DOI: 10.4149/BLL_2015_020

Bratisl Lek Listy 2015; 116 (2)

104 – 108

EXPERIMENTAL STUDY

Supplementation of apelin increase plasma levels of nesfatin-1 in normal and DOCA-salt hypertensive rats

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Abstract: *Aims:* We aimed to observe the effects of apelin supplementation on the plasma levels of nesfatin-1 in DOCA-salt hypertensive and normal rats.
Methods: For this purpose, 28 young Wistar albino male rats were divided into four groups; Control (C), Control + Apelin (C+A), Hypertension (HT) and Hypertension + Apelin (HT+A). Hypertension was induced by injection of DOCA-salt (25 mg/kg, s.c.) twice weekly, 4 weeks, whereas intraperitoneal apelin was administered (200 $\mu g.kg^{-1}$) for 17 days. Plasma nesfatin-1 and apelin levels were measured with ELISA. Systolic blood pressure was monitored using a tail cuff system. The relationships between plasma nesfatin levels and blood pressure were assessed.
Results: Plasma nesfatin-1 levels was found lower in control animals compared to C+A, HT and HT+A groups ($p = 0.002$, $p = 0.026$ and $p = 0.011$, respectively). Systolic blood pressures were similar in the C and C+A groups, but systolic blood pressures of the HT and HT+A groups was found significantly higher than the C and C+A groups.
Conclusions: In conclusion, apelin administration induced an increment of nesfatin-1 in normal rats and plasma levels of nesfatin-1 increase in DOCA-salt hypertension rats. But apelin addition in hypertension did not cause an extra increase in nesfatin-1 levels. This is the first report to investigate the effect of apelin administration on plasma nesfatin levels of normal and hypertensive rats *(Fig. 2, Ref. 44)*. Text in PDF *www.elis.sk.*
Key words: nesfatin, apelin, hypertensive rats, blood pressure, body weight.

Introduction

Hypertension, occuring due to many genetic and environmental factors, is a complex and multi factorial disease (1). Many pathophysiological factors such as increase in sympathetic nervous system activity, overproduction of vasoconstrictors such as endothelin and thromboxane, overproduction of sodium retaining hormones, insufficient intake of potassium and calcium, increased and inappropriate renin secretion, deficiency of vasodilators such as prostaglandins and nitric oxide, congenital abnormalities of resistance vessels, diabetes mellitus, insulin resistance, obesity, increased activity of vascular growth factors and changes of cellular ion transport play a role in the formation of hypertension (2).

Apelin was firstly isolated from bovine stomach by Tatemoto et al in 1998 (3). Apelin, secreted and produced by mature adipocyte, is described as a new adipocytokines (4). The peptide, expressed in human and rat various tissues including heart, lungs, testes, ovary, mammary glands, brain, liver, skeletal muscle, kidney and plasma (5, 6), has been identified as the endogenous ligand of the G-protein-coupled receptor APJ (3). The apelin gene encodes a pre-proprotein of 77 amino acids with a signal peptidein the N-terminal region. The pre-proprotein was fragmented in endoplasmic reticulum to compose of a pro-protein of 55 amino acid (3). Four active isoforms as apelin-12, 13, 17 and 36, each showing different receptor binding affinities, were produced from prepropeptide (7). It's thought to be that the 13 amino acid in the C terminal region of the pro-protein is responsible for biological activity of the peptide (8). Increasing evidence suggests that apelin regulates multiple physiological functions, including food intake, cell proliferation, regulation of blood pressure and vascular tonus, glucose utilization, angiogenesis and fluid homeostasis. Therefore reported that is multifunctional neuropeptide to play role in diabetes, obesity, hypertension and cardiovascular diseases (9, 10).

Nesfatin-1 is a newly discovered by Oh-I et al in 2006 (11). Nesfatin-1, 82-amino-terminal fragment derived from larger protein NUCB2 (*nonesterified fatty acid/nucleobinding* 2), is very high amino acid sequence homology among rat, mouse, and human species (> 85 %) (12). NUCB2 is at times referred to as NEFA/NUCB2 but in this case it is likely that NEFA is defined as "DNA binding/EF hand/acid amino acid region" (13). NUCB2 has many regions for posttranslational modification. Structural analyses revealed the presence of several conserved cleavage recognition sites for prohormone convertases (PC3/1 and PC2) within rat NUCB2/nesfatin sequence, thus suggesting this to be a precursor that gives rise, by differential proteolytic processing, to several active pep-

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Acknowledgements: This study was supported by Pamukkale University Research Fund (Project No. 2011SBE001 and 2011SBE005).

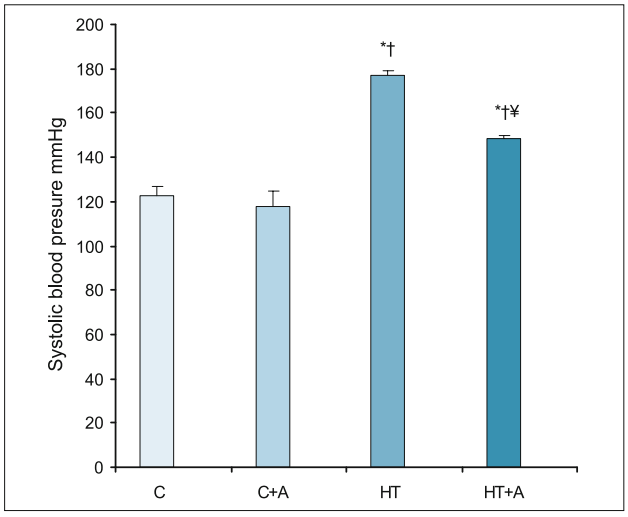

Fig. 1. Systolic blood pressure of all groups. * − shows significance between control group and HT and HT+A groups (p < 0.01), † − shows significance between C+A group and HT and HT+A groups (p < 0.01), ¥ − shows significance between HT and HT+A group (p < 0.01).

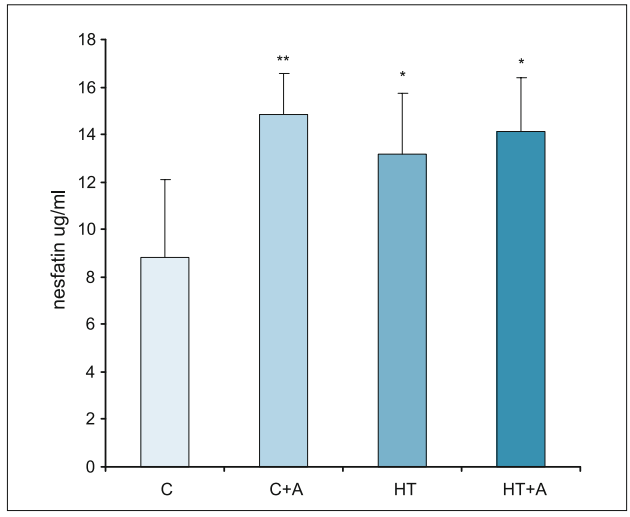

Fig. 2. The plasma levels of nesfatin in groups. * − shows significance between the control group and C+A, HT and HT+A (* p < 0.01, ** p < 0.05).

tides. The predicted (major) fragments of such processing were termed nesfatin-1, nesfatin-2, and nesfatin-3 (14, 15). Nesfatin-2 and nesfatin-3, the fragments of NUCB2, have the same structure as the DNA structure of calcium binding proteins However, there is no information about the biological activity of these peptides (14, 16, 17, 18). In particular, nesfatin-1 seems to be released by the concerted actions of PC3/1 and PC2. Functional analyses of three potential fragments of nesfatin-1 molecule (namely, the N-terminal (N23), the C-terminal (C29), and the central (M30) fragments) revealed that the mid-fragment contains the active site for the anorectic effects of nesfatin-1 (15, 19). It's reported that nesfatin-1 is a peptide secreted in the tissues of central and peripheral nervous system and involved in the regulation of homeostasis. Nesfatin1 is able to cross the blood brain barrier in both the blood-to-brain and brain-to-blood directions (20, 21) and was discovered a hypothalamus secreted protein that conduces to a decrease on food and water intake and to an increase on energetic waste (22). Recently studies have been shown an increasing blood pressure with administration of nesfatin-1. It has also been shown to play an important roles in the control of cardiovascular function (23, 24). However, the effect mechanism of nesfatin-1 on the blood pressure is not clear.

Although these peptides, apelin and nesfatin-1, were expressed various tissues and organs, the physiological effects of them were not fully elucidated. Apelin and nesfatin-1 are multifunctional peptides that have been studied in similar disease or physiologic states that there may be a relationship between these two peptides. Recently studies have been suggested that apelin can be used as a therapeutic and protective agent on diseases as hypertension and type II diabetes in the future. For that reason, we aimed to investigate the effects of the apelin administration on the blood levels of nesfatin-1 and blood pressure in DOCA-salt hypertensive and normal rats.

Materials and methods

Animals and study design

Twenty-eight Wistar Albino 8–10 week old male rats (180–300 g) were housed in the Experimental Research Unit of the University of Pamukkale, Denizli, Turkey. They were reared under the supervision of a veterinarian, kept in a well-ventilated, noiseless environment, and allowed free access to food and water. They were housed in plastic cages (42 x 26 x 15 cm), each containing three to four rats, under standard laboratory conditions (ambient temperature of 22 ± 1 °C, 12 hour light-dark cycle). The study was approved by the Pamukkale University Ethics Committee for Experimental Animals.

Rats were randomly divided into four groups; control group (C) (n = 7), control + apelin group (C+A) (n = 7), DOCA-salt hypertensive groups (HT) (n = 7), DOCA-salt hypertensive group + apelin (HT+A) (n = 7). The body weights of rats were measured at the beginning and at the end of the experiment.

Hypertension modeling

Hypertension was induced by DOCA-salt treatment as previously described (25). DOCA was injected (25 mg/kg of body weight in 0.4 ml of dimethylformamide subcutaneously), twice weekly for 4 weeks, and tap water for drinking was replaced by 1 % NaCl during the treatment period. In the control groups, the same volume of serum physiologic was injected. The rats of C+A and HT+A groups were treated with pyroglutamylated apelin-13 (Pyr-AP13; 200 $\mu g.kg^{-1}.day^{-1}$ ip) during 17 days (26). Apelin administration initiated in HT+A group after than hypertension developed.

Blood pressure monitoring

Systolic blood pressure was measured in awake rats using a non-invasive tail cuff blood pressure measuring system (Power

Lab/8SP data acquisition system, ADInstruments Co.) before DOCA treatment and on the 14th, 21th, 28th days of DOCA treatment. Blood pressure of rats treated with DOCA started to rise after one week and reached high systolic values (≥ 160 mmHg) after four weeks. Rats were placed in a plastic restrainer (Kursunluoglu Metal Co., Denizli, Turkey). All measurements were performed without anesthesia at room temperature in a silent room. The physiological data was analyzed using the LabChart 6.1 Pro software (AD Instruments Co.) (27).

Blood samples and measurements

At the end of the experimental period, all of the animals were anesthetized with Ketamin/Xylazine HCl (75 mg/kg/10 mg/kg intraperitoneally). Blood was collected in heparinezed tubes. After centrifugation, plasma was stored at −80 °C until analysis. Plasma concentration of nesfatin-1 was analyzed with rat ELISA assay kit using the chemiluminescence method (Diagnostic Product Corporation, USA) by an ELISA microplate reader (das, Digital and Analog Systems, Italy).

Statistical analysis

Statistical analysis was done with the SPSS (Statistical Package for Social Sciences) 16.0 pocket program. Results were expressed as the means ± standard deviation (M±SD). The Mann–Whitney U test was used for statistics, with p values ≤ 0.05 accepted as statistically significant.

Results

The effects of apelin administration in healthy and hypertensive rats investigated in this study, the plasma levels of nesfatin and blood pressure were compared between groups. Systolic blood pressure (SBP) measured at the end of the study in HT and HT+A groups were found significantly higher than C and C+A groups ($p < 0.01$) (Fig. 1). In HT+A group, SBP was observed significantly lower than HT group ($p = 0.001$) (Fig. 1). The nesfatin plasma levels of HT, HT+A and C+A groups were found significantly increase compared to control group ($p < 0.05$, $p < 0.05$, $p < 0.01$, respectively), shown in Figure 2.

Discussion

Hypertension can occur in many genetic and environmental factors and is a complex, multifactorial and major health problem (1). Acute positive inotropic effects of apelin have been shown in both healthy and heart failure rats (28, 29). Apelin have been reported as a marker of heart failure (28) and the vasodilatory effects of apelin have been shown in various studies (30, 31). However, it is worthwhile to keep it in mind that the reports on apelin's effects on blood pressure (BP) are varied. While there are studies, which showed decreased arterial pressure via a nitric oxide (NO)-dependent mechanism (32, 33, 34), there were reports showing an increased arterial pressure (33), biphasic change of mean arterial blood pressure (32), and lack of BP change following apelin administration (35). Dose, method of administration, experimental subject, could contribute to this variation. It is suggested that apelin, reported the effects on cardiovascular regulation, can be a potential therapeutic target in hypertension (36). Nesfatin-1, secreted in hypothalamus and brain stem, has been reported to be a peptide efficacy in the control of appetite (anorexigenic effects) regardless of leptin pathway (16, 11). There is a limited study about the effects of nesfatin-1 on blood pressure. The previous studies reported that nesfatin-1 can increase the mean arterial blood pressure in rats (25) and modulates blood pressure through directly acting on peripheral arterial resistance (23).

In the present study, we examined the effects of intraperitoneally injected apelin on plasma nesfatin levels and relationship between blood pressure and nesfatin-1 in normal hypertensive rats. The major finding of this study is that apelin administration increase plasma nesfatin-1 levels in normal rats. We cannot discuss a potential mechanism of how apelin might regulate nesfatin-1. Because this is the first report on the effect of apelin administration on plasma nesfatin-1 levels in normal rats. On the other hand, systolic blood pressure has not changed. We did not find a previous research about effect of apelin administration on plasma nesfatin levels. Maternal serum and cord blood apelin and nesfatin-1 concentrations in pregnant women with and without gestational diabetes mellitus (GDM) were investigated by Aslan et al (38). They reports increased apelin decreased nesfatin-1 levels in pregnant women with GDM compared to healthy pregnant women. However, a significant correlation was not found between the levels of nesfatin and Apelin (38). In addition, the correlation between apelin and nesfatin-1 levels in breast milk of lactating women and maternal serum were investigated in a study performed by Aydin et al They did not report significantly relationship (39).

In the current study, the plasma levels of nesfatin in hypertension group significantly increased compared to normal rats. In recent studies, increasing blood pressure effect of nesfatin-1 has been demonstrated (14, 24, 22). Yosten et al reported that intracerebroventricular injection of nesfatin 1 caused an increased blood pressure in rats (24). The hypertensive effect of nesfatin-1 is thought to be mediated via the activation of sympathetic nerves through acting on melanocortin-3/4 receptors. It was further shown that the hypertensive effect of nesfatin-1 was mediated via acting on hypothalamus oxytocin receptor, which is thought to be downstream of the melanocortin system (22). It is well known that sodium nitroprusside (SNP) (NO) induces smooth muscle relaxations via producing cGMP. Recently, study reported that nesfatin-1 affected rat isolated mesenteric artery and specifically impaired the SNP-induced smooth muscle relaxations through the inhibition of cGMP production (23). It is not mentioned by nesfatin administration in the current study. On the contrary, the increased plasma concentration of nesfatin was observed in hypertensive rats developed with DOCA-salt hypertension models. The DOCA-salt model including low-renin form of hypertension is similar to other experimental models that depend on high salt intake and reduced renal mass leading to hypervolemia. Increased ET-1 was shown both in vessel and in different tissues (39, 40). It is reported that excess vasoconstrictor substances such as Ang II and ET-1 or vasodilatation as a result of the formation of deletion

of genes encoding G protein-coupled receptors played an important role in the maintenance of hypertension (41). However, the release of aldosterone, antidiuretic hormone synthesis, sympathetic activation and salt absorption from renal tubules leads to the development of hypertension (42). We did not found any study on the relationship between nesfatin and ANG II, ET1, aldosterone, antidiuretic hormone. In this study, an increased plasma nesfatine-1 concentration was probably due to an increased ET-1 as well as it could be increased independent of ET-1. It was first demonstrated that intraperitoneal apelin injection in healthy animals increased the plasma levels of nesfatin-1, but this high level of endogen nesfatin-1 did not affect the blood pressure. On the contrary, previous studies reported that exogen nesfatin administration, giving intracerebraventricular (43) and intravenous (23) caused a high blood pressure. The results of the current study demonstrated that apelin treatment resulted in an increment nesfatin-1 levels in DOCA-salt hypertansive+apelin rats compared to control animals. The previous research indicated that the addition of intracerabral nesfatin induced hypertension (43) and intravenous administration of nesfatin-1 increased vascular contraction by inhibiting NO production (23) Also the low blood pressure was observed in HT+A compared to HT group despite the same nesfatin level shown in these groups. The reason for this situation could be apelin administration. Because in our previous study we demonstrated that addition of apelin reduces blood pressure in DOCA-salt rats (44). There are no studies reported in literature for plasma levels nesfatin in hypertension. In this sense, this study is the first study on plasma levels of nesfatin in experimental hypertension.

As a result, according to our findings, apelin administration increased nesfatin-1 blood level in normal rats, and there was an increased nesfatin-1 level in the hypertensive rats with or without administration of apelin. However, we can say this increment is not sufficient to increase the blood pressure. The first report on the effect of apelin administration on plasma nesfatin-1 levels in normal and DOCA-salt hypertensive rats. Apelin considered to be used for the prevention and treatment in hypertension that reason further detailed studies are needed to explain the relationship between apelin and nesfatin.

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Received November 5, 2013.
Accepted November 25, 2013.