Vascular smooth muscle cell polyploidy and cardiomyocyte hypertrophy due to chronic NOS inhibition in vivo

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J. Physiol. 274 (Heart Circ. Physiol. 43): H52–H59, 1998.—To assess the vascular and cardiac response to NO (nitric oxide) synthase (NOS) blockade in vivo, Wistar-Kyoto rats (WKY) were treated for 3 wk with Nω-nitro-L-arginine methyl ester (L-NAME; 10 mg·kg⁻¹·day⁻¹). L-NAME treatment induced hypertension that was associated with increased plasma renin activity. Flow cytometry cell cycle DNA analysis showed that aortic vascular smooth muscle cells (VSMC) from L-NAME-treated WKY had a significantly higher polyploid population compared with WKY controls. Using organ bath experiments, we have shown that aortic rings from L-NAME-treated WKY have an increased contractile response to phenylephrine and impaired relaxation to carbachol compared with control rings. NOS blockade in vivo caused a significant increase in cardiac and left ventricular hypertrophy. Northern mRNA analysis of the myocardium showed that L-NAME treatment caused reexpression of the fetal skeletal α-actin isoform without alterations in collagen type I expression, a pattern indicating true hypertrophy of the cardiomyocytes. These studies provide further insight to confirm that NO deficiency in vivo results in the development of vascular and cardiac hypertrophy.

cell cycle; hypertension; renin-angiotensin system; Nω-nitro-L-arginine methyl ester; skeletal α-actin

NITRIC OXIDE (NO) plays an important role in the regulation of blood pressure and vascular tone (14, 19). The short-term and long-term in vivo administration of NO synthase (NOS) inhibitors such as Nω-nitro-L-arginine methyl ester (L-NAME) produces an elevation in arterial pressure and an increase in peripheral vascular resistance that may be associated with a change in plasma renin activity (15, 21). However, the lack of a close and parallel relationship between arterial pressure and the degree of vascular and cardiac hypertrophy was demonstrated in previous studies (1, 2, 13). Analysis of the processes involved in vascular smooth muscle and cardiac growth at the cellular level implies that angiotensin II (ANG II) is one of the most important growth factors for vascular and cardiac myocytes (24, 27). Vascular smooth muscle cell (VSMC) hypertrophy occurs in large capacitance arteries such as the aorta of chronically hypertensive animals or humans and is accompanied by the development of polyploidy, which occurs when cells replicate their DNA but do not complete mitosis (24, 25). We have previ-ously demonstrated a role for ANG II in the development of aortic VSMC polyploidy in the stroke-prone spontaneously hypertensive rat (SHRSP; Refs. 10, 11). However, the role of basal NO release in the pathophysiology of VSMC polyploidy has not been previously assessed.

Cardiac hypertrophy was measured in previous studies by heart weight-to-body weight (HW:BW) and left ventricle plus septum weight-to-body weight (LV+S:BW) ratios (2, 21). Such data, although informative, do not indicate which of the components of myocardial tissue are involved in the hypertrophic response. Left ventricular hypertrophy involves changes in cardiac gene expression with an adaptive switch from the adult to the fetal pattern. This response includes changes in the expression of myosin heavy chain and α-actin isoforms in cardiomyocytes (4) as well as collagen genes in cardiac fibroblasts (6). The exact pattern of gene expression in the myocardium indicates whether left ventricular hypertrophy is caused by cardiomyocyte hypertrophy and/or interstitial myocardial fibrosis and remodeling.

Recent in vivo studies showed that impaired endothelial function is associated with L-NAME-induced hypertension (12, 29). However, there are no studies to our knowledge that integrate aortic function with cellular aspects of vascular and cardiac hypertrophy in L-NAME-treated Wistar-Kyoto rats (WKY). Furthermore, in vivo NOS inhibition has been associated with pathological changes, with previous studies focusing on hypertensive renal damage (3, 5) rather than incorporating aortic and cardiac histopathology.

The aim of the present investigation was to determine whether true vascular and cardiac myocyte hypertrophy is the underlying cellular response to NOS blockade in vivo. This was done in an attempt to resolve the controversy over the development and role of vascular and cardiac hypertrophy in L-NAME-induced hypertension. The effect of in vivo L-NAME treatment on aortic VSMC polyploidy, which is an indicator of vascular hypertrophy, was studied for the first time. Additional studies of vascular structure and function were performed using histology, immunocytochemistry, and classic organ bath experiments. The traditional HW:BW and LV+S:BW ratios of cardiac hypertrophy were supplemented by Northern mRNA studies of skeletal and cardiac α-actin isoforms and collagen type I expression to ascertain cardiac myocyte and fibroblast cellular responses. Finally, plasma renin activity, angiotensin-converting enzyme (ACE) activity, and ANG II levels were determined, because few studies in the past
have assessed all three components of the circulating renin-angiotensin system in relation to NOS blockade in vivo.

**METHODS**

Experimental Animals

WKY were obtained from colonies established in the Department of Medicine and Therapeutics, University of Glasgow. The breeding stocks were obtained from the colonies maintained at the University of Michigan, which in turn had obtained their breeding stocks from the National Institutes of Health (11). Rats were housed under controlled conditions of temperature (21°C) and light (12:12-h light-dark cycle; 7 AM to 7 PM) and were maintained on normal rat chow (rat and mouse no. 1 maintenance diet, Special Diet Services, UK) and water ad libitum.

The following experimental groups were used: L-NAME-treated WKY (n = 15; 10 mg·kg⁻¹·day⁻¹) and an age- and sex-matched WKY control group (n = 16). L-NAME was administered for 3 wk in the animals’ drinking water, starting at 8 wk of age. The untreated control group received vehicle alone.

Blood pressure was measured by tail-cuff plethysmography in conscious, restrained rats as previously described (10). Blood pressure was measured before treatment and then once a week during the treatment period. At the end of the treatment period the animals were killed by halothane overbreathing. At the end of the treatment period the animals were killed by halothane overbreathing. For Northern analysis, total RNA was isolated from the myocardium of L-NAME-treated WKY and control WKY (4). Ten micrograms of RNA and molecular weight markers were glyoxylated at 50°C for 1 h and size fractionated on a 1.2% agarose gel and then transferred to Hybond nylon membrane overnight. Hybridizations were performed overnight at 42°C in 50% formamide using [³²P]MCP-labeled skeletal α-actin cDNA, cardiac α-actin cDNA, collagen type I cDNA, or glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA. Probes were prepared using the Random Prime Kit (Gibco BRL) Autoradiographs were quantified by densitometric scanning by an independent observer. The filter was stripped with 0.1% sodium dodecyl sulfate in boiling water, and stripping between probes was confirmed by exposure to film. The GAPDH signal was used as the internal standard to normalize the skeletal α-actin, cardiac α-actin, and collagen type I signals.

Aorta: Isometric Tension Recording

After being carefully dissected, the thoracic aorta was cleaned of connective tissue and cut into 2- to 3-mm rings. The rings were suspended under 1-g tension in individual 10-ml muscle baths containing physiological saline solution (PSS) of the following composition (mmol/l): 130 NaCl, 4.7 KCl, 14.9 NaHCO₃, 1.18 KH₂PO₄, 1.75 MgSO₄ ·7H₂O, 1.6 CaCl₂ ·2H₂O, 5.5 glucose, and 0.03 CaNa₂-EDTA. The PSS was aerated with 95% O₂-5% CO₂, and the proteoglycan: collagen (P: C) ratio was added to final bath concentration of 10 µmol/l. Isometric tension was measured by a force transducer and recorded by a multichannel pen recorder. After a 1-h equilibration period, two protocols were followed on 5–15 rings from control rats and 5–14 rings from L-NAME-treated rats.

**Protocol 1** is illustrated in Fig. 1A. An initial response to KCl (100 mmol/l) was recorded. After a washout period, a concentration-response curve to phenylephrine (PE) (10⁻⁴–10⁻⁵ mol/l) was constructed. After a further washout, the rings were preconstricted to the half-maximal effective concentration (EC₅₀) of PE and a concentration-response curve to carbachol (10⁻⁴–10⁻⁵ mol/l) was constructed. PE and carbachol were washed out, the rings were again preconstricted to the EC₅₀ of PE, and a concentration-response curve to the endothelium-independent vasorelaxant sodium nitroprusside (SNP) (10⁻¹⁰–10⁻⁶ mol/l) was constructed to determine the vascular smooth muscle responsiveness to NO. In some rings from L-NAME-treated and control rats, the construction of the PE and carbachol concentration-response curves was repeated after addition of L-arginine (0.5 mmol/l).

**Protocol 2** is illustrated in Fig. 1B. Parallel rings to those used in protocol 1 were incubated with the superoxide dismutase (SOD) inhibitor diethyldithiocarbamic acid (DETCA; 10 mmol/l) for 45 min. Concentration-response curves for PE and carbachol were then constructed as for protocol 1.

Histology and Immunocytochemical Detection of Endothelial NOS

Aortas and hearts from L-NAME-treated and control WKY were fixed in 10% phosphate-buffered formal saline followed by dehydration and embedding in paraffin wax. Sections (4 µm thick) were cut, rehydrated, and stained with hematoxylin and eosin before light microscopic examination was used to establish the pathological changes present.

Immunocytochemistry for endothelial NOS (eNOS) was performed on serial 4-µm sections as described previously (17). Briefly, sections from aortas and hearts of L-NAME-treated and control WKY were incubated in succession with the rabbit anti-endothelial constitutive NOS polyclonal antibody (Transduction Laboratories) and a biotinylated goat anti-rabbit immunoglobulin followed by incubation in peroxidase-labeled streptavidin. The slides were developed in a

Evaluation of Cardiac Hypertrophy

Immediately after exsanguination, the thorax was opened and the heart was removed, blotted with tissue paper, and weighed. The atria and right ventricle were then removed, and the left ventricle and septum were weighed. HW:BW and LV+S:BW were then determined.

Northern mRNA Analysis

For Northern analysis, total RNA was isolated from the myocardium of L-NAME-treated WKY and control WKY (n = 3 each group) using RNAzol B (8). Ten micrograms of RNA and molecular weight markers were glyoxylated at 50°C for 1 h and size fractionated on a 1.2% agarose gel and then transferred to Hybond nylon membrane overnight. Hybridizations were performed overnight at 42°C in 50% formamide using [³²P]MCP-labeled skeletal α-actin cDNA, cardiac α-actin cDNA, collagen type I cDNA, or glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA. Probes were prepared using the Random Prime Kit (Gibco BRL). Autoradiographs were quantified by densitometric scanning by an independent observer. The filter was stripped with 0.1% sodium dodecyl sulfate in boiling water, and stripping between probes was confirmed by exposure to film. The GAPDH signal was used as the internal standard to normalize the skeletal α-actin, cardiac α-actin, and collagen type I signals.

Preparation of Aortic VSMC Nuclei and Flow Cytometric Analysis

The aorta was carefully dissected, and a section was used for preparation of aortic rings for functional studies (see Aorta: Isometric Tension Recording). The other section of the thoracic aorta was collected under sterile conditions for preparation of primary VSMC. VSMC were prepared by enzymatic dissociation of rat aorta as described previously (10, 11). From each aorta, ~10⁶ primary VSMC were obtained for analysis by flow cytometry. Nuclei for the flow cytometry DNA analysis were prepared and stained according to the method of Vindelov et al. (31) with some minor modifications as previously described (10). Human peripheral blood lymphocytes were stained in parallel to provide a diploid profile for DNA peak standardization. The DNA flow cytometry signal was used as the internal standard to normalize the skeletal α-actin, cardiac α-actin, and collagen type I signals.

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mixture of 0.03% H₂O₂ and 0.03% 3,3'-diaminobenzidine. Appropriate negative controls were run in parallel as described previously (17).

Plasma Renin-Angiotensin System

At the end of the in vivo L-NAME treatment period, rats were anesthetized with halothane, and blood was drawn from the left ventricle for measurements of plasma renin activity, ANG II concentration, and ACE activity. Plasma renin activity was measured using an antibody-trapping method adapted for use with rat plasma (22). Plasma ANG II was measured by radioimmunoassay after Sep-Pak C₁₈ (Waters Associates, Milford, MA) extraction, after which high-performance liquid chromatography of the Sep-Pak extract was performed as a check for its direct assay (23). Plasma ACE activity was measured directly using a color kit (Fujirebio, Tokyo, Japan; distributed in the UK by Mast Diagnostics, Bootle, UK).

Drugs and Materials

Enzymes, trypsin inhibitor, spermine tetrahydrochloride, propidium iodide, and L-NAME were purchased from Sigma Chemical (Poole, Dorset, UK). Agarose was purchased from Gibco, and RNAZol B was obtained from Biogenesis. Nylon membrane (Hybond) was from Amersham International (Amersham, UK).

Statistical Analysis

Data were analyzed using repeated-measures analysis of variance, with Wald's test in BMDP and paired and unpaired Student's t-test where appropriate. Values are expressed as means ± SE, and P < 0.05 was considered statistically significant.

RESULTS

Blood Pressure, Heart Rate, and Body Weight

There was a 40-mmHg difference in systolic blood pressure (SBP) between the control group (untreated WKY) and the L-NAME-treated WKY (Fig. 2A). The L-NAME-induced hypertension was rapid in onset, with significant increases in SBP as early as 1 wk after the initiation of treatment. Blood pressure, as measured using tail-cuff plethysmography, increased in a time-dependent fashion until, after 3 wk of treatment, the blood pressure of the L-NAME-treated group (n = 15) was 172.4 ± 5.68 mmHg compared with 132 ± 4 mmHg in the untreated control group (n = 16). Average SBPs were significantly different between the L-NAME-treated and control WKY groups (F = 62.1, P < 0.0001).
and there was a significant group × time interaction (F = 38.4, P < 0.0001; Fig. 2A). Analysis of heart rate and body weight for the control and L-NAME treatment groups showed no statistically significant differences (data not shown).

Flow Cytometry DNA Analysis

Flow cytometry DNA ploidy analysis of primary aortic VSMC showed that the percentage of cells in the G2 + M phase of the cell cycle was significantly higher in the L-NAME-treated group (24.12 ± 1.1%, n = 14) compared with the untreated WKY control group [16.58 ± 3.5%; n = 16; P < 0.0001, 95% confidence interval (CI) −9.94 to −5.1; Fig. 2B]. Therefore, there is a significant increase in the polyploid VSMC population in the aortas from L-NAME-treated WKY compared with untreated control WKY.

Cardiac Mass Indexes

Measurements of heart weight and left ventricular weight are expressed as HW:BW (mg/g) and LV+S:BW (mg/g). Body weight did not differ between the control and L-NAME-treated groups.

HW:BW was significantly higher in the L-NAME-treated group (4.20 ± 0.11 mg/g; n = 15) than in the control WKY group (3.095 ± 0.063 mg/g; n = 16; P < 0.0001, 95% CI 1.370 to −0.85; Fig. 3). LV+S:BW was also significantly increased in the L-NAME-treated group (3.42 ± 0.089 mg/g; n = 15) compared with the control WKY group (2.36 ± 0.037 mg/g; n = 16; P < 0.0001, 95% CI −1.259 to −0.85; Fig. 3).

Expression of Skeletal and Cardiac α-Actin mRNA

Expression of skeletal α-actin mRNA in myocytes from the heart was observed in vehicle-treated WKY (Fig. 4). Administration of L-NAME markedly enhanced the expression of skeletal α-actin mRNA and significantly increased the expression ratio of skeletal α-actin mRNA in the WKY myocardium (L-NAME: 0.463 ± 0.07, control: 0.173 ± 0.04; P = 0.03).

![Graph showing heart weight to body weight and left ventricle + septum (LV+S) weight to body weight for WKY(C) (n = 16) and WKY(L-NAME) (n = 15). Unpaired t-test: ***P < 0.0001 vs. WKY(C). Values are means ± SE.](Image)

Fig. 3. Ratios of heart weight to body weight and left ventricle + septum (LV+S) weight to body weight in WKY(C) (n = 16) and WKY(L-NAME) (n = 15). Unpaired t-test: ***P < 0.0001 vs. WKY(C). Values are means ± SE.

![Graph showing L-NAME administration enhanced expression of skeletal α-actin mRNA. Representative bands from Northern blots are presented for ventricles from WKY(L-NAME) and WKY(C) groups. First band, skeletal α-actin; second band, cardiac α-actin; third band, collagen type I; fourth band, housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH).](Image)

Fig. 4. L-NAME administration enhanced expression of skeletal α-actin mRNA. Representative bands from Northern blots are presented for ventricles from WKY(L-NAME) and WKY(C) groups. First band, skeletal α-actin; second band, cardiac α-actin; third band, collagen type I; fourth band, housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH).

![Graph showing PE response. The contraction to PE (relative to KCl) was significantly greater in rings from L-NAME-treated WKY [maximal contraction (E max) = 2.1 ± 0.17; n = 14] compared with rings from control WKY (E max = 1.6 ± 0.12; n = 7; P = 0.017, 95% CI 0.11 to 0.99; Fig. 5A). The addition of L-arginine did not significantly alter the contraction to PE in rings from L-NAME-treated WKY (E max = 2.3 ± 1.8; n = 6; P = 0.43, 95% CI −0.74 to 0.33; Fig. 5A). Carbachol response. The relaxation to carbachol (% of PE) was significantly attenuated in rings from L-NAME-treated rats (E max = 43 ± 4.7; n = 15) compared with rings from control rats (E max = 89 ± 3.8; n = 9; P < 0.0001, 95% CI 32.9–58.3; Fig. 5B). The addition of L-arginine significantly improved the relaxation of rings from L-NAME-treated rats (E max = 73 ± 7.3; n = 9; P < 0.0001, 95% CI 29.8–57.8), causing the relaxation to be not significantly different from control rat aortic rings (n = 9; P = 0.09, 95% CI −2.7 to 33.2; Fig. 5B). SNP response. There was no significant difference in the maximum relaxation to SNP (E max % of PE) between L-NAME-treated animals (99.8 ± 3.7; n = 5) and control (101.4 ± 1.4; n = 5; P = 0.70, 95% CI −8.5 to 11.7) animals. However, the EC50 (×10−8 mol/l) was significantly lower in L-NAME-treated animals (0.5 ± 0.14; n = 5) than in control animals (2.38 ± 0.61; n = 5; P = 0.04, 95% CI 0.13–3.59; data not shown). Effect of DETCA. In rings from control rats, the maximum relaxation to carbachol (% of PE) was significantly reduced in the presence of DETCA (E max = 56 ± 7.9; n = 5) compared with this response in the absence of DETCA (E max = 89 ± 3.8; n = 5; P = 0.036, 95% CI 3.7–66.7; Fig. 5C). In rings from rats treated with DETCA, the maximum relaxation to carbachol (% of PE) was also significantly reduced in the presence of DETCA (E max = 26 ± 1.4; n = 5; P = 0.0001, 95% CI 0.03–0.33).](Image)
L-NAME, the maximum relaxation to carbachol was similarly reduced in the presence of DETCA ($E_{\text{max}} = 18 \pm 2.6; n = 10$) compared with the relaxation in the absence of DETCA ($E_{\text{max}} = 43 \pm 4.7; n = 10; P = 0.017, 95\% \text{CI} 5.46$–$43.7$; Fig. 5D). The attenuation by DETCA (change in $E_{\text{max}}$) in rings from L-NAME-treated WKY ($25 \pm 8.5; n = 10$) did not differ significantly from the attenuation by DETCA in rings from control WKY ($35 \pm 11; n = 5; P = 0.48, 95\% \text{CI} -22$–$43$; Fig. 5, C and D).

**Histology**

Aorta. Structural integrity of the endothelial lining and underlying aortic wall was confirmed in both control and L-NAME-treated groups (Fig. 6, A and B).
Nuclear hypertrophy was clearly present in the aortic medial smooth muscle cells from the L-NAME-treated group of WKY (Fig. 6B) compared with the aortic smooth muscle cells in the untreated control WKY (Fig. 6A).

Heart. Cardiac myocyte hypertrophy was present in the L-NAME-treated group of animals. In addition, scattered foci of recent cardiac myocyte necrosis, with an associated reparative response including a mild, acute inflammatory infiltrate, were identified (Fig. 6, C and D). These foci of myocardial damage were identified in both the left ventricular and right ventricular walls, the damage involving ~3% of the left ventricular myocardium and 5% of the right ventricle. The coronary arteries appeared intact with no evidence of thrombosis or intrinsic arteritis (data not shown). Hearts from the control group of animals were normal by light microscopy.

Immunocytochemical Detection of eNOS

Endothelial immunocytochemical staining was positive for eNOS in the aortas and hearts from both the control and L-NAME-treated animals. No detectable difference in staining intensity or distribution was identified by light microscopy (data not shown). In the aorta, all endothelial cells were positive. The heart demonstrated endothelial positivity within the epicardial and larger intramyocardial coronary artery branches, with staining intensity markedly diminishing within the small subendocardial coronary arterioles and an absence of staining in the myocardial capillary plexus.

Plasma Renin-Angiotensin System

Treatment with L-NAME resulted in a significant increase in plasma renin activity compared with the untreated control group (P = 0.038, 95% CI −23.9 to −0.9; Table 1). Plasma ANG II concentration was also significantly increased in the L-NAME-treated group compared with the untreated control WKY group (P = 0.0095, 95% CI −313.2 to −66; Table 1). However, there was no significant difference in plasma ACE activity between the L-NAME-treated group and the controls (P = 0.33, 95% CI −1.8 to 5.08; Table 1).

**Table 1. Plasma renin-angiotensin system**

<table>
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<th>PRA, ng ml⁻¹·h⁻¹</th>
<th>ANG II, pg ml⁻¹</th>
<th>ACE, nmd·ml⁻¹·min⁻¹</th>
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<tr>
<td>WKY (C)</td>
<td>12.2 ± 1.5</td>
<td>103 ± 9.3</td>
<td>18.3 ± 1.2</td>
</tr>
<tr>
<td>WKY (L-NAME)</td>
<td>24.6 ± 4.6</td>
<td>292.5 ± 49.7</td>
<td>16.7 ± 1</td>
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Values are means ± SE for plasma renin activity (PRA), ANG II concentration, and angiotensin-converting enzyme (ACE) activity in control [WKY (C); n = 8] and in N⁵-nitro-L-arginine methyl ester (L-NAME)-treated [WKY (L-NAME); n = 7] Wistar-Kyoto animals. Unpaired t-test: *P < 0.05, †P < 0.01 vs. WKY (C).
permissive effect for cardiac hypertrophy in L-NAME-induced hypertension. Indeed, the results of the present study provide further evidence to support this explanation. We have also investigated cardiac gene expression to further assess the responses of cardiac myocytes and interstitial fibroblasts to NOS blockade in vivo. Using Northern analysis, we studied the expression of collagen type I and skeletal α-actin, a fetal form of sarcomeric actin that is reexpressed in the hypertrophied cardiomyocyte (4). Chronic administration of L-NAME increased the expression ratio of skeletal to cardiac α-actin mRNA, but cardiac collagen type I expression was unchanged. The absence of changes in collagen and reexpression of skeletal α-actin indicates the absence of interstitial fibrosis or myocardial collagen matrix remodeling combined with the presence of myocyte hypertrophy. The present results demonstrate true cardiomyocyte hypertrophy in L-NAME-induced hypertension, a characteristic that is shared with SHRSP but not necessarily with spontaneously hypertensive rats (SHR) (30).

Functional organ bath experiments showed that chronic L-NAME treatment in vivo resulted in increased vascular reactivity of the aorta in vitro to exogenously added PE. This could be caused by decreased NO availability and/or the presence of aortic hypertrophy. In the present study, endothelium-dependent relaxations in the aorta were reduced in L-NAME-hypertensive rats, which is in agreement with previous studies (18, 29). We found no significant difference in the maximum relaxation to SNP; however, the EC50 in L-NAME-treated animals was shifted significantly to the left compared with controls, suggesting that the former are more sensitive to NO donors. Such increased sensitivity to SNP after inhibition of vascular NO synthesis in vivo may be the consequence of enhanced guanylate cyclase sensitivity to chemical NO donors (20). Therefore, the impaired endothelium-dependent relaxations in L-NAME-treated animals in the current study cannot be explained by reduced sensitivity of VSMC to NO. The impaired relaxation to carbachol may be corrected by treatment with L-arginine in vitro. We also investigated the role of superoxide anion (O$_2^-$) in L-NAME-induced functional changes by blocking intracellular SOD activity, using DETCA. When SOD, the endogenous pathway for the disposal of O$_2^-$, is blocked, the accumulation of this anion impairs VSMC relaxation (16). However, we found that the impairment of relaxation was similar in aortic rings from both groups, suggesting that O$_2^-$ accumulation does not differ as a result of L-NAME treatment. Direct measurements of O$_2^-$ release could help in understanding the functional changes observed here.

Using immunocytochemistry, we detected no significant difference in eNOS expression in the aorta or heart despite the marked functional and structural changes that were present. However, more quantitative studies of eNOS expression are necessary to confirm this. Previous studies that addressed the histopathological changes in L-NAME-induced hypertension characterized renal vessel pathology, which was shown to consist primarily of glomerular ischemia, glomerulosclerosis, and interstitial expansion (3, 5). We noted similar renal pathological changes in the present study (data not shown). In addition, we noted aortic VSMC hypertrophy, which confirms our VSMC polyploidy results. Furthermore, cardiomyocyte hypertrophy and regions of myocardial necrosis with an associated focal reparative response were noted in sections of the myocardium from L-NAME-treated WKY, but these features were not present in sections from control WKY. Such marked cardiac pathology may result from the direct effect of NO inhibition or indirectly as a result of L-NAME-induced hypertension.

In summary, the current study combines aortic and cardiac functional and structural studies with analysis of the underlying cellular and molecular mechanisms involved in the response to NO blockade in vivo. The changes observed may be the direct result of NO deficiency or an indirect enhanced sensitivity to prevailing ANG II levels. The present data provide further evidence to support the role of basal NO production as being counterregulatory to the pressor and trophic actions of the renin-angiotensin system. In conclusion, we have shown that deficient vascular and cardiac synthesis of NO results in VSMC polyploidy and cardiomyocyte hypertrophy with concomitant vascular and cardiac abnormalities that are involved in the pathogenesis of L-NAME-induced hypertension.

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