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UROCORTIN 2 IN PATIENTS WITH HYPERTENSION TREATED WITH ANGIOTENSIN CONVERTING ENZYME INHIBITORS OR ANGIOTENSIN RECEPTOR BLOCKERS

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Urocortin 2 (Ucn2) - corticotropin-releasing hormone receptor 2 signalling has favourable effects in the cardiovascular system, including vasodilation, lowering of blood pressure and systemic peripheral resistance, increase in cardiac output and cardiac contractility, as well as cardioprotection against ischemia-reperfusion injury. Vasodilation and lowering of blood pressure seem to be very interesting and important effects, but their mechanism and interaction with the antihypertensive drugs have not been evaluated. The aim of the present study was to assess the relationship between Ucn2 concentration and antihypertensive therapy in patients with primary hypertension. We examined a group of 65 patients with primary hypertension receiving at least 3 antihypertensive drugs. In all of them plasma level of Ucn2, anthropometric measurements, biochemical tests, ambulatory blood pressure monitoring (ABPM), and echocardiography were performed. There were no differences in Ucn2 level related to beta-blockers, calcium channel blockers or diuretics, but we observed that in patients treated with angiotensin converting enzyme inhibitors (ACEI) (n = 52) serum Ucn2 levels were significantly higher than in patients treated with angiotensin-receptor blockers (ARBs) (n = 13) (10.93 versus 5.56 ng/mL; $P < 0.05$). Moreover, we did not observe any differences in terms of blood pressure on ABPM, biochemical measurements, left ventricular mass index, or presence of diabetes. In addition, in a small subgroup receiving alpha-blockers we also found a lower level of Ucn2, with coexisting higher systolic blood pressure at night, higher left ventricle mass index (LVMI) and more frequent occurrences of diabetes compared to non-alpha-blockers. Our findings suggest that the hypotensive action of renin-angiotensin-aldosterone system blockade may be related to the urocortin system. Ucn2 may be an important element in the mosaic of blood pressure-lowering factors in patients treated for essential hypertension.

Key words: *urocortin 2, angiotensin converting enzyme inhibitor, angiotensin receptor blocker, blood presurre, arterial hypertension, antihypertensive pharmacotherapy, renin-angiotensin-aldosterone system*

INTRODUCTION

Urocortin 2 (Ucn2) is a 38aa vasoactive peptide belonging to the corticotropin-releasing hormone (CRH) family of peptides, together with Ucn1 and Ucn3. These peptides share structural similarities with CRH - a hormone playing a crucial role in regulating the body response to stress. Urocortins were first discovered in rats, and later found also in humans (1-3). The effects of urocortins are mediated by the G-protein -coupled CRH receptor 1 (CRH-R1) and CRH-R2 and Ucn2 binds selectively to CRH-R2 (4). Both the ligand and the receptor are widely distributed throughout the brain and peripheral tissues, with strong cardiac and vascular expression (5). A range of diverse biological effects of Ucns has been identified since their discovery, including protective cardiovascular effects (6, 7).

Recent studies have shown that urocortins cause vasodilation (8) and have positive inotropic and lusitropic effects (9, 10). Moreover, they have cardioprotective effects against ischemia-reperfusion injury (11, 12). Beneficial hemodynamic effects such as the lowering of blood pressure and systemic peripheral resistance, an increase in cardiac output and cardiac contractility were also reported (13, 14). Moreover, Ucn2 causes suppression of the renin-angiotensin system and may have effects on the sympathetic nervous system (15-17). This was documented in experimental animal models, in healthy subjects and in patients with heart failure (18).

Among these effects, the vasodilation caused by urocortins is considered the most important and prospective one (19-21). Chen *et al*. demonstrated that urocortins produced both endothelium-dependent and endothelium-independent smooth muscle relaxation in the human internal mammary artery (22). Though largely unknown, the mechanisms of urocortin-mediated vasodilation may be diverse (19, 20, 23, 24). It has also been reported that urocortins reduced plasma angiotensin II levels in a sheep heart failure model (15, 16). Furthermore, similarly to angiotensin-converting enzyme inhibitors (25), urocortins also showed protective effects on endothelial function (20, 26). These reports led to the hypothesis that there might be a relationship between urocortins and blood and/or tissue angiotensin converting enzyme (ACE) activity, thus suggesting that vasodilation caused by urocortins may be in part due to their effects on ACE activity and therefore on angiotensin II levels.

To our knowledge, no studies published to date have investigated the relationship between antihypertensive therapy and the level of Ucn2 in patients with essential hypertension.

The aim of our study was to explore the relationship between levels of Ucn2 and selected antihypertensive agents in patients treated for primary hypertension.

MATERIALS AND METHODS

Study population

Our study included 65 patients with essential hypertension. All participants were recruited from the Hypertension Outpatient Clinic and Primary Care Clinic of the University Hospital in Cracow. The inclusion criteria were: age over 18 years old, written informed consent, willingness for cooperation, essential hypertension currently treated with at least three antihypertensive agents in optimal doses including a drug acting on renin-angiotensin-aldosterone-system (RAAS) (an ACEI or an ARB). The exclusion criteria were: secondary hypertension, systolic heart failure, chronic kidney disease, inflammation, neoplasm.

The study protocol was approved by the Bioethical Committee of Jagiellonian University Medical College (nr. 151/B/2012).

Study protocol

All patients completed a questionnaire including history of hypertension, current antihypertensive treatment, other medications, comorbidities, and tobacco smoking. The examination consisted of anthropometric measurements, and subsequently body mass index (BMI) and waist to hip ratio (WHR) were calculated.

Blood pressure measurements

Office blood pressure (BP) measurements and 24-hours ambulatory blood pressure monitoring (ABPM) were performed according to the ESH guidelines (27). The validated devices for ABPM (Spacelabs 90207 and 90217; Spacelabs Healthcare Inc., Washington, USA) were programmed to obtain BP readings every 20 minutes during the day (from 6 am to 10 pm) and every 30 minutes during the night (from 10 pm to 6 am). ABPM data were valid if the number of successful readings exceed 70%. Daytime and nighttime periods were defined based on the subject's diary. Hypertension was defined as treatment-resistant if averaged BP level was ≥ 130/80 mmHg during 24-hour and/or ≥ 135/85 mmHg during the daytime and/or ≥ 120/70 mmHg during the nighttime.

Office BP measurements were performed twice during one visit in 1 – 2-minute interval, in a sitting position, after a 10 minute rest, using a validated device (OMRON 705 IT, Omron Healthcare, Japan).

Blood analyses

We measured serum biochemical parameters after ten hours of fasting. The measurements included plasma aldosterone and plasma renin activity (PRA). We also assessed: a complete blood count with differential count, serum levels of glucose, creatinine, cholesterol, glycated haemoglobin (HbA1c), insulin, high sensitivity C-reactive protein (hsCRP), and N-terminal prohormone of brain natriuretic peptide (NTproBNP). Glomerular filtration rate was estimated using the MDRD equation. Diabetes was defined on medical history and a presence of hypoglycaemic therapy.

Urocortin 2 measurements

Duplicate measurements of serum Ucn2 levels were performed using Ucn2 ELISA Kit according to the manufacturer's instructions (Cloud-Clone, USA). Ucn2 serum (100 µl) was used per well. The readings were made at 450 nm and 2 Parametric Linear fit was used for the calculation of the results. The minimum detectable amount of Ucn2 was less than 48.6 pg/mL.

Left ventricle assessment

Standard echocardiography was performed in each patient using the Vivid 3 device (General Electric, Fairfield, Connecticut). Left ventricular volume was measured in apical 4-chamber view using a modified Simpson's rule. Data were stored digitally for subsequent analysis of left ventricle ejection fraction (LVEF), left ventricle mass index (LVMI) and left atrium volume index (LAVI).

Statistical analysis

The data were summarised as mean ± standard deviations (SD) for variables with normal distribution and as median (25^{th} – 75^{th} percentiles) for variables with skewed distribution or ordinal variables. The normality of distribution was confirmed using the Shapiro-Wilk test. Categorical data were presented as numbers and percentages. Independent groups were compared using Student's t-test or the Wilcoxon rank-sum test. To compare proportions, the Chi-square and Fisher exact tests were used. The relationships between Ucn2 concentrations and other measurements were assessed using the Spearman correlation coefficient. Two-sided P-value < 0.05 was considered significant. All statistical analyses were performed using SAS v.9.3 software (SAS Institute Inc., Cary, NC, USA) licensed to the Jagiellonian University, Cracow, Poland.

RESULTS

Baseline characteristic of the studied population

The median age of the 65 studied patients was 58 years, and men accounted for 49% of the subjects (*Table 1*). The median period of hypertension duration was 10 years. Almost 39% of the group had treatment-resistant hypertension. In the studied population, the most commonly used antihypertensive agents were, in order of occurrence: diuretics, ACEIs and calcium-channel blockers. Only 15% were treated with alpha-blockers (*Table 1*).

Correlation analysis revealed no correlations between Ucn2 levels and analysed variables including age, duration of hypertension, BP values measured using ABPM, LVEF, LVMI. Moreover, no significant correlations were found between Ucn2 and RAS parameters (PRA, aldosterone) as well as other biochemical parameters (glucose, HbA1C, insulin, eGFR, NTproBNP, hsCRP).

Relationship between urocortin 2 level and type of pharmacotherapy

Each participant of the study received a drug acting on RAAS - it was ACEI (n = 52) or ARB (n = 13). When the concentration of Ucn2 was analysed according to antihypertensive therapy (*Table 2*), we observed that in patients treated with ACEIs serum Ucn2 levels were significantly higher than in patients treated with ARBs (10.93 versus 5.56 ng/mL; $P < 0.05$). We also observed

Table 1. Baseline characteristic and antihypertensive therapy of studied group.

Parameter	**n = 65**
Cardiovascular risk factors	
Age [years]	58 (50 – 63)
Male [n, %]	32 (49.2)
BMI [kg/m^2]	29.5 ± 4.2
WHR	0.94 ± 0.08
Tobacco smoking [n, %]	12 (18.5)
SBP-24h [mmHg]	126 (119 – 134)
DBP-24h [mmHg]	75.5 ± 8.4
Diabetes [n, %]	18 (27.7)
Pharmacotherapy	
Statin [n, %]	36 (55.4)
ACEi [n, %]	52 (80)
ARB [n, %]	13 (20)
Calcium-channel blocker [n, %]	45 (69.2)
Beta-blocker [n, %]	40 (61.5)
Diuretic [n, %]	61 (93.8)
Alpha-blocker [n, %]	10 (15.4)
Echocardiography	
EF [%]	70.2 ± 8.8
LVMI [g/m^2]	99.7 ± 20.4
LAVI [mL/m^2]	21.6 ± 5.5
Biochemistry	
Ucn2 [ng/ml]	8.75 (2.99 – 14.16)
NTproBNP [pg/mL]	56.2 (36.4 – 128.8)
PRA [ng/mL/h]	2.1 (0.77 – 4.45)
Aldosterone [pg/mL]	143.5 (86.5 – 218.3)
Glucose [mmol/L]	5.6 (5.1 – 6.3)
Total cholesterol [mmol/L]	4.9 ± 0.97
Insulin [mmol/L]	12.0 (8.2 – 17.8)
hsCRP [mg/dL]	1.2 (0.75 – 3.07)
HbA1c [%]	5.9 (5.5 – 6.2)
Cortisol [µg/dL]	15.7 (12.8 – 20.5)
eGFR [MDRD]	86.8 ± 15.7

Data are presented as means ± standard deviation, median and (upper-lower quartile) or numbers (percentages).
Abbreviations: ACEI, angiotensin converting enzyme inhibitor; ARB, angiotensin II receptor blocker; BMI, body mass index; DBP-24h, 24hours diastolic blood pressure; eGFR, estimated glomerular filtration rate (using the MDRD, Modification of Diet in Renal Diseases equation); HbA1c, glycated haemoglobin; hsCRP, high sensitivity C-reactive protein; LAVI, left atrium volume index; LVEF, left ventricular ejection fraction; LVMI, left ventricular mass index; NTproBNP, N-terminal prohormone of brain natriuretic peptide; PRA, plasma renin activity; SBP-24h, 24 hours systolic blood pressure; Ucn2, urocortin 2; WHR, waist to hip ratio.

differences in Ucn2 levels according to the usage of alpha-blockers. However, only 15% of the studied group were treated with alpha-blockers.

There were no differences in Ucn2 levels depending on use of beta-blockers, calcium channel blockers or diuretics.

It should be noted that there was no patient who would not receive a drug acting on RAAS, each participant received ACEI (n = 52) or ARB (n = 13). In view of the observed significant difference in the concentration of Ucn2 between patients receiving ARB and ACEI, a comparative analysis of these groups was conducted. No differences of analysed parameters were found between such isolated groups (*Table 3*).

Moreover, no differences between these groups were found with respect to LVEF, LAVI, level of NTproBNP, CRP, HbA1C, presence of diabetes, use of statins and other antihypertensive agents. The proportions of patients with treatment-resistant hypertension were similar in both groups.

The most commonly used drugs from the ACEI group were ramipril 10 mg and perindopril 10 mg. Amongst ARB, valsartan 160 mg was the most widely used. (*Table 4* and *Table 5*).

Considering the significant difference in Ucn2 concentration, we turned our attention to the group of alpha-blocker users and compared them with patients who did not receive this medication.

It was a small group of patients (n = 10, which is 15% of the analysed population), in which there was a significantly lower level of Ucn2. In addition, subjects treated with alpha-blockers exhibited resistant hypertension more often, and took more

Table 2. Ucn2 level according to antihypertensive therapy.

Medication		**Median Ucn 2** (ng/ml)	**P**
ACEI (n = 52)		10.93 (4.17 – 16.46)	0.027
ARB (n = 13)		5.56 (1.76 – 10.42)	
Calcium-channel blocker	Yes (n = 45)	8.17 (2.88 – 14.16)	0.532
	No (n = 20)	9.67 (5.7 – 14.48)	
Beta-blocker	Yes (n = 40)	6.91 (2.97 – 14.09)	0.420
	No (n = 25)	11.14 (4.17 – 15.34)	
Diuretic	Yes (n = 61)	8.16 (2.91 – 14.15)	0.353
	No (n = 4)	12.52 (7.55 – 19.13)	
Alpha-blocker	Yes (n = 10)	2.29 (1.39 – 8.57)	0.009
	No (n = 55)	10.67 (4.90 – 14.86)	

Abbreviations: ACEI, angiotensin converting enzyme inhibitor; ARB, angiotensin II receptor blocker.

Table 3. Characteristics of patients with hypertension treated ACEIs and ARBs.

Parameter	**ACEI** (n = 52)	**ARB** (n = 13)	**P**
Age [years]	58.5 (46.5 – 63.0)	57.5 (53.5 – 63.0)	0.531
Male [n, %]	29 (56)	3 (23)	0.925
BMI [kg/m^2]	29.3 ± 4.4	30.0 ± 3.3	0.322
SBP-24h [mmHg]	126 (119 – 133.5)	124 (118 – 141)	0.971
DBP-24h [mmHg]	75.65 ± 8.11	74.77 ± 9.96	0.345
LVEF [%]	69.5 ± 9.0	73.1 ±7.8	0.651
LVMI [g/m^2]	100.0 ± 20.5	98.8 ± 20.9	0.697
Glucose [mmol/L]	5.6 (5.1 – 6.2)	5.57 (4.74 – 6.25)	0.618
Insulin [mmol/L]	11.76 (7.65 – 17.76)	12.57 (10.12 – 17.42)	0.866
Total cholesterol [mmol/L]	4.9 ± 1.02	4.94 ± 0.77	0.495
eGFR [MDRD]	87.37 ± 15.4	84.3 ± 17.3	0.678
Cortisol [µg/dL]	16.22 (13.2 – 20.4)	15.69 (11.0 – 21.06)	0.491
Aldosterone [pg/mL]	140.8 (88.7 – 201.8)	179.4 (113.8 – 286.4)	0.446
PRA [ng/mL/h]	2.3 (0.77 – 5.17)	1.78 (0.57 – 3.78)	0.423

Data are presented as mean ± standard deviation (SD) or median (upper-lower quartile).
Abbreviations: ACEI, angiotensin converting enzyme inhibitor; ARB, angiotensin II receptor blocker; BMI, body mass index; DBP-24h, 24hours diastolic blood pressure; eGFR, estimated glomerular filtration rate (using the MDRD equation); LVEF, left ventricle ejection fraction; LVMI, left ventricular mass index; PRA, plasma renin activity; SBP-24h, 24hours systolic blood pressure.

Table 4. ACEI and ARB used in the study group.

Medication	**Median dose** [mg]	N = 65
ACEI		N = 52
ramipril	10	26
perindopril	10	16
enalapril	20	1
trandolapril	2	1
lisinopril	20	5
zofenopril	30	3
ARB		N = 13
valsartan	160	11
losartan	100	2

Abbreviations: ACEI, angiotensin converting enzyme inhibitor; ARB, angiotensin II receptor blocker.

hypotensive drugs. In addition, they were characterised by higher SBP at night, higher LVMI, and diabetes was more common, compared to non-alpha-blockers.

No differences were observed in the scope of other analysed variables.

DISCUSSION

In principle, there are no data in the literature regarding the effects and levels of Ucn2 and antihypertensive therapy in patients treated for cardiovascular diseases. The results that are presented are sparse and concern only the animal model of heart failure, whereas there is a lack of such data in individuals with hypertension.

A New Zealand Christchurch research group has revealed that Ucn2 cotreatment with furosemide administered in sheep with heart failure enhanced hemodynamic and renal function and diuretic responsiveness (without additional potassium depletion) better than furosemide alone (28).

Table 5. Other hypotensive drugs used in the ACEI and ARB groups.

Medication	**ACEI** (n = 52)	**ARB** (n = 13)	**P**
Statins [n(%)]	27 (52)	9 (69)	0.322
Calcium antagonists [n (%)]	36 (69)	9 (69)	1.000
Beta-blockers [n (%)]	32 (62)	8 (62)	1.000
Metoprolol [n (%)]	10 (21)	2 (15)	1.000
Bisoprolol [n (%)]	14 (27)	3 (23)	1.000
Nebivolol [n (%)]	6 (12)	2 (23)	0.368
Diuretics [n (%)]	48 (92)	13 (100)	0.697
Hydrochlorothiazide [n (%)]	18 (35)	7 (54)	0.234
Indapamide [n (%)]	29 (56)	7 (54)	0.852
Furosemide [n (%)]	1 (2)	1 (8)	0.363
Spironolactone [n (%)]	2 (4)	1 (8)	0.471
Alpha-blocker	6 (12)	4 (31)	0.101

Abbreviations: ACEI, angiotensin converting enzyme inhibitor; ARB, angiotensin II receptor blocker.

Table 6. Characteristics of patients with hypertension treated with alpha-blockers and without alpha- blockers.

Parameter	**Alpha-blockers (+)** (n = 10)	**Alpha-blockers (–)** (n = 55)	**P**
Age [years]	49.7 ± 7.65	55.7 ± 11.0	0.123
Male [n, %]	6 (60)	26 (47)	0.511
BMI [kg/m^2]	32.1 ± 3.32	29.0 ± 4.19	0.038
SBP-24h [mmHg]	134.3 ± 12.13	126 ± 12.44	0.077
DBP-24h [mmHg]	77.5 ± 7.70	75.10 ± 8.57	0.164
SBP-night24h [mmHg]	124.3 ± 11.33	113.6 ± 13.95	0.026
LVMI [g/m^2]	112.8 ± 15.15	97.6 ± 20.45	0.049
Glucose [mmol/L]	5.70 (5.3 – 6.8)	5.53 (4.91 – 6.25)	0.300
Total cholesterol [mmol/L]	5.14 ± 1.26	4.87 ± 0.91	0.435
eGFR [MDRD]	88.60 ± 17.21	86.47 ± 15.57	0.709
Diabetes [n, %]	6 (60)	12 (22.6)	0.026
Aldosterone [pg/mL]	249.5 ± 190	168.7 ± 125.6	0.100
PRA [ng/mL/h]	1.9 (0.2 – 4.7)	2.14 (0.8 – 4.3)	0.423
Resistant hypertension [n, %]	8 (80)	17 (30.9)	0.005
Number of hypotensive agents	4.5 (4 – 5)	3 (3 – 3)	0.000
Ucn2 [ng/ml]	2.29 (1.39 – 8.57)	10.67 (4.90 – 14.86)	0.009

Data are presented as means ± standard deviation, median and (upper-lower quartile) or numbers (percentages).
Abbreviations: BMI, body mass index; eGFR, estimated glomerular filtration rate (using the MDRD, Modification of Diet in Renal Diseases equation); LVMI, left ventricular mass index; DBP-24h, 24hours diastolic blood pressure; SBP-24h, 24 hours systolic blood pressure; SBP-night24h, mean night systolic blood pressure; PRA, plasma renin activity; Ucn2, urocortin 2.

Rademaker *et al*. studied the combined administration of Ucn2 and captopril, an ACEI, in an experimental heart failure model in sheep. The effects of Ucn2 and captopril were assessed both jointly and separately. Ucn2 and captopril alone increased cardiac output (CO) and reduced BP, left atrial pressure (LAP) and peripheral vascular resistance (PVR) compared with controls. Compared with either treatment alone, the combined treatment further improved cardiac CO and reduced cardiac preload, with no further falls in BP (29). The same research group has also found that Ucn2 cotreatment with a mineralocorticosteroid receptor antagonist - canrenoic acid in animal heart failure model further improved hemodynamics relative to that achieved by canrenoic acid alone, while also reducing PRA, angiotensin II, aldosterone and vasopressin levels, and enhancing renal function (30).

Ucn2 coupled with beta-blockade in experimental heart failure improves CO and mean arterial pressure (MAP), sustains HR, reduces PVR, LAP, aldosterone level and augments renal function (31).

The *Table 7* presents a summary of the studies carried out on animal models assessing the effects of Ucn2 and drugs acting on various parameters of the cardiovascular system. Available data refer to the animal model of heart failure.

To our knowledge, it is the first study analysing Ucn2 concentration in patients treated for hypertension. Our study demonstrated that Ucn2 levels in patients with hypertension

Table 7. Combination of Ucn2 and other medications in animal models with cardiovascular disease.

Combination	Actions	Ref.
Ucn2 + ACEI	BP ↓; vasopressin ↓; renin ↓; aldosterone ↓; endothelin 1↓	(30)
Ucn2 + ARI	SVR ↓; right atrium pressure ↓; diuresis ↑; sodium excretion ↑; potassium levels ↓; vasopressin ↓;renin ↓;aldosterone ↓; Ang II ↓	(31)
Ucn2 + metoprolol	Cardiac output ↑; SVR ↓	(32)
Ucn2 + furosemide	BP ↓; vasopressin ↓; renin ↓; natriuretic peptides ↓; diuresis ↑, sodium excretion ↑	(29)

Abbreviations: ACEI, angiotensin-converting enzyme inhibitor; Ang II, angiotensin II; ARI, aldosterone receptor inhibitor; BP, blood pressure; RA, right atrium; SVR, systemic vascular resistance, Ucn2, urocortin2.

treated with ACEIs were higher than in those treated with ARBs. Also the group of patients receiving alpha-blockers was characterised by a lower level of Ucn2 comparing to subject without alpha-blockers. No other antihypertensive drugs such as diuretics, calcium channel blockers or beta-blockers were related to the Ucn2 concentration.

Urocortin 2 and renin-angiotensin-aldosterone-system

Interestingly, the groups treated with ACEIs and ARBs did not show any significant differences with respect to age, sex, biochemical parameters, BP values, severity of organ damages, concomitant medications or comorbidities except the Ucn2 level just mentioned.

This may suggest a possible association between the Ucn2 effects and RAAS activation/suppression.

It has been demonstrated that urocortin produced both endothelium-dependent and -independent vasodilation (22). The endothelium-dependent component of vasodilation is primarily mediated through endothelial nitric oxide (NO) that most likely causes cGMP-dependent activation of potassium and calcium channels in vascular smooth muscle cells (20, 22, 32).

The endothelium-independent component of relaxation is produced by urocortins *via* different mechanisms, namely Ca^{2+} dependent potassium channel activation (22, 33), nickel-sensitive Na^{+}-Ca^{2+} exchanger (19), cAMP-dependent signalling pathway (23), and activation of MAPK pathway (34).

Interesting data is provided by research conducted by Yang *et al*. demonstrating the interaction of Ucn2 and ACEIs. The results of their study suggest that effects of ACEIs and Ucn2 may be similar in many aspects. The authors investigated the relationship between urocortin and the activity of ACE. They assessed short-term and long-term administration of urocortin to Sprague-Dawley (SD) rats and measured serum ACE levels. Urocortin reduced serum ACE levels one hour after administration. Long-term administration of urocortin was associated with an increase in ACE activity, but serum ACE concentrations continued to be low. Corticotropin releasing factor (CRF) receptor blocker, astressin, abolished the effects of urocortin. Extracellular signal-regulated kinase 1/2 (ERK1/2) pathway blocker, PD98059, also markedly inhibited these effects, suggesting that in rats urocortin affects the activity of ACE through the ERK1/2 pathway. These findings support the changes in MAP following short-term and longer administration of urocortin (35). This suggests that alterations in ACE activity and angiotensin II production may be in part responsible for vasodilation caused by urocortin.

Another study by this group of researchers investigated the effects of chronic administration of urocortin on ACE and other components of RAAS. The authors administered urocortin to spontaneously hypertensive rats (SHR) for 8 weeks and measured serum and tissue ACE, angiotensin II, nitric oxide, angiotensin(1-7), and tissue chymase levels. They found reduced serum ACE levels and elevated tissue ACE levels. Administration of urocortin caused reduction in serum and tissue angiotensin II levels, but NO and angiotensin (1-7) levels increased in a concentration-dependent manner. Moreover, an essential decrease in systolic BP was seen after a long-term administration of urocortin and a significant positive correlation between serum ACE level and systolic BP was found. Pretreatment with astressin, a CRF blocker, and PD98059, an ERK1/2 pathway blocker, abolished these effects of urocortin. These findings also support the hypothesis that changes of ACE activity and other RAAS components may play a role in vasodilation caused by urocortin *via* the activation of the ERK1/2 pathway (36).

The half-life of Ucn-2 is approximately 10 min in healthy humans (1). Administration of Ucn-2 was associated with an increase in ACE activity (35). We hypothesize that inhibition of ACE by ACEI may be connected with decrease inactivation of endogenic Ucn-2, because our patients treated with ARB presented low levels Ucn2. However, it needs further studies.

It is worth noting that RAAS blockade, at various stages, leads to beneficial haemodynamic effects, but also improves oxidative stress parameters as well as antithrombotic parameters. In the experimental study in hypertensive rats authors considered the beneficial antiplatelet and antioxidative effects of combined treatment of spironolactone and quinapril administration on thrombosis, hemostasis and oxidative stress (37). In turn, Deska and Nowicki demonstrated in their work the interesting effect of treatment with ACEI administered alone or in a combination with statin on changes in serum potassium concentration suggesting that these treatments are safe and do not increase the potential risk of hyperkaliemia in hypertensive patients (38). Honjo *et al*. reported in their research that endothelial urocortin was upregulated by inflammatory cytokines and pitavastatin and suppressed reactive oxygen species production in human umbilical vein endothelial cells. Treatment with pitavastatin (healthy volunteers) increased the serum urocortin level in human subjects. Thus, endothelial urocortin might protect cardiomyocytes in inflammatory damages. Urocortin might partly explain the mechanisms of various pleiotropic effects of statins (39). The significance of urocortin in cardioprotection regarding the area of hemodynamics, as well as oxidative stress and hemostasis is presented in numerous reviews (1, 18).

Urocortin 2 and alpha-blockers

A comparative analysis of only ten patients receiving alpha-blockers, with others not taking these medications, revealed that apart from a significantly lower Ucn2 level, subjects treated with alpha-blockers more often had resistant hypertension, and took

more hypotensive drugs. They were also characterised by higher systolic blood pressure at night, higher LVMI and a more frequent occurrence of diabetes compared to non-alpha blockers. The explanation of this phenomenon may be multifactorial. We can consider a possible relationship between the mechanism of alpha blockade and plasma Ucn2 concentration. Also, the low Ucn2 level in this group may be secondary to a very high global cardiovascular risk, advanced hypertensive organ damages, difficult to control hypertension, and multidrug therapy. The alpha-blocker in these patients was another added antihypertensive agent - as a third-line therapy for resistant hypertension.

The blocking of post-synaptic alpha1 receptors causes diastole both of arterial vessels and capacitive veins. Alpha-adrenergic blockers inhibit the reaction of adrenergic receptors to catecholamines. This class of drugs is particularly beneficial to patients in whom increased vascular resistance is due to increased smooth muscle tone (40).

In von Kanel's study, the blockade of α-adrenergic receptors in normotensive humans partly attenuated norepinephrine cardiovascular effects during mental stress and could be implied to have preventive potential in susceptible individuals (41). Charles *et al*. in their study on animal model have reported the effects of Ucn2 on sympathetic nerve activity. Intravenous Ucn2 administration to conscious sheep induced a potent inhibition of sympathetic traffic (17). Fetterly and coworkers in their work on an experimental animal model showed that it is possible to influence the adrenergic alpha receptor on stress axis activity. Activation of alpha receptors inhibits the activation of corticotropin releasing factor (CRF) signaling and CRF neurons in the bed nucleus of the stria terminalis. It can therefore be assumed that their inhibition of alpha receptors will be associated with the activation of the CRF system and the same may modulate the urocortin system (42). Determining whether the low level of Ucn2 in the group of alpha-blockers results from the effect of the drug, or is secondary to the higher percentage of refractory hypertension, diabetes, worse control of systolic blood pressure at night, greater LVMI or more antihypertensive drugs co-existing in this group is not possible in such a small sample and requires further investigations.

Other hypotensive treatment and urocortin 2

To date, no studies were identified assessing relationships between angiotensin AT1 receptor blocker and Ucn2 effects. A hypothesis assuming that different points of blockade of RAAS by ACEIs and ARBs is related to changes in plasma Ucn2 levels needs further study and verification. Also, no studies have been identified analysing the relationship between calcium blocker and Ucn2 nor thiazide diuretics and Ucn2.

Limitations and strength of this study

Our study has several limitations. The population of patients was small, and the number of patients treated with ACEIs was significantly higher than those treated with ARBs. Our results need to be confirmed in a larger population.

It should also be noted that apart from the agents affecting RAAS the patients also received other drugs, including diuretics, calcium channel blockers, beta-blockers, alpha-blockers, and statins (*Tables 5* and *6*). Possible effects of these agents on the urocortin system must be taken under consideration, although in our study there were no differences between the groups with respect to therapy using these drugs.

This study was designed as cross-sectional. In this type of study, it is impossible to assess the direction of causality between measured variables. The cross-sectional design of the current study, although cost-effective and convenient, precludes the determination of causality. Unfortunately, revealing cause-and-effect relationships between measured variables seems to be crucial to fully understand results. Therefore, we are convinced that future research in this field should consider longitudinal data.

We are aware that the results should be treated with caution, but at the same time we pay attention to the growing number of publications on the beneficial effects of Ucn2 and its involvement in the pathophysiology of the cardiovascular system.

Our study is the first to present Ucn2 serum concentration in a group of patients treated for hypertension. The observed phenomenon of higher Ucn2 plasma level in the group treated with ACEIs versus ARB is an interesting element in the understanding and broadening of knowledge about the effects of Ucn2 in the group of hypertensive patients. Different levels of serum Ucn2 in ACEI and ARB groups may suggest a significant role of Ucn2 in the mechanisms of RAAS blockade in patients with hypertension. The observed lower levels of Ucn2 in the group of patients on alpha-blockers indicate a need for further research, as it is not possible to determine whether the low level of Ucn2 can be attributed to taking alpha-blockers or to the advanced stage of arterial hypertension.

Our findings support the results of previously published experimental studies on the role of Ucn2 and RAAS system blockade. Ucn2 may be an important element in the mosaic of blood pressure-lowering factors in patients treated for essential hypertension.

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REFERENCES

1. Rademaker MT, Richards AM. Urocortins: actions in health and heart failure. *Clin Chim Acta* 2017; 474: 76-87.
2. Vaughan J, Donaldson C, Bittencourt J, *et al*. Urocortin, a mammalian neuropeptide related to fish urotensin I and to corticotropin-releasing factor. *Nature* 1995; 378: 287-292.
3. Donaldson CJ, Sutton SW, Perrin MH, *et al*. Cloning and characterization of human urocortin. *Endocrinology* 1996; 137: 2167-2170.
4. Hsu SY, Hsueh AJ. Human stresscopin and stresscopin-related peptide are selective ligands for the type 2 corticotropin-releasing hormone receptor. *Nat Med* 2001; 7: 605-611.
5. Wiley KE, Davenport AP. CRF2 receptors are highly expressed in the human cardiovascular system and their cognate ligands urocortins 2 and 3 are potent vasodilators. *Br J Pharmacol* 2004; 143: 508-514.
6. Parkes DG, May CN. Urocortin: a novel player in cardiac control. *News Physiol Sci* 2000; 15: 264-268.
7. Huang Y, Yao X, Lau C, Chan Y, Tsang S, Chan FL. Urocortin and cardiovascular protection. *Acta Pharmacol Sin* 2004; 25: 257-265.
8. Terui K, Higashiyama A, Horiba N, Furukawa KI, Motomura S, Suda T. Coronary vasodilation and positive inotropism by urocortin in the isolated rat heart. *J Endocrinol* 2001; 169: 177-183.
9. Davis ME, Pemberton CJ, Yandle TG, *et al*. Urocortin 2 infusion in healthy humans. Hemodynamic, neurohormonal, and renal responses. *J Am Coll Cardiol* 2007; 49: 461-471.
10. Davis ME, Pemberton CJ, Yandle TG, *et al*. Urocortin 2 infusion in human heart failure. *Eur Heart J* 2007; 28: 2589-2597.

11. Scarabelli TM, Pasini E, Stephanou A, *et al*. Urocortin promotes hemodynamic and bioenergetic recovery and improves cell survival in the isolated rat heart exposed to ischemia/reperfusion. *J Am Coll Cardiol* 2002; 40: 155-161.
12. Barry SP, Lawrence KM, McCormick J, *et al*. New targets of urocortin-mediated cardioprotection. *J Mol Endocrinol* 2010; 45: 69-85.
13. Gu Y, Zhang K, Biswas N, *et al*. Urocortin 2 lowers blood pressure and reduces plasma catecholamine levels in mice with hyperadrenergic activity. *Endocrinology* 2010; 151: 4820-4829.
14. Dieterle T, Meili-Butz S, Buhler K, *et al*. Immediate and sustained blood pressure lowering by urocortin 2 a novel approach to antihypertensive therapy? *Hypertension* 2009; 53: 739-744.
15. Rademaker MT, Charles CJ, Espiner EA, *et al*. Urocortin has beneficial hemodynamic, endocrine and renal effects in experimental heart failure. *J Hypertens* 2002; 20: S306-S306.
16. Rademaker MT, Charles CJ, Espiner EA, Frampton CM, Lainchbury JG, Richards AM. Endogenous urocortins reduce vascular tone and renin-aldosterone/endothelin activity in experimental heart failure. *Eur Heart J* 2005; 26: 2046-2054.
17. Charles CJ, Jardine DL, Rademaker MT, Richards AM. Urocortin 2 induces potent long-lasting inhibition of cardiac sympathetic drive despite baroreflex activation in conscious sheep. *J Endocrinol* 2010; 204: 181–189.
18. Walczewska J, Dzieza-Grudnik A, Siga O, Grodzicki T. The role of urocortins in the cardiovascular system. *J Physiol Pharmacol* 2014; 65: 753-766.
19. Lau CW, Chan YC, Yao X, Chan FL, Chen ZY, Huang Y. Nickel inhibits urocortin-induced relaxation in the rat pulmonary artery. *Eur J Pharmacol* 2004; 488: 169-172.
20. Huang Y, Chan FL, Lau CW, *et al*. Urocortin-induced endothelium-dependent relaxation of rat coronary artery: role of nitric oxide and K^+ channels. *Br J Pharmacol* 2002; 135: 1467-1476.
21. Miki I, Seya K, Motomura S, Furukawa KI. Role of corticotropin-releasing factor receptor type 2 beta in urocortin-induced vasodilation of rat aortas. *J Pharmacol Sci* 2004; 96: 170-176.
22. Chen ZW, Huang Y, Yang Q, Li X, Wei W, He GW. Urocortin-induced relaxation in the human internal mammary artery. *Cardiovasc Res* 2005; 65: 913-920.
23. Huang Y, Chan FL, Lau CW, *et al*. Roles of cyclic AMP and Ca^{2+}-activated K^+ channels in endothelium-independent relaxation by urocortin in the rat coronary artery. *Cardiovasc Res* 2003; 57: 824-833.
24. Schilling L, Kanzler C, Schmiedek P, Ehrenreich H. Characterization of the relaxant action of urocortin, a new peptide related to corticotropin-releasing factor in the rat isolated basilar artery. *Br J Pharmacol* 1998; 125: 1164-1171.
25. Chappell MC. Biochemical evaluation of the renin-angiotensin system: the good, bad, and absolute? *Am J Physiol Heart Circ Physiol* 2016; 310: H137-H152.
26. Brar BK, Jonassen AK, Egorina EM, *et al*. Urocortin-II and urocortin-III are cardioprotective against ischemia reperfusion injury: an essential endogenous cardioprotective role for corticotropin releasing factor receptor type 2 in the murine heart. *Endocrinology* 2004; 145: 24-35.
27. Mancia G, Fagard R, Narkiewicz K, *et al*. 2013 ESH/ESC guidelines for the management of arterial hypertension: the Task Force for the Management of Arterial Hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). *Eur Heart J* 2013; 34: 2159-21219.
28. Rademaker MT, Charles CJ, Nicholls MG, Richards AM. Urocortin 2 inhibits furosemide-induced activation of renin and enhances renal function and diuretic responsiveness in experimental heart failure. *Circ Heart Fail* 2009; 2: 532-540.
29. Rademaker MT, Charles CJ, Nicholls MG, Richards AM. Urocortin 2 combined with angiotensin-converting enzyme inhibition in experimental heart failure. *Clin Sci* 2008; 114: 635-642.
30. Rademaker MT, Charles CJ, Nicholls MG, Richards AM. Interactions of enhanced urocortin 2 and mineralocorticoid receptor antagonism in experimental heart failure. *Circ Heart Fail* 2013; 6: 825-832.
31. Rademaker MT, Charles CJ, Nicholls G, Richards M. Urocortin 2 sustains haemodynamic and renal function during introduction of beta-blockade in experimental heart failure. *J Hypertens* 2011; 29: 1787-1795.
32. Grossini E, Molinari C, Mary DA, *et al*. Urocortin II induces nitric oxide production through cAMP and Ca2+ related pathways in endothelial cells. *Cell Physiol Biochem* 2009; 23: 87-96.
33. Sanz E, Monge L, Fernandez N, *et al*. Relaxation by urocortin of human saphenous veins. *Br J Pharmacol* 2002; 136: 90-94.
34. Kageyama K, Furukawa KI, Miki I, Terui K, Motomura S, Suda T. Vasodilative effects of urocortin II via protein kinase A and a mitogen-activated protein kinase in rat thoracic aorta. *J Cardiovasc Pharmacol* 2003; 42: 561-565.
35. Yang C, Xu Y, Mendez T, Wang F, Yang Q, Li S. Effects of intravenous urocortin on angiotensin-converting enzyme in rats. *Vascul Pharmacol* 2006; 44: 238-246.
36. Yang C, Liu X, Li S. Effect of long-term treatment with urocortin on the activity of somatic angiotensin-converting enzyme in spontaneously hypertensive rats. *Can J Physiol Pharmacol* 2010; 88: 168-176.
37. Gromotowicz-Poplawska A, Stankiewicz A, Mikita J, *et al*. Beneficial effect of combined spironolactone and quinapril treatment on thrombosis and hemostasis in 2K1C hypertensive rats. *J Physiol Pharmacol* 2018; 69: 245-253.
38. Deska P, Nowicki M. Short-term changes of serum potassium concentration induced by physical exercise in patient with arterial hypertension treated with angiotensin-converting enzyme inhibitor alone or in combination with statin. *J Physiol Pharmacol* 2017; 68: 133-138.
39. Honjo T, Inoue N, Shiraki R, *et al*. Endothelial urocortin has potent antioxidative properties and is upregulated by inflammatory cytokines and pitavastatin. *J Vasc Res* 2006; 43: 131-138.
40. Michel MC, Brodde OE, Insel PA. Peripheral adrenergic receptors in hypertension. *Hypertension* 1990; 16: 107-120.
41. von Kanel R, Heimgartner N, Stutz M, *et al*. Prothrombotic response to norepinephrine infusion, mimicking norepinephrine stress-reactivity effects, is partly mediated by α-adrenergic mechanisms. *Psychoneuroendocrinology* 2019; 105: 44-50.
42. Fetterly TL, Basu A, Nabit BP, *et al*. α2A-Adrenergic receptor activation decreases parabrachial nucleus excitatory drive onto BNST CRF neurons and reduces their activity in vivo. *J Neurosci* 2019; 39: 472-484.

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