Introduction

Albuminuria, defined as the urinary excretion of >30 mg of albumin per day in humans, is considered a hallmark of kidney diseases of various etiologies. Furthermore, the magnitude of albumin excretion is a prognostic marker for the progression of kidney diseases. Inadequate function of the glomerular filtration barrier is the most likely cause for the development of albuminuria (Haraldsson et al., 2008), but compromised albumin retrieval by the tubular system may also be involved (Russo et al., 2002). In addition to being a symptom of various glomerular diseases, there is accumulating evidence that enhanced filtration and excretion of albumin per se acts a risk factor for the progression of renal diseases (Remuzzi and Bertani, 1998; Remuzzi et al., 2005) and the occurrence of cardio-vascular events (Wagner et al., 1994). Thus, when the filtration of albumin exceeds the normal level, it is partly compensated for by tubular uptake processes (Christensen and Birn, 2001), and the intracellular accumulation of albumin and its degradation products causes a detrimental local inflammatory response (Bertani et al., 1989).

Independent of the confounding causes for albuminuria, inhibitors of the renin-angiotensin system (RAS) in many cases reduce the degree of albuminuria (Barnett, 2005). This beneficial effect is presumably related to hemodynamic (i.e., blood pressure–lowering) effects and to direct effects on the filtration barrier (Taal and Brenner, 2000). Thus, angiotensin II alters the function of the glomerular filtration barrier, resulting in increased leakiness for proteins, even when the renal perfusion pressure is kept constant (Axelsson et al., 2012; Schießl and Castrop, 2013).

Despite the compelling clinical evidence for an antialbuminuric effect of RAS inhibitors, a direct evaluation of their effect on the filtration barrier is difficult. Thus, evaluation of the morphologic effects of RAS inhibitors on the filtration barrier in kidney specimens, such as those prepared for use in electron microscopy, is an indirect approach and is aggravated by possible fixation artifacts. Whole-organ approaches, such as measurement of albumin in the urine, do not allow direct conclusions in terms of the function of the filtration barrier because the effects may be, at least partially, masked by tubular uptake and degradation. These technical obstacles contribute to the recent controversy regarding the amount of...
filtered albumin in the healthy glomerulus and the contribution of proximal tubular dysfunction in the pathogenesis of albuminuria (Russo et al., 2002, 2007; Peti-Peterdi, 2009; Tanner, 2009; Schießl and Castrop, 2013; Castrop, 2014; Sandoval and Molitoris, 2014).

In this study, we used intravital multiphoton microscopy (MPM) to assess the effects of the angiotensin-converting enzyme (ACE) inhibitor enalapril on albumin filtration in an animal model of chronic albuminuria. Enalapril was used because of its well established renoprotective effects and to modulate the effects of angiotensin II on both angiotensin II AT1 receptor and angiotensin II AT2 receptor receptors (Schießl and Castrop, 2013). Munich Wistar Frömter (MWF) rats were used as a hypertensive model with age-dependent albuminuria and deterioration of kidney function, and the influence of enalapril on the glomerular sieving coefficient for albumin (GSCA) was determined in the live animal. We hypothesized that enalapril would reduce the leakiness of the filtration barrier and, consequently, the amount of albumin excreted in the urine. We further aimed to determine whether the antiproteinuric effect of enalapril was related to changes in the glomerular filtration rate (GFR).

We found that enalapril markedly reduced the GSCA, which was paralleled by a similar decline in urinary albumin excretion. The reduction in the GSCA during enalapril treatment was accompanied by considerable improvement of the GFR. Our data suggest that the antiproteinuric effect of ACE inhibitors is primarily mediated by modulation of the permeability of the glomerular filtration barrier.

Materials and Methods

Animals. Animal experiments were performed using male MWF rats from a breeding colony at the University of Regensburg (Regensburg, Germany). For the experiments, we examined rats from the age of 6–8 weeks until 52 weeks. The animals were fed standard rodent chow (no. E15000; Iliff Spezialdiäten, Soest, Germany) and kept on a 12-hour light/dark cycle. The animals had free access to tap water. All of the animal care procedures and experiments were approved by the University of Regensburg Institutional Animal Care and Use Committee and were conducted according to the National Institutes of Health’s Guide for the Care and Use of Laboratory Animals.

MWF rats were used in the experiments because this strain has numerous surface glomeruli in the region of <100 μm below the kidney capsule and these glomeruli are readily accessible for MPM. The tubular system of the surface glomeruli of MWF rats is characterized by short loops of Henle. Animals were selected using visible (green) light from a mercury arc lamp. All of the measurements were taken at a depth of 30 μm underneath the kidney capsule (identified by the second harmonic generation of the collagen) to keep the intensity values between measurements comparable. For each measurement, six images were collected with a time lapse of 10 seconds to minimize laser-induced tissue damage. A z-stack was constructed for each of the selected glomeruli to assess the three-dimensional structure of glomerular capillaries and to ensure that the fluorescent values measured in the Bowman’s space were not artificially increased by out-of-focus fluorescence from the capillaries in the immediate vicinity below or above the focal plane. To prevent the tissue from photobleaching, the laser power used to generate the z-stacks was reduced to 18%.

To determine the GSCA, the fluorescent intensity in three regions of interest (ROIs) (150 pixels each) within the glomerular capillary loops and two ROIs in the Bowman’s space were measured for each of the six images. For the fluorescent intensity in the plasma, the ROIs were placed within the outer margins of the capillaries showing the brightest fluorescence in the field. The ROIs in the Bowman’s space were selected in regions away from capillaries.

To correct for background noise, six background images for each glomerulus were collected, before the dyes were injected, using identical recording settings. To measure the background fluorescence intensities, four ROIs were selected in Bowman’s space and two ROIs were selected within the capillary loops of each analyzed image.

GSC calculations were based on the average intensity values after subtracting the background fluorescence for the plasma and Bowman’s space using the following formula: GSCA = (intensity Bowman’s space – intensity background Bowman’s space)/(intensity capillaries – intensity background capillaries). was achieved using a Chameleon Ultra-II MP laser (Coherent Deutschland GmbH, Dieburg, Germany) at 860 nm with a laser power of 22% of 3200 mW. Sixteen-bit 512 × 512-pixel images (providing a theoretical dynamic range for intensities measurements of 0- to 65,536-pixel intensity) were obtained using a pixel dwell time of 1.27 microseconds and a line average of one by applying a 40× long-distance C-Apochromat 40/1.1 water objective. The emissions were collected using external detectors (nondescanned detectors with filter set 1 (green channel): beamsplitter 500–550, long pass 555; and filter set 2 (red channel): beamsplitter 565–610 including mirror) and internal detectors (blue channel: 420–440/main beamsplitter: 458).

The detector settings were kept constant for all measurements: for the green, red, and blue channels, respectively, the master gain was 500/500/500, the digital gain was 10/10/10, and the offset was −4000−3500−3000. The photodetector offset was chosen to reduce background fluorescence and, at the same time, keep an adequate detector sensitivity. For an analysis of the offset/GSCA ratios, see Supplemental Fig. 1.

To label the vasculature, a 5 mg/ml solution of Alexa Fluor 594–bovine serum albumin (BSA) conjugate (Invitrogen GmbH, Darmstadt, Germany) dissolved in phosphate-buffered saline was first purified by dialysis for 3 days (Spectra/Por Float-A-Lyzer, 50 kDa; Spectrum Europe B.V., Breda, The Netherlands), and then concentrated using Nanosep Centrifugal (VWR International GmbH, Darmstadt, Germany) and injected intravenously (1.3 μg). The fluorescence was detected using the red channel. Consequently, only the labeled exogenously administered albumin was measured in the experiments. The proximal tubules were visualized by collecting autofluorescence (green channel), and the collagen was visualized using second harmonic generation (blue channel).

Deterrmination of GSCA. The GSCA was defined as the ratio of the albumin concentration in the filtrate (in Bowman’s space) to the albumin concentration in the plasma (in the glomerular capillaries). In this study, the GSCA was determined using MPM to determine the fluorescence intensity ratio of the Alexa Fluor 594–BSA conjugate.

For each animal, two to four superficial glomeruli were selected using visible (green) light from a mercury arc lamp. All of the measurements were taken at a depth of 30 μm underneath the kidney capsule (identified by the second harmonic generation of the collagen) to keep the intensity values between measurements comparable. For each measurement, six images were collected with a time lapse of 10 seconds to minimize laser-induced tissue damage. A z-stack was constructed for each of the selected glomeruli to assess the three-dimensional structure of glomerular capillaries and to ensure that the fluorescent values measured in the Bowman’s space were not artificially increased by out-of-focus fluorescence from the capillaries in the immediate vicinity below or above the focal plane. To prevent the tissue from photobleaching, the laser power used to generate the z-stacks was reduced to 18%.

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GSC calculations were based on the average intensity values after subtracting the background fluorescence for the plasma and Bowman’s space using the following formula: GSCA = (intensity Bowman’s space – intensity background Bowman’s space)/(intensity capillaries – intensity background capillaries). Lower Limit of Detection for Fluorescent Intensity in Bowman’s Space. The lower limit of detection for the intensity values in the Bowman’s space was calculated for each experiment as

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previously described (Anderson, 1989; Tanner, 2009). The calculated values for GSCA were only used in further analyses if the mean fluorescence intensity value of the Alexa Fluor 594–BSA in the Bowman’s space exceeded the value of the lower limit of detection.

**Imaging of Glomerular Morphology Using MPM.** The vasculature was stained by injecting rhodamine B, which binds to plasma proteins. To visualize podocytes, negative imaging was performed during a continuous infusion of 3 μl/min of a 5 mg/ml Lucifer yellow solution (Peti-Peterdi and Sipos, 2010). This low-molecular-weight dye is freely filtered into Bowman’s space and stains the primary filtrate. Podocytes are excluded from this staining and appear as dark objects at the outer margins of the glomerular capillaries. By contrast, mesangial cells take up Lucifer yellow.

**Proximal Tubular Albumin Uptake.** Proximal tubular albumin uptake was quantified as the intensity of Alexa Fluor 594–BSA per area. In detail, three to four images of S1 proximal tubule segments were acquired 20 minutes after the injection of Alexa Fluor 594–BSA and proximal tubular fluorescence intensities were measured in three to four ROI’s for each image. The values were corrected for background autofluorescence. The values are given as intensities/area of the ROI. The average ROI was 360 ± 14 μm².

**Determination of GFR.** To monitor the GFR during enalapril treatment of 12-month-old MWF rats, GFR was determined as the creatinine clearance. To gain the required plasma samples, blood was obtained by tail-bleed at baseline and after 2 and 4 weeks of enalapril administration. Urine samples were collected in metabolic cages (6 hours) after animals were accustomed to the metabolic cages for 1 week. Urine collection was done between 9:00 AM and 3:00 PM (6 hours) to eliminate possible circadian variations. After measurement of creatinine concentrations in urine and plasma (conducted by the IDEXX [Regensburg, Germany] veterinarian laboratory by the use of the CREAplus enzymatic assay; Roche, Penzberg, Germany), the GFR was calculated using the following formula: GFR= (creatinine concentration in urine × urinary volume per minute)/creatinine concentration in plasma.

**Quantification of Urinary Albumin Excretion.** To quantify the urinary albumin content, the urine samples were collected in metabolic cages and diluted 1:10. The diluted samples were separated on a 10% SDS-PAGE gel. Albumin BSA standard solutions (0.25, 0.5, 1.0, and 2.0 μg/μl) were prepared and separated on the gel. A Coomassie staining was performed to visualize the albumin content, and the amount of albumin in the samples was analyzed by densitometry. Because the urinary albumin concentration for a given albumin filtration and tubular reuptake depends on the degree of volume reabsorption along the tubular system, the urine albumin content was normalized against the urine osmolalities, which were determined using the freezing point depression method, as described (Oppermann et al., 2010). This normalized concentration of albumin in the urine was used as a surrogate for renal albumin excretion per time point.

**Determination of Plasma Renin Activity.** Plasma renin activity (PRA) was measured in seven young MWF rats (aged 8 weeks) and six old MWF rats (aged 52 weeks). Blood samples were collected from rats by tail bleeding. Approximately 60 μl blood was collected into an EDTA-containing microhematocrit tube. Red blood cells and plasma were separated by centrifugation; the plasma was frozen until used for renin measurements. Using a 2-fold dilution of 12 μl plasma, renin activity was measured by radioimmunoassay (DiaSorin, Stillwater, MN), as described in detail elsewhere (Mederle et al., 2013).

**Experimental Protocols.** To characterize urinary albumin excretion in aging rats, urine samples from four MWF rats and two control Wistar rats were collected in metabolic cages at ages 9, 12, 14, 16, 18, 22, 25, and 52 weeks and the albumin/osmolality ratio was analyzed.

To analyze glomerular albumin filtration in young and aged MWF rats, the GSCA was determined in five young male MWF rats (aged <9 weeks) and in five 52-week-old MWF rats.

**Results**

**Changes in Urinary Albumin Excretion in MWF Rats and Wistar Control Rats during Aging.** As a model of low nephron number, MWF rats gradually develop glomerulosclerosis, hypertension, and proteinuria (Remuzzi et al., 1988; Hackbarth et al., 1991). An in vivo inspection of the morphology of the glomeruli of 9-week-old MWF rats revealed no apparent abnormalities (Fig. 1). By contrast, the glomeruli of 52-week-old MWF rats showed marked morphologic alterations, as shown in Fig. 2. Thus, in some glomeruli, podocyte detachment from the basement membrane was
observed, which was accompanied by an intense fluorescence of rhodamine B–labeled plasma proteins in the urinary space, indicating massive local protein filtration. In addition, the subpodocyte space occasionally was expanded, forming pseudocysts (Peti-Peterdi and Sipos, 2010) (Fig. 2). Furthermore, as shown in Fig. 2, we found evidence of mesangial matrix expansion.

To determine the progression of proteinuria in MWF rats in our colony, we characterized urinary albumin excretion during aging. As a control, the urinary albumin content of Wistar rats was measured. Urine from MWF rats and control Wistar rats was collected in metabolic cages at age 9, 12, 14, 16, 18, 22, 25, and 52 weeks and the albumin/osmolality ratio was determined. Urinary albumin excretion of Wistar rats did not change over time and was low at all investigated time points, averaging $0.00079 \pm 6.5 \times 10^{-7}$ [mg/ml]/[mOsmol/kg] ($n = 16$). There was no difference in urinary albumin excretion of the 9-week-old MWF rats compared with the nonproteinuric Wistar rats. However, as shown in Fig. 3, the albumin/osmolality ratio in the MWF rats increased from $0.00062 \pm 0.0001$ at age 9 weeks to $0.0054 \pm 0.0003$ [mg/ml]/[mOsmol/kg] at age 52 weeks (linear regression: $P < 0.0001$; $r^2 = 0.59$; $n = 5$). Compared with the Wistar rats, the urinary albumin content was significantly higher in the MWF rats at age 18, 22, 25, and 52 weeks ($P = 0.0189$, $P = 0.0001$, $P = 0.0015$, and $P < 0.0001$, respectively).

**Effect of Enalapril on Urinary Albumin Excretion in Aged MWF Rats.** Because angiotensin II is known to be involved in the progression of renal failure and proteinuria (Remuzzi et al., 2005), the PRA was determined in 8- and 52-week-old MWF rats. PRA in the old rats was lower compared with the young animals, averaging $5.4 \pm 1.2$ and $1.3 \pm 0.1$ ng angiotensin I/ml per hour, respectively ($P = 0.008$; $n = 7$ and $n = 6$, respectively; Fig. 4).

To address the antiproteinuric effects of ACE inhibition on urinary albumin excretion, 12-month-old proteinuric MWF rats received enalapril (100 mg/l) in their drinking water over a period of 4 weeks. Enalapril treatment caused a significant reduction in mean arterial blood pressure (MAP) in 12-month-old MWF rats. MAP in untreated MWF rats was $144.6 \pm 6.5$ mm Hg ($n = 5$). After 4 weeks of enalapril treatment, MAP was reduced to $110.9 \pm 0.6$ mm Hg ($n = 6$; $P = 0.0011$). To determine the effects of enalapril on urinary albumin excretion, urine was collected every week in metabolic cages, and albumin excretion was determined. As shown in Fig. 5, urinary albumin excretion decreased significantly in response to enalapril. The albumin/osmolality ratio was $0.0051 \pm 0.0003$ [mg/ml]/[mOsmol/kg] ($n = 6$) during baseline conditions, and declined to $0.0023 \pm 0.0004$, $0.0033 \pm 0.0003$, $0.0041 \pm 0.0003$, and $0.0036 \pm 0.0005$ [mg/ml]/[mOsmol/kg] after 1, 2, 3, and 4 weeks of enalapril administration, respectively ($P = 0.0002$, $P = 0.005$, $P = 0.030$, and $P = 0.0089$, respectively). Thus, albumin excretion was reduced by approximately 50% after 1 week of enalapril treatment and remained almost constant during the following 3 weeks.

![Fig. 2.](image-url) Glomeruli of old MWF rats showed marked morphologic alterations. (A) In a few glomeruli, extended areas of the glomerular basement membrane lacked podocytes (tag) and this was accompanied by a massively increased permeability of the filtration barrier for rhodamine B–labeled plasma proteins (marked red fluorescence in the urinary space). (B) In some areas, the typical green staining of mesangial cells by Lucifer yellow was expanded along the capillary wall indicating expansion of the mesangium (arrows). (C) Glomerular pseudocyst containing rhodamine B–labeled plasma proteins (asterisk).

![Fig. 3.](image-url) Characterization of the urinary albumin excretion in aging MWF and Wistar rats. Young MWF rats (aged <9 weeks) are nonproteinuric compared with Wistar rats. Urinary albumin content in MWF rats increases linearly with age.
GSCA in Young and Old MWF Rats. Considering the ongoing discussion regarding the physiologic extent of glomerular albumin filtration (Russo et al., 2007), we aimed to determine whether the observed proteinuria in 12-month-old MWF rats was due to increased glomerular albumin filtration or an insufficient proximal tubular reabsorption of the filtered albumin. To investigate glomerular albumin filtration in young (aged <9 weeks) and old (aged 12 months) MWF rats, the GSCA was determined using MPM. We found that the GSCA in young MWF rats (aged <9 weeks) was low, averaging 0.00057 ± 4.7 × 10^{-5} (n = 25). At age 12 months, the GSCA rose to 0.0027 ± 0.00036 (n = 36, P < 0.0001) in the MWF rats, an increase of almost 400% (Fig. 6).

Effect of Enalapril on the GSCA of Proteinuric Old MWF Rats. Because the urinary albumin excretion in old MWF rats was significantly reduced during administration of enalapril, we next investigated whether the antiproteinuric effects of enalapril are related to changes in the permeability of the glomerular filtration barrier. As shown in Fig. 7, after 4 weeks of enalapril treatment, the GSCA of 12-month-old MWF rats decreased by approximately 50%, averaging 0.0027 ± 0.00036 before enalapril administration and 0.00139 ± 0.00013 after enalapril administration (n = 36 and n = 39 glomeruli, respectively; P = 0.0005). Thus, the relative reduction of the GSCA caused by the enalapril treatment was quantitatively similar to what was observed for urinary albumin excretion.

Effect of Enalapril on Proximal Tubular Albumin Uptake in Old MWF Rats. Urinary albumin excretion depends on albumin filtration and tubular reuptake. Therefore, we next determined the effect of enalapril on the uptake of filtered albumin in the S1 segment of the proximal tubule. Tubular Alexa Fluor 594–BSA was markedly reduced after enalapril treatment, averaging 44.6 ± 4.9 and 13.7 ± 1.9 μm^2 in untreated and treated old MWF rats, respectively (P < 0.0001; Fig. 8).

Effect of Enalapril on the GFR of Old MWF Rats. Because glomerular albumin filtration is dependent on the GFR (Haraldsson et al., 2008), we next evaluated changes in the GFR during enalapril treatment. The GFR of the animals was estimated by the creatinine clearance method. The GFR of 12-month-old MWF rats was determined before and after 2 and 4 weeks of enalapril therapy. The GFR was 1.64 ± 0.3 ml/min before the enalapril administration and increased to 3.44 ± 0.1 ml/min (n = 6; P = 0.0022 versus baseline) after 2 weeks of enalapril administration. After 4 weeks of enalapril treatment, the GFR averaged 3.58 ± 0.3 ml/min (n = 6; P = 0.0025 versus baseline). Thus, RAS inhibition significantly improved the GFR in 12-month-old MWF rats (Fig. 9).

Discussion

In this study, we investigated glomerular albumin filtration and the antiproteinuric effects of ACE inhibitor treatment in aged MWF rats using intravital MPM. MWF rats were used in this study as a disease model of hypertension with chronic albuminuria. We found that enalapril markedly reduced albuminuria in aged MWF rats and that this antiproteinuric effect was directly related to changes in glomerular albumin filtration.

To address the antiproteinuric effect of enalapril, the GSCA was determined in vivo using MPM. This technique allows for the simultaneous determination of glomerular function and morphology in the live animal (Peti-Peterdi et al., 2012). A limitation is that the majority of glomeruli in mammalian kidneys are too deep to be visualized by MPM (Schleiß et al., 2013). Because of their high number of superficial glomeruli, MWF rats are particularly suited for the investigation of glomerular function by MPM, and, similarly, by micropuncture (Hackbarth et al., 1983). For the investigation of the normal function of the glomerulus, however, only young MWF rats appear to be suitable because aged MWF rats develop hypertension, proteinuria, and glomerulosclerosis (Hackbarth et al., 1991). This pathology is most likely related to the low nephron number of this rat strain (Rothermund et al., 2011). In accordance with recent ex vivo evaluations of the glomerular morphology of aged MWF rats (Macconi et al., 2006), we found marked structural alterations in vivo, including the detachment of podocytes from the basement membrane, formation of pseudocysts, and evidence for mesangial matrix expansion. As a limitation of the MWF strain, the surface glomeruli of MWF rats have very short loops of Henle and the contribution of these nephrons to total kidney albuminuria remains to be determined.
The GSCA was very low in young MWF rats (<0.0006), suggesting that the glomerular filtration of albumin in the normal kidney is minimal. The limited glomerular filtration of large proteins, such as albumin, is generally considered to be related to the charge- and size-selective properties of the glomerular filtration barrier (Haraldsson et al., 2008; Jeansson et al., 2009). The low GSCA values are in agreement with recent data from our group and others using MPM to determine the GSCA (Peti-Peterdi, 2009; Tanner, 2009; Schießl and Castrop, 2013). Similar low values for the GSCA were determined using micropuncture techniques. Thus, the GSCA in the rat was found to range from 0.0003 to 0.0006 (Oken and Flamenbaum, 1971; Stolte et al., 1979; Tojo and Endou, 1992). These data are in contrast with recent reports, which challenged the classic view that albumin is largely restricted from glomerular filtration. Thus, Russo et al. (2007) reported, using MPM, that the normal kidney filters albumin at near nephrotic levels, with GSCA values of approximately 2 orders of magnitude higher than what was found in our study. It was suggested that this massive filtration of albumin is subsequently compensated for by proximal tubular uptake and transcytosis of intact albumin (Dickson et al., 2014). These findings sparked a heated debate regarding the magnitude of glomerular albumin filtration in the normal kidney and its implications for albuminuria (Christensen et al., 2007; Russo et al., 2007; Peti-Peterdi, 2009; Tanner, 2009; Schießl and Castrop, 2013; Castrop, 2014; Sandø and Molitoris, 2014). The common understanding of the integrity of the filtration barrier implies that albumin is largely restricted from glomerular filtration (Haraldsson and Sörensson, 2004). Albuminuria, therefore, is usually considered to be caused by alterations in the properties of the glomerular filtration barrier (Salvetti et al., 1999; Haraldsson et al., 2008). Conversely, other researchers have proposed extensive glomerular albumin filtration suggesting that albuminuria is primarily an issue of inadequate tubular albumin retrieval (Dickson et al., 2014). Our data from the aging MWF rats, however, do not support this assumption because the increase in urinary albumin excretion in the aged rats was paralleled by a similar increment in the GSCA, suggesting that the primary cause for albuminuria is a glomerular one.

The antiproteinuric effects of ACE inhibitors are well known, but the underlying mechanisms responsible for these effects have not yet been fully elucidated. As expected, the administration of enalapril to aged MWF reduced MAP and caused a considerable reduction in urinary albumin excretion. It should be noted that in this context, the PRA of the aged MWF rats was low compared with young animals, confirming the clinical observation that ACE inhibitors also have antiproteinuric effects in patients with low-renin hypertension (Jafar et al., 2003; Arnold et al., 2013). Apparently, when the function of the glomerular filtration barrier is compromised due to various insults, even low angiotensin II levels are sufficient to promote albuminuria. In this context, an acute increase in the filtration of macromolecules in response to angiotensin II infusion without preexisting glomerular structural impairments has been shown (Axelsson et al., 2012; Schießl and Castrop, 2013). These acute effects of angiotensin II on the GSCA were largely blunted in the presence of the AT1 receptor antagonist losartan, suggesting that the effect of enalapril observed in our study was primarily related to an alteration of AT1 rather than AT2 receptor signaling (Schießl and Castrop, 2013).

The reduction of albuminuria in aged MWF rats during ACE inhibition was accompanied by a similar decrease in GSCA. Thus, during enalapril treatment, GSCA and urinary albumin excretion were similarly reduced by approximately 50%, again suggesting that the proteinuric effect of angiotensin II is related to glomerular rather than tubular effects. The proximal tubular uptake of Alexa-albumin was markedly reduced after administration of enalapril, suggesting that the ACE inhibition reduced the tubular load of albumin. As an alternative explanation, enalapril administration may have inhibited the proximal tubular albumin uptake. The latter explanation, however, appears less likely, because it was recently shown that blockade of the RAS promotes the tubular uptake of plasma proteins (Tojo et al., 2003); furthermore, reduced tubular albumin uptake would lead to increased, not decreased, albuminuria.

AT1 receptors are expressed in several structures of the glomerulus, including podocytes and mesangial cells (Sharma et al., 2004). GSCA was determined in young (aged <9 weeks) and old MWF rats (aged 52 weeks). Glomerular albumin filtration was significantly elevated in old MWF rats.

![Fig. 6. GSCA was determined in young (aged <9 weeks) and old MWF rats (aged 52 weeks). Glomerular albumin filtration was significantly elevated in old MWF rats.](image)

![Fig. 7. Effects of enalapril on the GSCA in old MWF rats. The GSCA was reduced by the application of enalapril.](image)
et al., 1998; Miyata et al., 1999). AT1 receptors have been shown to regulate the intracellular calcium level, cAMP concentration, and membrane potential of cultured podocytes, and angiotensin II may modulate the podocytes’ cytoskeleton and, consequently, the structure and function of the foot processes (Gloy et al., 1997, 1998; Gekle, 2007). Contraction of podocytes may increase the leakiness of the filtration barrier for macromolecules such as albumin (Lapinski et al., 1996; Kriz, 2004). In addition, when podocytes contract, the dimensions of the subpodocyte space will be reduced. The subpodocyte space is the area confined by the basement membrane and the podocytes, and this area restricts the movement of large molecules, such as albumin, across the glomerular capillary wall (Neal et al., 2005; Salmon et al., 2007). Similar to what has been observed for podocytes, there is considerable evidence that cultured mesangial cells constrict in response to AT1 receptor activation (Stockand and Sansom, 1998). Angiotensin II, therefore, may reduce the size of the effective filtration area and this would cause a reduction in the single nephron GFR for a given net filtration pressure. Changes in the single nephron GFR, in turn, may influence the GSCA, as outlined below.

The inhibition of the RAS by ACE inhibitors and angiotensin receptor blockers occasionally causes a transient reduction of the GFR, and this appears to be particularly relevant in patients with an activated RAS, such as during severe hypovolemia or renal artery stenosis (Campanacci et al., 1989; Bakris and Weir, 2000). Nevertheless, in the long run, ACE inhibitors and angiotensin receptor blockers preserve the magnitude of renal filtration and delay the progression of the deterioration of renal function in several diseases, such as diabetes and hypertension (Barnett, 2005; Ruggenenti et al., 2012). In our model of low-renin hypertension, treatment of the aged MWF rats with enalapril over 4 weeks even increased the GFR. Considering the effect of changes in the GFR on the filtration of albumin, Lund et al. (2003) reported that the glomerular GSC of macromolecules rose when the single nephron GFR was markedly reduced. The authors suggested that the increased GSC was related to the dominant contribution of diffusion to the transport of macromolecules across the filtration barrier, when the single nephron GFR approaches values of zero (Lund et al., 2003). According to these data, the rise in GFR observed during enalapril treatment may have contributed to the reduction of the GSCA. The exact quantitative contribution of the increased GFR to changes in the GSCA remains to be determined. It appears likely that the increase in

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**Fig. 8.** Effect of enalapril on proximal tubular albumin uptake in old MWF rats. (A) Proximal tubular uptake of albumin (red, arrows) in an untreated old MWF rat (aged 52 weeks). (B) Proximal tubular albumin uptake (S1 segment) was reduced by the application of enalapril. (C) Proximal tubular Alexa-albumin fluorescence intensities in untreated and enalapril-treated old MWF rats.
total kidney GFR during enalapril treatment was not mediated homogeneously by all glomeruli. Thus, a correlation between GSCA and the GFR would need to be established at the single nephron level.

In summary, albuminuria in aged MWF rats was related to an increased glomerular albumin filtration. The ACE inhibitor enalapril reduced the degree of albuminuria, and this was accompanied by a quantitatively similar decrease in the GSCA. The increase in GFR during enalapril treatment may have contributed to the decrease in the GSCA. Furthermore, enalapril treatment reduced the proximal tubular uptake of albumin, suggesting a reduced tubular albumin load. These data suggest that the antiproteinuric effects of the ACE inhibitor are related to changes in glomerular albumin filtration rather than to a modulation of the tubular albumin retrieval.

Authorship Contributions

Participated in research design: Schießl, Castrop.
Conducted experiments: Schießl, Kattler, Castrop.
Performed data analysis: Schießl, Kattler, Castrop.
Wrote or contributed to the writing of the manuscript: Schießl, Castrop.

References

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