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ORIGINAL ARTICLE

Selective association of endogenous ouabain with subclinical organ damage in treated hypertensive patients

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According to previous studies endogenous ouabain (EO) closely correlates with high blood pressure, congestive heart failure and kidney disease in humans. Our aims were to analyse associations between plasma, urinary EO level and various markers of cardiovascular damage in treated hypertensive patients. Forty-one adult patients with hypertension and/or diabetes mellitus (DM) and/or chronic kidney disease (CKD) were studied. We assessed plasma and urinary EO, pro-brain natriuretic peptide and catecholamines, profile of ambulatory blood pressure monitor and cardiovascular status by echocardiography and echo-tracking. The highest level of plasma EO (19.7 ± 9.5 pmol l^{-1}) was measured in hypertensive patients with DM and CKD. The nighttime mean arterial blood pressure independently correlated with the level of plasma EO ($P = 0.004$), while independent predictor of the β-stiffness of carotid artery was the urinary EO ($P = 0.011$). Elevated level of EO was associated with nighttime blood pressure and subclinical organ damage in treated hypertensive patients, suggesting possible role of EO in the pathogenesis of impaired diurnal blood pressure rhythm and arterial stiffness.

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Introduction

Over the past 20 years, many investigators have reported extraction of endogenous digitalis-like factors (EDLFs) including endogenous ouabain (EO) and/or marinobufagenin from blood and other tissue sources, and these factors have been linked to the pathophysiology of essential hypertension, congestive heart failure (extracellular volume overload), end-stage renal failure and pre-eclampsia.[1–3]

Previous studies have shown that EO is mostly produced in the adrenal cortex and circulating EO activate the sympathetic nervous system.[3,4] Their results suggested that these activations can be inhibited with β-blocker, angiotensin converting enzyme inhibitor and digoxin immune fab (antigen-binding fragments), Digi-Bind.[5,6]

The increased level of EO may have direct and indirect (via marinobufagenin) vasoconstrictor (hypertensive) effects by blocking sodium/potassium pump (Na^+/K^+-ATPase), consecutively increasing intracellular calcium level by the activation of the sodium–calcium exchanger, but this effect of EO is not homogenous, that is different Na^+/K^+-ATPase isoforms respond differently to EO.[7–9] Experimental use of low doses of ouabain increased arterial blood pressure in rats.[10] Beyond the classical effects of EO, the digitalis-like factors have genomic effects inducing hypertrophy in myocardial and vascular smooth muscle cells because of the activation of numerous known intracellular signaling pathways.[11–13]

The digitalis-like factors also stimulate fibroblast causing collagen overproduction and fibrosis in rats, which is perhaps important in the development of cardiomyopathy.[14] Some human studies have shown that the elevated plasma EO positively correlates with systolic and diastolic blood pressure in untreated hypertensive individuals and further studies have found positive correlation with left

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ventricular mass index, and left ventricular end-diastolic volume and cardiac dysfunction.[15,16] Elevated EO plasma level also correlated with total peripheral resistance in humans emphasizing possible role of EO in the development of arterial stiffness, which is a strong predictor of the early atherosclerosis, subclinical organ damage and cardiovascular diseases.[17]

The orally administered cardiac glycoside (digoxin) in the therapeutic range decreased the nighttime diastolic blood pressure in healthy volunteers as well as it had in patients with heart failure a diminishing effect on the nighttime diastolic blood pressure and elevated the nighttime systolic blood pressure but it did not have an effect on daytime blood pressure profile because it is overregulated by sympathetic effects of daytime activities.[18–20] Production of EDLF in humans is not constant; a decreased nighttime urinary EDLF excretion has been shown in healthy volunteers.[21]

Our goal was to analyse associations between plasma and urinary EO and several markers of cardiovascular status including results of echocardiography, echo-tracking and ABPM as well as other parameters as brain natriuretic peptide (BNP), nighttime catecholamines excretion, intima-media thickness (IMT) in patients with treated primary hypertension (HT), type 2 diabetes mellitus (DM) and/or chronic kidney disease (CKD).

Methods

In a cross-sectional clinical study, 41 adult patients were investigated, who were divided into four groups: (1) group of primary hypertensive, non-diabetic patients without CKD (HT, $N=10$); (2) group of hypertensive patients with type 2 DM (HT + DM, $N=11$); (3) group of hypertensive patients with type 2 DM and CKD (creatinine clearance under 90 ml min^{-1}) (HT + DM + CKD, $N=10$); and (4) group of hypertensive patients with CKD (HT + CKD, $N=10$). Diagnoses of patients with CKD were diabetic nephropathy ($N=10$) in HT + DM + CKD group and chronic pyelonephritis ($N=3$), nephrosclerosis ($N=7$) in the HT + CKD group.

None of the patients were enrolled in this study with hyper/hypothyroidism, adrenal gland adenoma, tumour, acute infection, atrial fibrillation or pacemaker therapy. All subjects were treated for hypertension, type 2 DM and CKD in our outpatient clinic and had no symptoms of severe congestive heart failure. None of the subjects was taking cardiac glycoside, spironolactone, prednisolone or other steroids.

The study was approved by the ethical committee of the Medical Faculty of the University of Pécs and all participants gave written informed consent before taking part in the study. The study was conducted in agreement with the Declaration of Helsinki.

The blood pressure monitoring was carried out with ABPM-04 (Meditech, Meditech Ltd, Budapest, Hungary), based on a validated oscillometric technique. During a 24-h monitoring period, the blood pressure was registered in 15-min intervals in daytime (0800–2200 h) and 30-min intervals during nighttime (2200–0800 h). The results of blood pressure monitoring were analysed by the proprietary software of ABPM-04. Hypertensive time index was expressed as a percentage of blood pressure values that exceed the upper level of normal range of blood pressure during daytime (systolic/diastolic: 140/90 mm Hg) and nighttime (systolic/diastolic: 130/80 mm Hg).

The therapy of the patients is summarized in Table 1.

The antihypertensive and insulin/antidiabetic therapies were not interrupted during the study.

There were no significant differences in serum chloride, calcium, liver enzymes (ALAT, ASAT, GGT, lactate dehydrogenase), serum triglyceride, high-density lipoprotein, low-density lipoprotein cholesterol, C-reactive protein, haematological parameters (red blood cell count, white blood cell, platelet) between groups (data not shown).

Patients' morning fresh urine was assessed by routine examination including pH, glucose, protein, pus, acetone, urobilinogen and level of creatinine. There were no significant differences between groups in the results of routine fresh urinary examination (data not shown).

Plasma and urinary EO determination

All patients had a 6-ml morning blood draw from the cubital vein in EDTA tubes between 0700 and 0800 h after 40 min of strict supine position. There blood samples were centrifuged with $3000 \times g$ within 10 min, and plasma was separated and transferred into plastic tubes and stored at $-20\,^{\circ}$C until assayed. Ten millilitres of urinary samples were taken out of 24-h collected urine and were stored at $-20\,^{\circ}$C. Plasma and urinary EO levels were determined by radioimmunoassay Ouabain 125 I radioimmunoassay kit (Biotop OY, Medipolis Center, Oulu, Finland), as described earlier.[22] Briefly, 1 ml samples were acidified with 0.2 ml 1 mol l^{-1} HCL then eluted with previously preconditioned (5 ml 2-propanol then 5 ml 0.1% aqueous trifluoroacetic acid) Sep-Pak C_{18} cartridges (Waters, Waters Corp., Waxford, Ireland). The first and second elutes (with 5 ml 0.1% trifluoroacetic acid) were discarded, then the ouabain compounds were dissolved using 2 ml 40% acetonitrile in 0.1% trifluoroacetic acid. The final elutes were evaporated in vacuum centrifuge and extracts were redissolved in 250 µl radioimmunoassay buffer. The intra- and interassay coefficients were 7.4 and 18.6%, respectively, linearity 10–100 pmol l^{-1}. The levels of urinary EO are presented as the daily (24-h) excreted level of EO. The average concentration of urinary EO was

Table 1 Characteristics of groups

	(1) HT (n = *10*)	*(2) HT+DM* (n = *11*)	*(3) HT+DM+CKD* (n = *10*)	*(4) HT+CKD* (n = *10*)
Sex (Male/female)	5/5	4/7	5/5	4/6
Age (years)	59.1 ± 6.8*	65.81 ± 8.6	69.5 ± 3.6	60.4 ± 7.9
BMI (kg m^{-2})	29.9 ± 5.9	29.53 ± 5.7	28.9 ± 3.9	28.7 ± 4.7
Duration of hypertension (years)	5.6 ± 2.1*	8.3 ± 2.3	9.6 ± 3.8	7.1 ± 3.1
Duration of type 2 DM (years)	—	6.1 ± 3.1	7.0 ± 2.7	—
Duration of CKD (years)	—	—	9.7 ± 3.2	8.6 ± 3.4
Se. Na^+ (mmol l^{-1})	139.8 ± 2.3	140.9 ± 2.8	140.9 ± 2.0	140.0 ± 2.2
Se. K^+ (mmol l^{-1})	4.3 ± 0.3	4.1 ± 0.4	4.4 ± 0.7	4.3 ± 0.4
Hb A_{1c} (%)	5.9 ± 0.25**	6.5 ± 1.0	6.2 ± 0.5**	5.5 ± 0.2
Creatinine clearance 24 h (ml min^{-1})	109.3 ± 31.7*,**	131.8 ± 24.3*,**	62.4 ± 18.9	66.9 ± 26.6
Urinary norepinephrine (nmol mmol^{-1} creat.)[a]	24.65 ± 13.41	27.63 ± 15.07	23.11 ± 14.89	23.94 ± 14.57
Urinary epinephrine (nmol mmol^{-1} creat.)[a]	6.85 ± 4.04	7.37 ± 3.41	6.31 ± 3.82	6.23 ± 2.80
Urinary dopamine (nmol mmol^{-1} creat.)[a]	154.07 ± 58.74*,**	111.98 ± 47.64	91.61 ± 46.23	86.18 ± 24.94
Plasma EO (pmol l^{-1})	14.76 ± 5.72	14.73 ± 5.13	19.79 ± 9.52	10.28 ± 5.32*
Urinary EO (pmol day^{-1})	102.46 ± 40.98	99.11 ± 25.60	97.67 ± 52.51	97.30 ± 21.17
EO clearance (ml min^{-1})	12.66 (16.97)	10.20 (20.59)	8.97 (10.11)	14.59 (17.34)
EO fractional excretion (%)	14.3 ± 8.7**	11.5 ± 9.0**	19.3 ± 17.6	31.2 ± 15.6
Se. pro-BNP (pg ml^{-1})	25.3 (78.4)*	76.9 (86.5)*	268.4 (228.4)	131.6 (354.7)
Intima-media thickness (mm)	0.64 ± 0.17	0.74 ± 0.22	0.88 ± 0.09	0.79 ± 0.27
Antihypertensive therapy				
ACE inhibitors (%)	80.0	72.7	100.0	90.0
ARB (%)	70.0	54.5	50.0	20.0
β- Blocker (%)	40.0	54.5	70.0	70.0
Ca channel blocker (%)	70.0	54.5	70.0	60.0
Diuretics (%)	40.0	54.5	80.0	40.0
Other therapy				
Oral antidiabetics (%)***	0.0	54.5	60.0	0.0
Insulin (%)***	0.0	45.5	50.0	0.0
Lipid-lowering drugs (%)***	40.0	90.9	90.0	30.0
Platelet aggregation blocker (%)***	20.0	81.8	80.0	70.0

Abbreviations: BMI, body mass index; BNP, brain natriuretic peptide; CKD, chronic kidney disease; creat., creatinine; DM, diabetes mellitus; EO, endogenous ouabain; Se., serum.
Data are presented as mean ± s.d., or as median and (interquartile range). The therapy of patients is presented with frequency of medication using during this study.
[a]Urinary catecholamines and creatinine were measured in morning fresh urine.
*$P<0.05$ compared with HT+DM+CKD.
**$P<0.05$ compared with HT+CKD.
***$P<0.05$ between groups by χ^2-test.

60.5 ± 17.9 pmol l^{-1}. All samples were measured in duplicates.

Urinary catecholamine and serum pro-BNP measurements

The morning urinary catecholamines (epinephrine, norepinephrine and dopamine) were measured by BIO-RAD Clinical HPLC System (BIO-RAD Laboratories, Ivry sur Seine, France). Briefly, the acidified urinary samples were prepared by Analytical Micro-Guard Cartridges and 20 µl prepared samples were analysed by HPLC with Model 1340C Electrochemical Detector (at 0.55 mV). Urinary catecholamines/creatinine (assessed by Jaffe methods) ratios were calculated.

Serum pro-BNP levels were measured on fully automatized (Elecsys 2010) system (Roche Diagnostics, Mannheim, Germany), which is an electrochemiluminescent sandwich immunoassay using polyclonal antibodies. Intraassay and interassay variabilities are 1.3–4.2 and 1.8–4.6%, respectively.[23]

Echocardiography and echo-tracking examination

Echocardiograms were recorded with an Aloka SSD 5500 (Aloka, Aloka Co. Ltd, Tokyo, Japan) ultrasound imaging system equipped with a 3.5 MHz transducer. All patients were assessed by the same cardiologist and examined within 1 h (between 0730 and 0830 h) after EO samples collecting and ABPM taking off. Before echocardiography and echo-tracking, all patients were supine position at least for 5 min. Operators were blinded to the status of the patients. Left ventricle (LV) internal diameter, septal and posterior wall thickness were measured at end-diastole and end-systole, according to the American Society of Echocardiography guidelines.[24] Left ventricular mass was calculated at end-diastole by using of Devereux correction according to the American Society of Echocardiography cube left ventricular formula. Individual values for LV mass were indexed by body surface area. LV end-diastolic and end-systolic volumes were obtained by using the Teicholz formula. Stroke volume was calculated as the differ-

ence between end-diastolic and end-systolic volumes. Ejection fraction was calculated as the ratio of stroke volume to LV end-diastolic volume. Individual values were indexed by body surface area (LV end-diastolic volume index (LVEDVI), left ventricular mass index (LVMI), LV end-systolic volume index (LVESVI), stroke index (SI)). Stroke index was multiplied by heart rate to obtain cardiac index. Blood pressure was recorded at the time of echocardiography. LV diastolic dysfunction was defined using transmitral Doppler parameters. Transmitral Doppler peak early diastolic E-wave velocity ($cm\,sec^{-1}$), peak late A-wave diastolic velocity ($cm\,sec^{-1}$), E/A ratio, deceleration time (msec) were recorded. The atrial areas (mm^2) were measured by the product of two atrial diameters.

Arterial stiffness parameters as the β-stiffness index (β), pulse wave velocity, and augmentation index were assessed by the characteristics of pulse wave intensity using echo-tracking system (Aloka SSD-5500, Aloka Co. Ltd) with a 10 MHz linear array probe. We used the right common carotid arterial diameter change waveforms to obtain pressure waveforms noninvasively. The peak and bottom values of a diameter change waveform were calibrated using systolic and diastolic pressure measured with a cuff-type manometer applied to the upper arm, and the diameter change waveform was used as a blood pressure waveform.[25]

IMT measurement

The IMT was measured in supine position by real-time ultrasound scanner equipped with a 10 MHz linear transducer (ALOKA SSD 4000, Aloka Co. Ltd). The measuring of IMT was made in B mode on carotid artery under the bifurcation of carotid artery in anterolateral longitudinal view.

Statistical analyses

Data are expressed as mean values ± s.d., or as median values and interquartile range. The distributions of variables were analysed using Kolmogorov–Smirnov non-parametric test. The normal variables were compared using one-way analysis of variance and the non-normal variables were compared with Kruskal–Wallis and Mann–Whitney *U*-test. The non-continuous parameters were analysed by χ^2 test. The correlations between variables were analysed using Pearson's (in cases of normal variables) and Spearmann's (non-normal variables) correlations. Stepwise multiple regression models were used to investigate the independent relationship between plasma, urinary EO and other parameters. The statistical analyses were made by SPSS 13.0 (SPSS Inc., Chicago, IL, USA) and two-tailed *P*-value of <0.05 was considered statistically significant.

Results

The basic characteristics of the patients are presented in Table 1.

There was no significant difference between the groups in the antihypertensive therapy of patients but the frequencies of the use of lipid-lowering drugs and the platelet aggregation blocker were different.

We found that the level of plasma EO was significantly higher in HT + DM + CKD group than in the HT + CKD group, and the difference remained significant ($P=0.046$) after adjusting for age. There were no differences in daytime or nighttime systolic, diastolic or mean arterial pressures or hypertensive time indices, echocardiographic or arterial stiffness parameters among the four groups. In all patients analysed together, plasma and urinary EO did not correlate with gender, age, body mass index and IMT or pro-BNP levels. In contrast, plasma EO showed significant positive correlations with nighttime blood pressures and hypertensive indices (*R*-values between 0.37 and 0.43, graph for nighttime mean arterial pressure is shown in Figure 1a) and a negative correlation with carotid artery augmentation index is depicted in Figure 1b.

The correlation between plasma EO and nighttime blood pressure was mainly attributable to the strong correlation in HT + DM + CKD group ($R=0.72$ for nighttime systolic blood pressure). In this group, there was also a strong negative correlation between plasma EO and the echocardiographic E/A ratio ($R=-0.67$).

Urinary EO had positive correlations with carotid artery pulse wave velocity (Figure 1c.), carotid artery β-stiffness (Figure 1d) and urinary norepinephrine excretion ($R=0.39$, $P=0.011$, not shown). The levels of plasma and urinary level of EO did not correlate with daytime blood pressure parameters either in the whole population or in the groups.

EO clearance and the fractional excretion rate of EO are presented in Table 1. Fractional excretion of EO was significantly higher in the HT + CKD group than in the HT and HT + DM groups.

Finally, we searched for independent predictors of the nighttime mean arterial pressure and β-stiffness of carotid artery in a multiple linear regression model. The independent predictors of nighttime mean arterial pressures were age and plasma EO. The independent predictor of carotid artery β-stiffness was urinary EO in the whole population (Table 2).

Discussion

In our study, the samples collection, echocardiography and echo-tracking measurements were completed in a short time, because of the levels of plasma EO strongly mirror the patient's current (night and early morning) cardiovascular status. The pathophysiological abnormalities of this period especially nighttime and morning hypertension are major risk factors for acute cardiovascular diseases, for example, myocardial infarction and stroke.[26]

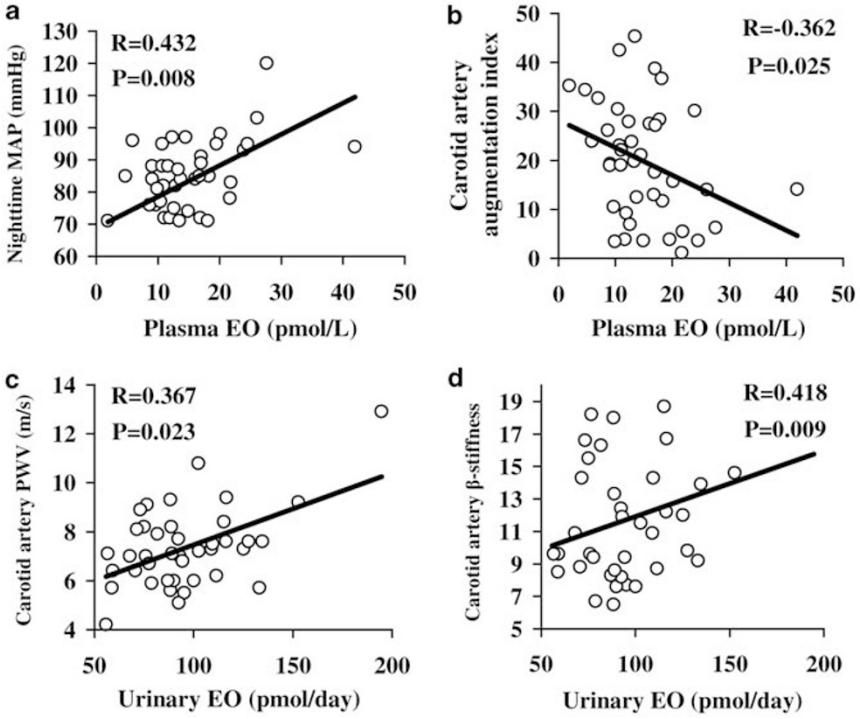

Figure 1 Correlations between plasma level of EO and nighttime mean arterial blood pressure (MAP) (**a**) and inverse correlation with augmentation index (Aix) of carotid artery (**b**). Correlations between daily urinary EO excretion and carotid artery pulse wave velocity (PWV) (**c**), and carotid artery β-stiffness (**d**) in the whole population.

Table 2 Results of stepwise linear regression of nighttime MAP, β-stiffness of carotid artery

Dependent predictor: nighttime MAP	*Independent predictor*	*Standard beta*	*Sig.*
All patients	Age	−0.426	0.005
	LVMI	0.375	0.016
	Plasma EO	0.426	0.004
Dependent predictor: β-stiffness of carotid artery	*Independent predictor*	*Standard beta*	*Sig.*
All patients	Urinary EO	0.412	0.011

Abbreviations: BMI, body mass index; E/A, the ratio between the early and late peak flow velocity; EO, endogenous ouabain; GFR, glomerular filtration rate; IMT, intima media thickness; LVMI, left ventricular mass index; MAP, mean arterial blood pressure; PWV, pulse wave velocity; TPR, total peripheral resistance.
The components of the model with dependent predictor of nighttime MAP were: LVMI, stroke index, TPR index, ejection fraction, E/A, urinary norepinephrine, IMT, β-stiffness, augmentation index and PWV of carotid artery, age, BMI, GFR, plasma EO and urinary EO.
The components of the model with dependent predictor of β-stiffness of carotid artery were: 24-h systolic and diastolic blood pressure, LVMI, stroke index, TPR index, ejection fraction, urinary norepinephrine, IMT, age, BMI, GFR, plasma EO and urinary EO.

The first main finding of this study is that the level of plasma EO associates with several parameters of nighttime blood pressure, therefore it may have a role in the pathogenesis of the nighttime hypertension. Significant correlations between nighttime blood pressure profile measured by ABPM, which is a more exact predictor of cardiovascular status than office blood pressure measurement,[27] and the level of EO were not verified previously in treated hypertensive patients. Similar significant correlations were found between plasma EO and nighttime blood pressure in untreated hypertensive patients.[28] One of the previous studies suggested that the production of digitalis-like substances is probably not constant during the day, but the physiological circadian regulation of EO production is poorly understood.[21]

The fractional excretions of EO were higher in CKD groups. Our results may suggest a role of natriuretic factor, EDLF in generation of higher nighttime blood pressure. In our study, the patients' daytime and nighttime urinary samples were not collected separately, so exact changes of EO excretion during the day remain unclear.

The level of plasma EO in healthy individuals was 9–12 pmol l^{-1}, assessed previously by the same method as we used in hypertensives.[29] In our study, the average level of plasma EO was 14.7 ± 5.7 pmol l^{-1}, nevertheless our patients were treated with RAAS blockers and/or β-blockers. In our study, the levels of EO were lower than in some studies in literature using other immunoassay methods (enzyme-linked immunosorbent assay, EIA), which are, however, not comparable with our results because of the use of different antibody and methods.[3]

Some papers have shown a modified nighttime blood pressure pattern in healthy persons and

patients with heart failure treated by exogenous cardiac glycoside presuming an elevated sympathetic tone in this population.[18–20] In our study, the excreted level of norepinephrine assessed in morning fresh urine, positively correlates with urinary level of EO. These observations support the theory that the level of plasma EO may have a regulatory role in nighttime blood pressure profile, probably with increased sympathetic activation in hypertensive patients untreated with cardiac glycosides.

EO take part in the development of hypertension through increased sympathetic tone, and in direct prohypertrophic effects on myocytes of heart and arteries, as well as causing collagen overproduction and cardiomyopathy in uraemic rat heart.[13,14] These processes can lead to cardiac hypertrophy *in vivo*.[17] In our study, we have found association between left ventricular diastolic dysfunction (E/A) and level of plasma EO in HT + DM + CKD group. Similar correlation was proved within normotensive offspring of parents with hypertension suggesting that the prolonged elevation of plasma EO level may have an effect on left ventricular diastolic dysfunction.[30]

A former study had shown that the plasma EO strongly correlates with several left ventricular parameters in patient with end-stage kidney disease,[2,3] and similar associations were proved in our study with urinary EO level indicating the role of kidney in the development of cardiac hypertrophy.

It is known that in rats ANP regulates brain and/or plasma level of Na^+/K^+-ATPase inhibitors.[31] On the basis of this observation, we hypothesized that the level of pro-BNP associates with the level of EO, but this direct relationship was not proved in treated hypertensives.

The other main results of our study suggest that the elevated plasma level of EO is associated with increased arterial stiffness. Relationship between non-invasive measurement of carotid artery stiffness (echo-tracking) including mainly pulse wave velocity (as a marker of aorta stiffness) and augmentation index (as a marker of resistance arteries vasoconstriction) and EO was not studied previously in treated hypertensive patients. It is known that the increased arterial stiffness is associated with insulin resistance and chronic renal failure and it is a risk factor for cardiovascular disease, including heart failure, myocardial infarction and stroke.[32–34] EDLF increases the calcium influx in vascular smooth muscle cells by blocking Na^+/K^+-ATPase and long-term ouabain administration and MBG stimulates fibroblast, which in turn leads to collagen accumulation in the wall of artery and in rats it causes stiffness of artery.[14,35] We found an inverse relationship between plasma EO and the augmentation index of carotid artery as well as positive correlations between levels of urinary EO and β-stiffness index and pulse wave velocity of carotid artery (Figure 1), which suggests that EO have a role in development of artery stiffness. Some studies have shown that the arterial stiffness relates with age, body mass index, systolic blood pressure, diabetes and hypertension and independently associates decreased glomerular filtration rate.[36,37] A decreased excretion of EO in the patients with diminished glomerular filtration rate may be a further component of relationship between the declining glomerular filtration rate and increased arterial stiffness.

According to our results, we conclude that the morning plasma EO level correlates with nighttime blood pressure profile and cardiac diastolic dysfunction, while the level of urinary EO correlates mainly with arterial stiffness.

A limitation of our study is its cross-sectional nature.

In conclusion, our results suggest that the elevated plasma EO may have an important role in nighttime blood pressure regulation, cardiac diastolic dysfunction and increased arterial stiffness. Correlations between the level of plasma and urinary EO and altered nighttime blood pressure profile suggest a new approach for investigators to focus on circadian rhythm of secretion of EDLFs in healthy individuals and hypertensive patients. Elevated level of EO in hypertensive patients with complications suggests that the conventional therapies of hypertension do not normalize the level of EO so that the antagonist of EO may be a new completion of hypertension therapy to reduce the subclinical organ damages.

What is known about this topic

- Endogenous ouabain is a predictor of essential hypertension in patients without treatment.
- Associations of the level of urinary endogenous ouabain were not investigated.
- Associations of endogenous ouabain and arterial stiffness were not studied previously.

What this study adds

- The independent predictor of nighttime blood pressure was level of plasma endogenous ouabain in treated hypertensive patients.
- Level of urinary endogenous ouabain independently associated with the parameters of arterial stiffness.

Conflict of interest

The authors declare no conflict of interest.

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