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ORIGINAL ARTICLE

The effects of maternal diabetes on expression of insulin-like growth factor-1 and insulin receptors in male developing rat hippocampus

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Abstract Diabetes during pregnancy causes neurodevelopmental and neurocognitive abnormalities in offspring. Insulin and insulin-like growth factor-1 (IGF-1) are important regulators of developmental and cognitive functions in the central nervous system. We examined the effects of maternal diabetes on insulin-like growth factor-1 receptor (IGF-1R) and insulin receptor (InsR) expression in the developing rat hippocampus. Female rats were maintained diabetic from a week before pregnancy through parturition and male offspring was killed at P0, P7, and P14. We found a significant bilateral upregulation of both IGF-1R and InsR transcripts in the hippocampus of pups born to diabetic mothers at P0, as compared to controls. However, at the same time point, the results of western blot analysis revealed only a slight change in their protein levels. At P7, there was a marked bilateral reduction in mRNA expression and protein levels of IGF-1R, although not of InsR in the diabetic group. We also found a downregulation in IGF1-R transcripts, especially in left hippocampus of the diabetic group at P14. Moreover, at the same time point, InsR expression was significantly decreased in both hippocampi of diabetic newborns. When compared with controls, we did not find any difference in hippocampal IGF-1R or InsR mRNA and protein levels in the insulin-treated group. The present study revealed that diabetes during pregnancy strongly influences the regulation of both IGF-1R and InsR in the right/left developing hippocampi. Furthermore, the rigid control of maternal glycaemia by insulin administration normalized these effects.

Keywords Maternal diabetes · Insulin-like growth factor-1 receptor · Insulin receptor · Hippocampus · Rat newborn

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Introduction:

Diabetes in pregnancy is the most common and most important metabolic condition, since it can result in several fetal malformations including neurodevelopmental and neurocognitive defects (Lopes et al. 2011; Persaud 2007; Ornoy 2005; Nelson et al. 2000; Ornoy et al. 1998; Stenninger et al. 1998). Several lines of evidence indicate that the effect of maternal diabetes on the fetal developing central nervous system (CNS) may be teratogenic, although the exact mechanisms are not yet understood (Meur and Mann 2007; Eidelman and Samueloff 2002; Schwartz and Teramo 2000; Styrud et al. 1995). Together, these studies not only suggest the teratogenic effect of diabetes in pregnancy on fetal CNS development, but also provide the perhaps earliest indicator of postnatal CNS problems reflected in intellectual, educational and behavioral problems exhibited by children of diabetic mothers. However, no report can fully explore the molecular mechanisms of maternal diabetes-induced neurodevelopmental defects

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because the CNS development is a complex process and is regulated by a number of signaling molecules and transcription factors.

Increasing evidence reveals that the offspring of diabetic pregnancies exhibit disturbances in behavioral and intellectual functioning, suggesting that the changes in maternal metabolism during pregnancy affect higher aspects of brain functioning in the fetus by altering brain development (Rizzo et al. 1997; Yamashita et al. 1996; Rizzo et al. 1994, 1995; Sells et al. 1994; Rizzo et al. 1991; Haworth et al. 1976; Churchill et al. 1969). Simán and Eriksson (1997a, b) showed that maternal diabetes is associated with a slight reduction in fetal brain weight at gestation day 20 (GD20). Moreover, the prospective study by Haworth et al. (1976) reported a 30% incidence of either neurologic abnormalities or impaired intellectual development in infants of diabetic mothers. Rizzo et al. (1991) noted significant correlations between diabetic pregnancies and lower IQ in offspring. Stehbens et al. (1977), following a cohort of 50 infants born to diabetic mothers, also reported that three infants had major neurologic abnormalities and six had IQs <80. Recent reports also have demonstrated a close relationship between maternal diabetes and an increased risk of psychological disorders such as schizophrenia in offspring (Van Lieshout and Voruganti 2008; Becerra et al. 1990; Lewis et al. 1989; Parnas et al. 1982).

Furthermore, in a cohort study, Gunnell et al. (2005) investigated the association of circulating levels of insulin-like growth factor-1 (IGF-1) in 8 to 9-year-old children with subsequent measures of IQ; their results indicated a positive correlation between IGF-1 levels and the intelligence measured. A study by Venkatasubramanian et al. (2007) also suggests that IGF-1 and insulin might be potentially involved in the pathogenesis of schizophrenia. In addition, there is also considerable evidence from in vitro, in vivo, transgenic animal, and human studies implicating defects in IGF-1R and InsR signaling due to diabetes neuropathies and also congenital abnormalities observed in offspring of diabetic mothers (Novitskaya et al. 2011; Kruis et al. 2010; Jian-bo et al. 2010; Chu et al. 2008; Lauszus 2007; Sullivan et al. 2008; Russo et al. 2005; Brussee et al. 2004; Li and Sima 2004; Sima et al. 2004; Leinninger et al. 2004; Pierson et al. 2003; Sima et al. 2003; Craner et al. 2002; Migdalis et al. 1995; Ekström et al. 1989). Ramsay et al. (1994) reported that brain IGF-1 mRNA levels were depressed in swine fetuses born to diabetic mothers compared with fetuses of controls.

Insulin and IGF-1 belong to the same protein family and a have wide variety of biological actions in the CNS development. These include neural cell proliferation, survival, differentiation, neuronal apoptosis inhibition, synaptogenesis, longevity, energy metabolism, neuroprotection and neuroregeneration (Agrawal et al. 2011; Liu et al. 2009; Nelson et al. 2008; Plum et al. 2005; Popken et al. 2004; Anlar et al. 1999; de Pablo and de la Rosa 1995; Baron-Van Evercooren et al. 1991; Baskin et al. 1988). Most of these actions are mediated by their two closely related members of transmembrane receptors, the insulin receptor (InsR) and the IGF-1 receptor (IGF-1R). The molecular structures of InsR and IGF-1R consist of two α- and two β-subunits linked by disulfide bonds. The α-subunit contains the ligand-binding domains, and the β-subunit contains the transmembrane as well as the protein tyrosine kinase domains, suggesting that the molecular mechanism of action of the two receptors is similar. The binding of either insulin or IGF-1 to their respective receptor serves to activate the protein tyrosine kinase activity of the β subunits. The receptors' kinase activity is required to elicit the biological responses to these hormones (Nakae et al. 2001; Zhao and Alkon 2001; Zhao et al. 2004; Bondy and Cheng 2004; Entingh-Pearsall and Kahn 2004; Navarro et al. 1999).

In rats, previous reports demonstrated that IGF-1R mRNA is expressed in the brain as early as embryonic day 13 (E13) and InsR mRNA is expressed abundantly at embryonic day 20 (E20) and at the day of birth (Kar et al. 1993; Marks et al. 1991; Breese et al. 1991; Werther et al. 1990; Marks et al. 1990; Hill et al. 1986). Several human and animal studies have reported that IGF-1 and insulin receptors are concentrated in different cell populations of the hippocampus, using a variety of techniques, including in situ hybridization and ligand-binding autoradiography (Zemva and Schubert 2011; Muller et al. 2011; Chiu and Cline 2010; Zhang et al. 2007; Dou et al. 2005).

The hippocampal formation—a brain structure particularly vulnerable to changes in glucose concentration—subserves important behavioral and physiological functions, such as spatial learning and memory (DeCarolis and Eisch 2010; Thompson et al. 2008; Förster et al. 2006; McNay et al. 2000; McNay and Gold 1999; Bayer 1980; Reagan et al. 1999; Hine and Das 1974). Tehranipour and Khakzad (2008) assessed the effect of STZ-induced maternal diabetes on neuronal density in rat neonate's hippocampus immediately after birth. Their data demonstrated that diabetes in pregnancy can reduce the number of hippocampal neurons, especially in CA3.

It has been shown that fetal hyperglycemia alters the expression of genes that are involved in the proliferation and differentiation of neural cells (Gao and Gao 2007; Fu et al. 2006; Loeken 2005), indicating the basis for neurodevelopmental and neurocognitive anomalies which are observed in infants of diabetic mothers. Because insulin and IGF-1 play an important role in the control of neuronal proliferation and differentiation, we hypothesized that the alteration in IGF-1R and InsR mRNA expression may be part of the cascade of events through which maternal

diabetes affects the newborn's hippocampal structure. Therefore, the goal of the present study was to examine the effect of STZ-induced maternal type-1 diabetes and the role of insulin therapy on the expression of IGF-1R and InsR genes in the developing rat hippocampus during the first 2 postnatal weeks—a very active hippocampal/dentate neurogenesis period—using quantitative real-time PCR and western blot analysis.

Materials and methods

Animals

All procedures involving animals were in accordance with the Guide for the Care and Use of Laboratory Animals of Mashhad University of Medical Sciences, Mashhad, Iran. Thirty virgin female Wistar rats (200–250 g body weight, 6–8 weeks) were purchased from Mashhad University of Medical Sciences Experimental Animal House (Mashhad, Iran). Animals were housed in individual cages under controlled temperature conditions (21–23°C) and had free access to food pellets and drinking water through the experiment.

Treatment

Animals were subdivided into three groups as follows:

1. Diabetic (STZ-D) group ($n = 11$)
2. Diabetic treated with insulin (STZ-INS) group ($n = 11$)
3. Controls (CON, $n = 8$)

To induce diabetes, females were injected intraperitoneally (ip) with streptozotocin (STZ) (Sigma, 45 mg/kg body weight) freshly dissolved in normal saline. STZ is one of the most commonly used diabetogens, inducing insulin-dependent diabetes mellitus in rodents by causing selective destruction of insulin-secreting beta cells in pancreatic islets. These experimental diabetic animal models have been widely used to study the molecular mechanisms of maternal diabetes-induced malformations in various organs, including the neural tissues (Lopes et al. 2011; Jawerbaum and White 2010; Zhou et al. 2007; Cederberg et al. 2003; Chang et al. 2003).

The treatment of diabetic animals was conducted after the verification of diabetes. Four to six units of protamine-zinc insulin (NPH) (EXIR Pharmaceutical Company, Iran) were delivered subcutaneously (SC). The dose of insulin was determined on the basis of a daily blood glucose test.

Control animals were injected with normal saline only. Animals were mated with non-diabetic males overnight starting a week after treatments. The presence of a vaginal plug the following morning was designated as day 1 of pregnancy (GD1).

Blood glucose concentration (BGC) in animals was measured using a commercially available digital glucometer (BIONIME, Switzerland). Only STZ-treated females exhibiting BGC > 350 mg/dl on the day of plug observation and on delivery day were used in the present study.

Tissue preparation

At the end of pregnancy, animals were allowed to deliver naturally; the day of birth was defined as postnatal day 0 (P0). Newborn rats born to diabetic and insulin-treated diabetic mothers were fostered onto control mothers to exclude other effects by the milk of diabetic rats, and thus enable to focus only on the environment of the fetal period. Male offspring were randomly assigned to three age groups, P0 ($n = 7$), P7 ($n = 7$), and P14 ($n = 7$). These time points coincide with the period during which IGF-1R and InsR mRNA expression peaks in the brain.

Pups were anaesthetized with chloroform and killed by cervical dislocation at P0, P7, and P14; brains were rapidly removed and hippocampi carefully dissected.

RNA isolation and cDNA synthesis

Newborn's hippocampi were collected to 1 ml of RNA stabilization reagent (RNAlater, Qiagen, Germany) and stored at −80°C until further analysis. Total RNA was isolated from hippocampal tissue using Trizol reagent (Invitrogen, Carlsbad, CA). Final RNA pellets were dissolved in diethylpyrocarbonate-treated water, and the yield of RNA was quantified by measuring the optical density at 260 nm. The integrity of the RNA was checked by visualization of 18S and 28S ribosomal bands on 1% agarose gel with ethidium bromide.

We used 1 μg of total RNA for reverse transcription to synthesize the first strand cDNA using oligo(dT)18 primers following the instructions of the RevertAid First Strand cDNA synthesis kit (Fermentas Life Science, Vilnius, Lithuania). cDNA samples were stored at −20°C.

Real-time reverse transcription polymerase chain reaction (RT-PCR)

Real-time PCR was performed to analyze hippocampal expression of IGF-1R and InsR genes. The primer sets were designed based on sequences from the NCBI database and checked for specificity using the NCBI BLAST tool (http://www.ncbi.nlm.nih.gov/BLAST); primers with no significant similarity to other loci were selected. The following primers were used: 5′-GCCGTGCTGTG-CCTGTCCTAAAAC -3′ (forward) and 5′-GCTACCGTG GTGTTCCTGCTTCG-3′ (reverse) for IGF-1R, 5′-GGCCC GATGCTGAGAACA-3′ (forward) and 5′-CGTCATTCCA

AAGTCTCCGA-3′ (reverse) for InsR, and 5′-AACTCC CATT CTTCCACCTTTG-3′ (forward) and 5′-CTGTAGC CATATTCATTGTCATACCAG-3′ (reverse) for GAPDH. Each 25 μL real-time PCR reaction containing 2 μL cDNA was performed with SYBR Premix Ex. Taq™ Kit (TaKaRa, Biotechnology Co., LTD, Dalian, China) and PCR parameters were 95°C for 30 s, 40 cycles of 95°C for 5 s, 60°C for 15 s, and 72°C for 15 s.

The real-time detection of emission intensity of SYBR Green bound to double-stranded DNAs was performed using Corbett Research Rotor-Gene 6000 real-time DNA analysis system (Corbett Research, Sydney, Australia). At the end of the runs, melting curves were obtained to make sure there were no primer–dimmer artifacts.

Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA was used as an internal control to measure the relative quantitation of the expression of the target genes. Relative differences in the expression of target genes were calculated using the Relative Expression Software Tool (REST-384). Efficiency of primers was also calculated and used for REST analysis.

Western blot analysis

Western blotting was used to detect changes in the protein levels of IGF-1R and InsR in newborn's hippocampi. Hippocampal protein was extracted as previously described (Klugmann et al. 1997), and stored at −80°C until use.

Aliquots of 50 μg protein were separated on 7.5% sodium dodecyl sulfate–polyacrylamide gels (SDS–PAGE) and transferred onto polyvinylidene difluoride (PVDF) membranes (Millipore, USA). Proteins of interest were detected using their specific antibodies: rabbit anti-IGF-1R (antibody) (1:500, Signalway Antibody SAB, Pearland, USA); rabbit anti-InsR (antibody) (1:500, Abnova, Taipei, Taiwan), and anti-B-actin (antibody) (1:1,000, Sigma-Aldrich, USA).

Following four washes in TBST, membranes were incubated with HRP conjugated secondary antibody (1:5,000, SAB, Signalway Antibody SAB, Pearland, USA). The IGF-1R protein immunoreactive bands were detected using the enhanced chemiluminescence (ECL) detection system from Amersham Corporation (Bioimaging, System, Syngene, UK) and quantified using ImageJ software.

Statistical analysis

Statistical analyses were performed using the SPSS statistical package, version 15.0 (SPSS). Differences between groups were determined using an independent sample *t* test and an one-way analysis of variance (ANOVA) followed by Tukey's tests. The results are expressed as mean ± SE and were regarded as being significant at $P \leq 0.05$.

Results

Maternal and neonatal glucose concentrations

Table 1 shows maternal BGC at the beginning and end of pregnancy, and also neonatal BGCs at the postnatal time points studied. As indicated in Table 1, maternal BGC in the STZ-D group was significantly higher (three- to fourfold) than levels in the STZ-INS or vehicle control groups ($P < 0.001$). These data reveal that diabetic rats remained hyperglycemic throughout gestation. No difference between the BGC of the STZ-INS and CON groups was observed at any time point studied ($P > 0.05$). In addition, as noted in Table 1, at P0, BGCs were markedly (approximately fourfold) higher in the STZ-D group as compared to those in the STZ-INS and control groups ($P < 0.001$). However, BGCs were not different among all three groups at P7 ($P = 0.49$) or P14 ($P = 0.93$). Our data show a significant reduction in BGC from P0 until P7 in the diabetic group ($P = 0.001$).

In all groups, we found a significant correlation between maternal and neonatal BGCs at P0 ($P = 0.001$). In the current study, there was no evidence of any increase in gross congenital abnormalities in newborns of STZ-treated diabetic group.

Table 1 Maternal and neonatal BGCs of STZ-D, STZ-INS, and control groups

Group	Maternal BGC (mg/dl)		Newborns BGC (mg/dl)		
	GD1	GD21	P0	P7	P14
STZ-D	494.25 ± 45.4*	559.67 ± 35*	389.87 ± 25.9*	135.57 ± 7.4	138.48 ± 5.4
STZ-INS	169.33 ± 18.7	156.25 ± 10.9	91.93 ± 5.3	146.47 ± 7.9	136.56 ± 4.6
Control	107 ± 13.6	127.34 ± 9.3	83.48 ± 7.7	110.35 ± 4.1	126.3 ± 4.7

Maternal BGC shown at the beginning (GD1) and end (GD21) of pregnancy; *asterisks* indicate statistically significant different between STZ-D group's mothers with two other groups ($P \leq 0.001$)

BGC in newborns born to diabetic dams at P0 is significantly increased in comparison to newborns in other groups ($P \leq 0.001$)

Data are expressed as mean ± SE

* Significant differences ($P \leq 0.001$)

Real-time quantitative PCR

We used quantitative real-time PCR to evaluate the effect of maternal diabetes and insulin therapy on IGF-1R and InsR gene expression in hippocampi of rat newborns at P0, P7 and P14.

Hippocampal IGF-1R expression

Figure 1 demonstrates the differential expression of the IGF-1R gene in right (A) and left (B) newborns' hippocampi in all three studied groups, separately. As shown in Fig. 1a, in offspring born to STZ-diabetic dams, IGF-1R mRNA expression was markedly increased at P0, in the right hippocampus in comparison to the two other groups' newborns one ($P < 0.05$). In the right hippocampus, our data also shows that the expression of IGF-1R was downregulated significantly at P7 in the STZ-D group when compared with STZ-INS and CON hippocampi ($P < 0.05$). Furthermore, the hippocampus of STZ-INS animals showed a lower expression of IGF-1R than that of CON offspring's hippocampi, although this difference did not reach the level of significance.

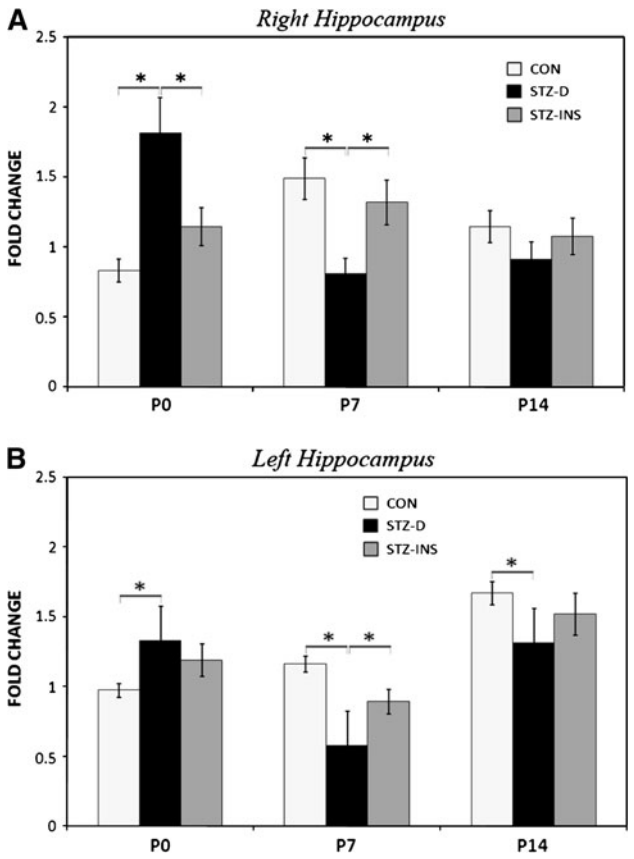

Fig. 1 Effect of STZ-induced maternal diabetes and insulin treatment on IGF-1R mRNA expression in rat newborn hippocampus at P0, P7, and P14. Right hippocampus (**a**), left hippocampus (**b**). Values represent the mean ± SE ($n = 7$, for each time point). *Significant differences ($P \leq 0.05$)

The results concerning hippocampal expression of IGF-1R mRNA in the left hemisphere are shown in Fig. 1b. We found a significant increase in the expression of IGF-1R mRNA in the left hippocampus of the STZ-D group at P0 ($P < 0.05$) when compared with control neonates. When compared with STZ-INS and CON, in the left hippocampus of STZ-D, neonates expression of IGF-1R gene was significantly downregulated at P7 ($P < 0.05$). Two weeks after birth, in contrast to the right hippocampus, a marked downregulation in expression of IGF-1R gene was found in the left one between STZ-diabetic born offspring as compared to controls ($P < 0.05$).

Concerning the STZ-INS group neonates, in this study, there were no significant differences in hippocampal IGF-1R gene expression between the insulin-treated diabetic animals with controls.

Hippocampal InsR expression

The differential expression of InsR mRNA in right and left hippocampi at P0, P7, and P14 in the three studied groups is shown in Fig. 2. Figure 2a demonstrates a significant increase in the expression of InsR mRNA at P0 in the right hippocampus of the STZ-D group in comparison to STZ-INS and CON newborns ($P < 0.05$). At P7, our data shows no differences in the expression of InsR mRNA in the right hippocampus in each group of neonates ($P > 0.05$). However, as compared to control newborns, the expression of InsR gene markedly decreased in offspring born to diabetic dams at P14 ($P < 0.05$).

The results concerning the expression of InsR mRNA in the left hippocampus are shown in Fig. 2b. As noted, in STZ-D group newborns, the hippocampal expression of InsR mRNA was significantly upregulated at P0 ($P < 0.05$), followed by a non-significant downregulation at P7 ($P > 0.05$), when compared with the other groups of newborns. At P14, our data also demonstrates a significant reduction in InsR expression in offspring of diabetic animals in comparison to STZ-INS and CON ($P < 0.05$).

Regarding InsR and IGF-1R, we did not find any significant changes in their hippocampal expression between STZ-INS and CON group neonates at any time point studied.

Western blot analysis

To determine the amount of protein of the IGF-1R and InsR in left and right hippocampi of STZ-D, STZ-INS and control neonatal rats, we performed western blot analysis. The analysis for IGF-1R and InsR revealed single immunoreactive bands at approximately 97 and 83 kDa, respectively. Using ImageJ software, we analyzed our data

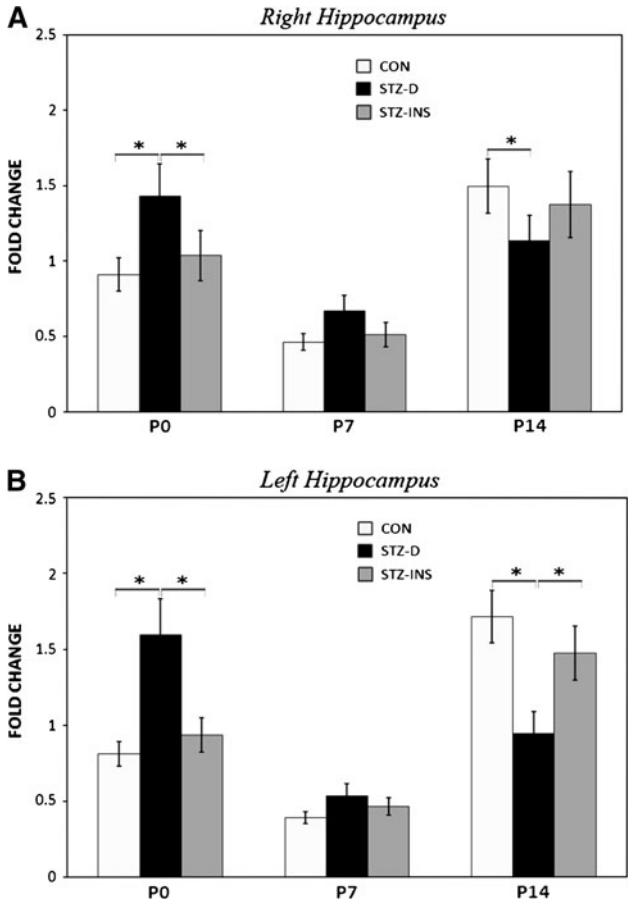

Fig. 2 Effect of STZ-induced maternal diabetes and insulin treatment on InsR mRNA expression in rat newborn hippocampus at P0, P7, and P14. Right hippocampus (**a**), left hippocampus (**b**). Values represent the mean ± SE ($n = 7$, for each time point). *Significant differences ($P \leq 0.05$)

densitometrically. The results of this analysis are presented in Fig. 3.

In the right hippocampus of the STZ-D group, despite the above-reported data that shows a significant upregulation in both IGF-1R and InsR mRNA expression at P0, we found that their protein levels were only slightly changed in comparison to STZ-INS and controls ($P > 0.05$) (Fig. 3a–e). At P7, paralleling the mRNA expression changes, we found a marked reduction in IGF-1R protein levels in right and left side hippocampi of the STZ-D group as compared to other groups' neonates ($P < 0.05$) (Fig. 3a–c). In contrast to the right hippocampus, our data show that IGF-1R protein level was significantly decreased in the left one in newborns of diabetic dams comparing to CON groups at P14 ($P < 0.05$) (Fig. 3a–c).

There were no differences in InsR protein levels in the left or right hippocampi at P7, in all three groups' neonates ($P > 0.05$) (Fig. 3a, d, e). Conversely, as compared to STZ-INS and CON offspring, we found a considerable reduction in InsR protein level in both hippocampi of the STZ-D group at P14 ($P < 0.05$) (Fig. 3a, d, e).

Concerning the STZ-INS and CON groups, the changes we found for IGF-1R and InsR protein levels followed the same pattern as those described for mRNA expression. This was true for both the right and left hippocampus comparisons (Fig. 3a–e).

Discussion

The present study utilized real-time quantitative PCR and western blot techniques to investigate the effects of maternal diabetes and the role of treatment by insulin on the expression of IGF-1R and InsR mRNA genes in the developing hippocampus. IGF-1 and insulin have been shown to affect CNS development/growth, including that of the hippocampus, since they promote neural cell proliferation, survival, differentiation and maturation as well as synaptogenesis and myelination, and also shorten the length of the cell cycle in neuron progenitors (Agrawal et al. 2011; Liu et al. 2009; Nelson et al. 2008; Popken et al. 2004; Nakae et al. 2001; Russo et al. 2005; Plum et al. 2005; Bondy and Cheng 2004; Navarro et al. 1999; Anlar et al. 1999; de Pablo and de la Rosa 1995; Baron-Van Evercooren et al. 1991; Hepler et al. 1990; Baskin et al. 1988). According to the previous evidence, alterations in the metabolic state and the supply of glucose and insulin in both mother and fetus are reflected in changes in the IGF system during diabetic pregnancies, which may contribute to changes in fetal growth and development (Singh et al. 1997; Higgins et al. 2010). In addition, the developmental regulation of insulin and IGF-1 receptor transcripts is partially coordinated with the appearance of their ligand transcripts (Baron-Van Evercooren et al.1991). Therefore, in this work, we hypothesized that the neurodevelopmental and neurocognitive impairments observed in diabetic mothers' neonates may be mediated, at least in part, via alterations in IGF-1R and InsR mRNA and/or protein levels. We focused on the first two postnatal weeks, because it is widely shown that this period is very active for hippocampal-dentate neurogenesis (Schulingkamp et al. 2000; Bach et al. 1991; Bartlett et al. 1991; Bondy 1991), and corresponds approximately to the mid-second through the mid-third trimester of human gestation (Humphrey 1967). We also compared the contents of IGF-1R and InsR mRNA and protein in right and left hippocampi of male offspring, since we formerly reported a differential pattern in the expression of these genes in right/left and male/female rat newborn hippocampi (Hami et al. 2011).

To induce diabetes in the current study we used a moderate dose of STZ (45 mg/kg). In agreement with the literature, this STZ dose-induced moderate diabetes with

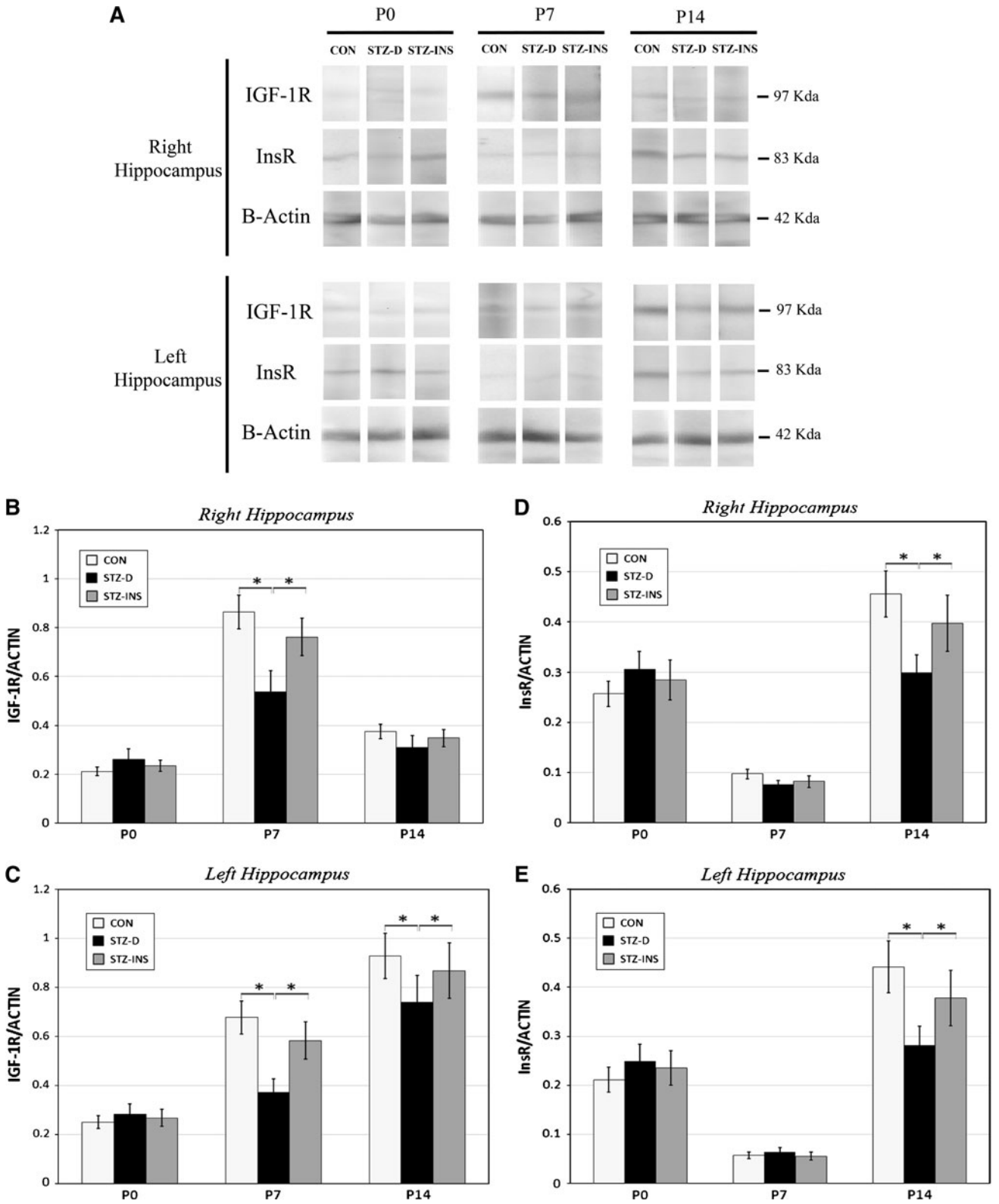

Fig. 3 IGF-1R and InsR protein levels were analyzed by western blot analysis and quantified using ImageJ analysis software and normalized to β-actin expression in right (**b**, **d**) and left (**c**, **e**) hippocampus. Data are presented as mean ± SE, *significant differences ($P \leq 0.05$)

maternal and fetal hyperglycemia, maternal insulinopenia, neonatal hyperinsulinemia, and also had a marginal effect on litter size, which was (Kervran et al. 1978; Aerts and van Assche 1977; Pitkin et al. 1970).

In contrast to insulin, glucose freely transfers from the maternal bloodstream into the fetal circulation across the placental barrier (Takata et al. 1997; Shin et al. 1997; Castellucci and Kaufmann 1995); thus, maternal hyperglycemia in pregnancy can cause fetal hyperglycemia, and the fetus' pancreatic insulin secretion is also solely determined by the maternal blood glucose level (Singh et al. 1997). Cardell (1953) and D'Agostino and Bahn (1963) determined that the maternal hyperglycemia was paralleled by fetal hyperglycemia, which stimulated the fetal pancreas, resulting in B cell hypertrophy and hyperplasia with increased insulin content and secretion. In utero hyperinsulinemia has also been shown by cordocentesis and at birth (Schwartz and Teramo 2000).

In our study, there were no differences in BGCs of mothers and newborns at any time point between the insulin-treated diabetic group and controls. These results demonstrated that we can successfully control the hyperglycemic condition in STZ-induced diabetic mothers by insulin administration. No evidence of increase in gross congenital malformations or fetal growth retardation in the diabetic group was observed in our investigation.

The differences in IGF-1R and InsR expression/translation in newborn's hippocampi reported in this study are unlikely to be a direct effect of STZ because this agent is cleared from the mother's circulation within 6 h (Karunanayake et al. 1976) and control of maternal glucose level by insulin treatment, in most cases, also prevented this effect of maternal diabetes on hippocampal expression/translation of these genes.

Taken together, the current results showed that, in comparison to the CON neonates, both IGF-1 and insulin receptor transcripts were markedly increased bilaterally at P0 in the hippocampus of male offspring born to STZ-diabetic mothers. Because, at the same time, the results of western blot analysis revealed a slight difference in either IGF-1R or InsR protein levels, especially in the right side hippocampus of STZ-D neonates, we conclude that there was a disturbance in the translational pathway for these genes.

In a previous study, Lindsay et al. (2007) indicated that, in addition to fetal hyperinsulinemia, umbilical cord IGF-1 levels are also increased in infants of diabetic mothers. Although, under normal conditions, IGF factors can only slowly cross the blood–brain barrier (Riikonen 2006; Pulford and Ishii 2001; Banks et al. 1997; Reinhardt and Bondy 1994), it has been shown that the distribution of the IGF-1 and insulin receptor in the CNS may be the target of circulating insulin and IGF-1, which are secreted from the bloodstream into the cerebrospinal fluid by the choroid plexus (Salehi et al. 2008; Russo et al. 2005). This could explain the significant upregulation of hippocampal IGF-1R and InsR transcripts in rats born to diabetic mothers immediately after birth reported in this study.

In agreement with our results, Tehranipour and Khakzad (2008) found a significant reduction in hippocampal neuronal density at postnatal day 0, induced by STZ-induced maternal diabetes. Another report indicated that increased hippocampal expression of IGF-1R mRNA is associated with aging and cognitive decline (Stenvers et al. 1996). Although the researchers did not measure the protein level of the IGF-1 receptor, they believed that IGF-1R upregulation may be part of an atrophic response to the degenerative and regenerative events that occur within the hippocampal formation during aging.

In contrast to InsR, in our study, the expression of IGF-1R mRNA and protein levels were markedly downregulated at P7 in both hippocampi of STZ-D group newborns. The considerable decrease in glucose blood levels occurring between P0 and P7 in neonates born to diabetic females could explain the significant reduction in IGF-1R mRNA in both hippocampi at 7-day-old newborns.

After 2 weeks of birth, we found a marked reduction in hippocampal IGF1-R gene expression and protein levels in offspring of the STZ-D group. Moreover, at the same time point, the expression of InsR transcript and protein levels were also significantly downregulated in both hippocampi of neonates born to diabetic mothers. Our results also showed a normoglycemic condition in offspring at P14. Furthermore, in an earlier report, we showed that the highest expression of IGF-1R in the left hippocampus, as well as of InsR in the both hippocampi occurred at 14-day-old male newborns (Hami et al. 2011), and may be the reason for the marked reduction in IGF-1R and InsR transcripts of the STZ-D group offspring at this time point.

In all three groups, our study results obtained from western blot analyses indicated that the changes found for protein levels of both IGF-1R and InsR followed the same time course as those described for mRNA expression at P7 and P14. Hence, we can conclude that the translation of IGF-1R and of InsR in the neonates' hippocampi is not subject to any kind of intermediary effects at this time.

We did not find any differences in hippocampal IGF-1R or InsR mRNA and protein levels between the STZ-INS group newborns and controls. These results confirm the previous reports and emphasize the effects of rigid control of maternal glycaemia during pregnancy for the prevention of fetal dysmorphogenesis (Lapolla et al. 2005; Eriksson et al. 1982, Eriksson et al. 1989a, b, c).

Although the exact mechanisms by which diabetes in pregnancy disrupts fetal development are not completely known, the conclusion from several studies is that

hyperglycemia may be a major teratogenic factor in the diabetic pregnancy. In addition, some researchers believe that the maternal metabolic condition (i.e. triglycerides and b-hydroxybutyrate levels, and branched chain amino acids), in combination with a disturbed fetal metabolism of sorbitol, inositol, arachidonic acid and prostaglandins, which was demonstrated in experimental diabetic pregnancy, could have teratologic significance and be important for the occurrence of fetal abnormalities (Styrud et al. 1995; Rizzo et al. 1991). Excess fetal reactive oxygen species (ROS) have also been implicated in the etiology of diabetes-induced congenital malformations. In vitro and in vivo studies have shown that the disturbed development of embryos in a diabetic milieu can be normalized by treating with different antioxidants, including vit C, vit E, among others (Wentzel et al. 1997; Simán and Eriksson 1997a, b; Viana et al. 1996; Sivan et al. 1996; Eriksson and Simán 1996; Hagay et al. 1995; Eriksson and Borg 1991, 1993; Som et al. 1981).

Taken together, our results indicated that maternal hyperglycemia, in combination with neonatal hyperinsulinemia may result in developmentally induced bilateral disturbances in the expression/translation of IGF-1 and insulin receptors in the offspring's hippocampus. These alterations may result in a delay in normal hippocampal development, and could be a reason for the structural, behavioral, and cognitive abnormalities observed in offspring of diabetic mothers. Future studies will be needed to determine the effects of diabetes during pregnancy on the expression of other related neurotrophic factors in other brain regions, in addition to the clinical importance of the current study's results.

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