Effects of Infantile Repeated Hyperglycemia on Behavioral Alterations in Adult Male and Female Rats

Malihe Moghadami [1,2], Ali Moghimi [3], Emad Ahangar [2,4] , Razieh Jalal [5], Morteza Behnam Rassouli [1], Naser Mahdavi Shahri [1]

1. Department of Biology, Faculty of Science, Ferdowsi University of Mashhad, Mashhad, Iran.
2. Applied Research and Production Center of Lab Animals; (Radman Pajoohan Jam Co.,), Roshd Center of Ferdowsi University of Mashhad(FUM), Iran.
3. Applied Research Center for Neurofeedback and Neurobehavioral Sciences (Aren) , Department of Biology, Faculty of Science, Ferdowsi University of Mashhad(FUM), Iran.
4. Department of Civil, Kashmar Branch, Islamic Azad University, Kashmar, Iran.
5. Department of Chemistry, Faculty of Science, Ferdowsi University of Mashhad, Mashhad, Iran.

Article info:
Received: 28 April 2012
First Revision: 26 June 2012
Accepted: 25 July 2012

Key Words:
Anxiety,
Elevated Plus Maze,
Exploratory,
Hyperglycemia,
Locomotive,
Open Field.

ABSTRACT

Anxiety symptoms have been reported to be present in many patients with diabetes mellitus. However, little is known about the effects of hyperglycemia in critical periods of the central nervous system development. We assessed locomotive, exploratory, and anxiety behaviors in adult rats that remained from infantile repeated hyperglycemia by the open field and elevated plus maze tests. Our findings showed significant hypo activity, reduced locomotive/exploratory activities, increased fear related behaviors, and anxiety state between hyperglycemic and control adult males and the same differences were observed among females. In addition, no significant behavioral alterations between male and female animals were observed. This study determined that repeated increments in daily blood sugar levels in newborns may affect neuronal functions and provide behavioral abnormalities in adults.

1. Introduction

Diabetes mellitus (DM) is a common metabolic disorder that is characterized by hyperglycemia and other complications (Popovic, Biessels, Isaacson, & Gispen, 2001). Elevated blood glucose levels in diabetic patients involve alternations in neurotransmission, electrophysiological abnormalities, structural changes, and cognitive deficits (Biessels et al.,, 1996, 1994; Monaghan, 1995; McCall, 1992). It has been reported that anxiety symptoms increased and were common among types 1 and 2 diabetic patients (Grigsby, Andersona, Freedlanda, Clouse, & Lustmana, 2002; Skenazy & Bigler, 1984; Lustman & Clouse, 1990), and such patients certainly suffer from reduced motor activity and increased risk of dementia and cognitive dysfunction (Gispen & Biessels, 2000). Such that, diabetic individuals performed poorly on tasks requiring motor efficacy and somatosensory discrimination (Lustman, 1988). Continuously, it has been suggested that anxiety is associated with poor control of glycemia (Lloyd, Dyert, & Barnett, 2000; McGrady & Horner, 2001; Rubin & Peyrot, 2001). Also, poor glycemic control can be a risk factor for various diseases, including myocardial infarction, stroke, Alzheimer's disease, bone fractures, and colorectal, liver, pancreas, bladder, and breast cancers (Croft & Hannaford, 1989; Coughlin, Calle, Teras, Pe-

**** Corresponding Author:***
Malihe Moghadami, PhD,
Department of Biology, Faculty of Science, Ferdowsi University of Mashhad, Mashhad, Iran.
E-mail: malihe.moghadami@gmail.com

trelli, & Thun, 2004; Stegmayr & Asplund, 1995; Ott et al., 1999; Vestergaard, Rejnmark, & Mosekilde, 2005).

In recent years, more attention has been focused on the relationship between neurochemistry, neuroanatomy and behavior. That is, our previous studies determined that hippocampal neuronal density decreased significantly and the susceptibility to Pentylentetrazol (PTZ) induced convulsions increased in experimental animals that were exposed to repeated acute hyperglycemia in infancy period (Moghadami, Moghimi, Jalal, Behnam-Rasouli, & Mahdavi-Shahri, 2011). Furthermore, several studies have demonstrated that diabetes is associated with behavioral changes in animals (Mooradian, 1988; Tomlinson, Gardiner, Hebden, & Bennett, 1992). So, it has been shown increased grooming activity in a novel environment (Ahmad & Merali, 1988), more anxiogenic activity (Ramanathan, Jaiswal, & Bhattacharya, 1998) in rats, decreased locomotor activity, increased immobilization time and anxiety behavior in diabetic mice (Asakawa, Toyoshima, Inoue, & Koizumi, 2007), reduced locomotor and exploratory behaviors in hyperglycemic females (Inhasz Kiss et al., 2009). Also cognitive deficits (Singh, Chopra, & RajatSandhir, 2008) and decreased behavioral activity were observed in diabetic animals using elevated plus maze, open field tests (Volchegorskii, Tseilikman, Ship, Bubnov, & Sinitskii, 2003), and hole-board test (Junzo Kamei, Ohsawa, Tsuji, Takeda, & Matsumiya, 2001). Likewise, offspring of diabetic dams showed hyperactivity and anxious behavior (Ramanathan et al., 2000).

Thus, these data indicate that hyperglycemia is enough to impair behavioral activity. But little is known about the effects of hyperglycemia in immature infants and its influence on behavioral alternations in adults. Furthermore, in the present study, hyperglycemia was induced in newborn rats and behavioral alternations in adult life were examined.

2. Methods

2.1. Subjects

Wistar rats (n=40) which were bred in the animal house of Ferdowsi University of Mashhad, Iran, were randomly divided into 4 groups (n=10 in each): hyperglycemic and control males and females. They were housed in the standard Plexiglas cages in temperature-controlled rooms (20°C) with a 12:12-hr light:dark cycle and free access to water and standard rodent diet (Javaneh Khorasan Co., Mashhad). The offspring were obtained by mating a normal father with a normal mother and birth day take into 0 day. Body weights of rats were monitored daily during the experiments. All experimental procedures were approved by the Institutional Animal Care and Use Committee and were conducted in accordance with the National Principle for the care and use of laboratory animals.

2.2. Hyperglycemia Induction and Blood Glucose Measurements

Hyperglycemia was induced by intraperitoneal injection of dextrose solution (Pasteur Institute of Iran; 50%, 2 g/kg i.p., 2 times/day) in hyperglycemic male and female groups at 10 days of age (n=10). Repeated intraperitoneal administrations of dextrose solution were continued for 15 days (Moghadami et al., 2011). To determine the glycemic state of the rats, blood glucose levels were measured in the blood samples, obtained by tail prick, using a strip operated glucometer (BIONIME), before injection (0 min), 30, 60, and 120 min after injection. Male and female control animals were injected with saline only (n=10). And then animals were kept to reach 60 days old.

2.3. Behavioral Tests

Adult animals (60 days old) were tested in two types of behavioral tasks, elevated plus-maze (EPM) and open field (OF) tests to assess differences in emotionality and anxiety in a random order. Immediately before the testing, animals were brought into the testing room in their home cage, and testing started immediately with no acclimatization period.

2.4. Elevated Plus Maze Test

Adult rats were subjected to elevated plus-maze and were recorded on various behavioral parameters. The experimental procedure was similar to that described by Pellow, Chopin, File, and Briley (1985). Subjects were placed one at a time in the central area of the apparatus facing a closed arm, and a timer was started. The apparatus consisted of two open arms (50 x 10 cm) and two closed arms (50 x 10 x 20 cm). The arms extended from a central platform (5 x 5 cm), and the maze was elevated to a height of 50 cm from the floor. During the 5 min test period, the following parameters were measured to analyze the behavioral changes of the experimental rats: open arm entry, closed arm entry, percentage of arm entry, total arm entry, time spent in open arms, time spent in closed arms, and percentage of time spent in open arm and closed arm (Espejo, 1997; Guimaraes, Del Bel, Padovan, MendonçaNetto, & Titze-de-Almeida, 1993).

An entry was defined as entering with all limbs into one arm. A decrease in open arm entries and also the time spent in these arms are indicative of anxiogenic activity shown by experimental rats. So that, the more anxious animals enter the open arms less frequently and spend less time in open arms. Overall animal locomotor activity can be measured by the total number of entries into both open and closed arms. Once the test was completed, each rat was returned to its home cage, the apparatus was cleaned with 75% Ethanol, and fully dried before performing the next experiment.

2.5. Open Field Test

To evaluate locomotor and exploratory activities in a novel environment, adult animals were subjected to an open-field activity test. Rats were placed in an open field box (40 × 100 x 100 cm) marked off into 25 equal squares, 9 holes were inserted in each square (on the center, corners and middles of square's ribs). All experiments were conducted in a quiet room under normal light. Each animal was tested in the apparatus once. To determine open field activity, each animal was placed for the first time in a fixed corner of the open field box. During a 5 minute period animals were permitted to move and explore the new environment (open field box). The number of rows of crossed squares (outer and inner) with all four paws, number and duration of grooming and rearing (defined as standing upright on hind limbs), the frequency of defecations, total locomotor activity and number of head dipping into holes were recorded using a video camera placed on the box. (Hallam, Horgan, McGrath, & Norman, 2004; Kennett, Dickinson, & Curzon, 1985). The animal exploratory performance in outer rows of squares adjacent to the walls of the box is considered as less anxiety. Total movement in the apparatus reflects general activity and the relative movement or crossing the central or peripheral squares are correlated to the anxiety state of rats. Therefore, exploratory activities determined by frequency of head-dipping into holes, fear-related behaviors are expressed by rearing and frequency of defecation. So, more defecation and less locomotor activity imply fear behavior in rats or mice (Bronikowski et al., 2001).

2.6. Statistical Analysis

All data are expressed as group means ± SEM. Statistical significance between groups was determined by one-way or two-way ANOVA followed by Dunnett's post-hoc test, or Student's t-test, as appropriate. A $P<0.05$ was considered significant.

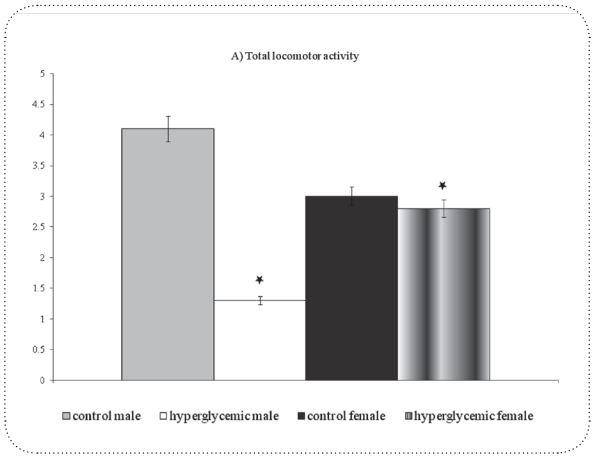
3. Results

3.1. Anxiety Level in the EPM Apparatus

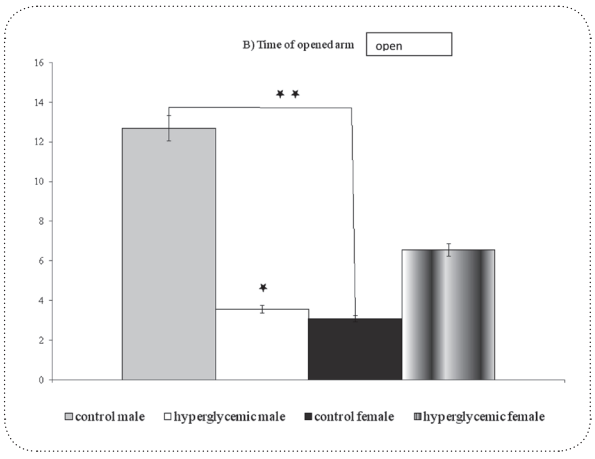
In the elevated plus maze test, hyperglycemic males showed a significant decrease in locomotor activity (Fig. 1A, $p<0.05$), increased numbers of entry and time spent in the closed arms (Fig. 1C, 1E, $p<0.05$), compared to controls. Also, the percentage of the total number (% entry) and the total time (% time) of entries in the open arms were reduced compared to control rats (Fig. 1D, 1B, $p<0.05$). Likewise, in experimental females, a significant decrease in the total locomotor activity (Fig. 1A, $p<0.01$), and closed arm entry (Fig. 1C, $p<0.05$) was observed. Also, results revealed no differences in performance between male and female hyperglycemic animals on these experiments but there were significant differences between controls at closed arm entry, the spent time in opened arm and open arm entry parameters (Fig. 1B, 1C, 1D).

3.2. Exploratory & Emotional Behaviors in the Open Field Apparatus

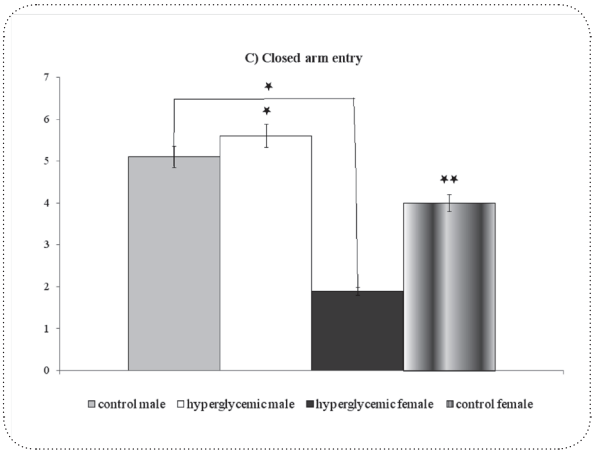
Open-field testing is used to assess locomotion, exploration, and anxiogenic-like behavior of rats. Compared to controls, hyperglycemic males spent less time in the outer rows of the field (Fig. 2A, $p<0.01$). Results showed that they crossed more inner rows (Fig. 2B, $p<0.05$), defecated less frequently (Fig. 2F, $p<0.001$), groomed more frequently (Fig. 2D, $p<0.001$), and their total locomotor activity was reduced significantly (Fig. 2H, $p<0.01$). However, there were no significant differences in the number of rearing and head-dipping behaviors between control and experimental male groups. Also, similar tests comparing locomotor and exploratory activities in female rats determined a significant increase in times of inner squares entry (Fig. 2B, $p<0.05$). Hyperglycemic females crossed less outer rows of field (Fig. 2A, $p<0.05$), with a reduced head-dipping frequency (Fig. 2G, $p<0.05$), and a decreased defecation frequency (Fig. 2F, $p<0.05$), and groomed more frequently (Fig. 2D, $p<0.01$). But there were no significant differences in the number of rearing behaviors and total locomotor activity between control and hyperglycemic females. Also, there were no differences between male and female experimental groups but there were significant differences between controls at head dipping, duration of grooming, inner rows entry parameters (Fig. 2G, 2C, 2B). As that implies, the control animals were more active in the open field (Fig. 2).



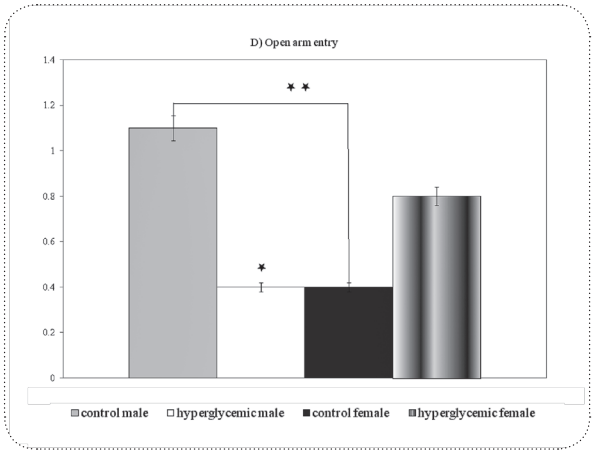
Panel A.



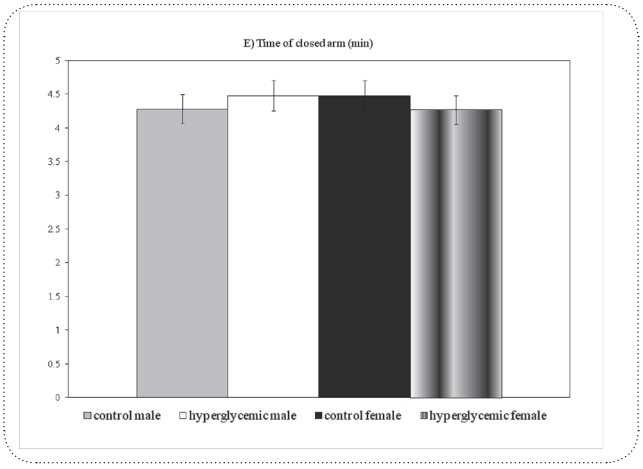
Panel B.



Panel C.



Panel D.



Panel E.

NEUROSCIENCE

Figure 1. Effects of infantile repeated hyperglycemia on anxiety state in the elevated plus maze. During the 5 min test period the following parameters were measured to analyze the behavioral changes of the experimental groups: A) Total locomotor activity, B) Time spent in open arms, C) Closed arm entry, D) Open arm entry, E) Time spent in closed arms. Results are expressed as mean ± S.E.M. n=10 for each group. *: P < 0.05, **: P< 0.01, ***: P< 0.001 compared to the control group.

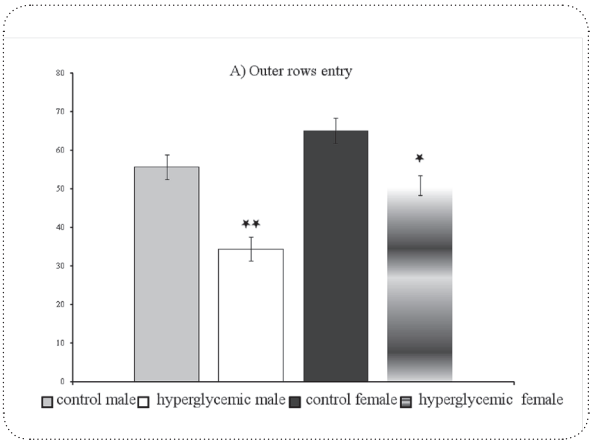


Figure 2A.

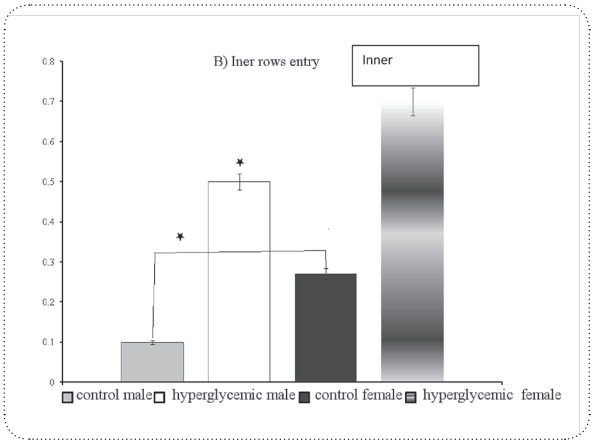


Figure 2B.

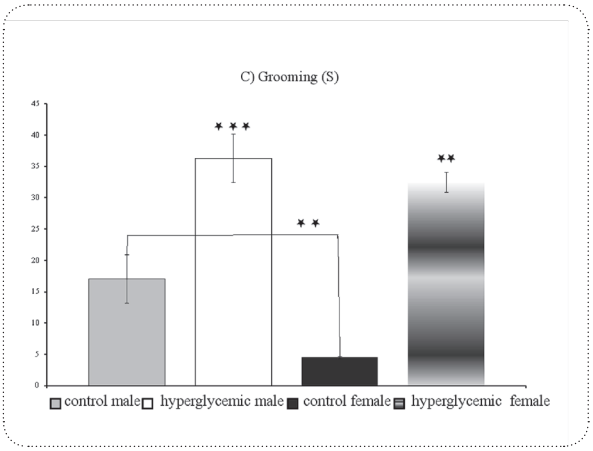


Figure 2C.

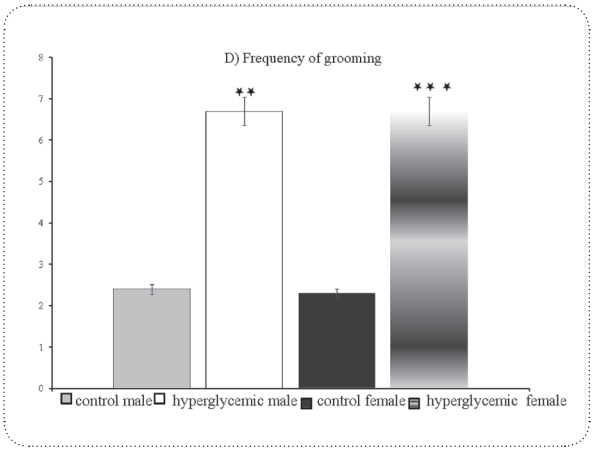


Figure 2D.

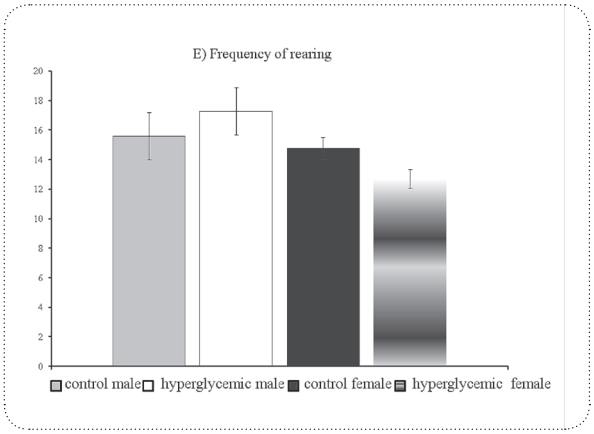


Figure 2E.

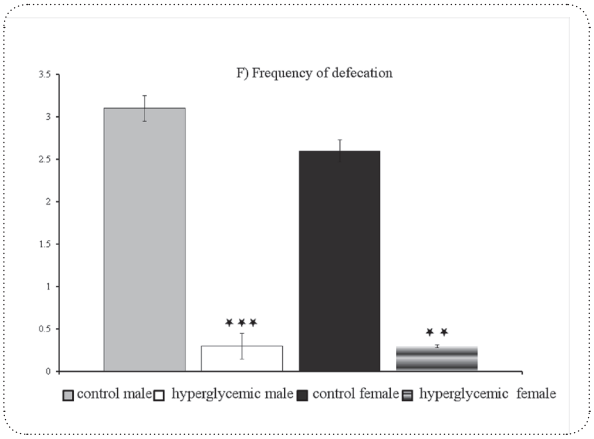


Figure 2F.

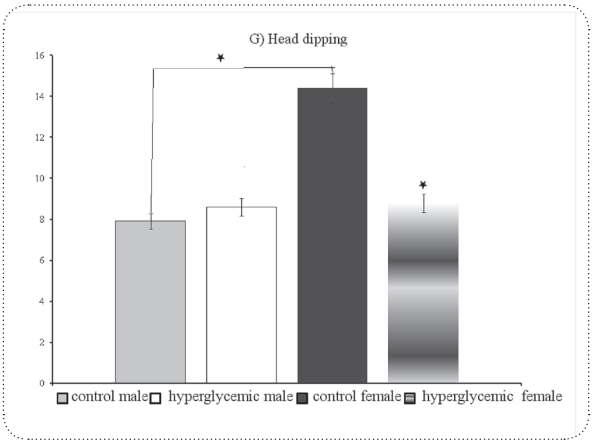


Figure 1G.

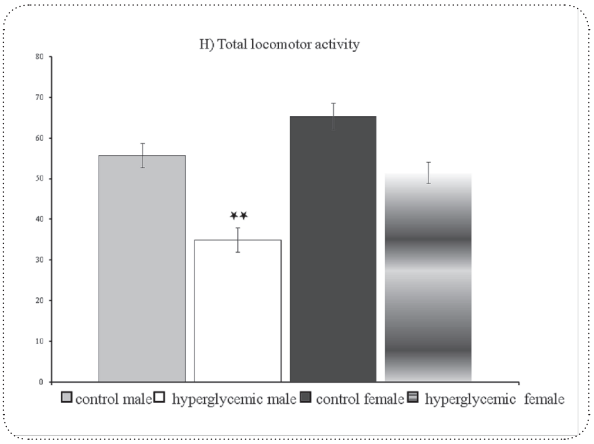


Figure 1H.

NEUROSCIENCE

Figure 2. Effects of infantile repeated hyperglycemia on exploratory and emotional behaviors in the open field test. During the 5 min test period the following parameters were measured to analyze the behavioral alternations of the experimental animals: Outer rows entry (A), Inner rows entry (B), Time spent of grooming (C), Frequency of grooming (D), Frequency of rearing (E), Frequency of defecation (F), Frequency of head dipping (G), Total locomotor activity (H). Results are expressed as mean ± S.E.M. n=10 for each group. *: P < 0.05, **: P< 0.01, ***: P< 0.001 compared to the control group.

4. Discussion

Our results indicated that adult rats with infantile repeated hyperglycemia showed anxiogenic activity on the EPM and hypo activity and less exploratory behaviors in the open field test. Although no such gender differences were found in activity and anxiety levels in the present study among hyperglycemic adult rats. We can suggest that although in normal males and females, abovementioned behaviors are significantly different, maybe hyperglycemia during infantile period of brain development will prevent the sexual dimorphism of brain anatomical and biochemical mechanisms and functions.

The time an animal spends in the open arm of the EPM apparatus reflects its anxiety level. Thus anxious animals spent most of the time in the closed arms while less anxious animals explored open areas longer. Hyperglycemic rats showed an increased percentage in the attempt made towards closed arm entry and the animals also remained in closed arms of EPM for a longer period, thereby causing hypo locomotion in experimental rats. Besides, reduced overall locomotor activity and elevated inner squares entry are exhibited in the open field test that is related to increased fear and anxiety state. Therefore, infantile repeated administration of dextrose solution may be correlated to increased anxiety state in adults.

Just as suggested, anxiety is a neurological problem associated with diabetes mellitus (Grigsby et al., 2002; Skenazy & Bigler, 1984; Gispen Biessels, 2000). Previous studies have reported that STZ-induced diabetic rats exhibited heightened anxiety, reduced locomotor activity on the EPM and open-field tests (Lustman, 1988; Inhasz Kiss et al., 2009; Ramanathan et al., 2000) and increased expression of fear-related behavior (Miyata, 2007) in various experimental paradigms. Moreover, rat offspring of diabetic mothers showed anxiogenic activity in the EPM test (Ramanathan et al., 2000). Additionally, offspring of control and diabetic dams did not differ in the amount of time spent in the open or closed arms (Sandrini, Vitale, Vergoni, Ottani, & Bertolini, 1997).

These results are in contrast with each other. Taken as a group, these studies and others have not yet yielded reliable conclusions as to whether neurodevelopmental differences exist between male and female groups. To our knowledge, no human or animal studies have been done specifically comparing anxiety and exploratory behaviors in the animals that have been exposed to elevated blood glucose concentrations in infancy period. Therefore, we set out to assess hyperglycemic effect in immature newborns on the exploratory and anxiety behaviors in the adult rats.

Taken together, previous studies (Muneoka et al., 1997; Thomas, Suzanne, Michael, Caroline, & Austin, 1995) revealed the correlation between diabetic anxiety and some neurochemical systems such as serotonergic mechanisms. Therefore, that is possible, anxiogenic effect shown in this research, is related to its effect on serotonergic transmission. Afterwards, further stud-

ies should be done to investigate this relationship and brain areas for possible alterations resulting from elevated levels of glucose and insulin. Also, the underlying mechanisms which indicate that diabetes mellitus influences behavior, remain to be determined. Then, it is clear that neurological and neurodevelopmental tests on effects of hyperglycemia are needed to begin to resolve the controversy and further studies will be required to elucidate how these functional alterations of nervous system and behavioral abnormalities occur.

5. Conclusion

These findings determined that hyperglycemic rats may behave differently from controls when subjected to a novel environment and the change in blood glucose rate in infantile period is related to the various behavioral and psychological changes in adults. It may be concluded that exposure of offspring to repeated hyperglycemia can lead to anxiogenic/emotional behaviors in adult life.

Acknowledgment

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

References

Ahmad, Q., & Merali, Z. (1988). The spontaneously diabetic Wister BB rat manifests altered grooming and catalepsy responses: implication of impaired dopamine function. Proc-Neuro psycho pharma col Biol Psychiatry, 12, 291-298.

Asakawa, A., Toyoshima, M., Inoue, K., & Koizumi, A. (2007). Ins2Akita mice exhibit hyperphagia and anxiety behavior via the melanocortin system. IJMM, 19, 649-652.

Biessels, G. J., Kamal, A., Ramakers, G. M., Urban, I. J., Spruijt, B. M., Erkelens, D. W., & Gispen, W. H. (1996). Place learning and hippocampal synaptic plasticity in streptozotocin-induced diabetic rats. Diabetes, 45, 1259-1266.

Biessels, G. J., Kapelle, A. C., Bravenboer, B., Erkelens, D. W., & Gispen, W. H. (1994). Cerebral function in diabetes mellitus. Diabetologia, 37, 643-650.

Bronikowski, A. M., Carter, P. A., Swallow, J. G., Girard, I. A., Rhodes. J. S., & Garland, T. (2001). Open-Field Behavior of House Mice Selectively Bred for High Voluntary Wheel-Running. Behavior Genetics, 31(3), 309-316.

Coughlin, S. S., Calle, E. E., Teras, L. R., Petrelli, J., & Thun, M. J. (2004). Diabetes mellitus as a predictor of cancer mortality in a large cohort of US adults. Am J Epidemiol, 159, 1160-1167.

Croft, P., & Hannaford, P. C. (1989). Risk factors for acute myocardial infarction in women: evidence from the Royal College of General Practitioners' oral contraception study. BMJ, 298, 165-168.

Espejo, E. F. (1997). Effects of weekly or daily exposure to the elevated plus-maze in male mice. Behav Brain Res, 87, 233-238.

Gispen, W. H., & Biessels, G. J. (2000). Cognition and synaptic plasticity in diabetes mellitus. Trends Neurosci, 23, 542-549.

Grigsby, A. B., Andersona, R. J., Freedlanda, K. E., Clouse, R. E., & Lustmana, P. J. (2002). Prevalence of anxiety in adults with diabetes: A systematic review. J Psychosom Res, 53, 1053-1060.

Guimaraes, F. S., Del Bel, E. A., Padovan, C. M., MendonçaNetto, S., & Titze-de-Almeida, R. (1993). Hippocampal 5-HT receptors and consolidation of stressful memories. Beh Brain Res, 58, 133-139.

Hallam, K. T., Horgan, J. E., McGrath, C., & Norman, T. R. (2004). An investigation of the effect of tacrine and physostigmine on spatial working memory deficits in the olfactory bulbectomised rat.Behav Brain Res, 153(2), 481-486.

Inhasz Kiss, A. C., Sinzato, Y. K., Machado Spada, A. P., Bueno, A., Iessi, I., Sakamoto, S., Saito, F., Kempinas, W. G., Cunha Rudge, M. V., & Damasceno, D. C. (2009). Neonatal induced mild diabetes: influence on rat development and behavioral activity. FASEB J, 962-967.

Junzo Kamei, J., Ohsawa, M., Tsuji, M., Takeda, H., & Matsumiya, T. (2001). Modification of the Effects of Benzodiazepines on the Exploratory Behaviors of Mice on a Hole-Board by Diabetes. Jpn. J. Pharmacol, 86, 47-54.

Kennett, G. A., Dickinson, S. L., & Curzon, G. (1985). Enhacement of some 5-HT depressant behavioral responses following repeated immobolization in rats. Brain Res, 330, 253-263.

Lloyd, C. E., Dyert, P. H., & Barnett, A. H. (2000). Prevalence of symptoms of depression and anxiety in a diabetes clinic population.Diabet Med, 17, 198-202.

Lustman, L. J. (1988). Anxiety disorders in adults with diabetes mellitus. PsychiatrClin North Am, 11, 419-432.

Lustman, L. J., & Clouse, R. E. (1990). Relationship of psychiatric illness to importance in men with diabetes. Diabetes Care, 13, 893-895.

McCall, A. L. (1992). The impact of diabetes on the CNS. Diabetes, 41, 557-570.

McGrady, A., & Horner, J. (2001). Role of mood in outcome of biofeedback assisted relaxation therapy in insulin dependent mellitus. Appl Psychophysiol Biofeedback, 24, 79-88.

Miyata, S., Yamada, N., Hirano, S., Tanaka, S., & Kamei, J. (2007). Diabetes attenuates psychological stress-elicited 5-HT secretion in the prefrontal cortex but not in the amygdala of mice. Brain Res, 147, 233-239.

Moghadami, M., Moghimi, A., Jalal, R., Behnam-Rasouli, M., & Mahdavi-Shahri, N. (2011). Effects of infantile repeated hyperglycemia on neuronal density of hippocampus and pentylentetrazol induced convulsions in male Wistar rats. IJBMS, 15 (4), 787-794.

Monaghan, D. T. (1995). Insulin potentiates N-methyl-D-aspartate receptor activity in Xenopus oocytes and rat hippocampus. NeurosciLett, 192, 5-8.

Mooradian, A. D. (1988). Diabetic complications of the central nervous system.Endocrinol Rev, 9, 346-356.

Muneoka, K., Mikuni, M., Ogawa, T., Kitera, K., Kamei, K., Takigawa, M., & Takahashi, K. (1997). Prenatal dexamethasone exposure alters brain monoamine metabolism and adrenocortical response in rat offspring. Am J Physiol Regulatory Integrative Comp Physiol, 273, 1669-1675.

Ott, A., Stolk, R. P., van Harskamp, F., Pols, H. A., Hofman, A., & Breteler, M. M. (1999). Diabetes mellitus and the risk of dementia: The Rotterdam study. Neurology, 53, 1937-1942.

Pellow, S., Chopin, P., File, S. E., & Briley, M. (1985). Validation of open:closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. J Neurosci Methods, 14:149-167.

Popovic, M., Biessels, G. J., Isaacson, R. L., & Gispen, W. H. (2001). Learning and memory in streptozotocin-induced diabetic rats in a novel spatial/object discrimination task. Behav Brain Res, 122, 201-207.

Ramanathan, M., Jaiswal, A. K., & Bhattacharya, S. K. (1998). Differential effects of diazepam on anxiety in streptozotocin induced diabetic and non-diabetic rats. Psychopharmacology (Berl), 135, 361-367.

Ramanathan, M., Jaiswal, A. K., & Bhattacharya, S. K. (2000). Hyperglycaemia in pregnancy: effects on the offspring behaviour with special reference to anxiety paradigms. Indian J ExpBiol, 38, 231-236.

Rubin, R. R., & Peyrot, M. (2001). Psychological issues and treatments for people with diabetes. J ClinPsychol, 57, 457-478.

Sandrini, M., Vitale, G., Vergoni, A. V., Ottani, A., & Bertolini, A. (1997). Streptozotocin-induced diabetes provokes changes in 5-HT concentration and on 5-HT1A and 5-HT2A receptor in rat brain. Life Science, 60, 1393-1397.

Singh, S. K., Chopra, K., & RajatSandhir, R. (2008). Neuroprotective effect of N-acetylcysteine in the development of diabetic encephalopathy in streptozotocin-induced diabetes. Met Brain Dis,. 23(4), 427-443.

Skenazy, J. A., & Bigler, E. D. (1984). Neuropsychopharmacological findings in diabetic mellitus. J Clin Psychol, 40, 246 -258.

Stegmayr, B., & Asplund, K. (1995). Diabetes as a risk factor for stroke. A population perspective. Diabetologia, 38, 1061-1068.

Thomas, S., Suzanne, L., Michael, L., Caroline, J., & Austin, W. (1995). Deficits in Radial Arm Maze Performance in Kindled Rats: evidence for long-lasting memory dysfunction induced by repeated brief seizures. J Neuroscience, 15, 8295-8301.

Tomlinson, K. C., Gardiner, S. M., Hebden, R. A., & Bennett, J. (1992). Functional consequences of streptozotocin induced diabetes mellitus, with particular reference to the cardiovascular system. Pharmacol Rev, 44, 103-150.

Vestergaard, P., Rejnmark, L., & Mosekilde, L. (2005). Relative fracture risk in patients with diabetes mellitus, and the impact of insulin and oral antidiabetic medication on relative fracture risk. Diabetologia, 48, 1292-1299.

Volchegorskii, I. A., Tseilikman, V. E., Ship, S. A., Bubnov, N. V., & Sinitskii, A. I. (2003). The Effects of Anxiogenic Stress on Glucocorticoid Sensitivity, Glucose Tolerance, and Alloxan Resistance in Rats. NeurosciBehav Physiol, 33(6), 595-599.