, as well as an increase in body weight and blood glucose were found, confirming induction of type 2 diabetes (Table 1 and Table 2). In type 1 diabetic group, increase in blood glucose, appetite, urination, thirst and body weight during experimental period can be indicative for type 1 diabetes induction.

Comparison between the results of neuronal density in CA1 and CA3 regions showed that CA1 neuronal density has been significantly diminished in both diabetic groups compare to control, whereas CA3 neuronal density exhibited a meaningful reduction only in type 1 diabetic group in comparison with control, and the reduction was not meaningful in type 2 diabetes (Fig. 1 and 2).

Regarding the results of previous studies on the effects of diabetes on different hippocampal regions, reports are varied and even contradictory. For example, findings of a study on eight-month old diabetic BB/Wor rats showed 37% and 24% reduction of hippocampal pyramidal cells respectively in CA1 and CA2, but not in other hippocampal regions [19]. On the other hand, neuronal density was remarkably decreased in CA2 pyramidal layer on the third day and in CA1 and CA3 layers on the seventh day of diabetes [12], and the decrement included all hippocampal regions (CA1, CA2, CA3, DG) on the tenth week of type I diabetes induction [20]. However, some studies have reported neuronal loss only in CA1 region of type I diabetic rats [21, 22].

Given the effects of type 2 diabetes on different hippocampal regions, it has been revealed that reduction in hippocampal volume first occurs in CA1, and then in other hippocampal regions, especially CA3, over time and with increasing age [23]; however, it has not been clarified whether the reduced volume is due to cells atrophy or degeneration. The results of the present study indicate that decreased neuronal density is more significant in CA1 than CA3 regions in both diabetic groups compared to the control. Thus, it seems that CA1 neurons react more severely to diabetes complications than CA3. CA1 region of the hippocampus is one of the most sensitive areas to hazardous conditions including ischemia, epilepsy and diabetes [24]. So, it can be concluded that inhibition of mitochondrial respiratory chain complex I, reduced access to oxygen and glucose and increased production of free radicals, which are probably more intense in early stages of
diabetes, are more significant in CA1, and, thus, exacerbate the cell vulnerability in this region [25]. Neuronal vulnerability of the CA1 region may be due to reduced levels of oxygen free radicals scavenging enzymes, especially superoxide dismutase [26], as antioxidant enzymes production requires high energy consumption, while ATP production is considerably low in this region due to defects in mitochondrial respiratory chain [27]. Moreover, oxidative stress accelerates neuronal death through increase in extracellular glutamate and NMDA receptor activity and subsequent augmentation in calcium influx to the neurons. Oxidative stress also leads to release of inflammatory factors from astrocytes, which in turn enhances the process of neuronal death through free radicals overproduction [27].

Comparison of neuronal density in the whole hippocampus pyramidal cells showed that neuronal density has diminished in experimental groups compared to the control, and such a reduction has been statistically meaningful in type 1 diabetes. Reduced neuronal density in the hippocampus of experimental groups is in consistence with the results of similar studies on diabetic rats [19-23]. It can be explained that decreased neuronal number is mainly brought about by hyperglycemia-induced oxidative stress and free radicals overproduction [14]. Oxidative stress mediates activation of stress-sensitive intracellular signaling pathways, and activation of specific genes and gene products resulting in neuronal damage [14]. Defects in insulin/C-peptide and insulin-like growth factors can be another reason for neuronal loss in experimental groups, since these factors are supposed to have anti-apoptotic effects, eventuating in neuronal death due to their dramatic reduction in diabetic conditions [28]. On the other hand, glucocorticoids augmentation may be another possible factor playing a role in hippocampus neuronal degeneration [29]. Under diabetic conditions, excess in glucocorticoids level contributes to increase in the level and decrease in the uptake of extracellular glutamate, changes in glutamate receptors expression and activity, and excessive calcium level and cell death [30].

On the explanation of insignificant difference between type 2 diabetic group and control, it can be mentioned that remarkable incidence of type 2 diabetes complications is somehow dependent on aging and diabetes duration [31], as complications of type 2 diabetes are enhanced by age and history of hyperglycemia [14]. In this regard, it has been shown that exacerbation possibility of diabetes neurological complications such as learning and memory deficits and cognitive dysfunction is considerably high in older people with long duration of the disease [32], and the risk of dementia is twice in elderly diabetic patients than others [33]. These findings indicate that type 2 diabetes increases the risk of central nervous system complication especially in the elderly [32].

Taken together, comparison of hippocampal neuronal density between type 1 and type 2 diabetic groups demonstrates that neuronal loss has been more significant in type 1 diabetic group. Since incidence rate of hyperglycemia and/or lack of insulin availability occur more promptly in type 1 than type 2 diabetes, significant neuronal alterations in type 2 diabetes depends on age and diabetes duration; hence, it is recommended that the effects of variables such as age, disease duration, antioxidant administration and insulin injection be further investigated on neuronal structure and hippocampal volume among diabetic rats.

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References


