Neuroscience 318 (2016) 122–133

THE IMPACTS OF DIABETES IN PREGNANCY ON HIPPOCAMPAL SYNAPTOGENESIS IN RAT NEONATES

S. VAFAEI-NEZHAD,[a] J. HAMI,[a]* A. SADEGHI,[b] K. GHAEMI,[c] M. HOSSEINI,[d] M. R. ABEDINI[e,h] AND H. HAGHIR[f,g]

[a] *Department of Anatomical Sciences, School of Medicine, Birjand University of Medical Sciences, Birjand, Iran*

[b] *Department of Anatomy and Molecular Biology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran*

[c] *Department of Neurosurgery, School of Medicine, Birjand University of Medical Sciences, Birjand, Iran*

[d] *Department of Public Health, Deputy of Research and Technology, Research Centre of Experimental Medicine, Birjand, Iran*

[e] *Department of Pharmacology, School of Medicine, Birjand University of Medical Sciences, Birjand, Iran*

[f] *Department of Anatomy and Cell Biology, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran*

[g] *Medical Genetic Research Center (MGRC), School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran*

[h] *Cellular & Molecular Research Center, Birjand University of Medical Sciences, Birjand, Iran*

Abstract—Diabetes during the pregnancy period impairs hippocampal development, and is associated with neurocognitive and neurobehavioral problems in the offspring. Synaptogenesis is one of the most important events in the development of the nervous system, and is known as a mechanism by which the memory process takes place. Synaptophysin (SYP) is an integral membrane protein of synaptic vesicles in the hippocampus involved also in learning and memory. The present study aimed to examine the effects of maternal diabetes on the expression and distribution pattern of SYP, as a marker of synaptogenesis, in the developing rat hippocampus using Immunofluorescence staining and real-time PCR. Wistar female rats were maintained as diabetic from a week before pregnancy through parturition and male offspring was euthanized at postnatal day (P) 0, 7, and 14. Our results showed a significant down-regulation in mRNA expression of SYP in the offspring born to diabetic animals at P7, and P14 ($P \leqslant 0.05$ each). Regarding to the density of SYP expressing hippocampal neurons, we found a marked decrease in the distribution pattern of SYP in all hippocampal subfields of Streptozotocin (STZ)-D group rat neonates, especially in one and two weeks of age ($P \leqslant 0.05$ each). Moreover, the results revealed no significant changes in either gene expression or distribution pattern of SYP – positive neurons in insulin-treated group compared with the controls. The present study demonstrated that diabetes in pregnancy has negative impacts on synaptogenesis in the offspring's hippocampus. Furthermore, the rigid maternal glycaemia control by insulin treatment in most cases normalized these effects.

Key words: maternal diabetes, hippocampus, synaptogenesis, synaptophysin, rat newborn.

INTRODUCTION

Diabetes is one of the common metabolic complications in pregnancy period whose prevalence is increasing among women of reproductive age and results in both long-and short-term adverse outcomes for the offspring (Aerts et al., 1990; Pettitt and Bennett, 1995; Eidelman and Samueloff, 2002; ter Braak et al., 2002; Persaud, 2007; Lynch et al., 2015). This common metabolic disease complicates two to three percent of all pregnancies in developed countries; but this range varies between 1 and 14 percent in different societies (Bell et al., 2008; Bhat et al., 2012; DeSisto et al., 2014; Mahalakshmi et al., 2014; Lynch et al., 2015; Mwanri et al., 2015). Diabetes in pregnancy may be divided into pre-gestational diabetes (type 1 or type 2 diabetes diagnosed before pregnancy) and gestational diabetes defined as diabetes with onset or first recognition in pregnancy. Approximately 87.5% of pregnancies complicated by diabetes are estimated to be due to gestational diabetes, with 7.5% being due to type 1 diabetes and the remaining 5% being due to type 2 diabetes (Reece and Homko, 1998; Wiznitzer and Reece, 1999; Lawrence et al., 2008).

Previous studies have clearly documented that diabetes in pregnancy is associated with an increased risk of maternal and child mortality and morbidity as well as major congenital anomalies (Schwartz and Teramo, 2000; ter Braak et al., 2002; Farooq et al., 2007; Persaud, 2007), including central nervous system (CNS) malformation in their offspring (Sells et al., 1994; Ornoy et al., 2001; Georgieff, 2006; Hami et al., 2015c). Moreover, multiple lines of evidence indicated that offspring of diabetic mothers exhibit disturbances in behavioral and intellectual functioning (Sells et al., 1994; Yamashita et al., 1996; Rizzo et al., 1997; Ornoy et al., 1998; Nomura et al., 2012)

*Correspondence to: J. Hami, Department of Anatomical Sciences, School of Medicine, Birjand University of Medical Sciences, Ghaffari Street, Birjand, Iran. Tel/fax: +98-56-32395361.
E-mail address: javadhami@gmail.com (J. Hami).

Abbreviations: BGC, blood glucose concentration; DAPI, 4′,6-diami dino-2-phenylindole dihydrochloride hydrate; DG, dentate gyrus; P, postnatal day; RT-PCR, real-time reverse transcription polymerase chain reaction; SC, subcutaneous; STZ, Streptozotocin; SYP, synaptophysin.

http://dx.doi.org/10.1016/j.neuroscience.2016.01.025
0306-4522/

although, the majority of the investigations did not differentiate between type of diabetes. In a study by Rizzo et al. (1991), the researchers compared the effect of pregestational (without diabetes type discrimination) and gestational diabetes exposure in children of age 1 year and 3–5 years. They found that both diabetic exposed-children showed similar defective behavioral and intellectual development (Rizzo et al., 1991). When pregestational and gestational diabetes-exposed children were grouped together in the study of DeBoer et al. (2005), it was demonstrated a negative link between maternal diabetes and development of memory, circuitry, and behavioral mnemonic performance in children at 1-year of age. Moreover, they showed that the metabolic abnormalities due to diabetes during pregnancy alters prenatal development, which can influence memory performance on a delay recall task (DeBoer et al., 2005). On the other hand, experimental studies revealed that maternal hyperglycemia can cause a significant reduction in gray matter volume rather than to white matter and reduces the number of neurons in the gray matter of the CNS in their offspring (Khaksar et al., 2011). In general, it can be concluded that the effect of maternal diabetes on the fetal CNS structure and function may be teratogenic (Styrud et al., 1995; Schwartz and Teramo, 2000; Eidelman and Samueloff, 2002; Meur and Mann, 2007; Hami et al., 2015c).

Although, the exact mechanism by which maternal diabetes increases the risk of congenital abnormalities in the developing CNS is not completely understood, the conclusion from several studies is that hyperglycemia may be a major teratogenic factor in the diabetic pregnancies (Allen et al., 2007; Hami et al., 2013, 2015c; Gabbay-Benziv et al., 2015); and therefore the risks to the developing fetal CNS may result from type 1 diabetes, type 2 diabetes or gestational diabetes. Results from previous studies showed that the increased placental transport of glucose from the hyperglycemic mother to fetus increases the risk of congenital abnormalities in offspring born to diabetic mothers (Schwartz and Teramo, 2000; Allen et al., 2007; Hami et al., 2013, 2015c; Gabbay-Benziv et al., 2015). Thereafter, fetal hyperglycemia stimulates the pancreatic β-cell hypertrophy and hyperplasia in the developing fetus resulting in increased insulin secretion and consequently, *in utero* hyperinsulinemia affects different parts of the developing CNS (Westgate et al., 2006; Persaud, 2007). Other biological alterations, including oxidative stress, hypoxia, and iron deficiency that occur in pregnancies with diabetes can also affect the development of CNS of the fetus (Rizzo et al., 1991; Styrud et al., 1995; Simán and Eriksson, 1997; Ziegler et al., 2004).

Synaptogenesis is one of the most important events taking place during the development of the CNS (Bourgeois, 1997; Huttenlocher and Dabholkar, 1997; Stiles and Jernigan, 2010; Tau and Peterson, 2010; Bury and Sabo, 2015). This process involves the formation of a neurotransmitter release site in the presynaptic neuron and a receptive field at the postsynaptic partners, and the precise alignment of pre- and post-synaptic specializations (Niell et al., 2004; Luikart and Parada, 2006). In the recent decades, with the advancement of neuroscience, the critical roles of the synaptic vesicles membrane structure and also their containing neurotransmitter have been found in synaptogenesis (Goldman et al., 2013; Bury and Sabo, 2015). In the earlier studies, various family of proteins with specific actions were identified in the membrane of the synaptic vesicles, including synaptophysin (SYP), synaptoporin, and synaptobrevin that effectively involved in the neurotransmitters exocytosis process (Knaus et al., 1990; Südhof and Jahn, 1991; Valtorta et al., 2004). SYP, a 38-kDa glycoprotein, is a major integral membrane protein and is the most abundant protein in the small synaptic vesicles membrane (Ozcelik et al., 1990; Gincel and Shoshan-Barmatz, 2002; Arthur and Stowell, 2007; Kwon and Chapman, 2011). This glycoprotein accounts for about 7–10% of total synaptic vesicle proteins which is suspected to be involved in regulating synaptic vesicle exocytosis (Elferink and Scheller, 1993; Bajjalieh and Scheller, 1995; McMahon et al., 1996; Arthur and Stowell, 2007). Expression of SYP occurs prior as well as parallel to the formation of synapses, and is considered as a marker of synaptogenesis, distribution and density of synapses (Valtorta et al., 2004; Joca et al., 2007). Evidence has also been suggested that SYP plays a key role in synaptic plasticity in aging (McGahon et al., 1997; Davies et al., 2003; Frick and Fernandez, 2003). Altered SYP levels have also been described in pathological brain conditions such as Alzheimer's disease (Masliah et al., 1989; Heinonen et al., 1995; Sze et al., 1997), Parkinson's diseases (Zhan et al., 1993), schizophrenia (Eastwood et al., 1994, 1995) and bipolar disorder (Vawter et al., 2002). Ishimaru et al. (2001) showed an alteration in SYP protein distribution in various hippocampal subdivisions especially in the CA1 region in ischemic rats (Ishimaru et al., 2001).

Hippocampal formation is a major component of the brains in humans and rodents that was located in the medial temporal lobe (Braak et al., 1996; El Falougy and Benuska, 2006; Tabassum and Frey, 2013). It belongs to the limbic system and believed that it plays important roles in the consolidation of information from short-term to long-term memory and spatial navigation (Nadel, 1991; Eichenbaum et al., 1992; Jarrard, 1993; Day and Good, 2005). The four main regions of the hippocampus include the CA1, CA2, CA3, and dentate gyrus (DG) (Sickmann et al., 2014; Weeden et al., 2014). These regions differ in terms of efferents, afferents and major cell types, neurogenesis, and synaptic plasticity mechanisms (Derrick et al., 2000; Ming and Song, 2011). This brain structure is particularly vulnerable to change in glucose concentration (Kamal et al., 1999; Youssef et al., 2009; Hami et al., 2015c). Experimental investigations in animals indicate a reduction in the numerical densities of neurons in some portions of fetal CNS, especially in the hippocampus due to diabetes during pregnancy which reflects in lower memory and learning skills and defect in memory storage and recall information (Tehranipour and Khakzad, 2008; Khaksar et al., 2011; Golalipour et al., 2012; Razi et al., 2014; Ghafari et al., 2015). A study by Tehranipour and Khakzad (2008) assessed the effect of Streptozotocin (STZ)-induced maternal diabetes on neuronal density in rat neonate's hippocampus immediately