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Research Article

Antenatal blockade of N-methyl-D-aspartate receptors by Memantine reduces the susceptibility to diabetes induced by a high-fat diet in rats with intrauterine growth restriction†

Xiao-Ting Huang[1], Shao-Jie Yue[2], Chen Li[3], Jia Guo[4], Yan-Hong Huang[1], Jian-Zhong Han[1], Dan-Dan Feng[1,*] and Zi-Qiang Luo[1,*]

[1]Department of Physiology, Xiangya School of Medicine, Central South University, Changsha, Hunan, China; [2]Department of Pediatrics, Xiangya Hospital, Central South University, Changsha, Hunan, China; [3]Department of Physiology, Changzhi Medical College, Changzhi, Shanxi, China and [4]Xiangya Nursing School, Central South University, Changsha, Hunan, China

*__Correspondence__: Department of Physiology, Central South University Xiangya Medical School, Changsha, Hunan 410078, China. Tel: +86-731-82355051; Fax: +86-731-82355056. E-mail: luoziqiang@csu.edu.cn; fengdandanph@163.com

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Abstract

Intrauterine growth retardation (IUGR) is closely related to the later development of type 2 diabetes in adulthood. Excessive activation of N-methly-D-aspartate receptors (NMDARs) causes excitatory neurotoxicity, resulting in neuronal injury or death. Inhibition of NMDARs enhances the glucose-stimulated insulin secretion and survival of islet cells in type 2 diabetic mouse and human islets. Here, we examined whether antenatal blockade of NMDARs by Memantine could decrease the risk of diabetes induced by a high-fat (HF) diet at adulthood in IUGR rats. Pregnant SD rats were assigned to four groups: control, IUGR, Memantine, and Memantine + IUGR. The pregnant rats were exposed to hypoxic conditions ($FiO_2 = 0.105$) for 8 h/day (IUGR group) or given a daily Memantine injection (5 mg/kg, i.p.) before hypoxia exposure from embryonic day (E) 14.5 to E 20.5 (Memantine + IUGR). The offspring were fed an HF diet with 60% of the calories from age 4 to 12 weeks. We found that *NMDAR* mRNAs were expressed in the fetal rat pancreas. An HF diet resulted in a high rate of diabetes at adulthood in the IUGR group. Antenatal Memantine treatment decreased the risk of diabetes at adulthood of rats with IUGR, which was associated with rescued glucose tolerance, increased insulin release, improved the insulin sensitivity, and increased expression of genes related to beta-cell function in the pancreas. Together, our results suggest that antenatal blockade of NMDARs by Memantine in pregnant rats improves fetal development and reduces the susceptibility to diabetes at adulthood in offspring.

Summary Sentence

The excessive activation of NMDARs is involved in the restriction of fetal pancreas development induced by intrauterine hypoxia, and antenatal Memantine treatment attenuates the susceptibility to diabetes induced by an HF diet in IUGR rats.

Key words: NMDA receptor, intrauterine growth retardation, intrauterine hypoxia, diabetes, Memantine, beta cells.

Introduction

Intrauterine growth and development are affected by many factors, such as oxygen content, low feed intake, nutrient imbalance, stress, and disturbances in maternal or fetal metabolism [1]. Intrauterine growth retardation (IUGR) is a condition in which the fetus does not reach its growth potential during the gestation period [2]. IUGR is a public health issue in human medicine and a concern in animal production with a range of adverse short- and long-term effects [1], such as a several-fold increase in neonatal morbidity and mortality [3]. In addition, IUGR has been closely related to later development of many diseases in adulthood, including type 2 diabetes, hypertension, obesity, coronary artery disease, and cancer among others, known as the Developmental Origins of Adult Disease hypothesis [4–8]. Recently, obesity and diabetes have been of interest to researchers worldwide. Ravelli and colleagues have reported that famine during pregnancy induces higher obesity rates at age 19 [9]. Another study also demonstrated that low birth weight is closely related to impaired glucose tolerance and increased risk of type 2 diabetes in adulthood [10]. Because there is currently no curative treatment for IUGR, designing strategies to prevent or treat IUGR in utero would be highly desirable.

Glutamate is an important excitatory neurotransmitter [11]. Excessive activation of glutamate receptors causes excitatory neurotoxicity, resulting in neuronal injury or death, through intracellular mitochondrial dysfunction and oxidative stress [12, 13]. Glutamate neurotoxicity is primarily mediated by N-methyl-D-aspartate receptors (NMDARs), a subset of ionotropic glutamate receptors [14]. In the rat brain, NMDARs are expressed as early as embryonic day (E) 14, peaking around the third postnatal week and then declining slightly to adult levels [15, 16]. NMDARs are involved in the normal development of the central nervous system (CNS), including neurogenesis, migration, proliferation, neuronal apoptosis, axonal outgrowth, and synapse formation [17]. Increased glutamate not only harms the CNS in adult animals but is also toxic to immature neuronal cells [18]. Furthermore, NMDARs are expressed in many peripheral tissues and cells, including the lung and islets [19, 20]. We have reported that the excessive activation of NMDARs contributes to hypoxia-induced fetal lung development retardation, and appropriate blockade of NMDARs might minimize the negative outcomes of prenatal hypoxia on lung development [21]. However, little information is available about the role of NMDARs in the fetal islets in rats with IUGR.

Recently, it has been reported that inhibition of NMDARs enhances the glucose-stimulated insulin secretion and survival of islet cells in adult mouse and human islets [22]. This indicates that antagonists of NMDARs may be a potential treatment strategy for diabetes. We therefore hypothesize that excessive activation of NMDARs in the fetal pancreas of rats with IUGR contributes to the dysfunction of islets and increases of the susceptibility of rats to diabetes induced by a high-fat (HF) diet in adulthood. Memantine, an NMDAR blocker, possesses the unique ability to prevent the excitotoxic effect of NMDAR stimulation without interfering with the normal synaptic activity [23]. Since 1982 it has been used as a moderate-affinity NMDAR blocker without clinically relevant side effects [24, 25]. This study was therefore designed to examine whether antenatal blockade of NMDARs by Memantine reduces the risk of type 2 diabetes at adulthood induced by an HF diet in rats with IUGR.

Materials and methods

Ethics statement

The Ethics Committee of the Center for Scientific Research with Animal Models at Central South University (Changsha, China) approved the experiments (No. 2012-06-03) in this study, which were performed in accordance with the guidelines of the National Institutes of Health. Rats were anesthetized with chloral hydrate (400 mg/kg, intraperitoneal injection), and necessary efforts were taken to minimize suffering before operation.

Animals

Eight-week-old male and female SD rats were obtained (Hunan SJA Laboratory Animal Co., Ltd, Changsha, China), acclimatized, and then mated within the animal facility. Throughout pregnancy, the rats were given a free access to water and standard laboratory murine food (LabDiet Ref. 5001; 3.02 kcal/mg, 3% protein, 4.5% fat, 6% fiber). The day on which vaginal plugs were observed was considered E0.5 [21]. On E14.5, the rats were randomly assigned into four groups: (1) control group: the rats were maintained in normoxic conditions and received daily intraperitoneal injections of saline; (2) Memantine group: the rats were maintained in normoxic conditions and received daily intraperitoneal injections of Memantine (5 mg/kg body weight, Sigma, USA); (3) IUGR group: the rats were exposed to hypoxic conditions ($FiO_2 = 0.105$) for 8 h/day until E20.5 and received daily intraperitoneal injections of normal saline; and (4) Memantine + IUGR group: the rats received a daily Memantine injection (5 mg/kg, i.p.) before hypoxia exposure [21]. After birth, eight newborns per litter were kept. Three weeks later, the offspring were weaned and were fed an HF diet with 60% of the calories from fat (Figure 1) [26].

Intraperitoneal glucose tolerance test and insulin releasing test

The rats were fasted for 12 h and then intraperitoneally injected with glucose (2 g/kg). Glucose concentrations were measured in blood collected from the tail at 0, 15, 30, 60, and 120 min after intraperitoneal injection. The area under the blood glucose curve (AUC) was calculated as AUC = 7.5 × (0 min glucose + 30 min glucose) + 15 × (15 min glucose + 30 min glucose + 60 min glucose) + 30 × (60 min glucose + 120 min glucose). Glucose concentrations were measured twice at each time point using an automatic glucometer (Roche, Germany). Insulin concentrations were measured 10 min after an intraperitoneal glucose injection by an ELISA (Alpco, USA).

Insulin tolerance test

Four groups of rats were fasted for 6 h. Human insulin (0.75 unit/kg, Sigma, USA) was injected intraperitoneally. Blood samples were collected via tail vein at 0, 15, 30, 60, and 120 min subsequently to measure glucose concentrations. The area under the blood glucose curve after insulin injection (AUCi) was calculated as AUCi = 7.5 × (0 min insulin + 30 min insulin) + 15 × (15 min insulin + 30 min insulin + 60 min insulin) + 30 × (60 min insulin + 120 min insulin).

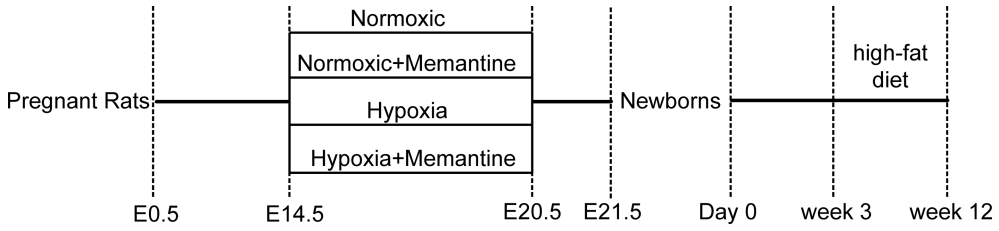

Figure 1. The experimental design for the animal model.

Table 1. Sequences of specific primers (rat) used in this study.

Gene	Forward primer sequence (5′ to 3′)	Reverse primer sequence (5′ to 3′)	Length (bp)
NMDAR1	CGGCTCTTGGAAGATACAGC	GTGGGAGTGAAGTGGTCGTT	156
NMDAR2A	ACATTGCAGAAGCTGCCTTT	TTCTGTGACCAGTCCTGCTG	186
NMDAR2B	GTGAGAGCTCCTTTGCCAAC	TGAAGCAAGCACTGGTCATC	157
NMDAR2C	AGACCAATACCCACCCTTCC	GTTGAGCACAGCAGCATCAT	187
NMDAR2D	TAGTGTCAGTGCGCAGATCC	ACCATGAACCAGACGTAGCC	114
Insulin	GCTTCTGCCAAGACCTTCAC	TAGGACAGGGTCCCAGACAC	159
Pdx1	ACACAGCTCTACAAGGACCC	GGCACTTCGTATGGGGAGAT	182
Mafa	AAATACGAGAAGTTGGCGGG	CACAGAAAGAAGTCGGGTGC	125
Beta-*actin*	GTCGTACCACTGGCATTGTG	CTCTCAGCTGTGGTGGTGAA	181
18S rRNA	AAACGGCTACCACATCCAAG	CCTCCAATGGATCCTCGTTA	155
28S rRNA	AAATGTGGCGTACGGAAGAC	CGTGCCGGTATTTAGCCTTA	199

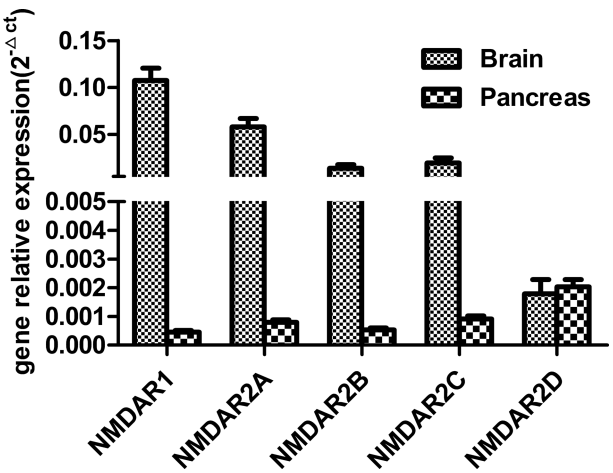

Figure 2. Real-time PCR analysis of NMDAR mRNA expression in the rat brain and fetal pancreas of rats. Values are shown as the mean ± SEM (n = 4).

RNA extraction and quantitative real-time PCR

Total RNA from pancreatic cells was extracted using TRIzol reagent (Invitrogen, USA) and reverse transcribed with random primers (Invitrogen, USA). The RNA concentration and quality were determined by measuring the optical density at 260 and 280 nm. The cDNA was stored at –20°C. Real-time PCR was performed using SYBR green master mix (Bio-Rad, USA). The fluorescence spectra were monitored by a CFX-96 Real-Time PCR detection system (Bio-Rad, USA). We used the average expression of 18S rRNA (Rn18s), 28S rRNA (Rn28s), and beta-actin as the references. Primers were designed using Primer 3. The specificity of the primers was determined using Primer-BLAST. The primers for the target genes are detailed in Table 1. Primer efficiency between 1.8 and 2.2 was acceptable. All samples were run in triplicate. To quantify the relative gene expression, the comparative threshold cycle (CT) method was employed. For each sample, the average CT value was subtracted from the corresponding average of the reference value, calculating the ΔCT. The ΔΔCT value was calculated by subtracting the average control ΔCT value from the average experimental ΔCT. The fold increase or decrease of the experimental sample relative to the control was calculated using the formula $2^{-\Delta\Delta CT}$ [27].

Immunohistochemistry

Briefly, paraffin-embedded sections (4 μm thick) were deparaffinized, treated with 3% H_2O_2 in methanol for 10 min to inactivate any endogenous peroxidase, washed with TBST (0.1% Tween 20 in TBS), and then incubated for 60 min in blocking buffer (3% bovine serum albumin in TBST). The sections were incubated overnight at 4°C with a rabbit anti-insulin antibody (1:50, Abcam, USA), followed by incubation with the a horseradish peroxidase (HRP)-conjugated secondary antibody (Sigma) diluted in 1% BSA at 37°C, and finally incubated for 3 min at room temperature with 3, 3′-diaminobenzidine for color development. All experiments were

Table 2. Initial maternal body weight and litter size date.

	Initial maternal body weight (g)	Mean litter size (n)	Male (%)
Control	280.1 ± 12.6	13.5 ± 1.4	59.1 ± 6.4
Memantine	283.1 ± 14.2	12.4 ± 1.0	56.7 ± 4.6
IUGR	279.9 ± 14.5	12.2 ± 1.1	54.7 ± 5.1
Memantine + IUGR	281.5 ± 9.7	13.1 ± 1.6	55.6 ± 6.9

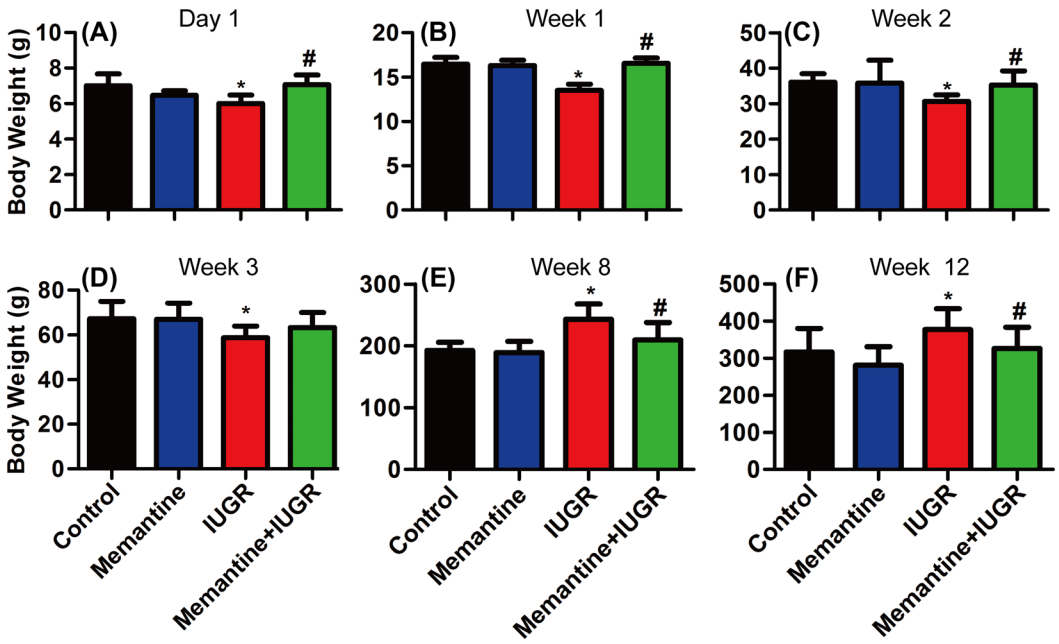

Figure 3. Antenatal Memantine treatment improved the body weight gain of rats with intrauterine hypoxia-induced IUGR. Body weights of rats at postnatal day 1(A), 1 week (B), 2 weeks (C), 3 weeks (D), 8 weeks (E), and 12 weeks (F). Values are shown as the mean ± SEM (n = 12-37). $^{*}P < 0.05$ vs control, $^{\#}P < 0.05$ vs IUGR.

repeated three times. The sections were viewed with a microscope (Thermo, USA).

Immunofluorescence

Pancreatic tissues were fixed overnight in 4% paraformaldehyde and then embedded in paraffin. Serial 4-μm sections were mounted on slides. The localization of insulin and cleaved caspase-3 in pancreatic islets was determined by a double-labeled immunofluorescence method. The slides were deparaffinized with sequential incubations in xylene and rehydrated with descending concentrations of ethanol. This was followed by antigen retrieval, conducted by boiling the slides in citrate buffer followed by gradual cooling. After being washed in PBS, the sections were treated with a blocking agent (0.5% bovine serum albumin, Sigma, USA) for 45 min at room temperature. The sections were incubated overnight at 4°C with a cocktail of two antibodies: rabbit anti-insulin antibody (1:50, Abcam, USA) and rat anti-cleaved caspase-3 antibody (1:20, Abcam, USA). Then, the sections were incubated with FITC- and Cy3-conjugated secondary antibodies after washing in PBS. The sections were viewed with a fluorescence microscope (Thermo, USA).

Statistical analysis

Data were expressed as the mean ± SEM. The statistical analysis was performed with SPSS17.0. Statistical comparisons among the groups were assessed by a one-way analysis of variance. When F ratios were significant ($P < 0.05$), the Tukey-Kramer post hoc test was performed (between group comparison). A P-value of <0.05 was considered statistically significant.

Results

Expression of N-methyl-D-aspartate receptor subtypes in the fetal pancreas

NMDARs are assembled as heteromers that differ in subunit composition [28]. To ascertain whether NMDARs were expressed in the fetal pancreas, the mRNA expression of NMDAR subtypes in the pancreases of fetal rats at E20.5 was examined by qPCR and compared to brain tissue, which expresses a significant level of glutamate receptors. We found that NMDARs were expressed in the fetal rat pancreas. *NMDAR2D* mRNA was expressed at a similar level compared to that in the rat brain (Figure 2).

Antenatal Memantine treatment improved body weight gain induced by a high-fat diet in intrauterine growth retardation rats

Initial maternal body weight and litter size did not differ between groups (Table 2). In the first 3 weeks after birth, the body weight of IUGR rats was much lower than that in the control group (Figure 3A–D, $P < 0.05$). After receiving an HF diet, the growth of the IUGR rats accelerated, and caught up with the control rats quickly. The body weight of the IUGR rats surpassed that of the control group at the 8th week and 12th week (Figure 3E and F, $P < 0.05$). Antenatal Memantine treatment attenuated the weight loss induced by hypoxia at postnatal day 1, 1 week, 2 weeks, and 3 weeks ($P < 0.05$). At the 8th week and 12th week, antenatal Memantine treatment reduced the increase of weight (Figure 3E and F, $P < 0.05$), and was not significant compared to the control group ($P > 0.05$). These data indicate that antenatal Memantine treatment rescues the body weight loss induced by intrauterine hypoxia in early stages of development and reduces the excessive increase in weight induced by an HF diet in adulthood.

Antenatal Memantine treatment rescued glucose tolerance induced by a high-fat diet in intrauterine growth retardation rats at adulthood

To evaluate the glucose metabolism function of the pancreas islet, glucose tolerance was investigated. After an intraperitoneal injection of glucose, no significant differences between IUGR rats and control rats were observed in blood glucose levels and AUCs at the first week and third week (Figure 4A–D, $P > 0.05$). The glucose tolerance of IUGR rats was impaired from the 8th to 12th week. IUGR rats exhibited significantly increased glucose levels at 15 and 30 min after glucose injection compared to the control rats at the 8th week, and a more significantly increase at 15, 30, and 60 min at the 12th week (Figure 4E and G, $P < 0.05$). The AUC of the IUGR group was markedly elevated at the 8th week and 12th week (Figure 4F and H, $P < 0.05$). Antenatal Memantine treatment prevented the impaired glucose tolerance in the hypoxia-induced IUGR rats at the 8th week and 12th week (Figure 4E–H, $P < 0.05$). These results indicate that a long-term intrauterine hypoxia environment during fetal pancreatic development increases the susceptibility of offspring to develop diabetes induced by an HF diet in adulthood. Antenatal Memantine treatment attenuates the glucose tolerance damage at adulthood in rats with intrauterine hypoxia-induced IUGR.

Antenatal Memantine treatment improved insulin release induced by a high-fat diet in intrauterine growth retardation rats at adulthood

To further evaluate the glucose metabolism function of the pancreas islet, insulin release was measured in response to glucose stimulation. At the first week and third week, rats in both the control and IUGR groups showed a nearly threefold increase in insulin secretion 10 min after intraperitoneal glucose injection, but there was no difference between the groups (Figure 5A–C, $P > 0.05$). At the 8th week and 12th week, the insulin secretory capacity was impaired in rats with intrauterine hypoxia-induced IUGR. The insulin secretion response to glucose was reduced at the 8th week and 12th week (Figure 5D–G, $P < 0.05$). Antenatal Memantine treatment significantly improved the insulin secretion in rats with intrauterine hypoxia-induced IUGR (Figure 5E and H, $P < 0.05$). These results indicate that antenatal Memantine treatment improves insulin release at adulthood in IUGR rats fed an HF diet.

Antenatal Memantine treatment improved the morphology of pancreas islets induced by a high-fat diet in intrauterine growth retardation rats at adulthood

Since insulin secretion was significantly decreased at the 12th week in IUGR rats, we determined whether the decrease in islet insulin release was due to alterations in beta-cell mass size in the pancreas at the 12th week. We found that rats with intrauterine hypoxia-induced IUGR had a smaller islet size than control rats, and insulin-positive cells were decreased in the IUGR group. Antenatal Memantine treatment significantly attenuated the reduction of islet size at adulthood in intrauterine hypoxia-induced IUGR rats fed an HF diet (Figure 6, $P < 0.05$).

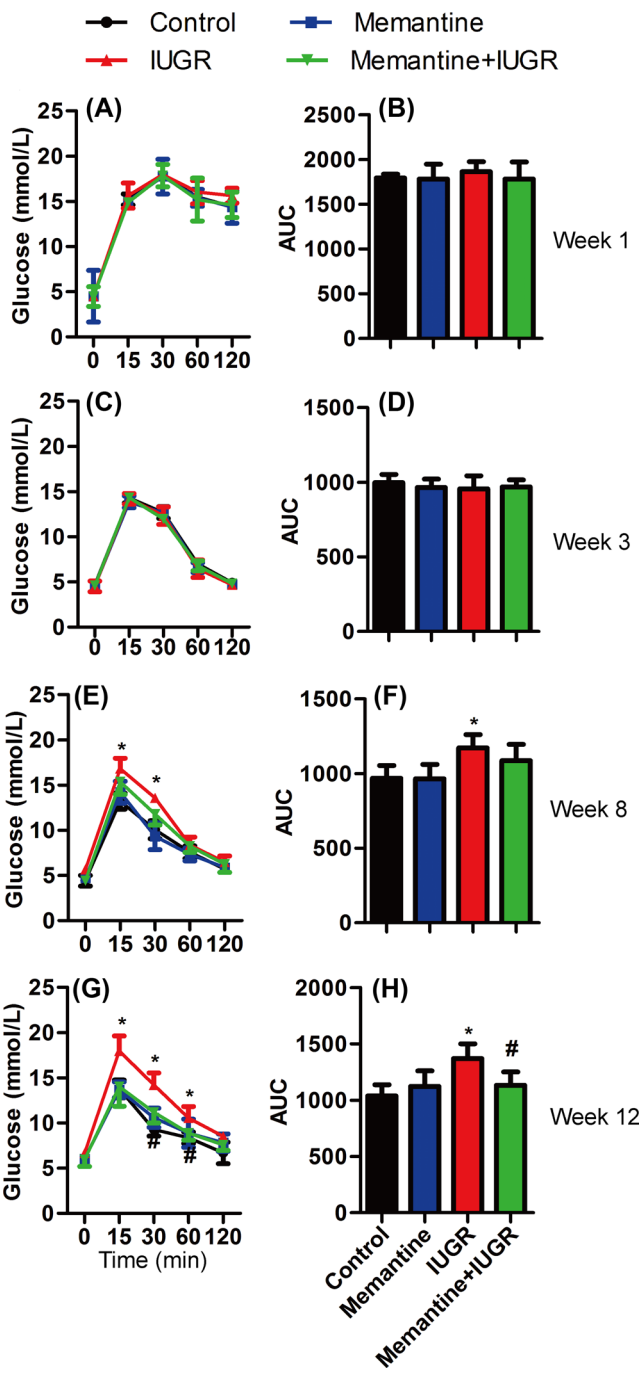

Figure 4. Antenatal Memantine treatment rescued the glucose tolerance of rats with intrauterine hypoxia-induced IUGR fed an HF diet. Blood glucose levels during an intraperitoneal glucose tolerance test on postnatal week 1 (A), week 3 (C), week 8 (E), and week 12 (G). Comparison of AUC in rats at week 1 (B), week 3 (D), week 8 (F), and week 12 (H). Values are shown as the mean ± SEM (n = 6). $^{*}P < 0.05$ vs control, $^{\#}P < 0.05$ vs IUGR.

Antenatal Memantine treatment improved insulin tolerance induced by a high-fat diet in intrauterine growth retardation rats at adulthood

It has been reported that the insulin sensitivity of IUGR rats is deteriorated at adulthood [29, 30]. To determine whether insulin resistance was mitigated by antenatal Memantine treatment

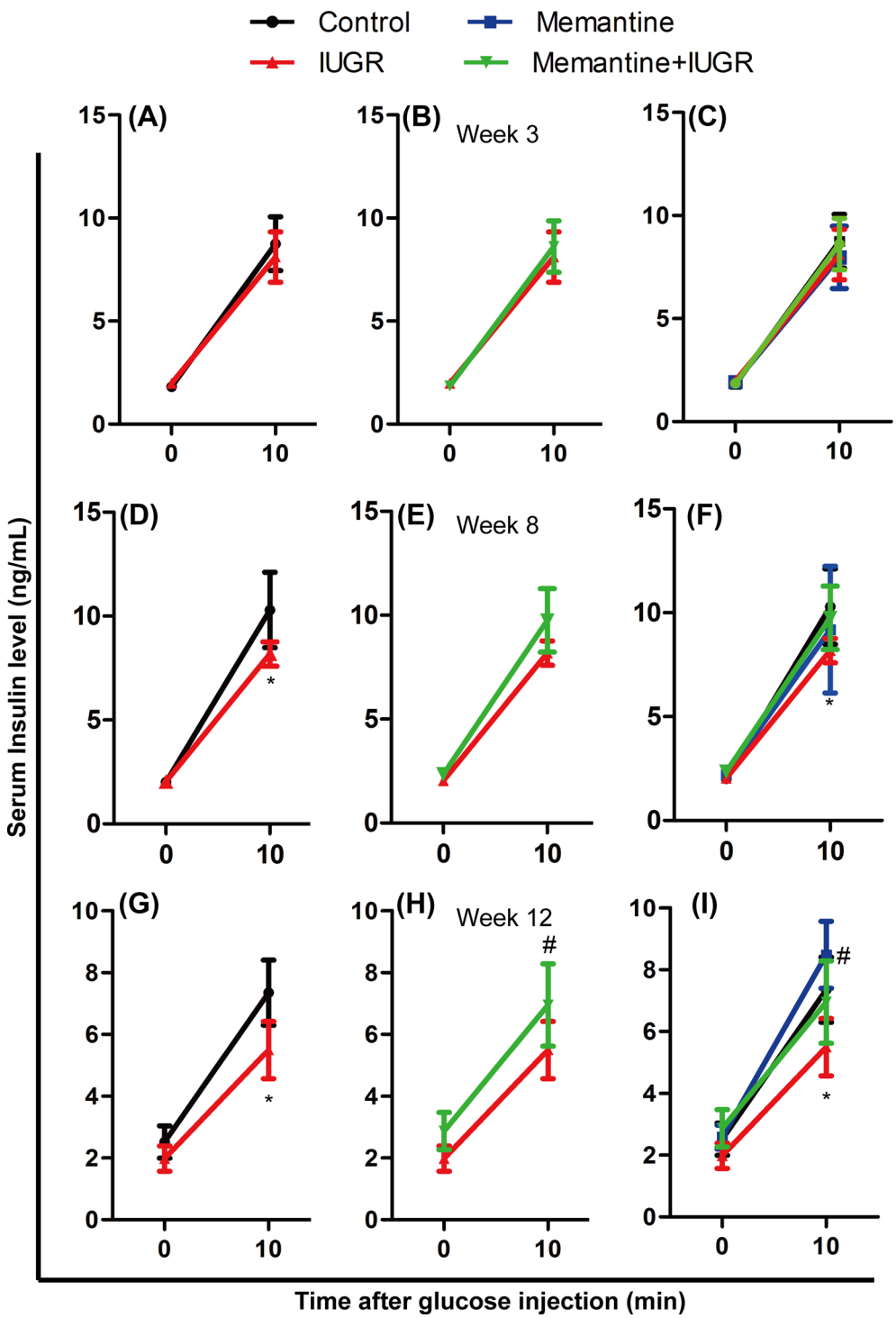

Figure 5. Antenatal Memantine treatment improved the insulin release of rats with intrauterine hypoxia-induced IUGR fed an HF diet. Serum insulin levels during intraperitoneal glucose tolerance test at 3 weeks (A–C), 8 weeks (D–F), and 12 weeks (G–I). Values are the mean ± SEM from each group (n = 6-8). $^{*}P < 0.05$ vs control, $^{\#}P < 0.05$ vs IUGR.

in IUGR rats, we performed insulin tolerance tests at the 8th week and 12th week. IUGR rats showed impaired insulin sensitivity at the 8th week, which deteriorated at the 12th week. The glucose levels in the IUGR group were higher followed by an injection of insulin than that in the control group. Antenatal Memantine treatment significantly improved the insulin sensitivity of IUGR rats at the 8th week and 12th week (Figure 7, $P < 0.05$).

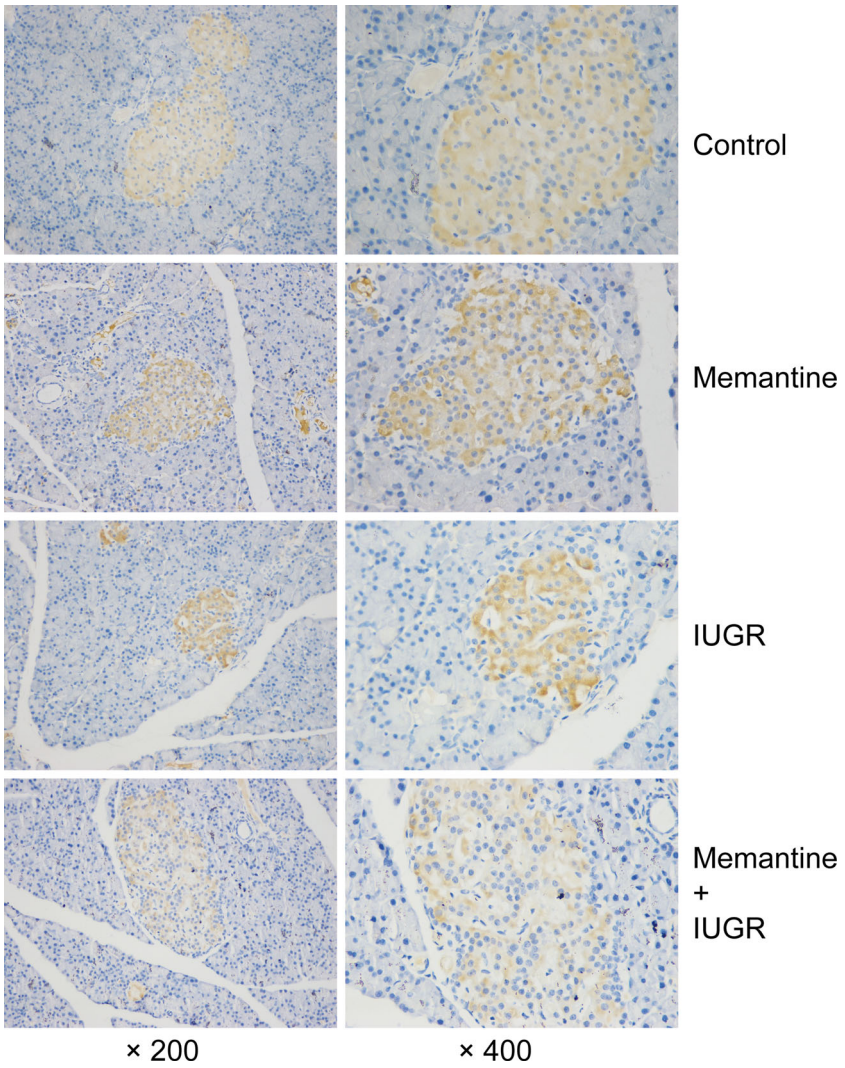

Figure 6. Antenatal Memantine treatment improved the morphology of pancreas islets of rats with intrauterine hypoxia-induced IUGR fed an HF diet. Immunohistochemistry analysis of islet beta cells using anti-insulin antibody. Representative figures are presented from the analyses of five different mice per group.

Antenatal Memantine treatment improved the expression of genes associated with beta-cell function and decreased cleaved caspase-3 protein expression in the pancreas induced by a high-fat diet in intrauterine growth retardation rats at adulthood

We further detected the expression of the *Insulin* gene and other transcription factors that regulate the transcriptional activity of the insulin gene, including *Pdx-1* and *Mafa* [31]. Rats with intrauterine hypoxia-induced IUGR expressed lower levels of *insulin*, *Pdx-1*, and *Mafa* mRNA in the pancreas at the 12th week (Figure 8, $P < 0.05$). Antenatal Memantine treatment significantly alleviated the decreased expression of these mRNAs (Figure 8, $P < 0.05$). Furthermore, immunofluorescence confirmed that IUGR rats with antenatal Memantine treatment expressed a lower level of cleaved caspase-3 in islets at the 12th week compared to IUGR rats without antenatal Memantine injection (Figure 9). Thus, antenatal Memantine treatment improves the expression of genes related to beta-cell function and survival in the pancreases of IUGR rats at adulthood.

Discussion

The salient findings of this study can be summarized as follows: (1) NMDARs were expressed in the fetal rat pancreas of rat, and *NMDAR2D* mRNA was expressed at a relativly high level; (2) rats with intrauterine hypoxia-induced IUGR exhibited a high rate of diabetes after being fed an HF diet at adulthood; (3) antenatal blocking of NMDARs by Memantine rescued glucose tolerance, increased insulin release, and improved the insulin sensitivity of IUGR rats at adulthood; and (4) lower mRNA levels of *Insulin*, *Pdx-1*, and *Mafa* and a higher level of cleaved caspase-3 protein were found in the pancreases of 12-week-old IUGR rats, and these levels were reversed by antenatal treatment with Memantine. The results are consistent with growing evidence that different types of maternal insults can lead to the development of diabetes in adult offspring [26, 29, 30, 32, 33]. Our results support the Developmental Origins of Adult Disease hypothesis and indicate that antenatal blocking of NMDARs by Memantine is a potential approach to prevent this effect.

NMDARs play an important role in the development of the CNS, including neurogenesis, migration, proliferation, neuronal apoptosis,

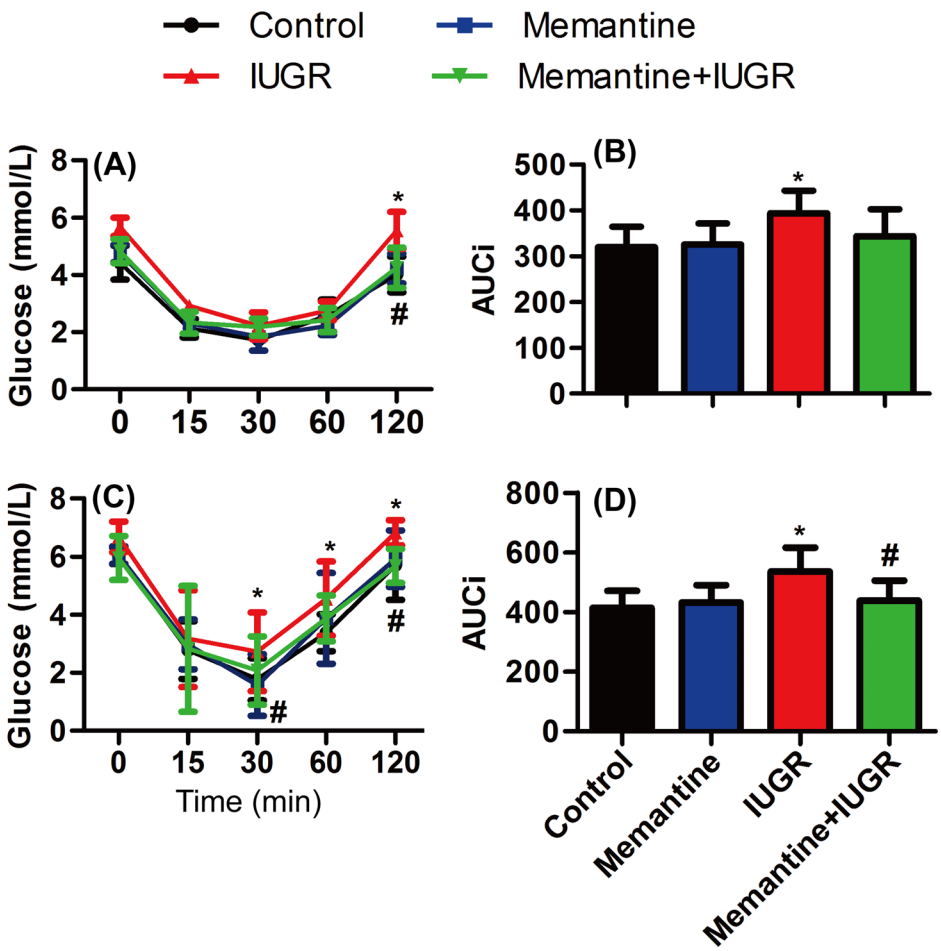

Figure 7. Antenatal Memantine treatment improved the insulin tolerance of rats with intrauterine hypoxia-induced IUGR fed an HF diet. Blood glucose levels during an intraperitoneal insulin tolerance test on postnatal week 8 (A) and week 12 (C). Comparison of AUCi in rats on week 8 (B) and week 12 (D). Values are shown as the mean ± SEM (n = 6). $^{*}P < 0.05$ vs control, $^{\#}P < 0.05$ vs IUGR.

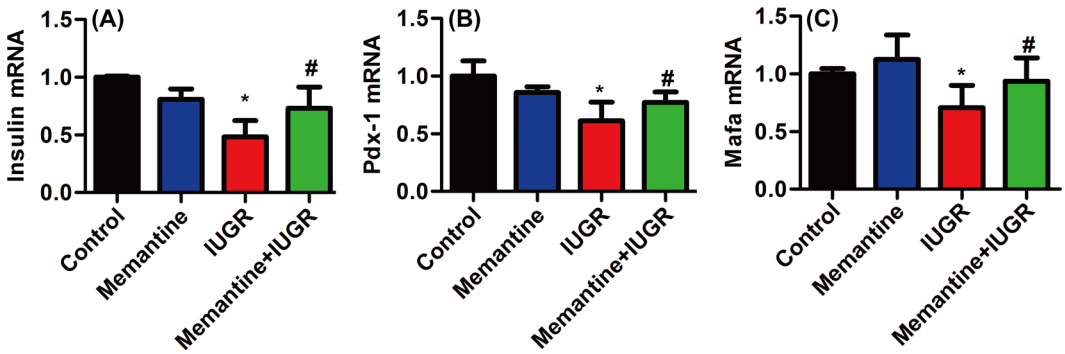

Figure 8. Antenatal Memantine treatment increased the expression of genes associated with beta-cell function and survival in the pancreases of rats with intrauterine hypoxia-induced IUGR fed an HF diet. The mRNA levels of *Insulin, Pdx-1,* and *Mafa* were normalized to average of *18S rRNA, 28S rRNA,* and beta-*actin* in the same sample. Values are shown as the mean ± SEM (n = 6). $^{*}P < 0.05$ vs control, $^{\#}P < 0.05$ vs IUGR.

axonal outgrowth, and synapse formation [17]. NMDARs are expressed as early as E14, peaking around the third postnatal week and then declining slightly to adult levels in the rat brain [15, 16]. Our previous study has found that NMDARs are also expressed in fetal rat lung. From E15.5 to E21.5, the expressions of *NMDAR2B* and *NMDAR2C* remained steady, while the expression of *NMDAR1*, *NMDAR2A*, and *NMDAR2D* mRNA increased during fetal lung development [21]. Similar to the function of NMDARs in fetal brain development, NMDARs play an important role in fetal lung development [21]. Since pancreatic islet beta cells share many cell biology features with neurons [49], NMDARs may play an important role in beta-cell viability and function. NMDA elicits a rise of $[Ca^{2+}]_i$ in single beta cells in vitro [18] and causes transient insulin secretion [50]. These effects are suppressed by the NMDAR antagonist,

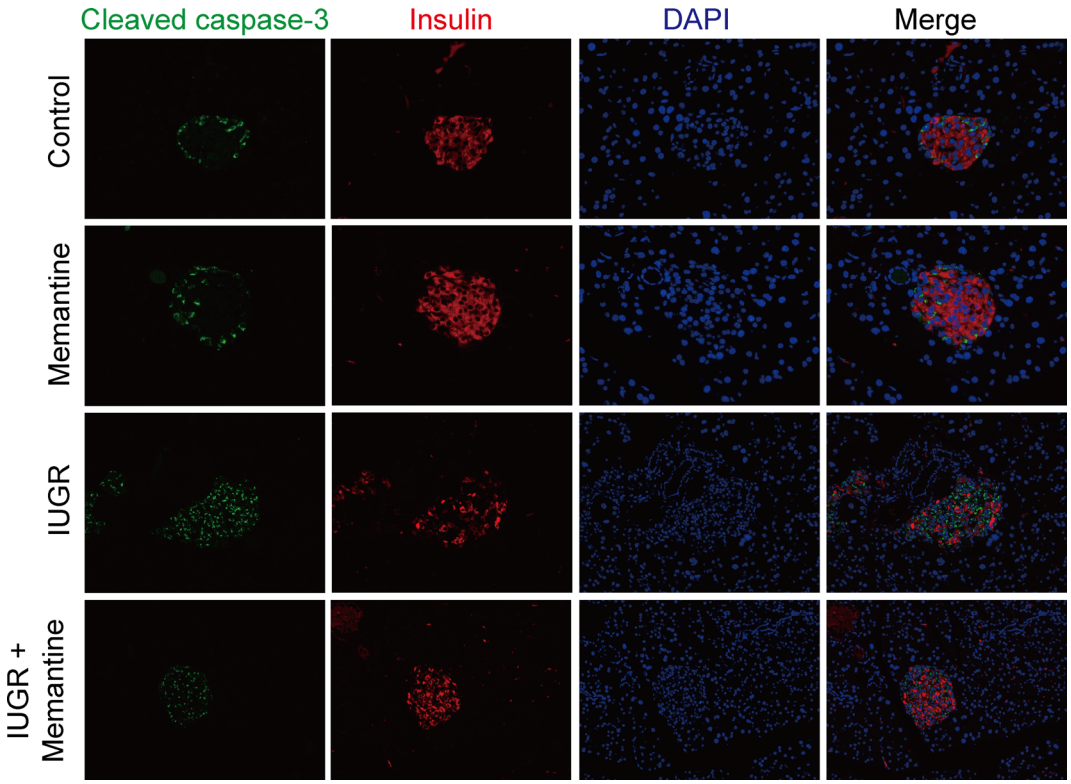

Figure 9. Memantine treatment inhibited the cleaved caspase-3 expression in islets of IUGR rats. Immunofluorescence staining of pancreatic sections from control and IUGR rats treated with or without Memantine (n = 5). Representative immunofluorescence images are shown of islets were stained by immunofluorescence for insulin (Red) and cleaved caspase-3 (Green).

MK-801 [51]. To our knowledge, this study is the first to confirm that NMDARs are expressed in the fetal rat pancreas and that *NMDAR2D* mRNA is expressed at a high level. These results indicate that NMDARs might play a role in the development of the fetal pancreas.

IUGR is a common complication of pregnancy with a high incidence rate. It has been linked to later development of many diseases in adulthood, including type 2 diabetes [5]. We used a chronic intrauterine hypoxia model in our study, because it exists worldwide and is the predominate cause of IUGR in China [26]. We found that after being fed an HF diet, rats with intrauterine hypoxia-induced IUGR exhibited reduced abilities of insulin secretion, glucose tolerance, and insulin sensitivity at adulthood. These results indicate that environmental changes caused by intrauterine hypoxia result in islet beta-cell function and insulin sensitivity alterations after birth, which have life-long effects and predispose the animal to glucose intolerance and diabetes in later life. Glutamate is a predominantly excitatory amino acid and the primary neurotransmitter in the CNS [11]. The excessive activation of glutamate receptors causes excitatory neurotoxicity, through intracellular calcium overloading, resulting in neuronal injury or death [12]. Maternal glutamate can pass into the placenta through transport systems and enter the fetal circulation [47]. The oral administration of monosodium glutamate (MSG) maintains its toxicity in neuronal cells during pregnancy up to the end of the weaning period of rats [34]. In addition, intraperitoneal injection of MSG at the neonatal stage of rodents results in obesity, lower pancreatic beta-cell mass and diabetes [35–37]. These date indicate that excessive glutamate could not only evoke toxic effects in fetal neurons but also cause pancreatic beta-cell dysfunction in neonatal rats. In addition, in a mouse model of type 2 diabetes mellitus, long-term treatment with the NMDAR antagonist dextromethorphan (DXM) improved islet insulin content, islet cell mass, and blood glucose control. Individuals with T2DM treated with DXM showed enhanced serum insulin concentrations and glucose tolerance in a small clinical trial [22]. This indicates that excessive activation of NMDARs may play an important role in the development of adult diabetes. A fetal hyperglutamate state may be observed in IUGR fetuses with reduced umbilical flow and impaired elimination of the fetal glutamate production [48]. Our previous study found that the excessive activation of NMDARs contributes to hypoxia-induced fetal lung development retardation, and appropriate blockade of NMDARs minimized the negative outcomes of prenatal hypoxia on lung development [21]. To investigate whether excessive activation of NMDARs in the fetal pancreas of IUGR rats contributes to the dysfunction of islets in adulthood, pregnant rats were treated with Memantine. We found that antenatal Memantine treatment rescued glucose tolerance, improved insulin release, mitigated the decrease in islet size, improved the expression of genes associated with beta-cell function, and decreased cleaved caspase-3 protein expression in IUGR rats after receiving an HF diet at adulthood. These results show for the first time that antenatal Memantine treatment attenuates the functional alterations of the islet beta cells occurring in adulthood of offspring exposed to intrauterine hypoxia and then fed an HF diet.

In the CNS, the excessive activation of NMDARs leads to mitochondrial dysfunction and oxidative damage, occurring along with decreased mitochondrial membrane potential and increased reactive oxygen species (ROS) production, causing excitatory neurotoxicity

in neurons [13, 38, 39]. In regard to the pathogenesis of the thrifty phenotype, mitochondrial dysfunction has been proposed as a link between poor nutrition early in life and diabetes as an adult [40]. Low levels of oxygen in growth-retarded fetuses decrease the activity of electron transport chain complexes and then increase ROS levels, leading to oxidative damage in the mitochondria [41]. The mitochondrial dysfunction in beta cells would have deleterious effects, especially in cells that have a high energy requirement, such as in response to an HF diet. The mitochondrial dysfunction also reduces *Pdx-1* expression, which plays a pivotal role in the development of diabetes in IUGR animals [42, 43], via the production of ROS in beta cells [44]. The adaptive reprogramming of mitochondrial function enables the fetus to survive in a limited energy environment [45]. However, ROS production and oxidative stress gradually increase in IUGR islets; ATP production, insulin secretion, and beta-cell mass are impaired and deteriorated with age and HF diet [41, 46]. In our study, the glucose tolerance, insulin sensitivity, expression of the *Pdx-1* gene, and beta-cell mass were impaired in later life in IUGR rats after receiving an HF diet. Antenatal Memantine treatment could attenuate the functional alterations of the islet beta cells at adulthood. This evidence is in accordance with previous data showing that Memantine impedes calcium influx, improves the mitochondrial function, and then blocks neurotoxicity [25, 47, 48]. These findings indicate that excessive activation of NMDARs impairs mitochondrial function in beta cells of IUGR fetuses through an excessive calcium influx. Antenatal blockade of NMDARs by Memantine may suppress these effects and protect beta cells of IUGR rats against the stress of an HF diet in later life.

One limitation in this study is that we did not examine the effect of gender difference on the susceptibility to diabetes of IUGR rats. We acknowledge the importance of gender in the development of diabetes, and this is worthy of further study in the future. Nonetheless, the data presented provide solid evidence for the effect of IUGR on diabetes. A second limitation is that we did not go to confirm that excessive activation of NMDARs altered mitochondrial function in fetal pancreas development. We believe this is a very important topic. Thus, we would like to carry out a separate but more extensive study on this topic. Finally, whether early antenatal Memantine therapy alone elicits an adverse programming effect on healthy people in clinical practice awaits further evaluation.

In conclusion, the data presented in our research support the Developmental Origin of Adult Disease hypothesis that intrauterine hypoxia causes an increased susceptibility of diabetes in adult rats fed an HF diet. Antenatal Memantine treatment attenuates the susceptibility to diabetes induced by an HF diet in IUGR rats, which was associated with reduced loss of beta-cell mass, rescued glucose tolerance, and improved insulin resistance. The excessive activation of NMDARs is involved in the restriction of fetal pancreas development induced by intrauterine hypoxia. Our results indicate that NMDAR activation plays an important role in pregnancy and contributes to the understanding of basic reproductive biology. We expect that the findings from this study to provide a strong scientific basis for antenatal blockade of NMDARs in pregnant women to improve fetal development and to reduce the susceptibility to diabetes in adulthood.

Contribution statement: X-TH designed and performed the experiments, analyzed, and interpreted the data and wrote the manuscript. S-JY, CL, JG, J-ZH, Y-HH, and D-DF assisted during the acquisition, analysis, and interpretation of data and revised the manuscript. All authors approved the final version of the manuscript. Z-QL and D-DF are responsible for the integrity of the work as a whole. All authors reviewed the manuscript.

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