Adducin Polymorphism Affects Renal Proximal Tubule Reabsorption in Hypertension

Paolo Manunta, Michel Burnier, Marco D’Amico, Laura Buzzi, Marc Maillard, Cristina Barlassina, Giovanna Lanella, Daniele Cusi, Giuseppe Bianchi

Abstract—Abnormalities in renal sodium reabsorption may be involved in the development and maintenance of experimental and clinical hypertension. Adducin polymorphism is thought to regulate ion transport in the renal tubule. It has recently been shown that there is a significant linkage of \(\alpha\)-adducin locus to essential hypertension and that the 460Trp allele is associated with hypertension. Patients with this allele display larger blood pressure changes with body sodium variation. The aim of this study was to test whether \(\alpha\)-adducin polymorphism is involved in abnormalities of renal function. Because proximal tubular reabsorption has been shown to be tightly coupled to renal perfusion pressure, this segmental tubular function was investigated in 54 (29 Gly/Gly and 25 Gly/Trp) untreated hypertensive patients in basal conditions with the use of endogenous lithium concentration and uric acid. Fractional excretions of lithium and uric acid were significantly decreased in the Gly/Trp hypertensive patients compared with the Gly/Gly hypertensives. The contribution of \(\alpha\)-adducin to fractional excretion of lithium was investigated by multiple regression analysis. Adducin genotype was significantly \(R^2=0.11, F=6.5; P<0.01\) and directly related to fraction excretion of lithium; gender, age, urinary Na\(^+\), urinary uric acid, mean blood pressure, and plasma renin activity were not related. In conclusion, the adducin gene can be considered to be a ‘renal hypertensive gene’ that modulates the capacity of tubular epithelial cells to transport Na\(^+\) and hence contributes to the level of blood pressure. (Hypertension. 1999;33:694-697.)

Key Words: lithium ■ genes ■ human ■ renal function ■ blood pressure ■ adducin ■ hypertension, genetic

Cross-transplantation studies in rat models of primary hypertension have shown that at least a portion of hypertension follows the kidney. This finding has been obtained in all strains in which such an experiment has been carried out, suggesting that the kidney contributes significantly to hypertension.\(^1\) Data consistent with those in rats have also been obtained in humans.\(^2\) Many abnormalities in kidney function and cell membrane ion transport have been described in hypertensive rats and humans.\(^3\) The logical sequence of events going from a genetic-molecular abnormality to a cellular abnormality that causes hypertension via a modification of kidney function is difficult to prove. In Milan hypertensive (MHS) rats we showed that (1) hypertension develops because of a primary increase in renal tubular sodium reabsorption; (2) a significant portion of blood pressure difference between MHS and normotensive animals (MNS) is due to a functional point mutation within the gene coding for \(\alpha\)-adducin;\(^4\) (3) transfection of either MHS or MNS \(\alpha\)-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;\(^5\) and (4) studies in a cell-free system have shown that mutated adducin affects actin polymerization and bundling.\(^6\) Furthermore, the mutated adducin from rats and humans binds to the Na-K ATPase at lower concentrations than wild type adducin.\(^7\)

Therefore, there appears to be a link between molecular variants of adducin and tubular Na\(^+\) reabsorption. In humans, the results of 2 case-control studies and a sib-pair analysis showed that a functional point mutation in the \(\alpha\)-adducin coding region (460Trp) was associated with hypertension.\(^8\) This association was confirmed by some investigators\(^9\) but not by others.\(^10\) Moreover, the slope of the pressure-natriuresis relationship is reduced in hypertensives carrying the \(\alpha\)-adducin variant, suggesting that adducin may affect blood pressure through changes in renal Na handling.\(^11\)

So far, an increase in tubular sodium reabsorption leading to sodium retention has been difficult to show in hypertensive patients. Recent studies in animals have examined the contribution of each tubular segment to renal sodium delivery indirectly with the use of endogenous lithium clearance, which reflects the volume of tubular fluid delivery from the proximal tubule to Henle’s loop.\(^11\) Lithium is freely filtered at the glomerulus and reabsorbed in the proximal tubule in parallel with sodium and water. In humans, an association between high serum uric acid levels, an increased proximal

Received August 10, 1998; first decision September 2, 1998; revision accepted October 30, 1998.

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Hypertension is available at http://www.hypertensionaha.org
tubular reabsorption, and a reduction of fractional uric acid excretion has been reported in hypertensive patients.\textsuperscript{12,13} These findings seem to suggest a role for endogenous lithium and uric acid as markers of proximal tubular function.

The purpose of the present study was to investigate the relationship between renal sodium handling and α-adducin polymorphism in untreated hypertensive patients, with endogenous lithium and uric acid used as markers of proximal tubular sodium reabsorption.

Methods

Subjects

The study was conducted in 54 untreated hypertensive patients. All patients were newly diagnosed, had never received drug treatment for hypertension, and had no evidence of renal or heart disease or secondary form of hypertension. When measured with a mercury sphygmanometer (phases I through V), their office blood pressures were $>140/95$ when measured in the sitting position at least 3 occasions. A 24-hour ambulatory blood pressure recording (SpaceLabs 90207) was performed in all patients to confirm the diagnosis of hypertension (defined as daytime blood pressure $>140/90$ mm Hg). All patients were advised how to eat a diet with stable Na\textsuperscript+ content of about 150 mmol/day for at least 1 month before the study. The aims of the study were explained to each patient and informed consent was obtained. The protocol was approved by the San Raffaele Hospital ethical committee.

Experimental Methods

All participants in the study were investigated at the San Raffaele Hospital between 7:00 and 9:00 AM. Twenty-four-hour urine and blood samples were obtained at about 7:00 AM, when the patients arrived at the hospital. After 1 hour in the supine position, blood was drawn and a urine sample was taken for the determination of plasma sodium, potassium, endogenous trace lithium, uric acid, and creatinine levels. Sodium, potassium, uric acid, and creatinine concentration were determined by an autoanalyzer. Endogenous lithium in plasma and urine was determined with an electrothermal atomic absorption spectrophotometer (model 1100B with HGA-700 graphite furnace; Perkin-Elmer) as previously described.\textsuperscript{14} The fractional excretion of lithium (FE\textsubscript{Li}), sodium (FE\textsubscript{Na}), and uric acid (FE\textsubscript{UA}) were calculated by the standard formula: FE\textsubscript{x} = $U\times P_{\text{x}} / P_{\text{crea}} \times U_{\text{crea}}$, where $U_{\text{crea}}$ and $P_{\text{crea}}$ represent urinary and plasma concentrations of lithium, sodium, and uric acid; and $P_{\text{crea}}$ and $U_{\text{crea}}$ are urinary and plasma concentrations of creatinine, respectively. The fractional reabsorption of sodium in the proximal tubules was calculated as [(FE\textsubscript{Na} - FE\textsubscript{Na}0) / FE\textsubscript{Na}] x 100. Plasma renin activity (PRA) and aldosterone were measured by radioimmunoassay.

Genotyping for Adducin Variant

All selected hypertensive patients were characterized for the α-adducin polymorphisms by individuals who were unaware of clinical characteristics of the patients. Genomic DNA was isolated from 3 mL of whole blood by a modified standard procedure. The Gly460Trp polymorphism was investigated by PCR amplification of genomic DNA followed by allele-specific oligonucleotide hybridization as previously described.\textsuperscript{15} Because in our population the frequency of the Gly460Trp genotype in hypertensives is 25% to 30%, we genotyped a large group of hypertensive patients (160) to study the phenotypic differences between Gly-Gly and Gly-Trp. From this large cohort, we randomly selected 29 Gly/Gly and 25 Gly/Trp who were most similar to the former for the major anthropometric parameters: age, gender, body mass index, and body surface area. Because the Trp/Trp genotype is relatively rare, the 3 persons homozygous for the α-adducin variant were included in the analysis of the Gly/Trp group. Samples were sent to Dr. M. Burnier and colleagues who were blinded to lithium determinations, and clinical researchers were also unaware of α-adducin genotype.

Clinical Characteristics of the 2 Groups of Hypertensive Patients Divided According to α-Adducin Genotype

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Gly/Gly\textsubscript{(n=29)}</th>
<th>Gly/Trp\textsubscript{(n=25)}</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>41.5±1.5</td>
<td>44.1±1.54</td>
<td>NS</td>
</tr>
<tr>
<td>Gender, M/F</td>
<td>27/2</td>
<td>22/3</td>
<td>NS</td>
</tr>
<tr>
<td>BMI, kg/m\textsuperscript{2}</td>
<td>26.1±0.5</td>
<td>25.8±0.6</td>
<td>NS</td>
</tr>
<tr>
<td>CREAT, ml/min</td>
<td>123.1±8</td>
<td>119±7</td>
<td>NS</td>
</tr>
<tr>
<td>PL K, mmol/L</td>
<td>4.15±0.05</td>
<td>4.13±0.08</td>
<td>NS</td>
</tr>
<tr>
<td>PL uric acid, μmol/L</td>
<td>291.45±11.89</td>
<td>303.35±11.91</td>
<td>NS</td>
</tr>
<tr>
<td>Uric acid, μmol/24 h</td>
<td>380.67±29.74</td>
<td>452.04±41.63</td>
<td>NS</td>
</tr>
<tr>
<td>PI Li, μmol/L</td>
<td>0.19±0.07</td>
<td>0.12±0.01</td>
<td>NS</td>
</tr>
<tr>
<td>Ur Li, mmol/24 h</td>
<td>6.1±1.62</td>
<td>2.6±0.46</td>
<td>NS</td>
</tr>
<tr>
<td>Ur Na, mmol/24 h</td>
<td>132±9.1</td>
<td>120±10</td>
<td>NS</td>
</tr>
<tr>
<td>Ur K, mmol/24 h</td>
<td>60±5.1</td>
<td>56±3.7</td>
<td>NS</td>
</tr>
<tr>
<td>PRA, ng·ml\textsuperscript{-1}·h\textsuperscript{-1}</td>
<td>1.13±0.13</td>
<td>0.66±0.06</td>
<td>0.004</td>
</tr>
<tr>
<td>PT Aldo, nmol/dL</td>
<td>9.04±0.86</td>
<td>7.10±0.75</td>
<td>NS</td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td>140.1±2.5</td>
<td>139.4±3.5</td>
<td>NS</td>
</tr>
<tr>
<td>DBP, mm Hg</td>
<td>97.1±1.7</td>
<td>95.3±1.8</td>
<td>NS</td>
</tr>
</tbody>
</table>

BMI indicates body mass index; CREAT, creatinine clearance; PL, plasma concentration of element shown; Ur, urinary concentration of element shown; SBP, systolic blood pressure; and DBP, diastolic blood pressure.

Statistical Analysis

All results are expressed as mean±SEM. The statistical significance of differences was evaluated by 1-way ANOVA followed by Fisher’s least significant differences test, with a value of P<0.05 as the minimum level of significance. A multiple regression analysis was performed using a probability of inclusion criterion of 0.05. Statistical analysis was performed using SPSS statistical software (Version 6; SPSS Inc).

Results

The characteristics of the 2 groups, divided according to α-adducin genotype, are presented in the Table. The 25 hypertensive patients with Trp adducin variants had lower PRA, although urinary electrolytes, aldosterone, and all other parameters considered were similar to those of the 29 Gly/Gly patients.

FE\textsubscript{Li} and FE\textsubscript{UA}, which reflect indirectly the proximal sodium reabsorption, are presented in the Figure. FE\textsubscript{Li} and FE\textsubscript{UA} both were significantly lower in the Gly/Trp hypertensive patients than in the Gly/Gly hypertensives. Similarly, the FE\textsubscript{Na}/FE\textsubscript{Li} ratio was significantly lower in the Gly/Trp group (P=0.029). The ratio of urinary Li to Na was also lower in Gly/Trp (0.02±0.003 versus 0.068±0.022, P=0.052). FE\textsubscript{Na} was similar in Gly/Trp and Gly/Gly patients (0.56±0.04% versus 0.58±0.05%). Finally, the contribution of the fractional reabsorption of sodium in the postproximal tubules to Na reabsorption was 95.24±0.6% in Gly/Gly patients and was reduced to 93.2±0.97% in Gly/Trp patients (P=NS), suggesting no distal compensation. Furthermore, the relationships between FE\textsubscript{Li} as dependent variable and gender, body mass index, mean blood pressure, urinary sodium and uric acid, PRA, and adducin genotype were investigated by multiple regression analysis. Adducin genotype was significantly (R\textsuperscript{2}=0.11; F=6.5; P<0.01) and directly related to FE\textsubscript{Li}, whereas gender, age, U\textsubscript{Na}, U\textsubscript{UA}, and PRA were not.
related. Finally, when the 3 Trp/Trp patients were analyzed separately, the results were similar to those of Gly/Gly genotype. Gly/Gly or Trp/Trp hypertensive patients are characterized by reduced fractional excretions of lithium and uric acid, which suggests an increased proximal sodium reabsorption. Lithium is indeed reabsorbed in parallel to sodium and water in the proximal tubule.14 At the present time, therefore, lithium clearance rather than the clearance of lithium and uric acid can be ruled out as a confounding variable because systemic arterial pressure was similar in the 2 groups of patients. The reduced FEUA in Gly/Trp hypertensive patients is also consistent with a reduced reabsorption in the proximal tubule. Several studies indicate that the bulk of urate reabsorption and secretion occurs in the proximal tubule.16 There may be additional transport sites in the pars recta and the more distal portion of the nephron, but the contribution of nephron segments other than the proximal tubule is small. Uric acid has been shown in multivariate analyses to predict the future onset of hypertension17,18 and has been strongly related to an elevated level of sodium-lithium countertransport.19 It has also been suggested that an increase in plasma uric acid level and a reduced excretion of uric acid are early indices of renal vascular involvement in essential hypertension.18

The finding of reduced fractional excretions of lithium and uric acid, markers of proximal tubular function, supports the hypothesis that humans bearing 1 Trp α-adducin variant display an increased proximal Na reabsorption. The molecular mechanism underlying this increase in Na reabsorption could be (1) a primary increase of the Na pump activity on the basolateral tubular cell as supported by the experiment both in transfected cells and in a cell free-system3,6; (2) mutated adducin that may induce an alteration on actin-spectrin–based membrane skeleton,19 which may affect the regulation of other factors in the Na transport system, such as anion exchanger, epithelial Na channels,20,21 and Na-K-Cl cotransport22 in the luminal part of the cell; or (3) a possible combination of these 2 mechanisms.

In conclusion, the findings presented here are consistent with the hypothesis that adducin can be considered as a ‘renal hypertensive gene’ that affects the capacity of the tubular epithelial cell to transport Na and, hence, affects blood pressure.

**Acknowledgments**

This work was supported, in part, by Ministero Università e Ricerca Scientifica of Italy (ex MPI 60% years 1995–1998 to DC and GB; ex MPI 40% years 1994–1996 to DC); by SIGMA TAU/MURST, National Research Project on Genetic and Molecular Analysis of Physiologic and Pathologic Response of Endocellular Receptors; by Telethon Grant No. E.C. 516, and by the Swiss National Research Fund (Grant No. 32-42543.94 to MB).

**References**


