Vascular smooth muscle function of renal glomerular and interlobar arteries predicts renal damage in rats

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Vavrinec P, Henning RH, Goris M, Vavrincova-Yaghi D, Buikema H, van Dokkum RP. Vascular smooth muscle function of renal glomerular and interlobar arteries predicts renal damage in rats. Am J Physiol Renal Physiol 303: F1187–F1195, 2012. First published July 11, 2012; doi:10.1152/ajprenal.00653.2011.—Previously, it was shown that individuals with good baseline (a priori) endothelial function in isolated (in vitro) renal arteries developed less renal damage after 5⁄6 nephrectomy (5⁄6Nx; Gschwend S, Buikema H, Van der Graaf P, van Dokkum RP. J Am Soc Nephrol 13: 2909–2915, 2002). In this study, we investigated whether preexisting glomerular vascular integrity predicts subsequent renal damage after 5⁄6Nx, using in vivo intravital microscopy and in vitro myogenic constriction of small renal arteries. Moreover, we aimed to elucidate the role of renal ANG II type 1 receptor (AT1R) expression in this model. Anesthetized rats underwent intravital microscopy to visualize constriction to ANG II of glomerular afferent and efferent arterioles, with continuous measurement of blood pressure, heart rate, and renal blood flow. Thereafter, 5⁄6Nx was performed, interlobar arteries were isolated from the extirpated kidney, and myogenic constriction was assessed in a perfused vessel setup. Blood pressure and proteinuria were assessed weekly for 12 wk, and focal glomerulosclerosis (FGS) was determined at the end of study. Relative expression AT1R in the kidney cortex obtained at 5⁄6Nx was determined by PCR. Infusion of ANG II induced significant constriction of both afferent and efferent glomerular arterioles, which strongly positively correlated with proteinuria and FGS at 12 wk after 5⁄6Nx. Furthermore, in vitro measured myogenic constriction of small renal arteries negatively correlated with proteinuria 12 wk after 5⁄6Nx. Moreover, in vivo vascular reactivity negatively correlated with in vitro reactivity. Additionally, relative expression of AT1R positively correlated with responses of glomerular arterioles and with markers of renal damage. Both in vivo afferent and efferent responses to ANG II and in vitro myogenic constriction of small renal arteries in the healthy rat predict the severity of renal damage induced by 5⁄6Nx. This vascular responsiveness is highly dependent on AT1R expression. Intraorgan vascular integrity may provide a useful tool to guide the prevention and treatment of renal end-organ damage.

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WHILE THE SUSCEPTIBILITY TO develop renal damage varies considerably among individuals, its determinants are still incompletely understood. The extent of existing systemic factors, such as diabetes or hypertension, cannot fully explain the predisposition to renal failure, suggesting additional factors to be involved (9). In general, the factors governing this individual vulnerability to renal damage are thought to be intrinsic to the kidney and probably largely genetically determined.

We have previously shown that individual differences in the endothelial dilative capacity of isolated small renal arteries in a healthy animal strongly predict the extent of renal damage in various models of experimental renal disease, i.e., 5⁄6 nephrectomy (5⁄6Nx) (3), unilateral nephrectomy combined with myocardial infarction (10), and adriamycin nephrosis (11). These observations suggest that the patency of renal endothelial function is a factor determining the severity of damage after induction of the disease.

Control of intraglomerular pressure is essential in maintaining kidney health. Intraglomerular pressure is controlled mainly by the renin-angiotensin-aldosterone system (RAAS), tubuloglomerular feedback, and myogenic constriction (MC), all acting on the regulation of the vascular tone of the afferent and efferent arteriole of the glomerulus. On the other hand, the RAAS plays a key role by its action on the angiotensin type 1 receptor (AT1R) in the progression of renal damage such as after 5⁄6Nx, through elevation of systemic blood pressure thereby increasing intraglomerular pressure by unbalanced constriction of the efferent and afferent arteriole. Whether contractility of the afferent or efferent arteriole to ANG II determines development of renal damage in this model remains unclear.

The 5⁄6Nx model of reduced nephron number is a model of progressive renal damage, where the initial reduction of nephrons ultimately leads to damage to the remaining ones (2). The plasma renin levels in 5⁄6Nx rats suggest that the secretory rates for renin may be increased for remnant nephrons, and furthermore with nephron reduction, the renin clearance rate falls (14). Taken together, these phenomena lead to elevated renin and consequently ANG II levels as well as subsequent development of renal damage after 5⁄6Nx. Intervention in the RAAS by both angiotensin-converting enzyme inhibition or by blocking AT1R is effective in slowing down the progression of renal damage in this model, which collectively point to the pathogenic role of ANG II in this model (15, 16). The importance of RAAS is further substantiated by experiments showing that sustained ANG II administration dose dependently induces proteinuria accompanied by progressive glomerular damage in otherwise healthy individuals (8), while short-term ANG II infusion, sufficient to affect renal hemodynamics, does not elicit proteinuria (13).

Previous studies showing the relationship between healthy interlobar artery function and renal damage employed ex vivo isolated preglobular renal arteries. To investigate whether functional aspects of glomerular afferent and efferent arterioles can determine/predict the damage thereafter, we studied their...
responsiveness to ANG II in in vivo experimental conditions (7, 18, 19) before the induction of renal damage upon 5/6Nx. Furthermore, we sought to test whether MC, assessed in vitro in vessels obtained at 5/6Nx, predicts the damage thereafter. Moreover, we aimed to find a relationship between these entities, i.e., in vivo glomerular vascular contractility and ex vivo interlobar artery myogenic contractility to pressure. Additionally, we investigated the relationship between the expression of AT1R, the main effector of ANG II, and vascular responses and renal damage.

MATERIALS AND METHODS

Experimental Animals

A single group of male Wistar rats [Hsd.Cpb.Wu (n = 11); 200–250 g; Harlan, Zeist, The Netherlands] was used for the study. Rats were used and housed under standard conditions (day-night rhythm, 12:12-h, group housing in macrolon cages) approved by the institutional animal ethical committee. The rats had free access to food and drinking water and received a normal diet (RMH-B, 2181; ABDiets, Woerden, The Netherlands). After arrival in our laboratory, the rats had an acclimatization period for 1 wk to get used to their new environment. After this period, the rats were trained for systolic blood pressure measurements (i.e., accustomed to immobility in a warmed restrainer for at least 10 min), because stress would cause an elevation of blood pressure. All animal experiments were conducted in accord with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals and approved by the Committee for Animal Experiments of the University of Groningen.

In Vivo Study

Intravital microscopy. The experimental setup (Fig. 1) consisted of a pencil video microscope with a cone-shaped lens (optical magnification ×3.5) and a charge-coupled device (CCD) camera (Nikon Kohnen, Tokyo, Japan), a micromanipulator, a xenon light source (LB-18; Welch Allyn, Tokyo, Japan), a monitor (KLV-17HR1; Sony, Badhoevedorp, The Netherlands), a DVD recorder (RDR-GX7), and a computer for image analysis (Intel Pa). The lens was fitted with a 12.7-mm grayscale CCD image sensor (XC ES55L; Toshiba, Tokyo, Japan) at the focal length (200 mm) of the lens. A green filter to complement red was placed in front of a CCD image sensor to enhance the contrast on the monitor between vessels and peripheral tissue. The CCD image sensor was connected to the camera module (DC700; Sony, Tokyo, Japan), and images were recorded as stacked image film (60 frames/min). The final spatial resolution of the video microscope was confirmed to be 0.86 mm with magnification of ×520. This technique thus allows for full visualization of one glomerulus and its relevant surrounding region in each experimental protocol (18, 19).

Analysis of vascular diameters. The image at each time point was captured as a stack of 60 frames using an image capture board (LG-3; Scion Computer Service, Frederick, MD) installed on the image-analysis computer. At least 3 clear frames, which were not influenced by respiration and heartbeat, were selected from the 60 frames in the captured stacks and analyzed. At the beginning of the experiment, one glomerulus per rat was selected for the sequential measurements of internal diameter changes in afferent and efferent arterioles. Diameters of afferent and efferent glomerular arteries were measured using image software (Scion) after calibration of the number of pixels (scale 2.42 pixels/m), measuring manually the distances between the interior vascular walls at two different locations, which were constant throughout the whole experiment. Then, we averaged the distances to get a single value of the inner vascular diameter.

Experimental protocol. Before the experiment started, the rats were anesthetized with isoflurane/O2 (2.5% isoflurane; Pharmachemie, Haarlem, The Netherlands) to insert a catheter (BD Insyte-W24 GA0, 75IN-0, 7 × 19 mm) in the tail vein. This catheter was used to administer a bolus injection of 36 mg/kg pentobarbital sodium (Hospital Pharmacy UMC Groningen, Groningen, The Netherlands) once isoflurane administration was ended and the animal started to

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**Fig. 1.** Schematic of experimental intravital microscopy consisting of a pencil video microscope with cone-shaped lens, micromanipulator, xenon light source, monitor, and DVD recorder and computer for image analysis and recording.
show reflexes. The operation region was anesthetized with lidocaine (20 mg/ml; Fresenius Kabi).

The carotid artery was cannulated for measurements of systolic blood pressure, diastolic blood pressure, and heart rate using a pressure transducer (Edwards Life Sciences, Saint-Prez, Switzerland) and an amplifier (model AP641G; Nihon Kohden, Tokyo, Japan). The jugular vein was cannulated for infusion of saline and ANG II. Then, the abdomen was opened by a midline incision and the left renal artery was equipped with an ultrasonic flow probe (model T106; Transonic Systems, Ithaca, NY) to measure renal blood flow, continuously registered with a flowmeter (model 1RB; Transonic Systems). To expose glomeruli, the capsule of the renal cortex was removed, and a thin slice of the renal surface was removed (maximum depth 0.5 mm) using a scalpel. The tip of the pencil-probe charge-coupled device (CCD) video microscope was guided to the bottom of the excision. Glomeruli in which the afferent and efferent arterioles could be visualized without affecting the blood flow were used. Per experiment, one glomerulus of the left kidney was monitored.

ANG II showed in pilot experiments as serving as the best constrictor of glomerular vessels. We tested phenylephrine; however, constriction of glomerular arterioles was minimal, although with a strong change in hemodynamic parameters. As the primary objective was to focus on vascular changes, we refrained from experiments with phenylephrine.

The protocol consisted of a baseline of 10-min saline infusion. Thereupon, ANG II infusion (10 min, 30 ng·kg⁻¹·min⁻¹; Bachem Bioscience) was started. Movies were recorded during steady-state saline and ANG II infusion for later analysis. After this period, the ANG II infusion was switched to saline for 30 min (to allow for return to baseline values) after which 5/6Nx was performed by removing the right kidney and by ligating two or three branches of the renal artery of the left kidney, leading to infarction of approximately two-thirds of this kidney. Animals received a subcutaneous injection of buprenorphine (10 μg/kg) postoperatively.

In Vitro Study

In vitro perfusion setup. Small renal interlobar arteries with an intraluminal diameter of 261 ± 2 μm were cleaned from perivascular tissue and transferred to an arteriograph system for pressurized arteries (Living System Instrumentation, Burlington, VT) as described previously (3). Artery segments were cannulated on glass micropipettes, and the vessel chamber was continuously recirculated with warmed (37°C) and oxygenated (5% CO₂ in O₂) Krebs solution with a pH of 7.4. An inverted light microscope attached to a video camera and video dimension analyzer were used to continuously register lumen diameter.

Myogenic reactivity of small renal resistance arteries. Intraluminal pressure was set at 60 mmHg, arteries were allowed to equilibrate for 45 min and checked for smooth muscle and endothelial viability by a single dose of phenylephrine (3 × 10⁻⁷ mol/l) and acetylcholine (3 × 10⁻⁵ mol/l), respectively. To exclude possible any influence of endothelium, arteries were mechanically denuded of endothelium. Removal of endothelium was confirmed by absence of dilative response to acetylcholine (3 × 10⁻⁵ mol/l) following a submaximal preconstriction with phenylephrine (3 × 10⁻⁷ mol/l).

Following a washout, intraluminal pressure was decreased to 20 mmHg and myogenic reactivity was studied by obtaining active pressure–diameter curves over a pressure range of 20–160 mmHg in steps of 20 mmHg. Each pressure step was maintained for 5 min to reach the stable contractile response. Thereafter, calcium containing Krebs solution was exchanged for calcium-free Krebs solution supplemented with EGTA (2 mmol/l) and passive pressure–diameter curves were obtained over the same 20- to 160-mmHg pressure range.

Real-Time PCR

RNA isolation and real-time PCR. Frozen kidney cortex samples were homogenized, and RNA was isolated using a kit (Qiagen, Venlo, The Netherlands), which included a DNase step. Integrity of RNA was determined using agarose gel electrophoresis, and RNA concentration was measured spectrophotometrically at 260 nm. RNA (1 μg) was reverse-transcribed, and cDNA was further used to analyze AT1R gene expression using a real-time PCR protocol of 35 cycles of 1 min, denaturation at 94°C, 1 min annealing at 56°C, and 1-min extension at 72°C. Sequence-specific PCR primers were purchased from Biologio (Nijmegen, The Netherlands). Relative AT1R gene expression was expressed as relative to GAPDH gene expression. The sequences of the primers used were as follows: rat AT1R: forward AGGTGTCTCAGCATGCACCCTACCC, reverse AGAATGATAAGGAAGGGAACAGAAGGCC; and rat GAPDH: forward CCCATACCATTTCTCCAGGAGCG, reverse ATGCAGGGATGATGTTCTGGCTGCC.

Follow-Up Measurements of Proteinuria and Blood Pressure

Urinary protein excretion was measured in samples of 24-h urine collected when the rats were put in metabolic cages for 24 h on a weekly basis for 12 wk. Urinary protein concentration was determined by nephelometry (Dade Behring III). Weekly systolic blood pressure measurements were carried out in conscious animals by tail-cuff plethysmography (IITC Life Science, Woodland Hills, CA).

Renal Histology

Paraffin-embedded kidneys were cut in 3-μm sections, stained with periodic acid-Schiff (PAS), and the incidence of focal glomerulosclerosis (FGS) was microscopically evaluated according to standard procedures as described previously (3).

Statistical Analysis

The results are expressed as means ± SE. Differences between the time periods were calculated and for comparisons a paired-sample t-test and independent t-test were used (SPSS). Graphs were made using SigmaPlot, and the relationship between parameters was calculated using regression analysis (SPSS).

The area under the curve (AUC) of myogenic tone was determined in individual arteries (SigmaPlot) and expressed in arbitrary units. Differences were considered significant at P < 0.05.

RESULTS

Systolic Blood Pressure, Proteinuria, and FGS

Systolic blood pressure, as measured in conscious animals, significantly increased in all experimental animals (P < 0.05) from 124 ± 3 (week 0) to 176 ± 12 mmHg 12 wk after 5/6Nx (Fig. 2A). After 5/6Nx, proteinuria significantly increased (P < 0.05) from 37.5 ± 4.6 mg/24 h at baseline to 229.1 ± 43.2 mg/24 h 12 wk after 5/6Nx (Fig. 2B). Renal damage after 12 wk of 5/6Nx was evidenced further by a 42.8 ± 4.8% incidence of FGS.

Acute Changes in Hemodynamic Parameters and Renal Blood Flow

To assess the ANG II response, hemodynamic parameters were compared between baseline and at 10 min of ANG II infusion. All animals reacted to ANG II infusion; compared with baseline values, ANG II significantly (P < 0.05) increased systolic blood pressure (23.9 ± 2.2%) and diastolic blood pressure (20.1 ± 2.9%) and reduced renal blood flow by 24.2 ± 6.5%.
Intravitral Microscopic Analysis of Afferent and Efferent Glomerular Arterioles

To evaluate changes in glomerular vascular reactivity, the changes in diameter of the afferent and efferent arterioles of the glomerulus were assessed (Fig. 3A). Ten minutes of ANG II infusion caused a significant (P < 0.001) constriction in both afferent and efferent arterioles compared with saline infusion. Notably, no difference was observed in the reactivity of afferent and efferent arterioles in response to ANG II infusion, as both were constricted to the same extent (Fig. 3B).

MC

Renal arteries isolated from the extirpated kidney at 5/6Nx from different rats developed MC to a variable extend. Averaged MC was 15.1 ± 1.8% of maximal MC over the whole pressure range (AUC: 1,267 ± 114 arbitrary units), with individual values ranging from 5.8% max MC (AUC: 586 arbitrary units) to 23.7% max MC (AUC: 1,927 arbitrary units).

Correlation Analysis

The responsiveness of both afferent and efferent arterioles to ANG II measured before 5/6Nx in vivo predicted proteinuria and glomerulosclerosis (FGS) 12 wk thereafter. The responsiveness of both afferent and efferent arterioles strongly correlated with proteinuria at week 12 after 5/6Nx (afferent: r = 0.727, P = 0.011, Fig. 4A; efferent: r = 0.900, P = 0.010, Fig. 4B). Similarly, there was a strong correlation between ANG II responsiveness and FGS (afferent: r = 0.691, P = 0.019, Fig. 4C; efferent: r = 0.664; P = 0.026, Fig. 4D). Therefore, these data indicate that healthy animals with higher responsiveness of glomerular arterioles to ANG II develop excess renal damage following subsequent 5/6Nx.

Also, the MC measured in vitro from renal small arteries obtained at 5/6Nx predicted the proteinuria and FGS 12 wk thereafter. MC negatively correlated both with proteinuria (r = 0.700, P = 0.016, Fig. 5A) and glomerulosclerosis (r = 0.882, P = 0.01, Fig. 5B), indicating that animals with a pronounced baseline MC assessed at 5/6Nx developed lower proteinuria and FGS 12 wk thereafter.

To substantiate a possible relationship between ANG II sensitivity and the extent of MC, additional correlation analysis was performed. Importantly, a strong negative correlation was found between the in vivo reactivity of afferent and efferent arterioles and ex vivo assessed MC (afferent: r = −0.700, P = 0.016, Fig. 5A) and glomerulosclerosis (r = −0.882, P = 0.01, Fig. 5B), indicating that animals with a pronounced baseline MC assessed at 5/6Nx developed lower proteinuria and FGS 12 wk thereafter.

To demonstrate a possible relationship between changes in systolic blood pressure and contractile responses of afferent arterioles, a correlation analysis was performed. Interestingly, a strong negative correlation was found between the in vivo reactivity of afferent and efferent arterioles and ex vivo assessed MC (afferent: r = −0.682, P = 0.021, efferent: r = −0.618, P = 0.043, Fig. 6). Thus, animals with lower responsiveness to ANG II displayed higher MC.

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and efferent arterioles after ANG II infusion, correlation analysis was performed. No significant relationship was found (afferent: $r = 0.218$, $P = 0.519$; efferent: $r = 0.291$, $P = 0.385$).

To investigate whether contractile responses of afferent and efferent arterioles to ANG II predict the development of hypertension at 12 wk after 5/6Nx, correlation analysis was performed (Fig. 6), which showed no significant correlation (afferent: $r = -0.077$, $P = 0.821$; efferent: $r = 0.005$, $P = 0.989$). Moreover, MC of isolated arteries also did not predict the development of hypertension at 12 wk after 5/6Nx ($r = 0.068$, $P = 0.842$).

**Correlation Analysis Between Relative mRNA Expression of AT1R, Vascular Parameters, and Renal Damage**

Relative mRNA expression of AT1R from renal cortex tissue obtained at 5/6Nx differed considerably among the animals. Correlation analysis revealed that expression of AT1R positively correlated with afferent (Fig. 7A) ($r = 0.621$, $P =$

![Graph A](image1.png)

**Fig. 4.** Correlation between individual ANG II (30 ng·kg$^{-1}$·min$^{-1}$)-induced responses at the time of 5/6Nx of the afferent and efferent arterioles and proteinuria (mg/24 h; A and B) and incidence of focal glomerulosclerosis (FGS) 12 wk after 5/6Nx (C and D; $n = 11$).

![Graph B](image2.png)

**Fig. 5.** Correlation between individual myogenic constriction responses [expressed as area under the curve (AUC) from individual curves] at the time of 5/6Nx and proteinuria (mg/24 h; A) and incidence of FGS 12 wk after 5/6Nx (B; $n = 11$).
Role of Afferent and Efferent Arterioles in Glomerular Pressure Regulation

We observed increased renal damage in those animals that had reacted more to ANG II before 5/6Nx on the levels of both afferent and efferent glomerular arterioles. The most obvious explanation is that the relationship reflects the sensitivity of individuals to ANG II, particularly as renal damage in the 5/6Nx model is (partly) driven by renin-angiotensin-aldosterone activation. Glomerular pressure is a central determinant of renal damage development. In transgenic rats harboring the mouse Ren2 renin gene, increased AT1R binding was found in vascular smooth muscle of afferent and efferent arterioles. This might suggest that upregulation of AT1Rs contribute to the glomerular damage in these rats (21). Therefore, similarly to our situation, rats with higher AT1R expression that had reacted more to ANG II developed greater damage thereafter. We observed a positive correlation between AT1R expression and the response of afferent or efferent arteriole ANG II-mediated constriction. Based on this result, we conclude that reactivity of glomerular arterioles is driven by the amount of AT1R, which further implies that renal damage in the 5/6Nx model is highly dependent on the expression of AT1R. Moreover, the relationship between AT1R expression and efferent arteriole constriction was stronger compared with afferent arteriole constriction, which suggests the higher importance of efferent arteriole function in the development of renal damage; this is in line with the notion that renal damage in 5/6Nx is strongly dependent on increased intraglomerular pressure (5).

MC as a Predictor of Renal Damage in 5/6Nx

We additionally show that rats with higher MC assessed before 5/6Nx developed less proteinuria and glomerulosclerosis than those with lower basal MC. This observation is in keeping with the notion that MC protects from an increase in intraglomerular pressure in rats with elevated blood pressure.

Increased intraglomerular pressure is a key determinant of renal damage development dictated by the integrity of the preglomerular vasculature. This view is substantiated by observations in animal models of renal failure (fawn-hooded hypertensive rat), in which a loss of MC in interlobar arteries precedes renal damage which develops only when systemic blood pressure increases (12). In contrast, spontaneously hypertensive rats (SHR), who have high systemic blood pressure, do not show renal damage. As SHR display strong myogenic response of preglomerular vessels, this possibly is the reason why their kidneys are protected from hypertension (6).

Relationship Between ANG II-Mediated Constriction and MC

Interestingly, we found a strong inverse relationship between MC of ex vivo preglomerular vessels and in vivo ANG II-mediated contractility of the afferent and efferent arterioles. There may be several explanations. The systemic action of ANG II infusion in this study caused an increase in systemic blood pressure, which most likely led to an increase in renal...
perfusion pressure as well, thus activating MC. Moreover, nonpressor doses of ANG II strongly augment MC, as measured in isolated mesenteric arteries (17). Therefore, ANG II-mediated constriction of the afferent and efferent arterioles may partly reflect the vessels’ ability to generate MC. Our results, however, show the two parameters to be negatively rather than positively correlated, making this option unlikely. Second, AT1R has been implicated as a stretch sensor. Mechanical stretch induces association of the AT1R with Janus kinase 2, translocation of G proteins into the cytosol, activation of ERK, and production of inositolphosphates (22). These features imply that the AT1R may act as a stretch receptor in vascular smooth muscle and hence be involved in MC generation. Indeed, we previously showed interaction of AT1R and MC in a model of heart failure, where increased MC was restored by inverse agonists of the AT1R (4, 17). Moreover, we showed the increased MC to coincide with a decreased number of caveolae in vascular smooth muscle and that a similar increase in MC is induced by disruption of caveolae (17). Thus it appears that the membrane distribution of the AT1R affects its responsivity to stretch. We observed a negative correlation between AT1R expression and MC, which supports our hypothesis mentioned above. While being an attractive explanation, there are unfortunately too limited data to substantiate this hypothesis, and further research is needed, which could possibly explain the role of the AT1R in MC.

Fig. 7. Correlation between individual relative values of ANG II type 1 receptor (AT1R) expression (A.U.) in renal cortex at the time of 5/6Nx and percentage of afferent (A) and efferent constriction (B), proteinuria (C), FGS at week 12 (D), myogenic constriction (MC) responses (expressed as AUC from individual curves; E), and SBP at week 12 (F; n = 11).
Similar Responsiveness of Afferent and Efferent Arterioles to ANG II

We found no significant differences in the ANG II-mediated constriction between afferent and efferent arterioles. This observation is in line with the similar reactivity of afferent and efferent arterioles to ANG II found in a previous study employing intravital microscopy in similar experimental settings (18). Nevertheless, it is generally believed that the efferent arteriole displays stronger contraction to ANG II, resulting in an increased glomerular pressure to achieve a physiological glomerular filtration rate. In a review of the literature, a large discrepancy exists regarding the extent of ANG II-mediated constriction in afferent vs. efferent arterioles. There is substantial evidence that under physiological conditions ANG II predominantly constricts the efferent arteriole. Nevertheless, a similar amount of evidence identifies the afferent arteriole as the predominant glomerular vessel sensitive to ANG II (1, 20). Different experimental approaches or animal strain specificity may account for this discrepancy. However, in the current study, no significant differences in responses between the two arterioles were detected although the trend (borderline significance) showed slightly higher contractility of the afferent arteriole to ANG II in our in vivo settings, which supports the view that the afferent arteriole reacts more to ANG II than the efferent arteriole.

Reactivity of Afferent and Efferent Arterioles Did Not Predict Development of Hypertension

Hypertension is believed to importantly contribute to renal damage in the 5/6Nx model. Indeed, our current and previous data (3) demonstrate that hypertension precedes development of renal damage in the 5/6Nx model. This sequence suggests that (compensatory) mechanisms of renal autoregulation are able to protect glomeruli from damage caused by pressure overload. However, once blood pressure exceeds the autoregulatory range, it seems that renal damage develops, as evidenced by an increase in proteinuria starting at week 8. Should one adopt this explanation, a relationship between the level of hypertension and the extent of renal damage seems obvious. However, we did not observe such a relationship. Our finding that MC predicts renal damage suggests that the mechanism, which protects glomeruli from hypertension, is more important than hypertension itself in the development of kidney damage in this model.

Our study implies that frequent contraction of glomerular arterioles should lead to renal damage. Unfortunately, there is no optimal model, where we could test this hypothesis. SHR possess higher contractility of glomerular arterioles (6); however, this strain is very unique with regard to renal autoregulation. Moreover, because of their inbred nature and the early development of hypertension, they seem not to represent the “best” control for the purpose of our study (i.e., to study normal variations in responsiveness of in vivo vasculature in relation to future damage). Therefore, SHR seem not to be the “best” control to test this hypothesis. In transgenic rats harboring the mouse Ren2 renin gene, increased AT1R binding was found in afferent and efferent arterioles (21), which implies that frequent contraction of glomerular arterioles might contribute to the glomerular damage in these rats. This possibly elevated contractility is ANG II specific, and therefore this strain seems not to be the “best” to test our hypothesis. Recently, we are studying genetically modified animals, which possess elevated contractility of glomerular arterioles that is ANG II independent, where we are testing our hypothesis.

Conclusion

In the present study, we provide evidence that in vivo reactivity of afferent and efferent arterioles to systemic infusion of ANG II, largely mediated by the amount of AT1R, predicts the degree of renal damage induced by subsequent 5/6Nx. Furthermore, ex vivo MC of interlobar arteries isolated at the induction of damage, i.e., 5/6Nx, predicts subsequent renal damage as well. These observations imply that measurement of intrarenal vascular function may be used to identify individuals prone to renal impairment. The relationship between in vivo sensitivity to ANG II and future renal damage will facilitate the employment of renal vascular responsiveness in the clinical setting. Nevertheless, further research is needed to explain the molecular and/or genetic background of patent vascular function as a determinant of susceptibility to renal damage.

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DISCLOSURES

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

AUTHOR CONTRIBUTIONS

Author contributions: P.V., R.H.H., H.B., and R.P.V.D. provided conception and design of research; P.V., M.G., D.V.-Y., and R.P.V.D. performed experiments; P.V. analyzed data; P.V., R.H.H., H.B., and R.P.V.D. interpreted results of experiments; P.V. prepared figures; P.V. drafted manuscript; R.H.H., H.B., and R.P.V.D. edited and revised manuscript; R.H.H. and R.P.V.D. approved final version of manuscript.

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