

Chapter 6

Endothelial Dilatory Function Predicts Individual Susceptibility to Renal damage in the 5/6 Nephrectomized Rat

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Abstract

Background

In experimental animal models of renal disease the degree of renal damage varies between individuals. This could be caused by variation in the noxious event or by differences in individual susceptibility. Intact endothelial function is assumed to provide a defense mechanism against progressive renal damage. We hypothesized that interindividual differences in renal endothelial function might be involved in individual susceptibility to renal damage.

Methods

We investigated whether endothelial function of small renal arteries prior to induction of 5/6 nephrectomy (5/6 Nx) in rats was related to development of renal damage after 5/6 Nx. Wistar rats underwent 5/6 Nx and small renal arteries of the removed right kidney were investigated for endothelium-dependent relaxation to acetylcholine (ACh, 10^{-8} - 10^{-4} mol/L). The contribution of underlying endothelial dilative mediators NO, prostaglandins (PG) and EDHF was assessed using the inhibitors L-NMMA, indomethacin and charybdotoxin+apamin, respectively.

Results

After 5/6 Nx proteinuria developed in each rat ranging from 22 to 278 (84 ± 14) mg/24h at week 5 (n=23). Interestingly, a significant inverse correlation between individual ACh-relaxation (expressed as area under curve in arbitrary units) and proteinuria 5 weeks after 5/6 Nx was found ($r = -0.54$, $P = 0.008$, $n = 23$). An inverse correlation was also found between individual NO- as well as PG-contribution and proteinuria 5 weeks after 5/6 Nx ($r = -0.86$, $P = 0.001$, $n = 11$, and $r = -0.74$, $P = 0.01$, $n = 11$, respectively). In addition, individual ACh-relaxation was positively correlated with GFR measured 6 weeks after 5/6 Nx ($r = 0.58$, $P = 0.016$, $n = 17$).

Conclusion

This study demonstrates for the first time that individual renal endothelial dilatory function of the healthy rat predicts susceptibility to renal damage after 5/6 Nx, which seems to depend on individual endothelial NO- and PG-activity.

Introduction

Development of renal damage is highly variable between individuals, shows racial differences and may be determined genetically¹⁻⁴. In animal models of renal disease, it has been demonstrated that development of renal damage may vary considerably despite similar levels of blood pressure^{5,6}. In transplantation studies in rats, differential development of renal damage despite similar metabolic and (high) blood pressure environments was ascribed to be intrinsic to the (donor) kidney⁷. Even in normotensive environments the susceptibility of the graft to develop renal damage appears to be strain dependent⁸. The development of renal damage may also vary considerably among individuals of one rat strain. Accordingly, large interindividual differences in the development of renal functional deterioration after 5/6 nephrectomy (5/6 Nx) in normotensive rats have been found^{6,9}. It appears that some kidneys may be more susceptible to develop renal damage in response to the same noxe than others. However, the underlying mechanisms of this individual susceptibility are largely unknown.

An intact endothelial dilatory function is assumed to provide a powerful defense mechanism against progressive renal damage^{10,11}, as evidenced by the well described deleterious effect of NOS-blockade as well as COX-blockade on kidney function¹²⁻¹⁵. These studies indicate the importance of endothelium-derived vasodilators such as NOS-derived nitric oxide (NO), COX-derived prostaglandins (PGs) and endothelium-derived hyperpolarizing factor (EDHF) in renal integrity. Because renal endothelial dilatory function also displays a large interindividual variability we hypothesized that interindividual differences in renal endothelial dilatory function might be involved in individual susceptibility to renal damage.

The present study employed the 5/6 Nx rat model of renal damage to address this item. The main objective was to test whether individual renal endothelial dilatory function of the healthy rat predicts the subsequent renal damage induced by 5/6 Nx. Renal damage was assessed as the increase in proteinuria and deterioration in renal function (GFR) after 5/6 Nx. To this end, acetylcholine-induced endothelium-dependent relaxation was assessed in isolated small renal arteries at the time of 5/6 Nx in individual rats, and this was related, respectively, to urinary protein levels up to 5 weeks and GFR at 6 weeks after 5/6 Nx. Furthermore, because endothelial dilatory function involves several different dilative mediators, it appears of significant interest to also explore which underlying endothelial dilative mediators may be involved. The secondary objective therefore, was to assess the contribution of NO, PGs and EDHF in individual rats. To this end, dilatory responses to acetylcholine were additionally studied in presence of pharmacological inhibitors of the above dilator pathways.

Methods

Animals, surgery and in vivo measurements

Male Wistar rats (250 - 275 g, n=23) were obtained from Harlan (Zeist, The Netherlands) and housed under standard conditions at the animal facilities of the University of Groningen. Animals had free access to food (synthetic diet containing 0.3% sodium; Hope Farms, The Netherlands) and drinking water throughout the study. By laparotomy and under anesthesia with isoflurane 3% in N₂O/O₂ (1:2), rats underwent 5/6 nephrectomy (5/6 Nx) 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 2/3 of this kidney. The removed right kidney was put into cold Krebs solution and one small renal (interlobar) artery per kidney was immediately

prepared for assessment of vasomotor function. Postoperatively, rats received a subcutaneous injection of 1:10 diluted buprenorphin (Temgesic[®]) for analgetic purposes and were allowed to recover from surgery. Urinary protein excretion (Biuret assay) was determined weekly up to 5 weeks thereafter by placing the rats in metabolic cages for 24 h. Systolic blood pressure (SBP) was also measured weekly in awake animals by means of the tail-cuff method (IITC Inc, USA). At 5 weeks after 5/6 Nx, rats were instrumented with a jugular and carotid catheter under isoflurane anesthesia. Rats were allowed to recover for one week and measurement of glomerular filtration rate (GFR) and effective renal plasma flow (ERPF) was performed according to the continuous infusion method in conscious, freely moving and spontaneously voiding rats, as described previously¹⁶. Briefly, rats were infused with ¹²⁵I-iothalamate and ¹³¹I-hippuran and standard GFR was corrected for inaccurate urine collection by the method described by Donker et al. and Apperloo et al. in man, and adapted for rat studies by de Vries et al.¹⁶⁻¹⁸. Filtration fraction (FF) was calculated as the quotient of GFR and ERPF. GFR and ERPF could be successfully determined in 17 rats. Drop outs were due to technical failures such as occlusion of catheters. All animal experimentation was conducted in accord with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals.

In vitro perfusion set-up

Small renal (interlobar) arteries with an intraluminal diameter of $277 \pm 6 \mu\text{m}$ ($n=23$) were transferred to an arteriograph system for pressurized arteries¹⁹ (Living System Instrumentation, Burlington, VT, USA). Artery segments were cannulated at both ends on glass micropipettes, secured, and the lumen of the vessel was filled with Krebs solution through the micropipettes. Intraluminal pressure was set to 70 mmHg and held constant (blind sac) 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. The vessel chamber was transferred to the stage of an inverted light microscope with a video camera attached to a viewing tube. The video dimension analyzer (Living System Instrumentation, Burlington, VT, USA) was used to analyze the signal obtained from the video image and to continuously register lumen diameter.

ACh-induced relaxation

Arteries were allowed to equilibrate for one hour in regular Krebs solution before being pre-constricted with phenylephrine (PE) (3×10^{-7} - 10^{-6} mol/L) by 45-50% for subsequent relaxation studies. Preconstricted vessels were studied for endothelium-dependent relaxation by giving cumulative doses of acetylcholine (ACh; 10^{-8} mol/L - 10^{-4} mol/L) to the recirculating bath. In each individual rat ($n=23$) a full concentration-response curve to ACh was obtained for one small renal artery of the removed right kidney. In preliminary experiments we established that the endothelial dilatory function, as measured in the current study, may be regarded as representative for the investigated kidney as we demonstrated that ACh-induced relaxation a) did not differ between renal arteries of different size within the used size range of 230 μm – 310 μm within one kidney, i.e. ACh-response size (area under curve in arbitrary units) did not depend on (did not show any correlation with) artery diameter (μm) ($n=6$ arteries), and b) did not differ between renal arteries of different kidney areas within one kidney, i.e. branches of the superior and inferior segmental artery ($n=4$ arteries), and c) did not differ between (subsequent) vessel preparations ($n=3$ arteries).

After determination of full ACh concentration-response curves and a washing and equilibration time of 20 minutes, 12 of the 23 arteries were used for subsequent investigation

of the three underlying endothelial dilative mediators. The other 11 arteries were subsequently investigated for contractile and dilatory properties using PE and sodium nitroprusside (SNP).

Inhibition of the PG, NO and EDHF pathway in ACh-induced relaxation

To determine the contribution of vasoactive prostaglandins (PGs), nitric oxide (NO), and endothelium-derived hyperpolarizing factor (EDHF) to endothelium-dependent relaxation, the response to ACh was additionally studied in presence of various inhibitors added to the bath 20 minutes prior to addition of ACh. To this end, indomethacin (10^{-5} mol/L), given to the superfusion medium, was used to inhibit prostaglandin production. N^o-monomethyl-L-arginine (L-NMMA, 10^{-4} mol/L), given to the superfusion medium in presence of indomethacin, was used to inhibit NO production, and a combination of charybdotoxin (chtx, 10^{-7} mol/L) and apamin (apa, 5×10^{-7} mol/L), applied into the lumen of the artery as well as to the superfusion medium in presence of indomethacin and L-NMMA, was used to inhibit EDHF^{20,21}. The exact nature of EDHF has not yet been established - meaning that specific inhibitors are not yet available. Nevertheless, the inhibition of calcium-dependent potassium channels with the combination of charybdotoxin and apamin has consistently been shown to inhibit the L-NMMA- and indomethacin-resistant relaxation and hyperpolarization which is believed to be mediated by EDHF²¹.

It is important to mention that the way in which the endothelial mediators are determined may be critical as they may not be independent but may interact. In this context NO has been described to attenuate EDHF (release)^{22,23} and thus, EDHF may be fully active only when NO is inhibited or decreased. Furthermore, NO itself may in part mediate its vasodilatory effect via opening of potassium channels and hyperpolarization (similar to the mechanism of EDHF)²⁴. Therefore, if potassium channel blockers are used alone to determine EDHF not only EDHF-mediated relaxation but also a part of the NO-mediated relaxation may be measured at the same time. Because of that we determined the contribution of EDHF always in presence of NO inhibition. A total amount of 11 rats/arteries was successfully investigated for contribution of all underlying endothelial dilative mediators.

SNP-induced relaxation and PE-induced constriction

Concentration-response curves to sodium-nitroprusside (SNP, 10^{-9} - 10^{-3} mol/L) were performed to account for dilatory ability of arterial smooth muscle to NO. Concentration-response curves to phenylephrine (PE, 10^{-8} - 10^{-5} mol/L) were performed to control for a potential relation between contractile ability of individual arteries to PE and development of renal damage after 5/6 nephrectomy. A total amount of 10 rats/arteries was successfully studied for PE and SNP response curves.

Solutions and drugs

Vessel segments were superfused with Krebs solution containing (in mM): 120.4 NaCl, 5.9 KCl, 2.5 CaCl₂, 1.2 MgSO₄, 25.0 NaHCO₃, 1.2 NaH₂PO₄, 11.5 glucose (Merck, Darmstadt, Germany). Acetylcholine, apamin, charybdotoxin, indomethacin, L-NMMA, phenylephrine and sodium nitroprusside, were obtained from Sigma-Aldrich Chemie B.V., The Netherlands. They were dissolved in de-ionized water and diluted with Krebs solution. Stock solution (10^{-2} mol/L) for indomethacin was prepared in 96% ethanol.

Data analysis

Data are expressed as mean \pm standard error of the mean (SEM). Concentration-response curves to acetylcholine (ACh) and maximal relaxation (E_{max}) were expressed in percentage

of precontraction to phenylephrine (PE). The concentration of drugs causing half-maximal responses (EC_{50} values) were expressed as negative logarithm of the molar concentration (pD_2 values). The Area Under each individual Curve (AUC) was determined (Sigma Plot, Jandell Scientific) and expressed in arbitrary units. The AUC was used to present total (individual) ACh-relaxation, and for subsequent analysis of differences in ACh-relaxation with and without indomethacin, L-NMMA and chtx/apa to estimate the contribution of PG, NO and EDHF, respectively²⁵. Statistical differences for E_{max} , pD_2 , AUC values and for whole ACh-response curves were determined by student's independent t-test and ANOVA for repeated measures, respectively. Significance was accepted at $P < 0.05$. The relationship between *in vitro* and *in vivo* data was calculated using regression analysis (SPSS).

Results

Blood pressure, proteinuria and renal function

After 5/6 Nx proteinuria gradually increased with time reaching a mean-value of 84 ± 14 mg/24h at week 5, individual values ranging from 22 to 278 mg/24h ($n=23$). In contrast, systolic blood pressure (SBP) did not increase with time and had a mean-value of 138 ± 5 mmHg at week 5 ($n=23$). GFR was determined at 6 weeks after 5/6 Nx with a mean-value of 0.55 ± 0.07 ml/min/100g, ranging from 0.17 to 1.13 ml/min/100g ($n=17$). ERPF showed a mean-value of 1.7 ± 0.2 ml/min/100g ($n=17$) and FF was 0.31 ± 0.02 ($n=17$). Control values for GFR, ERPF and FF were determined concomitantly in a separate group of normal healthy control rats (1.14 ± 0.04 ml/min/100g, 2.9 ± 0.2 ml/min/100g and 0.41 ± 0.04 , respectively, $n=5$ for each).

Baseline endothelial dilatory function and development of proteinuria and renal function loss after 5/6 Nx

Administration of ACh dilated small renal arteries of individual rats at the time of 5/6 Nx to a variable extent. On average ($n=23$) E_{max} was 73 ± 3 % and pD_2 was 6.5 ± 0.1 , corresponding with a mean AUC of 179 ± 10 . Regression analysis between individual ACh-relaxation (expressed as AUC in arbitrary units) and individual development of proteinuria and GFR after 5/6 Nx was performed. This analysis revealed that ACh-relaxation was inversely related to the individual extent of proteinuria development from baseline to 5 weeks after 5/6 Nx ($r = -0.72$, $P = 0.001$, $n = 23$) as well as to individual proteinuria levels measured 5 weeks after 5/6 Nx ($r = -0.54$, $P = 0.008$, $n = 23$, *Figure 1A*). Furthermore, individual ACh-relaxation was positively related to GFR 6 weeks after 5/6 Nx ($r = 0.58$, $P = 0.016$, $n = 17$, *Figure 1B*), *i.e.* rats with more pronounced ACh-relaxation developed less proteinuria and showed higher GFR after 5/6 Nx. The relation between individual ACh-relaxation and ERPF was not significant ($r = 0.45$, $P = 0.08$, $n = 17$) whereas, individual ACh-relaxation was positively related to individual FF 6 weeks after 5/6 Nx ($r = 0.61$, $P = 0.009$, *Figure 1C*). Individual ACh-relaxation was not correlated with individual SBP levels 5 weeks after 5/6 Nx.

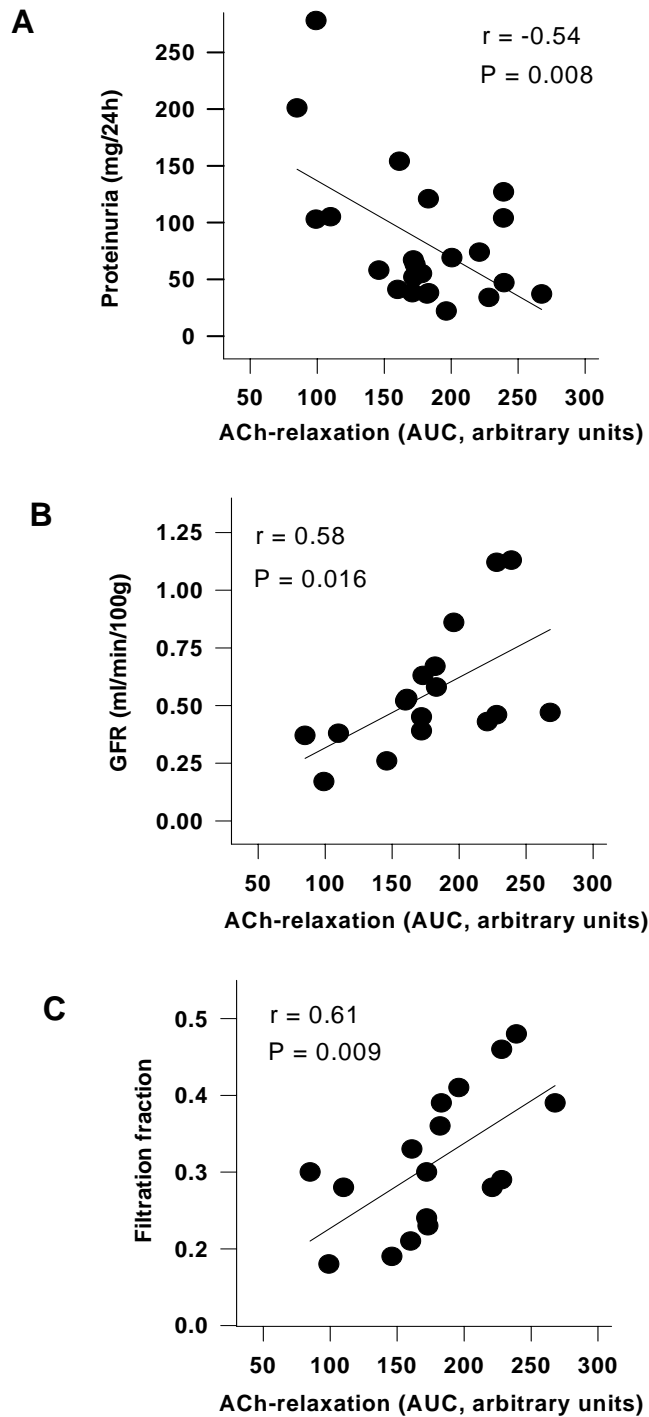


Figure 1 **A)** Relation between individual acetylcholine (ACh)-induced relaxation of small renal arteries at the time of 5/6 Nx operation and proteinuria (mg/24h) 5 weeks after 5/6 Nx (n=23). **B)** Relation between individual acetylcholine (ACh)-induced relaxation of small renal arteries at the time of 5/6 Nx operation and GFR (ml/min/100g) 6 weeks after 5/6 Nx (n=17). **C)** Relation between individual acetylcholine (ACh)-induced relaxation of small renal arteries at the time of 5/6 Nx operation and FF 6 weeks after 5/6 Nx (n=17).

Involvement of different endothelial mediators

Combined data on the three underlying endothelial vasodilator pathways together with total ACh-relaxation were successfully obtained in n=11 individual rats. Curve characteristics in absence and presence of inhibitors are given in *Table 1*.

Figure 2A shows the response to ACh in absence of inhibitors (control condition). Administration of indomethacin (10^{-5} mol/L) to inhibit PGs variably resulted in small increases or decreases in ACh-relaxation in individual rats. The effect on the group as a whole however was not significant (*Figure 2A, Table 1*). Additional administration of L-NMMA (10^{-4} mol/L) to inhibit NO consistently decreased ACh-relaxation but with a high interindividual variability. Overall, maximal ACh-relaxation was significantly decreased (*Figure 2A, Table 1*), demonstrating the significant contribution of NO in mediating ACh-relaxation in this vessel type. Subsequent additional inhibition of EDHF with charybdotoxin (chtx, 10^{-7} mol/L) and apamin (apa, 5×10^{-7} mol/L) nearly fully abolished the remaining ACh-relaxation (*Figure 2A, Table 1*). This indicates a major importance of EDHF in mediating ACh-relaxation in these arteries which is in line with previous studies²¹.

Correlation analysis was performed between individual contribution of different endothelial mediators and the level of proteinuria at 5 weeks after 5/6 Nx. This analysis revealed that individual PG contribution was inversely related to proteinuria 5 weeks after 5/6 Nx ($r=-0.74$, $P=0.01$, $n=11$, *Figure 2B*). Interestingly, this relation was based on the fact that in some rats the effect of PG inhibition with indomethacin was constrictive (indicating that the net effect of stimulated PGs in these arteries was dilative) and in other rats the effect of PG inhibition was dilative (indicating that the net effect of stimulated PGs was constrictive). Hence, rats with a predominance of dilative PGs developed less proteinuria compared to rats with a predominance of constrictive PGs, while the overall effect of PG inhibition on the population as a whole was minimal. Not only PG contribution, but also individual NO contribution was inversely related to proteinuria 5 weeks after 5/6 Nx ($r=-0.86$, $P=0.001$, $n=11$, *Figure 2C*), i.e. rats with more pronounced NO contribution displayed less proteinuria after 5/6 Nx. In contrast, individual EDHF contribution was positively related to development of proteinuria ($r=0.70$, $P=0.02$, $n=11$, *Figure 2D*).

SNP-induced relaxation and PE-induced contraction

Endothelium-independent relaxation to sodium nitroprusside (SNP) relaxed arteries to $97 \pm 2\%$ (E_{max}) with a pD_2 value of 6.4 ± 0.2 and an AUC of 261 ± 12 ($n=10$). Individual values obtained before induction of renal damage were not related to development of proteinuria after 5/6 Nx (data not shown), suggesting that the differences in ACh-relaxation were not due to alterations at the level of smooth muscle response to NO. Similarly, individual values for PE-induced contraction obtained before induction of renal damage were not related to development of proteinuria ($n=10$).

Table 1 Curve characteristics of ACh-induced relaxation and effect of inhibitors

n=11	E _{max} ^a	pD ₂ ^b	AUC ^c
control	83±3%	6.5±0.1	211±10
+ indomethacin	86±7%	6.2±0.2	191±19
+ indomethacin + L-NMMA	69±8% ^{d,e}	5.9±0.2 ^d	142±23 ^d
+ indomethacin + L-NMMA + chtx + apa ^g	3±1% ^{d,e,f}	-	4.7±1.0 ^{d,e,f}

^a E_{max} is the maximal relaxation to acetylcholine (ACh) in % of precontraction to phenylephrine.

^b pD₂ is the negative logarithm of the molar concentration of ACh causing half maximal responses (EC₅₀).

^c AUC is the area under the concentration-response curve expressed in arbitrary units.

^d P<0.05 versus control; ^e P<0.05 versus indomethacin; ^f P<0.05 versus indomethacin+L-NMMA.

^g chtx + apa, charybdotoxin + apamin.

Discussion

In the current study we found that renal endothelial dilatory function in the healthy rat predicts renal damage induced by 5/6 nephrectomy (5/6 Nx). The predictive value was determined by the different dilative mediators of the endothelium (NO, PG and EDHF) and not by differences in smooth muscle responses.

The 5/6 nephrectomy model

In the 5/6 Nx model, progressive renal damage and function loss of the initially normal remnant nephrons is described to develop with time^{9,26}. The initial reduction in nephron number progressively damages the remaining ones which suffer the consequences of (RAAS-mediated) adaptive increases in glomerular pressure and hyperfiltration leading to progressive proteinuria and decreases in GFR and ERPF which is in accordance with our present data. Our results show that the development of renal damage in this model of 5/6 Nx is largely variable between individuals as demonstrated previously^{6,9}. The occurrence of systemic hypertension in this model is described controversial and seems to depend on the renal ablation procedure, the used rat strain and sodium intake²⁶⁻²⁸. In our study, SBP did not increase over time indicating that blood pressure may not have had impact on the development of renal damage.

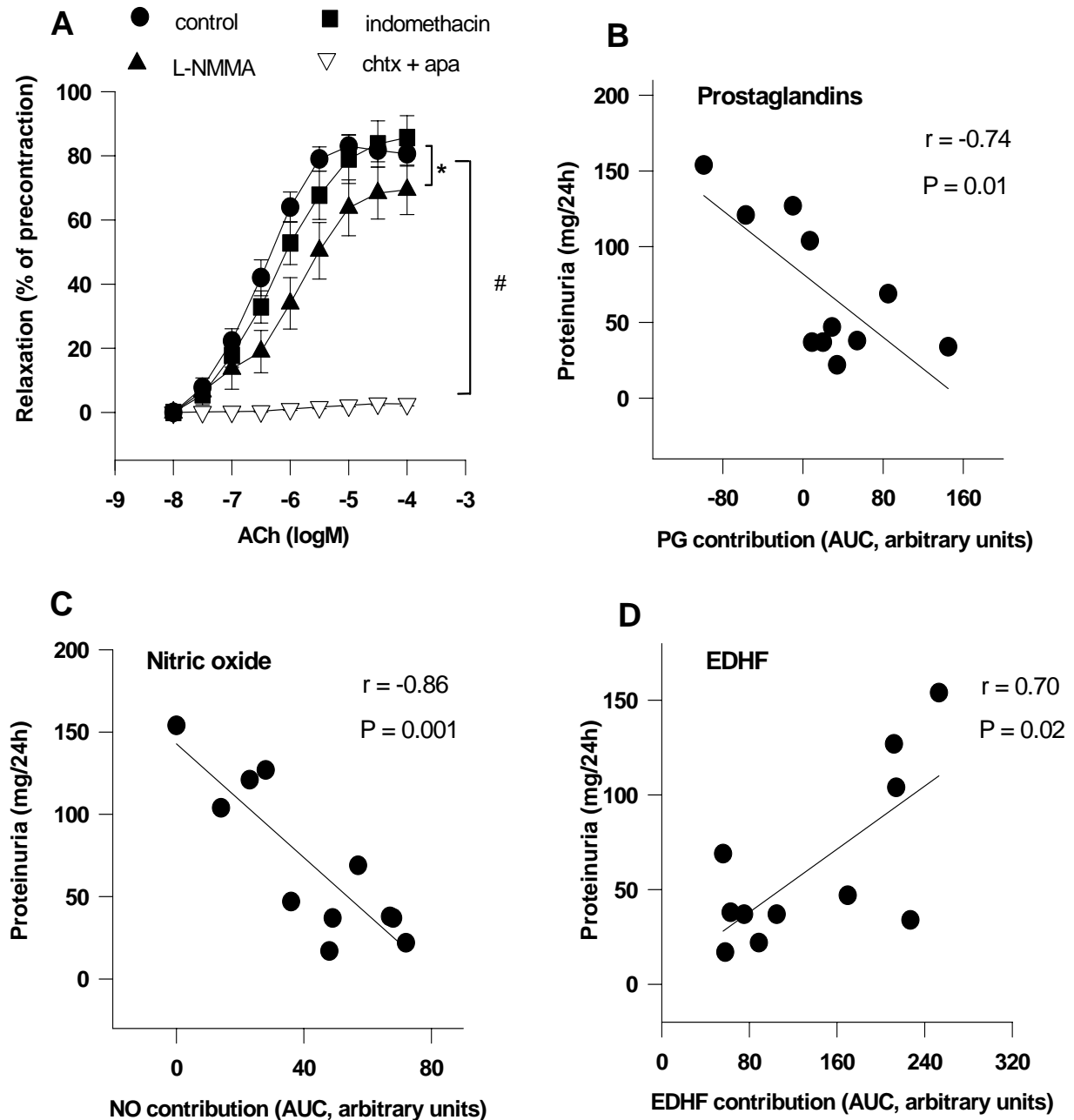


Figure 2 A) Concentration-response curves to acetylcholine (ACh) in small renal arteries (n=11) at the time of 5/6 Nx operation in absence of any inhibitor (control), in presence of indomethacin (10^{-5} mol/L), in additional presence of L-NMMA (10^{-4} mol/L) and in additional presence of charybdotoxin (chtx, 10^{-7} mol/L) and apamin (apa, 5×10^{-7} mol/L). * $P < 0.05$ for L-NMMA versus control and indomethacin; # $P < 0.05$ for chtx+apa versus control, indomethacin and L-NMMA. Relation between individual contribution of B) prostaglandins (PG), C) NO and D) EDHF to acetylcholine (ACh)-induced relaxation at the time of 5/6 Nx operation and proteinuria (mg/24h) 5 weeks after 5/6 Nx (n=11 for each).

Individual endothelial dilatory function predicts development of renal damage after 5/6 Nx

We found large interindividual variations in endothelium-dependent ACh-induced dilatory function of small renal arteries. Regression analysis revealed that individual ACh-relaxation measured before induction of 5/6 Nx predicted the development of renal damage and renal function loss, measured as proteinuria and GFR, after 5/6 Nx. In contrast, endothelium-independent relaxation to SNP and contractility to PE were neither related to proteinuria nor to GFR, demonstrating that the predictive value may be due to endothelium-dependent mechanisms rather than to differences in smooth muscle properties.

Our results may be in line with the concept that an intact endothelial dilatory function may be a powerful defense mechanism against progressive renal damage^{10,11,29-32}. In the 5/6 Nx model strong increases in glomerular pressure and hyperfiltration of the remnant nephrons are believed to largely contribute to the development of renal damage^{33,34} and in this respect it may be hypothesized that an intact vasomotor regulation with intact endothelial dilatory ability may protect the kidney from deleterious pressure effects after 5/6 Nx. However, whether endothelial dilatory function of small renal arteries exerts a direct protective effect on the development of renal damage in this model can not be determined from the present study. Alternatively, endothelial dilator function, as determined in the present study, may be a reflection of other protective properties of the endothelium. In this respect it may be interesting to know whether the predictability of renal damage by endothelial dilatory function may also be valid in other models of renal disease or if it may be specific to the hemodynamic component of renal disease progression in this 5/6 Nx model.

Role of nitric oxide, prostaglandins and EDHF in the development of renal damage

We found that individual NO contribution to ACh-relaxation was inversely related to development of proteinuria, *i.e.* rats with more pronounced NO contribution developed less proteinuria after 5/6 Nx. That NO may exert renoprotective functions is supported by a large amount of studies. NO is not only an important counteractor of constrictive mediators but also prevents leucocyte adhesion and mesangial cell hypertrophy/hyperplasia and may inhibit renal renin release. Genetic differences in NOS-activity are believed to account for different rates of occurrence of renal injury^{35,36}. A direct protective role of NO on renal hemodynamics is supported by the deleterious effects of NOS-inhibition. Independent of systemic blood pressure effects NOS-inhibition leads to increased intraglomerular pressure which may partly be due to a greater increase in efferent compared to afferent arteriolar resistance^{12,37-42}. In accordance with our data, it may be suggested that an intact NO-activity may protect the kidney from maladaptive effects such as increased glomerular resistance and glomerular hyperfiltration after 5/6 Nx. In the current study we have determined NO contribution in renal (interlobar) arteries which may not be directly involved in determining glomerular resistance. In this respect it is important to mention that (larger) renal arteries are believed to be a major source of renal vascular NO production which, transported by blood flow, may act on downstream arterioles to regulate renal vascular resistance³⁰.

Apart from NO contribution also individual PG contribution, measured at the time of 5/6 Nx was inversely related to development of renal damage after 5/6 Nx, *i.e.* rats with a predominant influence of dilative PGs developed less proteinuria compared to rats with a predominant influence of constrictive PGs. Inhibition of PGs with indomethacin had only a slight but not significant effect on ACh-relaxation in the group on average, as it has been described before²¹. Our study for the first time shows that the lack of effect of indomethacin in this artery type in the group on average may be explained by the fact that the response was

dilative in some individual rats as well as constrictive in other rats, minimizing the net effect. PGs (PGE₂, F_{2a}, I₂, D₂, TXA₂) with both vasodilative and vasoconstrictive actions are believed to play an important role in glomerular hemodynamics^{43,44} especially in “PG-dependent states” in which dilative PGs may counteract (Ang II-mediated) constriction. In accordance with our data it may be suggested that an individual PG balance with predominant dilative PG effects may protect the kidney from maladaptive hemodynamic effects after 5/6 Nx.

Apart from NO and PGs another endothelium-derived factor contributes to endothelium-dependent dilation, called EDHF, which mediates its effect via hyperpolarization of underlying smooth muscle cells. The exact nature of EDHF has not yet been elucidated⁴⁵ and the role of EDHF in the kidney is largely unknown. Recently, several studies suggested that EDHF may act as a dilative back-up system which may be upregulated under conditions when NO is decreased⁴⁶⁻⁴⁸. In the current study individual EDHF contribution, in contrast to NO contribution, was *positively* related to development of proteinuria. This may be best explained by its putative function as a dilative back-up mediator, *i.e.* in rats with low NO-activity EDHF may partly have compensated this NO-deficiency. This may also explain why the individual NO-activity is even stronger related than individual total ACh-relaxation because total ACh-relaxation may have partly been restored by (increased) EDHF.

In this study, we demonstrated for the first time that the degree of renal damage in this model can be predicted by testing endothelial dilatory function of small renal arteries prior to induction of renal damage. It provides a method to identify those individuals out of a group of normal rats with high individual risk to develop renal damage by a relatively easy measurement, *i.e.* the determination of renal ACh-induced relaxation. Furthermore, our study provides direction on underlying mechanisms of individual susceptibility to renal damage, namely individual NO-activity and the individual balance between constrictive and dilative PGs. Further research in animals, and especially in patients, may not only be crucial to explore exact underlying mechanisms of individual susceptibility to renal damage but may also provide new directions for the development of individual risk-assessment and for therapeutic risk management in the clinical setting such as renal transplantation.

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