J Am Soc Nephrol 11: 847–855, 2000

Impaired Renal Blood Flow Autoregulation in Two-Kidney, One-Clip Hypertensive Rats Is Caused by Enhanced Activity of Nitric Oxide

ERIKA TURKSTRA, BRANKO BRAAM, and HEIN A. KOOMANS
Department of Nephrology and Hypertension, University Hospital Utrecht, The Netherlands.

Abstract. Increases in renal perfusion pressure will induce shear stress-mediated nitric oxide (NO) release, which could oppose autoregulation of renal blood flow (RBF). Although cardiac, cerebral, and mesenteric autoregulation is enhanced during nitric oxide (NO) synthesis inhibition, this has not been reported for renal autoregulation of blood flow. In the present study, the lower limit and efficiency of RBF autoregulation (as assessed by the degree of compensation) were studied before and during NO inhibition in normotensive Sprague Dawley rats (control; $n = 9$) and in the non-clipped kidney of two-kidney, one-clip Goldblatt hypertensive animals (2K1C; $n = 9$; 3 wk; 0.25-mm silver clip). In both groups, renal autoregulation curves were obtained before and during infusion of N^G-nitro-L-arginine (L-NNA) (bolus 1.5 mg/kg intravenously, infusion 10 μg/kg per min intravenously), using a transit-time flow probe around the left renal artery. In control rats, mean arterial pressure (MAP) increased, RBF decreased, and renal vascular resistance (RVR) increased in response to L-NNA infusion. The lower limit of autoregulation in control animals did not significantly change during L-NNA infusion (78 ± 3 to 70 ± 2 mmHg). The degree of compensation in these rats slightly increased during L-NNA infusion, however, this was only significant below 90 mmHg. The 2K1C rats had elevated MAP under baseline conditions. L-NNA infusion resulted in a decrease in RBF and an increase in MAP and RVR. During L-NNA infusion, RVR in 2K1C rats greatly exceeded RVR in control rats. A significant decrease was observed in the lower limit of autoregulation from 85 ± 3 to 72 ± 5 mmHg ($P < 0.05$). In the contralateral kidney of 2K1C rats, the degree of compensation was lower than in control rats under baseline conditions. L-NNA infusion resulted in significantly higher degrees of compensation compared to baseline. In conclusion, the contralateral kidney displayed a high NO dependency, as RBF greatly decreased and RVR dramatically increased in response to L-NNA infusion. The contralateral kidney of 2K1C rats exhibited impaired RBF autoregulation, which was improved by NO inhibition, as judged from a decrease in the lower limit of autoregulation and an increase in the degree of compensation. This study indicates that perfusion pressure-dependent NO release can oppose autoregulation in the kidney. However, the enhanced influence of NO on pressure-dependent RBF may facilitate the preservation of renal function in the nonclipped kidney of 2K1C rats.

Nitric oxide (NO) tonically dilates the renal vasculature (1,2) and attenuates the responsiveness of the tubuloglomerular (TGF) feedback system (1,2), but supposedly does not alter the autoregulatory behavior of the kidney (2–5). The absence of an effect of NO on renal blood flow (RBF) autoregulation is surprising, considering that during increases in renal perfusion pressure (RPP) the shear stress-mediated release of NO is probably enhanced (6), which would oppose autoregulation. There have been several reports on blood flow autoregulation in the cerebral (7), mesenteric (8), and coronary vasculature (9), indicating that during NO synthesis inhibition, blood flow autoregulation in these vascular trees may well be more efficient, and extended to a lower range of perfusion pressures.

In our laboratory, combined peritubular infusion of angiotensin II (AngII) and the NO synthesis inhibitor N^G-nitro-L-arginine (L-NNA) led to extremely high TGF responses (10). Because the TGF system participates in RBF autoregulation, these enhanced responses during inhibition of NO formation could well lead to increased efficiency of RBF autoregulation, in particular under conditions of increased circulating AngII levels. RBF in the nonclipped kidney of two-kidney, one-clip (2K1C) Goldblatt hypertensive rats has been shown to be highly sensitive to NO inhibition (11,12). Furthermore, we have recently demonstrated that the intrarenal infusion rate of sodium nitroprusside needed to restore renal function to baseline after blockade of NO formation is much higher in the contralateral kidney of 2K1C rats than in sham-operated controls. TGF responses in this kidney are extremely dependent on NO in this model (11), suggesting high levels of ambient NO. Enhanced production and systemic delivery of AngII by the ipsilateral kidney forms the basic endocrine disturbance (13,14). Nevertheless, the reported defect in autoregulation of the contralateral kidney (15) is not compatible with actions of AngII (16).

Received August 11, 1999. Accepted September 13, 1999.
Correspondence to Dr. Branko Braam, Department of Nephrology and Hypertension, Room F03.226, University Hospital Utrecht, P.O. Box 85500, 3508 GA The Netherlands. Phone: +31 30 250 7329; Fax: +31 30 251 0964; E-mail: BrBraam@Knoware.nl
1046-6673/1105-0847
Journal of the American Society of Nephrology

The central hypothesis of the present investigation is that an adaptive increase of the actions of NO maximizes RBF at the cost of impaired autoregulation in the contralateral kidney of renovascular hypertensive rats. To address this hypothesis, the characteristics of renal autoregulation were studied in kidneys of normotensive rats and in the nonclipped kidney of 2K1C hypertensive rats before and during systemic infusion of the NO synthase (NOS) inhibitor L-NNA. A new mathematical analysis was developed and applied to identify the lower limit of autoregulation and to analyze the efficiency of renal autoregulation.

Materials and Methods

Animals and Clipping Procedure

Male Sprague Dawley rats (200 to 350 g; Harlan-Olac, Blackthorn, United Kingdom) received normal rat chow (sodium content 100 mmol/kg; Hope Farms, Woerden, The Netherlands) 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 negative throughout the course of the experiments (17). The studies were approved by the Utrecht University Board for studies in experimental animals. Two groups of rats were studied. One group consisted of 2K1C animals ($n = 9$). Three weeks before the autoregulation experiment, these rats were anesthetized with 0.19 mg/kg fentanyl citrate + 6 mg/kg fluanisone subcutaneously (Hypnorm®; Janssen Pharmaceutica, Beerse, Belgium) and 2.5 mg/kg midazolam intraperitoneally (Dormicum®; Roche, Mijdrecht, The Netherlands) and placed on a servo-controlled heated operating table. The right kidney was exposed through a flank incision, and a 0.25-mm silver clip was placed around the renal artery, as described previously (18). Normotensive animals served as controls ($n = 9$); three were sham-operated and six were left untreated. Comparison between the sham-operated and untreated controls revealed no differences and, thus, these animals were pooled.

Surgical Procedure and Infusions

On the day of the experiment, the animals were anesthetized with Inactin (110 mg/kg body wt intraperitoneally) and placed on a servo-controlled surgical table that maintained rectal temperature at 37°C. After intubation of the trachea, a catheter was placed into the left jugular vein (PE50) for infusion of solutions, and a second catheter (PE10) was introduced for bolus injections. The femoral artery was cannulated (PE50) to measure RPP and to collect blood samples. The left kidney was exposed through a flank incision, freed from surrounding tissue, and placed into a plastic holder; the left ureter was cannulated with PE-10 tubing. A 1RB ultrasonic flow probe was placed around the left renal artery and connected to a transit time blood flow meter (model T206; Transonics, Ithaca, NY). A sling was placed around the aorta between the renal arteries to enable reduction of the left RPP.

All animals received an intravenous infusion of a 150 mM NaCl solution containing 6% bovine serum albumin (Sigma Chemical Co., St. Louis, MO) at a rate of 10 μl/min per 100 g body wt. After surgery, the infusion was switched to a 150 mM NaCl solution with 1% bovine serum albumin at the same infusion rate. This infusion was maintained throughout the experiment. Experimental compounds were added to this standard solution. A 60-min equilibration period was observed before the start of the measurements. At the end of each experiment, the left kidney (and in the 2K1C rats also the right kidney) was removed, blotted dry, and weighed.

In normotensive rats (L-NNA, $n = 9$), an autoregulation curve was obtained under baseline conditions. Then, a bolus injection of L-NNA (1.5 mg/kg) was injected and an infusion of 10 μg/kg per min L-NNA was started. After equilibration at a new level of mean arterial pressure (MAP) and RBF, the autoregulation curve was repeated. In the 2K1C animals ($n = 9$), the protocol was identical. In previous experiments, we have shown that this infusion scheme leads to a stable increase in MAP and a stable reduction of RBF (19).

Autoregulation Protocol

First, a baseline measurement of RBF and MAP was obtained. Then, RPP was reduced in random order to 60, 70, 80, 90, 100, 110, 120, and, if applicable, to 130, 140, 150, and 160 mmHg by adjusting the sling around the aorta. After each reduction in RPP, the RBF was recorded for at least 20 to 30 s. After each measurement at reduced RPP, the sling was released and RPP and RBF were allowed to return to baseline values. After switching the infusion, at least 30 min was allowed to pass before another curve was recorded.

Statistical Analyses

The pressure system and the flow meter were connected to a personal computer by an analog-to-digital converter with a sample frequency of 3 Hz. The RBF autoregulation curve was generated by extrapolating the values of RBF at different levels of RPP by linear regression to exact values of 60, 70, 80, 90, 100, 120, and, if possible, to 130, 140, 150 and 160 mmHg. Renal vascular resistance (RVR) was calculated as RPP divided by RBF. Data are expressed as mean ± SEM. The lower limit of autoregulation and the degree of compensation were calculated as outlined in the appendix. The degree of compensation is a measure of autoregulatory efficiency. In case there is no change in RBF upon a decrease in RPP, the value is 100%; in case the change in RBF equals the change as expected if the vascular bed would not autoregulate, the value is 0. Data from control rats and 2K1C rats were compared with two-way ANOVA and two-way ANOVA for repeated measurements. If a variance ratio reached statistical significance, the Student-Newman-Keuls test was performed as *post hoc* test. In case the data were not normally distributed, a two-way repeated-measures ANOVA on ranks was used; this is indicated in the Results section.

A new method was developed to estimate the lower limit of autoregulation. The raw data of the autoregulation curves were subjected to nonlinear regression analysis using the logistic equation. The lower limit was then defined as the perfusion pressure, where the third derivative of the fitted curve was 0, which mathematically defines the shoulder in a sigmoidal curve. This method avoids the error introduced by the limited number of perfusion pressures at which RBF is evaluated. Also, if one uses an arbitrary decrease in RBF, one will incorrectly identify the lower limit of autoregulation in curves that show poor autoregulation. The exact mathematical derivation of the lower limit is outlined in the Appendix, and an example of a curve and its derivatives is depicted in Figure 1. The lower limit of autoregulation was also determined manually in all curves by six members of the department; the intercept of the lines drawn through the lower part and the upper part of the autoregulation curve was used as lower limit. It should be emphasized that the interpreters were given a brief instruction and that the curves were coded.

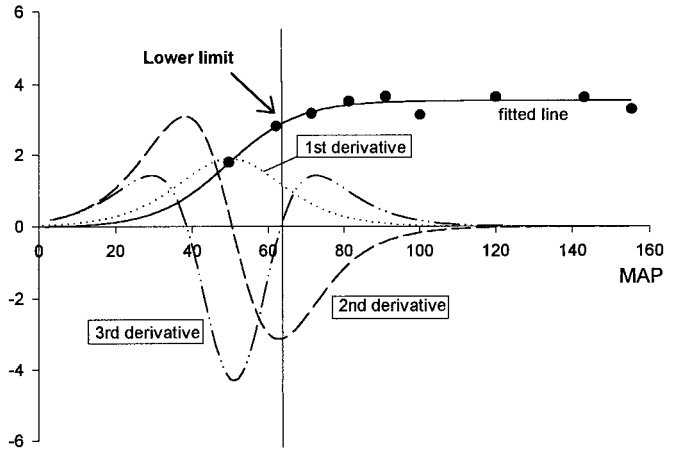

Figure 1. Example of the curve fitting procedure, and derivatives, using the logistic equation. The point where the third derivative equals 0 indicates the lower limit of autoregulation (arrow and straight vertical line).

Results

MAP, RBF, and RVR

General parameters of the rats are shown in Table 1. Under baseline conditions, MAP was significantly higher in the 2K1C hypertensive animals compared to the normotensive animals ($P < 0.05$) (Figure 2, top panel). During L-NNA infusion, MAP increased in both groups. Before infusion of L-NNA, RBF in the normotensive groups was significantly higher than the RBF in the nonclipped kidney of 2K1C hypertensive animals ($P < 0.05$) (Figure 2, middle panel). L-NNA infusion resulted in a significant decrease in RBF in the control and 2K1C animals. Under baseline conditions, RVR was significantly higher in 2K1C animals compared to control animals ($P < 0.05$) (Figure 2, bottom panel). RVR increased in both groups in response to L-NNA infusion. During L-NNA infusion, the RVR in the contralateral kidney of 2K1C rats greatly exceeded the RVR in control rats ($P < 0.05$).

Autoregulation Curves: RBF and RVR

Control animals demonstrated autoregulation of RBF before the infusion of L-NNA (Figure 3A). During the infusion of L-NNA, autoregulation was preserved, as RBF was lower at all RPP (Figure 3A). In the contralateral kidney of 2K1C rats, autoregulation under baseline conditions did not show such a clear plateau as observed in the control animals (Figure 3A). When expressed as percentage of control RBF (Figure 3B), this is even more pronounced. L-NNA infusion again decreased RBF at all perfusion pressures studied. Also, a plateau could now be observed in the autoregulation curves. It should be emphasized that the average curves presented in the figure underestimate the power of autoregulation of each individual rat, as not all lower limits of autoregulation are exactly similar.

Table 1. Baseline data of the normotensive (control) and 2K1C hypertensive rats administered L-NNA[a]

Parameter	Control	2K1C
	9	9
Body weight (g)	275 ± 8	318 ± 5[b]
Left kidney weight (g)	1.10 ± 0.06	1.37 ± 0.04[b,c]
Right kidney weight (g)	ND	1.09 ± 0.06

[a] 2K1C, two-kidney, one-clip; L-NNA, N^G-nitro-L-arginine; ND, not determined.
[b] $P < 0.05$ *versus* control.
[c] $P < 0.05$ *versus* right kidney.

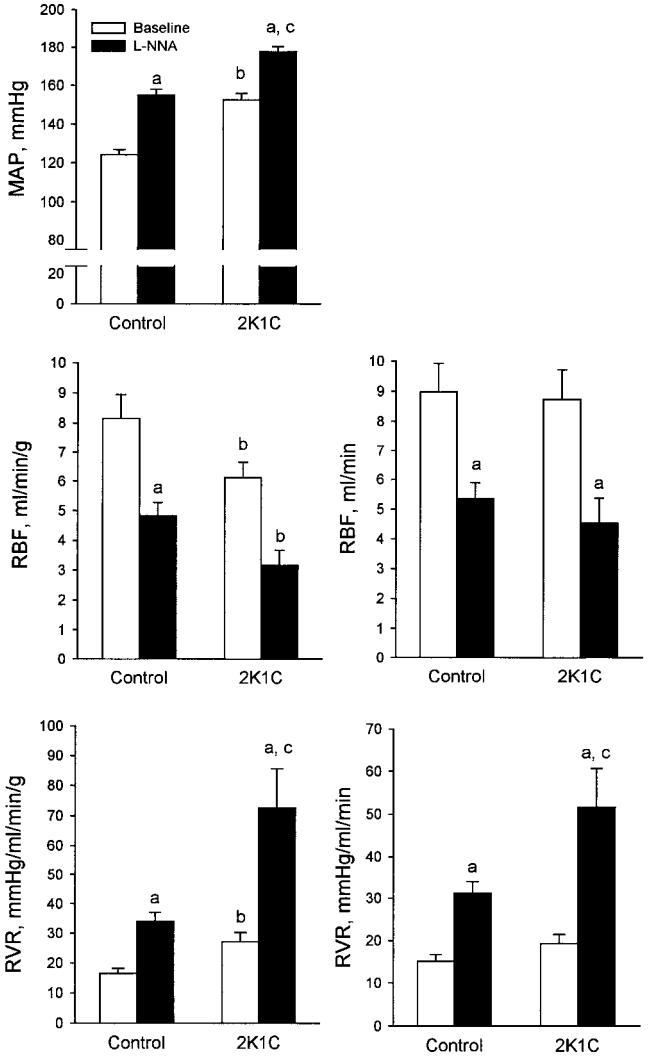

Figure 2. Mean arterial pressure (MAP; top panel), renal blood flow (RBF; middle panel), and renal vascular resistance (RVR; bottom panel) before (Baseline, □) and during N^G-nitro-L-arginine (L-NNA; ■) administration in control (Control; □, $n = 9$) rats and in nonclipped kidney of the two-kidney, one-clip (2K1C; ■, $n = 9$) rats. Because of the differences in kidney weight, RBF and RVR are also shown as corrected for kidney weight. [a]$P < 0.05$ *versus* baseline; [b]$P < 0.05$ *versus* control, baseline conditions; [c]$P < 0.05$ *versus* control, L-NNA infusion (ANOVA).

Autoregulation Curves: Lower Limit and Efficiency of RBF Autoregulation

The lower limit was calculated from the fitted sigmoidal curve and its third derivative as detailed in the Appendix.

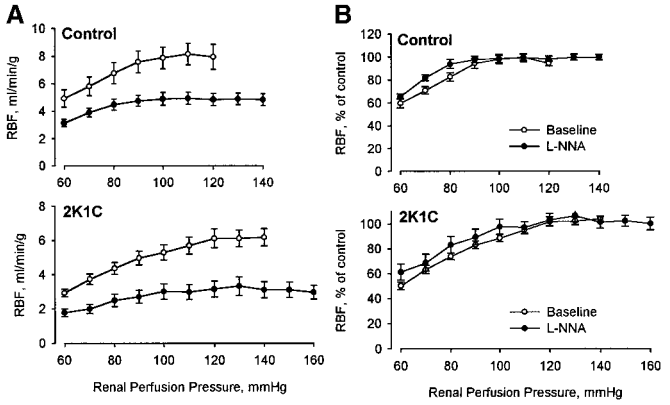

Figure 3. (A) RBF autoregulation in normotensive (Control) and in 2K1C hypertensive animals under baseline conditions (Baseline, ○) and during L-NNA infusion (L-NNA, ●). (B) RBF as expressed as percentage of control in the groups mentioned above.

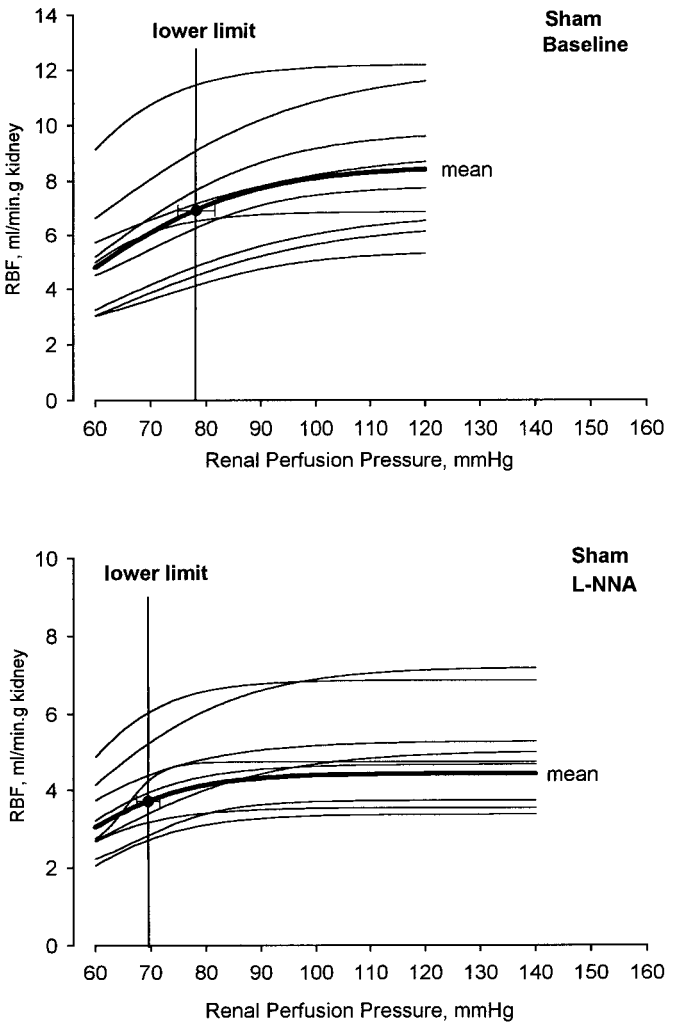

Figure 4. Reconstructed autoregulation curves in the normotensive animals before (top panel; baseline) and during (bottom panel) the infusion of L-NNA. The thick line indicates the average of the curves. The decrease in the lower limit was not significant.

Under baseline conditions, the lower limit was 78 ± 3 ($n = 9$) mmHg in the normotensive rats. During infusion of L-NNA, the lower limit decreased to 70 ± 2 mmHg, a value not significantly different from baseline (Figure 4). The degree of compensation under baseline conditions was not significantly different from the values observed during L-NNA infusion in the normotensive rats (Figure 5), except at a pressure below 90 mmHg. Nevertheless, the entire curve was shifted upward, indicating an overall tendency for better autoregulation during NO blockade.

In 2K1C hypertensive animals, the lower limit of autoregulation under baseline conditions was slightly higher than the values observed in the normotensive animals (85 ± 3 mmHg; $n = 9$; NS) (Figure 6). Under conditions of blockade of NO synthesis, however, the lower limit decreased significantly to 72 ± 5 mmHg ($P < 0.05$ *versus* baseline). At lower perfusion pressures, the degree of compensation was significantly lower in the 2K1C rats compared to the control animals. Also, the degree of compensation was less under baseline conditions compared to L-NNA infusion in the 2K1C animals (Figure 5). Thus, the contralateral kidney of 2K1C rats displayed impaired autoregulation under baseline conditions. A decrease in the lower limit and an increase in the efficiency of autoregulation followed infusion of L-NNA.

Manual Estimates of the Lower Limit of Autoregulation

Manually determined (as determined by taking the average for each curve of the six interpretations), the lower limit was 95 ± 3 mmHg under baseline conditions in left kidneys of the normotensive rats and 104 ± 3 mmHg in the nonclipped kidneys of the 2K1C rats. During L-NNA infusion, the lower limit decreased to 88 ± 3 and 93 ± 4 mmHg in normotensive and 2K1C rats, respectively. This decrease was only significant in the 2K1C rats.

The differences between the estimates of the six interpreters was quite high: On average, the difference between the highest and lowest estimate was 10 ± 2 mmHg under baseline conditions and 13 ± 4 mmHg during L-NNA infusion in the normotensive rats. In the 2K1C rats, these values were even higher and averaged 35 ± 3 and 30 ± 5 mmHg. This simple evaluation reveals considerable error in manual interpretation of the curves.

Discussion

The central hypothesis of the present investigation was that NO antagonizes autoregulation of RBF, in particular under conditions of enhanced AngII activity and enhanced ambient NO levels. To circumvent the possible errors of other methods, a new mathematical method to assess the lower limit and the efficiency of renal autoregulation was developed. Using this method, only a significant increase in efficiency of autoregulation was demonstrated at RPP values below 90 mmHg in normotensive rats. In the contralateral kidney of the 2K1C Goldblatt hypertensive rat, however, NO synthesis inhibition resulted in a decrease in the lower limit of autoregulation and an increase in autoregulatory efficiency. This indicates that NO

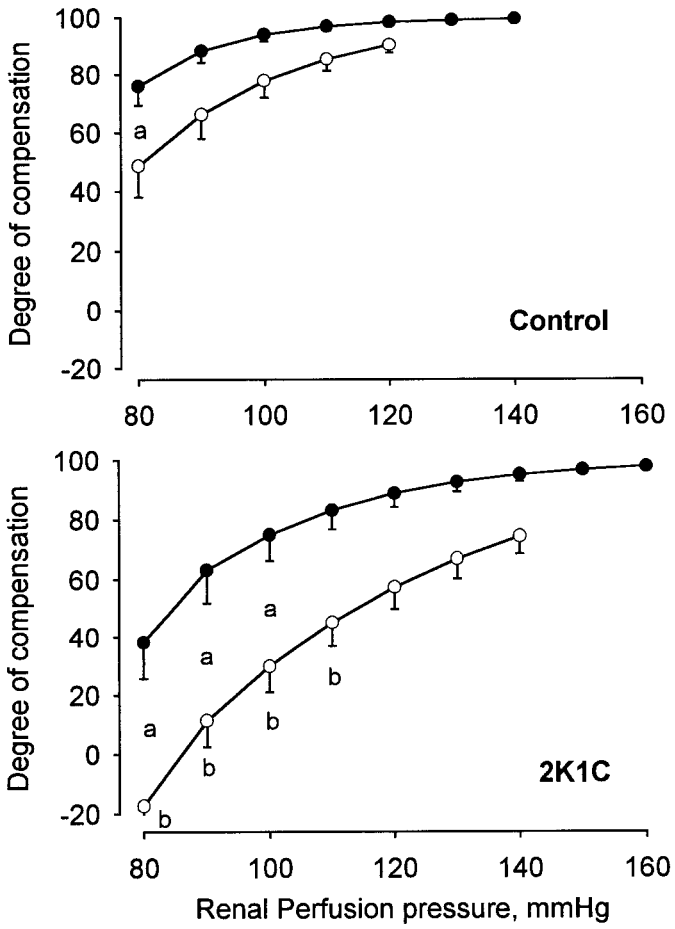

Figure 5. Efficiency of RBF autoregulation expressed as degree of compensation in control rats (Control; top panel) and in the contralateral kidney of the 2K1C animals (bottom panel) under baseline conditions (○) and during L-NNA infusion (●). $^{a}P < 0.05$ *versus* baseline; $^{b}P < 0.05$ *versus* normotensive rats (ANOVA).

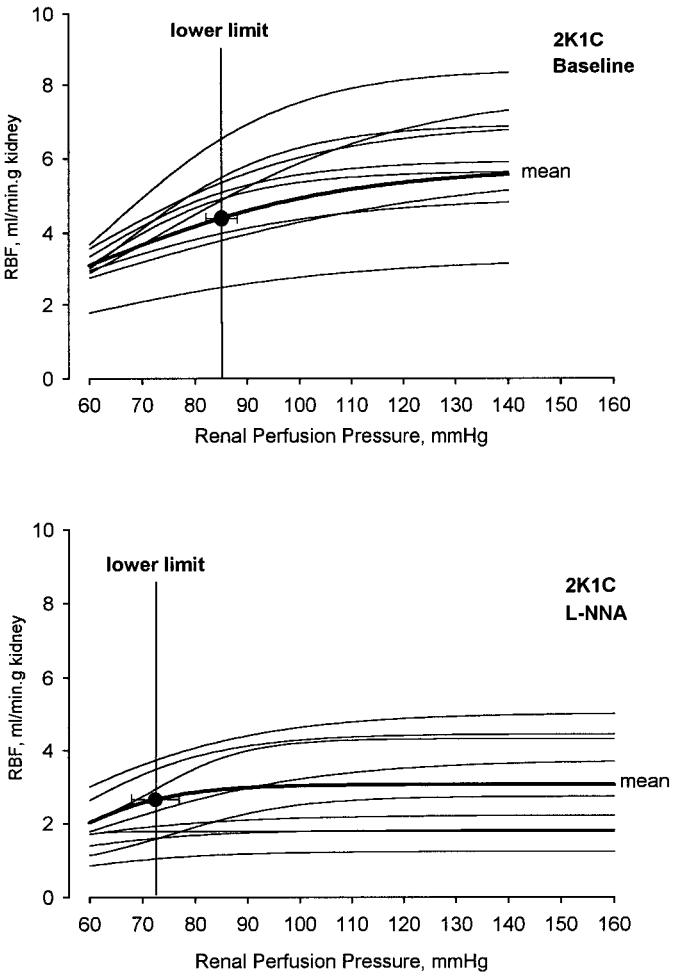

Figure 6. Reconstructed autoregulation curves in the 2K1C hypertensive animals before (top panel; baseline) and during (bottom panel) the infusion of L-NNA. L-NNA infusion resulted in a significant decrease in the lower limit of autoregulation ($P < 0.05$). The thick line indicates the average of the curves.

can oppose autoregulation of RBF in the contralateral kidneys of 2K1C Goldblatt hypertensive rats.

Previous studies have yielded contrasting results as to whether NO is involved in autoregulation of RBF in normotensive animals. In a study on the interaction of NO and prostaglandins on the RBF and papillary blood flow, Ortíz *et al.* demonstrated that blockade of NO formation clearly decreased the slope of the autoregulation curve. The curves of the average data revealed a decrease in the lower limit of autoregulation. Although suggesting an improvement of RBF autoregulation, the data were not analyzed to that purpose (20). In contrast, several studies have failed to show involvement of NO on autoregulation. NOS inhibition in normotensive rats and dogs failed to affect RBF autoregulation (4,5). Majid *et al.* have published several reports on the autoregulatory behavior of the kidney during NO synthesis inhibition in dogs (2,3) and concluded that NO is not involved in the autoregulatory component of RBF regulation. Finally, there have been reports that inhibition of NO synthesis leads to decreased efficacy of autoregulation, suggesting that NO is important in the vasodilator arm of the autoregulatory response (21). Why these studies have yielded contrasting results is obscure; however, this could be due to species differences, experimental setup, and different activities of NO and AngII in the various settings. Taken together, these studies failed to show a consistent influence of NO on RBF autoregulation, and the findings of the present study do not support an important influence of NO on autoregulation in normotensive rats.

Despite these findings, there are indications that NO modulates autoregulation in various other tissues (7–9). In coronary (9) and mesenteric (8) vessels, it has been demonstrated that blockade of NO synthesis leads to extension of the autoregulatory range and to a decrease in the slope of the autoregulation curve, respectively. Studies in the brain are less conclusive, as one report indicated impaired (22), and another improved, autoregulation (7) of cerebral blood flow during NO synthesis inhibition. It is surprising that no important influence of NO on renal autoregulation could be substantiated in normotensive rats. The renal vasculature is under strong influence of NO, as has been shown in many previous studies (1–4), and NOS has

been detected in the endothelium (23) and in the macula densa (24). At a functional level, Imig *et al.* demonstrated that L-NNA administration improves adjustments of the afferent arteriole to decreases in perfusion pressure in the isolated-perfused juxtamedullary nephrons (25). In the isolated-perfused hydronephrotic kidney, Hayashi *et al.* found a left-shift of the relationship between RPP and afferent arteriolar diameter during L-NNA administration (26). Furthermore, experiments in our laboratory (10,27) and experiments by others (24,28) have shown that systemic, intraluminal, and peritubular administration of L-NNA leads to pronounced enhancements of TGF responsiveness. This body of data on both the myogenic response and the TGF mechanism, which both participate in renal autoregulation, supports that renal autoregulatory efficiency will be enhanced during NO synthesis inhibition. Nevertheless, only at the lower perfusion pressures could a significant increase in autoregulatory efficiency be demonstrated, and no change in lower limit of autoregulation was observed.

A new method was developed and applied to analyze the autoregulation curves, using nonlinear curve fitting to the logistic equation. Correlation coefficients were between 0.90 and 0.99. The sigmoidal curve has the mathematical characteristics of one inflection point and two shoulders, which can be found by differentiation. The lower limit of autoregulation was determined as that perfusion pressure at which the third derivative of the curve was 0. With this method, one can mathematically identify the shoulder of the autoregulation curve in a standardized manner. It substantially differs from the method employed by others, which uses the intersection of two straight lines drawn through the lower and the upper part of the autoregulation curve. This analysis of the lower limit of autoregulation could introduce substantial interobserver error (4). We evaluated the accuracy of this manual interpretation and found considerable scatter between the determination of the lower limit among the interpreters of the curves. Another method that has been used in several reports identifies the lower limit of autoregulation by finding the RPP value that decreases RBF by more than 0.2 ml/min per g kidney wt from baseline levels (29). It is obvious that in case of poor autoregulation, this method will incorrectly identify the lower limit. The main advantage of the newly developed strategy is that no arbitrary choices have to be made. In the present study, this new method was successfully applied and enabled the detection of a change in the autoregulatory behavior of the kidney in renovascular hypertensive rats.

The contralateral kidney of 2K1C renovascular hypertensive rats displayed impaired autoregulatory efficiency under baseline conditions. Enhanced production and systemic delivery of AngII by the ipsilateral kidney forms the underlying endocrine disturbance (14). The defect in autoregulation of the contralateral kidney is not compatible with increased actions of AngII (16). NO synthesis inhibition decreased the lower limit and increased the efficiency (as assessed by the degree of compensation) in these kidneys. Several studies support that the contralateral kidney of the 2K1C rat is under enhanced influence of NO. First, AngII can directly stimulate the expression of endothelial NOS (30). Furthermore, Beierwaltes *et al.* showed that increasing degrees of stenosis in the ipsilateral kidney in the 2K1C model was associated with increasing depression of RBF during application of systemic NO inhibition (12). Finally, we have recently reported that NO production and activity in the contralateral kidney is increased, assessed by use of an intrarenal NO clamp, as well as by measuring NO_2/NO_3 excretion (11). It is noted that in this study, NO dependency of the TGF systems was also studied. Maximum TGF responses displayed an exaggerated increase in response to proximal tubular infusion of L-NNA, indicating that the TGF system was dampened by enhanced influence of NO (11). The very high RVR in the contralateral kidney of the 2K1C rats compared with the kidneys of normotensive rats indicates a major influence of NO in the present study. Thus, the present study supports that enhanced NO activity serves to optimize RBF, while it decreases the efficiency of autoregulation and shifts the lower limit of autoregulation to a higher level of RPP.

Impaired autoregulation of RBF has been observed in various other models of hypertension, such as in the spontaneously hypertensive rat (31) and in the Dahl salt-sensitive rat (32). In the adult spontaneously hypertensive rat, the myogenic response is impaired (26) and the TGF responsiveness is high (33). The myogenic response improves upon application of NO synthesis inhibition (26). The TGF response, however, does not show enhanced NO dependency as in the 2K1C rat (33). The response of overall autoregulation of RBF to NO synthesis inhibition has not been studied to our knowledge. In the Dahl rat, the myogenic response is impaired (34) and the TGF system seems to react slightly more efficiently than in control rats (35). Systematic study has not been performed on the influence of NO on TGF, myogenic response, and overall autoregulation of RBF in the Dahl rat. The present study adds to a further characterization of RBF regulation in the 2K1C hypertensive model. It is now evident that the myogenic response of the afferent arteriole of the contralateral kidney is diminished (36), that TGF responsiveness is normal (11,18), and that overall autoregulation of RBF is diminished (37). NO activity is enhanced in this kidney (11), and importantly contributes to the maintenance of RBF (11,12). Blockade of NO synthesis improves efficiency of autoregulation and strongly enhances TGF responses (11). Thus, in contrast to the spontaneously hypertensive rat, NO is involved in both axes of the autoregulatory process. Additional studies should be aimed at determining the influence of hypertension *per se* and the influence of the primary pathophysiologic disturbance in these models.

A decrease in the lower limit and an increase in efficiency of RBF autoregulation, serve, under physiologic conditions, to better protect the kidney from fluctuations in RPP (38). The adaptation of the NO system in the contralateral kidney of renovascular hypertensive rats allows the kidney to increase blood flow upon further increases in RPP, *i.e.*, upon further deterioration of systemic arterial pressure. In fact, NO synthesis inhibition unmasks the real actions of AngII on RBF autoregulation: reduction of RBF at all perfusion pressures and stabilization of RBF upon changes in RBF, both at the lower end and at the upper end of prevailing perfusion pressures. The

clinical relevance of the present findings is that intact endothelial function may relatively protect the contralateral kidney from sodium retention in renovascular hypertension. At present, it is unclear whether pharmacologic interventions improving endothelial dysfunction will lead to better control of arterial pressure in experimental or human renovascular hypertension.

In summary, the present study identified that in the nonclipped kidney of 2K1C hypertensive rats, the renal vasculature of the nonclipped kidney displays a high sensitivity to NO. Blockade of NO decreases the lower limit of autoregulation and increases the efficiency of RBF autoregulation. This change in autoregulatory behavior could be identified using a newly developed analytical method. The adaptive increase in NO activity in the contralateral kidney of the 2K1C rat may well protect the kidney from further increases in systemic arterial pressure.

Appendix

Finding the Point of Inflection and the Slope of the Autoregulation Curve

Assume a function $k(x)$: $k(x) = 1/f(x)$. In a general case, one can find the derivatives of this function.

First derivative:

$$k'(x) = -\frac{f'(x)}{f(x)^2}$$

Second derivative:

$$k''(x) = \frac{-f''(x)\,f(x)^2 + f'(x)2f'(x)\,f(x)}{f(x)^4} \Leftrightarrow k''(x) = \frac{-f''(x)}{f(x)^2} + \frac{2f'(x)^2}{f(x)^3}$$

Third derivative:

$$k'''(x) = -\frac{f'''(x)}{f(x)^2} + 2\frac{f'(x)\,f''(x)}{f(x)^3} + 4\frac{f'(x)\,f''(x)}{f(x)^3} - 6\frac{f'(x)^3}{f(x)^4} \Leftrightarrow k'''(x) = -\frac{f'''(x)}{f(x)^2} + 6\frac{f'(x)\,f''(x)}{f(x)^3} - 6\frac{f'(x)^3}{f(x)^4}$$

In the case that $f(x) = 1 + e^{b(x-c)}$, the sigmoidal curve, then

$$f'(x) = be^{b(x-c)}$$

$$f''(x) = b^2e^{b(x-c)}$$

$$f'''(x) = b^3e^{b(x-c)}$$

and

$$f(x)^2 = (1 + e^{b(x-c)})^2$$

$$f(x)^3 = (1 + e^{b(x-c)})^3$$

$$f(x)^4 = (1 + e^{b(x-c)})^4$$

Substituting yields:

$$k'''(x) = -\frac{e^{b(x-c)}b^3}{(1 + e^{b(x-c)})^2} + 6\frac{(e^{b(x-c)})^2b^3}{(1 + e^{b(x-c)})^3} - 6\frac{(e^{b(x-c)})^3b^3}{(1 + e^{b(x-c)})^4}$$

Replacing $e^{b(x-c)}$ by y yields:

$$k'''(x) = -\frac{yb^3}{(1 + y)^2} + 6\frac{y^2b^3}{(1 + y)^3} - 6\frac{y^3b^3}{(1 + y)^4}$$

By multiplying with $(1 + y)^4$ and dividing by b^3 the equation is simplified to:

$$k'''(x)(1 + y)^4/b^3 = -y(y^2 - 4y + 1)$$

Now find the values where $k'''(x) = 0$:

$$y^2 - 4y + 1 = 0 \Rightarrow y = \frac{+4 \pm \sqrt{16 - 4 \times 1}}{2} \Leftrightarrow y = +2 \pm \sqrt{3}$$

Remember, y replaced $e^{b(x-c)}$:

$$e^{b(x-c)} = +2 \pm \sqrt{3} \Leftrightarrow x = \frac{\ln(+2 \pm \sqrt{3}) + bc}{b}$$

Adding the other constants, a and d, in the logistic equation scales, the third derivative, however, does not influence value of x where the third derivative equals 0. Parameters for the logistic equation for each individual curve were calculated using a nonlinear curve fitting routine with SigmaPlot (Jandel Scientific®, Erkrath, Germany), and the result inspected in a graph. The lower limit of autoregulation was then calculated using the equation above. In the present study, this calculated value is used as the lower limit of autoregulation. An example of the application of this method is shown in Figure 1.

Using the first derivative of the logistic equation and the obtained parameters, the slope of each individual curve was estimated at an RPP of 80, 90, 100, 110, 120 mmHg and, if possible, at 130, 140, 150, and 160 mmHg. The first derivative of the logistic equation, as can be appreciated from the outline above, is:

$$k'(x) = -\frac{b(a - d)e^{b(x-c)}}{(1 + e^{b(x-c)})^2}$$

The degree of compensation can then be calculated as follows:

$$\text{Compensation} = 1 - \frac{k'(x)}{\text{RBF}(x)/\text{MAP}(x)}$$

The degree of compensation should be interpreted as follows. If there is no autoregulation, the observed slope of the curve, $k'(x)$, equals $\text{RBF}(x)/\text{MAP}(x)$, so the degree of com-

pensation is 0. If there is perfect autoregulation, the observed slope of the curve, $k'(x)$, is 0, and the degree of autoregulation is 1.

Acknowledgments

This study was supported by the Dutch Kidney Foundation (Grant C93.1321). The valuable mathematical advice of Dr. Peter Braam (Carnegie Mellon University, Pittsburgh, PA) and the comments on the methods by Dr. Niels Holstein-Rathlou (University of Copenhagen, Denmark) are greatly appreciated.

References

1. Navar LG, Inscho EW, Majid DSA, Imig JD, Harrison-Bernard LM, Mitchell KD: Paracrine regulation of the renal microcirculation. *Physiol Rev* 76: 425–536, 1996
2. Majid DS, Williams A, Navar LG: Inhibition of nitric oxide synthesis attenuates pressure-induced natriuretic responses in anesthetized dogs. *Am J Physiol* 264: F79–F87, 1993
3. Majid DS, Navar LG: Suppression of blood flow autoregulation plateau during nitric oxide blockade in canine kidney. *Am J Physiol* 262: F40–F46, 1992
4. Beierwaltes WH, Sigmon DH, Carretero OA: Endothelium modulates renal blood flow but not autoregulation. *Am J Physiol* 262: F943–F949, 1992
5. Naess PA, Christensen G, Kirkeboen KA, Kiil F: Effect on renin release of inhibiting renal nitric oxide synthesis in anaesthetized dogs. *Acta Physiol Scand* 148: 137–142, 1993
6. Malek AM, Izumo S: Molecular aspects of signal transduction of shear stress in the endothelial cell. *J Hypertension* 12: 989–999, 1994
7. Talman WT, Dragon DN: Inhibition of nitric oxide synthesis extends cerebrovascular autoregulation during hypertension. *Brain Res* 672: 48–54, 1995
8. Macedo MP, Lautt WW: Autoregulatory capacity in the superior mesenteric artery is attenuated by nitric oxide. *Am J Physiol* 271: G400–G404, 1996
9. Ueeda M, Silvia SK, Olsson RA: Nitric oxide modulates coronary autoregulation in the guinea pig. *Circ Res* 70: 1296–1303, 1992
10. Braam B, Koomans HA: Nitric oxide antagonizes the actions of angiotensin II to enhance tubuloglomerular feedback responsiveness. *Kidney Int* 48: 1406–1411, 1995
11. Turkstra E, Braam B, Koomans HA: 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. *Hypertension* 34: 679–684, 1999
12. Sigmon DH, Beierwaltes WH: Degree of renal artery stenosis alters nitric oxide regulation of renal hemodynamics. *J Am Soc Nephrol* 5: 1369–1377, 1994
13. Mitchell KD, Braam B, Navar LG: Hypertensinogenic mechanisms mediated by the intrarenal actions of renin-angiotensin system. *Hypertension* 19[Suppl I]: I-18–I-27, 1992
14. Ploth DW, Fitzgibbon W: Pathophysiology of altered renal function in renal vascular hypertension. *Am J Kidney Dis* 24: 652–659, 1994
15. Ploth DW, Roy RN, Huang WC, Navar LG: Impaired renal blood flow and cortical pressure autoregulation in contralateral kidneys of Goldblatt hypertensive rats. *Hypertension* 3: 67–74, 1981
16. Rosivall L, Youngblood P, Navar LG: Renal autoregulatory efficacy during angiotensin-converting enzyme inhibition in dogs on a low sodium diet. *Renal Physiol Biochem* 9: 18–28, 1986
17. Kraft V, Deeny AA, Blanchet HM, Boot R, Hansen AK, Hem S, Herck HV, Kunstyr I, Milite G, Needham JR, Nicklas W, Perrot A, Rehbinder C, Richard W,Vroey GD: Recommendations for the health monitoring of mouse, rat, hamster, guinea pig and rabbit breeding colonies. *Lab Anim* 28: 1–12, 1994
18. Braam B, Navar LG, Mitchell KD: Modulation of tubuloglomerular feedback by angiotensin type 1 receptors during the development of Goldblatt hypertension. *Hypertension* 25: 1232–1237, 1995
19. Turkstra E, Braam B, Koomans HA: Losartan attenuates modest but not strong renal vasoconstriction induced by nitric oxide inhibition. *J Cardiovasc Pharmacol* 32: 593–600, 1998
20. Ortíz MC, Atucha NM, Lahera V, Vargas F, Quesada T, Garcia-Estan J: Importance of nitric oxide and prostaglandins in the control of rat renal papillary blood flow. *Hypertension* 27: 377–381, 1996
21. Kiyomoto H, Matsuo H, Tamaki T, Aki Y, Hong H, Iwao H, Abe Y: Effect of L-N^G-nitro-arginine, inhibitor of nitric oxide synthesis, on autoregulation of renal blood flow in dogs. *Jpn J Pharmacol* 58: 147–155, 1992
22. Kobari M, Fukuuchi Y, Tomita M, Tanahashi N, Takeda H: Role of nitric oxide in regulation of cerebral microvascular tone and autoregulation of cerebral blood flow in cats. *Brain Res* 667: 255–262, 1994
23. Tojo A, Gross SS, Zhang L, Tisher CC, Schmidt HH, Wilcoxc CS, Madsen KM: Immunocytochemical localization of distinct isoforms of nitric oxide synthase in the juxtaglomerular apparatus of normal rat kidney. *J Am Soc Nephrol* 4: 1438–1447, 1994
24. Wilcox CS, Welch WJ, Murad F, Gross SS, Taylor G, Levi R, Schmidt HH: Nitric oxide synthase in macula densa regulates glomerular capillary pressure. *Proc Natl Acad Sci USA* 89: 11993–11997, 1992
25. Imig JD, Roman RJ: Nitric oxide modulates vascular tone in preglomerular arterioles. *Hypertension* 19: 770–774, 1992
26. Hayashi K, Suzuki H, Saruta T: Nitric oxide modulates but does not impair myogenic vasoconstriction of the afferent arteriole in spontaneously hypertensive rats. *Hypertension* 25: 1221–1219, 1995
27. Braam B, Koomans HA: Reabsorption of nitro-L-arginine infused into the late proximal tubule participates in modulation of TGF responsiveness. *Kidney Int* 47: 1252–1257, 1995
28. Thorup C, Persson AE: Inhibition of locally produced nitric oxide resets tubuloglomerular feedback mechanism. *Am J Physiol* 267: F606–F611, 1994
29. Iversen BM, Kvam FY, Morkrid L, Sekse I, Ofstad J: Effect of cyclooxygenase inhibition on renal blood flow autoregulation in SHR. *Am J Physiol* 263: F534–F539, 1992
30. Hennington BS, Zhang H, Miller MT, Granger JP, Reckelhoff JF: Angiotensin II stimulates synthesis of endothelial nitric oxide synthase. *Hypertension* 31: 283–288, 1998
31. Iversen BM, Sekse I, Ofstad J: Resetting of renal blood flow autoregulation in spontaneously hypertensive rats. *Am J Physiol* 252: F480–F486, 1987
32. Roman RJ, Kaldunski M: Pressure natriuresis and cortical and papillary blood flow in inbred Dahl rats. *Am J Physiol* 261: R595–R602, 1991
33. Thorup C, Persson AE: Impaired effect of nitric oxide synthesis inhibition on tubuloglomerular feedback in hypertensive rats. *Am J Physiol* 271: F246–F252, 1996
34. Takenaka T, Forster H, De Micheli A, Epstein M: Impaired

myogenic responsiveness of renal microvessels in Dahl salt-sensitive rats. *Circ Res* 71: 471–480, 1992
35. Karlsen FM, Leyssac PP, Holstein-Rathlou NH: Tubuloglomerular feedback in Dahl rats. *Am J Physiol* 274: R1561–R1569, 1998
36. Inscho EW, Carmines PK, Cook AK, Navar LG: Afferent arteriolar responsiveness to altered perfusion pressure in renal hypertension. *Hypertension* 15: 748–752, 1990
37. Ploth DW, Schnermann J, Dahlheim H, Hermle M, Schmidmeier E: Autoregulation and tubuloglomerular feedback in normotensive and hypertensive rats. *Kidney Int* 12: 253–267, 1977
38. Ofstad J, Aukland K: Renal circulation. In: *The Kidney: Physiology and Pathophysiology*, edited by Seldin DW, Giebisch G, New York, Raven, 1985, pp 471–498