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Effect of endogenous galanin on glucose transporter 4 expression in cardiac muscle of type 2 diabetic rats

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ABSTRACT

Although galanin has been shown to increase glucose transporter 4 (GLUT4) expression in skeletal muscle and adipocytes of rats, there is no literature available about the effect of galanin on GLUT4 expression in cardiac muscle of type 2 diabetic rats. In this study, we investigated the relationship between intracerebroventricular administration of M35, a galanin receptor antagonist, and GLUT4 expression in cardiac muscle of type 2 diabetic rats. The rats tested were divided into four groups: rats from healthy and type 2 diabetic drug groups were injected with 2 μM M35 for three weeks, while both control groups with 2 μl vehicle control. The euglycemic-hyperinsulinemic clamp test was conducted for an index of glucose infusion rates. The cardiac muscle was processed for determination of GLUT4 expression levels. The present study showed that the plasma insulin and retinol binding protein 4 (RBP4) levels were higher in both drug groups than controls respectively. Moreover, the results showed the inhibitive effect of central M35 treatment on glucose infusion rates in the euglycemic-hyperinsulinemic clamp test and GLUT4 expression levels in the cardiac muscle. These results demonstrate that endogenous galanin, acting through its central receptor, has an important attribute to increase GLUT4 expression, leading to enhance insulin sensitivity and glucose uptake in cardiac muscle of type 2 diabetic rats. Galanin and its fragment can play a significant role in regulation of glucose metabolic homeostasis in cardiac muscle and galanin is an important hormone relative to diabetic heart.

1. Introduction

Type 2 diabetes mellitus is a worldwide rapidly increasing disease, which especially is accompanied by high morbidity from cardiovascular disease [32,33]. The diabetic heart is closely associated with insulin resistance that causes glucose metabolism disorders [26,29], which result in cardiac ischemia, myocardial infarction and cell death [13,23,28]. Although the precise mechanism, by which diabetes and insulin resistance lead to heart lesions, remains poorly understood, the reduced capacity to utilize glucose could make great contributions to heart lesions [13,28]. Thus, therapeutic interventions focusing on reducing insulin resistance and enhancing myocardial glucose metabolism may greatly improve prevention and treatment of diabetic heart.

Glucose transporter 4 (GLUT4) is the most abundant glucose transporter isoform and primarily contributes to insulin-stimulated glucose uptake in the cardiac muscle [3,27]. Similar to skeletal muscle, GLUT4 levels in the myocardium were reduced in type 2 diabetic animals and patients [5,6,11,24,28]. The reduction in the GLUT4 levels contributes significantly to elevated insulin resistance and myocardial dysfunction of cardiac muscle [3,27]. Therefore, restoration of GLUT4 levels is very important to enhance glucose metabolism and to assuage myocardial dysfunction in the diabetic heart.

Galanin, a 29/30-amino-acid neuropeptide, was isolated in 1983 from porcine intestine by Tatemoto et al. [30]. Galanin distributes widely throughout the central and peripheral nervous system as well as other tissues [17]. The physiological effects of galanin occur through binding to one or more of the three identified receptor subtypes, GALR1-3. All of the subtype receptors are G-protein-coupled receptors and distribute in the hypothalamus, amygdala,

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hippocampus, thalamus, brainstem, spinal cord, dorsal root ganglia, heart, liver, lung, kidney and intestine [17]. Recently, there is increasing evidence focused on the important role of galanin as a critical factor to reduce insulin resistance and to benefit glucose uptake in insulin-independent diabetes [9]. Firstly, galanin may accelerate food intake, body weight and adiposity of subjects [10]. I.c.v. injection of galanin into paraventricular nucleus (PVN) significantly increased daily caloric intake, weight of fat depots, circulating non-esterified fatty acids content and lipoprotein lipase activity in adipose tissue, but reduced circulating glucose levels of animals [34]. Secondly, galanin gene knock out mice have impaired glucose disposal due to reduced insulin response and insulin-independent glucose elimination [2], whereas the homozygous galanin transgenic C57BL/6J mice of the obese phenotype show an increase in metabolic rates of lipid and carbohydrate [25]. Thirdly, galanin secretion in healthy volunteers and diabetic patients is positively correlative with the blood glucose level [7,19,20], which is strongly related to insulin sensitivity. And animals with metabolic disorder of galanin easily suffer from type 2 diabetes mellitus [18]. These results demonstrate that the plasma galanin contents are closely associated with blood glucose levels and insulin sensitivity in humans. Finally, our and other laboratories found that galanin can enhance insulin sensitivity via increasing GLUT4 expression and translocation to the plasma membrane in myocytes and adipocytes of type 2 diabetic animals [4,12,14,16,22,35]. Nevertheless, there are few reports about the effect of galanin on the glucose uptake in the myocardium by now. Accordingly, in this study we used intracerebroventricular (i.c.v.) administration of M35, a galanin receptor antagonist, to evaluate the putative relationship between endogenous galanin and GLUT4 expression levels in cardiac muscle of type 2 diabetic rats.

2. Materials and methods

2.1. Drugs and reagents

M35 and streptozotocin were purchased from Sigma Inc. (Sigma, USA). Anti-GLUT4 and Anti-β-actin were purchased from Santa Cruz Biotechnology Inc., USA. RIPA was purchased from Bioteke Corporation (Beijing, China). BCA™ protein assay kit was purchased from Pierce Chemical Company (Pierce, Rockford, USA). Trizol reagent from Gibco Invitrogen Inc. (Gibco Invitrogen, USA). Rat retinol binding protein 4 (RBP4) ELISA kit was purchased from Uscn Life Science, Inc. (Uscn Life Sci., China). Insulin radioimmunoassay kit was from China Institute of Atomic Energy (Beijing, China).

2.2. Type 2 diabetic models

Healthy male Wistar rats weighing 150 ± 10 g were employed in this study. Animals were kept in standard polypropylene cage and maintained under standard laboratory conditions of temperature 21 ± 2 °C, relative humidity 50 ± 15%, 12 h light–dark cycles, diet (59% fat, 21% protein and 20% carbohydrate) and water ad libitum. All animals used were closely monitored to ensure that none experienced undue stress or discomfort. Eight weeks later, forty of rats were treated with an i.p. of streptozotocin (35 mg/kg) in 0.1 mM citrate buffer (pH = 4.5) under a fasting state. The tail blood of the rats was weekly taken to determine the blood glucose level with a Glucometer (HMD Biomedical, Taiwan) during the study. After another four weeks, animals with fasting blood glucose concentration over 11.1 mmol/L were taken as models of type 2 diabetes. The thirty two diabetic rats were randomly distributed into diabetic control group (DC, $n = 16$) and diabetic drug group with M35 (D-M35, $n = 16$). In addition, thirty two healthy rats were attributed to healthy control group (HC, $n = 16$) and healthy drug group with M35 (H-M35, $n = 16$). All animal procedures used were performed in accordance to the Guiding Principles for Care and Use of Experimental Animals. The experiments were approved by the Animal Studies Committee of Yangzhou University.

2.3. Intracerebroventricular cannulation and injection

The method for animal preparation and i.c.v. injection is similar to that described previously [35]. In brief, animals were anesthetized with 3% amobarbital sodium (50 mg/kg i.p.) and stereotaxically implanted with a guide cannula into the lateral ventricle: anterior-posterior (AP), −0.8 mm; L, 1.4 mm; and V, 3.3 mm. The cannula was cemented to four jeweler's screws attached to the skull and closed with an obturator. Its location was judged by the flow of cerebrospinal fluid. All rats were allowed to recover from surgery for 7 days to minimize nonspecific stress responses. Rats from both drug groups were injected with 2 μM in 2 μl artificial cerebrospinal fluid (in mM: 133.3 NaCl, 3.4 KCl, 1.3 $CaCl_2$, 1.2 $MgCl_2$, 0.6 NaH_2PO_4, 32.0 $NaHCO_3$, and 3.4 glucose, pH 7.4 by 0.5 M hydrochloric acid) for three weeks, while rats from control group with 2 μl vehicle control. The injectors remained in place for an additional 3 min following the injections to assure complete drug delivery.

2.4. Blood sample handling and tissue collection

On the next day after the last i.c.v. injection half of rats in every group ($n = 8$) were anesthetized i.p. by 3% amobarbital sodium (50 mg/kg) dissolved in physiological saline. All animals were used to collected 1 ml of artery blood and 2 g cardiac muscle. The blood was centrifuged at 3500 r.p.m. for 10 min to obtain the plasma. Lastly, the plasma and cardiac muscle were stored at −80 °C. Once the above experiments were completed, each rat was humanely sacrificed under anesthesia, by infusion of amobarbital sodium and saturated potassium chloride.

2.5. Hyperinsulinemic euglycemic clamp tests

In the hyperglycemic clamp tests, other half of rats in every group ($n = 8$) were anesthetized and catheterized in the right carotid artery and left jugular vein after fasted 12 h as previously described [35]. The animals were infused with insulin at a constant rate of 2 mU/kg min into the jugular vein until the end of the test. And 10% glucose was infused at variable rates as needed to clamp glucose levels at 5 ± 0.5 mmol/L. The glucose infusion rates were calculated corresponding to the last 6 samplings at the clamp level. Once above experiments were completed, each rat was euthanized by infusion of amobarbital sodium and saturated potassium chloride. Their brains were checked to confirm the correct implantation of the cannulas.

2.6. Blood RBP4 and insulin assay

RBP4 was analyzed by an enzyme-linked immunosorbent assay (Uscn Life Sci., Inc. Wuhan, China). Insulin was analyzed by a radioimmunoassay. According to the manufacturer's specification, all measurements were performed in duplicate, and the mean of the two measurements was considered.

2.7. RT-PCR analysis

To determine the GLUT4 mRNA level, the total RNA from 1 g of the frozen cardiac muscle was isolated by Trizol according to the manufacturer's instructions. The concentration of the RNA was calculated by spectrophotometric assays of