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Original Article

Eugenosedin-A improves glucose metabolism and inhibits MAPKs expression in streptozotocin/nicotinamide-induced diabetic rats

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Abstract This study examined the effects of eugenosedin-A (Eu-A) in a streptozotocin (STZ)/nicotinamide-induced rat model of type II diabetes mellitus (T2DM). Six-week-old Sprague–Dawley rats were randomly divided into three groups: (1) RD group, normal rats fed a regular diet (RD), (2) DM group, T2DM rats fed a high-fat diet, and (3) Eu-A group, T2DM rats fed a high fat diet plus oral Eu-A (5 mg/kg/day). After 30 days, the DM group had higher body weight, higher blood glucose and lower insulin levels than the RD group. The DM group also had increased protein expression of glycogen synthase kinase (GSK) in liver and skeletal muscle and decreased protein expression of insulin receptor (IR), insulin receptor substrate-1 (IRS-1), IRS-2, AMP-activated protein kinase (AMPK), glucose transporter-4 (GLUT-4), glucokinase (GCK), and peroxisome proliferator-activated receptor γ (PPAR-γ). STZ/nicotinamide-induced T2DM increased the expression of mitogen-activated protein kinases (MAPKs: p38, ERK, JNK) and inflammatory p65 protein. In the Eu-A treated T2DM rats, however, blood

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glucose was attenuated and the insulin concentration stimulated. Changes in IR, IRS-1 and IRS-2 proteins as well as AMPK, GLUT-4, GCK, GSK, PPAR-γ, MAPKs, and inflammatory p65 proteins were ameliorated. These results suggested that Eu-A alleviates STZ/nicotinamide-induced hyperglycemia by improving insulin levels and glucose metabolism, and inhibiting the MAPKs- and p65-mediated inflammatory pathway.

Introduction

Type II diabetes mellitus (T2DM) has high morbidity and mortality associated with myocardial and cerebral infarctions [1]. In the early stage of T2DM, body weight and insulin secretion are normal, but peripheral tissues such as liver, muscle, and adipose tissues are not sensitive to insulin [2]. When insulin sensitive cells decreased, the pancreas secretes more insulin due to the continued hyperglycemia, resulting in hyperinsulinemia. In the advanced stage, insulin production gradually becomes insufficient, leading to more serious hyperglycemia [3].

Long-term consumption of a high carbohydrate/high fat diet increases postprandial levels of blood glucose and insulin, leading to insulin resistance. Insulin resistance influences the number and function of insulin receptors [4], the enzyme activity of phosphatidylinositide 3-kinase (PI3K)/Akt [5], glucose uptake [6] and glucagon synthesis [7]. Diminished glucose uptake, insulin deficiency, and insulin resistance contribute greatly to the development of DM and related cardiovascular diseases [8,9].

Eugenosedin-A (Eu-A), synthesized by our laboratory, is a 5-$HT_{1B/2A}$ and $\alpha_1/\alpha_2/\beta_1$-adrenergic blocker that can decrease blood pressure and heart rate [10], diminish inflammation, scavenge free radicals [11], and inhibit platelet aggregation [12]. Eu-A reduced obesity-related hyperglycemia, hyperinsulinemia, hyperlipidemia [13], inflammation, and adhesion molecules [14–16]. Eu-A also inhibited high-fat diet (HFD)-induced vascular and endothelial dysfunction and oxidative stress by attenuating α_1-adrenoceptor/5-HT activity and NADPH oxidase [16]. Based on our findings, Eu-A could be used to control the HFD-induced metabolic syndrome, which is associated with an increased risk of developing T2DM.

In this study, we further investigated the effects of Eu-A on the prevention of T2DM in a streptozotocin (STZ)/nicotinamide-induced diabetic rat model [17] and sought to better understand its mechanisms of action. This study is the first to establish that Eu-A, a 5-$HT_{1B/2A}$ and $\alpha_1/\alpha_2/\beta_1$-adrenergic blocker, can stimulate secretion of insulin and increase insulin receptor protein expression, glucose transporters, and glucose metabolism enzymes.

Materials and methods

Animals

Male Sprague–Dawley (SD) rats were provided by the National Laboratory Animal Breeding and Research Center (Taipei, Taiwan) and housed under constant temperature and illumination environmental conditions (light between 7:30 and 19:30). Water and regular diet were available ad libitum. After an acclimatization period, the 6-week-old rats were randomly divided into 3 groups. The control group was fed a regular diet for 30 days (RD group; $n = 8$). In our animal model, experimental T2DM rats were induced by intraperitoneal (i.p.) STZ (35 mg/kg) 15 min after i.p. administration of nicotinamide (200 mg/kg) continuously for 2 days, and then fed HFD (DM group; $n = 8$) for 30 days [17]. Some of STZ/nicotinamide treated HFD rats were supplemented with Eu-A (5 mg/kg/day, oral gavage) for 30 days (Eu-A group; $n = 8$). Body weight, fasting blood glucose and serum insulin levels were measured in all groups. At the end of the study, we excised the liver, and skeletal muscle from all rats. Tissues were stored in buffer solution at −80 °C until analysis. This study was approved by the Animal Care and Use Committee of Kaohsiung Medical University. The HFD (cat No. 58G9, TestDiet, Richmond, VA) contained 60% fat, 21.4% carbohydrates and 18.6% protein.

Chemicals and drugs

Eu-A (synthesized in our laboratory) was solubilized in 5% absolute alcohol. STZ (Sigma–Aldrich, St Louis, MO) was dissolved in 50 mM citric acid buffer, and nicotinamide (Sigma–Aldrich, St Louis, MO) was dissolved in physiological saline.

Measurement of serum biochemical parameters

Blood samples were collected for glucose measurement using a HITACHI Clinical Analyzer 7070. Insulin levels were measured using an ELISA kit (Mercodia, Uppsala, Sweden).

Western blot analysis of liver tissue

The homogenized liver tissues were identified by insulin receptor (IR, 95 kDa), insulin receptor substrate-1 (IRS-1, 180 kDa), IRS-2 (185 kDa), AMP-activated protein kinase (AMPK, 63 kDa), glucose transporter-4 (GLUT-4, 50 kDa), glucokinase (GCK, 56 kDa), glycogen synthase kinase (GSK, 51 kDa), peroxisome proliferator-activated receptor γ (PPAR-γ, 54 kDa), p38 (38 kDa), ERK (42 kDa), JNK (46 kDa), and p65 (65 kDa) antibodies (Santa Cruz Biotechnology, Santa Cruz, CA; 1:500 dilution) and IgG conjugated antibody (Santa Cruz Biotechnology, Santa Cruz, CA; 1:10,000 dilution). The relative protein expression in each tissue was

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