Vascular endothelial and myogenic function in renal disease
Ochodnicky, Peter

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Chapter 6

Altered myogenic constriction and EDHF-mediated relaxation in small mesenteric arteries of hypertensive subtotally nephrectomized rats

Simone Vettoretti*
Peter Ochodnický*
Hendrik Buikema
Robert H. Henning
C. Alex Kluppel
Dick de Zeeuw
Richard P.E. van Dokkum

*both authors contributed to this work equally

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Abstract

Objectives: Chronic renal failure (CRF) is associated with altered systemic arterial tone and hypertension. Myogenic constriction and endothelium-derived hyperpolarizing factor (EDHF)-dependent relaxation represent major vasoregulatory mechanisms in small systemic arteries. Elevated myogenic response and impaired EDHF might participate in the development of essential hypertension, however their role in CRF-related hypertension is unknown. We investigated whether myogenic response and EDHF are altered in subtotally nephrectomized (sNX) rats and whether these changes are modifiable by chronic treatment with ACE inhibitor.

Methods: In a pressure arteriograph, myogenic constriction and EDHF-mediated relaxation were evaluated in small mesenteric arteries isolated from male Wistar rats 15 weeks after either SHAM operation (n=7), sNX (n=12) or sNX followed by 9 week treatment with lisinopril (sNX+LIS, 2.5 mg/kg, n=13).

Results: Surprisingly, myogenic response was reduced in hypertensive CRF rats (maximal myogenic tone: 37±2 and 18±4%, p<0.01; peak myogenic index: -0.80±0.08 and -0.40±0.12 %/mmHg, p<0.05 in SHAM and sNX respectively). At the same time EDHF-mediated relaxation was impaired as well (maximal response: 92±2 and 77±5%, p<0.01; pD2: 6.9±0.1 and 5.3±0.1, p<0.05). Both myogenic response and EDHF were inversely related to the severity of renal failure and restored by treatment with lisinopril to levels found in SHAM animals.

Conclusion: Major constrictive (myogenic) and dilatory (EDHF) mechanisms of small systemic arteries are impaired in hypertensive CRF rats. These alterations do not seem to participate in the development hypertension, being rather directly related to the severity of renal impairment. Both systemic vascular changes might be restored by renoprotective treatment with ACE inhibitor.
Introduction

Chronic renal failure (CRF) is associated with increased cardiovascular morbidity and mortality. Structural and functional alterations of peripheral blood vessels are likely to be involved in many of the cardiovascular complications occurring in CRF. Abnormal function of large conduit arteries characterized by reduced arterial compliance may result in increased pulse pressure and the development of left ventricular hypertrophy, both independent prognostic factors for cardiovascular mortality in patients with end-stage renal disease. On the other hand, compromised reactivity of small systemic arteries may contribute to the increased peripheral resistance and development of hypertension in CRF. In fact, hypertension, the ultimate cardiovascular risk factor, occurs in 80-90% of patients with end-stage renal disease.

Several alterations in peripheral vasomotor mechanisms are implicated in CRF, among which, endothelial dysfunction have received a great deal of attention. Endothelial dysfunction due to oxidative stress-mediated loss of nitric oxide (NO) vasodilation, was proposed to account for hypertension in rats subjected to subtotal nephrectomy (sNX). However, apart from NO, other endothelium-derived mediators are involved in the regulation of peripheral vascular tone. Yet unknown factor termed endothelium-derived hyperpolarizing factor (EDHF) relaxes the underlying vascular smooth muscle cells by opening potassium channels and hyperpolarizing the cell membrane. Interestingly, in small resistance arteries such as small mesenteric artery, EDHF-mediated relaxation represents the principal endothelial vasomotor mechanism accounting for vast majority of endothelium-dependent vasodilation. Several authors have shown that EDHF might be compromised in large conduit arteries of rats with sNX. However EDHF-relaxation in small peripheral vasculature during hypertensive CRF has not been addressed thoroughly.

In addition to decreased local vasodilatory capacity, exaggerated contractile abilities of peripheral vessels have been reported in various forms of hypertension. Excessive contractile response of small arteries to increases in intravascular pressure, termed myogenic constriction, might contribute to elevated peripheral resistance in spontaneously hypertensive rats (SHR). It is unknown whether a similar mechanism occurs in hypertension associated with sNX. Interestingly, we previously found that the level of myogenic constriction may be inversely related to EDHF-mediated dilation, suggesting that both mechanisms share common signaling pathways, probably interacting at the level of vascular Ca\(^{2+}\)-regulated potassium channel. Therefore, the aim of the present study was to assess changes in myogenic contractility and EDHF-mediated vasodilation of small systemic arteries in rats with experimental CRF induced by sNX. We hypothesized that small mesenteric arteries of rats with experimental CRF display increased myogenic reactivity and decreased EDHF-mediated dilation as these mechanisms could be inversely related. Since systemic vascular alterations might play a role in the development of hypertension and cardiovascular damage in CRF our secondary aim was to explore whether...
these mechanisms are modifiable by renoprotective treatment with the angiotensin-converting enzyme inhibitor (ACEi) lisinopril.

**Methods**

**Animals**

Male Wistar rats, 12-13 weeks of age (n=40, Harlan, Zeist, The Netherlands) were housed under standard conditions at the animal facilities of the University of Groningen. Animals had free access to food (standard rat chow containing 0.3% sodium, Hope Farms, Woerden, The Netherlands) and drinking water throughout the study. Experiments were approved by the local Animal Ethical Committee.

**Surgery**

Under anaesthesia with isoflurane 3% in N\textsubscript{2}O/O\textsubscript{2} (2:1), sNX was performed by excision of the right kidney and subsequent infarction of approximately 2/3 of contralateral kidney by ligation of two to three branches of the left renal artery. SHAM animals underwent the same procedure and were closed after manipulation and decapsulation of the kidneys without being nephrectomized. Postoperatively, the rats were allowed to recover for one week.

**Experimental design and in vivo measurements**

Following surgery, systolic blood pressure (SBP) and urinary protein excretion were determined weekly. 6 weeks after the operation, nephrectomized rats were stratified based on proteinuria and divided in untreated (sNX, n=12) and treated (lisinopril; sNX+LIS, n=13) rats. SHAM rats (n=7) were left untreated. Lisinopril was dissolved in the drinking water at a dose of 2.5 mg/kg body weight. Subsequently, rats were followed up to 15 weeks after surgery. Shortly before sNX (baseline), at 6 weeks (stratification) and 15 weeks after sNX (termination), blood samples were collected for the determination of plasma creatinine. SBP was measured as the mean of three consecutive measurements in awake, restrained animals by means of the tail-cuff method (IITC Inc., Woodland Hills, CA, USA). Urinary protein excretion was determined by placing the rats in metabolic cages for 24 hours and protein concentration was analyzed by TCA (Nephelometer Analyzer II, Dade Behring, Marburg, Germany). Creatinine concentration was measured colorimetrically (Chema Diagnostica, Jesi (AN), Italy). Upon termination under isoflurane in N\textsubscript{2}O/O\textsubscript{2}, the remnant kidney and heart were removed and weighted. The mesenteric arterial bed was excised for investigation of vascular function.

**Preparation and cannulation of small mesenteric arteries**

Third-order branches in mesenteric arterial bed were isolated, cleaned from perivascular tissue and transferred to an arteriograph system for pressurized arteries (Living System
Instrumentation, Burlington, VT, USA). Each artery was cannulated at both ends with glass micropipettes, secured, and the lumen of the vessel was filled with Krebs solution through the micropipettes. Intraluminal pressure was set at 60 mmHg and was held constant (without flow) by a pressure servo system (Living System Instrumentation, Burlington, VT, USA). The vessel chamber was continuously recirculated with warmed (37°C) and oxygenated (5% CO₂ in O₂) Krebs solution with a pH of 7.4. Subsequently, the chamber was transferred to an inverted light microscope with a video camera attached to a viewing tube. Lumen diameter was continuously registered by a video dimension analyzer (Living System Instrumentation, Burlington, VT, USA).

**Determination of arterial reactivity and experimental protocol**

Prior to the experiments, arteries were allowed to equilibrate for 40 minutes. To test for viability of smooth muscle cells and endothelium, arteries were pre-constricted with a thromboxane A₂ analogue (U46619, 30 nmol/L) and relaxed with a single dose of acetylcholine (100 µmol/L). Following wash-out, myogenic reactivity was investigated by constructing active pressure-diameter curves in normal Krebs solution. These were obtained over a pressure range of 20–160 mmHg in steps of 20 mmHg. After each stepwise increase in intraluminal pressure, lumen diameter was registered when a stable diameter (contraction) was reached (~ 5 min). Subsequently, the pressure was set back to 60 mmHg and the arteries were allowed to stabilize for 20 minutes before the EDHF-mediated vasodilation was investigated. To address EDHF-dependent relaxation, the vessel was pre-contracted with U46619 (30 nmol/L). Thereafter, the concentration-response curve to acetylcholine (3.10⁻⁸- 3.10⁻⁵ mol/l) was determined in the presence of indomethacin (10 µmol/L) and Nω-monomethyl-L-arginine (L-NMMA, 100 µmol/L) in order to block cyclooxygenase and nitric oxide synthase, respectively. Previously, we showed that the remaining relaxation under these conditions is mediated by EDHF, since it is completely abrogated either by calcium-regulated potassium channels inhibitors charybdotoxin (100 nmol/L) and apamin (500 nmol/L)¹¹,¹⁸ or by high concentration (40 mmol) of KCl⁹. Finally, after perfusing the system with calcium-free Krebs solution supplemented with ethylene glycol-bis-(b-amino ethyl ether) tetraacetic acid (EGTA, 2 mmol/L), passive pressure-diameter step curves were obtained over a pressure range of 20-160 mmHg.

**Solutions and drugs**

Vessel segments were perfused with Krebs solution of the following composition (in mmol/L): NaCl 120.4, KCl 5.9, CaCl₂ 2.5, MgCl₂ 1.2, NaH₂PO₄ 1.2, glucose 11.5, NaHCO₃ 25.0). Lisinopril was purchased from Astra-Zeneca (The Netherlands). All other compounds were purchased from Sigma (St. Louis, MO, USA). Stock solution of indomethacin was prepared in 64 mmol/l NaHCO₃.
Data analysis
Data are expressed as mean ± SEM; n values represent the number of investigated rats as well as the number of investigated arteries since one artery segment per rat was used for the same protocol. To characterize myogenic responsiveness, the following parameters were calculated from the pressure-diameter curve of each individual artery:

1. **Myogenic tone**, describing myogenic behaviour of an artery at a given pressure, was expressed as percent decrease in active diameter from the maximally dilated (passive) diameter determined at the same pressure in calcium-free/EGTA solution, i.e., myogenic tone (%) = 100 \[(D_{Ca-free} – D_{Ca})/D_{Ca-free}\], where D is the diameter in calcium-free (D_{Ca-free}) or calcium-containing (D_{Ca}) Krebs. For every individual artery segment maximal myogenic tone was determined as the maximal value over the studied pressure range.

2. **Myogenic index**, describing myogenic reactivity of an artery in response to a pressure change, e.g. the slope of active pressure-diameter relationship, was calculated for every 20 mmHg pressure step (\(\Delta P\)) as a percentage change in corresponding active diameter D, i.e. myogenic index (%/mmHg) = 100 \[(\Delta D/D)/\Delta P\]. A negative value indicates active luminal reduction in response to an increase in pressure and provides the evidence of myogenic behaviour independently from passive diameters of the vessel^{19}. For each individual artery peak myogenic index denotes the largest value of all the pressure steps studied.

EDHF-dependent concentration-response curves to acetylcholine were expressed in percentage of pre-constriction to thromboxane A\(_2\) analogue U46619. The curves were characterized by maximal relaxation (\(E_{\text{max}}\)) and negative logarithm of acetylcholine molar concentration causing half-maximal relaxation (pD\(_2\)). Statistical differences for vascular parameters, proteinuria, systolic blood pressure, creatinine, body and organ weights, water intake and urinary output were determined by Student’s independent t-test. Differences in myogenic and EDHF curves among experimental groups were tested using Bonferroni post hoc multiple comparisons applied to ANOVA for repeated measures. The relationships between vascular reactivity and *in vivo* data were calculated using Spearman’s nonparametric correlation. Differences were considered significant at p<0.05 (two-tailed).

**Results**

**Survival**
Following the nephrectomy 6 rats died because of uremia before the stratification, and 2 animals of the sNX group died later. Consequently 32 rats completed the study and were eligible for the full protocol analysis at termination, (sNX, n=12; sNX+LIS, n=13 and SHAM, n=7). Prior to surgery, baseline values (week 0) of body weight, water intake, urinary output and plasma creatinine, proteinuria and blood pressure (*Table 1*) were similar in the three experimental groups.
Table 1. Clinical characteristics of experimental animals (SHAM-operated, subtotally nephrectomized- sNX and subtotally nephrectomized rats treated with lisinopril 2.5 mg/kg- sNX+LIS) measured at the baseline (week 0), stratification (week 6) and termination (week 15 after the operation).

<table>
<thead>
<tr>
<th>Variable</th>
<th>SHAM</th>
<th>sNX</th>
<th>sNX+LIS</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Body Weight (g)</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Baseline week 0</td>
<td>321±4</td>
<td>338±17</td>
<td>330±8</td>
</tr>
<tr>
<td>Stratification week 6</td>
<td>427±9</td>
<td>419±12</td>
<td>393±14</td>
</tr>
<tr>
<td>Termination week 15</td>
<td>512±10</td>
<td>490±15</td>
<td>472±14</td>
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<tr>
<td><strong>Water intake (ml/24h)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline week 0</td>
<td>21±2</td>
<td>28±3</td>
<td>34±2</td>
</tr>
<tr>
<td>Stratification week 6</td>
<td>26±2</td>
<td>45±3*</td>
<td>42±3*</td>
</tr>
<tr>
<td>Termination week 15</td>
<td>24±1</td>
<td>47±4*</td>
<td>47±4*</td>
</tr>
<tr>
<td><strong>Urine output (ml/24h)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline week 0</td>
<td>10±1</td>
<td>14±2</td>
<td>17±3</td>
</tr>
<tr>
<td>Stratification week 6</td>
<td>14±2</td>
<td>23±2*</td>
<td>24±2*</td>
</tr>
<tr>
<td>Termination week 15</td>
<td>13±1</td>
<td>33±4*</td>
<td>32±4*</td>
</tr>
<tr>
<td><strong>Proteinuria (mg/24h)</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Baseline week 0</td>
<td>20±3</td>
<td>20±2</td>
<td>30±7</td>
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<td>Stratification week 6</td>
<td>26±2</td>
<td>86±20*</td>
<td>117±25*</td>
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<tr>
<td>Termination week 15</td>
<td>39±9</td>
<td>354±38*</td>
<td>183±42*</td>
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<tr>
<td><strong>Plasma creatinine (µmol/l)</strong></td>
<td></td>
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<tr>
<td>Baseline week 0</td>
<td>46±5</td>
<td>44±2</td>
<td>47±4</td>
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<tr>
<td>Stratification week 6</td>
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<td>77±8*</td>
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<td>Termination week 15</td>
<td>57±5</td>
<td>100±16*</td>
<td>77±16</td>
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<tr>
<td><strong>Systolic blood pressure (mmHg)</strong></td>
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<td></td>
<td></td>
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<tr>
<td>Baseline week 0</td>
<td>124±6</td>
<td>122±3</td>
<td>124±3</td>
</tr>
<tr>
<td>Stratification week 6</td>
<td>134±4</td>
<td>163±9*</td>
<td>155±6*</td>
</tr>
<tr>
<td>Termination week 15</td>
<td>136±8</td>
<td>173±6*</td>
<td>139±9*</td>
</tr>
<tr>
<td><strong>Left ventricle weight (mg/g body weight)</strong></td>
<td></td>
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<tr>
<td>Termination week 15</td>
<td>2.1±0.1</td>
<td>2.7±0.2*</td>
<td>2.4±0.1*</td>
</tr>
<tr>
<td><strong>Wet kidney weight (mg/g body weight)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Termination week 15</td>
<td>3.3±0.1</td>
<td>4.7±0.2*</td>
<td>4.7±0.3*</td>
</tr>
</tbody>
</table>

* p<0.05 compared with SHAM at the same time point
# p<0.05 compared with sNX at the same time point
In vivo data
Within 6 weeks after sNX, the animals from both nephrectomized groups (sNX and sNX+LIS) developed severe renal failure characterized by increased SBP (Figure 1A), proteinuria (Figure 1B), elevated plasma creatinine levels and when compared to SHAM operated rats (Table 1, stratification). Additionally, water intake and urine output were higher in sNX and sNX+LIS group compared to SHAM animals (Table 1, stratification). At this time point, no differences were found in any of the measured parameters between the sNX and sNX+LIS groups.

Figure 1. The development of A) systolic blood pressure (mmHg) and B) proteinuria (mg/24h) in time after the subtotal nephrectomy. Note that after the development of the disease, the rats were stratified to different treatment groups at week 6. SHAM operated rats, sNX- subtotally nephrectomized and sNX+LIS- subtotally nephrectomized treated with lisinopril 2.5 mg/kg, * p<0.05 compared with SHAM, # p<0.05 compared with sNX
Renal disease progressed further in sNX animals, whereas the progression was slowed down by ACE-i. Upon sacrifice, 15 weeks after the operation, sNX rats displayed elevated SBP (Figure 1A), severe proteinuria (Figure 1B), double plasma creatinine levels, higher water intake, urine output, kidney and left ventricle mass (Table 1, termination) as compared to age-matched SHAM rats. ACE-i treatment substantially reduced proteinuria compared with sNX group, although the levels were still higher than in SHAM animals. Plasma creatinine levels and left ventricle mass tended to be lower in the sNX+LIS group, which was however not statistically significant. Increased SBP in the sNX group was restored by ACE-i to the levels measured in SHAMs (Table 1, termination) and had no effect on wet kidney weight, water intake and urine output.

**Vascular experiments**

*Myogenic constriction*

As shown in Figure 2A, passive diameters of small mesenteric arteries did not differ among the experimental groups over the whole pressure range, suggesting no apparent changes in maximal relaxant ability of the investigated arteries. Similarly, no differences were observed in the receptor-mediated contraction to the thromboxane A$_2$ analogue U46619 (data not shown). Small mesenteric arteries developed a substantial myogenic tone dependent on the intraluminal pressure applied to the vessel. Active diameters were significantly higher in the sNX group over the pressure range of 100-160 mmHg when compared with SHAM (Figure 2A). As a consequence, myogenic tone was blunted over this pressure range (Figure 3A). Threshold for the development of active myogenic constriction, indicated by negative myogenic index, was shifted in sNX rats to 80-100 mmHg pressure step as compared to 60-80 mmHg in SHAM rats (Figure 2B). Furthermore, the arteries of sNX rats displayed less pronounced negative values of myogenic index at pressure steps 60-80, 80-100 and 100-120 mmHg when compared with SHAM animals, indicating less steep slope of active pressure-diameter curve in this pressure range (Figure 2B). Maximal myogenic tone (p=0.001, Table 2) and peak myogenic index (p=0.004, Table 2) were also impaired in the vessels isolated from sNX rats. Interestingly, chronic treatment with lisinopril reversed active responses to pressure (Figure 2A). Consequently, myogenic tone was restored over the whole pressure range (Figure 3A) and maximal myogenic tone was comparable to the SHAM values (p=0.03 compared to sNX, Table 2). Additionally, the myogenic index was reversed to more negative values in sNX+LIS group at pressure steps 60-80, 80-100 mmHg (Figure 2B).

*EDHF-mediated vasodilation*

A single dose of acetylcholine induced significantly lower endothelium-dependent relaxation in the sNX group (100±3, 87±2 and 101±5 % of precontraction level for SHAM, sNX and sNX+LIS, respectively), suggesting an impairment in endothelium-mediated responses. EDHF-mediated relaxation of small mesenteric arteries was significantly diminished after sNX as compared to SHAM arteries (Figure 3B). The acetylcholine curve
performed under blockade of cyclooxygenase and nitric oxide synthase showed a reduction in both maximal response $E_{\text{max}}$ (p = 0.009, Table 2) and pD$_2$ values (p = 0.006, Table 2) in sNX rats when compared to SHAM.

**Figure 2.** A) Diameters of small mesenteric arteries in response to stepwise increase of intraluminal pressure in the presence (black symbols, active tone) or absence (white symbols, passive tone) of extracellular calcium and B) calculated myogenic index (in % of active diameter/mmHg) developed in response to stepwise increase of intraluminal pressure studied in SHAM operated, subtotally nephrectomized (sNX) and subtotally nephrectomized rats treated with lisinopril 2.5 mg/kg (sNX+LIS) 15 weeks after the operation. *p < 0.05 compared with SHAM; #p < 0.05 compared with sNX
ACE-i treatment effectively restored blunted EDHF-dependent dilation observed in vessels from sNx rats (Figure 3B). Rats from sNX+LIS displayed increased $E_{\text{max}}$ ($p=0.04$) and $pD_2$ ($p=0.04$) values of EDHF-acetylcholine curve when compared to sNX animals (Table 2).

**Figure 3.** Impaired A) myogenic tone (in % of passive diameter) and B) EDHF-dependent vasodilation (as acetylcholine-induced relaxation in % of precontraction) in small mesenteric arteries of subtotally nephrectomized (sNX) rats as compared to SHAM-operated, and subtotally nephrectomized rats treated with lisinopril 2.5 mg/kg (sNX+LIS).

* $p<0.05$ compared with SHAM; # $p<0.05$ compared with sNX

**Correlation analysis**
To characterize the role of blood pressure changes in the development of renal damage after sNX and in the renoprotective effects of ACE-i, we analyzed relationship between the clinical parameters. SBP correlated with both proteinuria and plasma creatinine ($r=0.64$, $p=$
0.02 and r= 0.66, p= 0.02, respectively) in sNX+LIS group, but not in sNX group (r= 0.18, p= NS and r= 0.12, p= NS, respectively).

EDHF-dependent relaxation positively correlated with acetylcholine-induced response, suggesting that EDHF represents principal endothelial vasodilatory mechanism in small mesenteric arteries (r= 0.61, p= 0.002, all groups included). To test our hypothesis on the relation between myogenic constriction and EDHF-dependent relaxation we performed correlation analysis in sNX animals. However, no relation was observed between these two parameters in small mesenteric arteries used in this study (r= 0.06, p= NS). When investigating whether beneficial effects of ACEi on both vascular parameters in sNX+LIS group might be interrelated, we did not find any relation between EDHF and myogenic tone either (r= 0.25, p= NS). Similarly, no correlation was found between maximal total acetylcholine-induced response and myogenic tone (r= 0.11, p= NS and r= 0.20, p= NS for sNX and sNX+LIS group respectively), suggesting no role for endothelium-mediated mechanisms in myogenic tone changes.

Table 2. Comparison of vascular reactivity in small mesenteric arteries of SHAM operated, subtotally nephrectomized (sNX) and subtotally nephrectomized rats treated with lisinopril 2.5 mg/kg (sNX+LIS) 15 weeks after the operation.

<table>
<thead>
<tr>
<th></th>
<th>SHAM</th>
<th>sNX</th>
<th>sNX+LIS</th>
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<tbody>
<tr>
<td><strong>Myogenic tone</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maximal myogenic tone</td>
<td>37±2</td>
<td>18±4*</td>
<td>33±5##</td>
</tr>
<tr>
<td>( % of passive diameter)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peak myogenic index</td>
<td>-0.80±0.08</td>
<td>-0.40±0.12*</td>
<td>-0.68±0.15</td>
</tr>
<tr>
<td>( % of active diameter/mmHg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>EDHF vasodilation</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$E_{\text{max}}$ ( % of precontraction)</td>
<td>92±2</td>
<td>77±5*</td>
<td>88±2##</td>
</tr>
<tr>
<td>$pD_2$</td>
<td>6.5±0.1</td>
<td>5.9±0.1*</td>
<td>6.3±0.1##</td>
</tr>
</tbody>
</table>

EDHF- endothelium-dependent hyperpolarizing factor, $E_{\text{max}}$- maximal relaxation, $pD_2$- negative logarithm of the acetylcholine concentration causing half maximal responses. * p<0.05 compared with SHAM; ‡ p<0.05 compared with sNX.

To address the role of renal failure and hypertension in the development or ACE-i mediated reversal of systemic vascular changes, additional correlation analyses were performed in sNX and sNX+LIS animals, respectively. Interestingly, in sNX rats maximal myogenic tone and maximal EDHF-dependent dilation of small mesenteric arteries inversely correlated or tended to correlate with proteinuria as a marker of renal damage, (Figure 4A, B; r= -0.60, p= 0.04 and r= -0.52, p= 0.08 for maximal myogenic tone and maximal EDHF dilation, respectively), but not with SBP (r= 0.06, p= NS and r= -0.02, p= NS, for maximal
myogenic tone, and maximal EDHF dilation, respectively). When the treated group was analyzed, an inverse correlation of borderline significance was found between myogenic tone and proteinuria (Figure 4A; \( r = -0.53, p = 0.06 \)), but not between EDHF dilation and proteinuria (Figure 4B, \( r = -0.04, p = \text{NS} \)). No significant correlations were observed between blood pressure and vascular parameters in sNX+LIS group.

**Discussion**

Chronic renal failure (CRF) is associated with altered arterial tone of the systemic vasculature, which may contribute to elevated blood pressure and accelerated rate of cardiovascular events observed in renal patients. In the current study, we demonstrate that two major vasoregulatory mechanisms of small mesenteric arteries, namely myogenic constriction and EDHF-mediated vasodilation, are impaired in a rat model of subtotal nephrectomy (sNX) with hypertension. Both vascular alterations in this experimental model might be reversed by renoprotective therapy with an ACE inhibitor.

Rats with sNX employed in the current study displayed characteristic features of CRF, as evidenced by severe proteinuria and almost doubled plasma creatinine levels, increased fluid intake, elevated urine production and increased renal mass, probably due to compensatory renal growth\(^{20}\). CRF was accompanied by an increase in blood pressure, a
complication frequently occurring in patients with end-stage renal disease and the ultimate risk factor for cardiovascular events\(^5\). The severity of hypertension did not correlate with the markers of renal damage, suggesting extrarenal factors may be involved in the increase of systemic blood pressure. Since hypertension in CRF is associated with increased peripheral resistance\(^{21}\), systemic vascular changes might well contribute to CRF-associated elevations in blood pressure. Furthermore, a significant body of evidence suggests that myogenic response of small peripheral arteries is enhanced in SHR rats\(^{15-17}\) implying that enhanced intrinsic reactivity of resistance arteries to increased pressure might be involved in elevated peripheral resistance in hypertension. However, as suggested by our current data this does not seem to be the case in CRF-associated hypertension. In contrast to our hypothesis, in sNX we observed a reduction in myogenic response of small mesenteric arteries, characterized by the impaired myogenic tone in the pressure range of 100-160 mmHg and altered myogenic index resulting in a shifted threshold for the development of active myogenic constriction to higher pressures. To our knowledge, the only other study investigating myogenic response of small resistance arteries in CRF did not find any difference between myogenic reactivity of small cremaster arteries in sNX and SHAM-operated rats\(^{22}\). Differences in anatomic localization, vascular diameter and function may account for the observed discrepancy with our data. Collectively however, both studies suggest that changes in systemic myogenic response do not contribute to the development of CRF-associated hypertension. Consequently, mechanisms other than excessive local myogenic reactivity, such as volume overload, overactivation of renin-angiotensin or sympathetic system, are probably responsible for increased peripheral resistance in CRF. The observed reduction of myogenic tone might represent a compensatory mechanism counteracting the increase in peripheral resistance in CRF. However, we found the level of myogenic tone not to be related with severity of hypertension, but rather with the severity of renal disease, suggesting that myogenic tone is specifically modulated by the state of renal insufficiency. Furthermore, the reduction of systemic myogenic tone was completely reversed after chronic treatment with lisinopril. Since ACE-i therapy resulted in a complete reversal of elevated systolic blood pressure and prevented the progression of renal damage, all antihypertensive, renoprotective and/or specific local effects of ACE-i (e.g. resulting from local ACE inhibition) might play a role in myogenic tone reversal. A positive correlation between blood pressure and severity of renal damage in treated animals suggests that antihypertensive and renoprotective effects of ACEi might be related. However, it is likely that kidney-related mechanisms modulate myogenic reactivity after ACE-i treatment, since the level myogenic tone was associated with renal damage rather than with blood pressure.

In addition to a reduction in smooth-muscle mediated myogenic mechanisms we also found an alteration in the major endothelium-dependent vasodilatory mechanism of small mesenteric artery, e.g. EDHF. Our data are in agreement with studies showing impaired EDHF-mediated relaxation in large conduit arteries of experimental animals with CRF\(^{12,23}\). However it is difficult to assess to which extent compromised EDHF relaxation of
resistance arteries contributes to CRF-associated hypertension, since impaired EDHF relaxation was also demonstrated in CRF-rats without hypertension, suggesting that CRF per se is associated with disturbed EDHF-mediated relaxation\(^{24}\). Our finding of a correlation between EDHF-mediated relaxation and markers of renal failure, but not hypertension, may support this view. Moreover, EDHF is differently modulated in various forms of hypertension ranging from altered\(^{25,26}\) to elevated EDHF relaxations in some animal models\(^{27-30}\). In latter cases, EDHF might compensate for reduced nitric oxide bioavailability. Therefore the role of altered EDHF relaxation in CRF-related hypertension will have to be defined further.

The mechanisms responsible for concomitant decrease of myogenic constriction and EDHF-mediated dilation of systemic arteries in CRF cannot be directly inferred from this study. In contrast to our hypothesis we could not confirm a direct antagonistic relation between these two parameters. Similarly, ACEi-induced changes in endothelium (EDHF)- and myogenic-mediated mechanisms do not seem to be correlated either, suggesting that endothelial and smooth-muscle mediated mechanisms are altered independently in CRF. Thus, rather than a result of endothelial dysfunction, myogenic alterations might be a consequence of inappropriate chronic stimulation of sympathetic\(^{31}\), endothelin\(^{32}\) or renin-angiotensin\(^{33}\) systems occurring in CRF. Myogenic tone is strongly potentiated by vasoconstricting agents, such as noradrenaline\(^{34}\), endothelin-1\(^{16}\), and angiotensin-II\(^{35,36}\) and the chronic overactivation might result in its adaptive downregulation. Involvement of renin-angiotensin system is also supported by the finding of complete reversal of systemic myogenic tone after chronic treatment with ACEi. Interestingly, the angiotensin AT\(_1\) receptor is involved in changes of myogenic reactivity observed in mesenteric arteries after experimental heart failure\(^{35}\). Yet, specific molecular mechanisms responsible for myogenic alterations will still have to be worked out. Although we cannot fully exclude the possibility of a generalized contractile defect on the level of smooth muscle cells, we found no differences in reactivity to the thromboxane A\(_2\) agonist and no change in general contractile ability was reported previously in this model and vascular bed\(^{24}\). Therefore, signaling mechanisms relatively specific for pressure-induced vasoconstriction are more likely to be involved, including altered activity or mechanosensitive, voltage- or calcium-regulated ion channels\(^{14}\). One may speculate on the role of smooth muscle membrane Ca\(^{2+}\)-regulated potassium large conductance channels (BK\(_{Ca}\)), closure of which is implicated in myogenic-induced depolarization\(^{14,37}\), whereas opening is involved in endothelium-induced hyperpolarization\(^{8,38}\). Reduced availability of BK\(_{Ca}\) channel might possibly explain the concomitant defect in myogenic tone and EDHF-mediated dilation in CRF. In line with renin-angiotensin system overactivity hypothesis, angiotensin II has been shown to inhibit vascular smooth muscle K\(_{Ca}\) channels\(^{39}\). However, further studies are needed to explore the mechanisms underlying vascular changes in CRF.

In conclusion, the present study revealed impaired myogenic constriction and EDHF-dependent dilation of small mesenteric arteries in a rat model of CRF with hypertension. The alterations in systemic vasoactive mechanisms do not seem to participate in the
development of CRF-associated hypertension, being rather directly related to the severity of renal impairment. Furthermore, both systemic vascular changes might be reversed by the renoprotective treatment with an ACE inhibitor.
References
