Research Article

Gestational Diabetes Induces Neuronal Loss in Dentate Gyrus in Rat Offspring

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Summary

Background: This study was conducted to determine the effect of gestational diabetes on neuronal density in the dentate gyrus (DG) subfields of hippocampus in rats offspring.
Methods: On day 1 of gestation, 10 dams randomly allocated into two control and diabetic groups. Five animals in diabetic group were received 40 mg/kg/BW of Streptozotocin (intraperitoneally) and control animals were received normal saline. Six offsprings of each gestational diabetes mellitus and controls were randomly selected at the day 7, 14 and 21. Infants were scarified and coronal sections were taken from the right dorsal hippocampus and stained with cresyl violet. The number of granular cells and thickness of layers of hippocampus in dentate gyrus lateral (DGl) and dentate gyrus media (DGm) were evaluated.
Results: In P7, P14, P21, granular cells numbers of DGm were significantly reduced from (107.6±6.2, 131.6±4.6, 143.5±4.0) in controls to(84.96±2.1, 109.8±7.3, 121.05±5.6)(P<0.05) in cases, respectively and Granular cells of DGl were significantly reduced from (98.76±4.4, 125.6±4.0, 149.9±4.2) in controls to (79.98±4.2, 107.07±8.5, 117.1±6.7)(P<0.05) of cases, respectively. In DGm and DGl, the thickness of the granular and polymorph layers in P7,14 and P21 significantly decreased in gestational diabetics in comparison with controls(p<0.05).
Conclusion: This study showed that the uncontrolled gestational diabetes reduces granular neurons of hippocampus in rats offspring.

Key words: Gestational diabetes, Dentate gyrus, granular cell, Rat

Gestasyonel Diabet Yavru Sıçanların Dentat Giruslarında Nöronal Yitime Yol Açar
Özet

Giriş: Bu çalışma gestasyonel diabetin sıçan yavrularının hipokampuslarındaki dentat girus (DG) alt-alanlarında nöronal yoğunluğa olan etkisini araştırmak üzere kurgulandı.
Yöntem: Gebeliğin 1. günü 10 eş gelişigüzel olarak kontrol ve diabetik grup olarak ayrıldılar. Diabetik gruptaki 5 denek periton içine 40mg/Kg/Bw dozunda Streptozotocin, kontrol grubundaki hayvanlar ise normal tuzlu su aldılar. Hem gestasyonal diabetik gruptan hem de kontrol grubundan 6 yavru 7.,14. ve 21. günlerde gelişigüzel seçildiler. Yavrular sakrifiye edilerek sağ dorsal hipokampuslarının koroner kesitleri alınadı ve kresil moru ile boyandı. Hipokampus lateral (DGl) ve medial (DGm) dentat girus kalınlıkları ve granüler hücreleri değerlendirildi.
Sonuç: DGm P7, P14 ve P21'de granüler hücre sayılarında kontrollere göre sırasıyla belirgin bir azalma (107.6±6.2, 131.6±4.6, 143.5±4.0) (84.96±2.1, 109.8±7.3, 121.05±5.6)(P<0.05) saptandı. DGl granüler hücrelerinde de (98.76±4.4, 125.6±4.0, 149.9±4.2) kontrollere göre (79.98±4.2, 107.07±8.5, 117.1±6.7)(P<0.05) önemli ölçüde azalma gözlendi. Gestasyonel diabetik DGm ve DGl granüler ve polimorf katmanlarının kalınlıklarında da P7, 14 ve P21'de , kontrollere göre önemli ölçüde azalma saptandı (p<0.05).

316

Yargı: Bu çalışma kontrol edilmemiş gestasyonel diabetin sıçanların yavrularının hipokampusunda granüler nöronlarda azalmaya yol açtığını gösterdi.

Anahtar Kelimeler: Gestasyonel diabet, Dentat girus, granüler hücre, Sıçan

INTRODUCTION

Diabetes mellitus is one of the most common endocrine disorders, affecting almost 6% of the world's population. More than 97% of diabetic patients have type II diabetes[23].

It has been demonstrated that diabetes results in subtle cerebral disorders1) including alterations in neurotransmission, electrophysiological abnormalities, and structural changes[29]. Diabetes mellitus, regardless of its type, is associated with cerebral alterations in both human and animal models of the disease[11,16,37].

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[8].

Recent studies has 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, nonhuman primates as well as humans[12,17-19,26].

Diabetes mellitus is associated with cerebral alterations in both human and animal models of the disease[9,11,16]. Hippocampal neurons are also sensitive to the effects of diabetes[16,36] and often show damage to presynaptic and postsynaptic structures, dysregulation of calcium homeostasis, neuronal loss, dendritic atrophy in CA3 neurons, reduced expression of insulin growth factors and their receptors, and decreased neurogenesis[26,30,34,36,50]. Several studies have demonstrated that neural progenitors in the dentate gyrus proliferate, migrate, and differentiate into granule cells, which extend their axons and contact the CA3 pyramidal neurons, becoming integrated into the hippocampal circuitry[23].

In the dentate gyrus of the hippocampus of mammals, including humans, new neurons have been shown to be generated during postnatal and adult periods[27]. Suggested that diabetes mellitus may induce functional and structural changes in the brain. In addition to the diabetic condition itself, secondary complications involving several organs, including the brain, occur[35].

In addition, it has been demonstrated that streptozotocin (STZ)-induced diabetes significantly reduces the number of proliferating cells in the dentate gyrus of rats[26]. New cell birth and neurogenesis have been demonstrated in the dentate gyrus of several adult mammals including humans.

Neurogenesis in the dentate gyrus of the hippocampus has been associated with learning and memory formation.

Previous studies have shown that several factors such as enriched environments, learning, seizure, N-methyl- D-aspartate (NMDA) receptor antagonists, serotonin, and physical exercise, and ischemia enhance the proliferation of granular cell precursors and/or neurogenesis in the dentate gyrus while adrenal steroids, opioid peptides, and stress inhibit it[29].

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[44], and involves an interaction between diabetic susceptibility genes and diabetogenic effects of pregnancy[25].

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[51], reduce visual and memory performance at 8 and 12 months[14], poorer performance on tests of general development in infants and toddlers[48] and inferior performance in elementary school children[42]. While motor delay may be a sign of mild, nonspecific brain damage, the abnormalities in memory processing suggest alterations in hippocampal development and function[39].

Although there are several studies regarding the adverse effects of type I and type II diabetes mellitus on CNS including hippocampus, hypothalamus, cerebellum and cerebrum[1,10,26,34,45], but there is no study about the effect of gestational diabetes on neuronal development of hippocampus which are important in spatial learning and memory. Therefore, this experimental study was design to assess the effect of gestational diabetes on neuronal density of dentate gyrus of subfield of hippocampus in the postnatal 7,14 and 21 days 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 ethic committee of Golestan University of medical sciences were obtained before study.

Experimental animals

Wistar rats, weighing 180-220 grams (12 weeks old) were used in this study. The animals were maintained in a climate-controlled room under a 12-hour 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 (STZ) (Sigma, St. Louis, MO, USA) dissolved in sterile saline solution (0.85%) to give 40 mg/kg dose intraperitoneally inject to female rats.

Animal groups and treatment

After 2 weeks of acclimation to the diet and the environment, female Wistar rats were placed with a proven breeder male overnight for breeding. Vaginal smears were done 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, pregnant females randomly divided into two control and diabetic groups.

Five female rats in diabetic group receiving 40 mg/kg/body weight of streptozotocin (STZ) and control groups (five rats) receiving an equivalent volume normal saline injection intraperitoneally (IP). Blood was sampled from the tail at 1 week after STZ injection. The mothers with blood glucose level 120-250 mg/dl known as gestational diabetic mothers. The pregnancy of dams was terminated physiologically.

In postnatal days of 7,14 and 21, from each mother in control and cases one or two male infant randomly selected. Totally six offspring of gestational diabetic mothers and control mothers at the day 7,14 and 21 were randomly selected and were scarified. For light microscope preparations brain was fixed in 10% neutral-buffered formalin for histological procedure. The coronal sections (6 micrometer) serially collected from bregma -3.30 mm to -6.04 mm of the hippocampal formation[43]. The sections were stained with cresyl violet.

Blood glucose measurements

Blood glucose level of mothers (before mating and after STZ injection) and offspring was obtained via tail vein and

was estimated with a glucometer (ACCU-CHEK® Active Glucometer, Roche Diagnostics, Mann-heim, Germany).

Morphometric techniques

For histomorphometric study, the sections were observed under the light microscope. In each postnatal pup, ten similar sections of anterior to posterior of hippocampal dentate gyrus subfield were selected and images by Olympus BX 51 microscope and DP12 digital camera attached to OLYSIA autobioreport software (Olympus Optical, Co. LTD, Tokyo, Japan). The number of granular cells was evaluated in 10000 μm^2 area of granular layer of dentate gyrus lateral and dentate gyrus Media subfield in 1000X magnification. The thickness (μm) of layers of hippocampus in dentate gyrus included granular, molecular, polymorph, were obtained from 200X magnification.

Statistical analysis

Morphometric data is expressed as the mean±SEM and analyzed by the Student's "t" test using SPSS 11.5 software. $P < 0.05$ was considered significant.

RESULTS

Morphometric results

The morphometric findings are depicted in Fig. 1,2 and Table 1.

The number of granular cells in DGm and DGl

The numbers of granular cells in 10000 μm^2 area of in DGm subfield of GDM significantly reduced from (107.6±6.2) in control group to 84.96±2.1. Neurons in GDM group in P7 ($P < 0.001$) and significantly decreased in control group(131.6±4.6) to GDM group(109.8±7.3) in P14 ($P < 0.001$) and significantly decreased in control group(143.5±4.0) to GDM group(121.05±5.6) in P21 ($P < 0.001$).

Also, the number of granular cells in DGl subfield of treatment group significantly reduced from 98.76±4.4 to 79.98±4.2 in P7

and from 125.6±4.0 to 107.07±8.5 in P14 and from 149.9±4.2 to 117.1±6.7cells in 10000 μm^2 area in P21 ($P < 0.001$).

Thickness of layers in granular

The mean thickness (μm) of the granular layer in DGm subfield in P7,P14, P21 significantly reduced in treatment group (40.47±2.0, 78.11±4.1, 94.14±2.9) comparing with the control group (33.83±1.2, 57.13±5.2, 71.78±3.6).

Also, The mean thickness (μm) of granular layer in DGl subfield in P7,P14, P21 significantly reduced in treatment group (48.11±2.8, 68.03±5.1, 82.35±5.7) comparing with the control group (41.60±1.3, 47.76±2.9, 64.10±4.1).

Thickness of layers in molecular

The mean thickness (μm) of the molecular layer in DGm subfield P7 significantly increased in GDM group (47.85±3.1) comparing with the control group (56.07±1.8).

in P14 significantly reduced in GDM group (98.62±2.2) comparing with the control group (82.33±4.1) and in P21 non significantly reduced in GDM group (107.8±2.9) comparing with the control group (99.11±5.0).

Also, The mean thickness (μm) of the molecular layer in DGl subfield P7 significantly increased in GDM group (73.05±4.8) comparing with the control group (86.56±3.4).in P14 non significantly reduced in GDM group (92.16±3.2) comparing with the control group (85.34±2.3) and in P21 significantly reduced in GDM group (102.8±3.8) comparing with the control group (90.63±3.6).

Dentate gyrus-media and dentate gyrus-lateral molecular layer significantly increased in P7 in cases in comparison with controls ($P < 0.05$), whereas other layers in cases had significant and non significant decrease when compared with control rats.

Thickness of layers in polymorph

The mean thickness (µm) of the polymorph layer in DGm subfield in P7, P14 and P21 significantly reduced in treatment group (88.63±3.1, 103.4±2.6, 116.6±2.9) comparing with the control group (73.80±2.7, 83.53±4.1, 92.18±6.8).

Also, the mean thickness (µm) of granular layer in DGl subfield in P7, P14, P21 significantly reduced in treatment group (93.92±3.2, 95.17±4.5, 112.4±3.2) comparing with the control group (74.84±6.0, 81.10±1.8, 98.99±3.8).
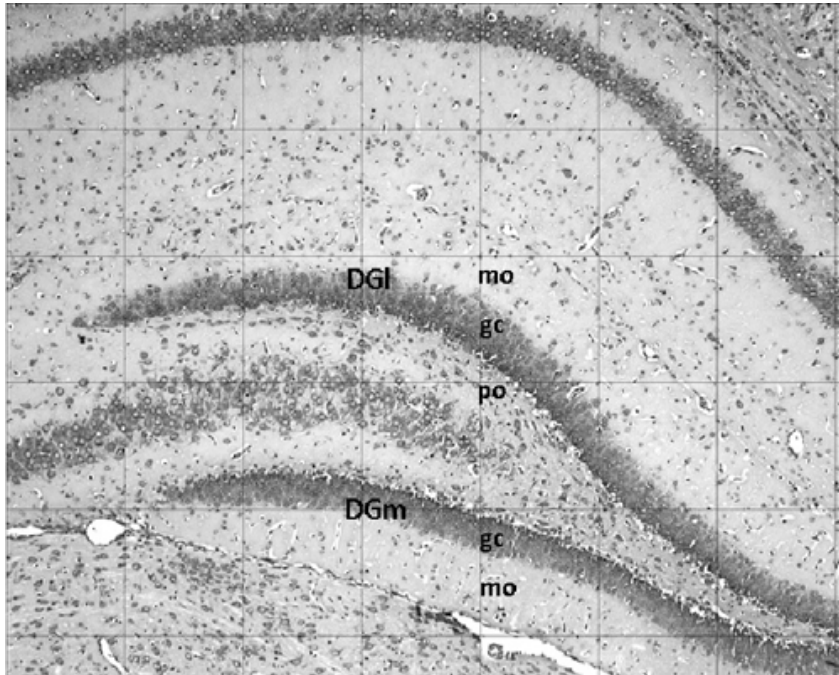
Fig 1: *Overview of dentate gyrus areas used for quantitative measurements from Wistar rat (P21) control animal. Coronal sections stained with cresyl violet. Quantification areas are: DGm, dentate gyrus medial blade; DGl, dentate gyrus lateral blade (×100 magnification)*
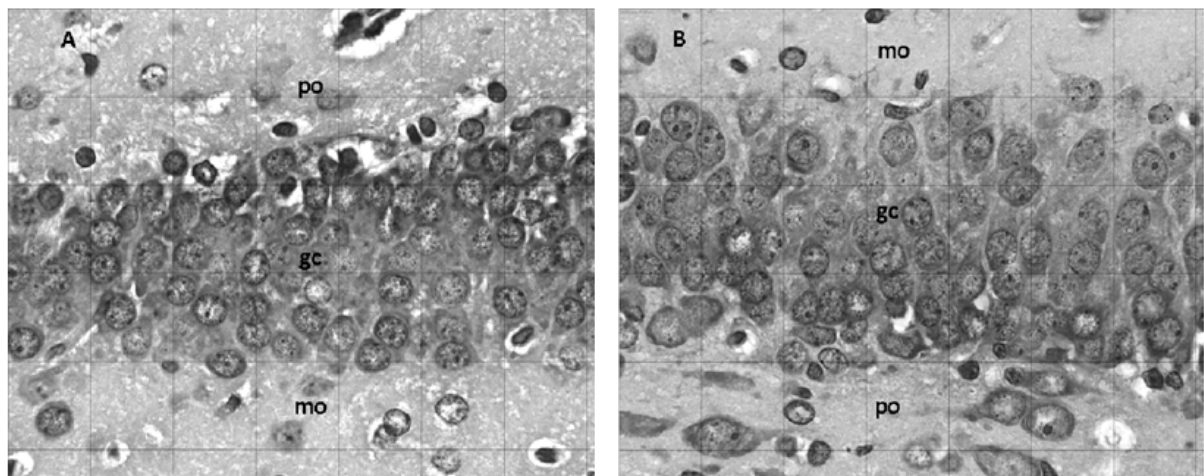
Fig 2: *Histological section of dentate gyrus in Wistar rat (P21) control animal. A: Dentate gyrus medial blade (DGm) and B: Dentate gyrus lateral blade (DGl) stained with cresyl violet (layers including: molecular layer (mo), granule cell layer (gc) and polymorph layer (po), ×1000 magnification)*

Table 1: The thickness of the various layers of dentate gyrus (μm) in postnatal day (P7,P14,P21) of Gestational diabetes mothers and control Wistar rats

P7		P14		P21	
Control	GD	Control	GD	Control	GD
47.85±3.1	56.07±1.8*	98.62±2.2	82.33±4.1*	107.87±2.9	99.11±5.0
40.47±2.0	33.83±1.2*	78.11±4.1	57.13±5.2*	94.14±2.9	71.78±3.6*
88.63±3.1	73.80±2.7*	103.43±2.6	83.53±4.1*	116.62±2.9	92.18±6.8*
73.05±4.8	86.56±3.4*	92.16±3.2	85.34±2.3	102.8±3.8	90.63±3.6*
48.11±2.8	41.60±1.3*	68.03±5.1	47.76±2.9*	82.35±5.7	64.10±4.1*
93.92±3.2	74.84±6.0*	95.17±4.5	81.10±1.8*	112.47±3.2	98.99±13.2*

Results are expressed as Mean±SEM of the mean (*compared with control group, P<0.05, n=6)

DISCUSSION

The present study demonstrated that gestational diabetes produces a significant reduction in the granular cell density of DGm and DGl hippocampal subfields in the postnatal 7,14 and 21 days of Wistar rats.

This reduction of neurons can be causes of disability of learning and memory which previously reported both in humans and animals newborns[55].

Previous studies have shown reduce neuronal density of dentate gyrus in animals with type 1 and 2 diabetes mellitus[23,29,35].

Also, animal model studies have shown that mothers with type 1 and 2 diabetes mellitus born offspring with low neuronal density in hippocampus[10,34,52], catecholaminergic systems of hypothalamus[46], granule layer of dentate gyrus[1] and cerebrum[28].

Hwang study showed that type II diabetes reduced hippocampal cell differentiation in the subgranular zone of the dentate gyrus in a rat model[23].

Also, Baek-Vin reported that streptozotocin-induced diabetes reduced neuronal density in in dentate gyrus of rats[35]. Indeed, Hong et al have shown that ginseng radix increases cell proliferation in dentate gyrus of Rats with streptozotocin-induced diabetes[29].

In spite of several studies regarding the effects of in diabetes I and II on CNS including hippocampus, there is no investigation about the effect of gestational diabetes on dentate gyrus neurons in offspring.

Our animal model study demonstrated that gestational diabetes similar to type I and II diabetes mellitus, has a neurotoxic effect on offspring dentate gyrus.

The reduction of neuronal density of dentate gyrus can be due to program cell death or block of neurogenesis[26].

Diabetes mellitus, regardless of its type, is associated with hyperglycemia. Several possible mechanisms are explained about cerebral alterations including neuronal loss due to hyperglycemia. Hyperglycemia induces multiple cellular responses. These can be considered to be neurologically passive or active cellular responses[30].

Diabetes mellitus is a chronic endogenous stressor that is associated with increased oxidative stress in central nervous system[2,20]. CNS complications of diabetes mellitus could be mediated through excessive free radicals generation[1,3,41,56].

These radicals contribute to increase neuronal death by oxidizing proteins, damaging DNA, and inducing the lipoperoxidation of cellular membranes[21].

Indeed, Several studies has shown that offspring of diabetic mothers have lower arachidonic acid (AA:20:4n-6) and docosahexaenoic acid (DHA:22:6n-3) in cord blood[15,38,54].

AA metabolite and prostaglandin E2 plays an important role in neurogenesis[53]. Zhao et al (2009) have reported that maternal arachidonic acid supplementation improves neurodevelopment in young adult offspring from rat dams with and without diabetes[55].

Also, other possible mechanism in cause of program cell deaths in diabetes mellitus[4,5,6,7,31,32,33,40] can be due to decrease insulin or insulin-like growth factor signaling[24], or an increase in cytokines such as TNFa[13].

Moreover, insulin-like growth factor has a neuroprotective anti-apoptotic effect 48 and down regulation of expression of insulin-like growth factor and its receptor in diabetes might also be expected to lead to neuronal loss[34,49].

Other factors in active response in hyperglycemia, is down regulation of nitric oxide synthase (NOS) mRNA and protein concentrations are within hippocampal CA1 and CA3 neurons[47].

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[22].

This study showed the uncontrolled gestational diabetes induces neurotoxic effects on hippocampal granular neurons in offspring, which remained persistent during postnatal period. Further studies are required for exploring the exact mechanism of CNS complications of gestational diabetes mellitus.

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