Asymmetrical dimethylarginine plasma clearance persists after acute total nephrectomy in rats

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Carello, Katari A., Steven E. Whitesall, Mary C. Lloyd, Scott S. Billecke, and Louis G. D’Aley. Asymmetrical dimethylarginine plasma clearance persists after acute total nephrectomy in rats. Am J Physiol Heart Circ Physiol 290: H209–H216, 2006. First published August 19, 2005; doi:10.1152/ajpheart.00208.2005.—Elevated plasma concentrations of asymmetrical dimethylarginine (SDMA) and asymmetrical dimethylarginine (ADMA) are repeatedly associated with kidney failure. Both ADMA and SDMA can be excreted in urine. We tested whether renal excretion is necessary for acute, short-term maintenance of plasma ADMA and SDMA. Sprague-Dawley rats underwent sham operation, bilateral nephrectomy (NPX), ureteral ligation, or ureteral section under isoflurane anesthesia. Tail-snip blood samples (250 μl) were taken before and at 6- or 12-h intervals for 72 h after operation. Plasma clearance was assessed in intact and NPX rats. High-performance liquid chromatography determined SDMA and ADMA concentrations. Sodium, potassium, creatinine, blood urea nitrogen (BUN), and body weight were also measured. Forty-eight hours after NPX, SDMA increased 25 times (0.23 ± 0.03 to 5.68 ± 0.30 μM), whereas ADMA decreased (1.17 ± 0.03 to 0.73 ± 0.08 μM) by 38%. Creatinine and BUN increased, paralleling SDMA. Sham-operated animals showed no significant changes. Increased SDMA confirms continuous systemic production of SDMA and its obligatory renal excretion, much like creatinine. In contrast, decreased plasma clearance of ADMA appeared unchanged 48 h after NPX. We conclude that renal excretory function is needed for SDMA elimination but not needed for acute, short-term ADMA elimination in that systemic hydrolysis is fully capable of clearing plasma ADMA.

nitric oxide synthase inhibitor; dimethylarginine dimethylaminohydrolase; source of asymmetrical dimethylarginine

AWARENESS OF THE PHYSIOLOGICAL IMPORTANCE of nitric oxide (NO) continues to grow as our basic understanding of the control of NO synthase (NOS) accumulates (12, 35). Endogenous inhibitors of NOS, such as asymmetrical (ADMA) dimethylarginine (DMA) and N6-monomethyl-l-arginine (L-NMMA) have been found to inhibit NOS (13, 61). In addition to the biological actions of ADMA in vivo, ADMA is also a reliable marker for cardiovascular disease (11). In selected populations it is an excellent predictor of cardiovascular events (62, 63) and mortality in the intensive care unit (43).

Elevated plasma ADMA levels have been reported in hypercholesterolemia (6), hypertension (57), hyperhomocyst(e)inemia (56), peripheral arterial occlusive disease (7), experimental hemorrhage (4), preeclampsia (17), hyperglycemia (37), and insulin resistance in patients (55). Even hindlimb ischemia may independently trigger the release of ADMA as suggested by indirect evidence for release of an endogenous NOS inhibitor in response to acute hindlimb ischemia (23–26). A recent review of Fliser et al. (19) traces the progression of ADMA, an innocent marker, to its current position as a leading candidate for a mediator of atherosclerotic complications in patients. In contrast, the physiological control, origins, and fates of this endogenous NOS inhibitor has yet to be fully defined.

In 1970, Kakimoto and Akazawa (27) first reported the natural occurrence of ADMA and symmetrical DMA (SDMA) in human urine and detected concentrations >30% higher than that of arginine in urine. In contrast, the plasma ADMA and SDMA concentrations they reported were <0.5% (0.3 μM) of the concentration of arginine (55.8 μM) in the plasma, reflecting a dramatic urinary concentrating effect on ADMA and SDMA and unequivocally demonstrating their renal excretion.

By 1976, intravenous injections of radiolabeled, methylated arginines were used to demonstrate that L-NMMA and ADMA were catabolized in some way, whereas SDMA was largely unchanged in urine excreted (39). Ogawa et al. (47) later identified an enzyme that hydrolyzed ADMA to citrulline. This enzyme, dimethylarginine dimethylaminohydrolase (DDAH), has been identified in the heart, brain, lung, liver, and skeletal muscle and has been shown to have particularly high activity in the kidney and pancreas (48). Although significant hydrolysis of SDMA in vivo has yet to be reported in the literature (47), Siroen et al. (53) have recently demonstrated that the human liver takes up SDMA as well as ADMA. Thus, for ADMA, the general capacity for both renal excretion and DDAH hydrolysis has been established.

The kidneys are a likely focal point for exploring the physiological control of plasma ADMA, because elevated ADMA has been often reported in association with renal failure (29, 64, 65, 68). Infusion of supressor doses of ADMA has been shown to decrease effective renal plasma flow in humans (33). With mildly pressor doses, ADMA has been shown to decrease effective renal plasma flow and to reduce cardiac output (32). Current literature, including a study of some investigators from our own laboratory (51), repeatedly correlates reduced renal function with elevated plasma ADMA. However, such correlations do not establish causality. Most recently, elevated plasma ADMA has been shown not only to be inversely related to glomerular filtration rate (GFR) but also to be a strong and independent risk marker for the progression of end-stage renal disease and mortality (49). Even in mild to...
moderate kidney disease, elevated plasma ADMA has been shown to be an independent predictor of disease progression (20). These latter authors speculated that elevated plasma ADMA may even promote progression of renal (vascular) disease. It is possible, then, that elevated plasma ADMA could be both caused by reduced renal function and contribute to the progressive loss of renal function, particularly GFR.

This study focuses on a presumed role of the kidney in determining the plasma concentrations of ADMA and SDMA (collectively DMA). Although our understanding of plasma clearance of ADMA continues to evolve, some authors have suggested that the loss of renal excretory function is responsible for elevated ADMA levels (57, 64). The experiments presented here were not designed to model end-stage renal disease but to test the hypothesis that acute loss of renal function is capable of, and indeed responsible for, increasing plasma ADMA and SDMA. In addition, we have used bilateral nephrectomy (2 × NPX), ureteral section, and ureteral occlusion protocols in rats as definitive mechanistic experiments to determine the necessity for renal excretory and synthetic roles in acutely maintaining plasma ADMA and SDMA. Plasma clearance, in contrast to renal clearance of ADMA in NPX rats, was used as a surrogate for post-NPX in vivo DDAH activity. Our novel observation is that removal of the kidneys acutely decreases plasma ADMA rather than increases plasma ADMA and that renal excretory function is needed for SDMA elimination but is not needed for ADMA clearance.

MATERIALS AND METHODS

Adult male Sprague-Dawley rats (200–400 g; Charles River) were anesthetized with isoflurane via chamber induction (5%) and maintenance (1–2.5%). Fed or 24-h fasted animals with free access to water underwent acute 2 × NPX through either bilateral flank incisions or a single dorsal midline incision. Separate groups underwent bilateral ureteral section or ligation through a 1-cm midline incision over the bladder. The ureters were isolated at their entrance to the bladder and either sectioned or ligated bilaterally. Sham-operated animals received anesthesia, incisions, and manipulation but no NPX, ureteral section, or ligation. Blood samples (250 μl into heparin-coated tubes) were taken before operation or sham operation and at 6- or 12-h intervals for 48 h in the ligation and ureteral section protocols and up to 72 h in the NPX protocol using a standard tail-snip method in a heated restraint. Animals surviving 72 h were anesthetized with isoflurane and euthanized with pentobarbital sodium (150 mg/kg ip). Peritoneal fluid from the ureteral-section rats was sampled for analysis, and total fluid volume was estimated by weighing sponges collected from the cavity. All animals were housed in metabolic cages controlled at 36°C. Standards, blanks, and samples (10 μl) were transferred to a low-volume insert and heated (55°C) for 20 min at 4°C. The filtrate was spiked 1:6 (20–100 μl) with l-NMMA (Novabiochem, San Diego, CA) as an internal standard for solid phase extraction. The spiked filtrate was extracted by loading 60 μl onto 50 mg of conditioned (1 ml 100% methanol) and equilibrated (1 ml 2% TCA) cation exchange, silica-based sorbent (Isolute SCX2, 50 mg/1 ml, International Sorbent Technology). Each loaded sample was sequentially gravity rinsed with 0.5 ml of the equilibration solution, 2 μl of a 15 mM (pH 8.00) sodium phosphate, and 0.5 ml of 100% methanol. Sample analytes were eluted by vacuum with 1.5 ml of eluant (10% TEA, 20% H2O, and balance methanol) and evaporated to dryness in air at 60°C. The dry residue was reconstituted and derivatized with 80 μl of a pH 8.2 borate buffer and 20 μl of fluorescent derivatizing agent (AccQ-Flour, Waters, Milford, MA). After 1 min of incubation, the 100 μl were transferred to a low-volume insert and heated (55°C) for 10 min to complete reaction before injection on the column.

High-performance liquid chromatography. The DMA ADMA and SDMA samples in plasma were quantified by reverse-phase liquid chromatography (Breeze System, Waters). A two-pump gradient system and column heater (Binary HPLC Pump 1525, Waters) delivered 88% to 86% (at 34 min) to 0% 10 mM sodium acetate trihydrate (pH 4.76) with balance methanol at 1 ml/min for 49 min. Separation was performed on a 4.6 mm × 150 mm, 3.5 μm column (XterraMS C18, Waters) with an identical 3.9 mm × 20 mm guard column, both controlled at 36°C. Standards, blanks, and samples (10 μl) were injected (717Plus Autosampler, Waters), and fluorescent peak height and area were evaluated at an excitation of 250 nm and an emission of 395 nm (2475 Multi-wavelength Fluorescence Detector, Waters) to 0.1 μM original concentrations. Standard curves were produced for 0, 0.37, 0.75, 1.5, and 3.0 μM ADMA and SDMA, and chromatograms showed stable retention times, and substantial peak separation were apparent. The average method detection limit (MDL; 46a) was calculated from 3 sets of 10 replicates in plasma and standards and is 0.07 μM for SDMA and 0.13 μM for ADMA. The average intra-assay coefficient of variation (CVs) (2 assays of n = 4 samples) for ADMA and SDMA (1.5 μM standard) were 2.4 and 3.3%, respectively. Intra-assay CVs (n = 8 samples) for ADMA and SDMA in pooled rat plasma (mean of 1.1 μM) were 2.2 and 3.2%, respectively. The inter-assay CV (n = 4 samples) for 1.5 μM was 2.6% for ADMA and 0.3% for SDMA. Assay results are presented in micromolars.

Statistical analysis. Measured variables are means ± SE and were considered statistically significant using the paired two-tailed Student’s t-test if P ≤ 0.05. The plasma ADMA and SDMA post-NPX time-course values were not statistically and significantly different.

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Nephrectomy reduces ADMA and increases SDMA

Blood urea nitrogen (BUN) was measured spectrophotometrically by a quantitative enzymatic colorimetric assay (Sigma kit 640-A; Beckman model 34 spectrophotometer). Sodium and potassium were measured by using standard flame photometry.

Plasma DMA clearance protocol. Three additional groups of anesthetized rats were used to assess plasma clearance of ADMA and SDMA. Each group (intact, acute 2 × NPX, and 48-h 2 × NPX) had the same protocol to establish an elevated steady-state concentration of ADMA and SDMA from which clearance (washout) was determined by stopping the infusion and repeatedly sampling as concentrations returned toward baseline. An intravenous bolus (1 mg/kg) of both ADMA and SDMA was followed immediately by an infusion of 0.05 mg·kg⁻¹·min⁻¹. To confirm steady-state plasma levels, arterial blood samples were taken at 60, 80, and 90 min during infusion after which the infusion was stopped. Precisely timed samples were taken at 3, 6, 9, 15, 27, and 51 min, thus allowing plasma concentration to decline from the steady-state levels toward baseline. Each sample was or below 100 μg/l such that <5% total blood volume was withdrawn and plasma was separated and frozen in HPLC vials.

Sample preparation for ADMA and SDMA assay. Heparinized plasma (100 μl) was ultrafiltered through a 10,000-mol wt cutoff membrane of regenerated cellulose (Ultracel MC, Millipore, Bedford, MA) at 16,000 g for 20 min at 4°C. The filtrate was spiked 1:6 (20–100 μl) with l-NMMA (Novabiochem, San Diego, CA) as an internal standard for solid phase extraction. The spiked filtrate was extracted by loading 60 μl onto 50 mg of conditioned (1 ml 100% methanol) and equilibrated (1 ml 2% TCA) cation exchange, silica-based sorbent (Isolute SCX2, 50 mg/1 ml, International Sorbent Technology). Each loaded sample was sequentially gravity rinsed with 0.5 ml of the equilibration solution, 2 μl of a 15 mM (pH 8.00) sodium phosphate, and 0.5 ml of 100% methanol. Sample analytes were eluted by vacuum with 1.5 ml of eluant (10% TEA, 10% NH4OH, 20% H2O, and balance methanol) and evaporated to dryness in air at 60°C. The dry residue was reconstituted and derivatized with 80 μl of a pH 8.2 borate buffer and 20 μl of fluorescent derivatizing agent (AccQ-Flour, Waters, Milford, MA). After 1 min of incubation, the 100 μl were transferred to a low-volume insert and heated (55°C) for 10 min to complete reaction before injection on the column.
Nephrectomy reduces ADMA and increases SDMA

RESULTS

2×NPX. Figure 1A shows a progressive decline in plasma ADMA to 53% of baseline values by 72 h after 2×NPX. Simultaneously, there was a dramatic increase in plasma SDMA (Fig. 1B) in contrast with sham-operated animals that showed no sustained change in either ADMA or SDMA. Although the kidney is capable of excreting both ADMA and SDMA, these data demonstrate that the removal of both kidneys caused opposite effects of decreasing plasma ADMA concentration and simultaneously increasing plasma SDMA concentration.

Only half the 2×NPX animals survived the full 72 h. Subsequent protocols and comparisons were done at 48 h after 2×NPX (Table 1). The average plasma concentrations at 48 h, when 9 of the 10 rats were still alive, show that SDMA increased by 25 times, whereas ADMA decreased to 62% of baseline values. These opposite effects on SDMA and ADMA were both statistically significant. Table 1 also shows data from a separate group of 2×NPX rats, which indicate the expected increase in plasma creatinine, BUN, and potassium and relatively stable plasma sodium, total protein, and hematocrit. The pH declined by 0.10 pH, and there was a <10% decline in body weight at 48-h post-NPX. The increase in SDMA is similar to the increase in plasma creatinine, suggesting that 2×NPX eliminates the excretory mechanism for both SDMA and creatinine.

Figure 2A contrasts the effects of 2×NPX, bilateral ureteral section, or bilateral ligation on plasma ADMA. There was ~50% reduction of plasma ADMA in 48 h due to either 2×NPX or ligation; however, plasma ADMA was maintained in rats undergoing bilateral ureteral section. When the mass (volume × concentration) of ADMA in the peritoneal cavity of the ureteral-sectioned rats is mathematically added to the ADMA mass in the plasma, the calculated plasma concentration would be 3.53 ± 0.45 μM or almost three times their actual measured plasma concentration. Thus the kidney with the sectioned ureters excreted, albeit into the peritoneum, large quantities of ADMA over the 2-day period. However, with ureteral ligation, there was apparently a reduction in formation of ADMA and/or an increase in systemic hydrolysis, because the plasma concentration of ADMA fell to levels similar to the 2×NPX group. Figure 2B contrasts the increase in SDMA due to 2×NPX with more modest increases due to ureteral ligation and ureteral section. When the mass of SDMA in the peritoneal cavity of the ureteral-sectioned rats is mathematically added to the mass in the plasma, the calculated SDMA plasma concentration would be 6.18 ± 0.41 μM or almost three times their actual measured plasma concentration. Thus the kidney in the

![Figure 1A](http://ajpheart.physiology.org/) and ![Figure 1B](http://ajpheart.physiology.org/)

**Table 1. Control values and values at 48-h postnephrectomy**

<table>
<thead>
<tr>
<th>n</th>
<th>ADMA, μM</th>
<th>SDMA, μM</th>
<th>Cr, mg/dl</th>
<th>BUN, mg/dl</th>
<th>Na⁺, mmol/l</th>
<th>K⁺, mmol/l</th>
<th>Plasma protein, mg/ml</th>
<th>Hematocrit, %</th>
<th>Whole blood, pH</th>
<th>Body weight, g</th>
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<td>CNT 10</td>
<td>1.17±0.08</td>
<td>0.73±0.08</td>
<td>P &lt; 0.009</td>
<td>2.36±1.06</td>
<td>174.0±25.6</td>
<td>P &lt; 0.001</td>
<td>1.12±0.7</td>
<td>42.3±0.1</td>
<td>7.41±0.02</td>
<td>253.4±5.7</td>
</tr>
<tr>
<td>NPX 9</td>
<td>0.03±0.03</td>
<td>0.23±0.03</td>
<td>P &lt; 0.001</td>
<td>0.23±0.03</td>
<td>5.68±0.31</td>
<td>P &lt; 0.001</td>
<td>0.03±0.03</td>
<td>0.03±0.03</td>
<td>0.03±0.03</td>
<td>234.7±5.6</td>
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**Table 1.** Control values and values at 48-h postnephrectomy

Values are means ± SE. CNT, control values; NPX, 48-h postnephrectomy values; Asymmetrical dimethylarginine (ADMA) decreases, whereas symmetrical dimethylarginine (SDMA), creatinine (Cr), blood urea nitrogen (BUN), and K⁺ increase.
ureteral-sectioned rats continues to excrete large quantities of SDMA into the peritoneal cavity.

**Plasma clearance of DMA.** The plasma steady-state concentrations of DMAs produced by the bolus plus infusion protocols were verified by three sequential samples at 60, 80, and 90 min (data not shown), just before the infusion pump was turned off. The steady-state average of these three time points is shown in Fig. 3 and is the starting point for the clearance curves in Fig. 4. The same bolus injection and infusion protocol was used in all three groups (intact, acute NPX, and 48-h NPX), such that the same steady-state plasma concentration would have been obtained if production, excretion, and/or hydrolysis rates had not changed. However, Fig. 3 shows a statistically significant increase (44%) in steady-state ADMA concentration within 2 h after 2×NPX. Given that identical bolus and infusion protocols were used in intact and 2×NPX animals, these data suggest either an increased production or a more likely acute decreased clearance due to acute loss of renal excretory function with acute 2×NPX. In contrast, the steady-state ADMA concentration produced by infusion at 48 h after 2×NPX was not statistically different from intact animals. Either production declined or there was a compensatory increase in any clearance mechanisms still remaining after 2×NPX that would presumably be from DDAH hydrolysis. With acute 2×NPX, the SDMA concentration increased dramatically (71%), suggesting a similar acute loss of renal excretory function for clearance. However, SDMA concentration continued to increase until 48 h, suggesting no compensatory change in production or hydrolysis as seen with ADMA.

The analysis of the profile of the ADMA clearance curves (Fig. 4A) failed to clearly identify an altered overall systemic clearance in either the acute 2×NPX or the 48-h 2×NPX group. A dominating role for DDAH in overall plasma clearance of ADMA is suggested. Rapid blood sampling in the first 3 min was not done, and that may have been able to resolve an initial clearance difference attributable to renal excretion of ADMA in the intact rats. The clearance curve data for SDMA (Fig. 4B) show little or no decline in plasma steady-state concentration for SDMA in both the acute and 48-h 2×NPX groups, thus confirming an absence of significant nonrenal clearance mechanisms for SDMA.

**DISCUSSION**

Acute 2×NPX is not a model of end-stage renal disease. Acute 2×NPX is, in the classical physiological tradition, a mechanistic experimental manipulation to definitively determine whether the kidneys are needed for the acute elimination or clearance of ADMA. The data presented here demonstrate that acute removal of the kidneys does not increase plasma ADMA. However, as predicted, SDMA markedly increased. These data call into question the previous conclusion of many investigators, including some of our own investigators (53),

![Fig. 2. A: plasma ADMA decreasing in response to bilateral NPX and ureteral ligation while remaining steady after ureteral section. B: plasma SDMA increasing in response to bilateral NPX and ureteral ligation and ureteral section.](http://ajpheart.physiology.org/)

![Fig. 3. ADMA (solid bars) and SDMA (open bars) concentrations at steady-state concentration produced by identical bolus plus infusion protocols in each group. Acute NPX increases ADMA but 48 h later ADMA has returned to intact levels.](http://ajpheart.physiology.org/)
that deterioration of renal excretory function observed in multiple disease states is responsible for elevated plasma ADMA. Although plasma ADMA unquestionably increases in virtually every renal disease state, this correlation with renal excretory failure does not establish causality.

The plasma concentrations of DMAs are the result of a balance between formation and elimination (11, 13). The only known mechanism for SDMA elimination in the rat is renal excretion (40, 44). The data from this study confirm this by showing an ongoing increase in plasma SDMA concentration after 2×NPX. Furthermore, with acute loss of renal excretory function, we observed parallel increases in plasma SDMA and creatinine concentration. This SDMA-creatinine correlation has been previously reported in fasting human subjects where no such correlation between ADMA and creatinine was observed (45).

In contrast with the increases in SDMA after NPX, we observed simultaneous decreases in ADMA after NPX. Although both ADMA and SDMA are presumably continuously formed, the elimination of plasma ADMA varies and appears to have both a renal excretory component and a component of hydrolysis to citrulline by DDAH. New evidence also suggests that the liver may contribute significantly to ADMA clearance in rats and humans (41, 54). The balance of formation and elimination (by excretion and hydrolysis) determines the plasma concentration. Bilateral NPX removes not only the renal excretory function but also an established tissue source of ADMA as indicated by the demonstration of protein arginine methyl transferases (PRMTs) in the kidney (58) and the high tissue levels of ADMA reported for renal tissues (3).

Several studies further support the idea that plasma ADMA levels may not correlate as closely with renal function as previously thought. Kielstein et al. (30) report that in patients with incipient renal disease, inulin clearance was strongly correlated with SDMA but poorly correlated with ADMA clearance. This study also showed that ADMA was elevated in renal failure patients with a normal GFR. Furthermore, after renal transplantation in chronic renal failure patients, SDMA returns to baseline, whereas ADMA remains elevated (18).

A growing body of evidence suggests that DDAH may be a significant route of elimination for ADMA. Of the total ADMA produced daily in humans, only 20% is excreted by the kidneys, whereas DDAH metabolizes the remaining 80% (1). This trend appears to be present in the rat where DDAH metabolizes >90% of ADMA (47). Thus a large fraction of the plasma clearance of ADMA is attributable to baseline DDAH activity. Furthermore, DDAH activity can be modulated in vivo and in vitro. DDAH activity decreased with exposure of endothelial cells to oxidized LDL (22) and was also decreased in rat models of Type 2 diabetes (37). Although these authors report a decrease in DDAH activity with an increase in ADMA, it does support the idea that systemic conditions can modify DDAH activity. Although DDAH has high activity in the kidney (48), there is also wide tissue distribution (36, 48, 59, 60). DDAH is even present in red blood cells (28). Thus, even with the removal of renal DDAH and ADMA excretion in the urine, a small increase in systemic DDAH activity could compensate for the loss of the kidneys and their relatively small excretory contribution to total ADMA clearance.

In the washout protocol (Fig. 4), steady-state SDMA remains elevated in 48-h 2×NPX animals, whereas ADMA decreased from a steady-state infusion level at a rate similar to the intact controls; i.e., the net plasma clearance of ADMA appears similar in these two cases. This suggests that at 48 h, the balance of infusion, endogenous formation, and endogenous hydrolytic activity (presumably by DDAH) produces the same plasma concentration that was observed in the intact group that had, in addition, renal excretion of DMAs. However, when plasma DMAs are examined immediately after 2×NPX (acute NPX group), there is a significant ($P = 0.002$) increase in plasma ADMA concentration despite identical infusion rates, suggesting an acute, and not yet compensated for, loss of plasma clearance mechanisms for ADMA. We conclude that this reflects the acute removal of renal excretory function before any systemic compensation. In the subsequent 48 h, other plasma clearance mechanisms compensate and take the place of renal clearance. By 48-h post-NPX, the same steady-state plasma concentration was obtained that was observed in the intact preparation, but this remained so by using a slightly different set of clearance mechanisms.

Plasma concentrations of ADMA can affect clearance rates. Any increase in DDAH specific activity (i.e., per mg DDAH protein) could be obscured (or mimicked) by the effect of the higher plasma levels of ADMA produced after acute NPX. A higher hydrolysis rate in the acute NPX group is expected due to higher initial steady-state substrate concentration. These concentrations (10–15 μM) are well below the $K_m$ for DDAH.

Fig. 4. A: washout of ADMA from steady-state concentration produced by identical bolus plus infusion protocols in each group. B: SDMA. For both panels, SE (not visible) are within the symbol.
contributing significantly to the observed reduction in plasma ADMA concentration is that creatinine, BUN, potassium, and SDMA all increased significantly \( (P < 0.001) \) after NPX, whereas body weight decreased by 7% (Table 1). If an expansion of the plasma volume had occurred, it would have needed to be of sufficient magnitude to reduce the plasma ADMA concentration by \( \sim 40\% \). Such a dilution effect would be expected to simultaneously reduce the concentration of other solutes in the plasma. However, this did not occur, and, in fact, the concentration of SDMA increased by 25 times, creatinine increased by 38 times, and BUN increased by 16 times rather than decreasing. Thus intravascular dilution is not a likely explanation for reduced plasma ADMA concentration.

The marked increase in plasma SDMA after NPX opens the possibility that there was a negative feedback of SDMA on ADMA formation. However, the literature suggests a negative feedback is unlikely in that simultaneous elevations of both plasma DMAs have been reported in peripheral arterial occlusive disease (7) and in end-stage renal disease (67, 68). The loss of a preferential ADMA reservoir by NPX is possible in that particularly high kidney tissue levels of ADMA have been reported (3). However, Nijveldt et al. reported net renal extraction, not synthesis, of ADMA in humans (45) and rats (42). Other sources of ADMA, such as isolated human endothelial cells in culture, have also been reported (16). Furthermore, Al Banchaabouchi et al. (3) report that plasma ADMA increased in rats subjected to 80% partial NPX. Thus the loss of a renal reservoir of ADMA is a much too simplistic explanation for our results.

Recent literature (13, 14) has linked the renin-angiotensin system (RAS) with ADMA levels. Given the central role that the kidney plays in the RAS, it is possible that renal manipulations (NPX, ligation, and section) could alter RAS and thus impact ADMA levels. A 50% reduction in plasma angiotensin II and an undetectable renin activity have been reported for rats 48 h after NPX (2, 9). The literature for RAS changes in ligation, and section remains less well defined (5, 14, 46), and significant variations of plasma renin activity has been reported for rats of Al Banchaabouchi et al. (3) report that plasma ADMA increased in rats subjected to 80% partial NPX. Thus the loss of a renal reservoir of ADMA is a much too simplistic explanation for our results.

With the data from this study we are definitely not questioning the ability of the kidneys to excrete ADMA. The kidneys are clearly capable of ADMA excretion. In fact, Al Banchaabouchi et al. (3) gave compelling evidence for the ability for ADMA excretion in rats, mice, and humans. In their subtotal NPX (40% or 80% NPX in rat), they actually showed an eightfold increase in excretion of ADMA. It is not surprising, however, that after a 3-wk recovery from an 80% NPX that the rats of Al Banchaabouchi et al. had significantly compensated renal excretory function. The control and subtotal NPX creatinine excretion \( (127.8 \pm 19.8 \text{ vs. } 137.6 \pm 26.7 \mu\text{mol}/24\text{ h, respectively}) \) were not even statistically different, illustrating the remarkable renal compensatory capacity.

Multiple laboratories (30, 64, 65, 67, 68), including Rajagopalan’s laboratory (51), have reported elevated plasma ADMA concentrations in renal failure patients. Renal dialysis has been reported to reduce plasma ADMA (31). Kielstein et al. (30) have even detected elevated ADMA in plasma of patients with incipient primary chronic renal disease. Puromycin nephrosis in the rat produces hypertension by focal and segmental glo-
meruliosclerosis (52), and Sato et al. (52) demonstrated a significant positive correlation between systolic blood pressure and plasma and urinary ADMA. With all of these correlative studies, including our own, none have demonstrated a cause and effect relationship between renal failure and increased plasma ADMA. It is often asserted that in renal failure there is a partial compromise of renal excretion of ADMA, and this failure is responsible for elevating plasma ADMA concentrations. The data presented here are the first to challenge these assertions. Having the ability to excrete ADMA does not mean that ADMA must be excreted to be cleared from the plasma. Furthermore, finding ADMA in the urine does not prove that failure of renal excretion causes the increase in plasma ADMA. In conclusion, based on our NPX and plasma clearance data, we conclude that control of systemic hydrolysis of ADMA (e.g., by DDAH) and/or the control of its formation (e.g., PRMT activity) can maintain essentially normal plasma clearance of ADMA in the absence of the kidneys. ADMA does not require the kidneys for its elimination from the plasma. By controlling plasma ADMA concentration, the balance of activity of synthetic and degradative pathways for ADMA presents yet another mechanism for modulating regional blood flow and arterial blood pressure by controlling NO and NO vascular smooth muscle relaxation in NO-dependent vascular beds.

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