Nitric Oxide Synthesis Inhibition Retards Surgical Reversal of One-Kidney Goldblatt Hypertension in Rats

Wann-Chu Huang, Ren-Yu Tsai

Abstract—Surgical correction of renal artery stenosis in Goldblatt hypertension rapidly normalizes blood pressure and increases renal function. This study was conducted in 1-kidney, 1 clip (1K1C) Goldblatt hypertensive rats to examine whether the unclipping-induced reversal of blood pressure and renal function is mediated by nitric oxide (NO). The 1K1C rats were prepared and given tap water with or without supplementation of \textit{N}^\text{G}-\textit{nitro-\textit{L}-arginine methyl ester} (L-NAME). Systolic blood pressure (SBP) before and after renal artery clipping was measured with the tail-cuff method. Four weeks later, surgical unclipping was performed while blood pressure and renal function responses were determined. The results show that clipping the renal artery for 4 weeks increased SBP from 140±5 to 183±6 mm Hg (\(P<0.05\)). Concurrent L-NAME treatment accelerated and aggravated the clipping-induced increases in SBP from 138±6 to 219±8 mm Hg (\(P<0.05\)). Surgical unclipping reduced blood pressure to normotensive levels within 2 hours in all hypertensive rats with and without chronic or acute L-NAME treatment. However, the magnitude of reductions in blood pressure in the initial 1 hour after unclipping was significantly less in L-NAME–treated rats than in nontreated rats (9±2% versus 16±1%, \(P<0.05\)). Despite reducing blood pressure, unclipping significantly increased glomerular filtration rate, urine flow, and sodium and potassium excretions, but the extent of the increases in these renal functions was significantly attenuated in L-NAME–treated rats. These data suggest that NO production partly contributes to the hypotensive and renal responses to unclipping but does not mediate the reversal of renovascular hypertension of this model. (Hypertension. 1998;32:534-540.)

Key Words: hypertension, one-kidney Goldblatt ■ renal artery obstruction ■ nitric oxide ■ diuresis ■ natriuresis

Constricting the renal artery of the remaining kidney in uninephrectomized rats produces 1-kidney, 1 clip (1K1C) Goldblatt hypertensive. This hypertensive model has been generally considered to be volume dependent.\(^1,2\) Surgical removal of the clip from the renal artery in this model of renovascular hypertensive rats precipitously decreases arterial blood pressure and enhances kidney function.\(^3-11\) The rapid depressor response to unclipping also occurs in chronic hypertension (>4 months) and is not a nonspecific effect of surgery.\(^10,11\) The precise mechanism responsible for such a major fall of blood pressure after renal artery clip removal is still not completely understood. It has been shown that the pattern of the depressor response to unclipping is not affected by indomethacin, aprotinine, platelet-activating factor receptor antagonist, or changes in the activity of the renin-angiotensin system.\(^7-11\) The salt and volume replacement during natriuresis and diuresis that accompanies renal artery clip removal attenuates but does not prevent the depressor response.\(^5,8\) Also, surgical correction of renovascular hypertension is not associated with changes in the sympathetic nerve activity and plasma noradrenalin level.\(^12,13\) Thus, changes in the most well-documented factors for blood pressure control and renal function regulation, such as the renin-angiotensin system, renal sympathetic nerve activity, prostaglandins, kinins, and sodium-volume balance, cannot account for the rapid reversal of hypertension by surgical correction of renal artery stenosis. On the other hand, medullipin, a polar lipid released from renal medulla, has been postulated to be one of the candidates for the marked fall of blood pressure after unclipping.\(^14-16\) However, the mediator role of renal medullipin in the reversal of hypertension is not unequivocal.\(^14,15,17\)

The vascular endothelium is capable of synthesizing nitric oxide (NO) from \textit{L}-arginine, a process that can be competitively inhibited by \textit{N}^\text{G}-\textit{nitro-\textit{L}-arginine methyl ester} (L-NAME) and other substituted \textit{L}-arginine compounds that compete for the NO synthase.\(^18-20\) NO acts on adjacent vascular smooth muscle to produce a cGMP-dependent relaxation. Acute or chronic inhibition of NO synthesis in experimental animals results in systemic hypertension and produces substantial influences on renal function.\(^21-26\) Because renal vascular NO synthesis is related to the degree of renal perfusion, and shear stress is the primary endogenous stimulus for endothelial NO production,\(^27,28\) it is likely that increases in renal perfusion pressure resulting from removal of the renal arterial clip may generate NO or an NO-associated mechanism for the rapid fall of blood pressure in...
the Goldblatt hypertensive model. Thus, we hypothesized that unclipping-induced rises in renal perfusion increase vascular shear stress and thereby stimulate the synthesis of NO, which in turn mediates the hypotensive and renal responses to unclipping, whereas inhibition of NO synthesis blunts the fall of blood pressure and the enhancement in renal function by unclipping in this hypertensive model. To test the hypothesis, a 1K1C model of Goldblatt hypertensive rats was produced, and surgical removal of the renal arterial clip was performed in the hypertensive rats with and without inhibition of NO synthesis by L-NAME. Our results show that sustained L-NAME treatment accelerated and aggravated the development of 1K1C Goldblatt hypertension in rats. Also, acute or chronic inhibition of NO synthesis by L-NAME delayed but did not prevent the normalization of blood pressure after unclipping, suggesting a modulator but not a mediator role of NO in the surgical reversal of hypertension in this hypertensive model.

Methods

Preparation of 1K1C Goldblatt Hypertensive Rats

Male Sprague-Dawley rats with an initial body weight of 200 to 220 g were used for preparation of 1K1C Goldblatt hypertensive rats. Rats were anesthetized with ketamine hydrochloride (60 mg/kg) and xylazine (7.5 mg/kg) intraperitoneally and underwent right nephrectomy. All rats were maintained in the animal care facilities of the school. After a 1-week recovery period, the nephrectomized rats underwent left renal artery constriction with an internal gap of 0.25 mm, silver clip. Penicillin G (25 000 U IM) was injected after surgery. Rats were fed a commercial rat chow (Fu-Sho Co) and allowed free access to tap water. SBP was measured twice a week with the tail-cuff method using a programmed electrosphygmonometer (Ueda, model UR-5000) as previously described. Acute unclipping experiments were carried out 4 weeks later, when the rats weighted 376±7 g. All procedures were conducted in accordance with the prior approval of the institutional animal care and use committee of this school.

Animal Groups

The 1K1C Goldblatt hypertensive rats were randomly divided into 5 groups and treated as follows: group 1 served as time control without unclipping or L-NAME treatment (n=5); group 2 was subjected to unclipping alone after 4 weeks of renal artery clipping (n=9); and group 3 was given chronic oral L-NAME (80 mg/100 mL drinking water, changed daily, approximately 100 mg/kg per day) immediately after renal artery clipping. Four weeks later, rats were subjected to acute removal of the renal arterial clip (n=11). Group 4 received acute intravenous infusion of L-NAME (10 mg/kg bolus followed by 1.0 mg/kg per minute) 4 weeks after renal artery clipping when the rats became hypertensive. Surgical unclipping was performed during L-NAME infusion (n=10). Group 5 received acute intravenous infusion of L-NAME without unclipping (n=6). The concentrations of L-NAME were chosen because they have been demonstrated to be effective in inhibiting endogenous NO synthesis in 1K1C rats and renal cortical and medullary NO production in normal rats.

Acute Unclipping Experiments

Rats were anesthetized with 100 mg/kg IP Inactin (5-ethyl-5-1-methyl-2-thiobarbiturate sodium). The rats were placed on a servocontrolled heated table, and the rectal temperature of each rat was maintained at 37±0.5°C. The trachea was intubated to keep the airways patent, and the right jugular vein was catheterized for the infusion of an isotonic NaCl solution and the drug. The right femoral artery was cannulated with a PE-50 tube for blood sampling and for continuous measurement of blood pressure via a Statham P23XL pressure transducer (Gould-Statham Instruments Inc) and recorded on an RS-3800 polygraph (Gould Inc). Through a flank incision, the clipped (left) kidney was exposed and placed in a Lucite cup. The fibrous tissue surrounding the silver clip on the left renal artery was carefully dissected free without damaging the renal artery or causing bleeding. This approach allowed the clip to be removed easily later in the experiments. Urine samples were sequentially collected by catheterizing the ureter.

During surgery, all rats were infused with an isotonic (154 mmol/L) NaCl solution at a rate of 0.02 mL/min. On completion of surgery, a priming dose of 0.3 mL 10% Inutest (polyfructosan; Laevosan-Gesellschaft) in normal saline was administered; this was followed by infusion of the same solution at a constant rate of 0.01 mL/min throughout the experiment. The saline infusion rate was reduced to 0.01 mL/min to maintain the total infusion rate constant. One hour was allowed for the animal to achieve a steady state, and then two 30-minute urine samples for the control

Figure 1. Effects of chronic oral administration of L-NAME on SBP in 1K1C rats in an unanesthetized state. *P<0.05 vs unclipping alone; †P<0.05 vs preclipping.

Figure 2. Effects of unclipping (UC) on mean blood pressure (MBP) of 1K1C Goldblatt hypertensive rats with and without chronic administration of L-NAME. Time control group of 1K1C Goldblatt hypertensive rats did not receive unclipping or L-NAME. *P<0.05 vs UC alone; †P<0.05 vs preclipping.
period were collected. The rats were subsequently subjected to unclipping (groups 2 and 3) or infused with L-NAME (10 mg/kg bolus, 1.0 mg/kg per minute, groups 4 and 5). For those rats receiving acute L-NAME infusion, unclipping was carried out after 2 postdrug urine collection periods. For the time control group (group 1), urine samples were sequentially collected without intervention. Arterial blood samples of 0.3 mL each were taken during the control, drug infusion, and postclipping periods. The plasma was immediately separated by centrifugation. The blood cells were resuspended in normal saline with a total volume of 0.3 mL and returned to the animal.

**Chemical Measurements and Statistical Analysis**

The concentration of Inutest in plasma and urine samples was measured with a semimicroanthrone colorimetric method as described previously. Plasma and urine sodium and potassium concentrations were determined with a flame photometer (model 943; Instrumentation Laboratory). Plasma osmolality was measured with an osmometer (model 3D3, Advanced Instrument). Glomerular filtration rate (GFR) and electrolyte excretion rates were calculated according to standard clearance formulas. The data were evaluated statistically by 2-way ANOVA. Bonferroni’s test and Student’s t test for paired or unpaired values, when relevant, were used. A value of $P<0.05$ was considered statistically significant. Results are expressed as mean±SEM.

**Results**

**Effects of Chronic L-NAME Treatment on Development of 1K1C Goldblatt Hypertension**

As shown in Figure 1, blood pressure increased gradually after the renal artery was constricted. Chronic L-NAME treatment accelerated and aggravated the increase of blood pressure. Two weeks after renal artery clipping, the SBP of L-NAME–treated rats significantly increased from 138±6 to 176±6 mm Hg ($P<0.05$), whereas that of the clipped rats without L-NAME treatment increased from 139±5 to 156±10 mm Hg ($P<0.05$) at the corresponding time points. The SBP of the L-NAME–treated group further increased to 196±6 mm Hg, which is significantly greater than that of the nontreated group (184±5 mm Hg, $P<0.05$) by the end of the 4th postclipping week.

**Blood Pressure and Renal Responses to Unclipping in 1K1C Rats With Chronic L-NAME Treatment**

Acute removal of the renal arterial clip alone significantly decreased blood pressure from 170±5 to 164±3 mm Hg ($P>0.05$) within 10 minutes in the hypertensive rats without L-NAME treatment (Figure 2). During the corresponding
time period, unclipping in the L-NAME–treated rats did not cause a significant change in blood pressure (from 178±3 to 176±3 mm Hg, \( P > 0.1 \)). Blood pressure was then further reduced toward the preunclipping levels in both groups (to 119±5 mm Hg in L-NAME–treated rats versus 113±6 mm Hg in nontreated group at 2 hours after unclipping). The magnitude of decreases in blood pressure in response to unclipping was significantly less in L-NAME–treated rats than in the nontreated rats at the first (9±2% versus 16±1%, \( P < 0.05 \)) but not the second postunclipping hour (32±2% versus 33±2%, \( P > 0.1 \)), as shown in Figure 6.

Removal of the renal arterial clip caused marked increases in GFR, urine flow, and sodium and potassium excretion rates (UNaV and UKV) in both L-NAME–treated and nontreated groups (Figure 3). However, the extent of increase in renal function after unclipping was significantly less in L-NAME–treated rats than in the nontreated rats at both the first postunclipping hour (13±1% versus 18±1% for GFR, 90±4% versus 174±5% for urine flow, and 26±3% versus 64±3% for UNaV; all \( P < 0.05 \)) and the second hour (12±1% versus 16±1% for GFR, 75±2% versus 130±3% for urine flow, and 35±3% versus 60±3% for UNaV; all \( P < 0.05 \)), as illustrated in Figure 6.

### Effects of Acute Infusion of L-NAME and Subsequent Unclipping on Blood Pressure and Renal Function

Acute administration of L-NAME for 40 minutes increased the mean blood pressure of 1K1C hypertensive rats by 12±3 mm Hg (\( P < 0.05 \)), as depicted in Figure 4. In the absence of unclipping (L-NAME treatment alone), the elevated blood pressure was maintained throughout the experiments. After the renal arterial clip had been removed in the presence of continuous infusion of L-NAME, blood pressure fell significantly from 189±3 to 178±2 mm Hg (\( P < 0.05 \)) over 40 minutes of unclipping and then further declined to 128±4 mm Hg by the end of the experiments. Acute administration of L-NAME did not significantly alter renal function. Subsequent unclipping induced dramatic increases in GFR, urine flow, and electrolyte excretion (Figure 5). The magnitudes of decrease in blood pressure and increase in renal function of the acute L-NAME–treated group were comparable to those of the chronic L-NAME–treated group but were significantly less than those of the nontreated group (Figure 6).

### Comparison of Body Weight, Kidney Weight, and Plasma Electrolytes and Osmolality Between Groups

As summarized in the Table, there were no significant differences in body weight, kidney weight, plasma osmolality, and plasma concentrations of sodium and potassium among groups of 1K1C hypertensive rats. Neither drug treatment nor unclipping altered plasma concentrations of electrolytes and osmolality.

### Discussion

The present study demonstrates that chronic oral administration of L-NAME to inhibit NO synthesis accelerated and aggravated the pressure increase in 1K1C Goldblatt hypertensive rats. Surgical correction of renal artery stenosis by unclipping caused a prompt decrease in blood pressure and a concurrent increase in renal function. Chronic or acute L-NAME treatment attenuated but did not prevent the unclipping-induced hypotensive and renal responses in the first 1 hour after unclipping. Blood pressure further declined to prehypertensive levels in the second hour after unclipping. The elevated renal function after unclipping was maintained until the end of the experiments, but the plateau was significantly lower in rats with L-NAME treatment than in rats without L-NAME administration. These results suggest that NO production contributes to the early reduction of blood pressure and the partial restoration of renal function due to renal artery clip removal but does not mediate the reversal of this renovascular hypertension after unclipping.

The unclipping-induced hypotensive response noted in the present study is in accordance with the observations of previous studies from this and other laboratories.\(^3\)\(^{–}\)\(^11\) The precise mechanism for the prompt and marked fall of blood pressure after surgical unclipping is still not fully understood. Earlier studies suggested that unclipping-induced depressor response occurred in the denervated kidney and was not attributable to prostanoids, kinins, platelet-activating factor, suppression of the renin-angiotensin system, or urinary loss of sodium and water.\(^3\)\(^\text{-}\)\(^13\) Observations on the role of NO in mediating the hypotensive response to unclipping and increased renal perfusion are inconsistent.\(^17\)\(^\text{-}\)\(^32\)\(^33\) Thomas et al\(^7\) demonstrated that NO synthesis inhibition did not prevent the hypotensive response to increased renal perfusion in rabbits. In contrast, Beierwaltes et al\(^32\) reported that acute blockade of NO synthesis by L-NAME abolished the reduction of blood pressure for 1 hour after unclipping in 2-kidney, 1 clip (2K1C) Goldblatt hypertensive rats. Also, Bergstrom et al\(^33\) showed that NO synthesis inhibition reduced the hypotensive response of an assay rat to high-pressure perfusion to an
isolated kidney. Our present results show that both chronic and acute administration of L-NAME attenuated but did not prevent the unclipping-induced hypotensive response in the first hour after unclipping, and blood pressure progressively declined to prehypertensive levels within 2 hours after unclipping. The inability of L-NAME treatment to completely prevent unclipping-induced normalization of blood pressure is not related to the effectiveness of the drug. On one hand, both the chronic and acute doses of L-NAME used in the present study have previously been shown to effectively inhibit endogenous NO production in 1K1C rats as well as renal cortical and medullary NO synthesis in normal rats as determined by plasma nitrite/nitrate and intrarenal NO levels. On the other hand, short-term and long-term treatment with these doses of L-NAME in 1K1C rats similarly caused an additional increase in blood pressure compared with the nontreated 1K1C rats (Figures 1 and 4), suggesting the effective inhibition of NO synthesis by L-NAME in the present study. Thus, the reason for the aforementioned disparities in observations on the mediating role of NO in the hypotensive response to unclipping and changes in renal perfusion is unclear, but differences in animal models used and the duration of experimental observations after unclipping probably all contributed. In the studies by Thomas et al and Bergstrom et al, an extracorporeal circuit rather than unclipping was used to increase renal perfusion in anesthetized normal rabbits or of the isolated kidney, whereas Beierwaltes et al used 2K1C Goldblatt hypertensive rats and did not continue to follow the blood pressure response to unclipping beyond 1 hour after unclipping, as we did in the present study. Nevertheless, the partial dependency on NO of the early but not late hypotensive response to unclipping as seen in the present study suggests that in addition to NO, other factors or mechanisms initiated by unclipping may also participate in the surgical reversal of hypertension of this model. Indeed, some studies have demonstrated that unclipping induces release of renal medullipin, a vasodepressor lipid, which may be partially responsible for the depressor response after unclipping, although the chemical nature of medullipin remains obscure.

It has been documented that acute or chronic inhibition of NO synthesis by L-NAME and other substituted L-arginine compounds that compete for the NO synthase in normal rats produces peripheral vasoconstriction, elevation of blood pressure, and decreases in renal blood flow, GFR, and sodium excretion. In the present study, chronic administration of L-NAME caused a faster and greater increase in blood pressure after clipping the renal artery in rats. Acute infusion
of L-NAME increased the blood pressure further in 1K1C hypertensive rats. These observations are consistent with previous studies and support the notion that NO is intimately involved in the regulation of blood pressure in normal and hypertensive conditions. Dubey et al. recently demonstrated that NO synthesis was increased 2 weeks after renal artery clipping and the elevated NO production then gradually declined to prehypertensive levels 5 weeks after renal artery clipping in 1K1C hypertensive rats. It is likely that the early increase in NO production is a compensatory mechanism that slows the initial rise in arterial pressure. Thus, blockade of NO synthesis by L-NAME accelerates the pressure increase after renal artery constriction, as observed in the present study (Figure 1). The later decrease in NO production could be the result of either an adaptation to the high blood pressure or endothelium damage caused by hypertension.

Despite the profound reductions of blood pressure, surgical removal of the renal arterial clip sharply increased GFR and renal excretions of water, sodium, and potassium. We have previously demonstrated that the function of the clipped kidney of 1K1C hypertensive rats is pressure dependent. It is likely that surgical correction of the renal artery stenosis after removal of the clip restored or increased renal perfusion and thereby stimulated NO production, which

**Comparisons of Body Weight, Kidney Weight, Plasma Osmolality, and Concentrations of Sodium and Potassium Among Various Groups of 1K1C Goldblatt Hypertensive Rats**

<table>
<thead>
<tr>
<th>Group</th>
<th>Rats, n</th>
<th>Body Weight, g</th>
<th>Kidney Weight, g</th>
<th>Pre-UC</th>
<th>Post-UC</th>
<th>Pre-UC</th>
<th>Post-UC</th>
<th>Pre-UC</th>
<th>Post-UC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time control</td>
<td>5</td>
<td>370 ± 16</td>
<td>1.9 ± 0.1</td>
<td>139 ± 1.4</td>
<td>138 ± 1.9</td>
<td>3.5 ± 0.2</td>
<td>3.6 ± 0.1</td>
<td>289 ± 1.1</td>
<td>288 ± 0.7</td>
</tr>
<tr>
<td>UC alone</td>
<td>9</td>
<td>367 ± 39</td>
<td>2.0 ± 0.2</td>
<td>138 ± 1.3</td>
<td>137 ± 2.0</td>
<td>3.7 ± 0.3</td>
<td>3.7 ± 0.2</td>
<td>288 ± 1.2</td>
<td>289 ± 0.8</td>
</tr>
<tr>
<td>Chronic L-NAME+UC</td>
<td>11</td>
<td>360 ± 40</td>
<td>2.0 ± 0.2</td>
<td>139 ± 1.6</td>
<td>138 ± 1.5</td>
<td>3.6 ± 0.2</td>
<td>3.7 ± 0.1</td>
<td>287 ± 1.1</td>
<td>287 ± 1.0</td>
</tr>
<tr>
<td>Acute L-NAME+UC</td>
<td>10</td>
<td>390 ± 49</td>
<td>1.6 ± 0.3</td>
<td>140 ± 1.5</td>
<td>140 ± 1.0</td>
<td>3.8 ± 0.1</td>
<td>3.7 ± 0.2</td>
<td>288 ± 0.5</td>
<td>287 ± 1.4</td>
</tr>
<tr>
<td>Acute L-NAME alone</td>
<td>6</td>
<td>404 ± 16</td>
<td>2.2 ± 0.5</td>
<td>138 ± 1.1</td>
<td>139 ± 1.5</td>
<td>3.6 ± 0.2</td>
<td>3.6 ± 0.3</td>
<td>289 ± 0.8</td>
<td>288 ± 1.1</td>
</tr>
</tbody>
</table>

UC indicates unclipping; P Na, plasma sodium concentration; P K, plasma potassium concentration; and P Osm, plasma osmolality.
in turn enhanced GFR and urinary excretions of sodium, potassium, and water. Thus, the smaller increases in GFR and renal excretory function after unclipping in the L-NAME–treated group compared with the rats with unclipping alone may be attributed to the absence of NO-mediated renal response under these experimental conditions.

In summary, chronic administration of L-NAME enhanced the development of 1KC Goldblatt hypertension in rats. Surgical correction of the renal artery stenosis rapidly normalized blood pressure within 2 hours. Marked increases in GFR and renal excretory function accompanied unclipping were also observed. Acute or chronic L-NAME treatment attenuated the hypotensive response to unclipping in the initial 1-hour period but did not prevent the subsequent decline of blood pressure toward prehypertensive levels. The unclipping-induced increase in renal function was also attenuated in L-NAME–treated rats. These results suggest that release of NO in response to an increased vascular shear stress due to removal of the renal arterial clip partially contributes to the early phase of reduction of blood pressure but is not responsible for the mediation of blood pressure normalization after unclipping in 1KC Goldblatt hypertensive rats.

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References

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