Mild renal ischemia-reperfusion reduces charge and size selectivity of the glomerular barrier

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Andersson M, Nilsson U, Hjalmarsson C, Haraldsson B, Nyström J. Mild renal ischemia-reperfusion reduces charge and size selectivity of the glomerular barrier. Am J Physiol Renal Physiol 292: F1802–F1809, 2007.—Despite recent discoveries of molecules in podocytes, the mechanisms behind most conditions of proteinuria are still poorly understood. To understand more about this delicate barrier, we studied the functional and morphological effects of mild (15 min) renal ischemia-reperfusion injury (IRI). Renal function was studied in rats in vivo, followed by a more detailed analysis of the glomerular barrier in cooled (8°C) isolated perfused kidneys (cIPK). Renal blood flow was quickly restored, whereas the glomerular filtration rate remained halved 30 min after IRI. Tubular cell activity was intact as judged from the unaffected Cr-EDTA U/P concentration ratio. In vivo, the fractional clearance (θ) for albumin increased 16 times. In rats subjected to cIPK starting 30 min after in vivo IRI, θalbumin was 15 times and θF_{crit.36Å} 1.8 times higher than in control cIPKs. According to the heterogeneous charged fiber model, IRI reduced the fiber charge density to 38% of control (P < 0.01, n = 7). Morphometric analysis with electron microscopy did not reveal any changes in the podocytes or the glomerular basement membrane (GBM) after IRI, suggesting more subtle changes of the GBM and/or the endothelial glycocalyx. We conclude that mild renal IRI induces formation of reactive oxygen species, massive proteinuria, and loss of charged fibers with no apparent change in morphology. These novel findings stress the importance of other components of the barrier, such as proteoglycans produced by the glomerular cells, and provide a tentative explanation for the mechanisms behind proteinuria in glomerulonephritis, for example.

endothelium; basement membrane; glycocalyx; kidney; oxidative stress; podocytes; proteinuria

THE NORMAL GLOMERULAR BARRIER is highly permselective, and proteinuria is a hallmark of kidney disease. In recent years, our knowledge of the intricate components of the glomerular barrier has expanded tremendously. This is mainly due to the discoveries of novel proteins in the podocyte slit membrane (26) and their association with hereditary nephrotic syndromes (18). However, the podocyte, the glomerular basement membrane (GBM), and the endothelium all are vital components of the glomerular barrier (7, 10), and defects in any of these layers will lead to impaired size and/or charge selectivity (33). The glomerular charge barrier has been questioned (30), but the controversy now seems to be settled in support of the classic view of charge selectivity (7, 10), albeit with slightly lower negative charge density than originally suggested (23, 33). Most of the charge is provided by proteoglycans and glycosaminoglycans (GAG) produced by glomerular cells themselves (3) to form GBM and glycocalyx cell surface layers (13). The pathophysiological roles of these intricate components of the glomerular barrier remain unclear.

Longer periods of warm ischemia (1–2 h) induce severe oliguria and/or anuria, which effectively eliminate our possibilities to study the glomerular barrier. This obstacle was ingeniously circumvented by Yoshioka et al. (35), as they infused hydrogen peroxide into renal arteries and found dose-dependent reversible proteinuria with no apparent change in morphology. The fractional clearance, θ, for dextrans increased, indicating impaired glomerular size selectivity (35) by the oxygen radicals. Also, during the first hours after renal transplantation, there is marked proteinuria (31), which gradually disappears after a day or two. Interestingly, the urinary loss of heparan sulfate follows a similar pattern (31). In vitro, hydroxyl radicals have been shown to cause depolymerization of heparan sulfate (27), which could explain the proteinuria. To our knowledge, there are no studies of glomerular size and charge selectivity of native kidneys after mild ischemia-reperfusion injury (IRI). The effects of renal IRI on tubular cells have been extensively studied, since acute tubular necrosis (ATN) is a common cause of renal failure in patients (21). Indeed, it is important to find new therapeutic tools for this condition that still today has a high mortality rate. Recently, focus has shifted to alterations in gene and protein expression induced by damage and/or repair. Practically all of these studies focus on tubular damage, which seems to require warm ischemia for 2 h or more.

In other organs, morphological studies have shown that the endothelium is highly sensitive to IRI. Thus cardiac endothelial glycocalyx is disrupted after hypoxia in rats (6, 34) and in guinea pigs (2). The loss of endothelial glycocalyx has been correlated to the appearance of free radical reaction products (6). Also, pretreatment with superoxide dismutase (SOD), an oxygen free radical scavenger, reduces the loss of the endothelial cell coat (2). In kidneys, the peritubular microvascularity is injured after 45 min of ischemia with a twofold increase in circulating vonWillebrand factor, alterations in F-actin, VE-cadherin, and increased permeability to FITC-dextran (32). These findings and other reports suggest endothelial damage to be important for the outcome after IRI (21).

In other organs, IRI has been shown to induce loss of proteoglycans including the endothelial glycocalyx, but little is known about how IRI affects the glomerulus. We wanted to explore whether a short period of ischemia, without apparent damage to the tubular cells, affects glomerular permeability. To obtain new insights about the intricate glomerular barrier, the costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
we used our previously developed experimental techniques (13, 14, 33) and theoretical models (16, 23) to study glomerular size and charge selectivity. Thus cooled isolated perfused kidneys, cIPK, allow for evaluation of glomerular permeability in the absence of tubular modification of the urine. The glomerular size selectivity can be assessed using neutral Ficoll, and charge effects are estimated by comparing size-matched Ficoll to the clearance for albumin. In addition, the glomerular ultrastructure was studied in a blinded fashion and a measure of oxidative stress was obtained by analysis of the endogenous ascorbyl radical concentration. Taken together, the data provide new information about proteinuria induced by mild IRI and suggest that delicate molecules such as proteoglycans are important for the glomerular barrier.

METHODS

Animal Preparation

Experiments were performed in 34 female rats (Sprague-Dawley; Mollegaard, Stensved, Denmark), 12 of which were studied solely in vivo, while cIPK were perfused in the remaining animals after ischemia-reperfusion (n = 7) or sham operation (n = 7) or used for perfusion fixation and electron microscopy (n = 8). The rats had free access to water and standard chow before the experiments. The local ethics committee approved the experiments.

Anesthesia was induced with pentobarbitone (60 mg/kg ip, Apoteksbolaget, Umeå, Sweden), a thermostatically controlled heating pad maintained the body temperature of the rat at 37°C, and the animals were prepared as previously described (14). In the 14 rats subsequently used for isolated, perfused kidney experiments, the animals were eviscerated and one kidney was prepared as described in detail previously (11, 24). The urine was collected in vials continuously weighed for assessment of urine flow, Qu. Arterial pressures, PΑ, were measured in vivo using the tail artery. In the cIPK, PΑ, were measured in vivo using the tail artery. Labview computer software monitored PΑ, urine weight changes, as well as urine flow and pump speed.

Tracers

51Cr-EDTA (Amersham Pharmacia Biotech, Buckinghamshire, UK) was used for calculation of glomerular filtration rate (GFR). Fluorescein isothiocyanate (FITC)-labeled neutral Ficoll molecules (Ficoll70, Bioflor, Uppsala, Sweden) with a molecular Stokes-Einstein radius distribution between 12 and 72 Å was utilized for analysis of glomerular permselectivity. FITC-Ficoll has no electrophoretic mobility, suggesting it to be uncharged (33). 125I-albumin (Isopharma, Kettel, Norway), eluted from an equilibrated desalting column (Sephadex G-25 PD-10; Amersham Pharmacia Biotech, Uppsala, Sweden) to reduce the free iodide content, was also added to the perfusate or injected into the rat.

Perfusate for the cIPK

A modified Tyrode solution containing human albumin (18 g/l, Albumin Immuno, Baxter Medical) 0.27 MBq/l 51Cr-EDTA, 0.5 g/l Ficoll70, and 1.5 MBq/l 125I-albumin was used at a pH of 7.4.

Experimental Protocol

In vivo experiments. A bolus dose (1 ml) of 51Cr-EDTA (0.076 MBq) and 125I-albumin (0.44 MBq) was given in the aortal catheter. Three blood samples (~40 µl in each sample) were aspirated from the caval vein for measurements of ascorbyl radicals, GFR, and fractional clearance (θ) of albumin. To make the left kidney ischemic, the aorta was ligated transiently proximal to the left renal artery for 15 min. The ligature was removed, and blood samples (~40 µl) were aspirated from the caval vein every 5 min for measurements of free radicals, GFR, and θ for albumin.

Total renal blood flow was measured in 10 female rats (Sprague-Dawley; Mollegaard) before and after 15-min ischemia by the use of a mean transit-time flow probe (1RB, Transonic Systems, Ithaca, NY) positioned around the renal artery. cIPK. In seven rats, 15 min of renal ischemia and 30 min of reperfusion in vivo were followed by isolated kidney perfusion. The kidneys were perfused at 8°C to inhibit tubular function, energy consumption, and myogenic tone (5) as well as protease activity (9). Other details can be found elsewhere (24, 33). The same protocol was used for the seven control rats, except for the ischemic period.

Fig. 1. A: glomerular capillary from a control kidney after oxygen-rich fluorocarbon-based perfusion fixation followed by tannic acid treatment. CL, capillary lumen; BM, basement membrane; P, podocyte; US, urinary space. Magnification ×16,000. The white line measures the glomerular BM (GBM) thickness, and the white squares mark arbitrarily chosen points in the lamina densa, where the density of the GBM was assessed. B: glomerular capillary from a kidney subjected to mild ischemia-reperfusion injury (IRI) under similar conditions as for A. The long white line indicates the position where the width of the podocyte foot processes was measured, and the short white lines by the white asterisks mark the diaphragms of the filtration slits.
Perfusion fixation for electron microscopy. A laparotomy was performed in eight rats, and the kidneys were exposed, decapsulated, and isolated in situ. One of the kidneys was subjected to 15-min ischemia and 30 min of reperfusion in vivo, after which both kidneys were fixed by perfusion fixation through a retrograde catheter in the abdominal aorta. The fixative contained an oxygen-carrying emulsion of fluorocarbons as previously described (13).

Sections from 140 glomerular capillaries (n = 8 rats) were examined in a LEO 912AB Omega electron microscope, equipped with a MegaView III Soft Imaging Systems camera by an investigator (C. Hjalmarsson) unaware of which sections were from ischemic kidneys. The density of the glomerular basement membrane (D-GBM) was measured in 10 arbitrarily chosen points of the lamina densa. In each capillary, the thickness of the basement membrane (W-GBM) was measured at five points; the “measuring line” was perpendicular to the podocyte slit diaphragm and the plasmalemma of the adjacent endothelial cell (see Fig. 1A). The width of the podocyte foot processes (nm; W-Podo) was assessed on the same pictures as the basement membrane parameters. The width was measured linearly at the base of the foot processes, following the interface with the basement membrane. The width of the podocyte filtration slits (nm; S-Podo) was assessed by measuring the slit diaphragms interconnecting the foot processes (see Fig. 1B).

Analysis of Tracer Concentrations

Perfusate and urine concentrations of $^{125}$I-albumin and $^{51}$Cr-EDTA were measured in a gamma-counter (Cobra, Auto-Gamma Counting systems, Packard Instrument, Meridan, CT). Corrections were made for background radioactivity and the crossover of $^{51}$Cr-EDTA radiation to the iodine channels.

For calculation of the sieving coefficients for FITC-Ficoll, both perfusate and urine samples were subjected to gel filtration (BioSep-SEC-S3000, Phenomenex, Torrance, CA) with fluorescence detection (RF 1002 Fluorescence HPLC Monitor, Gynko-tek, Germering, Germany) using Chromeleon (Gynko-tek) software. The eluent was 0.05 M phosphate buffer at pH 7.0 with 0.15 M NaCl. A 5- to 10-μl sample was analyzed at an excitation wavelength 492 nm and emission wavelength 520 nm. A calibration curve was obtained by analyzing five monodisperse samples of Ficoll with known molecular radii as previously described in detail (24).

Oxidative Stress Measurements

The measurements of ascorbyl radicals, an endogenous general indicator of oxidative stress, were performed with an Electron Spin Resonance (ESR) spectrometer (ECS 106, Bruker, Karlsruhe, Germany). The following spectrometer settings were used: field center
3.478.5 G, modulation amplitude 1.0 G, microwave power 10 mW, microwave frequency 9.74 GHz, scan range 5 G, scan rate 1 G/s. Each sample was scanned 20 times to improve the signal-to-noise ratio. The intensities of the ESR signals were converted into concentrations of ascoryl radicals by comparing them with a sample containing a known concentration of a stable nitroxide radical. The GFR is given by

\[ \text{GFR} = \left( \frac{C_U}{C_P} \right)_{\text{Cr-EDTA}} \cdot Q_U \]  

where \( C_P \) and \( C_U \) represent the concentrations of \( ^{51}\text{Cr-EDTA} \) in plasma and urine, respectively, and \( Q_U \) is urine flow.

The fractional clearance for a solute \( X \) equals

\[ \theta = \left( \frac{C_U}{C_P} \right)_{\text{X}} \cdot Q_U \]  

Heterogeneous charged fiber model. We have developed a heterogeneous charged fiber model (16) based on the work of Johnson and Deen (17) on partition coefficients for neutral and charged solutes in charged fiber gels. (For details of the model, please consult Ref. 16 or the detailed Mathcad appendix available on the internet at http://kidney.med.gu.se/9/MouseMod.pdf.) In brief, the model parameters were fitted to the experimental \( \theta \) for the neutral Ficolls of different radii (200 data points) and \( \theta \) for albumin using nonlinear regression analysis. The important parameters of this model are the charged fiber radii (200 data points) and the surface charge density of fiber (qf), the large-pore fraction of the glomerular filtrate (\( f_L \)), and the unrestricted exchange area over diffusion distance (\( A_0/\Delta x \)). The fiber radius (\( r_f \)) is constant at 5 Å, and the surface charge densities (q) of albumin and Ficoll are \(-0.022 \) and \( 0.0 \) C/m², respectively, and the large-pore radius is set to 120 Å. At the start of the nonlinear regression analysis, \( q_f \) is 8%, \( A_0/\Delta x \) is 1.000 m, \( f_L \) is 1%, and the \( q_f \) is \(-0.20 \) C/m².

Gel membrane model. To allow for comparisons with previous work, size selectivity was assessed using a two-pore model with four principal parameters: the small- and the large-pore radii (\( r_s, r_L \)), the large-pore fraction of the glomerular filtrate (\( f_L \)), and the unrestricted total exchange (pore) area over diffusion distance (\( A_0/\Delta x \)). The model parameters were fitted to the experimentally obtained fractional clearances of Ficolls in the molecular radius range 12–72 Å using nonlinear regression analysis as described above. The net fluxes of fluid and solutes were calculated separately for each pore pathway using nonlinear flux equations. Finally, the charge density was estimated from the clearance ratio of the anionic albumin over neutral Ficoll of similar size. The details of the gel membrane model have been extensively described in previous papers (23).

Statistics

Results are presented as means ± SE, and differences were tested using Student’s paired design t-test and Wilcoxon’s rank sum test where appropriate. In cases of skewed distribution of data, the values were log-transformed before analysis.

RESULTS

General

Arterial pressure was stable throughout the experiment at 113 ± 4 mmHg before and 105 ± 3 mmHg 30 min after renal ischemia-reperfusion. Preischemic renal blood flow was 2.6 ± 0.3 ml·min⁻¹·g kidney⁻¹ (\( n = 10 \)) in vivo. After 15 min of ischemia, renal blood flow rapidly returned toward normal levels as shown in Fig. 2, reaching 86% of control after 10 min of reperfusion [not significant (NS) compared with control].

In the cIPK experiments, the pump flow was 4.3 ± 0.4 ml/min, which gave \( P_A \) of 85 ± 4 mmHg for the sham-operated and 92 ± 7 mmHg for the kidneys previously exposed to ischemia-reperfusion (NS). The vascular resistance (PRU₁₀₀) was 0.24 ±

Table 1. Fractional clearance of albumin in vivo and in cIPK and \( \theta \) for Ficoll₁₅₅,₅Å in cIPK in control animals and rats subjected to mild ischemia-reperfusion injury

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>I/R</th>
<th>( P ) Value (( n ))</th>
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<tr>
<td>( \theta_{\text{albumin in vivo}} )</td>
<td>0.00081 ± 0.00033</td>
<td>0.0110 ± 0.0031</td>
<td>&lt;0.001 (( n = 11 ))</td>
</tr>
<tr>
<td>( \theta_{\text{albumin (cIPK)}} )</td>
<td>0.00189 ± 0.00046</td>
<td>0.0292 ± 0.0035</td>
<td>&lt;0.001 (( n = 7 ))</td>
</tr>
<tr>
<td>( \theta_{\text{Ficoll₁₅₅,₅Å (cIPK)}} )</td>
<td>0.106 ± 0.009</td>
<td>0.194 ± 0.027</td>
<td>&lt;0.05 (( n = 7 ))</td>
</tr>
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</table>

Values are means ± SE. \( n \), No. of rats; \( \theta \), fractional clearance; I/R, ischemia-reperfusion; cIPK, cooled isolated, perfused kidneys. *Control and I/R values in vivo are taken from the same kidneys during the course of the experiment, whereas the values for cIPK are from different sets of kidneys for control and I/R, respectively.
0.06 and 0.26 ± 0.03 mmHg·min·100 g wet wt⁻¹ for the two groups respectively (NS).

GFR. In vivo, GFR was 0.86 ± 0.07 ml·min⁻¹·100 g⁻¹. After ischemia, GFR was drastically reduced to 0.40 ± 0.11 ml·min⁻¹·100 g⁻¹ 26 min after start of reperfusion, i.e., 47% of control (P < 0.001 paired comparison, n = 11). Figure 3 contains more details. However, the U/P concentration ratio for $^{51}$Cr-EDTA was not significantly different before and after renal ischemia, being 15.6 ± 3.2 and 13.5 ± 2.8, respectively, reflecting intact tubular function after the mild ischemic insult.

In the cIPK, GFR was 0.15 ± 0.03 in the sham-operated kidneys and 0.16 ± 0.04 ml·min⁻¹·g kidney wet wt⁻¹ in the kidneys exposed to 15 min of ischemia and 30 min of warm reperfusion.

Ascorbyl Radical Measurements

In 17 rats, the ascorbyl radical concentration was 47 ± 7 nM before ischemia. There was a significant increase in the concentration of the ascorbyl radical after the ischemia compared with control, reaching 87 ± 14 nM at 5 min after start of reperfusion (P < 0.001) (see Fig. 4).

Fractional Clearance for Albumin and 36Å-Ficoll

The albumin θ increased 15–16 times in kidneys subjected to IRI compared with nonischemic controls, both in vivo and in the cIPK (Table 1). The fractional clearance for Ficoll$^{36\AA}$ (size similar to albumin, but no net charge) increased less in cIPK, 1.8 times, under the same conditions. In this study, θ for Ficoll was not measured in vivo. Thus the clearance ratio of Ficoll$^{36\AA}$ over albumin (the glomerular charge selectivity) in cIPK decreased significantly from 68.5 in the controls (99% confidence interval = 21–222) to 6.4 in the group subjected to IRI (99% confidence interval = 3.5–11.8).

Relationship Between Albumin Clearance and Radical Concentration

There was a significant correlation between individual values of albumin θ and the ascorbyl radical concentration in the animals subjected to IRI with a regression coefficient (β) of 1.44 ± 0.186 ± 5, P < 0.01, y = βx, where x = ascorbyl concentration in nM, and y = θ for albumin.

Analysis According to the Heterogeneous Charged Fiber Model

The kidneys subjected to IRI were characterized by a reduction of the net negative charge density of the fibers ($\theta_f$) to 38% of control (P < 0.01, n = 7) (Table 2). Moreover, there were tendencies toward a reduction of the fiber volume fraction, increased large-pore fraction, and reduced $\Delta A/\Delta x$ compared with control kidneys (NS for all). There was good agreement between measured and modeled data for Ficoll$^{36\AA}$ (Fig. 5) irrespective of the fiber charge density. However, analysis of the albumin data revealed that $\theta_f$ was reduced markedly by ischemia-reperfusion. The relationship between measured and modeled albumin data are shown in Fig. 6, and the graph shows that θ for albumin was markedly underestimated unless $\theta_f$ was reduced from −0.20 to −0.077 C/m² in the kidneys subjected to IRI.

Analysis According to the Gel Membrane Model

Compared with the nonischemic control group, the IRI group of kidneys had a significantly larger small-pore radius and a reduced charge density (Table 3). Changes in the other parameters were not statistically significant.
Morphometric Measurements of Glomerular Barrier Structures using Electron Microscopy

The density and the thickness of the GBM were similar in the two groups. The density was 1.618 ± 0.7 (95% confidence interval 1.450–1.780) pixels in normal kidneys and 1.570 ± 0.64 in ischemic kidneys (95% confidence interval 1.420–1.720 nm). The basement membrane thickness was 207 ± 10 nm in the control group and 198 ± 4.5 nm in the ischemic group. The mean foot process width was 292 nm (95% confidence interval 240–320 nm) in the control group and 321 nm (95% confidence interval 221–420 nm) in the ischemic group (NS.). The average width of the filtration slits was 43 nm (95% confidence interval 39–46 nm) in controls and 51 nm (95% confidence interval 41–60 nm) in the ischemic kidneys (NS.). There was no statistically significant difference between the two sets of data (Student’s t-test, paired design).

DISCUSSION

The main and novel finding of this study is that mild IRI causes profound effects on the glomerular charge barrier and to a lesser extent reduces the size selectivity. Also, IRI produced marked proteinuria without signs of ultrastructural alterations of the glomerulus, suggesting more subtle changes of less obvious structures, e.g., certain proteoglycans. One plausible mechanism behind the injury was increased oxidative stress as reflected by the 70% elevated concentrations of ascorbil radicals in the renal vein blood. The experimental data were obtained under highly controlled conditions in isolated kidneys perfused at a reduced temperature to abolish tubular modification of the composition of urine. It is a prerequisite for analysis of size and charge selectivity that the concentrations of proteins (and other tracers) in the urine sample resemble those in Bowman’s capsule (for further details, see Refs. 7, 8, 10, and 16). Furthermore, renal blood flow was rapidly restored during early reperfusion and GFR recovered somewhat but remained halved even after 40 min. The most dramatic effect was on the θ for albumin, which increased by more than one order of magnitude. This was not due to reduced tubular uptake of albumin, since the Cr-EDTA U/P concentration ratio in vivo was unaffected, indicating normal tubular cell polarity (20). Moreover, with respect to albumin clearance, ischemia caused similar effects in the cIPK, where tubular reabsorption and protease activity are completely abolished (9). The size barrier was affected, as reflected by the 83% increase in the fractional clearance for neutral Ficoll of similar size as albumin (36 Å). Indeed, the two-pore analysis revealed a 10% increase in the small-pore radius from the control value of 48 Å, a value similar to that previously reported for humans (4), mice (16), and rats in vivo (14, 25) and in vitro (23, 24). However, IRI increased θ for albumin considerably more than for θ for 36-Å Ficoll, reflecting markedly impaired charge selectivity (Table 1). Indeed, analysis of data using the heterogeneous charged fiber model indicated that the fiber net charge was reduced to 39% of control by ischemia (P < 0.01) (Table 2), and the gel membrane model gave similar results (Table 3). These alterations are in line with the in vitro observation of heparan sulfate depolymerization by hydroxyl radicals (27). In a previous report, renal artery infusions of H2O2 produced similar effects as in the present study in terms of proteinuria and impaired size selectivity (increased small-pore radius) without ultrastructural abnormality (35). The findings of this study are

### Table 3. Filtration parameter values calculated according to the gel membrane model based on data from cIPK for control animals and rats subjected to mild I/R injury

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>I/R</th>
<th>P Value</th>
</tr>
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<tbody>
<tr>
<td>Small-pore radius, Å</td>
<td>47.6±0.3</td>
<td>52.8±1.4</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Large-pore radius, Å</td>
<td>89.3±4.8</td>
<td>131±14</td>
<td>NS</td>
</tr>
<tr>
<td>Large-pore fraction, %</td>
<td>0.85±0.15</td>
<td>1.07±0.31</td>
<td>NS</td>
</tr>
<tr>
<td>Aθ/Δt, m</td>
<td>1.710±470</td>
<td>785±239</td>
<td>NS</td>
</tr>
<tr>
<td>Negative charge density, meq/l</td>
<td>62 (46–75)</td>
<td>30 (21–38)*</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 7. *Numbers within brackets denote 99% confidence interval.
also in qualitative agreement with a recent study using tissue uptake (28).

Electron microscopy did not reveal any significant changes in podocyte or basement membrane morphology after mild IRI. This would indicate that the marked alterations in charge and size selectivity observed in the present study are due to alterations in less visible structures such as proteoglycans and glycosaminoglycans covering the cells. This fits well with the previous observations that heparan sulfate containing proteoglycans are lost after IRI (31), after infusions of H2O2 (35), in diabetes (19), and are depolymerized in vitro (27). Moreover, the recent paper by Morita et al. (22) shows that perlecan is required for an intact glomerular filter. The latter study is important since previous knockout data seemed to indicate the proteoglycan to be less important for glomerular selectivity (29). Indeed, we have shown that perlecan is produced by the glomerular endothelium (3) and that the production is diminished by puromycin, an agent that induces nephrotic syndrome. In other organs, the endothelial glyocalyx has been suggested to contribute significant resistance to protein flux, since digestion of the glyocalyx with heparinase increases the permeability to albumin in coronary arterioles (15). In microvessels from muscle, enzymatic treatment of the endothelial glyocalyx with hyaluronidase increased the permeation of macromolecules (12). We have proposed that the endothelial (and epithelial) glyocalyx is important for the glomerular barrier (10, 13, 16, 24, 33), a view that is gaining support (7).

Albumin clearance remained high during the experimental period (1 h). However, from observations after renal transplantation (31) and from infusions of H2O2 (35), we know that the proteinuria following IRI is highly reversible. To a lesser degree, high albumin urinary excretion rates are found after living donor kidney transplantation as well (1), despite better renal preservation and shorter ischemia. It is therefore highly likely that the repair of the glomerular barrier after IRI involves protein synthesis.

We conclude that ischemia-reperfusion of the kidney induces oxidative stress and a dramatic increase in the θ for albumin. Mild ischemia markedly reduced glomerular charge selectivity, while the effect on size selectivity was less pronounced. Moreover, podocyte, endothelial cell, and basement membrane morphology was unaffected by IRI, suggesting loss of negatively charged components not easily visualized through conventional electron microscopic. Glomerular proteoglycans and/or glycosaminoglycans are likely to be such molecules that are important for the maintenance of an intact glomerular barrier. Moreover, our data suggest that, e.g., glomerulonephritis may cause proteinuria through local production of oxygen reactive species as part of an inflammatory process.

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