Stenosis-dependent role of nitric oxide and prostaglandins in chronic renal ischemia

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Tokuyama, Hiromitsu, Koichi Hayashi, Hiroto Matsuda, Eiji Kubota, Masanori Honda, Ken Okubo, Yuri Ozawa, and Takao Saruta. Stenosis-dependent role of nitric oxide and prostaglandins in chronic renal ischemia. Am J Physiol Renal Physiol 282: F859–F865, 2002. First published November 27, 2001; 10.1152/ajprenal.00012.2001.—The role of nitric oxide (NO) and prostaglandins (PG) in modifying renal hemodynamics was examined in clipped and nonclipped kidneys of unilateral renal artery stenosis. Chronic unilateral renal ischemia was established by 4-wk-clipping the left renal artery of canine kidneys, and renal interstitial nitrate+nitrite and PGE2 contents were evaluated by the microdialysis technique. Unilateral renal artery stenosis caused 45 ± 1 and 73 ± 1% decrements in renal plasma flow (RPF) in moderately and severely clipped kidneys and 21 ± 3% decrements in nonclipped kidneys with severe stenosis. Renal nitrate+nitrite decreased in moderately (−31 ± 1%) and severely clipped kidneys (−63 ±4%). Nω-nitro-L-arginine methyl ester reduced RPF (−56 ± 3%) and glomerular filtration rate (GFR; −54 ± 3%) in moderately clipped kidneys, whereas this inhibitory effect was abolished in severely clipped kidneys. In contrast, renal PGE2 contents increased modestly in moderate clipping and were markedly elevated in severely clipped kidneys (from 111 ± 7 to 377 ± 22 pg/ml); sulpyrine impaired renal hemodynamics only in severely clipped kidneys. In contralateral nonclipped kidneys, although renal PGE2 was not increased, sulpyrine reduced RPF (−32 ± 1%) and GFR (−33 ± 3%) in severe stenosis. Collectively, NO plays a substantial role in maintaining renal hemodynamics both under basal condition and in moderate renal artery stenosis, whereas the contributory role shifts from NO to PG as renal artery stenosis progresses. Furthermore, because intrarenal angiotensin II is reported to increase in nonclipped kidneys, unilateral severe ischemia may render the nonclipped kidney susceptible to PG inhibition.

ischemic nephropathy; renal artery stenosis; renal hemodynamics

Although renal artery stenosis constitutes an important disorder of secondary hypertension, the fundamental process of this disease is attributed to renal ischemia, which would potentially lead to impairment of renal function. Indeed, several lines of studies indicate that renal artery stenosis of any origin, including atherosclerosis, is associated with progressive loss of renal function due to renal ischemia, and a growing body of evidence has been provided that ischemic nephropathy occupies a substantial fraction of end-stage renal disease (8, 24). Undoubtedly, the renin-angiotensin system plays a major role in the development of hypertension, and the effect of this system on renal function has been extensively assessed (12). Nevertheless, the long-term influence of chronic renal artery stenosis on intrarenal vasodilator autocrine/paracrine factors and the adaptive renal hemodynamic action of these substances in stenotic and nonstenotic kidneys remain undetermined.

It has been established that, within the kidney, a variety of vasoactive substances are synthesized in situ and serve to maintain renal homeostasis in the face of the disturbance of renal function. Among these, nitric oxide (NO) and prostaglandins (PG) are important factors contributing to the preservation of renal hemodynamics (3, 4, 27). Thus the inhibition of NO synthesis results in a marked decrease in renal blood flow (RBF) (3, 4), and this observed effect would be the result of elimination of intrinsic NO-mediated renal vasodilation, allowing endogenous vasoconstrictors such as angiotensin II to predominate (32). Alternatively, chronic angiotensin II infusion is demonstrated to stimulate renal NO production (6), which would buffer the renal angiotensin II action. Thus chronic renal artery stenosis, where the renal renin-angiotensin system is activated, may affect the role of NO in the maintenance of renal hemodynamics, and the altered NO activity could counteract the deranged renal function in chronic renal ischemia (31). Additionally, although renal PG is generally acknowledged as a factor preserving renal homeostasis (25, 26, 34), the role of PG in mediating the renal function varies widely depending on the pathophysiological settings of underlying renal disease (14). For example, in renovascular hypertension, increased intrarenal PG may stimulate renal angiotensin II production (9), which would facilitate the development of hypertension. Nevertheless, no investigations have been conducted evaluating comprehensively the role of intrarenal vasodilator action of...
NO and PG in different severity of chronic renal ischemia. In the present study, we simultaneously assessed the changes in renal interstitial nitrate + nitrite (NOx) and PG contents in clipped and nonclipped dog kidneys after 4-wk unilateral renal artery stenosis and evaluated whether renal NO and PG contribute to the preservation of the renal hemodynamics in chronic renal ischemia. Additionally, whether the severity of stenosis influences the relative contribution of NO and PG to countervailing renal hemodynamics was also examined. The present study demonstrates contrasting roles of NO and PG; NO contributes more predominantly to the homeostasis of renal circulation in moderate renal ischemia, whereas in severe ischemia renal PG constitutes a pivotal determinant of renal function in maintaining the renal hemodynamics.

**METHODS**

**Measurements of Systemic and Renal Hemodynamics**

All experimental procedures in this study were conducted according to the guidelines of the Animal Care Committee of Keio University. Forty adult male mongrel dogs (8–13 kg) were fed a standard diet (Oriental Yeast, Tokyo, Japan) and were anesthetized with pentobarbital sodium (30 mg/kg). After intratracheal intubation, each animal was ventilated with an artificial respirator and placed on a heating blanket to maintain body temperature at 37°C. A 7-Fr catheter was inserted through the right femoral artery to measure mean arterial pressure (MAP), and the left radial vein was catheterized for infusion of drug. A 7-Fr catheter (Create Medic, Tokyo, Japan) was placed in the ureter for clearance study. Glomerular filtration rate (GFR) and renal plasma flow (RPF) of both kidneys were individually measured with the use of inulin and p-aminohippuric acid, respectively.

Data on MAP and RBF were analyzed with a Macintosh Laboratory System (Mac Lab, Analog-Digital Instruments, Castle Hill, Australia) (15, 16). The dogs were allowed to recover for 4 wk.

**Measurements of Renal Interstitial NOx and PG**

Renal interstitial NOx and PG levels were determined by using the microdialysis technique (2, 7, 13, 20, 33). A microdialysis tube (diameter, 0.5 mm; transmembrane diffusion cutoff, 10 kDa; Eicom, Kyoto, Japan) was inserted into the renal cortex at a depth of 2 mm from the renal surface (13). The microdialysis tube was perfused with lactated Ringer solution (147 meq/l Na, 4 meq/l K, 5 meq/l Ca, 156 meq/l Cl) at 2 μl/min. At this rate, in vitro recovery was 78 ± 3, 70 ± 4, and 72 ± 3% for nitrite, nitrate, and PGE₂, respectively. The effluent was collected at −20°C for NOx and PG measurement. Nitrite and nitrate concentrations were evaluated with the Griess reaction (20, 30), and the sum of these constituents was considered as a marker of renal NOx levels (7, 13). PG in dialysate samples were measured by radioimmunoassay (23, 29).

**Establishment of Unilateral Renal Ischemia**

Chronic renal ischemia was established by clipping the left renal artery. Through a retroperitoneal incision, the kidney was exposed, and an electromagnetic flow probe was placed around the left renal artery. After a 180-min stabilization period, two 30-min periods were allowed for sampling of renal interstitial fluid and evaluating renal clearance in each study protocol. Thereafter, a silver clip was placed around the renal artery. The internal diameter of the clip was adjusted to reduce RBF of the ipsilateral kidney to 50 and 10% of the prestenotic level by monitoring RBF with an electromagnetic flow probe. In sham-operated dogs, a clip was unattached; otherwise, the same manipulation was conducted.

**Experimental Protocols**

**Effect of renal ischemia on hemodynamics and renal interstitial parameters.** The effects of renal ischemia on systemic and renal clearance parameters were serially assessed before and after 4 wk of renal ischemia. Parameters of systemic (MAP) and renal clearance (RPF, GFR) (16), as well as renal interstitial NOx and PG contents were evaluated in each experimental period (13).

**Roles of NO and PG in renal hemodynamic response to ischemia.** The effects of NO and PG inhibition on renal hemodynamics were determined with the use of Nω-nitro-arginine methyl ester (L-NAME, Sigma, St. Louis, MO; n = 6), sulpyrine, an aspirin analog (21) (Daiichi Seiyaku, Tokyo, Japan; n = 6), and simultaneous injection of L-NAME and sulpyrine (n = 5), respectively. After the 60-min observation of basal renal and systemic hemodynamics, L-NAME (1 mg/kg), sulpyrine (1 mg/kg), or L-NAME (1 mg/kg) + sulpyrine (1 mg/kg) were administered intravenously. Thirty minutes after addition of these agents, the renal hemodynamic effects in clipped and nonclipped kidneys were assessed by clearance study, and the microdialysis technique was conducted consecutively.

**Statistics**

Results are expressed as means ± SE. Data were analyzed by two-way ANOVA with repeated measures, followed by Bonferroni’s post hoc test. P < 0.05 was considered statistically significant.

**RESULTS**

**Effect of Renal Ischemia on Systemic and Renal Hemodynamics**

After 4 wk of unilateral renal artery clipping, MAP did not change with moderate renal artery clipping (from 113 ± 3 to 115 ± 3 mmHg, P > 0.2, n = 17) but was slightly elevated with severe clipping (from 109 ± 2 to 131 ± 2 mmHg, P < 0.0001, n = 17). Plasma renin activity was elevated after 4 wk of renal artery clipping (moderate, from 1.7 ± 0.2 to 2.7 ± 0.2 ng·ml⁻¹·h⁻¹, P < 0.01, n = 17; severe, from 1.7 ± 0.2 to 9.4 ± 1.7 ng·ml⁻¹·h⁻¹, P < 0.0001, n = 17).

RPF in the clipped kidneys was reduced in a stenosis-dependent manner (Fig. 1); 45 ± 1 (from 53 ± 3 to 29 ± 1 ml/min, P < 0.0001, n = 17) and 73 ± 1% decrements (from 58 ± 2 to 16 ± 1 ml/min, P < 0.0001, n = 17) were observed in moderate and severe stenosis, respectively. GFR was also decreased in moderate-stenotic kidneys (from 15 ± 1 to 9 ± 1 ml/min, P < 0.0001, n = 17), but no further reduction was obtained in severe renal ischemia (from 16 ± 1 to 10 ± 1 ml/min, P < 0.0001, n = 17).

In nonclipped kidneys, 4-wk moderate renal artery clipping had no effect on RPF or GFR. Severe stenosis,
however, elicited significant reductions in RPF (from 58 ± 2 to 45 ± 2 ml/min, P < 0.0001, n = 17) without changes in GFR.

The filtration fraction (FF) did not change in moderate ischemia but was elevated in severe ischemia (clipped kidneys, from 0.29 ± 0.01 to 0.63 ± 0.05, P < 0.0001; nonclipped kidneys, from 0.29 ± 0.01 to 0.37 ± 0.03, P < 0.01, n = 17).

**Effect of Renal Ischemia on Renal Interstitial NOx/PG**

In clipped kidneys, renal interstitial NOx content was decreased in a stenosis-dependent manner (moderate, from 8.0 ± 0.4 to 5.6 ± 0.3, P < 0.0001, n = 17; severe, from 8.7 ± 0.5 to 3.0 ± 0.2 µmol/L, P < 0.0001, n = 17) (Fig. 2). In contrast, renal interstitial PGE2 was slightly increased in moderate stenosis (from 114 ± 13 to 197 ± 15 pg/ml, P < 0.001, n = 17) and more markedly elevated in severe ischemia (from 111 ± 7 to 377 ± 22 pg/ml, P < 0.0001, n = 17). In the contralateral, nonclipped kidneys, neither NOx nor PGE2 content was affected.

**Role of NOx/PG in Renal Hemodynamics in Renal Ischemia**

In sham-operated dogs, the inhibition of NO synthesis by L-NAME (1 mg/kg) caused 30 ± 3% reduction in RPF (from 60 ± 7 to 41 ± 4 ml/min, P < 0.05, n = 6) and 30 ± 6% reduction in GFR (from 18 ± 1 to 13 ± 2 ml/min, P < 0.05, n = 6) (Fig. 3). In chronic unilateral renal ischemia, the dose of L-NAME used had no effect on MAP (moderate, from 114 ± 6 to 124 ± 8 mmHg, P > 0.1; severe, from 127 ± 5 to 132 ± 4 mmHg, P > 0.1, n = 6). Renal ischemia markedly altered renal hemodynamic responses to L-NAME (Fig. 3). Thus, in clipped kidneys with moderate stenosis, the L-NAME-induced decreases in RPF and GFR were pronouncedly exaggerated, corresponding to 56 ± 3 and 54 ± 3% decrements from baseline, respectively (n = 6). With severe clipping, however, these inhibitory effects were abolished (P > 0.2, n = 6). In contralateral, nonclipped kidneys from dogs with moderate and severe renal artery stenosis, L-NAME reduced RPF (moderate, −25 ± 3%; severe, −31 ± 5%) and GFR (moderate, −27 ± 3%; severe, −35 ± 3%) to the levels similar in magnitude to those in sham-operated dogs.

The dose of sulpyrine used had no effect on MAP in sham-operated dogs (from 111 ± 3 to 110 ± 3 mmHg, P > 0.1, n = 6), dogs with moderate renal artery clipping (from 113 ± 5 to 112 ± 5 mmHg, P > 0.1, n = 6), or those with severe renal artery clipping (from 133 ± 4 to 132 ± 5 mmHg, P > 0.1, n = 6). In
sham-operated dogs (n = 6), the inhibition of PG production did not alter RPF or GFR (Fig. 4). Similarly, with moderate artery clipping (n = 6), sulpyrine had no effect on RPF (clipped kidneys, P > 0.2; nonclipped kidneys, P > 0.2) or GFR (clipped kidneys, P > 0.2; nonclipped kidneys, P > 0.2). In contrast, in severe unilateral clipping (n = 6), PG inhibition elicited marked reductions in RPF in both clipped (from 17 ± 1 to 6 ± 1 ml/min, P < 0.0001) and nonclipped kidneys (from 45 ± 4 to 31 ± 3 ml/min, P < 0.05). Similarly, GFR was reduced by 74 ± 3 and 33 ± 3% in clipped (from 10 ± 1 to 3 ± 1 ml/min, P < 0.0001) and nonclipped kidneys (from 16 ± 1 to 11 ± 1 ml/min, P < 0.01), respectively. Thus FF tended to decrease in clipped kidneys (from 0.59 ± 0.08 to 0.43 ± 0.04, P = 0.1) but was unaltered in nonclipped kidneys with severe unilateral ischemia (from 0.39 ± 0.06 to 0.38 ± 0.05, P > 0.5).

The alterations in renal interstitial NOx contents paralleled the changes in renal hemodynamic responses to NO inhibition (Fig. 5, top). In sham-operated dogs, the administration of L-NAME decreased renal cortical NOx contents by 29 ± 1% (P < 0.05, n = 6). In clipped kidneys, L-NAME reduced renal interstitial NOx contents in those with moderate (from 5.6 ± 0.4 to 2.1 ± 0.2 μmol/l, P < 0.0001, n = 6) and severe renal ischemia (from 3.1 ± 0.2 to 1.8 ± 0.1 μmol/l, P < 0.05, n = 6); after the L-NAME treatment, the renal NOx contents reached the same levels in moderately and severely clipped kidneys. In nonclipped kidneys, L-NAME elicited similar decrements in renal NOx contents in moderate (−31 ± 1%, n = 6) and severe ischemia (−29 ± 3%, n = 6).

In moderate ischemia, sulpyrine slightly decreased PGE2 contents in clipped kidneys (from 0.05, n = 6), but had no effect in nonclipped kidneys (Fig. 5, lower). In severe ischemia, the increase in renal interstitial PGE2 was suppressed in both clipped (P < 0.0001, n = 6) and nonclipped kidneys (P < 0.001, n = 6).

Finally, the effect of simultaneous treatment with L-NAME and sulpyrine on renal hemodynamics was examined in dogs with unilateral renal artery clipping. In clipped kidneys with moderate stenosis, the L-NAME + sulpyrine-induced decrements in RPF (−52 ± 2%, n = 5) and GFR (−54 ± 3%) were similar to those induced by L-NAME alone (P > 0.1; Fig. 6). In severe clipping, the inhibition by L-NAME + sulpyrine of RPF (−66 ± 1%, n = 5) and GFR (−76 ± 4%) was comparable to the sulpyrine-induced decrements (P > 0.1). In nonclipped kidneys, the L-NAME + sulpyrine-induced decreases in RPF and GFR did not differ from those induced by L-NAME alone in moderate stenosis (RPF, −26 ± 1%; GFR, −34 ± 3%, n = 5) or by sulpyrine alone in severe renal artery stenosis (RPF, −32 ± 2%; GFR, −35 ± 2%,

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**Fig. 4.** Effects of prostaglandins (PG) inhibition on RPF (top) and GFR (bottom) in sham-operated and kidneys with 4-wk renal artery clipping. ***P < 0.0001, **P < 0.01, *P < 0.05 vs. Control. †P < 0.05.

**Fig. 5.** Effects of NO/PG inhibition on renal interstitial NOx (top) and PGE2 (bottom) contents in sham-operated and kidneys with 4-wk renal artery clipping. ***P < 0.0001, **P < 0.01, *P < 0.05 vs. Control. †P < 0.05.

**Fig. 6.** Effects of simultaneous inhibition of PGE2 and NOx on RPF (top) and GFR (bottom) in sham-operated and kidneys with 4-wk renal artery clipping. **P < 0.01, *P < 0.05 vs. Control. †P < 0.05.
n = 5). Similarly, intrarenal contents of NOx and PGE2 simply reflected the sum of l-NNAME- and sulpyrine-induced changes in moderate and severe stenosis, respectively (Fig. 7).

DISCUSSION

Ischemic nephropathy has emerged as a significant clinical entity distinct from renovascular hypertension. Because chronic ischemic renal failure is probably underdiagnosed (24), ischemic nephropathy would constitute a significant subgroup of undiagnosed chronic renal failure among elderly patients. Indeed, it has recently been recognized that renal artery stenosis is responsible for the deterioration of renal function, possibly attributable to renal ischemia but not to blood pressure elevation per se (24). Renal ischemia, however, influences the intrarenal milieu where NO and PG are the most well-known factors contributing to the control of renal hemodynamics. Although the renin-angiotensin system undoubtedly participates importantly in the pathophysiology of ischemic renal disease, the role of intrarenal NO and PG in mediating the altered renal function in chronic ischemic renal disease has not been fully evaluated. Furthermore, there have been no investigations simultaneously evaluating the contribution of NO and PG in different stages (i.e., moderate vs. severe) of chronic renal ischemia.

Although renal artery clipping has been established as a tool for the investigation of renovascular hypertension, it is also well known that subtle changes in the clip size affect the renal hemodynamic consequence. Wiesel et al. (38) noted that clips with a luminal opening of 0.11 mm induced a high percentage of renal infarction, whereas clips with a 0.13-mm opening did not produce hypertension in murine models. To circumvent this technical problem, we used a silver clip by which a vessel diameter can be adjusted by monitoring RBF with an electromagnetic flow probe. With the use of this technique, we succeeded in establishing the quantification of chronic renal ischemia with varying degrees of renal artery stenosis (i.e., 45 ± 1 and 73 ± 1% reduction in RPF in clipped kidneys for moderate and severe stenosis, respectively). Because our chronic ischemic model manifests hypertension in severe renal artery stenosis, the observed effects in such a circumstance appear to be attributable to both renal ischemia and systemic hypertension.

The present study has demonstrated that chronic renal ischemia elicits stenosis-dependent decreases in renal NOx contents (Fig. 2). The contribution of NO to the regulation of renal hemodynamics, however, varies depending on the severity of renal artery stenosis. Thus, in moderately clipped kidneys, the l-NNAME-induced reductions in RPF (−56 ± 3%) and renal NOx contents (−62 ± 5%) were greater than those in kidneys with sham operation (RPF, −30 ± 3%; NOx, −29 ± 1%) or nonclipped kidneys (RPF, −25 ± 3%; NOx, −31 ± 1%; Figs. 3 and 5). In contrast, in severely clipped kidneys, l-NNAME had only a modest effect on RPF and renal interstitial NOx contents. These findings indicate that intrarenal NO plays an important role in the maintenance of renal circulation (25) but that this homeostatic mechanism is less active in severely clipped kidneys. Although the effect of l-NNAME is obviously mediated by direct renal vascular action, a part of its effect may be attributable to the systemic effect, including altered baroreceptor sensitivity (28). Of note, baseline contents of intrarenal NOx are stenosis dependently decreased. Furthermore, NOx levels after l-NNAME administration are the same in moderately and severely clipped kidneys, suggesting a near-maximal suppression of renal NO production. In concert, the differences in intrarenal NOx contents at baseline and after l-NNAME administration would determine the renal hemodynamic responses to this agent, i.e., greater susceptibility to NO inhibition in moderate renal ischemia, and the diminished role of NO in severe stenosis. In this regard, endogenous NO synthase blockers, such as Nω,Nω-dimethylarginine, have been shown to be accumulated in patients with impaired renal function (36) and hypercholesterolemia (5), which frequently coexist with renal artery stenosis (24). Thus, even in moderate renal artery stenosis, the coexistence of these precipitating conditions would be anticipated to be deleterious to renal function. Of course, this premise requires further investigation.

The renal protective role of NO remains a matter of controversy. Previous studies reported the countervailing action of NO when the kidney was exposed to a variety of humoral vasoconstrictor factors, including angiotensin II (39) and endothelin (19). Similarly, the present study demonstrates a beneficial role of NO in kidneys with moderate renal artery stenosis. Nakamoto et al. (17) and Sigmon et al. (32) have also reported that the inhibition of NO synthesis deteriorates the renal function in renovascularly hypertensive dogs and rats, respectively. Thus NO plays an important role as a factor in the preservation of renal circulatory...
homeostasis in the condition where RBF is reduced. Because renal interstitial NOx contents parallel the degree of renal artery stenosis, it appears that shear stress constitutes a determinant of renal NO production. In contrast, Nori et al. (18) demonstrated that in vivo targeting of inducible NO synthase with oligodeoxynucleotides protected against renal ischemia induced by complete renal artery clamping. Thus, when the renal perfusion is completely blocked, increased renal NO produced by inducible NO synthase is responsible for the development of ischemic renal injury. In concert, the role of NO in renal ischemia differs, depending on the severity of renal ischemia, and the divergent activity of endothelial vs. inducible NO synthase might be responsible for such difference.

Contrary to an important role of NO in normal and moderate ischemic kidneys, renal PGE2 contributes to renal homeostasis in a different manner. It has been reported that renal cyclooxygenase activity is enhanced in stenotic kidneys (9, 37), and PG within the ischemic kidney would serve to act as a vasodilator and may buffer the deleterious effect of renal ischemia (6, 10, 22). Nevertheless, the relationship between the severity of renal ischemia and renal PG production has not been elucidated hitherto. In the present study, we have demonstrated that moderate renal ischemia has only a modest effect on renal interstitial PGE2 levels. Moreover, the PG inhibition fails to alter renal hemodynamics (RPF and GFR) in normal or moderately ischemic kidneys. In striking contrast, in chronic severe renal artery clipping, renal PGE2 was pronouncedly increased, and the inhibition of PG synthesis markedly impaired renal hemodynamics with parallel decreases in intrarenal PGE2 production. These observations clearly indicate that the role of renal PG in renal hemodynamics is only modest in normal and moderately ischemic kidneys, but, in severe renal ischemia, renal PGE2 contributes more importantly to the preservation of renal hemodynamics. Thus the role of renal PG depends on the severity of clipping, with a greater contribution in severe stenosis. In this regard, we have recently reported that the inhibition of PG synthesis by NS-398, a specific cyclooxygenase-2 inhibitor, does not affect renal hemodynamics but reduces urinary Na excretion in unilateral renal artery clipping (35). Thus cyclooxygenase-1-mediated PGE2 might constitute an important determinant of renal hemodynamics in chronic unilateral renal ischemia.

Although previous studies demonstrated that both NO and vasodilatory PG contribute to the homeostasis of renal hemodynamics in a variety of renal injuries, no investigations have been conducted simultaneously examining the role of these two substances in renal homeostasis. In renal injury models, such as experimental glomerulonephritis and radiocontrast nephrotoxicity, both NO and PG participate in maintaining renal function (1, 11). The relative contribution of NO and PG to renal function, however, has not been determined in chronic renal ischemia. As demonstrated in the present study, NO plays a substantial role in maintaining renal hemodynamics in normal conditions and contributes importantly to countervailing deranged renal function in moderate renal artery stenosis. In the same experimental setting, the contribution of PG is only modest. In contrast, in severe renal artery clipping, the contributory action of NO is almost abolished. Alternatively, PG constitutes a more important determinant of renal hemodynamic regulation in chronic severe ischemic kidneys. These stenosis-dependent roles of NO and PG indicate a shift in the compensatory mechanisms from NO to PG as renal ischemia progresses. This formulation is supported by our present results that simultaneous administration of l-NAME and sulpyrine did not cause additive reduction in RPF and GFR (Fig. 6) as well as intrarenal NOx and PGE2 (Fig. 7).

Finally, the possible role of angiotensin II and its interaction with PG in renal ischemia merits comments. In the present study, we found that in clipped kidneys with moderate stenosis, RPF and GFR decreased in a parallel manner, with no changes in FF (Fig. 1, left). In contrast, in severe stenosis, GFR did not manifest further reduction, resulting in an elevated FF. Similarly, in nonclipped kidneys, RPF was reduced only in severe stenosis, whereas renal artery clipping with moderate or severe stenosis had no effect on GFR (Fig. 1, right); FF was again increased only in severe stenosis. Ostensibly, these responses mimic those induced by augmented angiotensin II activity, particularly in the efferent arteriole. Indeed, in our preliminary study, we have reported that renal interstitial angiotensin II content is markedly elevated in both clipped and nonclipped kidneys (35). In severe ischemia, therefore, PG inhibition would impair renal hemodynamics not only in clipped kidneys but also in nonclipped kidneys, where enhanced intrarenal angiotensin II action is unveiled during PG inhibition (Fig. 4). Alternatively, it is possible that in nonclipped kidneys with severe unilateral ischemia, elevated MAP impairs renal PG production (34), but this effect is countered by the stimulatory action on PG production of the increased intrarenal angiotensin II activity (6). Similarly, the greater GFR in l-NAME-treated dogs with severely clipped kidneys, compared with that in dogs with moderately clipped kidneys (Fig. 3), may reflect combined effects of enhanced intrarenal angiotensin II activity and increased PG production within the severely stenotic kidneys, both of which could preserve GFR. Thus, in renal ischemia, where intrarenal angiotensin II should be activated, renal PG contributes substantially to the preservation of renal hemodynamics, and the role of renal PG is closely associated with RPF and angiotensin II.

In conclusion, the present study demonstrates that chronic renal artery stenosis markedly alters renal homeostatic mechanisms. Both intrarenal NO and PG play an important role in maintaining renal hemodynamics to compensate for impaired renal function in renal artery stenosis. The contribution of NO and PG, however, differs, depending on the underlying renal hemodynamic circumstances. These two vasoactive autacoids exert renal homeostatic action, with the predominant contribution of NO in moderate renal ischemia but with an increasing role of PG as renal ischemia progresses. Such heterogeneity in renal homeostatic
mechanisms may be associated with the difficulty in characterizing ischemic renal disease.

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