# Increased nitric oxide activity in early renovascular hypertension

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*Department of Medicine, Center for Clinical Pharmacology, University of Pittsburgh Medical Center, Pittsburgh, Pennsylvania 15213–2582; Department of Physiology, West Virginia University, Morgantown, West Virginia; and Department of Obstetrics and Gynecology, Clinic of Endocrinology, University Hospital, 8091 Zurich, Switzerland*

**Dubey, Raghvendra K., Matthew A. Boegehold, Delbert G. Gillespie, and Marinella Rosselli.** Increased nitric oxide activity in early renovascular hypertension. *Am. J. Physiol.* 270 (*Regulatory Integrative Comp. Physiol.* 39): R118–R124, 1996.—A decreased influence of nitric oxide (NO) in the peripheral vasculature is associated with the pathophysiology of established hypertension, and some studies suggest that increased blood pressure positively correlates with decreased NO production. If so, then the increased arterial pressure in one-kidney, one-clip (1K1C) hypertensive rats should be associated with decreased circulating levels of nitrite/nitrate ($NO_2/NO_3$; stable metabolites of NO) and guanosine 3′,5′-cyclic monophosphate (cGMP; mediator of NO action). We measured serum $NO_2/NO_3$ and cGMP levels in early hypertensive 1K1C (2 wk after clipping) and sham-operated one-kidney (1K) normotensive rats, treated orally with or without the NO-synthase inhibitor $N^G$-nitro-L-arginine methyl ester (L-NAME, 2 wk). Compared with those in 1K rats, $NO_2/NO_3$ and cGMP levels were increased in 1K1C hypertensive rats but not in 1K1C rats treated with L-NAME. $NO_2/NO_3$ and cGMP levels in L-NAME-treated 1K and 1K1C rats were similar. Compared with that in 1K rats, systolic blood pressure (SBP) was increased in 1K1C rats and in L-NAME-treated 1K and 1K1C rats. The SBP increase in L-NAME-treated 1K1C rats was more rapid than in untreated 1K1C rats. In early hypertension, increases in SBP positively correlated with increases in serum $NO_2/NO_3$ and cGMP. After 2 wk of hypertension, circulating $NO_2/NO_3$ levels gradually declined and reached prehypertension levels by the fifth week of hypertension. These results provide evidence for increased NO synthesis in early hypertensive 1K1C rats, and this increased NO could be a compensatory mechanism to slow the development of hypertension in these animals.

nitrite/nitrate; guanosine 3′,5′-cyclic monophosphate

IN NORMAL BLOOD VESSELS, endothelium-derived nitric oxide (NO) is continuously released both luminally and abluminally (10, 14), protecting against platelet aggregation and adhesion (10, 14) as well as influencing vascular smooth muscle tone (10, 14). These effects of NO are mediated through the activation of cytosolic guanylate cyclase to elevate guanosine 3′,5′-cyclic monophosphate (cGMP) levels within the target cell (10, 14). NO is synthesized from L-arginine by the enzyme NO synthase (NOS) (14, 15), which can exist in either a constitutive or an inducible form (14, 15). Constitutive NOS, which is responsible for the continuous basal release of NO under normal conditions, requires $Ca^{2+}$/calmodulin, and its activity can be rapidly increased by mechanical forces associated with blood flow or by agonists such as acetylcholine (ACh) and bradykinin (15, 24). Inducible NOS, which is more slowly expressed in response to cytokines and lipopolysaccharides, can generate excessive amounts of NO even in the absence of $Ca^{2+}$ (15). The formation of NO by either enzyme can be inhibited by L-arginine analogues such as $N^G$-nitro-L-arginine methyl ester (L-NAME) (14, 15).

In hypertensive patients and in various animal models of hypertension, there is an impairment of the vascular relaxation response to agonists that act through NO release (3, 8, 11, 13, 16, 20, 30, 31). In contrast, nitrovasodilators produce a normal increase in vascular smooth muscle cGMP and a normal relaxation of these vessels (13, 21, 31), indicating that the impaired NO-dependent responses are not due to a deficit in smooth muscle cGMP activity. It is unclear whether this abnormal endothelial/smooth muscle communication is a primary defect that contributes to the genesis of hypertension or is a secondary response to increased pressure that contributes to the maintenance of elevated total peripheral resistance. In contrast to agonist-stimulated NO activity, the basal influence of NO on vascular tone is apparently not suppressed in at least one model of hypertension, the spontaneously hypertensive rat (SHR) (1, 6). It is not clear whether this preservation of basal NO activity is unique to the SHR strain or is a characteristic common to other forms of hypertension as well. If basal NO activity is not suppressed in other models of hypertension, this would suggest that only certain aspects of endothelial function may be adversely affected in hypertensive individuals.

Because NO is a labile substance with a short half-life, its direct measurement has proven to be difficult. NO decomposes rapidly into nitrite ($NO_2$) and nitrate ($NO_3$) in biological solutions (12), and these stable breakdown products have been measured as an index of NO production in various types of cultured cells (12, 14, 15, 25). Recently, circulating $NO_2/NO_3$ levels have been used as an index of NO activity in vivo (23, 26). In the current study, we reasoned that, if increased blood pressure per se causes a suppression of endothelium-derived NO synthesis/release, then hypertension should quickly lead to decreased circulating levels of $NO_2/NO_3$. Using the one-kidney, one-clip (1K1C) rat model of hypertension, we evaluated changes in NO synthesis over the development of hypertension (early and established stages) by comparing circulating $NO_2/NO_3$ levels in these animals with those in normotensive intact and sham-clipped one-kidney (1K) rats. The

effect of L-NAME treatment on circulating $NO_2/NO_3$ was also evaluated in each of these groups. Because any change in NO synthesis should also influence cGMP production (2, 26), we also measured plasma cGMP levels in each group as a secondary index of endogenous NO activity.

## MATERIALS AND METHODS

### *Material*

L-NAME, 3-isobutyl-1-methylxanthine (IBMX), and creatinine estimation kit 555 were purchased from Sigma Chemical (St. Louis, MO). Nitrate reductase (*Aspergillus niger*), nicotinamide-adenine dinucleotide phosphate (NADPH), and flavin adenine dinucleotide (FAD) were procured from Boehringer Mannheim (Rotkreuz, Switzerland). $NO_2/NO_3$ estimation kits (Greiss reagents) were purchased from Spectroquant, Merck (Darmstadt, Germany). Radioimmunoassay kit for cGMP was obtained from Immunotech International (Dianova-Immunotech, Hamburg, Germany). Thromboplastin reagent (Simplastin) was obtained from Organon Teknika (Durham, NC). Centrifugal microconcentrators (10,000-mol wt cutoff membrane, Centricon-10) were purchased from Amicon (Beverley, MA). Male Sprague-Dawley rats were obtained from Zivic-Miller Laboratories (Zelienople, PA).

### *Production of Hypertension*

For each set of experiments, involving five to six pairs of rats, we prepared paired 1K1C hypertensive and 1K normotensive control rats as we have previously reported (19).

Briefly, under ether anesthesia, all rats (150–190 g body wt) were unilaterally nephrectomized. Two to three weeks later, at body weights of 320–340 g, the contralateral (left) renal artery was partially constricted with a 0.44-mm ID silver clip in one-half of the rats. The remaining rats, destined to be normotensive control rats, were paired by body weight with the clipped rats and were sham clipped. Body weights and conscious systolic blood pressures (SBP) were measured by tail cuff (Rat Tail Manometer System) in the paired rats every other day. Rats were fed standard rat chow (Agway Prolab R-M-H3000; Agway, Syracuse, NY) and fluids ad libitum. Six clipped/sham-clipped pairs of rats drank normal water and another six pairs drank water containing 0.77 g/l L-NAME postoperatively until they were killed, 2 wk after clipping. Based on the amount of total water intake per day, the dose of L-NAME was calculated to be ~100 $mg \cdot kg^{-1} \cdot day^{-1}$. This dose of L-NAME has previously been shown to effectively inhibit endogenous NO production in rats (22). An additional 15 pairs of 1K and 1K1C rats were similarly prepared and maintained on standard rat chow and water, and 5 pairs of these rats were killed at 3, 4, and 5 wk after clipping.

At the time of death 2–5 wk after clipping or sham clipping, all rats were gaining weight or of stable weight and appeared healthy. Necropsies were performed to verify clip placement and condition of heart, kidney, lungs, and general health. Institutional guidelines on animal care and use were followed in all procedures.

*Protocol*. We obtained blood samples from all 1K and 1K1C rats after 2, 3, 4, or 5 wk of clipping. SBP of the 1K and 1K1C rats was monitored every second day for 2 wk in groups receiving water and L-NAME and before death in the groups that received water and were killed after 3, 4, or 5 wk of clipping.

### *Sample Collection and Platelet-Poor, Plasma-Derived Serum Preparation*

All rats were fasted overnight. Under ether anesthesia, groups of 1K1C hypertensive and 1K normotensive control rats were bled by aortic puncture into citrated plastic syringes (50 µl of 0.2 M tris(hydroxymethyl)aminomethane-citrate/ml blood). Because hemoglobin is known to interfere with the $NO_2/NO_3$ assay (23), the hemoglobin-free plasma-derived serum (PDS) in this study was prepared by our previously described method (19). Briefly, the blood was iced immediately and all subsequent manipulations (except as stated below) were done in plastic ware at 4°C. After an aliquot was taken for hematocrit, the blood was centrifuged for 18 min at 850 *g* and the supernatant was recentrifuged at 26,800 *g* for 30 min. One-milliliter samples were taken for the estimation of plasma $Na^+$, $K^+$, and creatinine. We added the phosphodiesterase inhibitor IBMX (2 mmol/l) and 5 µl/ml of 1.0 M $CaCl_2$ plus 33 µl/ml thromboplastin reagent to the remaining platelet-poor plasma in glass tubes and allowed the mixture to clot for 1 h. Hemoglobin-free serum was then obtained by breaking the clot and filtering it through a 10,000-mol wt cutoff membrane. Briefly, PDS samples were added to Amicon centrifugal microconcentrators and centrifuged on a fixed-angle rotor at 5,000 *g* for 30 min. Aliquots of filtered PDS were frozen at −70°C until levels of $NO_2/NO_3$ and cGMP were assayed.

### *Biochemical Estimations*

Plasma $Na^+$ and $K^+$ were measured by flame photometry and creatinine spectrophotometrically, respectively, with the use of Sigma kit 555.

### *$NO_2/NO_3$ Analysis*

Serum $NO_2/NO_3$ levels were measured with the use of the Greiss reagent as previously described (23). Briefly, aliquots (50 µl) of PDS samples prefiltered through a 10,000-mol wt cutoff membrane (to remove hemoglobin) were diluted with ultrapure water (50 µl; Seromed Biochrom, Berlin, Germany) and incubated at room temperature with 25 µl of substrate buffer (imidazole 0.1 mol/l, NADPH 210 µmol/l, FAD 3.8 µmol/l) in the presence of nitrate reductase (14 mU enzyme/50 µl) from *Aspergillus niger* to convert $NO_3$ to $NO_2$. Total $NO_2$ was then analyzed by reacting the samples with Greiss reagent (1% sulfanilamide, 0.1% naphthalene-ethylene-diamine dihydrochloride in 5% $H_3PO_4$) for 45 min at 27°C and measuring absorbance at 525 nm spectrophotometrically. Serum samples with no added Greiss reagent were used as sample blanks. Amounts of $NO_2$ in serum were estimated from a standard curve of $NaNO_2$ obtained by enzymatic conversion of $NaNO_3$ (0–32 µmol/l; >98% conversion achieved; Merck) prepared in dialyzed fetal calf serum that was free of $NO_2$ and $NO_3$ (Sigma). Because very little or no $NO_2$ is found in serum (23), we did not attempt to differentiate between $NO_2$ and $NO_3$ amounts, but rather enzymatically converted all $NO_3$ to $NO_2$, and therefore report our results as $NO_2/NO_3$. All samples were run in duplicate or triplicate.

### *cGMP Analysis*

cGMP levels in PDS were analyzed in samples by previously described radioimmunoassay methods (7) and by using a radioimmunoassay kit. Briefly, aliquots (20 µl) of sample (original or diluted) or standards and 200 µl of alkaline solution II were added to tubes containing succinic anhydride and vortexed. After the mixture was completely dissolved, 100 µl of succinylated solution was added to each tube, and

Table 1. *Blood pressures, weights, and plasma biochemical concentrations 2 wk after renal artery clipping or sham clipping*

| | 1K | 1K1C | 1K-L-NAME | 1K1C-L-NAME |
|---|---|---|---|---|
| BP, mmHg | 128 ± 1 | 188 ± 6* | 164 ± 5 | 196 ± 4* |
| Body wt, g | 394 ± 14 | 379 ± 11 | 395 ± 6 | 387 ± 10 |
| Heart wt, g | 1.08 ± 0.05 | 1.30 ± 0.05* | 1.04 ± 0.002 | 1.18 ± 0.09 |
| Heart wt/body wt, g/g · $10^2$ | 0.27 ± 0.005 | 0.30 ± 0.005* | 0.26 ± 0.001 | 0.30 ± 0.002 |
| Hct, vol% | 38 ± 0.5 | 38 ± 0.5 | 40 ± 0.4 | 40 ± 0.4 |
| $Na^+$, meq/l | 138 ± 1 | 136 ± 1 | 140 ± 1 | 139 ± 1 |
| $K^+$, meq/l | 3.4 ± 0.4 | 3.5 ± 0.1 | 3.5 ± 0.1 | 3.5 ± 0.1 |
| Creatinine, mg/dl | 0.7 ± 0.001 | 0.7 ± 0.003 | 0.6 ± 0.05 | 0.7 ± 0.04 |

Values represent means ± SE; $n = 6$. 1K and 1K1C, 1-kidney normotensive control rats and 1-kidney, 1-clip hypertensive rats, respectively, on normal drinking water; 1K-L-NAME and 1K1C-L-NAME, 1K rats and paired 1K1C rats drinking water with $N^G$-nitro-L-arginine methyl ester (L-NAME, 0.77 g/l). BP, tail systolic blood pressure; Hct, hematocrit. *$P < 0.05$ vs. 1K rats in same treatment group.

300 μl of tracer $^{125}$I-ScGMPTME was added to that and mixed gently. The tubes were incubated for 22 h at 4°C, after which the solution was aspirated, and binding in each tube was measured on a gamma counter by counting for 1 min. Estimations were then made by using the standard curve provided along with the kit. The cross-reactivity of this kit is 100% with succinyl cGMP and <0.003% for succinyl adenosine 3′,5′-cyclic monophosphate. The interassay variability was <4%, and the sensitivity of the assay defined as significantly different from the standard zero with a probability of 95% was 10 pmol/l. All samples were assayed in triplicate.

*Statistics*

For all analyses, the individual making measurements was unaware of the PDS source. Analyses were performed with the use of the Number Cruncher Statistical System software package (Kaysville, UT). Analysis of variance (ANOVA) was used to compare values among treated and untreated 1K and 1K1C rats for serum $NO_2/NO_3$ and cGMP, blood pressure, body weight, heart weight, and plasma electrolytes and creatinine. Between-group comparisons in the SBP increase over time were analyzed by two-factor ANOVA (factor 1: group; factor 2: time period), with repeated measures on factor 2. When evaluating treatment- and/or time-dependent effects within a group, raw data were analyzed by one-factor (factor: time) repeated-measures ANOVA followed by Fisher's least significant difference test for multiple comparison. All treatment-related effects within a group at a specific time point were compared by Student's unpaired *t*-test. Linear regression analysis was used to determine the correlation between SBP and serum $NO_2/NO_3$ or serum cGMP, as well as between the change in SBP and the $NO_2/NO_3$ levels. All data are presented as means ± SE, and a *P* value of <0.05 was considered statistically significant.

## RESULTS

*General*

Table 1 contains the SBPs, body weights, and heart (ventricular) weights on the day of death for each experimental group 2 wk after renal artery clipping or sham clipping. Plasma $Na^+$, $K^+$, and creatinine concentrations for each group are also shown. Compared with the 1K normotensive control rats, the 1K1C rats had increased blood pressures (see also Fig. 1*A*) and ventricular weights (both absolute and as a fraction of body wt). Body weights, hematocrits, and plasma electrolyte concentrations did not differ significantly between these groups. The SBP in 1K1C rats reached a maximum 2 wk after clipping; SBPs in 1K1C rats after 3, 4, and 5 wk of clipping were not statistically different from the 2-wk value (data not shown). For the groups of rats receiving L-NAME, the 1K1C rats had a significantly higher SBP than 1K rats (Fig. 1*B*), whereas body

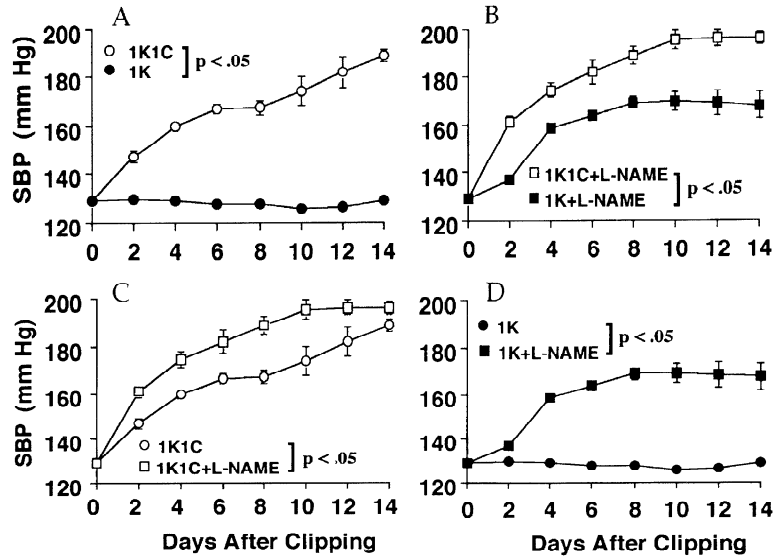


Fig. 1. Comparison of changes in systolic blood pressures (SBP) between untreated 1-kidney (1K) and 1-kidney, 1-clip (1K1C) rats (*A*); 1K and 1K1C rats on $N^G$-nitro-L-arginine methyl ester (L-NAME) (*B*); untreated 1K1C rats and 1K1C rats on L-NAME (*C*); and untreated 1K rats and 1K rats on L-NAME (*D*). Compared with untreated 1K rats, untreated 1K1C rats had a significant increase in SBP ($P < 0.05$). 1K1C rats on L-NAME had higher SBP than 1K rats on L-NAME ($P < 0.05$). Compared with that in untreated 1K1C rats, SBP increased more quickly in 1K1C rats on L-NAME ($P < 0.05$), although final pressure reached was not different between groups. SBP of untreated 1K rats did not change over time, whereas SBP in 1K1C rats on L-NAME increased significantly ($P < 0.05$). Data are expressed as means ± SE; $n = 6$ rats per group.

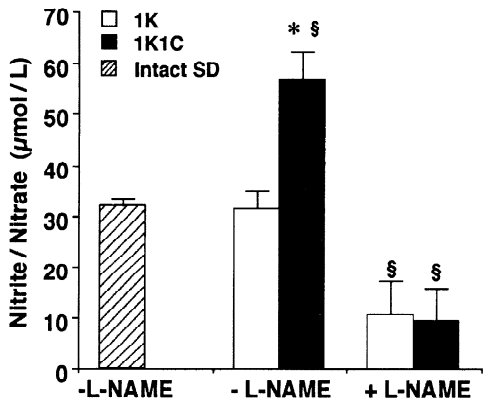


Fig. 2. Bar graph showing circulating nitrite/nitrate ($NO_2/NO_3$) levels in platelet-poor serum obtained from untreated Sprague-Dawley (intact SD) rats and from untreated and L-NAME-treated 1K and 1K1C rats. Two weeks after clipping, circulating $NO_2/NO_3$ levels were increased significantly in untreated 1K1C hypertensive rats compared with those in untreated 1K rats ($*P < 0.05$) and untreated Sprague-Dawley rats ($\S P < 0.05$). Circulating levels of $NO_2/NO_3$ were not different between untreated Sprague-Dawley and 1K rats, but $NO_2/NO_3$ levels in L-NAME-treated 1K and 1K1C rats were significantly decreased compared with those in their respective untreated groups ($\S P < 0.05$). Data are expressed as means ± SE; $n = 5$ rats per group.

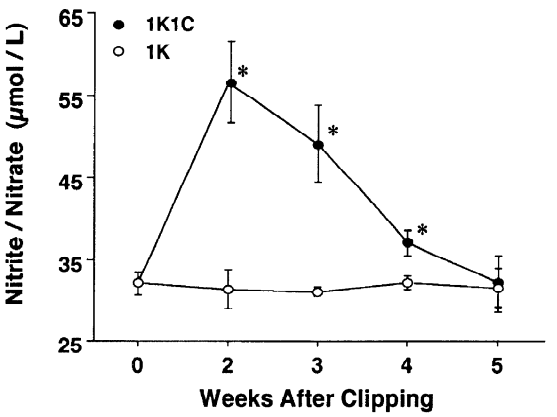


Fig. 3. Line graph showing circulating $NO_2/NO_3$ levels in platelet-poor serum obtained from paired groups of normotensive 1K rats and hypertensive 1K1C rats 2, 3, 4, and 5 wk after clipping. $*P < 0.05$ vs. corresponding value for 1K. Data are expressed as means ± SE; $n = 5$ rats per group.

weights, ventricular weights, and other measured variables were not different between these two groups.

*SBP in Untreated vs. L-NAME-Treated Rats*

Compared with that in untreated 1K rats, SBP was significantly elevated in 1K rats receiving L-NAME (Fig. 1*D*). This pressure elevation was significant after 2 days of L-NAME treatment and reached a maximum after 8 days of treatment. In hypertensive 1K1C rats receiving either normal water or L-NAME, SBP increased in a time-dependent fashion after the clipping (Fig. 1, *A* and *B*). However, the time course of the pressure increase in 1K1C rats receiving L-NAME was significantly faster than that in untreated 1K1C ($P < 0.05$; Fig. 1*C*). The maximal SBPs attained in both of these 1K1C groups were similar and not statistically different. Compared with that in untreated 1K1C rats, a significantly higher SBP in 1K1C rats on L-NAME was observed as early as 2 days after clipping ($P < 0.05$).

*Circulating Levels of $NO_2/NO_3$ and cGMP*

Compared with those in untreated 1K normotensive rats, serum $NO_2/NO_3$ levels were increased significantly in untreated 1K1C hypertensive rats 2 wk after clipping (57 ± 5 vs. 32 ± 3 μmol/l, $P < 0.01$) (Fig. 2). Serum $NO_2/NO_3$ levels in age-matched Sprague-Dawley rats and untreated 1K normotensive rats were similar. A maximal increase in serum $NO_2/NO_3$ levels was observed in untreated 1K1C rats 2 wk after clipping; the levels then gradually dropped in subsequent weeks until returning to the levels measured in normotensive 1K by the fifth week (Fig. 3). No changes in serum $NO_2/NO_3$ were observed in the normotensive 1K rats during this period. Compared with serum $NO_2/NO_3$ levels before clipping (32 ± 1 μmol/l), the respective levels in untreated 1K1C rats 2, 3, 4, and 5 wk postclipping were 54 ± 4 ($P < 0.01$), 49 ± 3 ($P < 0.05$), 37 ± 2 ($P < 0.05$), and 32 ± 3 μmol/l ($P > 0.05$). Two weeks after clipping, serum $NO_2/NO_3$ levels in the groups of 1K and 1K1C rats receiving L-NAME were similar (11 ± 6 and 10 ± 6 μmol/l, respectively) and were significantly lower than those in the paired untreated groups (Fig. 2). The groups receiving L-NAME also had significantly lower serum $NO_2/NO_3$ levels than normal Sprague-Dawley rats.

Compared with those in untreated 1K normotensive rats, serum cGMP levels increased significantly in untreated 1K1C hypertensive rats 2 wk after clipping (5.0 ± 0.6 vs. 2.4 ± 0.4 nmol/l, $P < 0.05$) (Fig. 4). The cGMP levels in paired 1K and 1K1C rats receiving L-NAME did not differ (1.7 ± 0.4 and 1.7 ± 0.7 nmol/l, respectively), but cGMP levels in L-NAME-treated 1K1C rats were significantly lower than those in untreated 1K1C rats ($P < 0.05$). Compared with those in un-

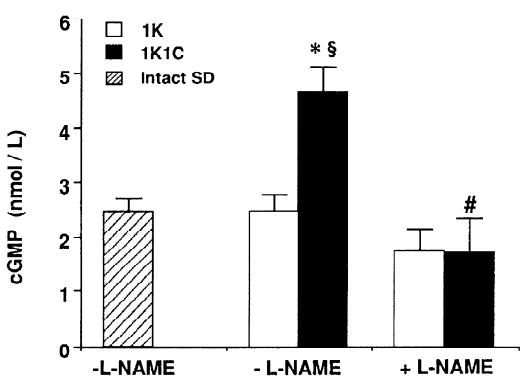


Fig. 4. Bar graph depicting circulating guanosine 3′,5′-cyclic monophosphate (cGMP) levels in platelet-poor serum obtained from untreated Sprague-Dawley rats and untreated or L-NAME-treated 1K and 1K1C rats. Two weeks after clipping, circulating cGMP levels were increased significantly in untreated 1K1C hypertensive rats compared with those in untreated 1K rats ($*P < 0.05$) and Sprague-Dawley rats ($\S P < 0.05$). There was no difference in cGMP levels between 1K rats and 1K1C rats treated with L-NAME or between Sprague-Dawley rats and untreated 1K rats. Circulating cGMP levels in L-NAME-treated 1K1C rats were significantly lower than in untreated 1K1C rats ($\#P < 0.05$). Data are expressed as means ± SE; $n = 5$ rats per group.

treated Sprague-Dawley and 1K rats, the cGMP levels in L-NAME-treated rats tended to be lower.

Linear regression analysis showed a significant correlation between $NO_2/NO_3$ levels and SBP ($r = 0.83$, $P < 0.01$; data not shown) as well as between cGMP levels and SBP ($r = 0.77$, $P < 0.05$; data not shown) in early 1K1C hypertension (2 wk postclipping). Although a significant correlation between $NO_2/NO_3$ and SBP was also evident after 3 wk of hypertension, no such correlation was found in rats with established hypertension (5 wk after clipping). A significant correlation was also found between $NO_2/NO_3$ or cGMP levels and the change in SBP from 1 to 2 wk postclipping and the change in SBP from 2 to 3 wk postclipping, but not between $NO_2/NO_3$ and cGMP levels and the SBP change from 3 to 5 wk postclipping. Furthermore, the correlations between $NO_2/NO_3$ and SBP observed in untreated early hypertensive 1K1C rats were absent in early hypertensive 1K1C rats receiving L-NAME.

## DISCUSSION

In this study, clipping of the contralateral renal artery in uninephrectomized rats caused a dramatic increase in arterial pressure that reached a plateau after 2 wk (Fig. 1*A*). There were also substantial increases in circulating $NO_2/NO_3$ and cGMP levels over this initial 2-wk period, and these increases were prevented by treatment with L-NAME. Although L-NAME treatment did not affect the steady-state level to which pressure rose after clipping, it did accelerate the pressure increase, thereby reducing the time to reach maximal pressure. The results of this study suggest that the onset of 1K1C hypertension in the rat is associated with a marked increase in NO production and that this early increase to some extent moderates the rapid rise in arterial pressure that occurs after renal artery clipping. Similar to our findings, Sigmon and Beierwaltes (27) have recently reported that NO activity is elevated 4 wk after renal artery clipping in rats with two-kidney, one-clip (2K1C) hypertension. Their observations indicate an enhanced influence of NO on resting vascular resistance throughout the periphery and suggest that this may be a normal compensatory mechanism to maintain adequate regional blood flows in the hypertensive state.

In the current study, circulating $NO_2/NO_3$ levels gradually declined after hypertension was fully established and had returned to control levels 5 wk after renal artery clipping (Fig. 3). The maintenance of elevated or normal NO activity during the first 5 wk of 1K1C hypertension, along with the findings of Sigmon and Beierwaltes in 2K1C hypertension, suggests that renovascular hypertension of moderate duration is not characterized by a suppressed influence of NO on vascular tone. However, because we did not study 1K1C rats beyond 5 wk postclipping, we do not know if NO activity may eventually become suppressed over longer periods of renovascular hypertension. In support of this possibility, Otsuka and co-workers (18) have reported that cGMP concentrations in the aortic wall of hypertensive 1K1C rats are decreased 6 wk after renal artery clipping.

In apparent contrast to our finding of elevated circulating $NO_2/NO_3$ levels in 1K1C rats 4 wk after clipping (Fig. 3), observations by Nakamura and Prewitt (16) in the striated muscle microvasculature of hypertensive 1K1C rats suggest that the basal influence of NO on arteriolar tone is reduced at this time. This discrepancy may be related to methodological differences between these two studies. In the current study, the renal artery was clipped when the rats were 10–12 wk old, and hypertension was fully developed 2 wk later. The 1K1C rats studied by Nakamura and Prewitt underwent renal artery clipping at 6–7 wk of age, and this approach apparently leads to a slower-developing but greater level of hypertension than that studied here (16, 28). It is possible that this more severe hypertension could exert an adverse effect on NO production by the fourth week after clipping, whereas the level of hypertension we studied did not.

Over the past decade, there has been extensive evidence indicating that hypertension is associated with morphological and functional alterations of the vascular endothelium (10). The animal model that has been most widely studied in this context is the SHR and its stroke-prone variant. In its established stage, this form of hypertension is associated with impaired arterial and arteriolar responses to the endothelium-dependent vasodilator ACh (13, 31), and this impairment has been attributed to the co-release of an endothelium-derived vasoconstrictor prostanoid along with NO (9, 13). This abnormality is not present in 3-wk-old normotensive SHR (8, 30) but is present in SHR by the fourth week of age, when arterial pressure is significantly increased (1, 30). Studies on other animal models and on human hypertensive patients have also revealed a correlation between the severity of hypertension and the extent to which endothelium-dependent responses are impaired (3, 6, 16, 18, 20), and this impairment often becomes more pronounced with prolonged hypertension (3, 11). Although these findings suggest that increased arterial pressure may diminish the NO-mediated interaction between endothelium and vascular smooth muscle, some aspects of this interaction may not be adversely affected. As in the 2K1C hypertensive rat (27) (and possibly in the 1K1C rats studied here), the basal influence of endothelium-derived NO on vascular tone is not suppressed in hypertensive SHR. Arnal and co-workers (2) have found no significant difference between 9-wk-old hypertensive SHR and normotensive Wistar-Kyoto (WKY) rats in arterial wall cGMP content, a variable considered to be a sensitive index of in vivo NOS activity (2). Furthermore, the effect on NOS inhibition on renal, hindquarters, and mesenteric vascular conductances in adult SHR is similar to that in age-matched WKY (6).

To our knowledge, the status of in vivo NO production at different stages of hypertension has not been previously explored. Serum and urinary $NO_2/NO_3$ have been used as markers for NO activity in vivo (23, 26), and the usefulness of serum $NO_2/NO_3$ levels as such a marker

in the current study is supported by our finding that L-NAME treatment significantly reduced these levels in all experimental groups (Fig. 2). Although NO is a source for circulating $NO_2/NO_3$, these levels could also be influenced by high-$NO_3$ diets. However, because the rats from different groups were pair-fed the same diet and consumed similar amounts, it is unlikely that the differences in $NO_2/NO_3$ levels among groups were due to diet. Tolins and co-workers (29) have demonstrated that, in the rat, hemodynamic responses to ACh correlate well with changes in urinary cGMP levels and that pretreatment with the NOS inhibitor $N^G$-monomethyl-L-arginine prevents ACh-induced increases in urinary cGMP excretion. Using urinary cGMP as one indicator of NO synthesis, Shultz and Tolins (26) provided evidence for increased in vivo NO synthesis in response to high dietary salt intake. However, Arnal and coworkers (1) have reported that serum cGMP levels do not reflect changes in aortic wall cGMP in SHR, but instead correlate closely with circulating levels of atrial natriuretic peptide. Although this finding suggests that serum cGMP levels may not be an accurate index of NO activity under all conditions, the reliability of serum cGMP as such an index in the current study is supported by two findings. First, elevated serum cGMP levels were found to correspond with elevated serum $NO_2/NO_3$ levels in 1K1C rats 2 wk after clipping (Figs. 2 and 4). Second, elevated cGMP levels, like elevated $NO_2/NO_3$ levels, were not found in 1K1C rats treated with L-NAME (Fig. 4).

It is not clear whether the apparent increase in endogenous NO activity in hypertensive 1K1C rats studied here is due to the actions of a constitutive or an inducible isoform of NOS. This study also provides no information on the site of increased NO production in these animals. In addition to endothelial cells, several other cell types are known to produce NO (10, 14, 25). Because a change in the hemodynamic forces associated with pressure and flow may change NO synthesis by influencing cyclic NOS activity in endothelial cells (24), it is possible that the increase in circulating levels of $NO_2/NO_3$ we observed is solely endothelium dependent. However, other possibilities cannot be ruled out and need to be further investigated.

As mentioned earlier, circulating $NO_2/NO_3$ levels in hypertensive 1K1C rats gradually decreased to prehypertension levels after 5 wk of hypertension. It is possible that this return to normal NO activity is due to some adaptation of the system to the hypertensive state or to an adverse effect of the hypertension on NO production. There is evidence that NO activity may actually decrease below normal in 1K1C rats after a more prolonged period of hypertension (18). Because NO normally inhibits both smooth muscle proliferation and migration (4, 5, 25), this secondary change could promote and/or facilitate other vascular adaptations associated with hypertension, such as vascular wall thickening or remodeling (10, 29). Factors such as platelet-derived growth factor and angiotensin II that may also promote vascular remodeling in hypertension (4, 5, 10, 25) would also be more effective in the presence of decreased NO synthesis (4, 5, 10, 25).

In conclusion, the results from the present study suggest that NO production is increased in early hypertensive 1K1C rats and that NO production then gradually decreases to prehypertension levels in established hypertension. The early increase in NO synthesis may represent an adaptation that slows the initial rise in arterial pressure, whereas the later decrease in NO synthesis could be the result of either a secondary adaptation of the system to the hypertensive environment or endothelial cell damage caused by a prolonged increase in blood pressure.

*Perspectives*

NO has previously been shown to play a protective role in the cardiovascular system by preventing platelet aggregation and adhesion in the peripheral vasculature. The current findings are consistent with a dramatic increase in NO production during the early stages of renovascular hypertension and suggest that the protective role of NO can be extended to include a slowing of hypertension development under these conditions. Further insight into the mechanisms that trigger increased NO production in early hypertension can be gained by the identification of the source of the NO (i.e., vascular endothelium or another cell type) and the determination of whether a constitutive or inducible NOS is involved. In addition, studies on other models of hypertension are needed to establish whether this potentially important counterregulatory mechanism is common to hypertension of different etiologies.

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