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Original Research Article

The effects of apelin treatment on a rat model of type 2 diabetes

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ABSTRACT

Purpose: Apelin is an adipokine that plays a role in the regulation of many biological functions in mammals including the neuroendocrine, cardiovascular, immune systems, glucose homeostasis and obesity. It can act via autocrine, paracrine, endocrine, and exocrine signaling. We aimed to identify the role of apelin pathophysiology of diabetes.
Material/methods: 37 male Wistar Albino rats aged 8–10 weeks were divided in four experimental groups as: control group (C) control + apelin group (C + A), diabetic group (D) diabetic + apelin group (D + A). Apelin and apelin receptor mRNA gene expressions in heart and aorta tissue were determined by real-time polymerase chain reaction. The plasma levels of insulin and plasma apelin were determined by ELISA.
Results: Plasma levels of insulin, glucose, blood pressure levels were significantly lower in D + A group. There was no statistically significant difference for level of apelin between diabetic groups. On the other hand, differences for apelin and APJ mRNA expression in heart and vascular tissue were found significant between groups.
Conclusions: Apelin can be used as a therapeutic agent in the treatment of type II diabetes in the future.

1. Introduction

Type 2 diabetes mellitus (T2DM) is a metabolic disorder which is characterized by hyperglycemia in the context of insulin resistance and relative lack of insulin [1]. Insulin resistance, which is the inability of cells to respond adequately to normal levels of insulin, occurs primarily within the muscles, liver, and fat tissue [2]. In the liver, insulin normally suppresses glucose release. However, in the setting of insulin resistance, the liver inappropriately releases glucose into the blood [3]. T2DM prevalence and incidence is rapidly increasing worldwide. T2DM is a chronic disease leading to macro- and microvascular complications, which results in severe illness and premature death, with elevated personal and economic costs [4].

Apelin is a newly identified adipokine which derives from a 77 amino acids precursor. It has several active forms including apelin-12, apelin-13, apelin-17, apelin-19 and apelin-36. Among them, apelin-13 has the highest abundancy and activity [5]. Apelin exerts its function by binding and activating the angiotensin receptor related G protein-coupled receptor, APJ [6]. Apelin and APJ are widely expressed in various tissues, including adipose, brain, lung and kidney [7]. Increasing evidence suggests apelin is involved in the regulation of multiple physiological functions, including food intake, blood pressure and glucose utilization [8,9].

Apelin signaling may have an important role in the physiopathology of diseases such as hypertension, heart failure, cardiovascular disease, type 2 diabetes, and obesity, although their effects and functions are still unclear. The physiological effects of apelin on diabetes are not fully known. Apelin and APJ have not been studied in high fat diet alloxan induced type 2 diabetes model before. Therefore, this study aimed to investigate the possible alterations in blood pressure, plasma insulin, blood glucose level and in the renin–angiotensin system in response to apelin in type II diabetic and healthy rats.

2. Materials and methods

2.1. Animals and experimental conditions

Thirty-seven Wistar Albino 8–10-week-old male rats (180–300 g) were obtained from the Experimental Research Unit of the University

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of Pamukkale (Denizli, Turkey). They were maintained on a 12/12 h light–dark cycle under controlled temperature and humidity. The animals were fed standard rat chow and given water ad libitum. All protocols used in this study were approved by the Pamukkale University Ethics Committee on animal research (2010/026).

2.2. Experimental design

The male animals were selected randomly and divided into four experimental groups:

Control group (C) (n = 10) received a single dose of saline given intraperitoneally (i.p.) for 17 days. (II) Control + apelin group (C + A) (n = 10) was treated with pyroglutamylated apelin-13 (200 μg kg^{-1} day^{-1} i.p.) for 17 days [10]. (III) Diabetic group (D) (n = 9) was administered with high fat emulsion (10 mL/kg) for 10 days and injected 120 mg/kg alloxan (Sigma–Aldrich, St. Louis, MO, USA) i.p. at day 11 and day 12, respectively [11,12]. At day 17, the blood glucose levels were determined. The animals with blood glucose levels greater than 300 mg/dL were accepted for inclusion as diabetic. (IV) Diabetic + apelin group (D + A) (n = 8) was given i.p. pyroglutamylated apelin-13 (200 μg kg^{-1} day^{-1}) to diabetic animals for 17 days [10].

2.3. Blood glucose and blood pressure measurement

Blood glucose and blood pressure levels were measured before starting the study and at the end of the experiment. Blood samples were collected from the tail of each animals after 12 h fasting. The tail was embedded in alcohol and about 1 mm of its end was cut and a drop of blood was used for the blood glucose test with the help of a glucometer (Plusmed Co., Izmir, Turkey). Systolic blood pressures were measured from rats as described previously [13].

2.4. Blood samples and measurements

At the end of the experimental period, all the animals were anesthetized with ketamin/xylazine HCl (75 mg/kg/10 mg/kg intraperitonealy). Blood samples were collected in heparinized tubes. After centrifugation, plasma samples were stored at −80 °C until analysis. The plasma ACE2, angiotension II, angiotensinogen, endothelin-1, apelin and insulin concentrations were measured by an enzyme-linked immunosorbent assay (ELISA) method using an ultra sensitive rat ELISA kit (Diagnostic Product Corporation, Los Angeles, CA, USA) in a multiplate ELISA reader (das, Digital and Analog Systems, Vimercate, MI, Italy). Homeostatic model assessment (HOMA-IR) score was calculated using fasting plasma insulin and fasting blood glucose concentrations measured at the end of the experimental period according to the following formula: HOMA-IR [(Fasting plasma insulin in U/L × fasting blood glucose in mmol/L)/22.5] [14,15].

2.5. Isolation of total RNA and synthesis of complementary DNA

Heart and aorta tissues of each rat were carefully cleaned of fat and connective tissue and were excised and weighed. The atrium was removed from the heart, and the right and the left ventricles were separated and weighed. Heart and left ventricle weights were normalized to body weight (left ventricle weight/body weight ratio).

Total RNA was isolated from fresh heart and aorta tissues using RNeasy kits (RNeasy Mini kit, Cat No. 74104, Qiagen, Germany) according to the manufacturer's instruction. The quantity and quality of RNA sample were determined with the spectrophotometer method (Biophotometer, Eppendorf, Hamburg, Germany). "Concentration, A260, A280, A260/280" values of the samples were recorded, and "stock concentrations" of each sample were calculated. Complementary DNAs (cDNA) were synthesized by QuantiTect Reverse Transcription Kit (Qiagen). Reverse transcription was carried out 42 °C for 15 min followed by incubation at 95 °C for 3 min. cDNAs were stored at −20 °C until used in the real-time polymerase chain reaction (RT-PCR).

2.6. Quantitative real-time reverse transcriptase-polymerase chain reaction

Relative quantitative analysis of target gene (APJ and apelin) and an internal reference gene (β-actin) was done using the RT-PCR system (Light-Cycler 480, Roche, Berlin, Germany). Primers and probes "Universal Probe Library (UPL) (Roche)" were designed for target gene. "Mouse β-actin Single Assay" (Assay ID: 500152, Roche) was used including both primers and original probes for the reference gene (Table 1). Final reaction volume for the analysis of apelin and APJ gene expression was performed in 20 μL volume; 0.5 μL from each primer (final concentration: 0.5 μM), 0.2 μL probe (final concentration: 0.2 μM), 10 μL of LightCycler probes Master Mix (Roche), 4 μL cDNA sample, and 4.8 μL PCR-grade water. The cycling conditions were 95 °C for 10 min, followed by 45 cycles at 95 °C for 10 s, 60 °C for 30 s, and 72 °C for 1 s. At the end of the cycles, a cooling step at 40 °C for 30 s was performed for each reaction. All runs included one negative-templated control consisting of PCR-grade water instead of cDNA. The RT-PCR phases of the samples were completed with optimized protocols, and relative quantitative expression levels of samples were determined. Both target gene and reference gene expression levels were performed with LightCycler Relative Quantitative software (version 3).

2.7. Statistical analysis

Statistical analysis was done with SPSS (Statistical Package for Social Sciences, Chicago, IL, USA) 10.0 pocket program. The results were expressed as means ± standard error (SE). "Kruskal–Wallis variance analysis" and "Mann–Whitney U test" were used for statistics, with p values ≤0.05 accepted as statistically significant.

3. Results

3.1. Blood pressure, blood glucose, percentage of body weight and heart weights

Significant differences were observed in the levels of percentage of body weight [(final body weight/initial body weight) × 100] among the groups, p = 0.000. The level of percentage of body weight in the D, D + A groups were observed to be significantly lower than in C and C + A (p = 0.000, p = 0.001, p = 0.001 and 0.003) (Table 2).

Table 1
Primers and probes used in analysis of expression APJ, apelin and β-actin mRNA (5′→3′).

APJ	
Primers	5′-CACCAAGACCCAGTGCTACAT-3′ (forward)
	5′-AGGCCCACTCTGAGTTTGAA-3′ (reverse)
UPL No.	Probe No.: #49 (04 688 104 001)
Apelin	
Primers	5′-CTATGTTGACTGGGCCCTTG-3′ (forward)
	5′-CCACTCTCCTTCCTTTCTTCG-3′ (reverse)
UPL No.	Probe No.: #58 (04 688 554 001)
β-Actin	
Single assay	ID: 500 152 (Cat. REF. 05 532 957 001)