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Research article

Pioglitazone improves the ability of learning and memory via activating ERK1/2 signaling pathway in the hippocampus of T2DM rats

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HIGHLIGHTS

- This study explains the function of pioglitazone on the ability of learning and memory.
- The mechanism of pioglitazone relies on ERK1/2 signaling pathway activation.
- ERK1/2 signaling pathway activation is due to RKIP reduction.

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ABSTRACT

OBJECTIVE: To explore the correlation between effect of PIO (pioglitazone, PIO) on learning as well as memory and ERK1/2 (extracellular signal regulated kinase 1/2, ERK1/2) pathway in T2DM (type 2 diabetes mellitus, T2DM) rats, further to elucidate the potential mechanism of PIO in improvement of learning and memory.

METHODS: 12-week-old male SD rats (number of 10 per group) were randomly divided into control group (CON), T2DM group (DM) and T2DM +PIO group (DM + PG). Rats in DM and DM + PG groups were given high fat diet for 20 weeks, then treated with Streptozotocin (27 mg/kg) by intraperitoneal injection at 21week. After 72 h, the FBG (fasting blood glucose, FBG) was greater than 7.0 mmol/L can considered T2DM rats. DM + PG group was treated with PIO (10 $mg{\cdot}kg^{-1}{\cdot}d^{-1}$) by gavage daily. After Hyperinsulinemic-Euglycemic Clamp Study and Morris water maze test at 30-week, all of animals were sacrificed. The expressions of RKIP (Raf-1 kinase inhibitor protein, RKIP) and ERK1/2 in hippocampus were detected using Western Blot and real-time PCR.

RESULTS: The FBG level: DM group (7.68 ± 0.54 mmol/L) was higher than CON group (5.35 ± 0.63 mmol/L) and DM + PG group (6.07 ± 0.84 mmol/L), the differences were considered statistically significant ($P < 0.05$). Hyperinsulinemic-Euglycemic Clamp Studies: GIR (glucose infusion rate, GIR) of DM group (21.02 ± 5.10 $mg{\cdot}kg^{-1}{\cdot}d^{-1}$) was less than CON group (27.64 ± 3.87 $mg{\cdot}kg^{-1}{\cdot}d^{-1}$) and DM + PG group (26.04 ± 5.41 $mg{\cdot}kg^{-1}{\cdot}d^{-1}$), the differences were considered statistically significant ($P < 0.05$). Morris water maze training: The escape latencies and searching platform performance of DM group (24.54 ± 5.02s) decreased significantly compared with CON group (16.73 ± 4.02s) and DM + PG group (18.05 ± 4.12s) ($P < 0.05$). Changes of RKIP, ERK, p-ERK protein relative content in rat hippocampus: Compared with CON groupand DM + PG group, the relative content of RKIP in DM group remarkably increased ($P < 0.01$); ERK protein levels were not considered statistically significant among the three groups ($P > 0.05$); The relative content of p-ERK1/2 protein in CON group and DM + PG group rats dorsal were higher than those in group DM, the difference was considered statistically significant ($P < 0.01$). Changes in hippocampus of rat RKIP and ERK gene relative content: Compared with CON group and DM + PG group, levels of RKIP mRNA in DM group were significantly increased ($P < 0.01$); ERK mRNA levels were not considered statistically significant among the three groups ($P > 0.05$).

CONCLUSION: Activation of ERK1/2 signal transduction pathway via reducing RKIP in the hippocampus may be one of the mechanisms of PIO to improve the learning and memory of the T2DM rats.

1. Introduction

T2DM (formerly called non-insulin-dependent or adult-onset diabetes) results from the body's ineffective use of insulin. Type

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2 diabetes accounts for the vast majority of people with diabetes around the world [1]. Globally, an estimated 422 million adults were living with diabetes in 2014. The global prevalence (age-standardized) of diabetes has nearly doubled since 1980, rising from 4.7% to 8.5% in the adult population [2]. Diabetes has been associated with increased rates of physical and cognitive disability [3], Which includes cognitive impairment and dementia. CID (Cognitive impairment in diabetes, CID) is one of the chronic complications in diabetes, which clinical manifestations were mild and moderate cognitive impairment, decreased learning and memory ability [4]. Dementia is a syndrome in which there is deterioration in memory, thinking, behavior and the ability to perform activities. Globally, the number of people living with dementia worldwide in 2015 was 47.47 million, reaching 75.63 million in 2030 and 135.46 million in 2050[5]. T2DM may be present in up to 80% of individuals with dementia who are aged 65 years or older [6]. Insulin resistance is believed to be an intrinsic link between diabetes and AD (Alzheimer's disease, AD). Longitudinal prospective studies support the link between T2DM and hyperinsulinaemia with the development of AD[7]. T2DM and Dementia are being recognized as a public health priority gradually, given its enormous socioeconomic burdens in the absence of effective treatments [8,2]. When the two kinds of chronic non-infectious diseases are combined together, it will increase the difficulty of treatment and social burden. The high glucose demand and insulin sensitivity of the hippocampus places it at particular risk for insulin resistance that is quintessential to aging and age-related disease states such as AD. Given that the hippocampus is a vital integrator for new memory formation, applying our understanding of the molecular processes. CID and Dementia pathogenic factors are mainly divided into insulin resistance, high glucose toxicity and inflammatory factors. The high blood glucose impaires the blood brain barrier, then induces inflammatory factors, directly damages the central nervous system. AGEs(Advanced Glycation end Products, AGEs) modify Tau protein and Amyloid beta protein [9–11]. Inflammatory factors mediate apoptosis of nerve cells and entanglement of nerve fibers [12,13]. There are plenty of insulin receptor in hippocampus, olfactory bulb and prefrontal cortex. Insulin possesses important neurotrophic properties, Insulin pathway blockade and insulin deficiency cause cognitive impairment [14]. ERK is one of the signal transduction pathways of MAPK (Mitogen-activated Protein Kinase, MAPK), which is the last step in the Ras-Raf-ERK cascade reaction, involving in many physiological and pathological processes such as cell growth, development, proliferation, differentiation and malignant transformation of cells. Recent studies have indicated that ERK signaling pathway plays a key role in learning and memory via participating in many physiological activities of cells [15]. RKIP can block the Ras-Raf-ERK cascade reaction, then inhibit the activation of ERK. As PPARγ receptor agonists, PIO not only reduces blood glucose by enhancing insulin sensitivity, but also plays a role in cognitive impairment in diabetes mellitus. It reduces the deposition of AGEs in the brain by activating PPAR, down regulates the expression level of receptor for AGEs, and suppresses the expression of pro-inflammatory response genes. In addition, pioglitazone indirectly plays a role in brain protection through ameliorating hypertension, hyperlipidemia and other metabolic disorders caused by insulin resistance [16,17]. Currently, it is worth researching whether this kind of drug is suitable for the management of mild cognitive impairment. Pioglitazone improves cognitive impairment on diabetic. However, the mechanism is stillunclear. In this study, we examined changes of ERK expression in the hippocampus of T2DM rats, observed the treatment effect of pioglitazone in MCI(mild cognitive impairment, MCI) of T2DM and explored possible mechanism.

2. Material and Methods

2.1. Animal grouping and treatment

'Healthy adult male SD rats, weight (300 ± 20) g and 12 weeks of age, were randomly divided into 3 groups, including CON group, DM group, DM + PG group. Animals were housed on a 12 hr-light/dark schedule in a room maintained at constant humidity (60 ± 5%), temperature (23 ± 1 °C). DM and DM + PG groups were given high fat diet (60% of calorie from fat) for 20 weeks to conducted intraperitoneal injection with 27 mg/kg Streptozotocin (SERVA Electrophoresis GmbH.). FBG was detected in 72 h, rats associated with FBG (>7 mmol/L) were identified as T2DM. Rats of DM + PG group were treated with pioglitazone hydrochloride(10 $mg{\cdot}kg^{-1}{\cdot}d^{-1}$, Takeda Pharmaceutical Company Limited.), once a day by gavage, CON group and DM group were given saline. Before sacrificed, rats were revealed spatial learning and memory ability by the Morris water maze. Hyperinsulinemic-Euglycemic Clamp experiments was to confirm insulin resistance. Protein and mRNA expressions of RKIP and ERK1/2 in hippocampus were detected by Western Blot and real-time PCR respectively.

2.2. FBG determination

Fasting blood glucose levels of rats were measured in blood sample collected from the their tail veins after 12 hours of fasting and water deprivation via glucose oxidase method [18].

2.3. Hyperinsulinemic-Euglycemic Clamp Studies

We chose this experiment because of Hyperinsulinemic-Euglycemic Clamp test is the gold standard for the detection of insulin resistance, the results were based on the glucose infusion rate. The euglycemic-hyperinsulinemic clamp was performed as previously described [19]. Briefly, Rats were anesthetized with 2% lidocaine(5 ml/kg, Sigma-Aldrich Co. LLC) administered intraperitoneally and the bilateral jugular veins were catheterized. Glucose and insulin were infused via left internal jugular vein, then blood glucose was measured on right internal jugular vein. Insulin (Novolin R; Novo Nordisk, Denmark) was inputted in the speed of 12 mU kg^{-1} d^{-1} by micro-pump in clamp experiments at the starting 10 min, then with the speed of 4 mU kg^{-1} d^{-1} in the following 110 min. Blood glucose was monitored by Abbott glucose meter every 5 min to maintained blood glucose level at 4.4-5.5 mmol/L with adjusting the rate of infusion of a 10% glucose solution. The steady-state glucose GIR was calculated in 60–120 min to reflect the degree of insulin resistance.

2.4. Morris water maze test

The spatial learning and memory ability of rats was measured by Morris water maze before rats were sacrificed for 4 consecutive days. The methods was performed as previously described [20]. Briefly, the experiment were carried out in a quiet, dark and constant temperature environment. The bucket (100 cm, diameter) was filled with water to a predetermined height about 40 cm before test. Black ink was added to make the water opaque black, and water was heated to 25°. Platform (9 cm, diameter)is located below the surface about 1.5 cm in the fourth quadrant of the central station. In the face of the pool wall, the rats were released into the pool from the first, second or third quadrant in turn. Time to find the escape platform (escape latency) was measured. Carring out the adaptive training for 3 days, Rats were trained for 6 consecutive times at 4-minute interval in formal behavioral training test.