Early Impairment of Renal Hemodynamic Reserve in Patients With Asymptomatic Heart Failure Is Restored by Angiotensin II Antagonism

Paola Magri, MD; Maria A.E. Rao, MD; Sara Cangianiello, MD; Vincenzo Bellizzi, MD; Rosaria Russo, MD; Alessandro F. Mele, MD; Michele Andreucci, MD; Bruno Memoli, MD; Luca De Nicola, MD; Massimo Volpe, MD

Background—The early/asymptomatic stages of heart failure (HF) are characterized by sodium retention secondary to derangement of sodium reabsorption at the proximal nephron level. Because this phenomenon is reversed by ACE inhibition, abnormalities of renal sodium handling may depend on intrarenal changes of angiotensin II (AII)/nitric oxide (NO) levels. Renal hemodynamic reserve (ie, the glomerular vasodilatory response to amino acid infusion) has been proposed as a reliable test to assess in vivo AII/NO balance.

Methods and Results—In this study, the effects of 6 weeks of treatment with 5 mg/d of enalapril or with 50 mg/d of losartan on systemic hemodynamics and renal function were assessed, at baseline and after amino acid infusion (AA), in patients with mild HF (NYHA class I) and in healthy volunteers. Untreated HF patients showed a basal renal function comparable to that of healthy subjects. After AA, glomerular filtration rate and renal plasma flow significantly increased in healthy subjects (129.0% and 130.4%, respectively), whereas no vasodilatory response was observed in HF. Although they did not affect basal renal hemodynamics, both enalapril and losartan restored a normal response to AA in HF patients. Blood pressure and heart rate were comparable in HF subjects and healthy subjects at baseline and were not modified by either treatment. Left ventricular ejection fraction was depressed in HF but did not change after either drug. Urinary excretions of cGMP and nitrate (indexes of NO activity in the kidney), comparable in healthy subjects and in HF patients, were unchanged by either enalapril or losartan and did not correlate with renal reserve.

Conclusions—(1) Renal functional reserve is absent in patients with early/asymptomatic HF and normal renal function and (2) both enalapril and losartan restore a normal vasodilatory response to AA in these patients without affecting basal systemic and renal hemodynamics. These data suggest a major role of AII in the development of early abnormalities in patients with HF. (Circulation. 1998;98:2849-2854.)

Key Words: angiotensin ■ heart failure ■ hemodynamics ■ kidney

Development of progressive renal dysfunction is a frequent complication of congestive heart failure (CHF) and is associated with a significant increment of mortality.1 Although exhaustive pathophysiological information has been provided on the advanced stage of CHF and the associated renal dysfunction, little is known about the initial phase of the disease. The evaluation of the early derangement of kidney function in these patients, however, is critical to improve prognosis. Previous studies from our laboratory have revealed that the early stage of CHF is characterized by sodium retention. Patients with dilated cardiomyopathy and mild heart failure (HF) showed reduced ability to increase natriuresis in response to both acute and chronic salt load, therefore exhibiting tendency to sodium retention.2,3 More recently, we have demonstrated that this phenomenon is secondary to derangement of sodium reabsorption at the proximal tubule level, which is not related to abnormalities of systemic or renal hemodynamics, but it is reversed by converting enzyme inhibitors (CEIs) despite normal plasma renin and aldosterone levels.4

CEIs decrease angiotensin II (AII) formation, but also increase the levels of nitric oxide (NO) through the kinin system.5,6 Thus, an abnormal intrarenal balance between AII and NO may contribute to these abnormalities of renal sodium handling. In fact, the renin-angiotensin system (RAS) is activated early in the course of CHF.7,8 In addition, AII is involved in the regulation of glomerular hemodynamics and proximal tubule reabsorption,5,9 and glomerulosclerosis and progression of renal failure develop when its levels are chronically high.10

On the other hand, NO, the most prominent vasorelaxant factor of endothelial origin, has an important pathophysiological role in cardiovascular disease,11 and its release in re-
response to various stimuli, including exercise, is impaired in patients with CHF.12,13 Thus, endothelial dysfunction has been associated with the progression of CHF.14,15 In addition to the cardiovascular effects, NO is a physiological antagonist of AII at the glomerular and proximal renal tubule level16 and inhibits smooth muscle and mesangial cell growth.17,18

Recent studies have proposed the assessment of renal functional reserve (RFR), that is, the glomerular vasodilatory response to amino acid infusion (AA),5,16 as an index of the intrarenal balance between AII and NO. In rats, a reduction of NO levels or an increment of AII levels diminishes the vasodilatory response. In fact, the absence of RFR has been observed in experimental models of renal damage, such as renovascular hypertension,19 diabetes,20 and glomerulonephritis.21 In all of these conditions, CEIs restored a normal RFR. Additional studies with humans have confirmed the reduction of RFR in conditions characterized by activation of RAS22 or by renal damage.23

The present study was designed to assess RFR in patients with mild HF. To gain insights into the pathophysiology and potential treatment of renal dysfunction in the initial stages of CHF, we performed the study in the absence of treatment and then after administration of the CEI, enalapril, or the AII AT1-receptor antagonist, losartan.

Methods

Study Subjects and Patients

The study was performed with 10 subjects who had chronic, stable, mild HF and no signs or symptoms of congestion. Eight healthy volunteers were also studied. All subjects gave written informed consent before participation in the study, which was approved by the ethical committee. Normal status was established by history, physical examination, laboratory analyses, chest radiograph, M- and B-mode echocardiograms, and ultrasonography of kidneys and bladder.

Table 1 shows the demographic, clinical and hormonal characteristics of the groups. The patients with HF were those fulfilling the inclusion criteria enrolled consecutively from the outpatient clinic. The cause of HF was idiopathic dilated cardiomyopathy in all patients, because no underlying causes of HF could be discovered during the clinical evaluation, which included coronary angiography. Exclusion criteria included angina pectoris, myocardial infarction within the previous 3 months, atrial fibrillation or severe ventricular arrhythmias, recent acute cardiac decompensation (as defined by the sudden onset of pulmonary congestion or peripheral edema, or previous treatment with diuretics), valvular disease or significant mitral regurgitation, cardiothoracic anatomy that does not allow satisfactory and reproducible recording of echocardiogram, diseases of kidneys or prostate or bladder, renal failure or serum creatinine levels ≥1.2 mg/dL, alteration of urinalysis, systemic arterial hypertension, and diabetes. The definition of mild HF was based on the criteria outlined below. Patients showed no reduction in their functional capacity (class I according to NYHA classification), and diabetes. The definition of mild HF was based on the criteria outlined below. Patients showed no reduction in their functional capacity (class I according to NYHA classification),

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age, y/Sex</th>
<th>LVEDD, mm</th>
<th>LVEF, %</th>
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<tbody>
<tr>
<td>1</td>
<td>44/M</td>
<td>66</td>
<td>25</td>
</tr>
<tr>
<td>2</td>
<td>46/M</td>
<td>55</td>
<td>48</td>
</tr>
<tr>
<td>3</td>
<td>47/M</td>
<td>61</td>
<td>35</td>
</tr>
<tr>
<td>4</td>
<td>24/F</td>
<td>57</td>
<td>48</td>
</tr>
<tr>
<td>5</td>
<td>35/F</td>
<td>63</td>
<td>40</td>
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<tr>
<td>6</td>
<td>45/M</td>
<td>59</td>
<td>32</td>
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<td>7</td>
<td>21/M</td>
<td>68</td>
<td>23</td>
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<tr>
<td>8</td>
<td>40/F</td>
<td>55</td>
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<tr>
<td>9</td>
<td>49/M</td>
<td>59</td>
<td>49</td>
</tr>
<tr>
<td>10</td>
<td>52/M</td>
<td>55</td>
<td>48</td>
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LVEDD indicates left ventricular end-diastolic diameter.
conditions and after AA, and (c) urinary cGMP and urinary nitrates (as indexes of NO activity in the kidney).

To evaluate the effects of CEI, we repeated the same experimental protocol in all the patients with mild HF after 6 weeks of treatment with enalapril (Merck & Co, Inc) (5 mg per 24 h PO), administered every night at 8 PM. To assess the effects of selective AII AT₁ subtype receptor antagonism, all the patients repeated the same experimental protocol after 6 weeks of treatment with losartan (Merck & Co, Inc) (50 mg per 24 h PO), administered every night at 8 PM. The sequence of the treatment with enalapril and losartan was randomized. Echocardiographic and biochemical parameters were recorded again after enalapril and losartan.

Renal Clearances
Subjects emptied their bladders at 8:00 AM, completing the 24-hour urine collection. Urinary volume was measured and samples were collected for determination of Na⁺ and urea nitrogen.

Studies of renal function started at 8:30 AM after overnight fasting under constant environmental conditions. As shown in Figure 1, renal clearances were performed to assess basal renal function and RFR. Clearance studies were performed during a state of water diuresis to assess the tubular function of the proximal nephron. The subjects received a loading dose of 10 mL/kg body wt of drinking water over a 60-minute period. At 9:30 AM, blood was taken via a cannula previously introduced into an antecubital vein for measurement of hematocrit, proteins, albumin, electrolytes, osmolality, basal para-amin hippurate sodium (PAH), and inulin (T₀). Subsequently, a priming bolus of PAH (0.05 mL/kg of 20%) and inulin (0.5 mL/kg of a 10%) diluted in 50 mL of 5% glucose, was given and was followed by constant infusion of a mixture (Solamin Forte, 7.5% Pierrel) was given at a rate of 240 mL/h. After 60 minutes equilibration, subjects were asked to void the bladder. Three 60-minute renal clearance periods were performed and urine and blood samples were collected at each interval. To measure RFR, a constant infusion of a standard AA mixture (Solamin Forte, 7.5% Pierrel) was given at a rate of 240 mL/h. After 60 minutes equilibration, subjects were asked to void the bladder. Three 60-minute renal clearance periods were performed and urine and blood samples were collected at each interval for determination of PAH, inulin, electrolytes, and osmolality. Urine and blood losses were replaced throughout the study via a cannula placed in the contralateral antecubital vein. Blood pressures (BP) were measured by sphygmomano- nometric technique, according to recommendations of the American Heart Association, and heart rates were assessed at 30-minute intervals.

Urinary samples for determination of NO₂ and cGMP were collected during the assessment of basal renal function and frozen at −80°C.

Calculations
GFR (mL/min) and renal plasma flow (RPF) (mL/min) were estimated from clearances of inulin and PAH, respectively, and corrected by body surface area. The renal parameters (effective RPF [ERPF], renal vascular resistance [RVR], free water clearance [CF₂H₂O], fractional clearance of free water [FC F₂H₂O], fractional excretion of potassium [FE K], and urine osmolality [U osm]) were calculated as previously reported. RFR was calculated as the difference between stimulated GFR (AAGFR) and baseline GFR (GFR): (AAGFR-GFR)/GFR, where GFR was the mean value of the 3 clearance periods of the resting phase and AAGFR was the mean value of the 3 clearance periods during AA infusion.

Laboratory Methods
Blood samples for baseline measurements of plasma atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP) concentrations were collected in prechilled tubes containing EDTA and spun immediately (within 10 minutes); blood samples for measurements of PRA and plasma aldosterone (PA) concentrations were collected at room temperature. Plasma was then separated and frozen until the time of the assay, which did not exceed 4 weeks. PRA was measured by enzymatic assay as previously described. Plasma immunoreactive ANP and BNP levels were determined by radioimmunoadassay (RIA) after plasma extraction, as described by our laboratory. PA concentrations were measured by RIA with the use of a commercial kit (DPC), K⁺ and Na⁺ levels by ion-selective electrodes (Beckman E2A Na/K system) and osmolality by a standard micro-osmometer. Calculations of all parameters by M- and B-mode echocardiograms were performed as previously described.

The exact timing of collection and urinary volume was used to calculate the urinary nitrate and cGMP excretion rate. Bacterial contamination was excluded in all samples by use of a negative nitrite strip test (Multistix 10SG, Bayer Diagnostics). Immediately after subjects voided, 5 mL of the sample was aspirated with a sterile syringe, filtered through a minifilter (0.22 μm) of cellulose acetate (Millipore) into a sterile evacuated tube (Vacutainer) (Becton Dickinson Vacutainer Systems), and stored at −80°C. The reagents used for the nitrate assay have been previously described. Urinary concentrations of cGMP were measured by RIA by use of a commercial kit (cGMP [125I] assay system), as previously described.

Statistical Analysis
Data are presented as mean±SEM. Comparisons of the basal data were performed by unpaired t test or Wilcoxon signed rank test, as appropri-
ate. One-way ANOVA for repeated measures, followed by post hoc analysis based on linear contrasts, was performed to detect changes over time within the same group. Between-group comparisons were tested by 2-way ANOVA (factoring by group and treatment).

Results

Characteristics of the Study Groups

As shown in Table 1, the study groups were comparable in terms of demographic and clinical characteristics and also in terms of hormonal and renal function profile. Also, mean BP was comparable in the two groups (90.6±3.5 versus 90.6±2.0 mm Hg). In patients with mild HF, LVEF was depressed and end-systolic and end-diastolic left ventricular diameters were elevated beyond the range of normality. Basal PRA and PA concentrations were not different in the two groups, whereas plasma ANP and BNP levels were significantly elevated in patients with mild HF compared with healthy subjects. Finally, as shown in Table 3, urinary NO₃ and cGMP excretion rate were comparable.

Renal Clearance Studies

All renal hemodynamic studies were performed with subjects in a state of water diuresis as indicated by the low values of U-osm (Table 4). No significant differences between the two groups were detected in urine volume and U-osm.

Figure 2 illustrates renal hemodynamics at baseline and during AA load in the control group and in the patients with mild HF before and during pharmacological treatment. Patients with mild HF showed a basal GFR comparable to that of healthy subjects. However, after AA infusion, GFR significantly increased only in healthy subjects (29.0±1.7%). In contrast, no vasodilatory response was observed in patients with mild HF. Similarly, ERPF was comparable in the two groups at baseline; however, after AA, ERPF increased only in healthy subjects (30.4±1.2%). Because the increases in GFR and ERPF were comparable, the filtration fraction, similar in the two groups at baseline, remained unchanged in both groups after AA. Because mean BP was unmodified in both groups during clearance studies, RVR was comparable in patients with mild HF and in healthy subjects at baseline and decreased only in healthy subjects after AA; in contrast, no significant change in RVR was induced by AA in patients with mild HF (Figure 2). C₁₂₀, F₁₂₀, and Fₑ, obtained with subjects in a state of water diuresis, were comparable in the basal state (Table 4). These values did not change in either group after AA.

Effects of ACE Inhibition and AII Receptor Antagonism

After the 6-week treatment with enalapril, no significant changes in heart rate (68.7±2.2 bpm), mean BP (84.7±2.9 mm Hg), and LVEF (40.8±1.7%) were observed. Similarly no differences in these parameters were detected after losartan (68.7±2.6 bpm, 88.3±2.8 mm Hg, and 41.0±1.8%, respectively).

As shown in Figure 2, GFR, ERPF, and RVR were not modified by enalapril and losartan at baseline. However, both drugs restored a renal vasodilatory response to AA comparable to those observed in healthy subjects. Normalization of RFR was associated with unchanged urinary nitrates and cGMP excretion rate (Table 3).

After both drugs, C₁₂₀, F₁₂₀, and Fₑ results were comparable to those observed in untreated patients in the basal state (Table 4), and did not change during AA.

Discussion

The present study provides the first evidence that the physiological renal vasodilatory response to amino acid infusion is absent in the early or mild stages of heart failure. A critical role in the pathogenesis of this abnormality is played by AII since normal vasodilatory responses were restored by both enalapril and losartan.

Recent experimental studies have demonstrated that the vasodilatory response to AA depends on the presence of a proper balance between NO and AII. The key role of AII as a modulator of the renal response to AA has also been described in different models of renal damage, thereby indicating that the lack of vasodilatory response to AA is not dependent on a specific renal abnormality. AII appears to be a critical factor in all these pathological conditions because CEIs normalized RFR.

Studies with humans have consistently confirmed a role of AII in the reduction of RFR in hypertensive patients kept on low salt diet; in fact, the consequent activation of the RAS blunted the hemodynamic

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<tr>
<th>TABLE 3. Urinary Nitrate and cGMP Excretion Rates in Healthy Subjects and in Patients With Mild HF Before and During Treatment With Enalapril or Losartan</th>
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<tbody>
<tr>
<td>Healthy Subjects</td>
</tr>
<tr>
<td>Uₙo₃V, pmol/min</td>
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<td>Uₙo₃V, pmol/min</td>
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<th>TABLE 4. Renal Tubular Function in Healthy Subjects and in Patients With Mild HF Before and During Treatment With Enalapril or Losartan</th>
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<td>Healthy Subjects</td>
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<tr>
<td>V, mL/min</td>
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<tr>
<td>Uₐ₉₀, mOsm/L</td>
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<tr>
<td>Gₑ₂₀, mL/min</td>
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<td>Fₑ₂₀, %</td>
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<td>Fₑ, %</td>
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response to AA that, again, was restored to normal by CEIs. In other pathophysiological conditions in humans, CEIs restored the hemodynamic response to AA. Therefore, in different states sharing the prevalence of AII over NO as a common feature, RFR is blunted or absent. This is likely to be caused by the overwhelming action of vasoconstrictor stimuli that offset the normal vasodilatory response to AA. Such a pathophysiological profile may occur also in CHF. In fact, in our study, in healthy subjects AA promoted increases of GFR and ERPF, whereas no renal vasodilatory response was elicited in patients with mild HF. Thus, the effects of pharmacological blockade of RAS on the renal abnormality observed in patients with mild HF were investigated.

The beneficial effects of ACE inhibitors on hemodynamics, progression of disease, and survival in patients with mild HF are widely recognized. Also, the efficacy of losartan in reducing mortality rate and preserving renal function in patients with CHF has been recently reported. In addition, we have recently demonstrated that enalapril is able to normalize sodium balance and prevent sodium retention during high salt intake in mild HF by counteracting the enhancement of sodium reabsorption at the proximal nephron level. In our previous studies, the beneficial effects of ACE inhibition on renal sodium handling were not coupled with significant changes of the circulating RAS components. Therefore, it was hypothesized that local changes of AII intrarenal concentrations play a permissive role in the increment of sodium proximal reabsorption.

In this study, we tested the effects of the same dosage of enalapril used in our previous work (5 mg/d) on the renal response to AA. Although the 6-week course of treatment with enalapril did not affect basal systemic and renal hemodynamics, it did normalize the vasodilatory response to AA. On the basis of these data, and of the similar circulating renin levels in the two groups, it can be speculated that the renal abnormalities observed in the early or mild stages of HF depend on altered balance between AII and NO at the local level.

To better discriminate between the role of intrarenal AII and NO in the same group of patients, we studied the effects of a 6-week treatment with losartan. The AII AT1-receptor antagonist did not modify basal systemic and renal hemodynamics but generated a normal renal response to AA, as observed with enalapril. These findings strongly indicate that the absence of RFR detected in the early phases of HF is related to local changes of AII intrarenal concentrations, although we cannot completely exclude that the beneficial effect of losartan on RFR was also partially determined by NO production due to agonistic AT2 subtype receptors. The indirect measurements of NO production, such as urinary excretion of nitrates and cGMP, further support the key role of AII. These parameters were similar in the study groups, regardless of the type of treatment, and did not correlate with the presence or absence of RFR.

Altogether, our studies indicate that in the mild/asymptomatic stages of heart failure, despite the normality of renal hemodynamics and tubular function in basal condition, renal alterations are unmasked by specific stimuli. A moderate increase of salt intake, in
fact, reveals a derangement of proximal tubular sodium handling, which is undetectable during normal sodium intake. A short course of treatment with CEIs, at a dosage that does not affect cardiac or systemic hemodynamics, is able to restore sodium balance. In addition, as demonstrated in the present study, AA does not produce the vasodilatory response in patients with mild HF. The loss of RFR that reflects initial glomerular hemodynamic alterations is completely reversed by either CEI or AII-antagonism. On the basis of both previous and current findings, it is therefore reasonable to postulate that local AII is responsible for the inadequate renal hemodynamic adaptations to AA and may also contribute to the proximal nephron derangement.

The absence of the renal vasodilatory response to AA is often coupled with a reduction of proximal tubule reabsorption in experimental models of renal disease, which suggests that the absence of RFR is, at least in part, caused by the activation of tubulo-glomerular feedback. In contrast, we could not identify any difference in tubular reabsorption of the proximal nephron after AA between HF patients and healthy subjects. The reason for this discrepancy is not readily apparent. However, our assessment of fractional free water excretion during water diuresis could provide information on the reabsorption at the level of the whole proximal nephron (ie, not limited at the proximal tubule). Alternatively, we may hypothesize that in mild HF patients, the glomerular response to AA is dissociated from the tubular response, as demonstrated in hypertensive and diabetic rats.

Although the present study cannot fully elucidate the exact mechanisms leading to renal dysfunction in the early phases of HF, we suggest that local AII plays a central role in mediating the renal hemodynamic and tubular abnormalities. Our findings further indicate the need for early identification of left ventricular dysfunction because pharmacological suppression of RAS from the initial stage of HF may prevent further deterioration of renal function.

References


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