Journal of Chemical Neuroanatomy 91 (2018) 27–34

Contents lists available at ScienceDirect

Journal of Chemical Neuroanatomy

journal homepage: www.elsevier.com/locate/jchemneu

The effects of maternal diabetes and insulin treatment on neurogenesis in the developing hippocampus of male rats

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ARTICLE INFO

Keywords:
Maternal diabetes
Neurogenesis
Hippocampus
Rat neonates

ABSTRACT

Diabetes in pregnancy is associated with an increasing risk of congenital malformations and central nervous system disorders (CNS) especially hippocampal neuronal circuitry disruption as a discreet region involved in neurogenesis phenomenon. This study aimed to investigate the effect of maternal diabetes and insulin treatment on the expression and distribution pattern of NeuN and DCX as two important markers of neurogenesis paradigm in developing rat hippocampus. All animals were randomly divided into three groups as follows: Control group, Diabetic (STZ-D), Diabetic treated with insulin (STZ-INS). Diabetes was induced in Wistar female rats by Sterptozotocin intraperitoneal injection (single does). Following confirmation of diabetes, animals were mated with non-diabetic males. Four to six units of protamine-Zinc insulin were delivered subcutaneously (SC) in insulin treated group. At the post-natal day 14 (P14), the brain of male offspring's were removed for further study. In fact Immunofluorescence staining and Real time − PCR assays are used for evaluation of neurogenesis phenomenon. Our results showed a significant higher level of hippocampal DCX expression and an increase in the mean number of DCX positive cells in the DG of diabetic group male offspring ($P < 0.05$). We also found an insignificant up-regulation in the expression of DCX and the mean number of positive cells in the insulin-treated diabetic group neonates as compared to control group ($P > 0.05$). Nevertheless the results of immunofluorescence staining for NeuN also indicated that the mean number of NeuN+ cells was significantly lower in dentate gyrus of diabetic group male offspring ($P < 0.05$). Besides, there were significant down- regulation in the hippocampal mRNA expression of NeuN in diabetic pups compare to control ($P < 0.05$ each). Our results revealed that diabetes during pregnancy has an adverse effect on the hippocampal neurogenesis in rat neonates. Furthermore, the control of glycemia by insulin is sufficient to prevent the alterations in expression of neurogenesis markers.

1. Introduction

1.1. Diabetes mellitus and CNS development

Diabetes mellitus is one of the most common disorders in pregnancy that is thought to affect up to 15 percent of pregnancies worldwide (Chiu and Cline, 2010; Hallschmid et al., 2007; Needleman and McAllister, 2008). This serious metabolic condition is associated with an increased risk of mortality and morbidity as well as congenital malformation in multiple vital systems such as urinary, respiratory and central nervous systems (CNS) in the offspring (Churchill et al., 1969; Georgieff, 2006; Schwartz and Teramo, 2000). The risk of the developing fetal abnormalities is due in part to increased placental transport of glucose and other nutrients from the mother to the fetus. Therefore, maternal hyperglycemia is paralleled by fetal hyperglycemia that stimulates the pancreatic β-cell hypertrophy and hyperplasia in developing fetus resulting in increased insulin secretion and consequently, in utero hyperinsulinemia which affects the developing organ systems at different developmental times (Pedersen et al., 1981; Salvesen et al., 1993; Susa et al., 1984; Widness et al., 1981). The multiple lines of

Abbreviations: STZ, Stereptomycin; DCX, doublecrtin; NeuN, Neuronal nuclear antigen; DG, dentate Gyrus; ROS, reactive oxygen species; IGF-1, Insulin like growth factor 1; CNS, Central nervous system

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https://doi.org/10.1016/j.jchemneu.2018.03.005
Received 30 January 2018; Received in revised form 17 March 2018; Accepted 21 March 2018
Available online 22 March 2018
0891-0618/

earlier human and animal investigations have shown that infants born to mothers with diabetes are at risk of having neurodevelopmental sequelae and neurocognitive deficits(Drapeau et al., 2003; Hami et al., 2015; Sells et al., 1994), Including cerebral dysfunction (Cannon et al., 2002), neurological abnormalities(Haworth et al., 1976), learning and memory impairments (Churchill et al., 1969; Delascio Lopes et al., 2011; Rizzo et al., 1991). In a study by Churchill et al. (1969), the researchers found that offspring of diabetic mothers had a significant lower mean Intelligence Quotient (IQ) scores than control infants (Churchill et al., 1969). Other studies have also revealed that hyperglycemia, during hippocampus development, decreases the maintenance of synaptic plasticity and reducing the durability of memory in male rats (Delascio Lopes et al., 2011; Hami et al., 2014). Interestingly, there are reports demonstrating a close relationship between maternal diabetes and an increased risk of psychological disorders such as schizophrenia in the male offspring (Brown, 2002; Cannon et al., 2002; Huttunen et al., 1994; Mazaeva, 2012; Meli et al., 2012). Despite several earlier studies, there is no conclusive evidence about the exact mechanisms by which the diabetes during pregnancy causes malformations in the offspring (Delascio Lopes et al., 2011; Hami et al., 2012) and little is known about its effects on the CNS development (Delascio Lopes et al., 2011). Nevertheless, it is clearly showed that in utero hyperglycemia may adversely affects the development of neurons and brain structures (Cannon et al., 2002). The previous study by Hami et al. (2013) indicated that maternal hyperglycemia, in combination with neonatal hyperinsulinemia, results in developmentally disturbances in the expression/translation of insulin-like growth factor −1 (IGF-1) and insulin receptors in the male offspring's hippocampus and cerebellum (Hami et al., 2013). Many studies have been proved the pivotal role of insulin like growth factor 1(IGF-1) on the regulation of central nervous system (CNS) development. A wide range of in vivo and in vitro studies showed that proliferation of neural progenitor cells and survival neurons and oligodendrocytes are affected by IGF_1 and it also inhibits neural cells apoptosis, besides it can induce neuronal differentiation. Hami et al. claimed that the alterations in hippocampal and cerebellum insulin and IGF-1 receptors may result in a delay in normal hippocampal development, and could be a reason for the structural, behavioural, and cognitive abnormalities observed in male offspring of diabetic mothers (Hami et al., 2013).

1.2. Hippocampus and neurogenesis

Hippocampus, a limbic structure important in spatial learning and memory, is particularly sensitive and vulnerable to changes in glucose concentration, especially during development (Hami et al., 2014; Hami et al., 2012). Neurogenesis has emerged as an integral and crucial process within the hippocampus, especially during development (Barcia et al., 2015; Beauquis et al., 2010; Gould et al., 1999; Lemaire et al., 2000; Parent et al., 1997; Tan et al., 2015) .This process is involving many stages. It can broadly be defined as the cell proliferation, migration, differentiation and ultimately the maturation of granule cells. The sub granular zone (SGZ) in the hippocampal dentate gyrus (DG) is identified as an endogenous resource of neural stem cells (Ming and Song, 2005; Taupin, 2005). New neurons that arise in the SGZ migrate into the adjacent DG granule cell layer (Beauquis et al., 2010; Esfandiary et al., 2014; Parent et al., 1997; Tan et al., 2015; Taupin, 2010; Zhao et al., 2008). Freshly formed neurons integrating in the granule layer of the DG are thought to play a key role in learning, memory and in some related hippocampal functions such as the regulation of emotions and stress (Brinton and Wang, 2006; Burke and Barnes, 2006; Casadesus et al., 2004). Data from studies of animal models further suggest that defective neurogenesis is present in individuals with diabetes; this condition is thus hypothesized to be an etiological factor of cognitive deficits in patients with diabetes (Clelland et al., 2009). In addition, some studies have demonstrated that behavioural and physiological stimuli such as learning, physical activity, and environmental enrichment as well as pharmacological agents enhance hippocampal neurogenesis (Caroni et al., 2012; Clelland et al., 2009). On the other hand, hippocampal neurogenesis is thought to modulate brain plasticity and repair, by providing neurotrophic support for neurons and a pool of neural progenitor cells (Caroni et al., 2012; Clelland et al., 2009; Drapeau et al., 2003). Accumulating evidence lead to a detailed classification of the new generated cells within the hippocampal DG which characterized by specific markers and their properties. Hippocampal neurons originate from a radial glia-like precursor cell (type-1) which has a proliferative capacity and is glial fibrous acid protein (GFAP) positive but negative to Doublecortin (DCX). Type-2 cell stage marks the transition between cells with astrocytic phenotype and cells with early features of the neuronal lineage. DCX, the marker of immature neurons, appear in type-2 cells and persist into post mitotic but immature granule cell precursors. The type-3 cells are the terminal post mitotic differentiation of granule cells that exits from the cell cycle. Finally, these cells mature into granule cell neurons in the DG that express specifically a neuronal nuclear protein (NeuN) (Filippov et al., 2003; Fukuda et al., 2003; Ming and Song, 2005; Steiner et al., 2004; Taupin, 2005). In the present study, we hypothesized that alterations in hippocampal neurogenesis may be part of the cascade of events through which maternal diabetes affects the offspring's hippocampus. Therefore, the goal of this study was to determine the effects of maternal streptozotocin- induced type-1 diabetes and insulin treatment on mRNA expression and neural density of NeuN and DCX markers involved in hippocampal DG neurogenesis of rat neonates at postnatal day 14(P14), a critical period in developing hippocampus in rodents (Muramatsu et al., 2007; Shors et al., 2001).

2. Materials and methods

2.1. Animals and ethical issue

Thirty six young adult female Wistar rats, weighing 200–250 g, were used in this study. They were purchased from the animal house of Isfahan University of Medical Sciences, Isfahan, Iran. Animals were housed in a temperature and humidity controlled colony room under diurnal lighting conditions (12 h of darkness and 12 h of light), and were allowed to adapt to human handling. Food and water were provided ad libitum. All of the animal experiments were conducted according to international principal guidelines which were approved by local ethic committee for Animal Experiments at Isfahan University of Medical Sciences, Isfahan, Iran (IR·MUI.REC.1393.3.26). All animals were randomly divided into three groups as follows: Control group (CON, n = 12), Diabetic (STZ-D) (n = 12), Diabetic treated with insulin (STZ-INS) (n = 12).

2.2. Induction of diabetic animal model and insulin treatment

In the current study, diabetes was induced by a single intraperitoneal (IP) injection of streptozotocin (STZ; Sigma, St. Louis, MA, USA; 45 mg/kg body weight) which was freshly dissolved in normal saline (Hami et al., 2015). Hyperglycaemia was assessed by the puncture of tail vein using a digital glucometer (BIONIME, Switzerland). Only rats with glycemic values equal to or higher than 350 mg/dL were considered as diabetics (Haghir et al., 2013). Control animals were injected with normal saline only. Treatment of diabetic animals was conducted after the verification of diabetes. Four to six units of protamine-Zinc insulin (Neutral Protamine Hagedorn; NPH; EXIR Pharmaceutical Company, Iran) were delivered subcutaneously (SC). The dose of insulin was determined on the basis of a daily blood glucose test. Animals in all groups were mated with non-diabetic males overnight and the following day in the event of a vaginal plug was considered as the first day of pregnancy (GD1). The pregnant rats were kept in individual cages until the birth of the offspring (20–22 days).