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Gestational diabetes induced neuronal loss in CA1 and CA3 subfields of rat hippocampus in early postnatal life

M.J. Golalipour[1], S. Kaboli Kafshgiri[2], S. Ghafari[3]

[1]*Gorgan Congenital Malformations Research Centre, Department of Anatomical Sciences, Golestan University of Medical Sciences, Gorgan, Iran*
[2]*Histology Laboratory, Golestan University of Medical Sciences, Gorgan, Iran*
[3]*Department of Anatomical Sciences, Golestan University of Medical Sciences, Gorgan, Iran*

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This study was conducted to determine the effect of gestational diabetes on the neuronal density of CA1 and CA3 subfields of the hippocampus in Wistar rat offspring. On day 1 of gestation, 10 dams were randomly allocated into two control and diabetic groups. Five animals in the diabetic group received 40 mg/kg/b.w. of streptozotocin (intraperitoneally) and the control animals were received normal saline. Six offspring of each of the gestational diabetics and controls were randomly selected in postnatal days 7 and 21. The infants were scarified and coronal sections were taken from the right dorsal hippocampus and stained with cresyl violet. The number of pyramidal cells per 10000 μm^2 area and the thickness of layers of hippocampus in CA1 and CA3 were evaluated. In postnatal day 7, the number of pyramidal neurons in CA1 significantly reduced from 118.82 ± 8.0 in the control group to 84.71 ± 3.3 neurons in gestational diabetic group, and in postnatal day 21 it significantly reduced from 112.71 ± 6.9 in the control group to 91.52 ± 8.5 in the gestational diabetic group. Also, the number of pyramidal cells of CA3 on postnatal day 7 significantly reduced from 90.33 ± 8.1 in the control group to 62.86 ± 7.2 in the gestational diabetic group, and in P21 the number of pyramidal cells significantly reduced from 78.33 ± 2.4 in the control group to 61.7 ± 9.5 cells in the diabetic group. In CA1 and CA3 the thickness of the pyramidal layer on postnatal days 7 and 21 non-significantly increased in gestational diabetics in comparison with the controls. This study showed that uncontrolled gestational diabetes reduces the pyramidal neurons of the hippocampus in rat offspring. (Folia Morphol 2012; 71, 2: 71–77)

Key words: gestational diabetes, hippocampus, pyramidal neuron, rat

INTRODUCTION

Diabetes mellitus is one of the most common serious metabolic disorders [23] characterised by hyperglycaemia and altered metabolism of lipids, carbohydrates, and proteins [67].

Type I or insulin dependent, type II or insulin independent, and gestational diabetes are the three general classifications of diabetes mellitus [50]. Diabetes mellitus, regardless of its type, is associated with cerebral alterations in both human and animal models of the disease [12, 23, 42].

These alterations include abnormal expression of hypothalamic neuropeptidase [18, 60], hippocampal astrogliosis [61], decreased hippocampal synaptic plasticity [34, 41], neurotoxicity, and changes in glutamate neurotransmission [15, 22, 65]. Dia-

Address for correspondence: Dr. M.J. Golalipour, Gorgan Congenital Malformations Research Centre, Department of Anatomical Sciences, Golestan University of Medical Sciences, Gorgan, Iran, P.O. Box: 49175-1141, tel/fax: +98(171) 4425165, 4421660, e-mail: mjgolalipour@yahoo.com

betic patients are prone to moderate alterations in memory and learning [21, 59].

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 [9].

Recent studies have reported that the process of neurogenesis including cell proliferation, survival, migration, and differentiation continues in the hippocampal formation well into adulthood in a variety of species, including rodents, non-human primates, as well as humans [14, 24–26, 33].

Evidence for brain disturbances were reported in the hypothalamus, cerebral cortex, and hippocampus of streptozotocin (STZ)-induced diabetic rats [11, 33, 53].

Gestational diabetes mellitus (GDM), defined as impaired glucose tolerance, affects approximately 4% of all pregnant women who have never before had diabetes but who do have high blood glucose levels during pregnancy [50], and involves an interaction between diabetic susceptibility genes and the diabetogenic effects of pregnancy [32].

Follow-up studies concerning the adverse effects of diabetic pregnancy on the developing brain have revealed neurobehavioral deficits in both sensory-cognitive and psychomotor functions. These include altered auditory recognition memory processing at birth [62], neurobehavioral deficits [55], reduce visual and memory performance at 8 and 12 months [17], poorer performance on tests of general development in infants and toddlers [56], and inferior performance in elementary school children [29, 48]. While motor delay may be a sign of mild, non-specific brain damage, the abnormalities in memory processing suggest alterations in hippocampal development and function [45].

Although there are several studies regarding the adverse effects of type I and type II diabetes mellitus on central nervous system (CNS) including the hippocampus, hypothalamus, cerebellum, and cerebrum [1, 10, 33, 40, 51], there is no study about the effect of gestational diabetes on neuronal development of the hippocampus, which is important in spatial learning and memory. Therefore, this experimental study was design to assess the effect of gestational diabetes on neuronal density of CA1 and CA3 of subfields of the hippocampus in postnatal days 7 and 21 of Wistar rats.

MATERIAL AND METHODS

This experimental study was performed at the Gorgan faculty of Medicine, Golestan University of medical sciences, Gorgan, Iran. Guidelines on the care and use of laboratory animals and approval of the ethics committee of Golestan University of Medical Sciences were obtained before the study.

Experimental animals

Wistar rats, weighing 180–220 g (12 weeks old) were used in this study. The animals were maintained in a climate-controlled room under a 12-h alternating light/dark cycle, 20°C to 22°C temperature, and 50% to 55% relative humidity. Dry food pellets and water were provided *ad libitum*.

Drug

Streptozotocin (Sigma, St. Louis, MO, USA) was dissolved in sterile saline solution (0.85%) to give a 40 mg/ /kg dose intraperitoneally injected to female rats.

Animal groups and treatment

After 2 weeks of acclimation to the diet and the environment, the female Wistar rats were placed with a proven breeder male overnight for breeding. Vaginal smears were taken the next morning to check for the presence of sperm. Once sperm was detected, that day was assigned as gestational day 1 (GD). On day 1 of gestation, the pregnant rats were randomly allocated into control and diabetic groups.

Five dams in the diabetic group were given 40 mg/kg body weight of STZ and the control groups (5 dams) were given an equivalent volume of normal saline, intraperitoneally. Blood was sampled from the tail at 1 week after STZ injection. The dams with blood glucose level 120–250 mg/dL were known as GDM. The pregnancy of dams was terminated physiologically.

On postnatal days 7 and 21, from each mother in the controls and cases, 1 or 2 male infants were randomly selected. In total 6 offspring of GDM and control mothers on days 7 and 21 (P7, P21) were randomly selected and scarificed. For light microscope preparations, the brain was fixed in 10% neutral-buffered formalin for histological procedure. The coronal sections (6 μm) were serially collected from bregma –3.30 mm to –6.04 mm of the hippocampal formation [49]. The sections were stained with cresyl violet.

Blood glucose measurements

Blood glucose levels before mating and after STZ injection were obtained via the tail vein and were estimated with a glucometer (ACCU-CHEK® Active Glucometer, Roche Diagnostics, Mann-heim, Germany).

Morphometric techniques

For histomorphometric study, the sections were observed under a light microscope.

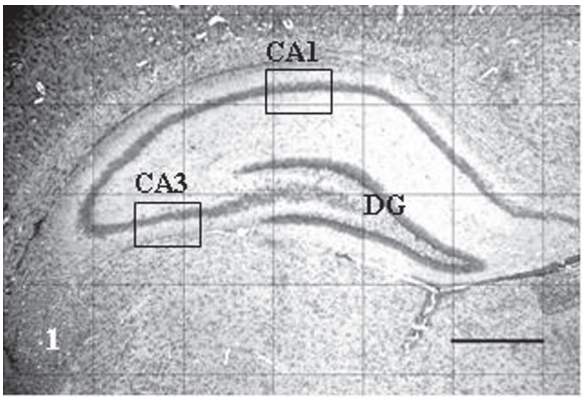

Figure 1. Presentation of hippocampal areas in a control infant rat (P21). Coronal sections stained with cresyl violet; CA1 — cornu ammonis 1; CA3 — cornu ammonis 3; DG — dentate gyrus; Grid: 500 μm ×500 μm, scale bar: 500 μm.

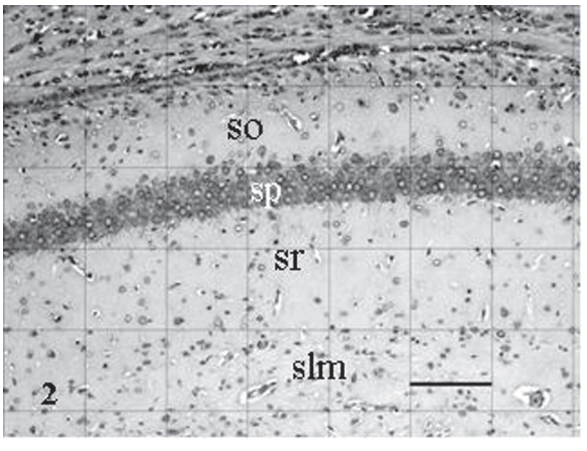

Figure 2. Hippocampal CA1 subfield in a control infant (P21). CA1 layers included stratum oriens (so), stratum pyramidal (sp), stratum radiatum (sr), and stratum lacunosum-moleculare (slm); Grid: 200 μm ×200 μm, scale bar: 200 μm.

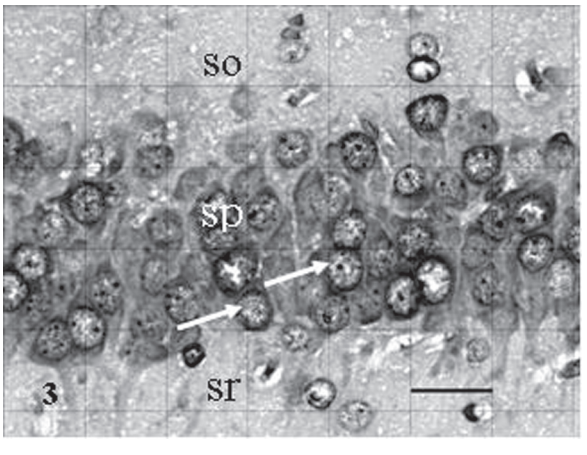

Figure 3. Hippocampal CA1 subfield in a control infant animal (P21). CA1 layers included stratum oriens (so), stratum pyramidal (sp) and stratum radiatum (sr). Arrow shows pyramidal cells; 1000× magnification; Grid: 20 μm ×20 μm, scale bar: 20 μm.

In each postnatal pup, ten similar sections of anterior to posterior of the hippocampal CA1 and CA3 subfield were selected and images were taken with an Olympus BX 51 microscope and DP12 digital camera attached to OLYSIA autobioreport software (Olympus Optical, Co. LTD, Tokyo, Japan). The number of pyramidal cells was evaluated in a 10000 μm^2 area of a pyramidal layer of the CA1 and CA3 subfield at 1000× magnification (Figs. 1–3). The thickness (μm) of layers of the hippocampus in CA1 included stratum oriens (so), stratum pyramidal (sp), stratum radiatum (sr), and stratum lacunosum-moleculare (slm) and in the CA3 field it included so, sp, stratum lucidum (slu), sr, and slm, obtained at 200× magnification.

Statistical analysis

Morphometric data is expressed as the mean ± SEM and analysed by the Student's "t" test using SPSS 11.5 software. A p value < 0.05 was considered significant.

RESULTS

Blood glucose concentrations and Body weight

The mean ± SEM of maternal blood glucose concentrations and body weight before mating and 7 and 21 days after delivery in the GDM and control groups are given in Tables 1 and 2. Blood glucose levels showed a significant increase after injection of STZ in the GDM group in comparison with the control group ($p < 0.05$). Maternal body weight 21 days after delivery significantly increased in GDM in comparison with the control group ($p < 0.05$).

Furthermore, infant body weight on the 21st postnatal day in GDM significantly increased in the GDM group compared to the controls ($p < 0.05$) (Table 3).

Morphometric results

The number of pyramidal neurons in CA1 and CA3. On postnatal day 7, the numbers of pyramidal neurons per 10000 μm^2 of the pyramidal layer in CA1 significantly reduced from 118.82 ± 8.0 in the control group to 84.71 ± 3.3 in the GDM group ($p < 0.001$).

Also, in P21, pyramidal neurons significantly reduced from 112.71 ± 6.9 in the control group to 91.52 ± 8.5 in the GDM group ($p < 0.001$).

The number of pyramidal cells of CA3 on postnatal day 7 significantly reduced from 90.33 ± 8.1 in the control group to 62.86 ± 7.2 in the GDM group

Table 1. Maternal blood glucose level (mg/dL; mean ± SEM) on the insemination day, 7 and 21 day after delivery in control and gestational diabetics (GDM)

Insemination Day		Day 7		Day 21	
Control	GDM	Control	GDM	Control	GDM
97.7 ± 2.3	97.35 ± 2.2	100.2 ± 2.1	143.2 ± 3.1*	97.5 ± 2.5	141.2 ± 3*

Results are expressed as mean ± SEM of the mean; *$p < 0.001$; $n = 5$

Table 2. Maternal body weight (gram; mean ± SEM) on the insemination day, 7 and 21 day after delivery in control and gestational diabetics (GDM)

Insemination Day		Day 7		Day 21	
Control	GDM	Control	GDM	Control	GDM
192.3 ± 11.8	194.6 ± 8.3	201.6 ± 1.2	197.6 ± 1.4	235.3 ± 7.5	262.5 ± 6.7*

Results are expressed as mean ± SEM of the mean; *$p < 0.05$; $n = 5$

Table 3. Infant body weight (gram; mean ± SEM) on the 7th and 21st postnatal days in gestational diabetics (GDM) and in controls

P7		P21	
Control	GDM	Control	GDM
12.67 ± 0.2	12.75 ± 0.6	40.84 ± 1.2	46 ± 1.3*

Results are expressed as mean ± SEM of the mean; *$p < 0.05$; $n = 6$

($p < 0.001$). In P21, the number of pyramidal cells significantly reduced from 78.33 ± 2.4 in the control group to 61.7 ± 9.5 cells in the GDM group ($p < 0.001$).

Thickness of layers in CA1

On postnatal days 7 and 21 the mean thickness (μm) of the pyramidal layer non-significantly increased in the GDM group compared to the control group. However, stratum oriens (so) and stratum radiatum (sr) in P7 were significantly increased in the GDM group in comparison with the controls ($p < 0.001$) (Table 4).

Thickness of layers in CA3

The mean thickness (μm) of the pyramidal layer in P7 and P21 non-significantly increased in the GDM group compared to the control group. The thickness of stratum oriens (so) and stratum lacunosum-moleculare (slm) significantly increased in P7 in cases in comparison with the controls ($p < 0.05$), whereas the thickness of other layers in cases non-significantly increased in comparison to the controls (Table 4).

DISCUSSION

The present study demonstrated that gestational diabetes produces a significant reduction in the pyramidal cell density of CA1 and CA3 hippocampal subfields on postnatal days 7 and 21 in Wistar rats.

This reduction of neurons can be a cause of disability of learning and memory, which has previously been reported both in human and animal newborns [68]. The hippocampus is necessary for cognitive function, especially in processing recognition memory and transforming short-term memory items into long-term storage [43].

Previous studies have shown reduced neuronal density in animals with type 1 and 2 diabetes mellitus [1, 10, 33, 40, 51].

Moreover, animal model studies have shown that mothers with type 1 and 2 diabetes mellitus born offspring with low neuronal density in the hippocampus [10, 40, 63], catecholaminergic systems of the hypothalamus [52], granule layer of dentate gyrus [1], and the cerebrum [35].

In spite of several studies regarding the effects of diabetes I and II on the CNS, including the hip-

Table 4. Mean ± SEM of the thickness (μm) of the various layers of hippocampal subfield (μm) of offspring in postnatal day (P7, P21) of gestational diabetics (GDM) and controls

Hippocampal region	P7		P21	
	Control	GDM	Control	GDM
CA1				
Stratum oriens (so)	95.73 ± 3.6	116.36 ± 2.8*	120.8 ± 5.7	138.3 ± 10.21
Stratum pyramidal (sp)	69.86 ± 5.7	77.15 ± 2.8	55.48 ± 2.9	58.57 ± 4.3
Stratum radiatum (sr)	144.46 ± 7.4	180.76 ± 6.1	149.6 ± 6.5	195.7 ± 15.7
Stratum lacunosum-moleculare (slm)	70.42 ± 4.2	106.42 ± 5.2*	84.26 ± 7.3	79.8 ± 4.1
Total	383.98 ± 13.41	490.6 ± 12.2*	412.26 ± 14.9	473.3 ± 0.5
CA3				
Stratum oriens (so)	97.75 ± 8.5	142.0 ± 4.4*	107.4 ± 9.3	141.9 ± 13.5
Stratum pyramidal (sp)	78.80 ± 12.7	84.62 ± 5.7	67.03 ± 8.3	68.53 ± 5.7
Stratum lucidum (slu)	35.29 ± 2.6	43.47 ± 3.1	32.65 ± 2.2	46.8 ± 6
Stratum radiatum (sr)	146.7 ± 13.85	143.1 ± 3.2	122.3 ± 3.8	179.4 ± 5.1
Stratum lacunosum-moleculare (slm)	35.3 ± 0.6	43.46 ± 2.1*	53.22 ± 3.2	46.4 ± 1.7
Total	393.86 ± 21	457.14 ± 12*	382.79 ± 14.8	483.14 ± 25.7

Results are expressed as mean ± SEM of the mean; *compared with control group; $p < 0.05$; $n = 6$

pocampus, there is no investigation about the effect of gestational diabetes on hippocampal structural neurons in offspring.

Our animal model study demonstrated that gestational diabetes similar to type I and II diabetes mellitus has a neurotoxic effect on the hippocampus of offspring. The neurotoxic effect of gestational diabetes is established as significant reduction in the pyramidal cell density of CA1 and CA3 hippocampal subfields in the postnatal 7 and 21 days of Wistar rats.

The reduction of pyramidal cell density of CA1 and CA3 hippocampal subfields can be due to program cell death or the block of neurogenesis in the hippocampus [33].

Diabetes mellitus, regardless of its type, is associated with hyperglycaemia. Several possible mechanisms have been explained regarding cerebral alterations, including neuronal loss of hippocampus due to hyperglycaemia. Hyperglycaemia induces multiple cellular responses. These can be considered to be neurologically passive or active cellular responses [36].

Diabetes mellitus is a chronic endogenous stressor that is associated with increased oxidative stress in CNS, in particular the hippocampus [2, 27]. The polyol pathway is activated during hyperglycaemia and leads to consumption of NADPH and depletion of glutathione, which lowers the threshold for intracellular oxidative injury [36].

CNS complications of diabetes mellitus could be mediated through excessive free radical generation [1, 3, 47, 69]. These radicals contribute to increased neuronal death by oxidising proteins, damaging DNA, and inducing the lipoperoxidation of cellular membranes [28].

Also, in other passive cellular responses, increased formation of advanced glycosylation end-products damages endothelial cells; therefore, it is contributed to vascular damage during hyperglycaemia. Diacylglycerol activation of protein kinase C has a negative effect on cerebral blood flow and vascular permeability [36].

Indeed, several studies have shown that offspring of diabetic mothers have lower arachidonic acid (AA:20:4n-6) and docosahexaenoic acid (DHA:22:6n-3) in cord blood [20, 44, 66].

AA metabolite and prostaglandin E2 play an important role in neurogenesis [64]. Zhao et al. (2009) [68] reported that maternal arachidonic acid supplementation improves neurodevelopment in young adult offspring from rat dams with and without diabetes.

Also, another possible mechanism in the cause of program cell death in diabetes mellitus [4–8, 37–39, 46] could be due to decreased insulin or insulin-like growth factor signalling [31] or an increase in cytokines such as TNFα [16].

Moreover, insulin-like growth factor has a neuroprotective anti-apoptotic effect [48], and down regulation of the expression of insulin-like growth

factor and its receptor in diabetes might also be expected to lead to neuronal loss [40, 57, 58].

Furthermore, several studies have shown that the damage to both presynaptic and postsynaptic structures in the hippocampus in diabetes from hyperglycaemia induced alterations in the handling and homoeostasis of intracellular calcium concentrations [13, 19, 21, 41, 42]. Also, up regulated GLUT-3 transporters, as one of the compensatory responses aimed at increasing neuronal glucose uptake and use, is low in diabetes [42, 47].

Anther factor in active response in hyperglycaemia is down regulation of nitric oxide synthase (NOS) mRNA and protein concentrations within hippocampal CA1 and CA3 neurons [54].

This down regulation of NOS mRNA may provide a partial explanation for the impaired long-term potentiation that is seen in the diabetic hippocampus because induction and maintenance of potentiation are dependent on NOS activity, and experimental inhibition of NOS decreases long-term potentiation and impairs cognitive learning and memory [30].

Limitations of the study. The blood glucose concentration was not measured in offspring. Although, this study was done on a small sample size of animals and thus the findings of this study cannot be directly used for human risk assessment, it should be mentioned that uncontrolled gestational diabetes might be related to CNS complications in human infants.

CONCLUSIONS

This study showed that uncontrolled gestational diabetes induces a neurotoxic effects on hippocampal pyramidal neurons in rat offspring, which remained persistent during postnatal period. Further studies are required to explore the exact mechanism of CNS complications of gestational diabetes.

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