Effect of ACE inhibition on glomerular permselectivity and tubular albumin concentration in the renal ablation model

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Submitted 9 November 2010; accepted in final form 27 March 2011

Sangalli F, Carrara F, Gaspari F, Corna D, Zoja C, Botti L, Remuzzi G, Remuzzi A. Effect of ACE inhibition on glomerular permselectivity and tubular albumin concentration in the renal ablation model. Am J Physiol Renal Physiol 300: F1291–F1300, 2011. First published March 30, 2011; doi:10.1152/ajprenal.00656.2010.—Despite the central role of tubular plasma proteins that characterize progressive kidney diseases, protein concentrations along the nephron in pathological conditions have not been quantified so far. We combined experimental techniques and theoretical analysis to estimate glomerular and tubular levels of albumin in the experimental model of 5⁄6 nephrectomy (Nx) in the rat, with or without angiotensin-converting enzyme (ACE) inhibition. We measured glomerular permselectivity by clearance of fluorescent Ficoll and albumin and used theoretical analysis to estimate tubular albumin. As expected, 5⁄6 Nx induced an elevation of the fractional clearance of the largest Ficoll molecules (radii >56 Å, P < 0.05), increasing the importance of the shunt pathway of the glomerular membrane and the albumin excretion rate (119 ± 41 vs. 0.6 ± 0.2 mg/24 h, P < 0.01). ACE inhibition normalized glomerular permselectivity and urinary albumin (0.5 ± 0.3 mg/24 h). Theoretical analysis indicates that with 5⁄6 Nx, an increased albumin filtration overcomes proximal tubule reabsorption, with a massive increase in average albumin concentration along the tubule, reaching the highest value of >2,500 μg/ml at the end of the collecting duct. ACE inhibition improved glomerular permselectivity, limiting albumin filtration under proximal tubule reabsorption capacity, with low albumin concentration along the entire nephron, averaging <13 μg/ml at the end of the collecting duct. These results reinforce our understanding of the mechanisms of renal disease progression and the effects of angiotensin II antagonism. They also suggest that evaluation of tubular protein concentration levels could help to identify patients at risk of kidney disease progression and to improve clinical management.

proteinuria; proximal tubule; protein reabsorption; fractional clearance; mathematical modeling

CURRENT THERAPIES FOR PROTEINURIC progressive renal diseases are based on angiotensin-converting enzyme (ACE) inhibition or angiotensin receptor (AT1) antagonism (31, 44). Beside reducing blood pressure, these therapies lower abnormal urinary protein excretion and retard the progression of the disease, at both the experimental and clinical levels (5, 42). However, there are still open questions about the mechanisms responsible for these beneficial effects of ANG II antagonism. Almost 30 years of experimental and clinical studies suggested a common mechanism responsible for degeneration of glomerular and tubular function in these diseases (1, 43), pointing out the central role of glomerular capillary hypertension in glomerular membrane dysfunction, including permselectivity changes (19). We and others have suggested that abnormal glomerular filtration of plasma proteins exposes tubular cells to excessive protein reabsorption, with generation of toxic mediators of tissue inflammation and scarring (23, 48, 51).

The evidence that ANG II antagonism strongly prevents renal disease progression and favors tissue repair (24, 34–36) has generated a number of clinical trials that confirmed important beneficial effects in patient populations and established the current clinical therapeutic approach (42). The effect of current therapies is monitored in terms of the reduction or prevention of protein excretion. It is generally accepted that ANG II antagonism improves glomerular permselectivity, lowers albumin filtration and, consequently, urinary albumin excretion. However, a quantitative evaluation of the effect of these treatments on albumin concentration in glomerular ultrafiltrate and in tubular fluid has not been formally addressed.

Original micropuncture studies showed that plasma albumin filtration is highly restricted by the glomerular capillary membrane, and its concentration in ultrafiltrate is three to four orders of magnitude lower than in circulating plasma (18). These data have been confirmed recently by in vivo two-photon microscopy studies (32, 47), and the average glomerular ultrafiltrate-to-plasma albumin concentration ratio was estimated to be ~0.002 under normal conditions in the rat. In proteinuric conditions, few experimental studies, still based on the micropuncture technique, suggested that glomerular albumin filtration is significantly increased and highly variable among nephrons, compared with normal conditions (6, 28). No data are available on the effect of ANG II inhibition on albumin concentration in glomerular ultrafiltrate, or for quantification of albumin concentration along the entire renal tubule.

These experimental estimations are difficult to obtain because tubular fluid is not easily accessible from the surface of the kidney and because albumin concentrations in tubular fluid microsamples are very low (41, 49). Thus indirect evaluations of glomerular permselectivity properties have been obtained, at the experimental and clinical levels, using exogenous trace macromolecules of graded size (9, 40), such as neutral dextran and Ficoll (7, 18), the latter being more globular in shape and more closely resembling the steric configuration of plasma proteins.
proteins. These studies showed that in experimental models of glomerular diseases, and in proteinuric clinical conditions, the selectivity of the glomerular membrane toward the largest neutral macromolecules is altered, and ANG II antagonism restores glomerular membrane size-selective function (9, 39). However, to what extent tubular albumin concentration is actually elevated along the nephron in proteinuric conditions is not known. Similarly, to what extent ACE inhibition induces amelioration of glomerular permeselectivity and restores tubular albumin concentration is not known. We therefore combined solute clearance techniques and theoretical modeling to estimate glomerular and tubular levels of albumin in proteinuric conditions in the rat with or without ACE inhibition. Specifically, we determined glomerular permeselective dysfunction with fluorescently labeled Ficoll molecules and quantified urinary albumin excretion in normal conditions in animals subjected to renal mass ablation and in partially nephrectomized animals treated with an ACE inhibitor. We then used a recently developed theoretical model (22) to compare glomerular permeselective changes with estimates of albumin concentration in tubular fluid, in the different experimental conditions.

METHODS

Study design. Twenty-two male Sprague-Dawley rats (Charles River Italia, Calco, Italy) with initial body weights of 275–350 g were used. Animal care and treatment were conducted according to current law (24). Renal mass reduction (RMR) by 5/6 nephrectomy (Nx) was carried out as previously described (2, 29). Our experimental design included three experimental groups: a sham-operated control group; an RMR group; and an RMR + lisinopril group. Lisinopril (AstraZeneca, Milan, Italy) was administered in the drinking water (12.5 mg/l) from day 7 after RMR. In all groups, systolic blood pressure (SBP) and urinary protein excretion were monitored throughout a 4-wk observation period. SBP was measured by tail-cuff plethysmography in awake animals (15), while 24-h urine samples were collected using metabolic cages and proteinuria determined for each animal level.

Urinary albumin excretion. For determination of albumin concentration, urine samples were subjected to size-exclusion HPLC (1100 HPLC system, Agilent Technologies, Milan, Italy). Aliquots of urine samples (20 μl) were injected onto a Zorbax GF-250 column (250 × 9.4 mm, CPS Analytica, Milan, Italy). The mobile phase consisting of 50 mM Na2HPO4 and 150 mM NaCl (adjusted at pH 7.0 with phosphoric acid) was pumped at a flow rate of 0.5 ml/min. The absorbance of emerging peaks was monitored at 214 nm. Calibration curves were generated using an albumin working solution (1,000 mg/ml) by serial dilutions with phosphate saline buffer. Albumin concentration was determined through calculation of the area under the peak.

Clearance studies. Four weeks after Nx, or sham operation, animals were anesthetized with pentothal sodium (60 mg/kg body wt, Hospira, Milan, Italy) and both the right femoral artery and vein were cannulated with a PE-50 cannula. Two saline solutions, one containing iohexol (Omnipaque 300, final concentration 16.2 mg/ml, GE Healthcare, Milan, Italy) and PAH (2 mg/ml, Sigma-Aldrich, St. Louis, MO), and the other containing fluorescently labeled Ficoll (FITC-Ficoll, 1 mg/ml, TdB Consultancy, Uppsala, Sweden) were used for infusion into the two vessels, respectively. The Ficoll mixture contained polydisperse molecules with molecular mass ranging from 17.5 to 132 kDa. Two bolus infusions of 0.35 ml of the iohexol and PAH solution and 0.5 ml of the FITC-Ficoll solution were slowly injected at 0.5 and 1.0 ml/min, respectively. Then, sustaining infusions of both solutions at 1 ml/h were maintained throughout the study. The bladder was cannulated, and three 30-min clearance periods were started after a 45-min equilibration period. Urine and plasma samples were used for determination of iohexol, PAH, and FITC-Ficoll concentrations, as described previously (17). Briefly, aliquots of plasma (50 μl) or urine (10 μl) were diluted up to 200 μl with distilled water and deproteinized by perchloric acid. Samples were then centrifuged and analyzed by HPLC with the detector wavelength set at 254 nm and a 250 × 4-mm column packed with LiChrosorb C-18 (Merck, Darmstadt, Germany). Iohexol and PAH were eluted by a mixture of deionized water/acetonitrile (96:4 vol/vol, adjusted at pH 2.5 with phosphoric acid), pumped at a rate of 1.5 ml/min. Internal calibration curves of both iohexol and PAH were prepared for each set of samples.

FITC-Ficoll molecules were separated by gel filtration by using System Gold HPLC (Beckman, Fullerton, CA) equipped with a fluorescence detector (FP-821, Jasco, Cremlenna, Italy) with a xenon lamp set at 492-nm excitation and 520-nm emission wavelength. Five microliters of plasma or urine (diluted 1:1 with distilled water) were injected into a 300 × 7.8-mm TSK G4000PWXL polymeric column (Tosoh, Brughiero, Italy) and eluted at a flow rate of 0.5 ml/min with 50 mM Na2HPO4 and 150 mM NaCl (adjusted at pH 7.0 with phosphoric acid). Detection of the FITC-Ficoll concentration during separation was digitally recorded. For column calibration, the FITC-Ficoll solution (Ficoll 70, 1 mg/ml) was separated on a gel filtration column (Sephacryl S300-HR, Pharmacia, Uppsala, Sweden), previously calibrated with Ficoll standards of known molecular weight, and 14 fractions collected with a molecular radius ranging from 16 to 120 Å were used as standards for HPLC column calibration. Effective molecular radii of Ficoll in these fractions was calculated as previously described (10). Preliminary analyses showed a complete recovery of FITC-Ficoll upon injection onto the chromatographic column and no carryover effects.

Theoretical analysis of glomerular size selectivity. Glomerular filtration rate (GFR) and renal plasma flow (RPF) were calculated from plasma clearance of iohexol and PAH, respectively. The fractional clearance of Ficoll molecules of graded sizes was calculated as the ratio between the urine-to-plasma concentration ratio of FITC-Ficoll and that of iohexol (8). To investigate the intrinsic changes in glomerular membrane size-selective properties, we analyzed Ficoll sieving coefficients using the established mathematical model described in detail previously (12, 33). We assumed the glomerular membrane to be perforated by cylindrical pores having a log-normal distribution of their radii, described by two parameters, the mean (μ) and standard deviation (σ) of the corresponding normal probability distribution. In parallel, we assumed that the membrane is perforated by large pores, not selective even for largest macromolecules (>76 Å). This shunt pathway parameter (ωω) represents the fraction of filtered water that would cross the glomerular membrane through nonelective pores if plasma proteins were absent (12). The model is also based on the glomerular ultrafiltration coefficient (Kf), the product of hydraulic permeability and filtering surface area of the glomerular membrane. We calculated Kf as extended to all glomerular population in both kidneys, using the model previously described (14) and mean glomerular hemodynamic parameters measured for each clearance study. For both theoretical models, we assumed the mean glomerular transmembrane hydraulic pressure difference to be in line with previous micropuncture data obtained in the RMR model (3). The intrinsic membrane permeability parameters were calculated, as previously reported (15), minimizing the sum of squared errors between experimental and calculated sieving coefficients, at the single-animal level.

Theoretical analysis of tubular albumin reabsorption. To estimate albumin concentration along the proximal tubule (PT), and the resulting fractional clearance of the protein in the three experimental conditions, we used the theoretical model developed by Lazzara and...
Deen (22). Briefly, this model assumes saturable endocytosis kinetics for filtered albumin at the tubular level, with a maximum reabsorptive capacity (\( V_{\text{max}} \)), and concentration at half saturation (\( K_m \)). The model considers the effects of fluid flow in the proximal tubular lumen and albumin diffusion in the microvillar space. The model allows estimation of albumin reabsorption along the PT and corresponding tubular fluid concentration (\( Calb \)) on the basis of geometrical parameters of the PT, assumed \( V_{\text{max}} \) and \( K_m \), and of the boundary conditions, albumin concentration in Bowman’s capsule (BC) and single-nephron GFR (SNGFR). We estimated an average value of SNGFR in our control animals, dividing measured GFR by an assumed number of 54,000 nephrons in both kidneys (15). Similarly, SNGFR in rats subjected to RMR was calculated assuming a reduction in number of functioning nephrons equal to five-sixths.

Since we measured the amount of albumin excreted, we used this theoretical model to derive, by an iterative procedure, the best estimate of albumin concentration in the BC (\( Calb_0 \)) on the basis of actual albumin excretion. To this purpose, we first estimated \( Calb \) at the end of the PT from albumin excretion assuming that PT fluid reabsorption equals two-thirds of the GFR in all animals and that albumin is neither reabsorbed nor secreted by the distal tubule and the collecting duct. We then calculated, by an iterative procedure, the value of \( Calb_0 \) that allows to obtain the estimated \( Calb \) at the end of the PT for assumed values of \( K_m \) and \( V_{\text{max}} \). We also assumed different values of \( K_m \) and \( V_{\text{max}} \) in line with the literature (22) to make a sensitivity analysis of these parameters on tubular \( Calb \) in the PT in different experimental conditions, as explained in detail in RESULTS.

Statistical analysis. Data are expressed as means ± SD. Statistical analysis was performed by two-way ANOVA (Prism, GraphPad Software, San Diego, CA). Differences between two groups were considered statistically significant for \( P < 0.05 \).

RESULTS

Results on SBP, total urinary proteins, and albumin excretion at the end of the observation period are reported in Fig. 1 and Table 1. As expected, ACE inhibition significantly prevented the rise in SBP observed in RMR animals. Urinary proteins were significantly higher in rats with RMR than in control sham-operated rats, and lisinopril completely prevented proteinuria. The same trend was observed for the albumin excretion rate, as shown in Fig. 1. Of note, the urinary albumin concentration peak was much higher in RMR than in controls (see Fig. 1), but in RMR animals treated with lisinopril very low albumin peaks were observed. As a result, urinary albumin excretion was negligible in sham-operated animals, significantly increased in RMR, and almost completely prevented in lisinopril-treated rats. Morphological analysis of kidney tissue by optical microscopy in RMR animals showed focal and segmental glomerulosclerosis in 34% of glomeruli on average and a mild presence of tubular protein casts. In lisinopril-treated animals, glomerulosclerosis was partially prevented (afflicting 12% of glomeruli on average) and protein casts in the tubules were absent (Remuzzi A, personal communication).

Both GFR and RPF were reduced in RMR animals, and lisinopril treatment partially but significantly prevented the fall in renal function as reported in Table 1. No statistically significant differences were found in filtration fraction. As reported in Fig. 2, in animals with intact kidneys the fractional clearance (\( \theta \)) of Ficoll molecules decreased uniformly with size, with a mean value of \( 9 \times 10^{-5} \) for 76-A-radius molecules. Ficoll \( \theta \) in animals with RMR was numerically lower than in controls for radii <30 Å and significantly higher for molecular size ≥54 Å. In RMR animals treated with lisinopril, Ficoll \( \theta \) values were comparable to normal rats or even numerically lower for largest macromolecules, ranging from 50 to 76 Å in radius.

Calculated best fit values of mean (\( u \)) and distribution (\( \sigma \)) of pore radii and the shunt parameter (\( \theta_0 \)) are reported in Table 2 and Fig. 3. On the basis of previously reported micropuncture data (19), we assumed that in the RMR group the mean glomerular membrane pressure difference was increased compared with control animals and that ACE inhibition prevented this increase. As expected, we calculated that \( 5/6 \) \( N_x \) is associated with an important reduction in total kidney \( K_t \), in line with previous observations (3, 4). In these animals \( K_t \) was almost equal to one-sixth of control animals, closely reflecting the extent of surgical mass reduction. In lisinopril-treated animals, mean calculated \( K_t \) was significantly lower than in the
control group, but with a reduction of only 50%. Thus we estimated that ACE inhibition increased \( K_0 \) compared with untreated RMR rats (20). The pore-size distribution parameters \( u \) and \( s \), calculated by best fitting of Ficoll fractional clearance data using the heteroporous model of size selectivity, were not significantly affected by either RMR or lisinopril treatment, and the pore-size distribution \( g(r) \) was only slightly shifted toward smaller pore dimensions in both RMR groups compared with control animals (see Fig. 3). By contrast, the calculated shunt parameter \( \omega_0 \) was significantly higher in RMR animals compared with controls. Of note, in RMR animals treated with lisinopril, \( \omega_0 \) was significantly lower than in the untreated RMR group (see Table 2 and Fig. 3).

The results of theoretical analysis of \( Calb \) along the PT are reported in Figs. 4 and 5. In the initial step, we determined the values of \( V_{\text{max}} \) and \( K_m \) for control rats, assuming a range of BC albumin concentration (\( Calb \)) and the estimated albumin concentration at the PT end. Using an iterative procedure, we assumed \( Calb \) in each animal and an initial guess of \( V_{\text{max}} \) and \( K_m \); then, we changed \( V_{\text{max}} \) or \( K_m \) until calculated \( Calb \) at the PT end was equal to that estimated from measured urinary excretion. We initially determined, for each animal in the control group, the values of \( V_{\text{max}} \) (assuming constant \( K_m = 31 \mu g/ml \)) that allowed for obtaining the measured albumin flow rate at the PT end with \( Calb \) values ranging from 10 to 66 \( \mu g/ml \). These values were assumed on the basis of available data from micropuncture (41, 49) and two-photon microscopy studies (32, 47). As reported in Fig. 4A, the mean values of calculated \( V_{\text{max}} \) ranged from 0.10 to 0.21 ng·s\(^{-1}\)·mm\(^{-2}\) for the assumed range of \( Calb \) values. As shown in Fig. 4A, for increasing values of \( Calb \), the tubular albumin flow rate is increased and higher values of \( V_{\text{max}} \) are achieved with increasing albumin receptor capacity required to meet the measured protein flow rate at the PT end. Subsequently, we calculated corresponding values of \( K_m \), keeping a constant value of \( V_{\text{max}} = 0.15\) ng·s\(^{-1}\)·mm\(^{-2}\) (Fig. 4B). The mean values of estimated \( K_m \) ranged from 59 to 26 \( \mu g/ml \). Upon an increase in \( Calb \), the calculated \( K_m \) values decreased as a lower concentration at half-saturation makes the albumin receptor more efficient.

We then calculated the values of \( Calb \) for the control and the two experimental groups, once again assuming a range of

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**Table 1. Kidney functional parameters at the end of the observation period**

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>BW, g</th>
<th>SBP, mmHg</th>
<th>UrProt, mg/24 h</th>
<th>UrAlb, mg/24 h</th>
<th>GFR ml/min</th>
<th>ml/min·100 g(^{-1})</th>
<th>RPF ml/min</th>
<th>ml/min·100 g(^{-1})</th>
<th>FF,%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham-operated</td>
<td>7</td>
<td>446 ± 21</td>
<td>119 ± 8</td>
<td>25 ± 4</td>
<td>0.62 ± 0.21</td>
<td>2.46 ± 0.75</td>
<td>0.55 ± 0.17</td>
<td>8.00 ± 1.58</td>
<td>1.80 ± 0.38</td>
<td>30.3 ± 6.17</td>
</tr>
<tr>
<td>RMR</td>
<td>7</td>
<td>365 ± 45</td>
<td>189 ± 17</td>
<td>141 ± 51</td>
<td>118.7 ± 40.8</td>
<td>0.93 ± 0.35</td>
<td>0.26 ± 0.11</td>
<td>2.89 ± 1.13</td>
<td>0.80 ± 0.35</td>
<td>33.0 ± 6.32</td>
</tr>
<tr>
<td>RMR + lisinopril</td>
<td>8</td>
<td>386 ± 57</td>
<td>117 ± 14</td>
<td>17 ± 11</td>
<td>0.45 ± 0.27</td>
<td>1.18 ± 0.20</td>
<td>0.31 ± 0.05</td>
<td>4.91 ± 1.07</td>
<td>1.28 ± 0.23</td>
<td>24.9 ± 6.44</td>
</tr>
<tr>
<td>Sham vs. RMR</td>
<td>P &lt; 0.01</td>
<td>P &lt; 0.01</td>
<td>P &lt; 0.01</td>
<td>P &lt; 0.01</td>
<td>P&lt;0.01</td>
<td>P &lt; 0.01</td>
<td>P &lt; 0.01</td>
<td>NS</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>RMR vs. RMR + lisinopril</td>
<td>NS</td>
<td>P &lt; 0.01</td>
<td>P &lt; 0.01</td>
<td>P &lt; 0.01</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
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<td></td>
</tr>
<tr>
<td>Sham vs. RMR + lisinopril</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
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<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
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</table>

Values are means ± SD. RMR, renal mass reduction; BW, body weight; SBP, systolic blood pressure; UrProt, urinary protein excretion rate; UrAlb, urinary albumin excretion rate; GFR, glomerular filtration rate; RPF, renal plasma flow; FF, filtration fraction; NS, not statistically significant.
Table 2. Calculated glomerular capillary membrane permeability parameters

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>ΔP (Assumed), mmHg</th>
<th>K_m, ml·min⁻¹·mmHg⁻¹</th>
<th>u, Å</th>
<th>σ</th>
<th>ω_uo ×10⁻⁵</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham-operated</td>
<td>7</td>
<td>45</td>
<td>0.15 ± 0.08</td>
<td>39.2 ± 2.0</td>
<td>1.16 ± 0.02</td>
<td>5.5 ± 5.4</td>
</tr>
<tr>
<td>RMR</td>
<td>5</td>
<td>55</td>
<td>0.02 ± 0.01</td>
<td>37.8 ± 0.6</td>
<td>1.17 ± 0.01</td>
<td>22.4 ± 16.4</td>
</tr>
<tr>
<td>RMR+lisinopril</td>
<td>7</td>
<td>45</td>
<td>0.07 ± 0.01</td>
<td>37.3 ± 3.3</td>
<td>1.17 ± 0.03</td>
<td>2.5 ± 1.0</td>
</tr>
<tr>
<td>Sham vs. RMR</td>
<td>P</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>RMR vs. RMR+lisinopril</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Sham vs. RMR+lisinopril</td>
<td>P</td>
<td>0.05</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are means ± SD. ΔP, mean transmembrane hydraulic pressure difference; K_m, glomerular ultrafiltration coefficient; u, mean radius of log-normal membrane pore size distribution; σ, SD of log-normal pore size distribution; ω_uo, shunt parameter.

V_max values and keeping K_m constant and vice versa. For these calculations we assumed the range of V_max and K_m to be in line with those estimated for the control group (V_max from 0.075 to 0.225 ng·s⁻¹·mm⁻² and K_m from 25 to 60 µg/ml). We reasoned that in the RMR group the V_max of surviving nephrons may be the same as in control rats, or eventually reduced due to the underlying pathological changes. Similarly, the range of K_m values was assumed as in the control group, based on the normal function of remaining nephrons or eventually increased, if in pathological conditions albumin concentration at normal function of remaining nephrons or eventually increased. Specifically, we assumed V_max values ranging from 0.050 to 0.225 ng·s⁻¹·mm⁻² and values of K_m from 25 to 75 µg/ml. As shown in Fig. 4C, for the RMR group Calb₀ ranged from 117 to 188 µg/ml for the entire range of V_max values and a constant K_m, suggesting that, independently from assumed V_max, albumin concentration in BC is consistently increased compared with control rats. On the contrary, in the lisinopril-treated group, calculated Calb₀ was remarkably low for the entire range of V_max assumed, even lower than in control rats, ranging from 0.5 to 4.0 µg/ml only. We obtained similar results when we assumed a range of V_max values with constant V_max to estimate Calb₀ in both experimental groups, as shown in Fig. 4D. For the RMR group, Calb₀ was almost constant upon variation of K_m (decreasing from 159 to 148 µg/ml only) and higher than values calculated from control rats. Calculated Calb₀, as a function of assumed K_m, was also importantly lower than controls in the lisinopril group, ranging from 2.8 to 0.6 µg/ml. The large differences in estimated Calb₀ in the two experimental groups, compared with the control group, for the entire range of assumed V_max and K_m indicate that an effective increase in glomerular albumin filtration must take place in RMR animals and that changes in tubular albumin uptake cannot completely explain increased urinary excretion. The same observation applies to animals treated with the ACE inhibitor, in which changes in tubular albumin uptake alone cannot explain the prevention of albuminuria.

The values of Calb at different axial positions along the PT (x), calculated for average values of assumed V_max and K_m, are reported in Fig. 5. As expected, in the control group Calb decreased along the PT from 34 µg/ml to very low values at the PT end (0.6 µg/ml). On the contrary in the RMR group, PT albumin concentration actually increased along the PT, reaching the average final value of 332 µg/ml. In the lisinopril-treated group, the already low albumin concentration in the BC (Calb₀ = 2.0 µg/ml) further decreased along the PT with a concentration of 0.7 µg/ml at the PT end. To evaluate the worst-case scenarios, we also considered the condition of lowest and highest albumin reabsorption by the PT. We assumed V_max = 0.050 ng·s⁻¹·mm⁻² and K_m = 75 µg/ml for the lowest albumin receptor kinetics and V_max = 0.225 ng·s⁻¹·mm⁻² and K_m = 25 µg/ml for the highest albumin receptor kinetics, respectively. Even in these extreme cases, the calculated values of Calb₀ are importantly higher in RMR animals compared with the control group and much lower in RMR animals treated with lisinopril.

On the basis of these estimates of PT Calb, we also calculated the expected Calb along the entire nephron. To this purpose, we assumed that no albumin reabsorption takes place after the PT, and we calculated Calb on the basis of tubular

fluid flow and measured albumin excretion. In line with the literature (45), we assumed that filtrate flow is reduced, due to water reabsorption, to a fixed level of 33, 20, and 10% of filtrate, respectively, at the end of the PT, at the end of Henle’s loop, and at the end of the distal tubule. Water reabsorption along the last part of the nephron was assumed to be variable, and the tubular fluid flow rate at the end of the collecting duct derived from measured diuresis, that averaged 19.6/11006 5.8, 45.3/11006 12.0, and 36.4/11006 9.7 ml/24 h, respectively, for the control, RMR, and RMR/11001 lisinopril group. The results are reported in graphical form in Fig. 6 for the three experimental groups on average. In control conditions, the estimated albumin filtration load is almost completely reabsorbed by the PT and the final Calb was 33/11011 g/ml at the end of the collecting duct. By contrast in the RMR group, for the elevated filtered albumin at glomerular level the process of tubular albumin uptake cannot reabsorb a large fraction of filtered albumin. Thus the final result is that in these animals, due to water reabsorption, Calb massively increases during the passage along the PT and the following segments of the nephron, with a very high final Calb (>2,500 µg/ml), >80 times that observed for the control group. In the lisinopril treatment group, this condition was completely prevented. Despite massive renal ablation, Calb in these animals was very low along the entire nephron, starting from the PT and reaching the maximum average Calb of only 13 µg/ml in the final urine.

DISCUSSION

The results of the present study demonstrate that the important increase in albumin excretion in 5/6 RMR in the rat is associated with effective changes in glomerular size-selective function, consisting of selective elevation of fractional clearance of very large neutral test macromolecules that escape from the circulation by a nonselective shunt pathway across the glomerular capillary membrane. In addition, our results show that the important preventive effect of ACE inhibition on albuminuria, in this experimental model of nephropathy, is associated with lower fractional clearance of the largest test macromolecules and a significant reduction in the membrane shunt pathway. The permselective function of the glomerular capillary wall is determined by the sieving properties of the three layers of the membrane, i.e., the endothelial layer, the glomerular basement membrane, and the epithelial layer, as recently reported (16). Selective changes in even one of the membrane components may result in elevated albumin filtration, and likely in protein excretion, as reviewed by Deen (11). Detailed theoretical analyses of the process of macromolecule filtration across individual membrane layers have been reported in the literature (13, 18, 21, 27); however, these models are based on several assumptions regarding the complex ultrastructure of the membrane components. The uncertainty of these dimensions, as well as the heterogeneity of the membrane ultrastructure, make the use of these theoretical models more difficult and their predictions harder to describe in simple/intuitive terms, compared with the classic heteroporous model of the glomerular size-selective function (12).

Our experimental results on glomerular permselective function are in line with previous observations (3, 4) and document more completely the effective changes in glomerular permeability function due to extended size range of test macromole-

Fig. 4. Results of the theoretical analysis of tubular albumin reabsorption. A: values of maximum reabsorptive capacity (V max) calculated for different assumed values of albumin concentration in Bowman’s capsule (Calb0) for control group. B: calculated values of concentration at half-saturation (K m) in the control rats for assumed values of Calb0. The bold lines represent mean group values, and dotted lines represent the range of values calculated for the control group. C and D: calculated Calb0 derived for the mean group values of urinary albumin excretion and glomerular hemodynamics of the 3 animal groups for different assumed values of V max or the K m. See METHODS for a detailed description of input parameters and theoretical analysis.
along the proximal tubule in the control, RMR, and RMR + lisinopril groups calculated for average values of $V_{\text{max}} = 0.15 \text{ng} \cdot \text{s}^{-1} \cdot \text{mm}^{-2}$ and $K_m = 31 \mu \text{g/ml}$. Grey area represent the value of $Calb$ calculated for the worst cases for lowest ($V_{\text{max}} = 0.050 \text{ng} \cdot \text{s}^{-1} \cdot \text{mm}^{-2}$ and $K_m = 75 \mu \text{g/ml}$, dotted line) and highest albumin reabsorptive capacity ($V_{\text{max}} = 0.225 \text{ng} \cdot \text{s}^{-1} \cdot \text{mm}^{-2}$ and $K_m = 25 \mu \text{g/ml}$, dashed line) in the RMR and RMR + lisinopril group, respectively.

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inopril was associated with reduced albumin excretion, but this does not indicate to what extent albumin concentration in glomerular ultrafiltrate is actually reduced. On the other hand, it is difficult to measure directly albumin concentration in tubular fluid along the nephron using the micropuncture technique or using renal imaging in this and other experimental models (49). We then adopted a theoretical approach to estimate changes in glomerular albumin filtration and in tubular fluid albumin concentration induced by RMR and the effects of the conventional antiproteinuric therapy (i.e., ACE inhibition), using a model of albumin reabsorption by the PT as recently reported (22). This model allows calculation of PT fluid albumin concentration assuming, as input parameters, the concentration of the protein in the BC, and two parameters of albumin receptor kinetics, $K_m$ and $V_{\text{max}}$.

On the basis of measured albumin excretion, we derived albumin concentration in the end-side of the PT and we adopted an iterative procedure for estimating corresponding albumin concentration in the BC. As the values of the receptor kinetic parameters have not been directly measured or derived in the rat in vivo, we first performed a sensitivity analysis of $V_{\text{max}}$ assuming a constant value of $K_m$. For control animals, we obtained expected mean values of $V_{\text{max}}$ ranging from 0.10 to 0.21 ng·s$^{-1}$·mm$^{-2}$. We derived this range on the basis of the two boundary conditions, at the opposite side of the PT, the albumin concentration in the BC reported in the literature in physiological conditions, and our estimate of albumin concentration from measured urine excretion. This range is in line with previous estimation of $V_{\text{max}}$ for normal rats (22). Subsequently, we assumed a constant value of $V_{\text{max}} = 0.15$ ng·s$^{-1}$·mm$^{-2}$ to calculate expected values of $K_m$ for the control group, and the estimated $K_m$ ranged from 26 to 59 μg/ml. These values are in good agreement with experimental results (30, 46).

For experimental groups, we assumed that $V_{\text{max}}$ may be unaffected by the experimental conditions, the RMR with or without lisinopril treatment, or that the kidney surgical injury might have reduced albumin receptor capacity. We then calculated albumin concentration in the BC ($Calb_0$) in these two groups assuming a wider range of $V_{\text{max}}$ (from 0.05 to 0.225 ng·s$^{-1}$·mm$^{-2}$) and of $K_m$ (from 25 to 75 μg/ml). Estimated $Calb_0$ was elevated in RMR animals, more than a threefold increase on average over control values. $Calb_0$ values were almost independent of assumed values for $V_{\text{max}}$ and of $K_m$ even for very low values of $V_{\text{max}}$ and high values of $K_m$ that would be expected in case of compromised albumin reabsorption due to kidney tissue injury. Calculation of $Calb_0$ in worst-case conditions confirmed this conclusion (see Fig. 5). These results indicate that effective elevation of albumin filtration at the glomerular level must take place in RMR and that changes in tubular albumin receptor kinetics alone cannot entirely explain the measured elevation in albumin excretion. On the contrary, in animals treated with lisinopril calculated $Calb_0$ values were markedly lower than in controls, with values <5 μg/ml, again independently of assumed values of $V_{\text{max}}$ and $K_m$. These results have two important implications. The first is that a rather small change in permeselectivity properties of the glomerular capillary wall, confined to the shunt pathways, may be responsible for important changes in urinary albumin excretion. The second is that ACE inhibitors exert a selective and efficient influence on glomerular capillary wall permeselectivity.
ity, while it is unlikely that they prevent albuminuria only by increased albumin reabsorption at the tubular level. The low reabsorption of albumin along the PT in the lisinopril group, even for the largest assumed value of $V_{\text{max}}$ and the lowest $K_m$ (see Fig. 5) may be due to low albumin uptake by the receptors of PT cells because of the low concentration of the protein in tubular fluid. Thus even higher values of $V_{\text{max}}$ and lower values of $K_m$ than those assumed in our calculation cannot account by themselves for measured urinary albumin excretion, but effective prevention of abnormal glomerular albumin filtration must take place in RMR animals upon treatment.

Theoretical modeling of albumin reabsorption by the PT allowed us to calculate also average albumin concentration along the entire nephron. As graphically represented in Fig. 6, in the normal rat tubular fluid albumin concentration is rapidly decreased by PT reabsorption in such a way that at the end of the collecting duct albumin concentration remains almost equal to the entrance concentration ($Calb_0$) despite $>99\%$ of water reabsorption. We estimated a completely different condition for animals subjected to RMR. In this group, albumin concentration did not decrease along the PT, but rather it increased, since the filtered albumin load saturated the reabsorptive capacity of PT cells. By the end of the PT, albumin concentration was estimated to double, compared with BC concentration, and in the following segments of the nephron albumin concentration further increased, with an $\sim 17$-fold increase in final concentration, compared with $Calb_0$. It is evident that these abnormally high albumin levels are likely a primary cause of nephron degeneration, in line with previously available evidence on toxic effects of albumin on tubular cells (1, 23, 48, 51).

Animals treated with lisinopril showed very low albumin concentration along the entire nephron despite RMR and independently of assumed $K_m$ and $V_{\text{max}}$. Thus our data provide evidence that ACE therapy reduced glomerular albumin filtration and the low filtered load was completely reabsorbed by the PT. Water reabsorption in distal parts of the nephron only modestly increased albumin concentration. This pattern of very low albumin concentration along the entire nephron (see Fig. 6) strongly supports the hypothesis that the mechanism through which ACE therapy exerts protection in human pathological conditions is likely related to low albumin tubular concentration, which prevents exposure of tubular cells to albumin overload. Even though these concepts have been suggested by us and others in previous investigations (42, 51), the present experimental results and the related theoretical analysis allow for the quantification of the important difference in tubular fluid albumin between normal and proteinuric conditions, as well as the effect of an antiproteinuric therapy.

Our present investigation strengthens the concept that changes in the amount of filtered albumin directly affect its concentration in the tubular fluid along the entire nephron, suggesting that when the amount filtered at the glomerular level exceeds the reabsorptive capacity of the PT, the concentration is elevated by water reabsorption, and the resulting high protein concentration may be deleterious for the nephron because of tubular cell activation, cell differentiation, the production of fibrotic and inflammation mediators, as well as formation of protein casts that block tubular fluid flow (48, 51).

It would be helpful to identify the experimental and clinical conditions in which this imbalance is reached. It would be even more important to identify early changes in glomerular permselective function that increase only slightly the amount of albumin filtered at the glomerular level that can be still completely reabsorbed by the PT and not revealed in the urine. New and more specific markers of glomerular selectivity func-

Fig. 6. Color-coded graphical representation of estimated $Calb$ along the entire nephron in the 3 animal groups. Numbers represent local group average albumin concentration in $\mu$g/ml calculated for the same input values assumed for calculation of average data reported in Fig. 5.
tion have yet to be identified and validated. If such tools were available, it would be possible to start therapeutic intervention before abnormal albumin is observed in the urine, with higher efficacy and specificity of current treatments. In addition, we must also take into consideration that impaired tubular reabsorption might contribute to proteinuria in some renal diseases, and the glomerular vs. tubular origin of proteinuria will be really settled only when reliable micropuncture and/or imaging studies are available.

In conclusion, our results show that in the renal ablation model changes in glomerular permselective function, involving only the shunt pathway, elevate the filtered load of plasma albumin and consequently the increased flow rate of the protein overcomes PT reabsorption, with consequent elevation of protein concentration along the entire nephron. This condition is completely reversed by ACE inhibitor treatment, likely because of improved selectivity of the glomerular membrane, which lowers albumin load to the nephron under the threshold of PT albumin reabsorption capacity, leaving tubular fluid almost devoid of albumin. Elucidation of these mechanisms in renal disease progression, and of the effects of pharmacological therapies, may further improve the outcome of clinical management of the large population of patients affected by proteinuric progressive kidney diseases.

ACKNOWLEDGMENTS

The authors thank Dr. Luca Antiga for help in computations and Silvia Noli for the preparation of the manuscript.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

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