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Apelin/APJ expression in the heart and kidneys of hypertensive rats

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ABSTRACT

Hypertension is an important health problem that is manifested by systemic arterial blood pressure being permanently elevated and leading to serious complications. Hypertension is the basis for coronary heart diseases, heart failure, kidney damage, cerebrovascular diseases. Due to ethical concerns, there is no detailed study of the mechanism, side effects and treatment of hypertension in humans. For this reason, specific studies related to the organ of hypertension are performed in experimental animals. The heart and kidney tissue, which are the most important organs that hypertension has damaged, have formed specific organs of our work.

In our experimental study, a total of 35 (hypertensive group: 20, control group: 15) *Rattus Norvegicus* Wistar albino rats were used. In order to obtain our hypertension model, our experimental animals were given L-NAME together with drinking water for six weeks. After six weeks, the experimental procedures were terminated. Heart and kidney tissues of the hypertensive and control group were obtained. Expression of apelin and apelin receptor (APJ) was demonstrated by immunohistochemical and Western Blot protocols.

Hypertrophic cardiac atrium of the hearts of the large cavities, interventricular septum and myocardium to the disintegration, as well as an increase in the diameter of the coronary artery has been observed. In general, kidney tissues of the hypertensive group showed narrowing in cortical renal structures and enlargement in structures in the renal medulla.

As a result, in hypertensive cases, there was an increase in expression of Apelin and APJ receptor in heart tissue, and a decrease in expression of Apelin and APJ receptor in kidney tissue. We think that our findings may contribute to experimental or clinical studies related to hypertension and apelin.

1. Introduction

O'Dowd et al. identified a gene very similar to angiotensin type-1 receptor in 1993 (O'Dowd et al., 1993). The specific selective ligand of this receptor called APJ receptor was determined by Tatemoto et al. in 1998 as apelin (Tatemoto et al., 1998). The high expression of apelin and APJ takes place in the central nervous system and peripheral tissues, which undertake significant functions in the cardiovascular system, such as decreasing blood pressure, regulating the cardiovascular tone., renal diseases and renin-angiotensin system (Chun et al., 2008; Ishida et al., 2004). It is increasingly evident that the cardiovascular system constitutes apelin's major target. Apelin and APJR are provided at a high level by the vascular smooth muscle, cardiac myocytes, and endothelial cells (Kawamata et al., 2001). It has been demonstrated that the nitric oxide release from endothelial cells is caused by apelin and therefore cardiac contractility is increased, and vascular tone is decreased (Szokodi et al., 2002; Tatemoto et al., 2001). Apelin is found in the myocardium and left ventricle, and an increase in the apelin mRNA levels is observed in case of the chronic heart failure (Kleinz and Davenport, 2005). The relationship of apelin/APJ system with hypertension has been currently examined in a number of studies (Yu et al., 2014).

At present, it is clearly demonstrated that hypertension represents a cardiovascular disease with a progressive course originating from complex and interdependent etiologies (Glasser et al., 2011). Since kidneys play a significant role in the blood pressure regulation in the long period of time, renal diseases constitute the major reasons for hypertension causing the damage of different organs, for example, heart failure (Badyal et al., 2003). Although renal and cardiovascular diseases are mutually related, fatal and highly epidemic pathologies, the renal apelinergic system and the role of apelin in the renal metabolism have not been investigated in detail. Malyszko et al. found the apelin-36 level in dialysis patients to be low (Małyszko et al., 2006). Although apelin levels were low in these patients, coronary artery disease was observed. Then, the same research team indicated that the apelin level decreased significantly in patients diagnosed with coronary artery disease and

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who underwent a renal transplant. It was anticipated that this decrease in the apelin level might be due to the presence of coronary artery disease, endothelial damage or inflammation (Malyszko et al., 2008). The studies conducted are mostly related to the apelin level in plasma. It is stated that the apelin level in plasma decreases in hypertensive cases (Sonmez et al., 2010). However, this is a general result for the organism, and there is very little specific information about the organs. Thus, in our study, we aimed to determine the apelin and apelin receptor expression with the immunohistochemical examination and Western Blot method specifically in the cardiac and renal tissue in hypertensive rats with L-NAME.

2. Material and methods

2.1. Animals

The Experimental Animal Unit of Akdeniz University, Faculty of Medicine, provided 35 adult male Wistar rats (Rattus norvegicus) in total to be used in the present study. 3–4-month-old male Wistar rats 250–300 g in weight were utilized in the control (n = 15, got normal drinking water in the course of the study) and experimental groups (n = 20, got L-NAME in drinking water in the course of the study).

Approval for the experimental protocols was received from the Animal Care and Usage Committee of Akdeniz University, and the above-mentioned protocols were consistent with the declaration of Helsinki and International Association for the study of pain guidelines. The experimental protocol was approved by the animal care and use committee of Akdeniz University (approval number: 06.06.2011/97).

2.2. L-NAME induced hypertension in rats

During the first two weeks of the current study, it was ensured that rats followed the standard feed and water consumption specified for them on a daily basis. On the 15th day from the beginning of the study, induction of experimental hypertension was performed by administering L-NAME (25 mg/kg/day), which is a non-selective NOS inhibitor, in the drinking water of rats for the 6 week period. The calculation of the L-NAME concentration in the drinking water was performed based on the rats' body weights and the volumes of water they consumed, and newly prepared every two days.

2.3. Measurement of the blood pressure

The tail-cuff method was employed for the non-invasive measurement of Systolic Blood Pressure (SBP) and Diastolic Blood Pressure (DBP) of all the examined rats included in the experimental groups, and it was regarded that rats of which mean blood pressure was higher than 140/90 mm Hg were hypertensive. The loop-shaped pressure probe, which was fixed on the tail, was used for the collection of signals, and they were transferred to the computer environment. After the basal values of each animal had been determined, the control of blood pressure was performed every 2 weeks. During the measurements performed on a periodic basis, minimum five pressure traces were recorded for every rat, and the mean of the above-mentioned three values determined was used for the calculation of blood pressure.

2.4. Tissue processing

Rats in the control and experimental groups were killed under deep anesthesia at the end of the sixth week of the study. The animals were anesthetized with a single intraperitoneal (i.p.) injection of xylazine–-ketamine (10 and 90 mg/kg, respectively, Alfasan International B.V., Woerden, Holland). The opening of the abdomens was performed by midline incision, and samples of the heart and kidney tissue were obtained, and their processing was carried out. Following the removal, the vertical division of the heart and kidney samples into two parts was performed. The routine processing of one part was carried out for the immune-localization of apelin, apelin receptor (APJR). The other part was placed in liquid nitrogen for its following utilization for the Western blotting of apelin. For the purpose of the immunohistochemical examination, processing of the tissue samples was performed for their routine submerging in paraffin wax. Fixation of the heart and kidney tissue samples was performed by immersion in 4% buffered formaldehyde (100 ml of 37% formalin, 900 ml of distilled water, pH, 6.8) at room temperature for the period of approximately 24 h. Tap water was used for the washing of the tissues for the period of 4 h. Afterwards, dehydration of the tissues was carried out using a graded series of ethanol, and the transverse sections submerged in paraffin wax were cut on the microtome.

2.5. Semi-quantitative evaluations

The distributions of positively immunoreactive cells in control and hypertensive groups were determined semi-quantitatively [–: negative, +: positive, ++: strong positive, +++: very strong positive.]

2.6. Immunohistochemistry

Formalin-fixed samples submerged in paraffin were cut into 5 µm pieces and put on poly-L-lysine-coated slides. Following the process of deparaffinization, slides were boiled in citrate buffer (pH 6.0) for the period of 10 min to retrieve antigen and left for cooling for the period of 20 min at room temperature. Afterwards, pieces were submerged in 3% hydrogen per-oxide for the period of 20 min in order to prevent endogenous peroxidase. Incubation of the slides was performed in a humidified chamber by using an UltraV block (Lab-vision, Fremont, CA, USA) for the period of 7 min at room temperature. The excess serum was removed, and the incubation of the sections was performed using primary antibodies apelin (NBP1-07130; Novus Biologicals, Littleton, CO,USA) rabbit polyclonal antibody at 1:150 dilution; apelin receptor (APJR) (bs-2430R; Bioss, Woburn, MA, USA) rabbit polyclonal antibody at 1:150 dilution for a night in a humidified chamber. The primary antibody was substituted with the suitable serum or non-immune IgG at the identical dilutions with the specific antibodies, and therefore, negative controls were carried out. Phosphate buffered saline (PBS) was used to wash the pieces three times for the period of 5 min and following this, their incubation was performed using biotinylated secondary antibody (HRPLSAB-2 system, K0609; DakoCytomation, Glostrup, Denmark) for the period of 30 min and afterwards using peroxidase labeled streptavidin (HRP LSAB-2 system, K0609; DakoCytomation, Glostrup, Denmark) for the period of 30 min. Following this, PBS was used to wash the slides, and visualization of peroxidase activity was accomplished by using 3,3_-diaminobenzidine (DAB) (Sigma–Aldrich, St.Louis, MO, USA; D4418) for the period of 3–5 min. Hematoxylin was utilized for the counterstaining of the pieces. Their dehydration was carried out, and they were placed in Kaiser's glycerin gelatin (OB514196; Merck, Whitehouse Station, NJ, USA) and investigated using Zeiss Axioplan 100 light microscope (Zeiss, Oberkochen, Germany), and their photographs were taken.

2.7. Sodium dodecyl sulfate polyacrylamide gel electrophoresis and western blotting

Protein extraction and immunoblot analysis were performed as described previously (Acar et al., 2012). Tissue samples for each group were weighed and put into a homogenization buffer supplemented with Complete protease inhibitor cocktail (Boehringer, Mannheim, Germany). After homogenization, samples were centrifuged at 10,000*g* for 10 min. Supernatants were collected and stored at −70 °C. The protein concentration was determined by Lowry assay (Lowry et al., 1951) and 50 mg of protein was applied per lane. Samples were subjected to SDS polyacrylamide gel electrophoresis (30% acrylamide in 7.5% gel) at