Effects of acute and chronic L-Arginine treatment in experimental hyperuricemia

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ABSTRACT

Experimental hyperuricemia (HU) results in preglomerular arteriolopathy, cortical vasoconstriction and glomerular hypertension. Recently uric acid has been shown to induce endothelial dysfunction. We therefore studied the effect of acute and chronic administration of L-Arginine (a substrate for endothelial nitric oxide synthase) on the renal hemodynamic and vascular structural alterations induced by HU. To induce HU, oxonic acid (OA, 750 mg/kg/day), was administered in male Sprague-Dawley rats. To study the acute effect of arginine, 9 rats received L-Arginine (L-Arg, 15 mg/kg/min) during micropuncture. To elucidate the chronic effect of L-Arg, OA+ 1% L-Arg (n=8) and OA+ 2.5% L-Arg (n=6) (drinking water) were evaluated throughout the 5 week period. Eight normal control (N), and 8 OA, rats were also studied. Kidneys were fixed by perfusion and afferent arteriole morphology was evaluated. HU rats developed the renal functional and structural alterations described and had suppressed urinary excretion of NO$_2$/NO$_3$. Acute stimulation of NO synthesis markedly increased urinary NO$_2$/NO$_3$, lowered systemic blood pressure and relieved cortical vasoconstriction despite a significant increment of glomerular hypertension and afferent arteriole damage. Increasing doses of chronic L-Arg were associated with increasing excretion of urinary NO$_2$/NO$_3$, reduction of systemic hypertension and prevention of cortical vasoconstriction (2.5% L-Arg). In addition, both doses prevented glomerular hypertension and preglomerular arteriolopathy. Thus an acute relief of renal vasoconstriction in the setting of afferent arteriole damage cannot reverse glomerular hypertension, likely due to impairment in preglomerular autoregulation.
On the other hand chronic L-Arg preserved arteriolar structures probably mediated by the antiproliferative effect of NO on VSMC.

**Key words:** endothelial dysfunction, hyperuricemia, renal cortical vasoconstriction, glomerular hypertension, arteriolopathy
INTRODUCTION

Recent studies suggest that hyperuricemia may be able to cause both hypertension and kidney disease. Subjects with elevated serum uric acid levels are at high risk for developing hypertension (17) and kidney disease (16). Hyperuricemia is also common in subjects with untreated hypertension (5; 12). Perhaps most importantly has been the observation that experimental hyperuricemia in rodents results in the development of hypertension and kidney disease (28; 29; 33). The kidney disease is characterized by the development of arteriolosclerosis (preglomerular arteriolopathy), glomerular hypertrophy and sclerosis, and progressive interstitial fibrosis in the absence of intrarenal crystal deposition (28; 29; 33).

The underlying hemodynamic mechanisms associated with uric acid-mediated hypertension and renal disease have been elucidated in micropuncture studies. Hyperuricemia is associated with the development of cortical vasoconstriction (37; 38) resulting in tubulointerstitial changes consistent with ischemia and which is thought to have a role in the development of salt-sensitivity and hypertension (45). Hyperuricemic rats also develop thickened preglomerular arterioles which along with the efferent arterioles vasoconstrict and mediate a fall in single nephron GFR and renal plasma flow (37). Interestingly, the diseased preglomerular arterioles do not constrict enough to prevent the transmission of the elevated systemic pressures into the glomerulus, and as a consequence glomerular hypertension also develops (37; 38). Hence, one observes an unusual situation in which both glomerular hypertension and reduced renal blood flow develop. We have suggested that these hemodynamic changes induced by
hyperuricemia may provide a mechanism for renal progression in subjects with hypertension (18).

Understanding the mechanisms driving the cortical vasoconstriction in response to hyperuricemia is thus of great importance. In this regard, experimental studies have shown that uric acid can reduce nitric oxide levels in endothelial cells (21; 23). Uric acid can also impair aortic ring vasodilation in response to acetylcholine, which is known to be mediated in part by endothelial nitric oxide (32). Furthermore, hyperuricemic rats have low plasma nitric oxide (as reflected by low nitrites and nitrates) which can be rescued by lowering uric acid levels (23). In addition, chronic treatment with the substrate of the nitric oxide synthase, L-Arginine to hyperuricemic rats lowered arterial hypertension, decreased renin and increased NOS-1 expression in the macula densa despite persistence of hyperuricemia (28). Most impressively, hyperuricemic individuals have endothelial dysfunction (46), and lowering uric acid has been shown to improve endothelial function in numerous studies (4; 6; 9; 11; 15; 30).

The mechanism by which uric acid limits the availability of NO is not known, however, it was recently showed that uric acid may increase the activity of NADPH oxidase in cultured adipocytes (39). Increased production of reactive oxygen species and oxidative stress was associated with a significant decrement of NO bioavailability and increase in protein nitrosylation (39). Thus, these findings support a direct role for uric acid in mediating endothelial dysfunction.

On the other hand, supplementation of L-Arginine as a therapeutic measure against endothelial dysfunction appears to have positive benefits for cardiovascular and renal disease (3; 7; 22; 35; 36). Despite the presence of potentially adequate
quantities of L-Arginine inside cells, previous studies have demonstrated that the administration of L-Arginine can increase endothelial NO production (42).

Since a lack of nitric oxide is known to cause both renal vasoconstriction as well as preglomerular arteriolopathy (34), it seems likely that endothelial dysfunction might be responsible for the effects of hyperuricemia on renal hemodynamics. We therefore tested the hypothesis that endothelial dysfunction may have a role in the renal hemodynamic response to hyperuricemia, by administered L-Arginine acutely to rats with hyperuricemia-induced hypertension. In addition we addressed the participation of endothelial dysfunction on the development of preglomerular vessels arteriolopathy by administering L-Arginine chronically to hyperuricemic rats.
METHODS

Five groups of male Sprague-Dawley rats were studied (290-350 gr, Harlan Mexico). All groups were fed with normal rat chow during the study (L-Arg 1.06% Cat 2018S Harlan Teklad, Indianapolis, IN, USA.). Eight rats served as Normal control group (N). Thirty one rats received oxonic acid once a day by gastric gavage (750 mg/kg BW). All groups were followed for 5 weeks. Experiments were approved by the Ethics Committee of Instituto Nacional de Cardiologia Ignacio Chavez.

In order to evaluate both acute and chronic affects of L-Arginine administration we divided oxonic acid treated rats in the following groups: OA control group (OA, n=8); OA + acute L-Arg group(OA+AA, n=9) in which rats received L-Arg (15 mg/kg/min) during the micropuncture experiment along with the infusion of polyfructosan; OA + chronic L-Arg 1% group (OA+CA-1, n=8) in which animals received 1% of L-Arg in drinking water during the 5 weeks of the follow-up; and OA + chronic L-Arg 2.5% group(OA+CA-2.5, n=6) in which rats received 2.5% of L-Arg in drinking water during the 5 weeks of the follow-up.

Measurements

At the end of five weeks systolic blood pressure (SBP) was measured in conscious rats by tail cuff sphyngomanometer (XBP-1000 Kent Scientific Corp, Torrington, CT USA). All animals were preconditioned for blood pressure measurements 1 week before each experiment. Plasma uric acid (Uricostat, Wiener. Argentina) was measured at the same time point. In urine samples taken during micropuncture, the excretion of the final products of NO metabolism (NO\(_{2^-}/NO_{3^-}\)) was measured. Samples were first incubated with E. coli nitrate
reductase to convert the NO$_3^-$ to NO$_2^-$, as described previously (1; 14). After incubation, total NO$_2^-$ was measured using the Griess reagent. Known concentrations of NaNO$_2$ and NaNO$_3$ were used as standards in each assay. Data were corrected by whole GFR and are expressed as nmol/ml.

**Micropuncture**

Animals were anesthetized with pentobarbital sodium (30 mg/kg ip) and placed on a thermoregulated table to maintain body temperature at 37°C. Trachea, jugular veins, femoral arteries and the left ureter were catheterized with polyethylene tubing (PE-240, PE-50, and PE-10). The left kidney was exposed, placed in a Lucite holder, sealed with agar, and covered with Ringer’s solution. Mean arterial pressure (MAP) was monitored with a pressure transducer (Model PT300; Grass Telefactor, Warwick, RI USA) connected to the catheter in the femoral artery and recorded on a polygraph (Grass Instruments, Quincy, MA, USA). Blood samples were taken periodically and replaced with blood from a donor rat. Rats were maintained under euvolemic conditions by infusion of 10 ml/kg of body weight of isotonic rat plasma during surgery, followed by an infusion of 25% polyfructosan, at 2.2 ml/h (Inutest, Fresenius Kabi, Linz, Austria) in Normal and 6 OA rats, the rest of OA group received L-Arginine (15 mg/kg/min) along with the polyfructosan. After 60 min, five to seven samples of proximal tubular fluid were obtained to determine flow rate and polyfructosan concentrations. Intratubular pressure under free-flow (FF) and stop-flow (SFP) conditions and peritubular capillary pressure (Pc) were measured in other proximal tubules with a servo-null device (Servo Nulling Pressure System; Instrumentation for Physiology and
Glomerular colloid osmotic pressure was estimated from protein concentrations obtained from blood of the femoral artery (Ca) and surface efferent arterioles (Ce). Polyfructosan was measured in plasma and urine samples by the anthrone based technique of Davidson and Sackner (8).

The volume of fluid collected from individual proximal tubules was estimated from the length of the fluid column in a constant bore capillary tube of known internal diameter. The concentration of tubular polyfructosan was measured by microfluorometoric method of Vurek and Pegram (43). Protein concentration in afferent and efferent samples was determined according to the method of Viets et al (41). GFR, single-nephron GFR, glomerular capillary hydrostatic pressure (PGC), single-nephron plasma flow (QA), afferent (AR), efferent (ER) resistances and Kf were calculated with equations previously reported (2).

Renal histology and quantification of morphology

After the micropuncture study, kidneys were washed by perfusion with phosphate-buffered saline (PBS) and fixed with 4% paraformaldehyde at approximately 100 mmHg of mean arterial pressure. Kidneys were then excised and weighed. Renal biopsies were embedded in paraffin. Four µm sections of fixed tissue were stained with periodic acid Schiff (PAS) reagent. Arteriolar morphology was assessed by indirect peroxidase immunostaining for alpha-smooth muscle actin (DAKO Corp. Carpinteria, CA, USA). Renal sections incubated with normal rabbit serum were used as negative controls for immunostaining against alpha smooth-muscle actin (37).
Only vessels situated in close proximity to the glomerulus were measured. For each arteriole, the outline of the vessel and its internal lumen (excluding the endothelium) were generated using computer analysis (Image Pro-Plus 5.0 Media Cybernetics, Silver Spring MS USA) to calculate the total medial area (outline – inline), in 10 arterioles per biopsy. The media/lumen ratio was calculated by the outline:inline relationship (37). Quantifications were performed blinded.

Statistical Analysis

Values are expressed as mean±standard error (SE). Groups were analyzed by one way ANOVA and the following set of comparisons were performed: N, OA, OA+AA and N, OA, OA+CA-1 and OA+CA-2.5. The relationship between variables was assessed by correlation analysis. Statistical analysis was performed with Prism version 3.03 (GraphPad Software, San Diego CA, USA). P < 0.05 was considered significant.
RESULTS

*General parameters.*

Body weight was comparable in all groups of rats at the end of the study (N: 342±7 gr; OA: 347±15 gr; OA+AA: 356±4 gr; OA+CA-1: 343±5 and OA+CA-2.5: 346±2 gr) suggesting a similar food consumption among the groups.

As we previously reported (28; 37; 38) after 5 weeks of OA administration rats developed hyperuricemia (N: 1.4±0.14 mg/dL; OA: 3.35±0.17 mg/dL; p<0.001 vs N) and systemic hypertension (SBP; N: 122±1.2 mmHg; OA: 148±4.3 mmHg; p<0.001 vs N) compared to normal control rats. (Fig 1). The group which received acute L-Arg during micropuncture had similar values of UA and SBP as the OA control group at the end of Week 4 (UA: 3.11±0.22 mg/dL, p<0.001 vs N, p= ns vs OA; SBP: 153±3.0 mmHg, p<0.001 vs N, p= ns vs OA).

Neither dose of L-Arginine administered chronically modified UA levels compared to OA group (OA+CA-1: 3.81±0.35 mg/ dL, p= ns vs OA; OA+CA-2.5: 3.21±0.37 mg/dL, p=ns vs OA). Chronic administration of 1% of L-Arg did not have a hypotensive effect in OA treated animals (147±3.4 mmHg, p= ns vs OA), however 2.5% of L-Arg mildly but significantly reduced SBP (135±4.2 mmHg, p<0.05 vs OA).

*Glomerular hemodynamics.*

Urinary excretion of NO$^{-2}$/NO$^{-3}$ is showed in figure 2. In OA group the excretion of NO stable end products was suppressed compared to normal control rats (N: 6.13±0.6 nMol/ml GFR; OA: 0.80 ±0.2 nMol/ml GFR). Although by ANOVA analysis this difference did not reach statistical significance, when we compared
these two groups with unpaired t-test the p value was statistically significant (p<0.0001).

Acute infusion of L-Arg in OA treated rats was associated with a considerable increment of urinary NO\textsubscript{2}/NO\textsubscript{3} excretion of almost 10 times compared to the normal control group and 75 times compared to OA rats (OA+AA: 59.0±13.0 nMol/ml GFR p<0.001 vs N and p<0.001 vs OA+V).

Increased doses of chronic L-Arg treatment was associated with augmented excretion of urinary NO\textsubscript{2}/NO\textsubscript{3}. Thus rats which received 1% L-Arg excreted 18.1±4.5 nMol/ml GFR (p< 0.05 vs OA) and animals which received 2.5% L-Arg excreted 33.5±10.3 nMol/ml GFR (p<0.001 vs OA, p<0.05 vs N, p<0.05 vs OA+CA-1)

In the hyperuricemic (OA) group, mean arterial pressure was significantly higher (N: 123±3 mmHg; OA: 149±2 mmHg; p<0.001) while whole GFR was similar compared to normal control animals (N: 0.85±0.1 ml/min; OA+V: 0.68±0.1 ml/min; p=ns). These findings are similar to our earlier studies of hyperuricemic rats (OA treated) at 5 weeks (37).

Acute stimulation of NO synthesis by acute L-Arg infusion decreased MAP (127±4 mmHg, p<0.01 vs OA) and raised whole GFR (1.2±0.2 ml/min, p<0.05).

As with systolic blood pressure, lower doses of chronic L-Arg (1%) did not modify MAP measured in anesthetized animals during micropuncture (150±7 mmHg, p=ns vs OA, p<0.01 vs N) neither did the 2.5% dose of L-Arg (141±3 mmHg; p=ns vs OA, p<0.01 vs N). Whole kidney GFR was similar in OA+CA-1 compared to the OA group (0.92±0.1 ml/min, p=ns vs OA), whereas the increment
in GFR observed with OA+CA-2.5 reached statistical significance compared to OA rats (1.2±0.2 ml/min, p<0.05 vs OA).

Previously we reported that OA-induced hyperuricemia was associated with cortical vasoconstriction, a decrease in single nephron GFR (SNGFR), and a reduction in the glomerular plasma flow and ultrafiltration coefficient; these changes were coupled with a rise in both afferent and efferent arteriolar resistances (37). In the present study, we confirmed these findings: OA rats had lower SNGFR (N: 28±1 nl/min; OA: 18±2 nl/min, p<0.05; Fig.3), reduced glomerular plasma flow (N: 91±10 nl/min; OA+V: 67±6 nl/min, p<0.01) and a decreased ultrafiltration coefficient (N: 0.035±0.003 nl/s/mmHg; OA: 0.015±0.002 nl/s/mmHg; p<0.01). Similarly, hyperuricemia was associated with an increase in afferent (N: 3.45±0.3 dyn*s/cm⁻⁵; OA: 6.0±0.6 dyn*s/cm⁻⁵, p<0.01 vs OA, Fig 3) and efferent resistances (N: 1.9±0.2 dyn*s/cm⁻⁵; OA+V: 3.2±0.3 dyn*s/cm⁻⁵, p<0.01 vs OA, Fig 3).

Stimulation of NO synthesis with acute L-Arg infusion completely reversed cortical vasoconstriction. Furthermore, SNGFR (OA+AA: 44±10 nl/min, p<0.05 vs OA, Fig 3), glomerular plasma flow (OA+AA: 183±38, p<0.01 vs OA, p<0.05 vs N), and ultrafiltration coefficient significantly rose (OA+AA: 0.031±0.006 nl/s/mmHg, p<0.05 vs OA). Acute L-Arginine-induced vasodilatation in OA treated rats was further evidenced by a significant decrement of afferent (OA+AA: 2.0±0.5 dyn*s/cm⁻⁵, p<0.001 vs OA, Fig 3) and efferent arteriolar resistances (OA+AA: 1.5±0.2 dyn*s/cm⁻⁵, p<0.001 vs OA, Fig 2).

One percent of L-Arg administered chronically to hyperuricemic rats partially alleviated cortical vasoconstriction. We observed slight increments of SNGFR
(26±1 nl/min, p= ns vs OA) glomerular plasma flow (101±5 nl/min, p=ns vs OA) and ultrafiltration coefficient (0.027±.001 nl/s/mmHg, p=ns vs OA) although they did not reach statistical significance. However the reduction in afferent (4.1±0.3 dyn*s/cm⁻⁵, p<0.01 vs OA) and efferent resistences (1.9±2 dyn*s/cm⁻⁵, p<0.001 vs OA) were significant compared to the OA group.

On the other hand, higher dose of chronic L-Arg (2.5%) fully prevented cortical vasoconstriction. Single nephron GFR (57±15 nl/min, p<0.001 vs OA; p<0.01 vs N), glomerular plasma flow (207±52 nl/min, p<0.001 vs OA; p<0.01 vs N) and ultrafiltration coefficient (0.069±.02 nl/s/mmHg, p<0.001 vs OA; p<0.01 vs N) reached values even greater than that observed in the normal group. In addition reductions of afferent (2.4±0.5 dyn*s/cm⁻⁵, p<0.001 vs OA, p<0.05 vs OA+CA-1) and efferent (1.2±0.2 dyn*s/cm⁻⁵, p<0.001 vs OA) resistances were significant.

In contrast, mild hyperuricemia results in glomerular hypertension despite cortical vasoconstriction (37; 38). In the present study we confirmed this effect (N: 49±1.1 mmHg; OA: 56±1.6 mmHg, p<0.05, Fig 2).

Although acute L-Arg infusion fully prevented cortical vasoconstriction, it induced a further increment of glomerular pressure (63±3 mmHg, p<0.05 vs OA; p<0.01 vs N. Fig 2). To better understand why glomerular pressure remained elevated in the setting of lower blood pressure and renal vasodilatation in OA administered acutely L-Arg, we calculated the ratio between efferent/afferent arteriolar resistances (ER/AR). This relationship was similar between normal and OA groups (N: 0.57± 0.1; OA: 0.55± 0.02), while in OA rats administered acutely L-Arg the ratio of ER/AR was significantly higher (OA+L-Arg: 0.86±0.1, p<0.01 vs OA; p<0.05 vs N) suggesting that in this group ER was relatively elevated
compared to the other two groups. In addition we found a positive correlation between ER/AR ratio and glomerular pressure (r= 0.8, p<0.0001, Fig 4 A).

In contrast, both doses of L-Arginine administered chronically prevented the rise of glomerular pressure (OA+CA-1: 51±2 mmHg, p<0.05 vs OA. OA+CA-2.5: 49±1 mmHg, p<0.01 vs OA).

Arteriolar morphology.

We also evaluated the effect of acute L-Arg infusion on the afferent arteriolar lesion which is known to be present in hyperuricemic rats (29; 37; 38). Similar to previous reports (29; 37; 38) OA-induced hyperuricemia was associated with arteriolar wall thickening (N: 2.2±0.3; OA: 3.7±0.4, p<0.01, Fig 4B). As expected, acute L-Arg infusion did not have any effect on this structural modification of the preglomerular vessels (OA+AA: 3.6±0.3, p<0.01 vs N, Fig 4B).

While acute L-Arg did not modify the preglomerular lesion, the chronic administration of L-Arg totally prevented vascular wall thickening (OA+CA-1: 2±0.1, p<0.001 vs OA. OA+CA-2.5: 2.7±0.2, p<0.05 vs OA).

Furthermore a positive correlation was demonstrated between M/L ratio and glomerular pressure when all groups were analyzed together (r= 0.61, p=0.0001, Fig 4C).
DISCUSSION

In this study we examined the effect of acute and chronic administration of L-Arg on the glomerular hemodynamic changes induced by mild hyperuricemia in rats. We first confirmed that after 5 weeks of OA treatment rats developed hyperuricemia, arterial and glomerular hypertension, cortical vasoconstriction and arteriolopathy of the afferent arteriole (37); in addition, as previously reported (23), OA-induced hyperuricemia was associated with a decreased synthesis of NO as indicated by the suppression of urinary excretion of NO\textsuperscript{2}/NO\textsuperscript{3}. On the other hand, acute and chronic L-Arginine administration in hyperuricemic rats stimulated NO synthesis, as reflected by a marked increment of NO\textsuperscript{2}/NO\textsuperscript{3} urinary excretion.

The primary finding was that the acute administration of L-Arginine could reverse the renal cortical vasoconstriction and that this was associated with an increase in single nephron GFR and a decrease in mean arterial pressure. However, glomerular hypertension was not improved, likely due to the presence of the preglomerular arteriolar lesion that we have previously shown to correlate with impaired autoregulation. In contrast, the chronic administration of L-Arg (mainly the high dose) from the initiation of the model was able to prevent both systemic and glomerular hypertension and the renal vasoconstriction, and this was associated with maintenance of normal afferent arteriolar morphology. Thus, these results support the notion that endothelial dysfunction contributes to the systemic hypertension, renal vasoconstriction, glomerular hypertension and arteriolopathy observed in rats with hyperuricemia-induced hypertension.
Nitric oxide is a labile substance with a short half-life which decomposes rapidly to NO$_2^-$ and NO$_3^-$ in biological solutions (27); these stable end-products have been measured as an index of NO production (31). In the present study we found that OA treated animals had an almost suppressed excretion of NO$_2^-/NO$_3^- coupled to systemic hypertension and renal cortical vasoconstriction. In this respect, a significant decrement of urinary excretion of NO$_2^-/NO$_3^- has been reported in other models of hypertension, endothelial dysfunction and renal vasoconstriction such as dTGR rats (30), lead induced hypertension (39), aging SHR (25), and rats administered with angiotensin II (13) and L-NAME (34).

The acute infusion of L-Arg was associated with a marked increase in urinary nitrites suggestive of an increase in endothelial nitric oxide production. Moreover increased doses of L-Arg administered chronically also induced increased excretion of urinary nitrites suggesting a dose-related effect. In concert with the rise in nitrite excretion we observed a significant reduction in cortical vasoconstriction and in systemic hypertension in acutely infused rats whereas during chronic treatment only the higher dose of L-Arg (2.5%) partially reduced blood pressure. This apparent paradox might be related to a higher plasma concentration of L-Arg that would have been predicted to occur during acute infusion.

An important observation in this study was that acute L-Arg administration corrected the renal vasoconstriction but not the glomerular hypertension, while chronic administration also prevented the rise in glomerular pressure. This is likely because the immediate correction of endothelial dysfunction could not reverse the
structural changes in the glomerular arterioles whereas chronic stimulation of NO synthesis with L-Arg was able to prevent the afferent arteriole hypertrophy. In this regard it is well known that NO has an antiproliferative effect on VSMC (10; 19).

We have previously reported that the induction of preglomerular arteriolar disease disrupts normal renal autoregulation and results in the development of glomerular hypertension (37; 38). In this study we observed that the renal vasodilatation induced by acute L-Arg acted to enhance glomerular pressures further, likely because the diseased arterioles could not constrict adequately in response to systemic pressure. Consistent with these findings was the presence of a positive correlation between the individual values of M/L ratio and glomerular pressure. In addition the ratio between ER/AR was higher in OA+ AA than in OA group (0.64±0.02 vs 0.86±0.13). Therefore efferent resistance, despite a significant decrement in L-Arg infused rats, is still elevated for the rise in glomerular plasma flow (175% higher in OA+AA); this effect could contribute to maintain glomerular hypertension. In support to this contention we found a positive linear relationship between ER/AR ratio and glomerular pressure when N, OA and OA+AA animals were included (r= 0.80, p<0.0001).

We do not know the reason why ER was proportionally more elevated in OA+AA rats; one possible explanation could be the preferential effect of NO on afferent arteriole (24); another possibility may relate to the proliferative effect of uric acid on VSMC (29). In renal vascular beds, hyperuricemia may induce vascular changes in a wide variety of arterial vessels (20); thus involvement of the postglomerular arteriole could impair its ability to vasodilatate in response to NO.
On the other hand, chronic L-Arg administration in addition to preventing renal vasoconstriction also preserved arteriolar structure and maintained glomerular pressure within normal levels. The effect of preserving afferent arteriole morphology on the renal autoregulatory response was better demonstrated in rats which received the lower dose of L-Arg. Thus although 1% of L-Arg did not reduced systemic hypertension, the increment of blood pressure was not transmitted to glomerular capillaries due to an adequate increment in afferent resistance in these animals.

The mechanism by which uric acid inhibits endothelial NO levels is an area of intense study. Cell culture studies have demonstrated that uric acid must enter the endothelial cell in order for an inhibition of endothelial NO levels to occur (21). The effect to reduce endothelial NO levels is also not immediate, and consistent with this observation is the finding that acute infusion of urate into humans does not cause endothelial dysfunction (44). There is some evidence that the effect of uric acid to induce endothelial dysfunction may involve an effect of uric acid to stimulate oxidant production, either NADPH oxidase (39) or via stimulation of C-Reactive Protein (21). In this regard, while uric acid may function as an antioxidant, it can also assume pro-oxidative effects under a variety of conditions (26).

In conclusion, both endothelial dysfunction and arteriolar damage participate in the glomerular hemodynamic alterations induced by mild hyperuricemia. Endothelial dysfunction appears to drive the renal vasoconstriction, whereas arteriolar damage predisposes to glomerular hypertension. The combination of these major mechanisms likely accounts for both the risk for hypertension and renal progression observed with experimental hyperuricemia.
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DISCLOSURES

Dr. Richard J. Johnson is consultant for TAP Pharmaceuticals.
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FIGURE LEGENDS

Figure 1. Values of plasma uric acid and systolic blood pressure in N, OA and OA+L-Arg groups at the end of the follow-up (In AO+AA group before acute infusion of L-Arg).

Figure 2. Urinary excretion of nitric oxide stable end products NO\(^{-2}\) and NO\(^{-3}\) measured in samples taken during micropuncture studies.

Figure 3. Glomerular hemodynamics in N, OA, OA+AA, OA+CA-1 and OA+CA-2.5 groups at the end of 5 weeks of follow-up.

Figure 4. A) Correlation between individual values of the afferent resistance/efferent resistance ratio (ER/AR) and glomerular pressure including N, OA and OA+AA groups. B) Afferent arteriole media to lumen (M/L) ratio in N, OA, OA+AA, OA+CA-1 and OA+CA-2.5. C) Correlation between individual values of M/L ratio and glomerular pressure including N, OA, OA+AA, OA+CA-1 and OA+CA-2.5 groups.
Plasma uric acid (mg/dL)

Systolic blood pressure (mmHg)

- Normal
- Oxonic acid
- OA+CA-1%
- OA+CA-2.5%
- OA+AA

a = p < 0.05 vs OA; b = p < 0.05 vs CA-1%; c = p < 0.05 vs CA-2.5%; d = p < 0.05 vs NI
Urinary NO\textsubscript{2}/NO\textsubscript{3} (nMol/ml GFR)

- Normal
- Oxonic acid
- OA+CA-1%
- OA+CA-2.5%
- OA+AA

a = p < 0.05 vs OA; b = p < 0.05 vs CA-1%; c = p < 0.05 vs CA-2.5%; d = p < 0.05 vs NI
Single nephron GFR
nl/min

Afferent resistance
dyn*s/cm²

Glomerular pressure
mmHg

Efferent resistance
dyn*s/cm²

Key:
- Normal
- Oxonic acid
- OA+CA-1%
- OA+CA-2.5%
- OA+AA

a: p<0.05 vs OA; b: p<0.05 vs CA-1%; c: p<0.05 vs CA-2.5%; d: p<0.05 vs NI