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Endogenous ouabain and the renin–angiotensin–aldosterone system: distinct effects on Na handling and blood pressure in human hypertension

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Abstract

Objective—To evaluate whether the renin–angiotensin–aldosterone system (RAAS) and endogenous ouabain system differently affect renal Na handling and blood pressure.

Methods—Three hundred and one patients in whom we compared blood pressure, and renal Na tubular reabsorption in the basal condition and 2 h (T₁₂₀) after saline infusion.

Results—Following multivariate-adjusted linear and quartiles analysis, baseline mean blood pressure (MBP) was significantly higher (113.7 ± 1.33 mmHg) in the fourth versus the first endogenous ouabain quartile (103.8 ± 1.04 mmHg) and the trend across the quartiles was highly significant ($\beta=0.23$, $P=3.53 \times 10^{-4}$). In contrast, an inverse relationship was present in the renin activity (PRA) quartiles with MBP highest in the first (112.5 ± 1.26) and lowest in the fourth PRA quartile (107.6 ± 1.48 , $P=0.039$). Following an acute saline load, changes in MBP and the slope of the pressure–natriuresis relationship were inversely related across the PRA quartiles. The fractional excretion of sodium (FENa) showed a negative linear trend going from the first to the third endogenous ouabain quartiles (2.35 ± 0.17 and $1.90 \pm 0.14\%$, $P=0.05$). Patients in the fourth endogenous ouabain quartile (>323 pmol/l) showed increased FENa T₁₂₀ ($2.78 \pm 0.18\%$, $P<0.01$) and increased Na tubular rejection fraction ($P=0.007$) after Na load. After the saline load, there was a biphasic relationship between plasma endogenous ouabain and FENa favoring Na retention at low endogenous ouabain and Na excretion at high endogenous ouabain levels.

Conclusion—The RAAS and endogenous ouabain system are two independent and complementary systems having an inverse (RAAS) or a direct (endogenous ouabain system) relationship with hemodynamic parameters.

Keywords

hormones; Na pump inhibitor; Na-K ATPase; salt; salt sensitivity

Introduction

Consumption of excess salt over many years leads to arterial hypertension [1,2]. This process is accompanied by the activation/modulation of a great number of genes involved in the remodeling and hypertrophy of the heart, kidneys and the wall of the arteries. These sodium (Na)-induced alterations lead to a higher incidence of stroke, greater stiffness of conduit arteries and enhanced activity of resistance arteries [3,4]. Both the renin–angiotensin–aldosterone system (RAAS) and endogenous ouabain system are involved in the regulation of renal Na handling and blood pressure [5]. Several differences have been detected between them. For example, within the RAAS, aldosterone preferentially regulates renal tubular luminal Na transport mechanisms, whereas the endogenous ouabain system at low concentration augments basolateral Na pump activity and in arteries raises myogenic tone [5–8]. In addition, both angiotensin II and endogenous ouabain have potent and generalized vascular effects within and outside the kidney; the vascular effects of endogenous ouabain include increased myogenic tone [6] and reactivity in mesenteric arteries and reduced renal blood flow [7–9]. Within the complex network of feedback loops regulating body Na, blood pressure and renal function [10], these apparent functional similarities have also been associated with clear differences. For example, acute saline infusions decrease the RAAS but do not change the plasma endogenous ouabain system [11]. Thus, we suspected that the RAAS and endogenous ouabain system might have complementary actions that mediate different physiological needs.

The aim of this study was to evaluate the behavior of these two systems and their relationships with renal Na handling and blood pressure in a large population of newly discovered and never-treated hypertensive patients. These patients were chosen as the most appropriate group in which to evaluate physiological mechanisms underlying the initial uncomplicated phase of hypertension.

We identified 301 patients for study. We compared blood pressure, and renal Na reabsorption in the basal condition and following saline infusion. The study results were analyzed at different plasma levels of endogenous ouabain, renin and aldosterone that were grouped in quartiles of their respective distributions. This study has some similarities with a previous study [12] using a smaller cohort of patients (155) in which we evaluated the behavior of endogenous ouabain in response to saline loads among patients with adducin polymorphisms. The present study differs in that it is an explicit comparison between endogenous ouabain system and the RAAS and employs a larger patient cohort that has allowed subdivision into quartiles as a more powerful means to probe for nonlinear relationships.

Methods

We enrolled 301, consecutive, newly discovered hypertensive patients in the ‘Outpatient Clinic for Hypertension’ of San Raffaele Hospital of Milan. The Ethics Committee of the San Raffaele Hospital approved the study and informed consent was obtained from each individual. Patients underwent clinical examination, routine biochemistry, and secondary hypertension was excluded by routine methods. All patients underwent 24-h ambulatory blood pressure monitoring (ABPM; Spacelab 90207; Spacelab Medical Inc., Redmond, Washington, USA). Blood samples for Na, K, creatinine, renin activity (PRA), aldosterone

(Aldo), endogenous ouabain, and 24-h urine collections for Na, K, and creatinine were obtained the day before the 24-h ABPM recording.

Mean blood pressure (MBP) was calculated as 1/3 difference systolic BP and diastolic BP.

Acute protocol saline infusion

The entire study group of 301 hypertensive patients, who had given signed informed consent, were included in this study. The acute Na loading protocol used was similar to that previously reported [12,13]. Briefly, the protocol began with a 2-h equilibration period during which patients were taken to a quiet room and a venous catheter was inserted into an antecubital vein. Patients remained in the supine position until the end of the Na loading except for voiding. Patients then received a light breakfast. Between 0800 and 1000 h, the patients drank a water load of 5 ml/kg body weight to ensure high urine output. They were asked to empty their bladders spontaneously. A steady state was considered to be achieved when the volume of urine collection and the values of the BP recordings varied by less than 1 ml/min and less than 3 mmHg, respectively. The average equilibration period lasted 2 h. After the equilibration period and achievement of a steady state, a constant-rate intravenous infusion of 2 l of 0.9% NaCl was carried out in 2 h. BP (mean of three measurements taken 3 min apart) was measured every 30 min during the 2 h of loading and three times at 3-min intervals at the end of the infusion. These last three BP values were averaged and used in the analysis.

Biochemical and renal parameters

Serum and urinary sodium (PNa and UNa) and potassium (PK and UK) were measured by flame photometry, serum and urinary creatinine (P_{crea} and U_{crea}) by an automated enzymatic method. Plasma renin activity and plasma aldosterone were measured by radioimmunoassay (RIA; Medical System, Genova, Italy). Plasma endogenous ouabain was determined by C-18-extracted samples using a specific antiserum as previously described [14]. Intraindividual variability is lower than 10%, as previously discussed [5].

The slope of the relationship between BP and Na excretion (mmHg/ μEq per min) was calculated, per patient, by plotting Na excretion on the Y-axis as a function of MBP, on the X-axis, observed both under basal conditions and after 2 h of saline infusion, as discussed elsewhere [12]. Clearances (C) were calculated as $C_x = U_x \times V/P_x$, when U_x and P_x are the urinary and plasma concentrations of the solute x, respectively, and V is the urine flow rate measured in milliliter per minute. Fractional excretion of sodium (FENa) was calculated as $100 \times P_x \times U_{\text{crea}}/P_{\text{crea}} \times U_x$, when U_x and P_x are the urinary and plasma concentrations of the solute x, respectively, and U_{crea} and P_{crea} are the urinary and plasma concentrations of creatinine, respectively [11].

Renal regulation of Na output is mediated through the rate of Na filtration and the fraction of filtered Na which escapes tubular reabsorption: the tubular rejection fraction (TRF_{Na}) is defined as:

$$TRF_{\text{Na}} = (U_{\text{Na}} * V / (GFR * P_{\text{Na}})) * 100$$

where U_{Na} = urinary Na concentration, V = urine flow, GFR = glomerular filtration rate measured with creatinine determination, and P_{Na} = plasma sodium concentration [15,16]. These parameters evaluate the percentage of Na that is not reabsorbed by the distal tubule, is strictly related to FENa ($r^2 = 0.856$), and more accurately define the overall tubular Na handling.

Statistical analysis

For database management and statistical analysis, we used the SPSS software package (SPSS Inc., Chicago, Illinois, USA), version 11. Some parameters (plasma endogenous ouabain, PRA and aldosterone) were not normally distributed (Kolmogorov–Smirnov test). Accordingly, we normalized the distributions of plasma endogenous ouabain, PRA and aldosterone by a logarithmic transformation. Quartile values corresponding to the 25th, 50th, and 75th percentiles were then calculated. We performed analysis of variance to compare means between groups. ANOVA for repeated measures was used to test the effect of Na load within and between patients. Our statistical methods also included trend analyses single and multiple linear regressions. We included in our models covariables with known physiological relevance for arterial BP and renal phenotypes including age, sex, body mass index (BMI), baseline PRA and aldosterone, and urinary Na excretion.

Results

The 301 participants included 52 (17.3%) women, and 249 (82.7%) men who were never-treated hypertensive patients. Table 1 shows the clinical characteristics of the study participants grouped by sex. As expected, BMI, urinary Na and K were lower in women. Circulating endogenous ouabain levels also were lower in women as previously reported[17]. As the distribution of PRA, plasma endogenous ouabain and Aldo departed from normality ($P < 0.0001$), the data were normalized with logarithmic transformation and are presented as geometric means, and quartile groups were used for subsequent analyses.

In multivariable-adjusted linear analyses after accounting for confounders, the baseline mean office blood pressure (MBP) values increased significantly ($P < 0.001$) with plasma endogenous ouabain and age (Table 2a). When we analyzed the effect of endogenous ouabain and PRA into the main component of MBP, based upon systolic and diastolic BP after the Na load we found two distinct effects (Table 2):

1. Plasma endogenous ouabain was directly related to diastolic BP both at baseline ($\beta = 0.265$, $P = 3.29 \times 10^{-5}$) and after the saline load (Table 2, panel c), in a multivariable-adjusted linear analyses.
2. PRA was the main determinant that was inversely related to systolic BP values after the saline load as shown in Table 2, panel b. Also diastolic BP (panel c) was inversely related to baseline PRA values.

Supplementary Tables 1 and 2, <http://links.lww.com/HJH/A42>, <http://links.lww.com/HJH/A43> report the clinical characteristics of the groups segregated by their endogenous ouabain, PRA, and aldosterone quartiles. Patients in the highest (fourth) PRA quartile were younger, lighter, had lower systolic BP and diastolic BP and higher plasma levels of endogenous ouabain and Aldo. Patients within the highest (fourth) baseline aldosterone quartile were also younger, and had higher PRA but otherwise showed no differences.

Figure 1 presents the quartile group subdivisions according to endogenous ouabain (left panel) and PRA (right panel) levels at steady state for office MBP values (panel a). Plasma Na (Fig. 1, panel b) concentration showed a negative trend with PRA ($P_{\text{Trend}} = 0.012$), and a positive trend for endogenous ouabain ($P_{\text{Trend}} = 0.05$) confirming a previous result [12]. Furthermore, baseline GFR was negatively related to endogenous ouabain, with reduced values in the fourth quartile (Fig. 1, panel c). FENa was significantly lower in the higher endogenous ouabain quartiles (Fig. 1, panel d), whereas the same negative trend was present across the PRA groups but did not reach statistical significance.

After saline infusion, PRA was suppressed in all quartiles (Table 3), expressed as changes (Δ) from baseline and the suppression of PRA was significantly greater in those patients with higher baseline PRA. Following an acute saline load, the rise in MBP (Fig. 2, MBP T_{120} , panel a) and the slope of the pressure–natriuresis relationship (slope P/Nat, panel b) showed linear negative changes across the first to the fourth PRA quartiles; those patients with low PRA showed greater increments in BP to the volume stimulus (Fig. 2, right panel). Further, when the Aldo quartile groups were considered, no significant associations were found either with BP changes or FENa (data not shown).

Conversely, the baseline endogenous ouabain bore no significant relationship to any of the changes evoked by the acute Na load (Fig. 2, panels a and b). Plasma endogenous ouabain levels remained unchanged by the acute volume load as previously reported [11] (Table 3). In a multivariable linear regression analysis the relationship of FENa with endogenous ouabain showed a positive trend that did not reach statistical significance, probably due to the nonlinear relationship shown in Fig. 2. Indeed, when we analyzed FENa T_{120} as the dependent variable with plasma endogenous ouabain of the first three quartiles (i.e. excluding those with the highest plasma values in the fourth quartile), endogenous ouabain was the only determinant ($\beta = -0.192$, $P = 0.014$) of FENa T_{120} . Moreover, the relationship of endogenous ouabain with FENa T_{120} was a negative linear trend across the first three endogenous ouabain quartiles (2.35 and 1.9% in the first and third endogenous ouabain quartiles, respectively). However, those patients in the fourth endogenous ouabain quartile (>323 pmol/l) showed significant increases in FENa T_{120} ($2.78 \pm 0.18\%$) that were opposite to the response in the other endogenous ouabain quartiles.

Finally, when plasma creatinine (P_{crea}) values were analyzed in response to the saline infusion, a significant trend toward increased P_{crea} was found in the higher endogenous ouabain quartiles (Fig. 3a, left panel). Those hypertensive patients within the normal range of creatinine values and with high plasma endogenous ouabain showed reduced renal function (supplementary Table 1, <http://links.lww.com/HJH/A42>) versus those in other quartiles ($P_{\text{TREND}} = 0.007$ at baseline). The dilution of plasma creatinine (T_{120} and T_{240}) due to the saline infusion did not mask this difference. However, PRA quartiles did not show any significant relationship with P_{crea} (Fig. 3a, right panel) and this result persisted irrespective of whether eGFR was used with or without sex and body surface area.

When the fraction of Na which escapes tubular reabsorption both at the end of acute saline infusion (T_{120}), and after recovery period (T_{240}) was evaluated, patients in the fourth endogenous ouabain quartile excreted more of the acute sodium load (Fig. 3b, left panel). Thus, patients with high levels of endogenous ouabain excreted more Na following the salt load in spite of their lower GFR (Fig. 1). For example, by the end of the recovery period, the output of Na had increased from 0.043 ± 0.004 (T_0), to 0.341 ± 0.02 (T_{120}) and to (T_{240}) 0.336 ± 0.019 mEq/min/m² ($P < 0.004$, Fig. 3b, left panel) in the high endogenous ouabain quartile versus the other three quartiles 0.041 ± 0.033 (T_0), 0.259 ± 0.028 (T_{120}) and 0.289 ± 0.021 (T_{240}).

Discussion

The main findings of these studies, carried out in patients with never-treated newly diagnosed uncomplicated essential hypertension, are the following:

1. Office diastolic BP was directly related to the circulating levels of endogenous ouabain both at baseline and after saline infusion. This effect may be due to the ability of endogenous ouabain to augment the myogenic mechanism in arteries as recently shown [5–9].

2. Under baseline conditions, the fractional excretion of Na trends lower across the endogenous ouabain quartiles suggesting that endogenous ouabain may promote Na retention. However, in response to the acute volume load, endogenous ouabain shows a biphasic relationship with tubular reabsorption. Net Na retention is favored at low plasma levels of endogenous ouabain, whereas patients with the highest endogenous ouabain show enhanced ability to excrete the Na load.
3. Systolic and diastolic BPs were inversely related to RAAS activity, reflecting body Na status. In addition, a positive relationship between baseline PRA and Aldo levels ($r = 0.406$) was observed. The baseline PRA status itself predicted salt sensitivity, and the marked relationship of baseline PRA to all the measured parameters following Na loading (Table 2) is entirely consistent with the classic relationship of this system to body Na and its known role in response to changes in body Na [18–20].
4. The plasma Na concentration was positively correlated with baseline plasma endogenous ouabain, whereas an inverse relationship was observed with PRA and there was no association with plasma ALDO levels. Increased tubular reabsorption of Na has been proposed as an independent determinant of plasma Na and hypertension [21]. Our finding suggests that endogenous ouabain should be included in this relationship. The clear implication that remains to be tested in humans is that small elevations of plasma Na in the range of 1–3 mEq/l stimulate endogenous ouabain secretion and help to raise blood pressure. In addition, the opposite relationships of endogenous ouabain and PRA with increased plasma Na (Fig. 1b) also raise the issue of whether there is a subtle, hitherto unrecognized, deficit in the circulating levels and/or renal response to antidiuretic hormone. We suggest that this possibility may be less likely because the statistically significant variation of plasma Na among the endogenous ouabain and PRA quartiles remains within the normal range for plasma Na. This suggests that any deficit in thirst and/or the antidiuretic axis if present is likely to be small.
5. Plasma creatinine increased across the endogenous ouabain and PRA quartiles, whereas overall tubular reabsorption, expressed as FENa, decreased across the endogenous ouabain quartiles, as previously shown [12]. The relationship between endogenous ouabain and creatinine observed in our patients may be a direct consequence of the increased level of plasma endogenous ouabain. Prolonged ouabain infusion in rats causes an increase in plasma creatinine, BP and tubular Na reabsorption (Ferrandi, unpublished).
6. The BP response to saline and the slope of the pressure natriuresis relationship was not related to variations in baseline endogenous ouabain. However, the relationship of endogenous ouabain to changes in BP evoked by long-term changes in dietary Na remains to be investigated.
7. There was a progressive decline in FENa T_{120} (during saline infusion) in patients across the first three endogenous ouabain quartiles. For reasons mentioned below, this result strongly suggests that variations in baseline plasma endogenous ouabain in the relevant ranges impair the ability of the body to excrete Na following a volume load. This phenomenon by definition would affect approximately 75% of the patients studied and contrasts with patients in the fourth endogenous ouabain quartile whose FENa T_{120} was significantly greater. In addition, the tubular rejection fraction of Na the fourth endogenous ouabain quartile was increased, whereas this parameter was reduced significantly in the fourth PRA quartile at T_{120} and T_{240} . Taken together, these findings suggest that those patients in the fourth endogenous ouabain quartile experience a more dramatic decrease in tubular Na

reabsorption following Na overload than their hypertensive counterparts. The biphasic relationship of FENa and TRF_{Na} with baseline endogenous ouabain is of interest because a similar biphasic dose–response relationship has been shown in cell systems. For example, subnanomolar concentrations of ouabain paradoxically activate Na transport in proximal tubular cells, whereas higher concentrations are inhibitory [7,22]. The latter result is especially intriguing because transgenic mice with highly ouabain-sensitive $\alpha 1$ renal Na pumps excrete acute salt loads faster than their ouabain-resistant (wild-type) counterparts [23]. Thus, (occupation of) the ouabain-binding site in some manner can influence salt excretion and this implies that the natriuresis in response to salt loads involves an interaction of the Na pump with several intrinsic as well as extrinsic factors that control renal tubular ion transport [24–26].

The significance of acute versus more chronic forms of Na administration is also important. For example, in a study involving increased dietary salt in humans, plasma endogenous ouabain rose maximally on the third day of the high salt intake indicating that the response of endogenous ouabain is clearly related to long-term as opposed to short-term changes in Na balance [26]. With patients in long-term steady-state Na balance, as the vast majority of our patients would have been in the present study, the baseline endogenous ouabain values are of most relevance. Among the first three endogenous ouabain quartiles, it seems likely that endogenous ouabain contributed to the elevated BP via enhanced tubular Na^+ reabsorption and directly by augmenting arterial myogenic tone. The endogenous ouabain-driven augmentation of renal Na retention may contribute to but seems less likely to explain the long-term pressor effect of endogenous ouabain; for example, in the fourth endogenous ouabain quartile in which BP was highest, both FENa and TRF_{Na} were increased. In rats, ouabain augments myogenic arterial tone with an EC_{50} of approximately 700 pmol/l [6]. Thus, concentrations of endogenous ouabain as low as 100 pmol/l (i.e. well within the first endogenous ouabain quartile) will almost certainly provide some direct stimulus to myogenic tone (assuming that human arteries show similar high sensitivity to ouabain as their rodent counterparts). Further, any increase in renal vascular myogenic tone evoked by the prolonged elevation of plasma endogenous ouabain (or ouabain) would be expected to reduce renal blood flow and further augment Na^+ retention [9]. Clearly, for those patients in the fourth endogenous ouabain quartile, the increase in myogenic tone must be the dominant hypertensive mechanism because renal Na excretion is augmented.

Limitations and perspectives

The discrepancies in the literature concerning the identification, source and potential significance of circulating endogenous ouabain in humans raise confusion concerning the physiological role of this hormone [5]. To clarify, the complexity of the relationships among endogenous ouabain, Na-K-ATPase, vascular tone and renal Na reabsorption, the genetic approach and the use of clear settings, including chronic Na depletion and loading, as well as diuretic therapy will help to better understand the physiological role of endogenous ouabain. For example, the involvement of the α -2 isoform of the Na pump and the vascular sodium calcium exchanger type 1 in endogenous ouabain and salt-dependent hypertension have been proven by transgenic technology [8,27,28]. The present results in patients again demonstrate a fundamental association between the plasma levels of endogenous ouabain and arterial BP and are consistent with the overall impression from the experimental models. The identification of the biosynthetic pathway for endogenous ouabain [26,29] may give further opportunity to study the relationships between the polymorphisms of genes coding for enzymes involved in endogenous ouabain metabolism and the clinical phenotypes related to vascular tone and renal Na handling [30].

In summary, our data show there are marked differences between endogenous ouabain and PRA in the regulation of renal Na handling and BP. These differences are consistent with the notion that PRA reflects body Na status and that this has primarily a compensatory role in the regulation of BP. Conversely, endogenous ouabain is linked with long-term BP and also has a complex biphasic relationship with tubular reabsorption during volume loading: favoring Na retention at low plasma levels (ranging from 50 to 325 pmol/l) while promoting Na excretion at the higher levels (>325 pmol/l). Therefore the relative contribution of the vascular and the renal Na tubular effects of endogenous ouabain on the regulation of BP can be expected to change as one moves across the range of plasma endogenous ouabain concentrations in hypertensive patients. Furthermore, the marked difference in the temporal characteristics between endogenous ouabain and PRA in response to the saline loading is consistent with the finding that normal variations in plasma endogenous ouabain typically have slow but persistent effects on BP in humans when compared with angiotensin II. We conclude that endogenous ouabain and RAAS are independent and complementary hormonal systems with distinctly different roles in and implications for renal Na handling and BP.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

EO	endogenous ouabain
FENa	fractional excretion of sodium
GFR	glomerular filtration rate
MBP	mean blood pressure
PRA	plasma renin activity
RAAS	renin–angiotensin–aldosterone system

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Fig. 1.

BASELINE values of office mean blood pressure (MBP, panel a), plasma Na (PNa, panel b), estimated glomerular filtration rate (eGFR, panel c), and fractional excretion of Na (FENa, panel d) in PRA (◆, right panel) and plasma endogenous ouabain (EO) (●, left panel) quartiles. The positive (+) or negative (–) trends are indicated where appropriate. Baseline MBP was significantly higher in the fourth versus the first endogenous ouabain quartile and the trend across the quartiles was highly significant (panel a, left). In contrast, the opposite relationship was present in the PRA quartiles (panel a, right): MBP was highest in the first and lowest in the fourth PRA quartile and again the negative trend across the quartiles was significant.

Fig. 2.

ACUTE Na Load: mean blood pressure after 2 h of saline infusion (MBP T_{120} , panel a), slope of the pressure–natriuresis relationship (slope P/Nat, panel b), and fractional excretion of Na (FENa, panel c) at the end of saline load (T_{120}) in plasma endogenous ouabain (EO) (◐, left panel) and PRA (◑, right panel) quartiles. The significant positive (+) or negative (–) trends are indicated when appropriate. See text for details.

Fig. 3.

Tubular rejection fraction of sodium. Plasma creatinine (P_{crea} , panel a) equilibration period (T₀), at the end of saline infusion (T₁₂₀) and recovery period (T₂₄₀) in endogenous ouabain (EO) (●, left panel) and PRA (▲, right panel) quartiles. Hypertensive patients within the normal range of creatinine values and with high plasma EO had increased P_{crea} versus those in other quartiles ($P = 0.05$ between patients). The dilution of plasma creatinine ($P < 0.001$ within patient) due to saline infusion (T₁₂₀ and T₂₄₀) was similar in each of the four groups. The renal regulation of Na output expressed as the rate of Na filtration and the fraction of filtered Na which escapes tubular reabsorption (TRF_{Na}), both at the end of acute saline infusion (T₁₂₀), and after recovery period (T₂₄₀) are represented in panel b: according to EO quartiles (● left panel) and to PRA quartiles (▲ right panel). Patients in the fourth EO quartile excreted more of the Na load; however, no similar relationships were apparent among the PRA quartiles.

Table 1

Clinical characteristics

	Men	Women	<i>P</i>
<i>n</i>	249	52	–
Age (years)	53.3 ± 9.5	52.2 ± 9.4	n.s.
BMI (kg/m ²)	26.0 ± 2.7	24.4 ± 3.8	<0.001
SBP day time (mmHg)	144.2 ± 11.6	142.9 ± 8.5	n.s.
DBP day time (mmHg)	95.3 ± 8.8	95.4 ± 6.4	n.s.
UNa (mEq/24 h)	143.2 ± 70	122.4 ± 52.5	0.046
UK (mEq/24 h)	56.5 ± 22.5	47.0 ± 17.6	0.005
Creat Cl (ml/min)	107.0 ± 25.6	103.3 ± 32.4	n.s.
PRA (ng/ml per h)	0.89 (0.53–1.62)	0.81 (0.52–1.52)	n.s.
Aldo (pmol/l)	41.8 (29–60)	34.8 (20–61)	n.s.
EO (pmol/l)	230.9 (173–357)	178.6 (124–296)	0.008

All values are expressed as mean ± SD. PRA, EO and Aldo geometric mean and CI intervals have been reported. Systolic (SBP) and diastolic (DBP) blood pressures are the average of all day time determinations (ABPM). Urinary Na and K (UNa and UK) were collected the same day of ABPM.

Table 2

Results of multivariable linear regression analysis

Independent variable	β	<i>P</i>
(a) Dependent variable: baseline mean blood pressure		
Endogenous ouabain	0.230	3.53e-04
Age	0.235	0.001
Out of the model: sex, BMI, PRA, plasma aldosterone, and urinary Na excretion.		
(b) Dependent variable: systolic blood pressure after saline		
PRA	-0.200	0.006
Age	0.283	2.8e-05
Out of the model: sex, BMI, EO, aldosterone and urinary Na excretion.		
(c) Dependent variable: diastolic blood pressure after saline		
Endogenous ouabain	0.241	9.5e-005
Age	0.224	0.001
PRA	-0.226	0.002
Out of the model: sex, BMI, and aldosterone, and urinary Na excretion.		

Data are expressed as standardized beta regression coefficient (β) and relative *P* value.

Table 3

Impact of acute Na load [changes (Δ) from baseline]

Parameters	EO <146 (n=77)	EO 146-211 (n=73)	EO 211-325 (n=76)	EO >325 (n=75)	P_{ANOVA}
Δ SBP (mmHg)	5.15 $\pm$ 1.03	5.18 $\pm$ 1.15	3.49 $\pm$ 1.19	5.97 $\pm$ 1.14	n.s.
Δ DBP (mmHg)	1.89 $\pm$ 0.68	2.46 $\pm$ 0.69	2.22 $\pm$ 0.76	2.96 $\pm$ 0.69	n.s.
Δ MBP (mmHg)	2.98 $\pm$ 0.69	3.37 $\pm$ 0.73	2.64 $\pm$ 0.76	3.96 $\pm$ 0.72	n.s.
Δ Plasma EO (%)	14.51 $\pm$ 11.4	-3.45 $\pm$ 7.60	-0.72 $\pm$ 7.19	-6.98 $\pm$ 5.68	n.s.
UNa exc. rate (μ Eq/l per min)	416.8 $\pm$ 25.0	373.1 $\pm$ 29.3	395.8 $\pm$ 29.9	496.6 $\pm$ 31.6	0.017
Slope Press/Nat (mmHg/ μ Eq per min)	0.013 $\pm$ 0.004	0.016 $\pm$ 0.004	0.011 $\pm$ 0.004	0.010 $\pm$ 0.005	n.s.
Δ FENa (%)	1.48 $\pm$ 0.29	1.36 $\pm$ 0.18	1.43 $\pm$ 0.15	2.22 $\pm$ 0.20	0.014
PRA <0.54 (n=76) PRA 0.54-0.93 (n=77) PRA 0.93-1.58 (n=73) PRA >1.58 (n=75)					P_{ANOVA}
Δ SBP (mmHg)	8.83 $\pm$ 1.45	6.40 $\pm$ 1.25	2.52 $\pm$ 0.86	2.57 $\pm$ 1.11	0.0003
Δ DBP (mmHg)	4.55 $\pm$ 0.74	3.79 $\pm$ 0.79	1.85 $\pm$ 0.73	0.83 $\pm$ 0.74	0.002
Δ MBP (mmHg)	5.98 $\pm$ 0.83	4.66 $\pm$ 0.84	2.07 $\pm$ 0.65	1.41 $\pm$ 0.69	<0.0001
Δ PRA (%)	-7.66 $\pm$ 8.34	-25.8 $\pm$ 6.1	-23.3 $\pm$ 6.3	-53.3 $\pm$ 3.7	0.006
UNa exc. rate (μ Eq/l per min)	432.0 $\pm$ 37	462.5 $\pm$ 31	482.3 $\pm$ 38	327.2 $\pm$ 23	0.005
Slope Press/Nat (mmHg/ μ Eq per min)	0.022 $\pm$ 0.005	0.016 $\pm$ 0.004	0.010 $\pm$ 0.003	0.008 $\pm$ 0.003	0.043
Δ FENa (%)	1.94 $\pm$ 0.21	1.62 $\pm$ 0.15	2.20 $\pm$ 0.26	1.28 $\pm$ 0.17	0.009

DBP, diastolic blood pressure; EO, endogenous ouabain; FENa, fractional excretion of sodium; MBP, mean blood pressure; SBP, systolic blood pressure.