کارگاه‌های آموزشی مرکز اطلاعات علمی

مقاله نویسی علوم انسانی

اصول تنظیم قراردادها

آموزش مهارت های کاربردی

در تدوین و چاپ مقاله
Effects of Infantile Repeated Hyperglycemia on Neuronal Density of Hippocampus and Pentylentetrazol Induced Convulsions in Male Wistar Rats

Malihe Moghadami*1,2, Ali Moghimi3, Razieh Jalal4, Morteza Behnam-Rasouli1, Naser Mahdavi-Shahri1

Abstract

Objective(s)
High blood glucose induces molecular, cellular, morphological and behavioral changes in the brain. Metabolic disturbances, contribute to the hippocampus injury and development of partial focal seizures. The aim of this study was to investigate the effects of infantile repeated hyperglycemia on neuronal density of hippocampal CA3 region in newborn Wistar male rats and its effect on chemoconvulsant pentylentetrazol (PTZ) induced generalized seizures in adults.

Materials and Methods
Ten days old male Wistar rats were randomly divided into 2 groups (n=20 for each): hyperglycemic and control. Hyperglycemia was induced by intraperitoneal injections of 2 g/kg dextrose solution, twice a day, for 2 weeks. Control animals received saline solution in the same manner. Blood glucose was regularly measured. After that, the brains of rats from each group (n=10) were removed for histological analysis of the CA3 region of hippocampus by stereological method. Other animals (n=10) were kept to grow older. Afterwards, seizure was induced in hyperglycemic and control adult rats, by an intraperitoneal injection of 45 mg/kg PTZ solution and then latency of convulsions onset and severity of seizures for each group were recorded.

Results
Results showed that hippocampal neuronal density decreased significantly and susceptibility to PTZ induced convulsions increased in experimental animals.

Conclusion
The result determined that repeated increments in daily blood sugar levels in infantile period may damage neuronal structures of hippocampus and also make adults more susceptible to PTZ induced convulsions in adulthood period.

Keywords: Convulsion, Hippocampus, Hyperglycemia, Neuronal density, PTZ, Stereological method

1 Department of Biology, Faculty of Sciences, Ferdowsi University of Mashhad, Iran
* Corresponding author: Tel: +98-915-1242819; Fax: +98-511-8762227; email:malihe.moghadami@gmail.com
2 Applied Research and Production Centre of Lab Animals, (Radman Pajoohan Jam Co.), Rosht Centre of Ferdowsi University of Mashhad(FUM), Mashhad, Iran
3 Applied Research Centre for Neurofeedback and Neurobehavioral Sciences”(Aren), Department of Biology, Faculty of Science, Ferdowsi University of Mashhad (FUM), Mashhad, Iran
4 Department of Chemistry, Faculty of Sciences, Ferdowsi University of Mashhad, Mashhad, Iran
Introduction
Hyperglycemia is one of the most common and most serious metabolic disorders (1, 2). Several studies have demonstrated the effects of hyperglycemia on neurophysiologic alterations (3), cognitive abnormalities (4) and functional impairments in the CNS (5-9). Previous investigations showed that abnormal glucose levels, whether too high or too low, can cause seizures. Clinical studies show that adults with hyperglycemia have an increased predisposition to seizure (10-14). Also, several data indicated that, in the adult rats, high glucose concentrations are associated with proconvulsant effects (15). In animal experiments, seizure susceptibility has been shown to be related to higher blood glucose levels (15, 16). Such, diabetic hyperglycemia (DH) aggravates seizures and status epilepticus-induced hippocampal damage (17). There are some case reports of people experienced very severe hyperglycemia and an epileptiform convulsion (10, 11, 18).

Thus, it can be suggested that, focal epileptic seizures can be the first manifestation of a diabetic disorder (19). Also clinically, hyperglycemia-related seizures generally are present as partial motor seizures that may or may not change into generalized form (12, 13, 20). However, we have learned much about anatomical and physiopathological effects related to hyperglycemia and diabetes in adults, while there is little information about effects of hyperglycemia on CNS of newborns. In present research, we studied effects of infantile repeated hyperglycemia on histological characteristics of hippocampal CA3 region in 25-day old rats.

Materials and Methods
Animals
Forty newborn (10-days old) male Wistar rats, bred in the animal house of Ferdowsi University of Mashhad, Iran, were randomly divided into 2 groups (n= 20 for each): hyperglycemic group and control group. The animals had free access to drinking water and standard rodent diet (Javaneh Khorasan Co., Mashhad). Rats were maintained in standard Plexiglas cages under a constant 12/12 light/dark cycle and at a temperature of 21-23 °C and 60% of humidity.

Hyperglycemia induction and blood glucose measurements
Hyperglycemia was induced by intraperitoneal injections of dextrose solution provided by Pasteur Institut, Iran (50% , 2 g/kg BW, 2 times/ day ) for a period of 15 days in hyperglycemic group (n= 20). Control animals were injected with saline only (n= 20). Blood glucose was measured in blood samples, obtained by tail prick, using a strip operated glucometer (BIONIME), before injection and 30, 60 and 120 min after injection.

Histological analysis
At the end of injection period, 25-days old male animals were euthanized by ether inhalation and their brains were removed and prepared for histological analysis. Twenty sections from each animal brain were selected using a uniform random sampling scheme and stained with toluidine blue (Merck). Then numerical density of neurons in CA3 region of hippocampus was estimated by stereological methods. Stereology is the study of three-dimensional objects through the interpretation of two dimensional images. This is useful not only because it allows us to study the structure of entire cells and tissues based on thin sections or photomicrographs of sections, but also because it allows us to study this structure quantitatively. Methods of unbiased estimation of the above stereological parameters were described previously in details (21, 22).
**Induction of generalized seizures**

Generalized convulsions were induced by a single intraperitoneal (i.p.) injection of PTZ at a dose of 45 mg/kg BW dissolved in saline in hyperglycemic and control rats at 60 days of age (200-250 g). After that, severity of PTZ induced generalized convulsions (from third stage to the end of seizures) and latency period of generalized tonic-clonic convulsions onset was recorded during 60 min for each group based on our previous work (23).

**Statistical analysis**

All statistical procedures were performed by two-way analysis of variance (ANOVA) with a post-hoc Tukey test or Student's t test. A \( P < 0.05 \) was considered significant. All data were expressed as mean ± SEM.

**Results**

The levels of blood glucose before and after dextrose solution injection are illustrated in Table 1. As it is shown, 30 min after injection, blood glucose level significantly increased in experimental group and then gradually decreased to normal level, 60 and 120 min after injection. Histological analysis (Figure 1) of brains belong to 25-old days male rats revealed that in comparison to control group, the numerical density of CA3 region in hyperglycemic animals significantly decreased (\( P < 0.01 \). Numerical density was estimated by stereological methods stained with toluidine blue (Figure 2). Images were obtained with light microscope. In addition, hyperglycemia increased the occurrence of seizures induced by intraperitoneal administration of chemoconvulsant PTZ in adult rats. Latent period of generalized seizures occurrence in experimental group reduced compared with control group (\( P < 0.05 \), Figure 3) and severity of PTZ induced generalized convulsions increased significantly (\( P < 0.05 \), Figure 4). These results demonstrate that infantile hyperglycemia has a proconvulsant effect on PTZ-induced seizures in the rat.

Figure 1. Effects of infantile repeated hyperglycemia on neuronal density of hippocampal CA3 region in 25 day old male Wistar rats. Results are expressed as mean±SEM. \( n=10 \) for each group. **\( P < 0.01 \) compared to control group by Student's t test.

Figure 2. Numerical density of CA3 area neurons was estimated by stereological methods as were described in the text. Neuronal density of hippocampal CA3 region in 25 day old hyperglycemic males are decreased in (up image) compared to controls (down image).
Figure 3. Effect of repeated infantile hyperglycemia on latent period of PTZ induced generalized seizures onset in hyperglycemic and control male groups. Results are expressed as mean ± SEM n=10 for each group. *P<0.05 compared to control group by Student's t test.

Figure 4. Effect of repeated infantile hyperglycemia on severity of PTZ induced generalized convulsions in hyperglycemic and control male groups. Results are expressed as mean±SEM (n=10, * P< 0.05) compared to control group by Student's t test.

Table 1. Effect of repeated administration of dextrose solution on blood glucose concentration (mg/dl)

| Day | Time 0  | Time 30  | Time60 |
|-----|---------|----------|--------|
| 1   | 109.65  | 169.89   | 142.67 |
| 3   | 126.88  | 187.65   | 146.89 |
| 5   | 132.76  | 176.54   | 153.76 |
| 7   | 139.78  | 190.32   | 157.89 |
| 9   | 145.76  | 224.87   | 158.9  |
| 11  | 136.65  | 226.87   | 156.43 |
| 13  | 141.75  | 231.79   | 158.33 |
| 15  | 146.87  | 234.65   | 159.75 |
| Mean| 135.01  | 205.32*  | 154.32 |

Values are mean ± SEM (n=10, P< 0.05)

Discussion

The present study, demonstrated that repeated increments of blood glucose levels in infantile period can play a remarkable role in appearance of generalized epileptic seizures in adulthood. Moreover, the structure of hippocampus is severely damaged in under-age rats that; a fact that may cause tonic clonic convulsions in adults. Likewise pathophysiology is still under investigation and is probably multi-factorial.

Previous studies claimed that the temporal lobe is susceptible to seizure activity so, temporal lobe structures, including the hippocampus, have the lowest seizure thresholds in the brain (24). Hyperglycemia-triggered seizures are thought to be due to the increased metabolism of γ-amino butyric acid (GABA) (25). As, increased GABA metabolism lowers the epileptogenic threshold and predisposes it to seizures (20). Homeostasis of electrolytes in the hippocampal extra cellular fluid may be important in preventing seizure activity in aged rats (26). Moreover, over expression of specific glutamate receptors would induce seizures and spontaneous nonsynaptic bursting in vitro, and so are associated with cellular changes seen in temporal lobe epilepsy (27). Both in vivo and in vitro experimental studies suggest that a threshold glucose concentration is necessary to support synaptic transmission. Conversely, it appears that elevated extracellular glucose is associated with neuronal hyper excitability. The importance of glucose balance has been identified in studies demonstrating that hyperglycemia exacerbates ischemia-induced brain damage (16). However, mechanisms underlying the pathogenesis of seizures caused by metabolic disturbances are poorly understood.

Other hypotheses suggest changes in the hydroelectric balance (28) and alterations in neurotransmitters (29). In addition, epileptiform EEG activity associated with ischemia can contribute to early damage of hippocampal neurons (30). Similarly, some studies revealed that hyperglycemia caused dramatic increase of neuronal alterations and
glial cell damage (31), oxidative damage in rat brain (32, 33), and furthermore, enhance oxygen free radical formation (34-36) that will cause damage to nervous tissue (37, 38) and leading to neuronal death (apoptosis), DNA impairment and lipoperoxidation of cell membrane (39).

Also, hyperglycemia itself is proconvulsant, in both diabetic and normal rats (16). As a result, high blood sugar leads to hyper-excitability of CNS neurons.

Neurons need a normal level of glucose, their main source of energy, to function correctly. With the brain's overexcited and imbalanced, hyperglycemic seizures can be triggered. In other words, too much sugar makes the neurons work too much, predisposing them to "short circuit," causing a seizure.

Schwechter et al (2003) highlighted the link between metabolism and neuronal excitability and emphasized the need for further research on the long-term effects of hyperglycemia on various aspects of brain functions (15).

As explained, hyperglycemia can affect the neuronal population of hippocampal CA3 region, so, there are many suggestions indicating the role of this region in the initiation of seizure. The present study emphasized these opinions. On the one hand hyperglycemia has oxidative effects resulting in cellular death and on the other hand, hippocampal neuronal population decrement will make the brain of rats more susceptible to convulsive effects of PTZ. Thus, susceptibility to clonic and tonic–clonic induced seizures positively correlated with blood glucose concentrations (16), as the increased blood glucose concentration was associated with proconvulsant effects. So, decreased neuronal population induced by infantile repeated hyperglycemia in our studies is in agreement with previous reports (31, 40, 41). Perhaps these results will confirm the cellular structural sensitivity and damage, because of changing in the cellular membranes rigidity as have been suggested (42).

Conclusion
The results of our study indicate that repeated hyperglycemia induced obvious cell death in hippocampal CA3 region that maybe due to serious changes in metabolic processes, such as abnormal amounts of free radicals formation. And so, hyperglycemia is proconvulsant and might aggravate epileptic seizures in adult rats.

Acknowledgment
This research financially was supported by the Ferdowsi University of Mashhad (Mashhad, Iran).

References
1. Weiss R, Dziura J, Burgert TS, Tamborlane WV, Taksali SE, Yeckel CW, et al. Obesity and the metabolic syndrome in children and adolescents. N Engl J Med 2004; 350:2362–2374.
2. Reynolds K, He J. Epidemiology of the metabolic syndrome. Am J Med Sci 2005; 330:273–279.
3. Dolu N, Ozesmi C, Comu N, Säker C, Gölgele A. Effect of hyperglycemia on electrodermal activity in diabetic rats. Int J Neurosci 2006; 116:715-729.
4. Ryan C, Geckle M, Orchard T. Cognitive efficiency declines over time in adults with type 1 diabetes: effects of micro- and macrovascular complications. Diabetologia 2003; 46:940–948.
5. Sima AA, Kamiya H, Li ZG. Insulin, C-peptide, hyperglycemia, and central nervous system complications in diabetes. Eur J Pharmacol 2004; 490:187-97.
6. Northam EA, Rankins D, Cameron FJ. Therapy Insight: the impact of type 1 diabetes on brain development and function. Nat Clin Pract Neurol 2006; 2:78-86.
7. Bilotta F, Giovannini F, Caramia R, Rosa G. Glycemia management in neurocritical care patients: a review. J Neurosurg Anesthesiol 2009; 21:2-9.
8. Makimattila S, Malmberg-Ceder K, Hakkinen AM, Vuori K, Salonen O, Summanen P, et al. Brain metabolic alterations in patients with type 1 diabetes-hyperglycemia-induced injury. J Cereb Blood Flow Metab 2004; 24:1393–1399.
9. Ferguson SC, Blane A, Perros P, McCrimmon RJ, Best JJ, Wardlaw J, et al. Cognitive ability and brain structure in type 1 diabetes: relation to microangiopathy and preceding severe hypoglycemia. Diabetes 2003; 52:149–156.
32. Singh BM, Strobas RJ. Epilepsia partials continua associated with nonketotic hyperglycemia: clinical and biochemical profile of 21 patients. Ann Neurol 1989; 8:155–160.
33. Tiamkao S, Pratipanawat T, Nitinavakarn B, Chotmangkol V, Jitpipolmard S. Seizures in nonketotic hyperglycemia. Seizure 2003; 12:409–410.
34. Karim C, Rosenbaum D, Daras M. Hyperglycemia presenting with occipital seizure. Epilepsia 1991; 32:215–220.
35. Schwechter EM, Veliskova J, Velisek L. Correlation between extracellular glucose and seizure susceptibility in adult rats. Ann Neurol 2003; 53:91–101.
36. Stafstrom CE. Hyperglycemia lowers seizure threshold. Epilepsy Curr 2003; 3:148–149.
37. Huang CW, Cheng JT, Tsai JJ, Wu SN, Huang CC. Diabetic hyperglycemia aggravates seizures and status epilepticus-induced hippocampal damage. Neurotox Res 2009; 15:71–81.
38. Moien-Afshari F, Téllez-Zenteno JF. Occipital seizures induced by hyperglycemia: A case report and review of literature. Seizure 2009; 18:382–385.
39. DeCarlo LJ, Reining M, Croft BT. Clonic focal seizure of the foot secondary to nonketotic hyperglycemia. J Am Podiatr Med Assoc 2002; 92:109–111.
40. Martinez-Fernández R, Gelabert A, Pablo MJ, Carmona O, Molins A. Status epilepticus with visual seizures in ketotic hyperglycemia. Epilepsia Behav 2009; 16:660–662.
41. Behnam-Rasouli M, Nikravesh M, Mahdavi N, TehraniPour M. Post-treatment time after sciatic nerve crush on the number of alpha motoneurons, using a stereological counting method (disector). Iran Biomed J 2000; 4:45–49.
42. Sundberg MD. An introduction to stereological analysis: morphometric techniques for beginning biologists. Chapter 3. Louisiana: Department of Botany Louisiana State University Baton Rouge; 70803-1705.
43. Kheirabadi M, Moghimi A, Rakshande H, Behnam Rasouli M. Evaluation of the anticonvulsant activities of Rosa Damascena on the PTZ induced seizures in Wistar rats. J Sci Biol 2008; 8:426–430.
44. Abdelmalik PA, Burnham WM, Carlen PL. Increased seizure susceptibility of the hippocampus compared with the neocortex of the immature mouse brain in vitro. Epilepsia 2005; 46:356–366.
45. Placidi F, Floris RA, Bozzao A. Ketotic hyperglycemia and epilepsy partialis continua. Neurology 2001; 57:534–537.
46. Takeda A, Sakurada N, Kanno S, Ando M, Oku N. Vulnerability to seizures induced by potassium dyshomeostasis in the hippocampus in aged rats. J Health Sci 2008; 54:37–42.
47. Telfeian AE, Federoff HJ, Leone P, During MJ, Williamson A. Overexpression of GluR6 in rat hippocampus produces seizures and spontaneous nonsynaptic bursting in vitro. Neurobiol Dis 2000; 7:362–374.
48. Bough KJ, Rho JM. Anticonvulsant mechanisms of the ketogenic diet. Epilepsia 2007; 48:43–58.
49. Nylen K, Likhodi S, Burnham WM. The ketogenic diet: proposed mechanisms of action. Neurotherapeutics 2009; 6:402–405.
50. Caruana DA, Nesbitt C, Munby DG, Chapman CA. Seizure activity in the rat hippocampus, perirhinal and prefrontal cortex associated with transient global cerebral ischemia. J Neural Transm 2008; ?115-113.
51. Li PA, Gisselsson L, Keuker J, Vogel J, Smith ML, Kuschinsky W, et al. Hyperglycemia-exaggerated ischemic brain damage following 30 min of middle cerebral artery occlusion is not due to capillary obstruction. Brain Res 1998; 804:36-44.
52. Aragno M, Brignardello E, Tamagno O, Boccuzzi G. Dehydroepiandrosterone administration prevents the oxidative damage induced by acute hyperglycemia in rats. J Endocrinol 1997; 155:233-240.
53. Aragno M, Parola S, Tamagno E, Brignardello E, Manti R, Danni O, et al. Oxidative derangement in rat synaptosomes induced by hyperglycemia: restorative effect of dehydroepiandrosterone treatment. Biochem Pharmacol 2000; 60:389-95.
40. Li C, Li PA, He QP, Ouyang YB, Siesjö B.K. Effects of streptozotocin-induced hyperglycemia on brain damage following transient ischemia. Neurobiol Dis 1998; 5:117-128.
41. Convit A. Links between cognitive impairment in insulin resistance: An explanatory model. Neurobiol Aging 2005; 2:31-35.
42. Pari L, Latha M. Protective role of scoparia dulcis plant extract on brain antioxidant status and lipidperoxidation in STZ diabetic male Wistar rats. BMC Complement Altern Med 2004; 2:4-16.
کارگاه‌های آموزشی مرکز اطلاعات علمی

مقاله نویسی علوم انسانی

اصول تنظیم قراردادها

آموزش مهارت های کاربردی در تدوین و چاپ مقاله