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

Rahime Sekerci[a], Nuray Acar[b], Filiz Tepekoy[c], Ismail Ustunel[b], Nigar Keles-Celik[a,*]

[a] Department of Anatomy, Akdeniz University, Faculty of Medicine Antalya, Turkey
[b] Department of Histology and Embryology, Akdeniz University, Faculty of Medicine, Antalya, Turkey
[c] Department of Histology and Embryology, Istinye University, Faculty of Medicine, Istanbul, Turkey

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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

* Corresponding author at: Department of Anatomy, Akdeniz University Faculty of Medicine, 07070 Antalya-Turkey.
E-mail address: nigarc@akdeniz.edu.tr (N. Keles-Celik).

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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

100 V for approximately 1.5 h and were then transferred onto nitrocellulose membranes (Pharmacia, Kirkland, Quebec, Canada) in a buffer containing 0.2 mol/L glycine, 25 mmol/L Tris and 20% methanol overnight, at +4 °C, under 32 V and 65 A. The membranes were blocked for 1 h with 5% non-fat dry milk (BioRad Laboratories, Hercules, CA, USA) and 0.1% Tween 20 (Sigma-Aldrich, St Louis, MO, USA) in 0.14 mol/L Tris-buffered saline (TBS; pH 7.2–7.4) at 4 °C. Membranes were incubated with a 1:200 dilution of rabbit polyclonal anti-apelin antibody (#sc33805, Santa Cruz Biotechnology, Santa Cruz, CA, USA), 1:200 dilution of rabbit polyclonal anti-apelin receptor antibody (#2430, Bioss, Woburn, MA, USA) overnight. After the washing steps, the membranes were further incubated with peroxidase goat anti-rabbit IgG antibody (Vector Laboratories, PI-1000, CA, USA), diluted 1:2000, for 1 h at room temperature. Immunolabeling was visualized by using the chemiluminescence-based SuperSignal CL HRP Substrate System (Pierce, Rockford, IL, USA) prepared according to the manufacturer's instructions, and the membranes were exposed to Hyperfilm (Amersham, Bucks, UK; 28906837). Membranes were also labeled by identical protocol for binding of a 1:6000 dilution of an anti-mouse b-actin antibody (Abcam, Cambridge, UK; ab 6276-100) as an internal control to confirm the equal loading of the samples. The bands were quantified using NIH image analysis software (Image J Version 1.36b, National Institutes of Health, USA).

2.8. Statistical analysis

Apelin and apelin receptor Western blotting band values of control and hypertensive group heart and kidneys were done by One-Way Anova tests followed by *post-hoc* Tukey tests. Probability values of less than 0.05 were considered significant. All statistical analyses were performed using SPSS (statistical package for the social sciences 10.0 for Windows, Chicago, IL, USA).

3. Results

3.1. Body weight results

The body weights of the control and hypertension groups were 238 ± 6.54 g and 242.9 ± 9,64 g respectively, at the end of the experiment. There wasn't a statistically significant difference between the groups.

3.2. Blood pressure results

Systolic blood pressure values measured 4 times at 2 week intervals throughout the 6 weeks experimental period are shown in Fig. 1. Basal blood pressure values were not different between groups. The values in the hypertension group were higher than the control group from the second week. While there was no statistical significance in the first measurements of the control group and the hypertension group ($p = 0.899$), there was a statistically significant difference between the second, third and fourth measurements $p < 0.05$, $p < 0.05$, $p < 0.05$, respectively (Fig. 1).

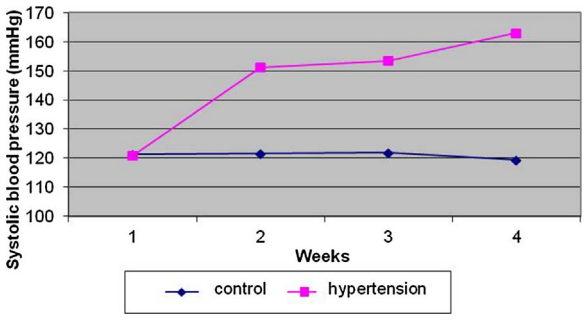


Fig. 1. Systolic blood pressure values of control and hypertension groups measured 4 times at 2 week intervals throughout the 6 weeks experimental period.

3.3. Histological evaluation of control and hypertensive hearts

Control and hypertension group heart sections stained with haematoxylin-eosin staining were evaluated. There were morphological differences between the control and the hypertension groups. There were wider gaps in the atriums of hypertension group compared to the control group (Fig. 2). Interventricular septum, myocardium and coronary artery width were wider in the hypertension group compared to the control group (Fig. 2).

3.4. Histological evaluation of control and hypertensive kidneys

Control and hypertensive group kidney sections stained with haematoxylin-eosin staining were evaluated. There were morphological differences between the control and the hypertension groups. Juxtaglomerular apparatus, proximal and distal tubules were shrunk in hypertension group compared to the control group (Fig. 3). Lumens of collecting ducts and Henle's loop were wider in hypertension group compared to the control group (Fig. 3).

3.5. Immunohistochemical findings

3.5.1. Apelin immunolocalization in control and hypertension group hearts

The distribution pattern of apelin immunolabeling intensity in the hearts of control and hypertension groups is summarized in Table 1.

There was no apelin expression in atria of control and hypertension group hearts. Apelin expression was strong positive and very strong positive in epicardium of control group and hypertension groups, respectively. Similarly apelin expression was strong positive and very strong positive in myocardium of control group and hypertension groups, respectively. And there was strong positive and very strong positive apelin expression in endocardium of control group and hypertension groups, respectively (Fig. 4).

3.5.2. Apelin immunolocalization in control and hypertension group kidneys

The distribution pattern of apelin immunolabeling intensity in the kidneys of control and hypertension groups is summarized in Table 1. Apelin expression was very strong positive and strong positive in visceral and parietal layers of Bowman's capsule in control and hypertension groups, respectively. There was very strong and strong positive apelin expressions on peritubuler capillaries in control and hypertension groups, respectively.

While there was very strong positive apelin expression in collecting ducts of control group, collecting ducts of hypertension group had strong positive expression. Apelin expression in medullary capillaries was very strong in control group and strong positive in hypertension group (Fig. 5).

3.5.3. Apelin receptor immunolocalization in control and hypertension group hearts

The distribution pattern of apelin receptor immunolabeling intensity in the hearts of control and hypertension groups is summarized in Table 1. Apelin receptor expression was strong positive and very strong positive in control group and hypertension atriums, respectively. Myocytes in epicardium, myocardium and endocardium layers had strong positive and very strong positive apelin receptor expression in control and hypertension groups, respectively (Fig. 6).

3.5.4. Apelin receptor immunolocalization in control and hypertension group kidneys

The distribution pattern of apelin receptor immunolabeling intensity in the kidneys of control and hypertension groups is summarized

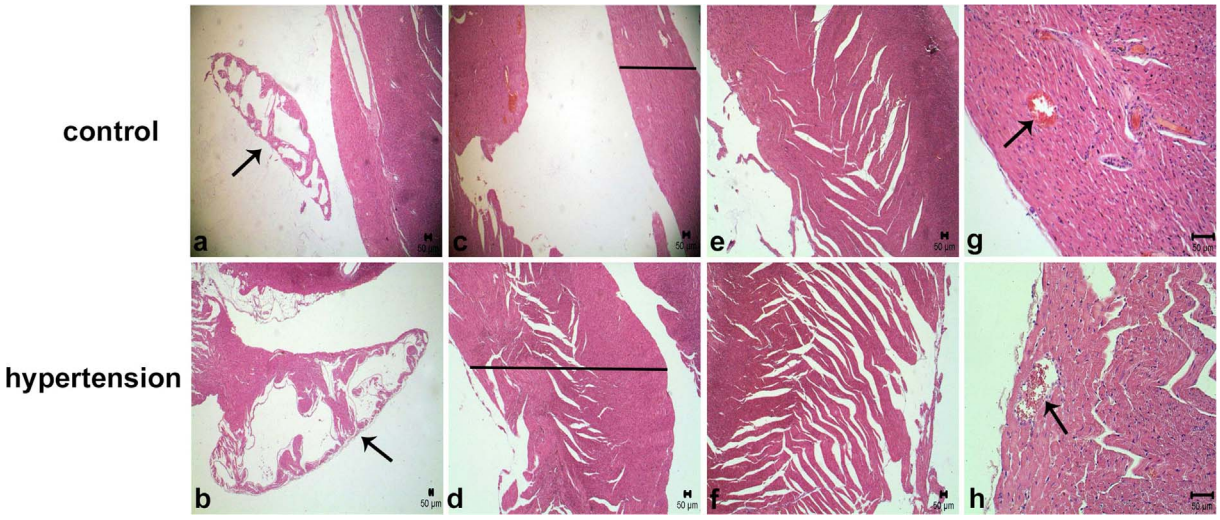


Fig. 2. Hematoxylin & eosin staining of control and hypertension group heart sections. a) control group atrium and b) hypertension group atrium are shown by arrows. c) control group interventricular septum and d) hypertension group interventricular septum are lined. e) control group myocardium and f) hypertension group myocardium. g) control group coronary artery and h) hypertension group coronary artery are shown by arrows. Scala bars: 50 µm.

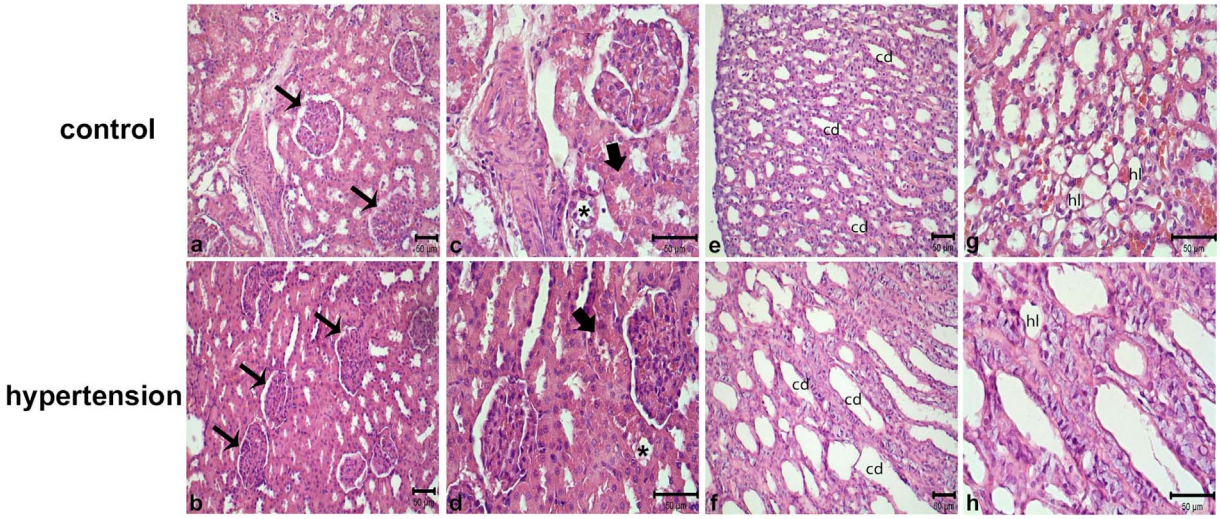


Fig. 3. Hematoxylin & eosin staining of control and hypertension group kidney sections. a) control group juxtaglomerular apparatus and b) hypertension group juxtaglomerular apparatus are shown by arrows. c) control group distal tubule (shown by asteriks) and proximal tubule (shown by arrow) d) hypertension group distal tubule (shown by asteriks) and proximal tubule (shown by arrow). e) control group collecting duct (written cd) and f) hypertension group collecting duct (written cd). g) control group Henle's loop (written hl) and h) hypertension group Henle's loop (written hl). Scala bars: 50 µm.

Table 1
Semi-quantitative scoring of apelin and apelin receptor immunolabeling intensities in the hearts and kidneys of control and hypertension groups. –: negative, +: positive, ++: strong positive, +++: very strong positive (EP;Epicardium, MY;Myocardium, EN;Endocardium, JA;Juxtaglomerular apparatus, DT; Distal tubule, CT; Collecting tubule, HL; Henle loop).

Tissue	Apelin		APJ	
	Control group	Hypertension group	Control group	Hypertension group
Heart atrium	–	–	++	+++
Heart ventricule (EP, MY, EN)	++	+++	++	+++
Kidney JA	+++	++	–	–
Kidney DT,CT,HL	+++	++	++	+

in Table 1. There were no apelin receptor expression in Bowman's capsule and glomerulus in control and hypertension groups. Apelin receptor expression in distal tubules were strong positive and positive in control and hypertension groups, respectively. Collecting duct and Henle's loop of control group kidneys had strong positive apelin receptor expression on the other hand collecting ducts and Henle's loop of hypertension group kidneys had positive apelin receptor expression (Fig. 7).

3.6. Western blotting findings

3.6.1. Apelin western blotting in control and hypertension group hearts

According to our Western blotting results apelin expression in heart was higher in hypertension group compared to the control group with a statistically significant difference ($p < 0.001$). Apelin expressions in heart were 0,890 ± 0,00578 and 1163 ± 0,0233 in control and hypertension groups, respectively (Fig. 8).

3.6.2. Apelin western blotting in control and hypertension group kidneys

Our Western blotting results showed that apelin expression in kidney was lower in hypertension group compared to the control group with a statistically significant difference ($p < 0.001$). Apelin

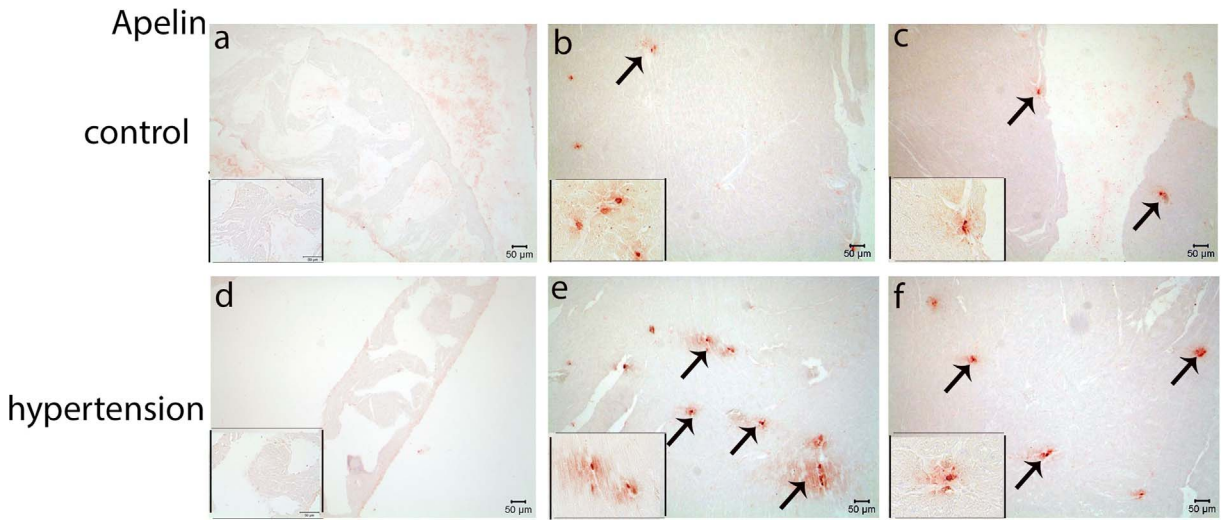


Fig. 4. Apelin immunohistochemistry in control and hypertension group heart sections. a) control group atrium, inset: larger magnification of atrium. b) control group myocardium inset: larger magnification of myocardium. c) control group endocardium inset: larger magnification of endocardium. d) hypertension group atrium, inset: larger magnification of atrium. e) hypertension group myocardium inset: larger magnification of myocardium. f) hypertension group endocardium inset: larger magnification of endocardium. Scala bars: 50 µm.

expressions in kidney were 10,592 ± 0,0075 and 8479 ± 0,0089 in control and hypertension groups, respectively (Fig. 9).

3.6.3. Apelin receptor western blotting in control and hypertension group hearts

According to our Western blotting results apelin receptor expression in heart was higher in hypertension group compared to the control group. But there wasn't a statistically significant difference ($p = 0.857$). Apelin receptor expressions in heart were 0,273 ± 0,00218 and 0,287 ± 0,0183 in control and hypertension groups, respectively (Fig. 10).

3.6.4. Apelin receptor western blotting in control and hypertension group kidneys

Our Western blotting results showed that apelin receptor expression in kidney was lower in hypertension group compared to the control group with a statistically significant difference ($p < 0.011$). Apelin receptor expressions in kidney were 2401 ± 0.290 and 0,913 ± 0,0714 in control and hypertension groups, respectively (Fig. 11).

4. Discussion

In this study, it was aimed to determine the apelin and APJR expressions in the heart and kidney samples of rats, which were administered with L-NAME for the purpose of making them hypertensive for the period of 4 weeks. The hypotensive effect of apelin is caused by endothelium-derived nitric oxide. In addition, apelin cause the increase of the plasma levels of nitric oxide metabolites. In mouse endothelial cell cultures, apelin was found to enhance the phosphorylation of endothelial-derived nitric oxide synthase enzyme. However, while apelin cause nitric oxide dependent vasodilatation in undamaged endothelium, it cause vasoconstriction in damaged endothelium via smooth muscle cells. Apelin shows positive inotropic effect in the heart. It is effective in vasodilatation (on undamaged endothelium), vasoconstriction (damaged endothelium), nitric oxide release, and angiogenesis in the vessels. It is known that there is a relationship between the increase in peripheral arterial resistance and nitric oxide (NO) in the pathophysiology of hypertension (Dunbar, 1992; Fregly, 1984). It was identified that peripheral arterial resistance increased and thus systemic hypertension occurred with the chronic inhibition of nitric oxide synthetase (NOS) enzyme (Fregly, 1984; Tipton et al., 1977). For the first time in 1992, two different research groups reported that

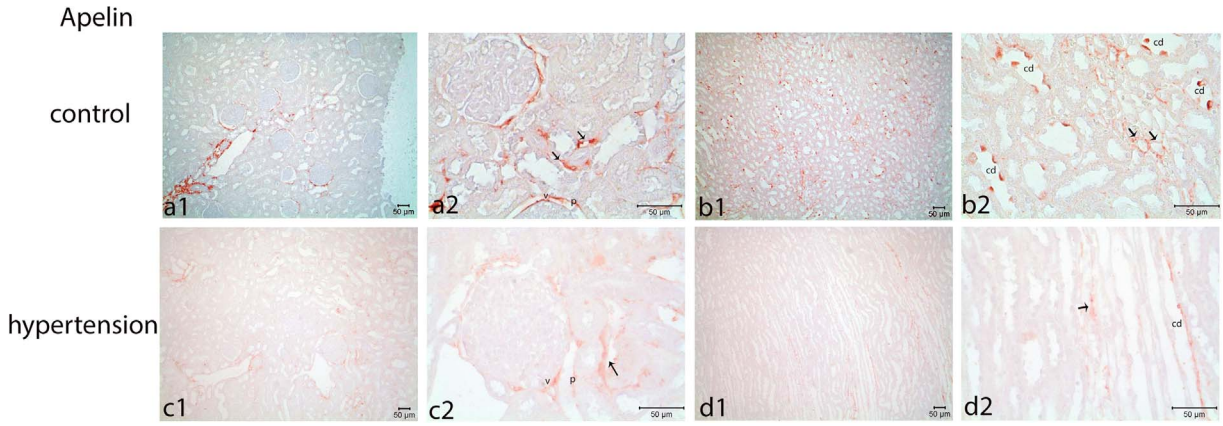


Fig. 5. Apelin immunohistochemistry in control and hypertension group kidney sections. a1) control group cortex a2) larger magnification of control group cortex, peritubuler capillaries are shown by arrows, v: visceral layer of Bowman's capsule, p: parietal layer of Bowman's capsule. b1) control group medulla a2) larger magnification of control group medulla, medullar capillaries are shown by arrows, cd: collecting duct. c1) hypertension group cortex c2) larger magnification of hypertension group cortex, peritubuler capillaries are shown by arrows, v: visceral layer of Bowman's capsule, p: parietal layer of Bowman's capsule. d1) hypertension group medulla d2) larger magnification of hypertension group medulla, medullar capillary is shown by arrow, cd: collecting duct.

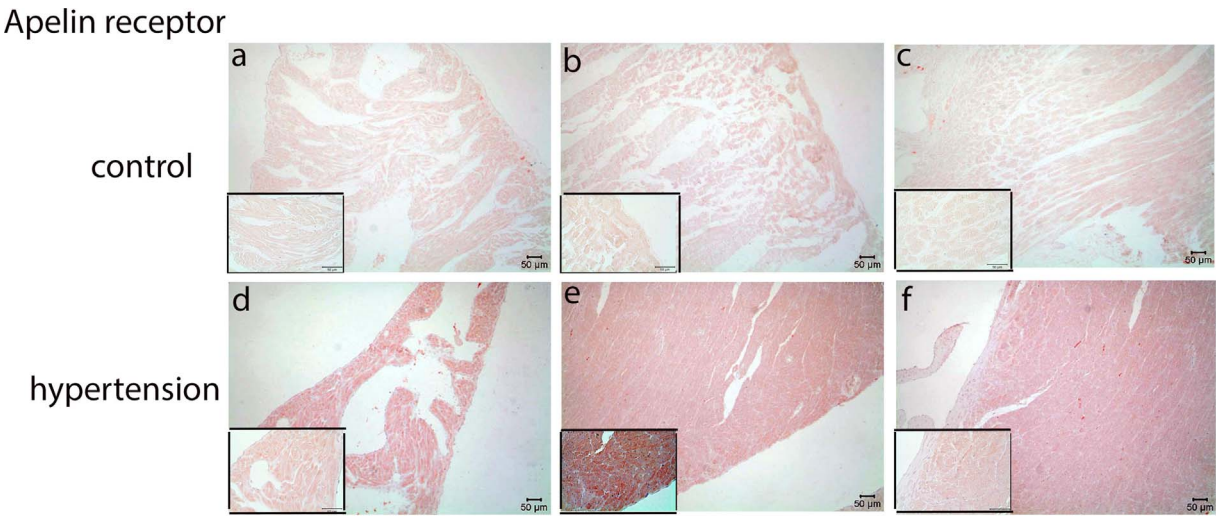


Fig. 6. Apelin receptor immunohistochemistry in control and hypertension group heart sections. a) control group atrium, inset: larger magnification of atrium. b) control group epicardium inset: larger magnification of epicardium. c) control group endocardium inset: larger magnification of endocardium. d) hypertension group atrium, inset: larger magnification of atrium. e) hypertension group epicardium inset: larger magnification of epicardium. f) hypertension group endocardium inset: larger magnification of endocardium. Scala bars: 50 µm.

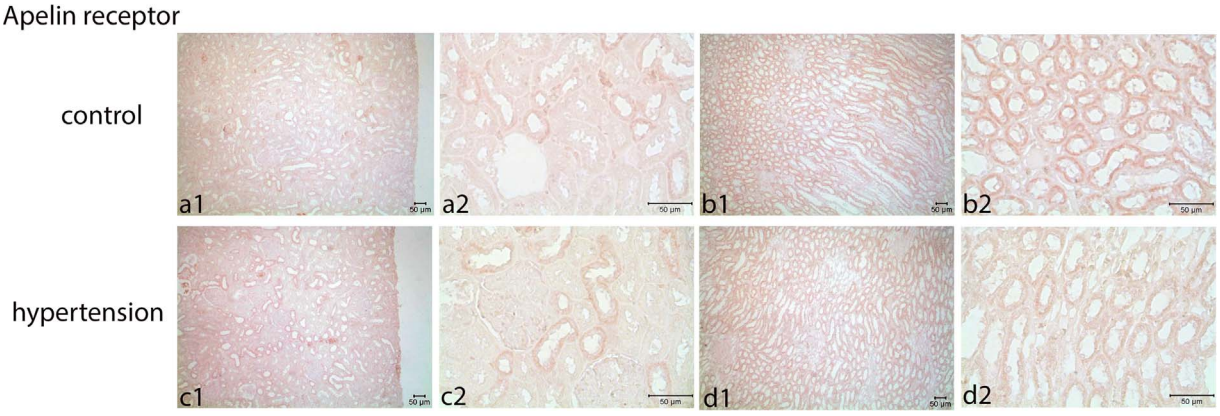
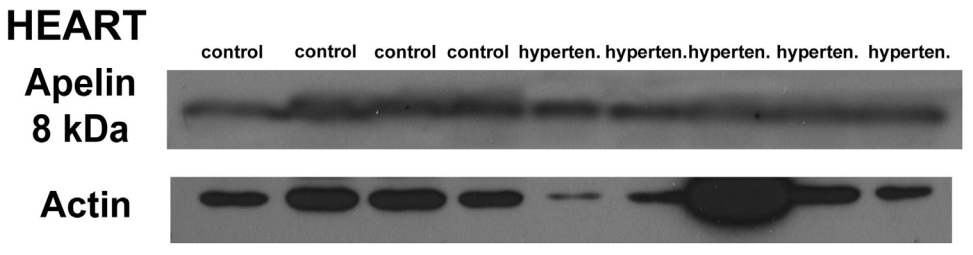


Fig. 7. Apelin receptor immunohistochemistry in control and hypertension group kidney sections. a1) control group cortex a2) larger magnification of control group cortex. b1) control group medulla a2) larger magnification of control group medulla. c1) hypertension group cortex c2) larger magnification of hypertension group cortex. d1) hypertension group medulla d2) larger magnification of hypertension group medulla.



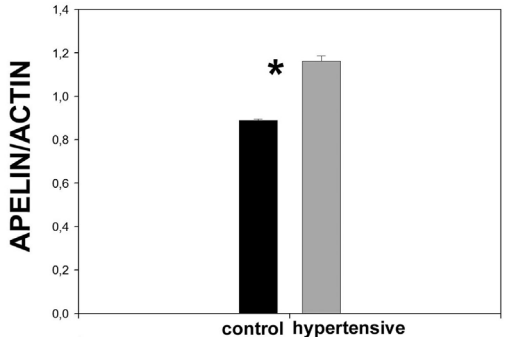


Fig. 8. a) Western blotting results of apelin in control and hypertension hearts. Beta-actin was used as an internal control. b) Quantitative analysis of apelin Western blotting data. The values in the bar graphs were obtained by dividing the protein intensity value by its corresponding beta-actin intensity value. The differences seen between control and hypertension group hearts were statistically significant (*). The image is representative of three independent experiments.

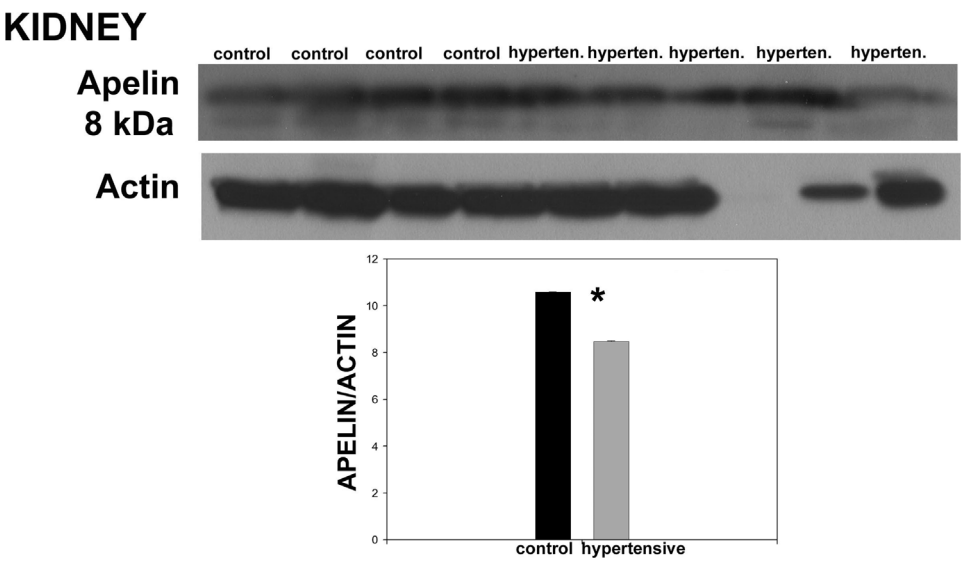


Fig. 9. a) Western blotting results of apelin in control and hypertension kidneys. Beta-actin was used as an internal control. b) Quantitative analysis of apelin Western blotting data. The values in the bar graphs were obtained by dividing the protein intensity value by its corresponding beta-actin intensity value. The differences seen between control and hypertension group kidneys were statistically significant (*). The image is representative of three independent experiments.

chronic NOS inhibition could be used as a new arterial hypertension model. This finding is consistent with the data that NO is necessary for the long-term regulation of blood pressure. In addition to the fact that NOS inhibitor administered to rats in different doses causes hypertension, high doses cause more severe hypertension and renal damage (Roberto and Baylis, 1998). The first NOS inhibitor used to induce hypertension in rats is L-NAME, which is an L-arginine analog. The fact that L-NAME dissolves in water and can be easily given to animals with drinking water has led to the widespread use of this model in recent years (Gardiner et al., 1992; Roberto and Baylis, 1998). We also added L-NAME concentration to drinking water to ensure that the subjects were hypertensive in our study. In various studies, it has been shown that apelin has an antihypertensive impact. Nevertheless, in the study recently performed by Nagano et al., a temporal increase in the BP has been observed as a reaction to apelin stimulation in NG-nitro-L-arginine methyl ester (LNAME)-induced peripheral vascular damaged hypertension mice, which demonstrates that differently from the normal conditions, apelin can operate as a vasopressor peptide under the vascular damaged circumstance (Nagano et al., 2013). Apelin and APJ represent a signaling pathway, and their wide expression is observed in different tissues, particularly in the cardiovascular system, such as the heart, vessels, and kidneys (Lv et al., 2013a,b). Various proofs show that the apelin/APJ pathway represents a crucial regulator of the cardiovascular function and takes a significant part in the emergence and development of cardiovascular diseases, such as heart failure, coronary heart disease (CAD), atherosclerosis, hypertension, myocardial ischemia–reperfusion injury (MIRI), pulmonary arterial hypertension (PAH) and atrial fibrillation (Barnes et al., 2010; Galanth et al., 2012; Tycinska et al., 2012).

The finding of the apelin/APJ axis represents an impressive event in cardiovascular studies. As a result of various experimental and clinical studies conducted in vivo and in vitro, it has been indicated that apelin/APJ is a crucial mediator of cardiovascular homeostasis, which takes part in the pathogenesis of a number of major cardiovascular diseases, for example, heart failure, atherosclerosis, hypertension, and atrial fibrillation. Nevertheless, disputes about the effect of apelin/APJ on atherosclerosis and hypertension are still continuing, and therefore, the role of the molecular pathway in the disease protection in humans

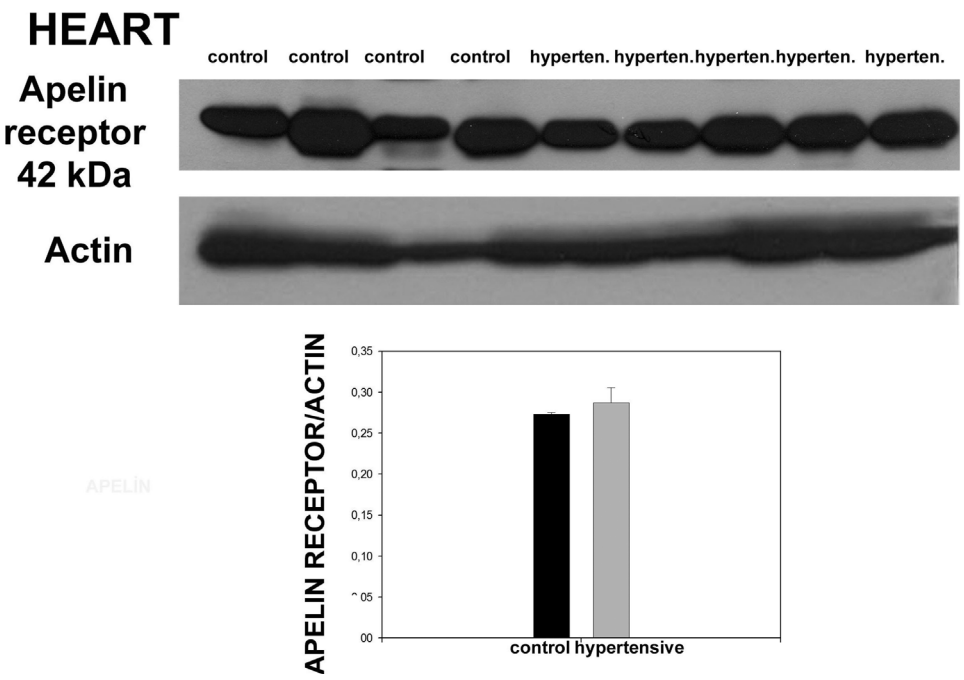


Fig. 10. a) Western blotting results of apelin receptor in control and hypertension hearts. Beta-actin was used as an internal control. b) Quantitative analysis of apelin Western blotting data. The values in the bar graphs were obtained by dividing the protein intensity value by its corresponding beta-actin intensity value. The differences seen between control and hypertension group hearts were not statistically significant. The image is representative of three independent experiments.

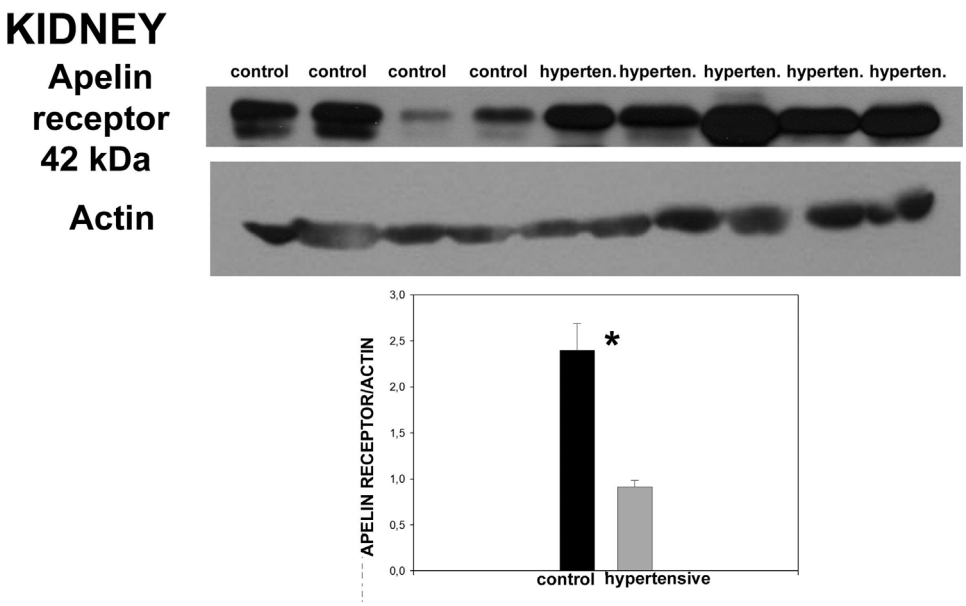


Fig. 11. a) Western blotting results of apelin receptor in control and hypertension kidneys. Beta-actin was used as an internal control. b) Quantitative analysis of apelin Western blotting data. The values in the bar graphs were obtained by dividing the protein intensity value by its corresponding beta-actin intensity value. The differences seen between control and hypertension group kidneys were statistically significant (*). The image is representative of three independent experiments.

should be examined more (Yu et al., 2014). The diverse status of hypertension, along with the action site of apelin, can lead to the difference in the levels of apelin change on the treatment drugs in question. However, the apelin/APJ system unquestionably represents a probable therapy target in the treatment of hypertension, and the laboratory findings of the present study have introduced a significant aspect of the apelin/APJ system as a therapeutic target in cardiovascular diseases (Lv et al., 2013a,b; Xie et al., 2013). The relationship of apelin/APJ system with hypertension has been currently examined in a number of studies. Masked hypertension represents a meaningful indicator of cardiovascular diseases, and plasma apelin levels are observed to be considerably lower in the masked hypertensive group in comparison with normotensive controls (Papadopoulos et al., 2013). In patients having significant hypertension, circulating apelin levels are decreased as well and related in an independent way with more serious left ventricular systolic and diastolic function deterioration (Przewlocka-Kosmala et al., 2011). Furthermore, it is observed that hypertensive rats have decreased plasma apelin levels and down-regulation of APJ in kidneys (Najafipour et al., 2012). Malyszkoj et al. (Małyszko et al., 2006) reported that the apelin levels decreased in chronic renal disease. It was also stated that the apelin levels decreased in coronary artery patients receiving hemodialysis. It is estimated that apelin has a function in the physiopathology of possible cardiovascular diseases as well as chronic renal failure. In the light of this information, it is indicated that apelin can be used as a treatment approach for uremic cardiomyopathy in the future (Ladeiras-Lopes et al., 2008).

It was observed that, in the kidneys, the highest level of APJ mRNA was in the inner strip of outer medulla, which represents an area in the kidney that undertakes a significant function in water and sodium balance. Furthermore, a direct tubular function of apelin has been demonstrated (Hus-Citharel et al., 2008). The endothelium and vasoactive substances originating from the endothelium play an important role in the local control of blood pressure. It was observed that the endothelial gene expression increased in the endothelial cells of some hypertensive patients and these findings support the possible role of the endothelium in the hypertension development (DeArtinano and Gonzalez, 1999; Kaplan, 1998; Kaplan, 1997; Kornitzer et al., 1999). As a consequence of the cardiovascular actions, which are observed for apelin, it is possible to imply that a future drug target for hypertension and heart failure treatment is presented by apelin (Kleinz and Davenport, 2005). The effects of apelin-APJ receptor on the regulation of blood pressure should be discussed. There are many studies indicating that the average blood pressure increases (Katugampola et al., 2001) and decreases after apelin application. It was interpreted that contradictory results in the literature might have originated from the fact that the researchers used different experimental animal models and from the differences in the types of apelin application. In spite of the contradictive results, the common finding of the researchers is the fact that the vascular effect of apelin is related to the endothelial integrity (Cheng et al., 2003; Salcedo et al., 2007; Tatemoto et al., 2001). Apelin causes endothelium-dependent vasodilatation by modulating NO or prostanoid release in the cases in which the endothelial integrity is preserved under in vivo conditions (Salcedo et al., 2007). However, if there is an endothelial dysfunction, apelin affects smooth muscle cells and causes the vasoconstrictive response (Katugampola et al., 2001). The effects of apelin on vascular tone may be due to the dynamic interaction between apelin-APJ system and angiotensinogen-AT-1. Studies performed on hypertensive rats showed that, all trans retinoic acid (aTRA) that regulates the G protein receptor signal decreases blood pressure and increases APJ-apelin expression. In hypertensive rats, apelin inhibits the renin angiotensin system, reducing blood pressure and cause decrease of APJ in the kidney and plasma apelin levels (Hus-Citharel et al., 2008, Nagano et al., 2013).

In our study, the expression of apelin and APJ receptor in heart and kidney tissues was examined immunohistochemically and Western Blot in rat hypertensive rats with L-NAME. 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.

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References

Acar, N., Korgun, E.T., Ustunel, I., 2012. Cell cycle inhibitor p57 expression in normal and diabetic rat placentas during some stages of preg- nancy. Histol. Histopathol. 27, 59–68.
Badyal, D.K., Lata, H., Dadhich, A.P., 2003. Animal models of hypertension and effect of drugs. Indian J. Pharmacol. 35, 349–362.
Barnes, G., Japp, A.G., Newby, D.E., 2010. Translational promise of the apelin–APJ system. Heart 96, 1011–1016.
Cheng, X., Cheng, X.S., Pang, C.C., 2003. Venous dilator effect of apelin, an endogenous

peptide ligand for the orphan APJ receptor, in conscious rats. Eur. J. Pharmacol. 470 (3), 171–175.
Chun, H.J., Ali, Z.A., Kojima, Y., Kundu, R.K., Sheikh, A.Y., Agrawal, R., Zheng, L., Leeper, N.J., Pearl, N.E., Patterson, A.J., Anderson, J.P., Tsao, P.S., Lenardo, M.J., Ashley, E.A., Quertermous, T., 2008. Apelin signaling antagonizes Ang II effects in mouse models of atherosclerosis. J. Clin. Invest. 118 (10), 3343–3354.
DeArtinano, A.A., Gonzalez, L.M., 1999. Endothelial dysfunction and hypertensive vasoconstriction. Pharmacol. Res. 2, 113–124.
Dunbar, C.C., 1992. The antihypertensive effects of exercise training. N. Y. State J. Med. 92 (6), 250–255.
Fregly, M.J., 1984. Effect of an exercise regimen on development of hypertension in rats. J. Appl. Physiol. 56 (2), 381–387.
Galanth, C., Hus-Citharel, A., Li, B., Llorens-Cortes, C., 2012. Apelin in the control of body fluid homeostasis and cardiovascular functions. Curr. Pharm. Des. 18, 789–798.
Gardiner, S.M., Kemp, P.A., Bennett, T., Palmer, R.M.J., Moncada, S., 1992. Nitric oxide synthase inhibitors cause sustained, but reversible, hypertension and hindquartes vasoconstriction in Brattieboro rats. Eur. J. Pharmacol. 213, 449–451.
Glasser, S.P., Judd, S., Basile, J., Lackland, D., Halanych, J., Cushman, M., Prineas, R., Howard, V., Howard, G., 2011. Prehypertension, racial prevalence and its association with risk factors: analysis of the reasons for geographic and racial differences in stroke (REGARDS) study. Am. J. Hypertens. 24 (2), 194–199.
Hus-Citharel, A., Bouby, N., Frugière, A., Bodineau, L., Gasc, J.M., Llorens-Cortes, C., 2008. Effect of apelin on glomerular hemodynamic function in the rat kidney. Kidney Int. 74, 486–494.
Ishida, J., Hashimoto, T., Hashimoto, Y., Nishiwaki, S., Iguchi, T., Harada, S., Sugaya, T., Matsuzaki, H., Yamamoto, R., Shiota, N., Okunishi, H., Kihara, M., Umemura, S., Sugiyama, F., Yagami, K., Kasuya, Y., Mochizuki, N., Fukamizu, A., 2004. Regulatory roles for APJ, a seven-transmembrane receptor related to angiotensin-type 1 receptor in blood pressure in vivo. J. Biol. Chem. 279 (25), 26274–26279.
Kaplan, N.M., 1997. Systemic Hypertension. Mechanisms and Diagnosis. Braunwald E. Heart Disease. WB Saunders Company, Philadelphia, pp. 811–816.
Kaplan, N.M., 1998. Primer Hypertension: Pathogenesis, in Clinical Hypertension, 7th. Williams&Wilkins, pp. 41–99.
Katugampola, S.D., Maguire, J.J., Matthewson, S.R., Davenport, A.P., 2001. [(125)I]-(Pyr (1))Apelin-13 is a novel radioligand for localizing the APJ orphan receptor in human and rat tissues with evidence for a vasoconstrictor role in man. Br. J. Pharmacol. 132 (6), 1255–1260.
Kawamata, Y., Habata, Y., Fukusumi, S., Hosoya, M., Fujii, R., Hinuma, S., Nishizawa, N., Kitada, C., Onda, H., Nishimura, O., Fujino, M., 2001. Molecular properties of apelin: tissue distribution and receptor binding? Biochim. Biophys. Acta 1538 (2–3), 162–171.
Kleinz, M.J., Davenport, A.P., 2005. Emerging roles of apelin in biology andmedicine. Pharmacol. Ther. 107, 198–211.
Kornitzer, M., Dramaix, M., De Backer, Guy., 1999. Epidemiology of risk factors for hypertension-Implication for prevention and therapy. Drugs 57 (5), 695–712.
Ladeiras-Lopes, R., Ferreira-Martins, J., Leite-Moreira, A.F., 2008. The apelinergic system: the role played in human physiology and pathology and potential therapeutic applications. Arq. Bras. Cardiol. 90 (5), 343–349.
Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193, 265–275.
Lv, D., Li, H., Chen, L., 2013a. Apelin and APJ, a novel critical factor and therapeutic target for atherosclerosis. Acta Biochim. Biophys. Sin. (Shanghai) 45, 527–533.
Lv, D., Lu, Q., Cao, J., Chen, L., 2013b. Unanticipated role of apelin: regulation of miRNA generation. Acta Biochim. Biophys. Sin. (Shanghai) 45 (10), 896–898.
Małyszko, J., Małyszko, J.S., Koźminski, P., Myśliwiec, M., 2006. Apelin and cardiac function in hemodialyzed patients: possible relations? Am. J. Nephrol. 26 (2), 121–126.
Malyszko, J., Malyszko, J.S., Pawlak, K., Wolczynski, S., Mysliwiec, M., 2008. Apelin, a novel adipocytokine, in relation to endothelial function and inflammation in kidney allograft recipients. Transplant. Proc. 40 (10), 3466–3469.
Nagano, K., Ishida, J., Unno, M., Matsukura, T., Fukamizu, A., 2013. Apelin elevates blood pressure in ICR mice with LNAMEinduced endothelial dysfunction. Mol. Med. Rep. 7 (5), 1371–1375.
Najafipour, H., Soltani Hekmat, A., Nekooian, A.A., Esmaeili-Mahani, S., 2012. Apelin receptor expressionin ischemic and non-ischemic kidneys and cardiovascular responses to apelin in chronic two-kidney-one-clip hypertension in rats. Regul. Pept. 178, 43–50.
O'Dowd, B.F., Heiber, M., Chan, A., Heng, H.H., Tsui, L.C., Kennedy, J.L., Shi, X., Petronis, A., George, S.R., Nguyen, T., 1993. A human gene that shows identity with the gene encoding the angiotensin receptor is located on chromosome 11? Gene 136 (1–2), 355–360.
Papadopoulos, D.P., Mourouzis, I., Faselis, C., Perrea, D., Makris, T., Tsioufis, C., Papademetriou, V., 2013. Masked hypertension and atherogenesis:the impact of apelin and relaxin plasma levels. J. Clin. Hypertens. (Greenwich) 15, 333–336.
Przewlocka-Kosmala, M., Kotwica, T., Mysiak, A., Kosmala, W., 2011. Reduced circulating apelin in essential hypertension and its association with cardiac dysfunction. J. Hypertens. 29, 971–979.
Roberto, Z., Baylis, C., 1998. Chronic nitric oxide inhibition model six years on. Hypertension 32 (6), 958–964.
Salcedo, A., Garijo, J., Monge, L., Fernández, N., Luis García-Villalón, A., Sánchez Turrión, V., Cuervas-Mons, V., Diéguez, G., 2007. Apelin effects in human splanchnic arteries; role of nitric oxide and prostanoids. Regul. Pept. 144 (1–3), 50–55.
Sonmez, A., Celebi, G., Erdem, G., Tapan, S., Genc, H., Tasci, I., Ercin, C.N., Dogru, T., Kilic, S., Uckaya, G., Yilmaz, M.I., Erbil, M.K., Kutlu, M., 2010. Plasma apelin and ADMA levels in patients with essential hypertension. Clin. Exp. Hypertens. 32 (3), 179–183.
Szokodi, I., Tavi, P., Földes, G., Voutilainen-Myllylä, S., Ilves, M., Tokola, H., Pikkarainen, S., Piuhola, J., Rysä, J., Tóth, M., Ruskoaho, H., 2002. Apelin, the novel endogenous ligand of the orphan receptor APJ, regulates cardiac contractility. Circ. Res. 91 (5), 434–440.
Tatemoto, K., Hosoya, M., Habata, Y., Fujii, R., Kakegawa, T., Zou, M.X., Kawamata, Y., Fukusumi, S., Hinuma, S., Kitada, C., Kurokawa, T., Onda, H., Fujino, M., 1998. Isolation and characterization of a novel endogenous peptide ligand for thehuman APJ receptor. Biochem. Biophys. Res. Commun. 251 (2), 471–476.
Tatemoto, K., Takayama, K., Zou, M.X., Kumaki, I., Zhang, W., Kumano, K., Fujimiya, M., 2001. The novel peptide apelin lowers blood pressure via a nitricoxide-dependent mechanism. Regul. Pept. 99, 87–92.
Tipton, C.M., Matthes, R.D., Callahan, A., Tcheng, T.K., Lias, L.T., 1977. The role of chronic exercise on resting blood pressures of normotensive and hypertensive rats. Med. Sci. Sports 9 (3), 168–177.
Tycinska, A.M., Lisowska, A., Musial, W.J., Sobkowicz, B., 2012. Apelin in acute myocardial infarction and heart failure induced by ischemia. Clin. Chim. Acta 413, 406–410.
Xie, F., Li, L.F., Chen, L.X., 2013. Apelin/APJ receptor as myocardial cell baroreceptor induced myocardial hypertrophy formation. Prog. Biochem. Biophys. 40 (01), 33–36.
Yu, X.H., Tang, Z.B., Liu, L.J., Qian, H., Tang, S.L., Zhang, D.W., Tian, G.P., Tang, C.K., 2014. Apelin and its receptor APJ in cardiovascular diseases. Clin. Chim. Acta 428, 1–8.