α-Adducin polymorphisms and renal sodium handling in essential hypertensive patients

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α-Adducin polymorphisms and renal sodium handling in essential hypertensive patients. The relationship between blood pressure and sodium (Na) excretion is less steep in hypertension caused by increased renal tubular reabsorption. We recently demonstrated that one mutation in rat α-adducin gene: (J) is responsible for approximately 50% of the hypertension of MHS rats, and (2) stimulates tubular Na-K pump activity when transfected in renal epithelial cell, suggesting that its pressor effect may occur because an increased tubular reabsorption. Linkage and association studies demonstrated that the α-adducin locus is relevant for human hypertension. A point mutation (G460W) was found in human α-adducin gene, the 460W variant (G/W) is more frequent in hypertensives than in normotensives. The aim of this study was to test whether acute changes in body Na may differently affect blood pressure in humans as a function of α-adducin genotype. The pressure-natriuresis relationship was analyzed in 108 hypertensives using two different acute maneuvers: Na removal (furosemide 25 mg p.o.) and, two days later, Na load (310 mmole i.v. in 2 hr). We found that 80 patients were wild-type homozygous (G/G), 26 were G/W heterozygous, and 2 were W/W homozygous with similar blood pressure, age, body mass index, gender, plasma and urinary sodium and potassium. In basal condition G/W-W/W patients showed a lower plasma renin activity and fractional excretion of Na. In either case the pressure-natriuresis relationship was less steep in G/W-W/W than in G/G patients, obviously negative for Na depletion with furosemide (−0.011 ± 0.004 vs. −0.002 ± 0.002 mm Hg/µmol/min, P < 0.03), and positive for Na load (0.086 ± 0.02 vs. 0.027 ± 0.007 mm Hg/µmol/min, P < 0.001). The finding of reduced slope after Na depletion or Na load supports the hypothesis that, as MHS rats, humans bearing one W α-adducin variant display an increased of renal tubular sodium reabsorption.

The renal pressure-natriuresis relationship is less steep or shifted rightward in arterial hypertension [1]. This abnormality may be caused by either intrarenal or extrarenal factors, which affect renal blood flow (RBF), glomerular filtration rate (GFR), or tubular reabsorption. The characteristics of this relationship are different according to the renal mechanisms involved [1]. In fact, the increase in tubular reabsorption, or reduced GFR, produce a decrease in the slope of the relationship, as it is observed in salt sensitive forms of primary hypertension or in patients with mineralocorticoid excess. Reduced RBF produces a rightward shift of this relationship. These characteristics may help in establishing the type of underlying renal function abnormality that is at work in a particular subset of patients [1–4].

During the last 20 years our group has carried out a series of studies comparing the Milan hypertensive rats (MHS) with the normotensive control strain (MNS) [5, 6]. These studies demonstrated that hypertension develops in MHS because of a primary increase in renal tubular sodium reabsorption [6–8], and that a functional point mutation within the gene coding for α-adducin may be responsible for increased tubular reabsorption and for a significant portion of the blood pressure difference between MHS and MNS [9–11]. Adducin is a cytoskeleton protein involved in the formation of actin-spectrin lattice, actin polymerization and cell signal transduction [12–14]. Transfection of either MHS or MNS α-adducin cDNA into rat renal epithelial cells showed that cells expressing MHS adducin had a significantly greater Na pump activity at Vmax and a larger number of Na pump units expressed on the cell surface [15]. Therefore, a link between molecular adducin variants and tubular Na reabsorption could be suggested. In humans, the results of two case-control studies and a sibling-pair analysis were consistent with the findings in rats [16, 17]. These studies showed that a functional point mutation in the α-adducin coding region G460W was associated with hypertension and with a greater decrease in blood pressure after long-term thiazide treatment or acute Weinberger’s test [18], thus supporting the notion that adducin may affect blood pressure through changes in renal Na handling. If the mutated human adducin variant causes hypertension by increasing tubular reabsorption, as it has been demonstrated for the rat variant, hypertensive individuals carrying at least one copy of the mutated variant should have a less steep pressure natriuresis curve than those with the wild variant. The pressure natriuresis relationship reflects the influence of blood pressure on sodium excretion. Therefore, animal data were produced by analyzing the changes of Na excretion as a function of blood pressure; however, in humans chronic and acute studies are performed by analyzing the blood pressure changes as a function of Na intake or excretion [1, 3, 4]. In this study our hypothesis was tested analyzing blood pressure changes after Na depletion (25 mg furosemide p.o.) and after Na load (310 mmole i.v.) as a function of α-adducin genotype in 108 patients with essential hypertension. The results showed that the

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**METHODS**

**Patient selection**

One hundred and twenty-three essential hypertensive patients were randomly recruited from one of our outpatient clinics by two of us (P.M. and M.R.) after having excluded: (i) patients with a history of myocardial infarction, congestive heart failure, stroke, creatinine clearance < 80 ml/min, diabetes mellitus, liver disease; (ii) severe hypertension requiring treatment; (iii) women on oral contraceptives; and (iv) individuals known to abuse drugs or alcohol. A large number of them (N = 65) had a recent diagnosis of hypertension, and had never been treated before with antihypertensive drugs. Treated patients discontinued any medication for at least four months. During this period, blood pressure was monitored every two to three weeks and therapy was started. Patients were consequently excluded from the study if diastolic blood pressure reached 110 mm Hg (N = 6). The office blood pressure was greater than 145/95 mm Hg, at least on three occasions, in the patients enrolled in the study. A 24-hour ambulatory blood pressure recording (24-hr ABPM, Spacelabs 90207) was performed in all patients to confirm the diagnosis of hypertension. Patients with mean daytime blood pressures > 140/90 mm Hg were included. All patients were advised on the appropriate diet to maintain a stable Na content of about 150 mmol/day.

The study was approved by the Ethical Committee of the San Raffaele Hospital. Informed consent to this study was always obtained from each individual enrolled. During the initial hospitalization, a diagnosis of secondary hypertension was ruled out by the presence of normal routine blood chemistry (including renal, thyroid, and liver function) urine analysis, plasma aldosterone, renin activity and urinary excretion of catecholamines and vanil mandelic acid. When clinically indicated, a three-dimensional phase contrast magnetic resonance angiography of the renal arteries and magnetic resonance of the suprarenal glands were also performed. Five patients showed evidence for a detectable secondary cause for hypertension (two primary aldosteronism, one renal parenchymal disease, and two renovascular hypertension), and were subsequently excluded from the study.

**Study protocols**

The study protocol is shown in Figure 1. Throughout the study, the subjects were on a hospital diet, which was similar to the controlled diet recommended during the previous month, containing a constant amount of protein (1.3 g/kg body wt), calories (30 kcal/kg body wt), calcium (800 mg), potassium (80 mEq) and sodium (150 mEq). Twenty-four-hour urine samples and blood were obtained at day 0 at about 8 a.m., when the patient arrived at the hospital with the urine of the last day of controlled diet in order to verify the compliance to the diet. Urine collection was also performed on days 2 (on hospital diet), 4 (the day after furosemide administration), and 5 (the day of Na load), for measurement of sodium (U_{Na,V}) and potassium (U_{K,V}) excretion. Creatinine clearance (C_{Cr}) on days 0 and 2, corrected to a body surface area of 1.73 m², was taken as an index of glomerular filtration rate (GFR). GFR and the clearance of sodium C_{Na} were used for the calculation of fractional excretion of sodium (the percentage of filtered sodium that escapes reabsorption) FE_{Na} = C_{Na}/GFR.

Blood pressure was measured with a standard mercury sphygmomanometer by the same investigator (M.D. or L.B.) in the individual patient throughout the study. Each data point of blood pressure is the mean of at least three consecutive readings. Mean arterial pressure (MAP) was calculated as the sum of diastolic pressure plus one third of pulse pressure.

Two patients with urinary sodium excretion on day 0 of 2 > 250 mEq/24 hr (325 ± 23 mEq/24 hr) and two patients with blood pressure values of < 130/85 during the hospitalization were excluded from the study.

All selected hypertensive patients were characterized for the α-adducin polymorphisms, but the clinical researchers were unaware of the α-adducin genotype status of the patients.

On days 3 and 5, the patients were asked to void the bladder, and assume the supine position between 7:00 and 8:00 a.m., in a
quiet comfortable room, and one venous catheter was inserted into an antecubital vein. They remained in the supine position throughout the studies except for voiding. Patients then received a light breakfast (no coffee or tea). Between 8 and 9:00 a.m. the patients drank an oral water load of 5 ml/kg. They were asked to empty their bladder spontaneously every 30 minutes. After voiding, an equivalent amount of water was given orally to ensure a high urine output. After a 60-minute equilibration period, several 30-minute baseline measurements were performed until the patients were in a steady state. Steady state was considered to be achieved when the volume of two consecutive 30-minute urine collections and the values of the mean blood pressure recordings varied less than 1 ml/min or 3 mm Hg, respectively. Thus, the average equilibration period lasted about two hours. The Na and K content of the urine collected during the last 30 minutes were measured.

Protocol 1: Furosemide test. This part of the study was carried out on day 3. After the equilibration period and steady state achievement, all subjects were given furosemide (25 mg p.o). Urine was collected at the end of equilibration period, immediately before furosemide, and for the following 240 minutes (t 240). Blood pressure was measured every 60 minutes, for three times at three minute intervals, throughout the study (t 240). Blood sample for serum sodium, and potassium determination were collected at time 240 minutes.

Protocol 2: Acute salt loading test. This part of the study was carried out on day 5. Following the equilibration period and steady state achievement, 2 liters of i.v. saline (Na 310 mmol) were infused in two hours. Blood pressure was measured every 30 minutes during the two hours of loading, and for three times at three minutes interval at the end of the infusion (t 120). These last three blood pressure values were averaged. The patient was then allowed to rest and have lunch. Blood and urinary samples were collected at the end of equilibration period, at the end of load, and until 8 a.m. of day 6, for sodium and potassium determination.

Determination of the pressure-natriuresis relationship

The pressure-natriuresis relationship was obtained by plotting the urinary sodium excretion rate (UNaV, in μmol/min) on the y axis as a function of MAP (mm Hg) on the x axis measured under two different sodium balance. Na excretion and the averaged blood pressure during the last 30-minute equilibration period were used as baseline values. Both variables were also obtained after 240 minutes (t 240) of furosemide administration in the first protocol, and at the end of the sodium load (t 120) in the second protocol. The urine collected during the entire period was used to calculate the sodium excretion rate, while the average of the three measurements at the end of the period was taken as a final blood pressure value achieved by the maneuvers. To compare the major characteristics of the relationship, namely, the shift of the relationship along the arterial pressure axis and the slope of the relationship, we calculated the extrapolated x intercept, A (mm Hg), and the slope, B (μmol/min/mm Hg) as follows [3, 19, 20]:

\[
A = \frac{[UNaV(H) \times [MAP(L)] - [UNaV(L) \times [MAP(H)]}{UNaV(H) - UNaV(L)}
\]

\[
B = \frac{UNaV(H) - UNaV(L)}{MAP(H) - MAP(L)}
\]

where L and H are the data obtained during the equilibration period and at the end of either test (time 240 for furosemide test and time 120 for Na load, respectively). As pointed in the introduction, animal data were produced by analyzing the changes of Na excretion as a function of blood pressure, however, in humans chronic and acute studies are performed by analyzing the blood pressure changes as a function of Na intake (and/or Na balance). For this reason the reciprocal of the slope (1/b in mm Hg/μmol Na) is most commonly used as a more correct index of the pressure-natriuresis relationship [3, 19, 20]. This calculation has been proposed to analyze the relationship between Na excretion and blood pressure in steady state, while in this study we use the cumulative Na excretion during the sodium load or furosemide action. The characteristics of the relationship should, however, not be qualitatively different from those obtained in steady state conditions. We deliberately chose this experimental protocol in order to avoid the influence of a variety of environmental and physiological factors on blood pressure, which may change from one week to the other, as required to reach the steady state after a change in Na intake, and to minimize the error in urine collections or other types of stress that may arise from more frequent collections of urine.

Analytic methods

Plasma renin activity and aldosterone were measured by radioimmunoassay. Urinary and plasma sodium, and potassium were determined by flame photometry. Creatinine was determined by autoanalyzer.

Genotyping for adducin variant

Genomic DNA was isolated from 3 ml of whole blood by a modified standard procedure [21]. The G460W polymorphism was investigated by PCR amplification of genomic DNA followed by allele specific oligonucleotide hybridization. Genomic DNA (100 ng) was subjected to amplification using the following primers: 5’GACAAGATGGCTGACTTG 3’ and 5’AGTCTTCGACCTGGGACTGC 3’ in a total volume of 30 μl containing 10 mm Tris-HCl (pH 8.8), 50 mm KCl, 1.5 mm MgCl2, 0.1% Triton X-100, 15 pmol of each primer, 200 μM of dNTP, 1U Taq Polymerase. The polymerase chain reaction (PCR) product was 79 bp. Allele specific oligonucleotide hybridization for the probe for the wild type allele was 5’ TTCTGCCCTTCTC 3’, and the probe for the mutated allele (W variant) was 5’ TTCTGCCATTCCTC 3’. They were labeled by T4 polynucleotide kinase and used for hybridization in 5 × SSC, 46°C, for two hours. The filters are washed in 3 × SSC for the wild type probe, at 46°C, and 5 × SSC for the mutated probe at 46°C.

Statistical analysis

All clinical parameters are expressed as mean (± SEM). Averages of blood pressure values and anthropometric variables were compared with Student’s t-test. Statistical analysis was performed using SPSS (version 6) statistical software.

RESULTS

The 108 patients studied were divided into distinct groups according to the α-adducin genotype, and the genotype frequencies observed were: 74% (N = 80) G/G, 24% (N = 26) G/W, and 2% (N = 2) W/W. This was similar to that expected from the allele frequencies according to the Hardy-Weinberg equilibrium
for a sample of the same ethnic origin [17]. Since the W/W genotype is relatively rare, the two homozygous for the α-adducin variant were included in the analysis in the G/W-W/W group.

The clinical characteristics of 80 G/G and 28 G/W-W/W hypertensive patients are described in Table 1. Homozygous and heterozygous patients were similar for all the anthropometric parameter considered. Blood pressure values both during 24-hour ABPM (MAP G/G 115.3 ± 1.9 mm Hg and G/W-W/W 119.3 ± 1.9 mm Hg) and during days 1 and 2 of hospitalization were similar in the two groups (Table 1). All the other clinical characteristics considered were not significantly different between the two groups.

On day 0, GFR was significantly higher while fractional Na excretion was significantly lower in G/W-W/W homozygous than in G/G wild-type homozygous patients. On day 2 these differences were in the same direction but were not statistically significant.

In spite of similar basal Na intake, measured as 24-hour urinary Na excretion, plasma renin activity was significantly lower in G/W-W/W heterozygous than in G/G wild-type homozygous patients. On day 2 these differences were in the same direction but were not statistically significant.

Urinary sodium and potassium excretion

Urinary sodium and potassium excretions are shown in Figure 2. Before furosemide test (day 2) both groups excreted the same amount of Na as at the end of the controlled Na diet (day 0). Vice versa, on the day preceding the sodium load (day 4) both groups had a significantly lower urinary sodium as a result of the sodium depletion with furosemide on day 3. Therefore, both groups of patients started the study protocols from a similar sodium balance. Of course, by day five, after high Na intake, both groups had an increased Na excretion. G/W-W/W patients increased their urinary K excretion more than G/G (52.3 ± 4.3 vs. 41.4 ± 1.5 mEq/day, P < 0.01) on day 4, after the furosemide test.

Protocol 1: Pressure-natriuresis curve during the furosemide test

At baseline of the furosemide test all of the parameters examined were similar in the two groups (Table 2). Urinary excretion of Na was similar in G/W-W/W and in G/G patients, while urinary excretion of K was lower in G/W-W/W patients. After furosemide the fall in plasma K (∆ = baseline – time 240 minutes), was greater in G/W-W/W patients. On the contrary, the changes in urinary K excretion tended to be lower in the G/W-W/W group, though the difference was not statistically significant (P = 0.09). We also observed a trend to a lower MAP after

Table 1. Major clinical variables of the patients who underwent the furosemide test and acute salt loading test divided according to their genotype for α-adducin (80 G/G, wild-type homozygous; 28 G/W-W/W, heterozygous)

<table>
<thead>
<tr>
<th>Variable</th>
<th>G/G</th>
<th>G/W-W/W</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age years</td>
<td>42.6 ± 1.1</td>
<td>44.1 ± 1.5</td>
<td>ns</td>
</tr>
<tr>
<td>Duration of hypertension years</td>
<td>3.7 ± 0.7</td>
<td>3.6 ± 0.7</td>
<td>ns</td>
</tr>
<tr>
<td>Sex male/female</td>
<td>71.9</td>
<td>23.5</td>
<td>ns</td>
</tr>
<tr>
<td>BMI kg/m²</td>
<td>25.4 ± 0.3</td>
<td>26.2 ± 0.6</td>
<td>ns</td>
</tr>
<tr>
<td>MAP mm Hg</td>
<td>115.0 ± 1.1</td>
<td>117.4 ± 2.3</td>
<td>ns</td>
</tr>
<tr>
<td>Plasma K mEq/liter</td>
<td>4.19 ± 0.03</td>
<td>4.15 ± 0.06</td>
<td>ns</td>
</tr>
<tr>
<td>U_kV mEq/24 hr</td>
<td>39.7 ± 2.3</td>
<td>65.5 ± 3.3</td>
<td>ns</td>
</tr>
<tr>
<td>U_kV day 0 mEq/24 hr</td>
<td>183.7 ± 8.9</td>
<td>158.9 ± 11.2</td>
<td>ns</td>
</tr>
<tr>
<td>U_kV day 2</td>
<td>161.8 ± 6.7</td>
<td>143.9 ± 8.1</td>
<td>ns</td>
</tr>
<tr>
<td>U_kV average</td>
<td>172.1 ± 6.6</td>
<td>151.4 ± 8.1</td>
<td>ns</td>
</tr>
<tr>
<td>Ccr, day 0 ml/min/1.73 m²</td>
<td>115 ± 2.4</td>
<td>126.3 ± 4.5</td>
<td>0.03</td>
</tr>
<tr>
<td>Ccr, day 2</td>
<td>131.9 ± 2.4</td>
<td>118.1 ± 5.0</td>
<td>ns</td>
</tr>
<tr>
<td>Ccr, average</td>
<td>114.4 ± 2.2</td>
<td>124.1 ± 2.9</td>
<td>0.05</td>
</tr>
<tr>
<td>FENA % day 0</td>
<td>0.66 ± 0.027</td>
<td>0.53 ± 0.04</td>
<td>0.02</td>
</tr>
<tr>
<td>FENA % day 2</td>
<td>0.59 ± 0.025</td>
<td>0.54 ± 0.025</td>
<td>ns</td>
</tr>
<tr>
<td>FENA % average</td>
<td>0.62 ± 0.022</td>
<td>0.53 ± 0.025</td>
<td>0.03</td>
</tr>
<tr>
<td>Plasma aldosterone, clino ng/dl</td>
<td>13.8 ± 1.2</td>
<td>14.5 ± 0.9</td>
<td>ns</td>
</tr>
<tr>
<td>PRA, clino ng/ml/hr</td>
<td>1.15 ± 0.10</td>
<td>0.74 ± 0.097</td>
<td>0.015</td>
</tr>
</tbody>
</table>

Abbreviations are: BMI, body mass index; MAP, mean arterial pressure; Ccr, creatinine clearance; FE Na, fractional clearance of sodium; clino, standing body position; PRA, plasma renin activity.

U_kV and U_kV, urinary sodium and potassium excretion; average is the average values of day 0 and day 2.

Fig. 2. Urinary sodium (A) and potassium (B) excretion during the six day hospitalization in the two groups of patients divided according to the α adducin genotype (G/G, N = 80, G/W-WW, N = 28). In both groups the U_kV was similar. However, U_kV was significantly higher (*P < 0.01) in the G/W-W/W patients on day 4 after the furosemide test. Data are expressed as mean ± SEM.
furosemide in the G/W-W/W patients (from 118.8 ± 2.1 to 115.1 ± 2.5 mm Hg, \( P = 0.109 \)).

Figure 3 shows the pressure-natriuresis relationship obtained, as indicated in the Methods section, by plotting UNaV before and after furosemide on the y axis as a function of MAP on the x axis, in G/W-W/W and G/G hypertensives. The reciprocal of the slope of G/W-W/W patients \((-0.011 ± 0.004 \text{ mm Hg/\mu mol/min})\) was significantly different \((P < 0.03)\) from that of G/G patients \((-0.002 ± 0.002 \text{ mm Hg/\mu mol/min})\).

Protocol 2: Pressure-natriuresis relationship during the acute salt loading test

MAP values and urinary sodium excretion were lower at baseline in both groups than those reported the first day, probably as a consequence of Na depletion in day 3 caused by furosemide administration (Table 3). Urinary sodium excretion and blood pressure were measured before and after saline infusion (t 120 min). The increase in MAP levels produced by sodium load was greater while the urine volume was lower in G/W-W/W than in G/G patients. The urinary sodium excretion after the Na load was significantly lower \((P < 0.03)\) in G/W-W/W (296 ± 22.6 \(\mu \text{mol/min}\)) than in G/G (393 ± 24.4 \(\mu \text{mol/min}\)).

The pressure natriuresis relationship was obtained, as indicated in the Methods section, by plotting urinary Na excretion on the ordinate as a function of MAP in the abscissa, in G/W-W/W and G/G hypertensives. As shown in Figure 4, the reciprocal of the slope of the renal function curve in G/W-W/W (0.086 ± 0.02 mm Hg/\(\mu \text{mol/min}\)) was significantly \((P < 0.001)\) different from that of G/G patients (0.020 ± 0.007 mm Hg/\(\mu \text{mol/min}\)). The extrapolate X intercept, A, was significantly \((P < 0.01)\) lower in G/W-W/W (99.8 ± 4.9 mm Hg) than in G/G (110.1 ± 1.8 mm Hg) patients. This indicates that the critical level of blood pressure below which the urinary Na excretion is halted, is lower in G/W-W/W than in G/G.

Relationship between changes in body sodium and blood pressure

After both maneuvers, the variation in body sodium may be calculated either by subtracting the sodium excreted from the sodium infused or simply by subtracting the amount of sodium excreted after furosemide.

Figure 5 shows the relationship between the variation in body sodium after either depletion (furosemide) or load and the corresponding blood pressure levels in the two groups of patients. The slopes obtained were significantly different in the two groups (G/W-W/W 0.029 ± 0.004 vs. G/G 0.007 ± 0.002, \( P = 0.0001 \)). For the same degree of variation in body sodium, the magnitude of blood pressure changes is greater in G/W-W/W than in G/G patients clearly demonstrating that the former have a regulation of blood pressure that is very sensitive to variations in total body sodium.

DISCUSSION

These findings show that the patients with the 460W ‘hypertensive’ variant when compared to the patients with G/G genotype display: (1) a lower plasma renin activity and fractional excretion of Na in basal conditions; (2) a decrease in the slope of the pressure natriuresis relationship after acute furosemide administration or saline load; and (3) a greater change in blood pressure for a given modification of body Na.

These studies were carried out at a sodium intake considered to be “normal” for these patients to avoid the fluctuations of the homeostatic mechanisms involved in body Na control [22–26].
Moreover, particular attention was devoted to minimizing the influence of the sympathetic drive to the kidney, which may affect renal function [27, 28]. This was achieved by performing the critical measurements of BP and Na excretion after a period of time that allowed good familiarization of the patients with the environment and the investigators during such measurements, and by avoiding clear differences in posture. Probably one of the causes responsible for the contrasting results on renal function changes in prehypertensive or hypertensive phases in the literature may be ascribed to variations in the experimental conditions, which may have caused different degrees of sympathetic drive to the kidney [29, 30]. We have to recognize that, when measuring the pressure-natriuresis relationship, the Na excretion was calculated in the urine collected during the entire period of load (120 min) or depletion (240 min), while the blood pressure value was taken at the end of this period. Clearly, this experimental procedure is not appropriate to measure this relationship at steady state conditions [1, 3], and it may underestimate the degree of changes in the pressure-natriuresis relationship. However, we chose these conditions to minimize the errors in the urine collection and the stimuli of postural changes [31]. The pressure natriuresis refers to the effect of blood pressure changes on a change in sodium excretion. Such a phenomenon cannot be demonstrated directly in humans. An attempt to circumvent this problem was to change the Na balance and observe the concomitant changes in blood pressure [3]. Our sole scope was to evaluate possible changes in blood pressure caused by acute variation in body volumes in patients with and without the 460W hypertensive variant.

The lower fractional Na excretion, slope of the pressure-natriuresis relationship, and plasma renin in the subjects with the “hypertensive” W variant indicate a faster tubular Na reabsorption as the cause of the renal function differences between the two groups of patients. The higher GFR in patients with the W variant is not a consistent finding [17]. However, in the present experimental conditions, where particular care was devoted to minimize

<table>
<thead>
<tr>
<th>Variable</th>
<th>G/G</th>
<th>Δ</th>
<th>G/W-W/W</th>
<th>Δ</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP mm Hg</td>
<td>112.8 ± 1.1</td>
<td>2.2 ± 0.7</td>
<td>110.5 ± 1.7</td>
<td>8.05 ± 1.2</td>
<td>ns</td>
</tr>
<tr>
<td>Urinary vol ml/min</td>
<td>7.2 ± 0.4</td>
<td>1.4 ± 0.4</td>
<td>8.6 ± 1.0</td>
<td>−1.8 ± 0.8</td>
<td>ns</td>
</tr>
<tr>
<td>U&lt;sub&gt;Na&lt;/sub&gt;V μmol/min</td>
<td>161.0 ± 8.5</td>
<td>239.6 ± 19</td>
<td>158.5 ± 17.6</td>
<td>137.6 ± 22</td>
<td>ns</td>
</tr>
<tr>
<td>U&lt;sub&gt;K&lt;/sub&gt;V μmol/min</td>
<td>71.5 ± 4.4</td>
<td>0.49 ± 4.3</td>
<td>77.4 ± 9.3</td>
<td>−11.2 ± 7.2</td>
<td>ns</td>
</tr>
<tr>
<td>PRA ng/ml/hr</td>
<td>1.3 ± 0.13</td>
<td>−0.19 ± 0.3</td>
<td>0.81 ± 0.10</td>
<td>−0.11 ± 0.1</td>
<td>0.05 n.s.</td>
</tr>
</tbody>
</table>

Changes in systemic and renal parameter in G/G and G/W-W/W hypertensive patients at baseline and at the end of the Na load (t 120). Δ = baseline − t 240 values. Data are mean ± SEM. Abbreviations are: P Baseline, significance for comparisons between the two groups at baseline; P Δ, significance for comparisons between the two groups after Na load; MAP, mean arterial pressure; U<sub>Na</sub>V and U<sub>K</sub>V, urinary sodium and potassium excretion; PRA, plasma renin excretion.
methodological errors or environmental variables, the results obtained exclude a primary decrease of glomerular filtration in patients with W variant as the cause of reduced slope of the pressure-natriuresis relationship [1].

Adducin is present in many tissues [12, 13]. Therefore, its variants may affect the function of such tissues, which in turn may modify the renal function (including tubular reabsorption) through some extrarenal mechanism. For instance, the brain and the heart are very rich in adducin and we cannot rule out a renal modification that is secondary to some changes in the function of these organs. In fact, the expression of the mutant α-adducin in these tissue may contribute to the regulation of long-term mechanisms involved in blood pressure control and/or contribute to renal sodium handling control. However, besides mineralocorticoid hormones, we are unaware of any extrarenal pressor factor or mechanism that may increase tubular reabsorption producing a concomitant increase in GFR [32]. On the other hand, in the Milan hypertensive rats, where hypertension can be transplanted with the kidney [33], the same pattern of renal function is present. Also, in humans we found an increase in GFR in the phase preceding the development of hypertension in a subgroup of subjects [29], together with the observation that a positive family history for hypertension of the donors affects the blood pressure of a kidney recipient [34], even though, for obvious reasons, this was not demonstrated in the same subjects. An additional argument supporting the notion of a primary increased tubular reabsorption is that the known extrarenal causes that may produce hypertension through changes in tubular reabsorption are very rare conditions and are very often accompanied by an increased level of aldosterone and/or low serum potassium [35, 36]. Therefore, they could not account for the difference in fractional Na excretion between the two groups of patients in the presence of similar levels of aldosterone and serum potassium.

We deliberately maintained the term of increased tubular reabsorption without distinguishing among the different portions of the nephron (proximal, loop of Henle, distal and collecting ducts), since we do not have sufficient data to address this issue. However, the salt sensitivity of blood pressure in these patients together with lower PRA favor the speculation that the distal tubule is the portion of nephron involved in the phenomena we are describing [1]. Moreover, renal K handling after furosemide or acute Na loading differ in the two groups of patients. After furosemide a greater fall in plasma K occurs during the four hours of the test and is accompanied by lower urinary K excretion during the period (Table 2). However, one day after furosemide (day 4, Fig. 2) urinary K excretion is greater in G/W-W/W patients. The most likely explanation for the changes in urinary K after furosemide is that the acute falls in plasma K decreased the tubular potassium load. The change in plasma K is very likely due to a shift from extracellular to intracellular compartment. The explanation for this effect of furosemide in K distribution within the fluid compartment is unknown at present and deserves further investigation. The slightly lower K excretion in G/W-W/W patients during Na loading (Table 3) is very likely secondary to a less distal Na delivery, as suggested by the lower Na excretion in this patients.

We did not measure all of the possible renal function changes during these tests, and therefore we cannot exclude that the mutated adducin variant produces changes in other renal districts. Glomerular pressure, for instance, which was suggested to be different between salt sensitive and salt resistant patients [37–40], was not measured here.

The large blood pressure variation for any given change in body Na in G/W-W/W patients (Fig. 5) is in keeping with previous data of higher sodium sensitivity in G/W-W/W compared to G/G patients [17]. We cannot clearly exclude the involvement of extrarenal factors in this process even though, for the reasons given above, we do think they are likely. We can, however, speculate about the possible cellular mechanism linking the adducin polymorphism to the pressure-natriuresis relationship as follows: it has been recently suggested that an increase in Na excretion after the rise of renal perfusion pressure is accompanied by a shift of the Na pump units from the basolateral plasma membrane of the tubular cells to an intracellular membrane pool [41]. This shift may then reduce the capacity of the proximal tube to reabsorb Na, thus leading to faster Na excretion and with a consequent reduction of blood pressure. Transfection of hypertensive adducin cDNA in tubular cells increases the \( V_{\text{max}} \) of the Na-K pump and the surface expression of Na pump units [15]. Therefore, it can be suggested that the hypertensive adducin variant might slow down this process of internalization of the Na pump units brought about by the increased perfusion pressure, thus leading to a decrease of the slope of the pressure-natriuresis relationship. However, human adducin variant is different from rat variant. Therefore, the assumption that human hypertensive W variant causes cellular effects similar to that of rat hypertensive adducin variant must be proved with experiments of cDNA transfection in the renal epithelial cell.

In conclusion, the findings discussed here are consistent with the hypothesis that the pressure changes caused by the adducin mutated variant may occur for an effect on tubular reabsorption. However, we admit that our results do not clearly distinguish between the intrarenal or extrarenal mechanisms responsible of these tubular function modifications.

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APPENDIX

Abbreviations used in this article are: ABPM, ambulatory blood pressure monitoring; BMI, body mass index; \( C_{\text{cr}} \), creatinine clearance; clino, standing position; \( C_{\text{s}} \), sodium clearance; \( \text{FE}_{\text{Na}} \), fractional excretion of sodium; GFR, glomerular filtration rate; K, potassium; MAP, mean arterial pressure; MHS, Milan hypertensive strain; MNS, Milan normotensive strain; Na, sodium; PRA, plasma renin activity; RBF, renal blood flow clearance; \( U_{\text{K}} \), urinary potassium excretion; \( U_{\text{Na}} \), urinary sodium excretion; \( U_{\text{Vol}} \), urinary volume; wt, weight.

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