Renal Response to Angiotensin After Short-term Angiotensin Converting Enzyme Inhibition

Thierry Hannedouche, François Schmitt, Achour Ikeni, Luis-Paulo Marques, Svetlozar Natov, Michelle Déchaux, Bernard Lacour, and Jean-Pierre Grünfeld

In 13 normotensive subjects on a normal sodium diet, we studied hormonal, blood pressure, and renal vascular changes and dextran sieving profiles induced by infusion of exogenous angiotensin II (Ang II) (5 ng kg\(^{-1}\) min\(^{-1}\)), during baseline conditions and after 5 days of administration of the angiotensin converting enzyme inhibitor cilazapril. Cilazapril induced a renal vasodilative effect without affecting supine blood pressure and glomerular filtration rate. Fractional dextran clearances were significantly decreased for dextran of effective radius ranging from 3.0 to 4.0 nm. This shift was primarily related to an increase in glomerular capillary plasma flow, because no change was observed in the transcapillary glomerular pressure gradient, the ultrafiltration coefficient, or the membrane parameters. Ang II elicited a slight pressor response accompanied by hormonal, antinatriuretic, and renal hemodynamic changes that were similar during and before short-term angiotensin converting enzyme inhibition. Dextran sieving curves were unchanged by a low dose of Ang II. However, the transcapillary glomerular pressure gradient and the ultrafiltration coefficient were computed to increase by 19.4% and to decrease by 44.2%, respectively, whereas membrane parameters were unaffected. When superimposed onto short-term angiotensin converting enzyme inhibition, glomerular response to this unique dose of Ang II was similar to that induced by Ang II alone. These findings indirectly suggest that most, if not all, of the renal effects of cilazapril are mediated through suppression of Ang II formation. (Hypertension 1993;21:261–266)

KEY WORDS • angiotensin II • angiotensin converting enzyme inhibitors • renal circulation • lithium • dextran

In normotensive subjects, angiotensin converting enzyme (ACE) inhibitors have been found to induce renal hemodynamic changes, including renal vasodilation and natriuresis, and less consistently to decrease blood pressure, depending on sodium intake and initial blood pressure.\(^1\)–\(^4\) However, during chronic ACE inhibitor treatment, vascular reactivity to endogenous angiotensin II (Ang II) could be affected by several factors, including upregulation in the number or density of vascular receptors to Ang II\(^5\) and dissociation between persistent ACE inhibition and hourly changes in plasma Ang II concentrations.\(^6\)–\(^7\) Indeed, rats treated with a 14-day course of captopril demonstrated significant upregulation of receptor number without changes in dissociation constant, \(K_d\), presumably because of inhibition of Ang II production by captopril.\(^8\) Furthermore, recent works using measurements of true plasma Ang II concentrations have clearly shown that plasma Ang II virtually disappears from the circulation after acute ACE inhibition, whereas it is always detected, albeit at low levels, with long-term therapy. Of note, Ang II levels were found to return to baseline 14–30 hours after initial drug intake of a long-acting ACE inhibitor such as enalapril or benazepril,\(^7\) although the in vitro measurements of plasma ACE activity indicated persistent inhibition. This return to baseline of Ang II in plasma seems to be entirely explained by the rise in plasma angiotensin I and active renin, presumably as a consequence of the acute interruption of the permanent negative feedback effect of Ang II on renin release.\(^7\) Moreover, Ang II concentrations may exhibit circadian changes in relation to protein meal\(^7\) and increased renin synthesis.\(^8\) Because renal vasculature is exquisitely sensitive to Ang II, chronic upregulation of the number of Ang II receptors may influence the renal response to acute changes in Ang II levels.

We therefore found it of interest to compare renal vascular reactivity to exogenous Ang II in healthy normotensive subjects in baseline conditions and after short-term ACE inhibition. Our overall results showed that exogenous Ang II had comparable effects on renal hemodynamics, sodium excretion, blood pressure, and hormonal changes whether administered at baseline conditions or superimposed to short-term ACE inhibition.

Methods

Study Population

Thirteen healthy normotensive subjects (seven males), aged 29±1 years, participated in the study after
informed consent and agreement of the local Ethics Committee. Renal or cardiovascular disease had been excluded by appropriate clinical and laboratory investigations. All subjects received a controlled normal sodium diet (100–120 mmol per 24 hours) for at least 1 week before the study, and none had any other drug during the study.

Study Protocol

Renal function was studied two times, immediately before and 5 days after oral administration of 2.5 mg cilazapril once a day (the last dose given 1 hour before the initiation of the second renal study). Subjects were given 750 mg lithium carbonate orally at 10 PM the night before the clearance studies. Water loading was sustained by 300 mL tap water orally every 30 minutes during the study. After a priming infusion of inulin (polyfructosan, Inutest Loevson, Linz, Austria), para-aminohippuric acid (PAH) (Nephrotest, Biologische Arbeitsgemeinschaft GmbH, Lich, FRG) and dextran 40 (Rheomacrodex, Pharmacia France; 130 mg/kg) clearances were performed as previously described3 using a constant-rate infusion of inulin and PAH and six consecutive clearance periods of 30 minutes each. Urine was collected every 30 minutes by spontaneous voiding, and peripheral venous blood was drawn from an indwelling cannula to bracket each urine collection. After the end of period 3, the synthetic analogue of Ang II, AngioII-amide (Hypertensin, Ciba, Basel, Switzerland), was infused at a constant rate of 5 ng • kg⁻¹ • min⁻¹. Mean arterial pressure (MAP) was measured with an automatic noninvasive oscillometric technique (Dinamap, Critikon, Tampa, Fla.; cuff size 23×13 cm) every 10 minutes during clearance evaluation. Samples for plasma renin activity, plasma aldosterone concentration, and ACE activity were drawn before and 60 minutes after Ang II administration and were immediately centrifuged and kept frozen at −80°C until assayed.

Clearances of various substances were calculated according to the standard clearance formula

\[ C = \frac{U \times V}{P} \]

where V is urine flow, U is urine concentration of the substance, and P is its plasma concentration, and were adjusted for a body surface of 1.73 m². The average of three 30-minute clearances of either inulin or PAH was calculated to evaluate glomerular filtration rate (GFR) and effective renal plasma flow (RPF), respectively.

MAP was calculated as the average of all MAPs recorded during the clearance procedure. Filtration fraction was calculated as GFR/RPF and renal vascular resistance as

\[ \text{MAP} \times (1 - \text{Ht}) / \text{RPF} \]

where Ht is hematocrit. Fractional excretion of sodium, \( \text{FE}_{\text{Na}} \), was calculated as \( \text{C}_{\text{Na}} / \text{GFR} \), where \( \text{C}_{\text{Na}} \) is sodium clearance.

Segmental tubular sodium handling was estimated from the lithium clearance (\( \text{C}_{\text{L}} \)), based on studies showing that lithium is reabsorbed almost exclusively in the proximal tubule and in parallel with sodium so that fractional lithium reabsorption reflects proximal sodium reabsorption.9 Fractional lithium reabsorption (\( \text{FR}_{\text{L}} \)) was calculated as \( 1 - \text{C}_{\text{L}} / \text{GFR} \) and fractional lithium excretion (\( \text{FE}_{\text{L}} \)) as \( \text{C}_{\text{L}} / \text{GFR} \). Other values were calculated as follows:

\[ \text{APR} = \text{GFR} - \text{C}_{\text{L}} \]

where APR is absolute isosmotic proximal reabsorption;

\[ \text{FDR}_{\text{Na}} = 1 - \text{C}_{\text{Na}} / \text{C}_{\text{L}} \]

where FDR is fractional distal reabsorption of sodium; and

\[ \text{ADR}_{\text{Na}} = (\text{C}_{\text{L}} - \text{C}_{\text{Na}}) \times \text{P}_{\text{Na}} \]

where \( \text{ADR}_{\text{Na}} \) is absolute distal reabsorption of sodium and \( \text{P}_{\text{Na}} \) is plasma sodium.

Fractional dextran clearances (\( \theta_{b} \)) were computed using the equation

\[ \theta_{b} = (U/P_{b}) / (U/P)_{\text{in}} \]

where \( (U/P)_{b} \) and \( (U/P)_{\text{in}} \) refer to urine-to-midpoint plasma concentration ratio of dextran and of inulin, respectively.

Afferent onotic pressure (\( \Pi_{a} \)) was calculated using the equation

\[ \Pi_{a} = a \times C + b \times C^{2} \]

where C is the plasma protein concentration and the coefficients \( a = 1.629 \) and \( b = 0.294 \) were derived by least \( \chi^{2} \) analysis of the quadratic relation between measured plasma onotic pressure and plasma protein concentration.10 Efferent onotic pressure (\( \Pi_{e} \)) was derived from afferent protein concentration and filtration fraction.

Laboratory Procedures

Plasma sodium was determined by specific electrodes on a multiparametric analyzer (Hitachi Inc., Tokyo) and urine sodium on an Autoanalyzer SMA II (Technicon Instrument Corp., Tarrytown, N.Y.); plasma and urine sodium by atomic absorption spectrophotometry (PU 900U, PYE Unicam Ltd., Cambridge, UK); plasma and urine concentrations of PAH and inulin by colorimetric methods11,12 performed on an AutoAnalyzer I (Technicon); and hematocrit by a routine Coulter counter (Coulter Corp., Hialeah, Fla.).

Separation of dextran in protein-free filtrates of plasma and urine into narrow fractions was achieved by high performance liquid chromatography using a pump (model 5000, Varian Associates, Inc., Palo Alto, Calif.) and two Micropak TSK columns in series (TSK 3000 and TSK 4000, Varian). The columns were calibrated with four narrowly dispersed dextrans, all of known molecular weight (51, 49, 24, and 12 kd purchased from Pharmacosmos, Denmark). Blue dextran and alanin were used to identify the void volume and the total volume, respectively. Dextran concentrations were measured with a refractive index detector (RI-4, Varian). An integrator (4400, Varian) was used to divide the chromatogram into three slices per minute. The integrated area of each slice was equated to the dextran concentration at the corresponding retention time. Molecular radius (\( r_{c} \)) was computed from the linear relation between retention time and molecular weight (MW), where

\[ r_{c} = 0.33 \times (\text{MW})^{0.463} \]
The fractional clearances of each discrete dextran fraction at 0.2-nm intervals over the molecular radius range of 3.0–5.6 nm were computed using the equation given above.

Plasma renin activity was determined as the rate of formation of angiotensin I, and plasma aldosterone concentration was determined by radioimmunoassay and ACE activity using an enzymatic kinetic method modified from Cushman et al. Results are expressed as nanomoles hippuric acid formed per minute and per milliliter of serum at 37°C under standard assay conditions. Normal values in our laboratory are 21.5±2.7 nmol • min⁻¹ • mL⁻¹. Plasma renin activity was determined as the rate of formation of angiotensin I, and plasma aldosterone concentration; ACEA, angiotensin converting enzyme activity. Values are mean±SEM; n = 13.

It has been demonstrated that transmembrane dextran flux is governed not only by the intrinsic properties of the membrane but also by the hemodynamic determinants of GFR, namely, the afferent oncocotic pressure (Ila), RPF, and the transcapillary hydraulic pressure gradient (ΔP). The isoporous+shunt model was used to search for the best baseline combination of ΔP, Kt, r0, and w0 that minimized the sum of χ² between calculated and observed sieving coefficients (LOGINSERM, INSERM, 1991). This was done over a ΔP range consistent with a state of filtration pressure disequilibrium, i.e., above the glomerular oncocotic pressure prevailing at the efferent end of the glomerular capillary network (Ile).

Theoretical Analysis of Glomerular Transcapillary Water and Solute Flux

Inulin and dextran are inert and uncharged polysaccharides excreted solely by glomerular filtration; neither is secreted or reabsorbed.

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Theoretical Analysis of Glomerular Transcapillary Water and Solute Flux

Inulin and dextran are inert and uncharged polysaccharides excreted solely by glomerular filtration; neither is secreted or reabsorbed. The urinary clearance of a dextran of given size relative to inulin, therefore, reflects its Bowman’s space-to-plasma concentration ratio or sieving coefficient. Applying sieving coefficients of permeant dextrans of radii of 3–5.6 nm to a theory of hindered solute transport through water-filled pores, the glomerular capillary wall was found to be represented most accurately as an isoporous+shunt membrane. In this model, the major portion of the capillary wall is assumed to be perforated by restrictive, cylindrical pores of identical radius (r0). The model assumes that there exists, in addition, a parallel “shunt pathway” that does not discriminate on the basis of dextran size and through which passes only a negligible fraction of the filtrate volume. The shunt pathway is characterized by a parameter ω0, which governs the fraction of filtrate passing through this nonrestrictive portion of the membrane. In addition to r0 and ω0, the membrane is characterized by an ultrafiltration coefficient (Kf), which is the product of hydraulic permeability and the filtration surface area of all glomerular capillaries in the two human kidneys.
activity was significantly increased and plasma aldosterone concentration decreased nonsignificantly after cilazapril administration.

Ang II infusion without prior ACE inhibition induced an expected pattern of hemodynamic and hormonal changes, including a modest decrease in GFR and a profound decrease in RPF, whereas filtration fraction, MAP, and renal vascular resistance rose markedly. Ang II induced a frank antinatriuretic effect, as indicated by the drop in absolute and fractional sodium excretions, due to an increase in both proximal and distal reabsorptions as suggested by the elevation in fractional reabsorption of sodium. Plasma renin activity was dramatically decreased and plasma aldosterone concentration stimulated by Ang II.

After 5 days of cilazapril administration, Ang II infusion induced renal hemodynamic and hormonal changes of similar magnitude to those noted when subjects were studied in baseline conditions.

On the whole, cilazapril treatment did not influence the whole-kidney effects of Ang II except for filtration fraction, which was slightly less increased after cilazapril.

Dextran sieving curves were evaluated in six subjects before and after 5 days of cilazapril treatment. Results, shown in Figure 1, indicated a decrease in fractional dextran clearance significantly different from the control curve for radii ranging from 3.0 to 4.0 nm \((p<0.05)\). Dextran sieving curves during Ang II infusion after either the control period or cilazapril administration were similar to the control curve over the entire range of dextran molecular radii, i.e., 3.0–5.6 nm.

Determinants of glomerular filtration and “intrinsic” membrane parameters are given in Table 3. Ang II increased \(\Delta P\) by 16.6% and decreased \(K_{f}\) by 49.4%, whereas intrinsic parameters \(\tau_{0}\) and \(\omega_{0}\) were unchanged. Cilazapril did not affect any of the hemodynamic or intrinsic parameters. When superimposed on cilazapril, the intraglomerular hypertension and the decrease in \(K_{f}\) induced by Ang II were similar to those induced by Ang II alone, i.e., an increase in \(\Delta P\) by 13.8% and a decrease in \(K_{f}\) by 45.3%.

In a model in which the glomerular capillary wall operates as an isoporous membrane hindering solute transport through water-filled pores, both diffusive and convective transports of the macromolecules are predicted to increase at high versus low intraluminal concentrations of the molecules and will thus change in parallel with the filtration fraction.\(^{16,18}\) Molecules filtering freely and therefore relatively permeant dextrans with molecular radii less than 4.0 nm are the most influenced by these hemodynamic factors.\(^{16,18}\) Our data therefore indicate that the effects of low-dose Ang II on glomerular permeselectivity are primarily dependent on changes in \(\Delta P\), \(K_{f}\), and glomerular plasma flow, whereas the changes in dextran sieving profiles induced by cilazapril are chiefly related to an increase in glomerular plasma flow.

**Discussion**

In normotensive subjects, ACE inhibitors have been found to induce renal hemodynamic changes, including renal vasodilation and natriuresis,\(^{1-4}\) essentially attributed to suppression of Ang II generation. In these previous studies, decrease in blood pressure was not consistent and depended greatly on supine position, sodium content of the diet, initial blood pressure, and duration of drug administration.\(^{1-4}\) In our normotensive
subjects on a normal sodium diet, cilazapril increased RPF and decreased renal vascular resistance but did not change GFR, blood pressure, or sodium excretion. Such postdose changes were noted in the 60–150 minutes after the last cilazapril administration, during the peak of ACE inhibition and Ang II blockade.\textsuperscript{19} As previously reported with other ACE inhibitors, plasma aldosterone concentration decreased slightly, whereas plasma renin activity increased, consistent with removal of Ang II–mediated permanent negative feedback of renin release.\textsuperscript{4,6}

Despite vasodilation and unchanged GFR, we found a significant decrease in fractional clearance of neutral dextran in the range of 3.0–4.0 nm effective radius. Applying sieving coefficient of permeant dextrans of radii of 3.0–6.0 nm to a theory of hindered solute transport through water-filled pores, researchers have found the glomerular capillary wall to operate as an isoporous membrane.\textsuperscript{15,16} In addition, a parallel “shunt pathway” has been postulated that does not discriminate on the basis of dextran size and through which passes a small fraction of the filtrate volume accounting for permeation of large dextrans with radii of more than 5.5 nm (isoporous+shunt model).\textsuperscript{15} In this isoporous model, both diffusive and convective transport of the macromolecules are predicted to increase at high versus low intraluminal concentrations of the molecules and will thus change in parallel with the filtration fraction. These hemodynamic factors have the greatest influence on molecules filtering freely, i.e., dextrans with molecular radii of less than 4.0 nm.\textsuperscript{16,18}

Although cilazapril slightly decreased sieving coefficient $\Theta_o$ for dextrans of low molecular weight, neither intrinsic membrane parameters $r_0$ and $\omega_o$ nor transcapillary glomerular pressure gradient $\Delta P$ and ultrafiltration coefficient $K_r$ were affected. These results indicate that most of the observed renal hemodynamic effects of angiotensin converting enzyme inhibition in normal subjects are actually related to an increase in glomerular plasma flow. However, these results in normotensive, healthy volunteers probably should not be extrapolated to pathological conditions. Indeed, in overt diabetic nephropathy, a 90-day course of ACE inhibition was found to improve both hemodynamic determinants and membrane size selectivity.\textsuperscript{20}

Long-term ACE inhibitor treatment has been found to upregulate the number or density of renal glomerular receptors to Ang II in rats,\textsuperscript{4} which could increase the renal vascular reactivity to angiotensin. Moreover, it has recently been shown that Ang II concentrations exhibit marked circadian variations even on long-term ACE inhibitor treatment. Indeed, inhibition of ACE and suppression of Ang II formation are dissociated, because the small amount of ACE activity not inhibited by ACE inhibitors and the increase in active renin and angiotensin I due to initial removal of Ang II–mediated negative feedback of renin release actually resulted in a significant recovery of Ang II concentration by 50% or more of initial values in the 4–6 hours after ACE inhibition.\textsuperscript{6,7}

The present study was designed to assess whether prior short-term ACE inhibition may affect the renal and hormonal responses to low-dose exogenous Ang II. In both settings, Ang II consistently exerted a profound antinatriuretic effect related to an increase in both proximal and distal fractional reabsorption of sodium as suggested by calculation from lithium clearance. This antinatriuretic effect is probably mediated through a direct tubular effect or hemodynamic changes. Accordingly, Ang II has been demonstrated to directly stimulate the sodium-hydrogen antiport in the proximal tubule.\textsuperscript{21} Moreover, a rise in filtration fraction and the ensuing increase in oncotic pressure in the peritubular capillary increase the driving force for proximal reabsorption. Ang II also decreases renal medullary blood flow and pressure, and the ensuing decrease in interstitial pressure was found to enhance sodium reabsorption in the distal part of the nephron.\textsuperscript{22}

Our results showed that a low pressor dose of Ang II had comparable effects on whole-kidney hemodynamic and peripheral hormonal changes whether it is administered in baseline conditions or superimposed onto short-term ACE inhibition. This suggests that acute changes in Ang II concentrations after repeated once-a-day ACE inhibitor administration does not significantly affect renal vascular reactivity, although Ang II receptors were found upregulated in the rat.

Dextran sieving curves during Ang II administration were similar to the control curve over the entire range of dextran radii, including low molecular weight dextran, whose filtration is primarily dependent on determinants of glomerular hemodynamics. Although RPF, and therefore glomerular plasma flow, was reduced by Ang II, fractional clearance of low-radii dextrans remained stable because of an increase of convective forces acting in the opposite direction. Using the isoporous+shunt model developed by Deen et al,\textsuperscript{15} we computed that Ang II increased the transcapillary glomerular pressure gradient $\Delta P$ by 16.6% while decreasing the ultrafiltration coefficient $K_r$. Conversely, intrinsic membrane parameters—namely, $r_0$, the mean radius of restrictive pores, and $\omega_o$, an index of the shunt pathway

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Placebo administration</th>
<th>Cilazapril administration</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\Delta P$ (mm Hg)</td>
<td>36</td>
<td>42</td>
</tr>
<tr>
<td>$K_r$ (mL/min per mm Hg)</td>
<td>26.9</td>
<td>13.6</td>
</tr>
<tr>
<td>$r_0$ (nm)</td>
<td>5.5</td>
<td>5.5</td>
</tr>
<tr>
<td>$\omega_o$</td>
<td>0.0008</td>
<td>0.0008</td>
</tr>
</tbody>
</table>

\textsuperscript{Ang II, angiotensin II; $\Delta P$, transcapillary glomerular pressure gradient; $K_r$, ultrafiltration coefficient; $r_0$, mean effective radius of restrictive pores; $\omega_o$, fraction of filtrate passing through the nonrestrictive shunt pathway. $n=6.$}
through large, nonrestrictive pores—were essentially unaffected by Ang II at the dose used in this study.

Of note, Loon et al\(^2\) found that Ang II increased fractional dexter clearance over the range of 3.4 to 5.4 nm, suggesting a shift of glomerular pores toward a larger size. Our results do not contradict the findings from Loon et al, who used a much higher dosage of Ang II (21±9 ng · kg\(^{-1}·\)min\(^{-1}\)), but indicate that Ang II–induced changes in permselectivity could be readily demonstrated at pharmacological concentrations when GFR is allowed to decrease and blood pressure to increase by an average of 15 mm Hg.

Although blockade of Ang II formation is responsible for a major share of the effects of ACE inhibitors on renal hemodynamics and sodium excretion, ACE inhibition, which also reduces degradation of kinins, may have additional effects through enhancing availability of this peptide. Because Ang II at this unique dose exerted similar effects on whole renal hemodynamics, sodium excretion, and blood pressure, whether or not superimposed on previous ACE inhibition, these findings indirectly suggest that most, if not all, of the renal effects of cilazapril are mediated through suppression of Ang II formation, as formerly suggested in dogs.\(^2,24\) Accordingly, the analysis of the determinants of transmembrane flux in the current study disclosed a similar Ang II–induced glomerular hypertension and drop in \(K_f\) when subjects were submitted to previous ACE inhibition.

Acknowledgments

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