Increased Availability of Nitric Oxide Leads to Enhanced Nitric Oxide Dependency of Tubuloglomerular Feedback in the Contralateral Kidney of Rats With 2-Kidney, 1-Clip Goldblatt Hypertension

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Abstract—The contralateral kidney of 2-kidney, 1-clip hypertensive (2K1C) rats is unable to escape the renal vasoconstrictive and sodium-retaining effects of increased circulating angiotensin II levels. Evidence is accumulating that renal function is relatively preserved by enhanced influence of NO in the contralateral kidney. In this study, we investigated (1) whether the high NO dependency of renal hemodynamics in the contralateral kidney is due to increased availability of NO or increased sensitivity to NO and (2) whether elevated NO activity dampens the actions of angiotensin II to enhance tubuloglomerular feedback (TGF) responses in the nonclipped kidney of 2K1C rats. To estimate whether the available NO is increased, the NO clamp technique was applied in rats that underwent sham operation (n=6) and in the contralateral kidney of 2K1C Sprague-Dawley rats (3 weeks old; 0.25-mm silver clip; n=6). During systemic infusion of nitro-L-arginine (L-NNA; 50 μg/kg · min⁻¹), sodium nitroprusside (SNP) was infused in the renal artery and the rate was adjusted so that renal vascular resistance (RVR) was restored to baseline levels. In sham rats, RVR increased during L-NNA treatment from 17.2±2.0 to 33.0±3.6 U (P<0.01) and was restored to baseline values during SNP infusion (17.1±2.3 U); 9.2±1.8 nmol/min of SNP was needed to restore RVR to baseline values. In 2K1C rats, RVR increased during L-NNA treatment from 16.7±1.1 to 53.4±3.5 U (P<0.01). This increase of RVR was significantly larger than in sham rats. RVR was restored to baseline values during SNP infusion (17.4±0.9 U); 26.0±4.3 nmol/min of SNP was needed to restore RVR to baseline values (P<0.05 versus sham). Furthermore, maximum TGF responses were assessed before and during late proximal tubular infusion of L-NNA in the kidneys of sham rats and the nonclipped kidneys of 2K1C rats. Control maximum TGF responses were 4.7±0.7 and 5.1±0.4 mm Hg in sham and 2K1C rats, respectively. During intraluminal L-NNA infusion, maximum TGF responses were 15.4±0.9 mm Hg in sham rats and 22.2±2.5 mm Hg in 2K1C rats (P<0.05 versus sham). Finally, urinary NO₂⁻+NO₃⁻ excretion in the nonclipped kidney was significantly higher than in the clipped kidney (P<0.05). In conclusion, (1) as assessed using the NO clamp, ambient intrarenal NO levels are increased in the contralateral kidney of 2K1C rats and (2) the NO dependency of the TGF system is enhanced. These experiments indicate that adaptations in NO activity lead to relatively low TGF responsiveness, which will offset the simultaneous sodium-retaining actions of angiotensin II on proximal tubular reabsorption and TGF responsiveness. (Hypertension. 1999;34:679-684.)

Key Words: hypertension, renal hemodynamics, nitric oxide, tubuloglomerular feedback

In 2-kidney, 1-clip (2K1C) Goldblatt hypertension, the nonclipped kidney is unable to escape the sodium-retaining effects of elevated circulating angiotensin (Ang) II produced by the stenosed kidney. Ang II has been shown to directly stimulate proximal tubular reabsorption and to enhance tubuloglomerular feedback (TGF) responsiveness. If the increase in proximal tubular reabsorption did not coincide with enhanced TGF responsiveness, the resulting decrease in distal delivery would lead to a TGF-mediated increase in single-nephron glomerular filtration rate, which would offset the increase in proximal reabsorption. Studies examining the TGF system in Goldblatt hypertension have not been able to consistently show enhanced TGF responsiveness, as is expected from the increased circulating Ang II levels.

An explanation for the failure to demonstrate enhanced TGF responses is that either the increase in circulating Ang II levels or the increase in systemic arterial pressure will evoke counteracting factors that will normalize TGF responsiveness. It is now clear that NO strongly depresses TGF responses and is probably released with increases in distal delivery. We have demonstrated that the actions of Ang II on TGF responsiveness are strongly enhanced during decreased
local NO activity. Furthermore, it has been demonstrated that renal blood flow (RBF) and renal autoregulation in the contralateral kidney in 2K1C rats are highly influenced by NO. Enhanced nitrotyrosine staining has been demonstrated in the cortical vascular structure of 2K1C rats, indicating enhanced NO activity. Therefore, the hypothesis of this study was that enhanced NO activity in the contralateral kidney of the 2K1C rat counteracts the actions of Ang II to enhance TGF responsiveness. In this scheme, blockade of the local, attenuating effects of NO on the TGF system will enhance TGF responsiveness. In this scheme, blockade of the local, attenuating effects of NO on the TGF system will enhance TGF responsiveness.

First, we investigated whether the high NO dependency of the contralateral kidney is due to increased availability of NO or increased sensitivity of the vasculature to NO. The delivery rate of sodium nitroprusside (SNP) necessary to restore renal vascular resistance (RVR) to control levels during systemic NO blockade using nitro-L-arginine (L-NNA) was used to estimate functional NO levels in the nonclipped kidney of 3-week-old 2K1C rats. Second, we assessed whether enhanced NO activity explains the normal TGF responses in the nonclipped kidney of 2K1C rats. Therefore, maximum TGF responses were assessed before and during intraluminal administration of L-NNA. Rats that underwent sham operation served as controls. The study was performed in rats 3 weeks after clipping or sham operation, because in this developmental phase of hypertension, alterations in renal hemodynamics will reflect functional rather than morphological changes.

**Methods**

**Animals and Clipping Procedure**

Male Sprague-Dawley rats (200 to 350 g; Harlan-Olac, Bicester, UK) received normal rat chow (Hope Farms) and had free access to tap water. Sentinel animals were monitored regularly for infection by nematodes and pathogenic bacteria, as well as antibodies for a large number of rodent viral pathogens, and were consistently free of infection throughout the course of the experiments. The Utrecht University Board for Studies in Experimental Animals approved the study protocol. Three weeks before acute experiments, rats were placed on a servo-controlled surgical table that maintained rectal temperature at 37°C. After an intravenous infusion of a 150-mmol/L NaCl solution containing 6% BSA (Sigma Chemical Company) at a rate of 10 μL/min · 100 g body weight. An intrarenal infusion of a 150-mmol/L NaCl solution was maintained throughout the experiment at a rate of 10 μL/min. The infusions were started after a blood sample was drawn from the femoral artery during surgery. After surgery, the infusion was switched to a 150-mmol/L NaCl solution with 1% BSA at the same infusion rate. This infusion was maintained throughout the experiment. Experimental compounds were added to this standard solution. A 60-minute equilibration period was observed before measurements were made. At the end of each experiment, the kidneys were removed, blotted dry, and weighed.

**Assessment of TGF Responses**

Stop-flow pressure (SFP) responses to the maximum increase in late proximal perfusion rate were obtained as described previously. In brief, a proximal tubule with several surface segments was localized by use of a micropipette with a 4- to 6-μm-diameter tip containing artificial tubular fluid (ATF; for composition, see Turkstra et al) stained with 0.2% Fast Green (Sigma). A wax block was inserted into an early proximal tubular segment. SFP was measured with a pressure pipette with a 3- to 4-μm-diameter tip that was filled with 2 mol/L NaCl and connected to a continuous recording servo-null pressure system (model 5a, Instruments for Physiology and Medicine). A perfusion pipette with a 5- to 7-μm-diameter tip, filled with stained (0.2% Fast Green) isotonic ATF, was introduced into a late proximal tubular segment and connected to a microperfusion pump system (Effenberger, Phaffing/Attel). Maximum TGF-mediated decreases in SFP were obtained by switching late proximal perfusion rates from 0 to 40 mL/min, after which recovery of zero-flow SFP was measured.

**NO Clamp Protocol**

To estimate available NO, an intrarenal NO clamp was established in sham and 2K1C animals. This method assumes that the amount of the NO donor SNP needed to restore RVR during administration of L-NNA correlates with the amount of NO present before administration of L-NNA. First, during a 30-minute control period, mean arterial pressure (MAP) and RBF were continuously measured, and RVR under control situations was calculated with the use of a computer program. After the control measurements were performed, an intravenous infusion of L-NNA (50 μg/kg · min−1) was started. In pilot experiments, it was determined that this dose results in maximal responses in MAP and RBF in both sham and 2K1C rats. When MAP and RBF had stabilized, an intrarenal SNP infusion was started. The rate of infusion was adjusted to decrease RVR to baseline levels, thereby changing the intrarenal perfusion between 8 and 30 μL/min. Again, the current RVR was calculated with a computer program.

**TGF Protocol**

To assess the influence of local NO synthesis inhibition in sham and 2K1C rats on maximum TGF responses, the following approach was used. First, a maximum TGF-mediated decrease in SFP was assessed by late proximal perfusion with ATF at a rate of 40 mL/min. Then, the perfusion pipette was replaced by a pipette containing ATF with 1 mmol/L L-NNA, and a late proximal perfusion was started and continued until SFP had stabilized for at least 2 minutes. On average, perfusion with L-NNA lasted 8 to 10 minutes before a plateau was reached. For time-control experiments, the following procedure was applied in different tubules. First, a control response was obtained by late proximal perfusion with ATF at 40 mL/min. Then, a second response with ATF without L-NNA was obtained during 10 minutes of perfusion at 40 mL/min. In the TGF experiments, at least 2 plasma samples were obtained to measure colloid osmotic pressure and 2 to 3 free-flow proximal tubular pressure measurements were obtained.

**Analyses and Statistics**

Total urinary NOx excretion (sum of urinary nitrate and nitrite) was measured after complete conversion of nitrate to nitrite by nitrate

All animals received an intravenous infusion of a 150-mmol/L NaCl solution containing 6% BSA (Sigma Chemical Company) at a rate of 10 μL/min · 100 g body weight. An intrarenal infusion of a 150-mmol/L NaCl solution was maintained throughout the experiment at a rate of 10 μL/min. The infusions were started after a blood sample was drawn from the femoral artery during surgery. After surgery, the infusion was switched to a 150-mmol/L NaCl solution with 1% BSA at the same infusion rate. This infusion was maintained throughout the experiment. Experimental compounds were added to this standard solution. A 60-minute equilibration period was observed before measurements were made. At the end of each experiment, the kidneys were removed, blotted dry, and weighed.
reductase. Total nitrite (representing reduced nitrate and endogenous nitrite) was analyzed colorimetrically by use of the Griess reaction (formation of a purple diazo dye by reaction of nitrite with sulfanilamide and N-naphthylethlenediamine) with a nitrate/nitrite assay kit (Cayan). The within- and between-assay variation coefficients are 4% and 7%, respectively. RVR was calculated as MAP divided by RBF and is expressed in millimeters of mercury per milliliter per minute per gram of kidney weight (KW) (hereafter referred to as units [U]). Glomerular capillary pressure (P_{GC}) under stop-flow conditions was calculated by the equation \( P_{GC}=SFP_{0}+\pi_{k} \), where \( \pi_{k} \) is the colloid osmotic pressure in femoral artery plasma samples, which is presumed to equal afferent arteriolar colloid osmotic pressure. Colloid osmotic pressure in arterial samples was measured with a strain gauge microcoulter. The glomerular transcapillary pressure gradient (\( \Delta P \)) was calculated from the equation \( \Delta P=P_{GC}-P_{t} \), where \( P_{t} \) is proximal tubular pressure. The fraction of \( \Delta P \) controlled by TGF was the maximum TGF-mediated decrease in RBF and is expressed in millimeters of mercury per gram of kidney weight (KW) (hereafter referred as to units [U]).

Results

NO Clamp Protocol

Findings of the NO clamp protocol are summarized in Figure 1. In sham rats, NO synthase (NOS) inhibition with l-NNA infusion decreased RBF from 6.6±0.5 to 4.6±0.5 mL/min·g^{-1}KW (P<0.01). Subsequent intrarenal SNP infusion increased RBF to 6.1±0.3 mL/min·g^{-1}KW. l-NNA infusion increased MAP from 123±2 to 161±5 mm Hg (P<0.01), and subsequent intrarenal SNP infusion reduced MAP to 112±4 mm Hg (P<0.05 versus baseline). In these rats, RVR increased from 17.2±2.0 to 33.0±3.6 U during l-NNA infusion (P<0.01). Subsequent intrarenal infusion of SNP restored RVR to 17.1±2.3 U (not different from baseline). An SNP delivery rate of 9.2±1.8 nmol/min was used to restore RVR in sham rats.

2K1C rats had a significantly higher MAP than sham rats (141±3 mm Hg; P<0.05). In the contralateral kidney of 2K1C rats, l-NNA infusion was followed by a decrease in RBF from 9.5±0.7 to 4.3±0.3 mL/min·g^{-1}KW (P<0.01). The change in RBF was significantly higher in these rats than in sham rats (-5.0±0.5 versus -2.1±0.2 mL/min·g^{-1}KW, respectively; P<0.05). Subsequent intrarenal SNP infusion increased RBF to 7.2±0.5 mL/min·g^{-1}KW. l-NNA infusion increased MAP from 141±3 to 187±5 mm Hg (P<0.01 versus baseline and P<0.05 versus sham). The increase in MAP was not different from that in sham rats. Subsequent intrarenal SNP infusion reduced MAP to 103±5 mm Hg (P<0.05 versus baseline). l-NNA infusion increased RVR from 16.7±1.1 to 53.4±3.5 U (P<0.01 versus baseline). The increase in RVR in response to l-NNA in 2K1C rats exceeded the values measured in sham rats (36.6±3.4 versus 18.7±4.0 U, respectively; P<0.01). Superimposed intrarenal infusion of SNP restored the RVR to 17.4±0.9 U (not different from baseline). Remarkably, the delivery rate of SNP necessary to restore RVR to baseline levels was 2- to 3-fold higher in 2K1C rats than in sham rats (26.0±4.3 nmol/min; P<0.05 versus sham).

Body weight was not different between sham and 2K1C animals, averaging 291±25 and 294±10 g, respectively. Weight of the left kidney was slightly, but not significantly, higher in 2K1C rats (1.27±0.06 versus 1.08±0.09 g in sham rats).

TGF Protocol

General parameters of the rats used in the TGF protocol are shown in the Table. MAP was significantly higher in 2K1C hypertensive rats than in normotensive control rats. RBF, RVR, colloid osmotic pressure, and proximal tubular pressure were not different between the 2 groups. Glomerular capillary pressure was significantly higher in 2K1C rats than in sham rats.

Maximum TGF responses in sham and 2K1C rats are shown in Figure 2. Baseline SFP in sham rats was 41.6±1.6 mm Hg (10 nephrons, 6 rats). The maximum SFP response during ATF infusion was 4.7±0.7 mm Hg. Intraluminal infusion of 1 mmol/L l-NNA increased maximum TGF responses to 15±2.9 mm Hg (P<0.01 versus ATF). Repeated measurements of maximum responses during ATF infusion (9 nephrons, 6 rats) revealed no time-dependent changes (4.7±1.0 versus 4.7±0.8 mm Hg). The percentage of glomerular transcapillary pressure gradient controlled by
the TGF system was 10.1%±1.2% during ATF infusion and 32.2%±1.8% during intraluminal L-NNA infusion (P, 0.05 versus ATF).

Baseline SFP in 2K1C rats was 49.3±1.5 mm Hg (7 nephrons, 6 rats; P, 0.05 versus sham). The maximum SFP response during ATF infusion was 5.1±0.4 mm Hg (NS versus sham). Maximum SFP decreases in the 2K1C rats were as high as 22.2±2.5 mm Hg during intraluminal infusion of L-NNA, which was significantly higher than those in sham rats (P, 0.05). Repeated measurements of maximum responses during ATF infusion (7 nephrons, 6 rats) revealed no time-dependent changes (5.2±0.9 versus 6.2±0.4 mm Hg). The percentage of the glomerular transcapillary pressure gradient controlled by the TGF system was 9.3%±0.6% during ATF infusion and 40.3%±3.8% during intraluminal L-NNA infusion (P, 0.05 versus ATF and sham).

**Urinary Excretion of NO Metabolites in TGF Experiments**

NO2±NO3 excretion was measured in the left and right kidneys of sham and 2K1C rats. Values in sham rats were not different for the right and left kidney. However, in 2K1C rats, NO2±NO3 excretion in the nonclipped kidney significantly exceeded that in the clipped kidney. Despite the fact that NO2±NO3 excretion in the nonclipped kidney of 2K1C rats was numerically higher than that in sham rats, this difference did not reach statistical significance. Data are summarized in Figure 3.

**Discussion**

The main hypothesis of this study was that increased NO availability in the nonclipped kidney of 2K1C hypertensive would lead to increased NO dependency of the TGF system. Systemic infusion of L-NNA resulted in a larger decrease in RBF and a larger increase in RVR in 2K1C rats than in sham rats. The NO clamp technique was applied to estimate the ambient NO level in both groups. During L-NNA administration, SNP was infused into the renal artery at a rate that could restore RVR to baseline levels. The method assumes that the amount of SNP administered correlates with the NO level under baseline conditions. The intrarenal infusion rate of the NO donor SNP necessary to restore RVR to baseline levels during L-NNA infusion was 2- to 3-fold higher in 2K1C rats than in sham rats. Under baseline conditions, maximum TGF-mediated decreases in glomerular capillary pressure were not different between 2K1C rats and normotensive control rats. However, augmentation of maximum TGF responses during intraluminal, late proximal infusion of L-NNA was more pronounced in 2K1C rats. The results of this study confirm the hypothesis that increased NO availability leads to
increased NO dependency of the TGF system in the contralateral kidney of 2K1C rats.

There is evidence that the contralateral kidney of 2K1C rats is under enhanced control of NO. Pronounced changes in RVR after NO blockade in the contralateral kidney have been observed in several studies, including this one.11,12,15 High intrarenal delivery rates for SNP in 2K1C animals were necessary to restore RVR to baseline levels during NO synthesis inhibition with L-NNA. This suggests that the actual ambient levels of NO, rather than the sensitivity of the vasculature to NO, are increased. It is unclear why no increases in inducible NOS (iNOS), endothelial NOS (eNOS), or brain-type NOS (bNOS) mRNA have been demonstrated in the contralateral kidney of 2K1C rats.16,17 In fact, bNOS mRNA expression has been demonstrated to be relatively decreased in the contralateral kidney of 2K1C rats as compared with the clipped kidney,17 and several other observations of coordinated changes in bNOS and renin mRNA during various stimuli have been reported. Increased Ang II levels can directly increase vascular NO production18 and cause changes in shear stress that induce increased NO synthesis.19 Nevertheless, it is unclear at present to what extent the altered hemodynamics in the nonclipped kidney of the 2K1C rat lead to increases in shear stress by itself. Together, the present data support the hypothesis that the vasculature in the nonclipped kidney is under increased influence of NO because of increased levels of NO rather than increased sensitivity of the vasculature to NO. At present, there is no conclusive evidence of whether eNOS or bNOS is responsible for increased NO activity in the nonclipped kidney of the 2K1C rat.

Supposedly, an increase in the ambient NO level in the kidney will also result in enhanced degradation. Bosse and Bachmann13 demonstrated increased nitrotyrosine staining (a footprint of ONOO−, the product of the reaction between superoxide and NO) in the cortical vasculature of the nonclipped kidney of 2K1C rats but not in the extraglomerular mesangium. It is tempting to speculate that the relatively high amounts of SNP needed to restore RVR in the 2K1C rats as compared with the differences in RVR responses to NOS inhibition could reflect enhanced degradation of NO by superoxide. Only more studies of the balance between NO and superoxide in 2K1C rats can resolve this issue. Nevertheless, the increased level of urinary NO2−/NO3− excretion in the nonclipped kidney as compared with the clipped kidney suggests that ambient NO levels are still increased.

Because we4 and others5,6 have failed to consistently show enhanced TGF responses, which would be expected from the increased circulating Ang II levels in this model, we investigated whether increased NO dependency of the TGF system was responsible for this observation. The data failed to show enhanced maximum TGF responses under baseline conditions, which is in agreement with results of previous studies.4–6 Intraluminal infusion of L-NNA in 2K1C rats resulted in a 3-fold increase in maximum TGF-mediated decreases in SFP. To more precisely estimate the impact of this enhancement of TGF responses on the control of net ultrafiltration pressure, we calculated the percentage of ultrafiltration pressure controlled by TGF. In fact, during NO inhibition, TGF controlled 40% of net ultrafiltration pressure. Both the maximum TGF responses and the percentage of net ultrafiltration pressure exceeded the values observed in sham rats. The present finding seems specific for 2K1C hypertensive rats, because enhancement of responses to NO synthesis inhibition were blunted in both spontaneously hypertensive and Milan hypertensive rats.20 The failure to demonstrate consistently increased TGF responses in the contralateral kidney of 2K1C rats may well be due to enhanced NO dependency of the TGF system.

In a previous study, we studied RBF autoregulation before and during NO synthesis inhibition. By use of mathematical analysis, we were able to demonstrate that in the nonclipped kidney, the lower limit of autoregulation significantly decreased and the efficacy (as assessed by the degree of compensation) increased during NO inhibition.12 The TGF system forms an integral part of autoregulation21 and controls distal delivery.22 Results of the previous study suggested that adaptive increases in NO activity contribute to relative maintenance of RBF. The results of this study add to this finding that NO opposes the action of Ang II to enhance TGF responsiveness, thus opposing this property of Ang II to limit distal delivery.23 It has been proposed that the synergistic actions of Ang II to enhance proximal tubular reabsorption and TGF responsiveness form a potent sodium-retaining mechanism.24 NO likely antagonizes both aspects of Ang II, because it decreases proximal reabsorption25 and attenuates TGF responsiveness.7,4 However, the adaptations are unable to fully counteract the actions of Ang II, because normal rather than attenuated TGF responses were observed at increased renal perfusion pressures.

In summary, the current data confirm that in the nonclipped kidney of 2K1C hypertensive rats, the vasculature is under enhanced influence of NO, probably as a result of increased ambient NO levels rather than increased sensitivity of the vasculature to NO. The TGF system is highly responsive during NO synthesis inhibition in the contralateral kidney as compared with kidneys of control rats. The results of these experiments indicate that adaptations in NO activity lead to relatively low TGF responsiveness, which enables increases in distal delivery with additional deterioration of systemic arterial pressure.

Acknowledgments

This study was supported by the Dutch Kidney Foundations (grant C93.1321). The expert technical assistance of Fatima El Khalouki, Nel Willekes-Kolschijn, and Remmert de Roos is greatly appreciated.

References

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Hypertension. 1999;34:679-684
doi: 10.1161/01.HYP.34.4.679

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