Parallel Renal and Extremity Blood Supply Abnormalities in Nonmodulation: Responses to ACE Inhibition

Ramiro A. Sánchez, Felipe Ramos, Carlos Giannone, Patricia Fischer, Lucas Masnatta, Hugo P. Baglivo, Agustín J. Ramírez, Norman K. Hollenberg

Abstract—The aim of this work was to ascertain, in nonmodulating essential hypertension, whether the abnormality in the renal blood supply is extended to the extremities and showed a similar response to ACE inhibition and whether these abnormalities could be identified in normotensive offspring of hypertensives, as non-modulation is a familial process with genetic underpinnings. We measured forearm vascular blood flow (FBF) and forearm vascular resistance (FVR) by plethysmography and urinary albumin excretion in 20 normotensive without family story of hypertension (NT: 25±9 years), 10 modulating offspring of hypertensive parents (MHO: 25±6 years), 10 nonmodulating offspring of hypertensive parents (NMHO: 26±5 years), 12 modulating essential hypertensives (MHT: 34±5 years), and 11 nonmodulating essential hypertensives (NMHT: 32±4 years). Measurements were repeated in hypertensives after 3-month treatment with ramipril (5 mg daily). Nonmodulating individuals showed lower maximum FBF (MHO: 41.96±3.3 mL/100 g per minute and NMHO: 35.6±9.0 mL/100 g per minute) than modulating subjects (MHT: 57.5±10.0 mL/100 g per minute and MHO: 51.8±7.0 mL/100 g per minute; P<0.003). Likewise, all nonmodulating subjects showed higher minimum FVR (NMHT: 2.5±0.2 AU; NMHO: 2.8±0.5 AU) than modulating individuals (MHT: 1.9±0.5 AU; MHO: 1.8±0.3 AU; P<0.025). Urinary albumin excretion was higher in NMHT and NMHO than MHT, MHO, and NT (P<0.05). Ramipril increased maximum FBF to 53.8±8.0 mL/100 g per minute and reduced minimum FVR to 1.9±0.5 AU in NMHT (P<0.01). Likewise, ramipril increased effective renal plasma flow and reduced renal vascular resistance and urinary albumin excretion only in NMHT (P<0.05). These results have shown an early involvement of the peripheral circulation in association with increased urinary albumin excretion not only in essential hypertensives but also in NMHO. The effectiveness of ramipril in reducing minimum FVR and urinary albumin excretion in NMHT also suggests a common mechanism. (Hypertension. 2003;41:919-924.)

Key Words: angiotensin-converting enzyme ■ plethysmography ■ hypertension, essential ■ ramipril

A number of renal abnormalities are prominent in the subset of patients with essential hypertension, in whom normal modulation of the responsiveness of the renal blood supply and the adrenal gland to Ang II with salt intake is absent.1 These abnormalities include renal vasoconstriction, inability to handle a salt load, salt-sensitive hypertension, and urinary albumin excretion,2-4 all of which are sensitive to treatment with an ACE inhibitor.2,3 Because these abnormalities are associated with a striking family history of hypertension,5 they are congruent in sibling pairs,6 and are associated with polymorphism of the angiotensinogen gene that increases substrate availability for renin system activity, there is strong evidence for a genetic component.7 In support, similar abnormalities have been documented in the normotensive offspring of hypertensives.8

Abnormalities of the blood supply to the extremity have also been found common in essential hypertension.9-11 These abnormalities include an increase in local vascular resistance and an increase in the resistance to blood flow that is present even during maximal local vasodilation.11

Thus, this study was designed to address several related issues. First, are the abnormalities in the extremity present preferentially in no modulators? Second, does an ACE inhibitor reverse these abnormalities in the extremity as they are in the kidney? Third, does the abnormality in the extremity extend to the offspring of hypertensives, as it does in the kidney?9,12

Methods
Twenty normotensives without a family history of hypertension (25±9 years; 10 female), 10 modulating offspring of hypertensive parents (25±6 years; 5 female), 10 nonmodulating offspring of hypertensive parents (26±5 years; 3 female), 12 modulating essential hypertensives (34±5 years; 6 female), and 11 nonmodulating hypertensives.
essential hypertensives (32 ± 4 years; 5 female) were evaluated in this study.

Hypertensive individuals were recruited from the outpatient clinic in our hospital.

Normotensive subjects and offspring of hypertensive parents were recruited from a larger population in our city. In these subjects, a very careful clinical examination was performed before the beginning of the study to exclude hypertension. History and physical examination, screening biochemical testing, renal echography, and isotopic radiorenographic studies excluded secondary forms of hypertension.

Renal function was normal in all the patients studied (serum creatinine range, 0.8 mg% to 1.0 mg%).

Low renin hypertension was characterized by identifying patients with values of PRA < 0.4 ng/mL per hour under a diet containing 20 to 40 mmol of sodium (7 days’ intake) and failed to increase the PRA after 2 hours of furosemide (40 mg PO). Those subjects were excluded from the study.

In hypertensive subjects, antihypertensive therapy was discontinued 5 weeks before the beginning of the study. No subjects received oral contraceptives or estrogen before or during the study.

The Institution Ethics and Research Committee approved the protocol. Written informed consent was required from all the subjects before the beginning of the study.

Low and High Sodium Diets

Renal hemodynamic studies were performed in all subjects after 10 days of low Na+ intake (20 mmol/24 h) and after 10 days of high Na+ intake (250 mmol/24 h) delivered in fixed order. Because all the subjects who received the low and high sodium diets were outpatients, 48-hour urine was collected the days before each study to measure 48-hour urinary Na+ excretion for confirmation of Na+ balance and dietary compliance.

Salt Sensitivity Characterization

To characterize salt sensitivity, essential hypertensives and offspring of hypertensives were submitted to the consecutive 2 periods of Na+ intake (20 mmol/24 h) and after 10 days of high Na+ intake. Subjects who failed to reach a minimum increase of 30% in effective renal plasma flow and a decrease of at least 30% in filtration fraction (FF) after moving from the low to the high sodium intake. The criteria used to define modulation status was, for a subject, to achieve these changes were considered nonmodulators.

Glomerular filtration rate (GFR) and effective renal plasma flow (ERPF) were calculated by the simultaneous clearances of inulin and para-aminomhippurate (PAH), as described elsewhere.

Filtration fraction was calculated as the relationship between GFR and ERPF (FF = GFR/ERPF) and intrarenal vascular resistance as the relationship between ERPF and mean blood pressure (IRVR = ERPF/MBP).

The ERPF, GFR, and FF values used in this report are the mean value calculated from the 3 values mentioned above.

After the renal hemodynamic study, hypertensive subjects were placed in a treatment with ramipril (5 mg daily) and maintained under a high sodium diet (180 to 200 mmol/24 h) during an average period of 3 months (2 to 8 months). At the end of this period, a new renal hemodynamic study was performed with the individuals receiving the high Na+ diet.

Forearm Blood Flow and Vascular Resistance

Studies were performed in a temperature-controlled laboratory (21° to 23°C), with the subjects previously informed about the study and instructed to be relaxed in the supine position. After a 15-minute resting period, forearm blood flow (FBF) was measured in the right arm with mercury-in-rubber strain-gauge plethysmography. The right forearm was supported to maintain an angle of 30° to 45° over the horizontal mid-axillary line, above the heart level, to ensure a rapid venous drainage after the arm cuff deflation. The strain gauge length, to correctly fit the patient’s forearm, was selected, subtracting 2 to 3 cm of the forearm larger circumference, and was placed ~5 cm below the antecubital fossa. The average of these readings was used to calculate vascular resistance. During the recording periods, the right hand was excluded from the circulation by inflating a wrist cuff to a suprasystolic pressure (200 mm Hg) or at least 40 mm Hg over the measured systolic patient’s blood pressure. The upper arm–congesting cuff was rapidly inflated to 40 mm Hg for 15 seconds repeatedly.

During this period of time, measurement of FBF (paper speed: 5 mm/s) was performed thrice, with time intervals between inflating periods enough to allow a complete venous depletion. This sequence was repeated 5 times throughout 10 to 15 minutes. Between each one of these measurements, the wrist cuff was deflated to avoid pain and discomfort. The FBF was expressed in milliliters per 100 g of forearm tissue per minute (mL/100 g per minute). The forearm vascular resistance (FVR) was calculated as the ratio of mean blood pressure to the average of FBF measurements. The resting FVR was the average of all FVR measurements. All FVR were expressed in arbitrary units (AU).

Maximal FBF and Maximal Vascular Resistance

After the measurement of resting FBF, the arm cuff was inflated to a suprasystolic pressure and maintained for 13 minutes. Throughout this period, blood pressure was recorded every 2 minutes. At the end of this time, before wrist cuff inflation, the arm cuff was deflated and subsequently FBF was measured 10 to 16 times during the first minute at a faster paper speed (24 mm/s). After that, the slope of the incremental rise in the plethysmograph waveform, in the first wave after venous occlusion, was then calculated. During these flow recordings, along the first minute after ischemia, 2 blood pressure measurements were recorded. The procedure was repeated at an intermediate paper speed (10 mm/s) at the third minute after ischemia.

Equipment

A Hokanson EC-10 rapid cuff inflator and a Gould TA 550 were used.

The coefficient of reproducibility of the measurement in our laboratory was 5%. In hypertensive patients, either modulator or nonmodulator, forearm blood flow studies were performed before and after an average period of 3 months (range, 2 to 8 months) under treatment with 5 mg ramipril daily with the patients maintained on a high Na+ diet (180 to 200 mmol/24 h). In a second group of modulator essential hypertensives (n = 9), forearm blood flow was also measured before and after 3 months (range, 2 to 8 months) of treatment with 1 tablet of placebo instead of ramipril, under a similar high sodium intake.

Urinary albumin excretion rate (UAE) was determined in duplicate, by radioimmunoassay, from the 48-hour urine collected after 10 days of low and 10 days of higher sodium intake and after treatment with ramipril, for an average period of 3 months. Values are expressed as micrograms per minute, as previously described.

The UAE intrasubject variability between both 24-hour determinations for the control (NT) group was expressed by the coefficient of variation, using the standard deviation resulting from the measurement error variance for replicate determinations. It ranged from 242% to 250% for the low and high sodium sets of samples, respectively.

Plasma Renin Activity Measurements

In all the subjects studied, plasma renin activity was measured under normal sodium intake (100 to 150 mmol/d) by radio immunoassay, as described previously.

Statistical Analysis

Normal distribution assumption was tested for all the variables, applying Kolmogorov-Smirnov D test. Figures were presented as mean ± SD. One-way ANOVA was applied to detect significant differences between means among the 5 groups considered. ANOVA
TABLE 1. General Characteristics of the Population Studied

<table>
<thead>
<tr>
<th>Group</th>
<th>Age</th>
<th>BMI</th>
<th>Low Na+</th>
<th>High Na+</th>
</tr>
</thead>
<tbody>
<tr>
<td>NT</td>
<td>25±9</td>
<td>22.3±2.0</td>
<td>126±5/82±3</td>
<td>125±4/80±2</td>
</tr>
<tr>
<td>MHO</td>
<td>25±6</td>
<td>23.5±3.0</td>
<td>129±5/80±7</td>
<td>130±4/82±2</td>
</tr>
<tr>
<td>NMHO</td>
<td>26±5</td>
<td>24.0±4.0</td>
<td>127±9/74±11</td>
<td>138±3/87±3†</td>
</tr>
<tr>
<td>MHT</td>
<td>34±5</td>
<td>25.4±3.0</td>
<td>144±9/96±7</td>
<td>145±5/98±3</td>
</tr>
<tr>
<td>NMHT</td>
<td>32±4</td>
<td>27.2±3.0</td>
<td>146±6/98±5</td>
<td>161±3/108±2†</td>
</tr>
</tbody>
</table>

Values are mean± SD for age (y), body mass index (BMI, kg/m²), and systolic/diastolic blood pressure (mm Hg) in all the groups studied. NT indicates normotensives; MHO, modulating hypertensive offspring; NMHO, nonmodulating hypertensive offspring; MHT, modulating hypertensives; and NMHT, nonmodulating hypertensives.

*P<0.05 compared with either NT, MHO, or MHT; †P<0.005 compared with the low Na⁺ intake.

was followed by a multigroup comparison of means using the Scheffé test. ANOVA fixed effects for repeated measurements was applied to assess between sample variability for both 24-hour samples of UAE. Multiple linear regression between UAE (as dependent variable) and mean blood pressure and renal parameters (glomerular filtration rate and effective renal plasma flow), both for the modulators subgroup and for the nonmodulators subgroup, were assessed to evaluate correlations. Significant differences were accepted at a value of P<0.05

Results

Characteristics of the studied population are shown in Table 1. As expected, blood pressure was significantly higher in hypertensives than NT and hypertensive offspring. The BP response after high Na⁺ diet showed a significant increase, >10 mm Hg, only in nonmodulating individuals, either hypertensives or hypertensive offspring’s, indicating the salt-sensitive status of these patients.

Ramipril significantly reduced blood pressure values in hypertensive patients, either modulators (137±0.6/02.5±4.0 mm Hg) or nonmodulators (141.5±6.094.3±4.0 mm Hg), compared with the pretreatment values (MHT: 145.0±5.098.5±3.0; NMHT: 161.5±3.0/108.0±2.0 mm Hg; P<0.002).

Resting FBF (Figure 1A) in NMHT was significantly lower than in MHO or NT subjects, whereas in NMHT it was only significantly lower than in NT subjects.

Resting FVR (Figure 1B) in NMHT was significantly higher than in NT. Although resting FVR in NMHO and MHT was higher than in NT or MHO, this increase was not statistically significant.

Maximum FBF (Figure 2A) in NMHO and in NMHT was significantly lower than either NT or their respective modulating groups. Conversely, minimum FVR (Figure 2B) in NMHO and NMHT was significantly higher than either NT or their respective modulating groups.

After an average 3 months of treatment with ramipril, resting (Figure 1A) and maximum FBF (Figure 2A) were significantly increased only in nonmodulating hypertensives. Likewise, a significant reduction in resting (Figure 1B) and minimum FVR (Figure 2B) after treatment was only observed in NMHT.

Placebo administration to 9 NMHT, for 3 months on average, failed to induce any change in resting FBF (before: 3.6±1.0; after: 4.3±1.0 mL/100 g per minute) or FVR (before: 30.9±11.0; after: 24.2±7.0 AU), maximal FBF (before: 45.3±7.0; after: 45.9±8.0 mL/100 g per minute), and minimal FVR (before: 2.2±0.4; after: 2.1±0.5 AU).
In normotensives without family-related hypertension, ERPF was, under low sodium intake, $690 \pm 11$ mL/min per 0.1.73 m² and after high sodium intake $930 \pm 19$ mL/min per 0.1.73 m²; GFR after low sodium intake was $130 \pm 15$ mL/min per 0.1.73 m² and after high sodium intake $123 \pm 10$ mL/min per 0.1.73 m²; FF, after low sodium intake, was $19 \pm 1\%$ and after high sodium intake was $13 \pm 1\%$.

As shown in Figure 3, modulating hypertensives had increased ERPF and decreased FF and IRVR without changes in GFR during high Na⁺ intake, whereas no changes were observed in nonmodulating hypertensives. After ramipril (Figure 3), nonmodulating hypertensives increased ERPF and reduced FF as well as intrarenal vascular resistance, whereas in modulating hypertensives, ramipril failed to induce any renal hemodynamic change.

As shown in Table 2, urinary albumin excretion was significantly higher in NMHO and NMHT than in NT, MHO, or MHT under either low or high Na⁺ intake. Ramipril was able to induce a significant decrease in UAE only in NMHT compared with the low or high Na⁺ status.

When changes induced by both sodium diets in UAE, blood pressure, and renal hemodynamic parameters were evaluated (Figure 4), only nonmodulators showed a significant correlation between changes in UAE and blood pressure and filtration fraction (multiple $R^2=0.72$; partial correlation UAE versus filtration fraction, $r=0.483$; $P=0.04$; partial correlation between changes in UAE and mean blood pressure, $r=0.46$, $P=0.05$).

Finally, nonmodulating individuals had higher plasma renin activity (NMHT: $5.4 \pm 1.2$ ng/mL per hour; NMHO: $5.8 \pm 0.9$ ng/mL per hour) than modulators (MHT: $2.3 \pm 0.9$ ng/mL per hour; MHO: $2.7 \pm 1.0$ ng/mL per hour; $P<0.01$).

**Discussion**

This study reports 3 new findings and confirms a number of others. The new findings are that abnormalities in the renal blood supply that occur in nonmodulators are paralleled by abnormalities in the blood supply to the extremity that as is the case with the kidney, are corrected by ACE inhibition. Moreover, these abnormalities all occur in the normotensive offspring of hypertensives, in support of other observations that suggest a genetic component.5,6,8 In addition, this study confirmed the salt sensitivity of blood pressure in nonmodulators, the association with UAE, which is responsive to ACE inhibition, and extends the evidence of urinary albumin excretion to normotensive offspring of hypertensives. Finally, our results showed for the first time early forearm vascular alterations and increased UAE in nonmodulating offspring of hypertensive parents, a nonhypertensive salt-sensitive population.

We have taken changes of minimal forearm vascular resistance as an indirect marker of vascular reactivity that in hypertension, could be secondary to a defective endothelial mediated relaxation, as it was suggested elsewhere.10 Nevertheless, these vascular alterations, found in our nonmodulating hypertensive subjects, could be either functional or structural, responding to changes in the arterial wall stiffness.
or to an optimized NO activity, which is known to be altered in hypertension.\textsuperscript{17–20} In this way, the administration of ramipril reversed the forearm and renal vasoconstriction, achieving a hemodynamic response similar to that observed in the modulating group. Moreover, ramipril increased maximum forearm blood flow and reduced minimum forearm vascular resistance only in NMHT, whereas the administration of placebo failed to achieve it. Because ACEI are able to reduce superoxide radical formation and favor an increased disposability of NO, the functional alteration could be supported. Nevertheless, the morphological changes in small arteries in hypertension have been previously documented.\textsuperscript{21} Eutrophic or hypertrophic remodeling develops in small arteries, and the nature of the determinants for this remodeling still unclear. Despite this, growth factors and vasoactive peptides such as angiotensin II and endothelin have been mentioned, among others, to exert a proliferative effect in the vascular smooth muscle cells.\textsuperscript{21} Additionally, the increased media-lumen ratio appears to be the main alteration found in all forms of small-artery hypertensive remodeling that is able to reduce forearm blood flow and to elevate peripheral vascular resistance.\textsuperscript{21} One possible limitation of our findings is the short time in which we have found changes with ramipril, since it lasted an average of 3 months. In fact, it is known that most of the morphometric studies performed with antihypertensive treatments including ACEI or AT\textsubscript{1} antago-

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|c|c|c|}
\hline
\textbf{Na\textsuperscript{+} Intake} & \textbf{NT} & \textbf{MHO} & \textbf{NMHO} & \textbf{MHT} & \textbf{NMHT} \\
\hline
Low & 8±3 & 12±2 & 46±6\textsuperscript{*} & 26±14 & 56±4\textsuperscript{*} \\
High & 10±2 & 10±2 & 53±4\textsuperscript{*} & 32±13 & 60±2\textsuperscript{*} \\
Ramipril & 21±7 & 20±8\textsuperscript{†} & & & \\
\hline
\end{tabular}
\caption{Effects of Na\textsuperscript{+} Intake and Ramipril on 24-Hour Urinary Albumin Excretion}
\end{table}

\textsuperscript{*}P\textless0.05 compared with either NT, MHO, or MHT; \textsuperscript{†}P\textless0.005 compared with high Na\textsuperscript{+} intake.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure3}
\caption{Effective renal plasma flow (ERPF, left upper panel), glomerular filtration rate (GFR, right upper panel), filtration fraction (FF, left bottom panel), and intrarenal vascular resistance (IRVR, right bottom panel) in modulating (MHT) and nonmodulating (NMHT) hypertensive subjects. In each group, black column represents value obtained under low Na\textsuperscript{+} intake; white column with crosshatches, values obtained under high Na\textsuperscript{+} intake; dark column with crosshatches, values obtained after treatment with ramipril. #P\textless0.01 compared with the low Na\textsuperscript{+} intake. *P\textless0.01 and **P\textless0.025 compared with pretreatment values.}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure4}
\caption{Relationship between \Delta UAE and \Delta MBP (upper panel) or \Delta FF (bottom panel) induced by moving from low to high sodium diet.}
\end{figure}
nists have lasted 1 year.\textsuperscript{22,23} However, we have previously shown that treatment with the same ACEI, ramipril, reduced carotid arterial thickening and improved arterial compliance in the same early 3-month period.\textsuperscript{24}

In addition to the forearm vascular alterations, we also found increased UAE in nonmodulating hypertensives and nonmodulating normotensive individuals with a family history of hypertension. It is known that an increased UAE is more frequently observed in individuals with severe hypertension than in individuals with mild hypertension.\textsuperscript{15} Notwithstanding, we found in our study higher levels of UAE even in normotensive nonmodulating individuals. When a multiple linear regression analysis was performed, a significant correlation between UAE and the changes in filtration fraction and MBP from low to high sodium intake was found only in nonmodulating essential hypertensives and hypertensive offspring subjects. This positive correlation is settled only in nonmodulating essential hypertensives and hypertensive offspring subjects. This positive correlation is settled despite overall unchanged mean values with the salt intervention caused by a large bidirectional intraindividual variability that could explain this apparent discrepancy. This result highlights that the interindividual variability of blood pressure, and, possibly, renal endothelial dysfunction caused by hyperfiltration, are involved in the increased UAE levels found in the nonmodulating subgroup.

Our results also showed, in nonmodulating individuals, an increased filtration fraction under the 2 conditions of sodium intake associated with high levels of urinary albumin excretion. However, we recognize the small sample size (21 individuals) of subjects studied, which emphasizes the need for a further study using a greater sampling of subjects to verify this result.

In conclusion, we showed for the first time a higher postischemic forearm vascular resistance and lower maximum forearm blood flow in nonmodulating offspring of hypertensive parents. This might reflect early systemic vascular functional and/or structural alterations in these individuals, suggesting that nonmodulation is not only a restricted alteration involving a renal hemodynamic dysfunction. In addition, these subjects could be at higher risk for development of salt-sensitivity hypertension and cardiovascular events, as has been recently shown.\textsuperscript{25}

\textbf{References}

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