Apoptosis, Coronary Arterial Remodeling, and Myocardial Infarction After Nitric Oxide Inhibition in SHR

Yuko Ono, Hidehiko Ono, Hiroaki Matsuoka, Takahiro Fujimori, Edward D. Frohlich

Abstract—This study was designed to investigate the relationship between apoptosis (programmed cell death) and coronary arterial remodeling in spontaneously hypertensive rats (SHR) following prolonged nitric oxide synthesis inhibition. In addition, we evaluated whether the development of coronary arterial smooth muscular cell apoptosis was related to hemodynamics or to vascular hypertrophy. Three groups of 20-week-old male SHR were investigated: controls, and two groups that received two doses of Nω-nitro-L-arginine (L-NAME, 50 mg/L and 80 mg/L) each for 3 weeks. Mean arterial pressure and total peripheral resistance index increased whereas cardiac index diminished with L-NAME. Pathohistological study demonstrated increased pericardiac fibrosis and coronary arterial injury score in the L-NAME group in a dose-dependent manner. The high dose of L-NAME (Group 3) produced myocardial infarction in 78% of the rats. The wall:lumen ratio of epicardial coronary arteries was greater in L-NAME treated SHR (0.23±0.02 versus 0.16±0.02; P<0.05) and was associated with markedly increased apoptosis (15.3±6 versus 1.9±1; P<0.05) without smooth muscle cell proliferation (PCNA positive cells). Apoptosis occurred predominantly in hypertrophic coronary arterial smooth muscular cells; myocardial infarction and ventricular fibrosis were exacerbated by impaired hemodynamics induced by L-NAME. These data suggest that coronary endothelial dysfunction and myocardial ischemic disease induced by L-NAME were responsible for apoptosis of coronary arterial smooth muscle cells, myocardial fibrosis, and infarction, all pathological findings that are consistent with what may be found in clinical hypertensive heart disease. (Hypertension. 1999;34:609-616.)

Key Words: apoptosis ■ nitric oxide ■ L-NAME ■ microvascular remodeling ■ myocardial infarction ■ ventricular fibrosis ■ myocardial hemodynamics ■ spontaneously hypertensive rat

Apoptosis is a tightly regulated and energy-requiring physiological process in which cell death follows a programmed sequence of events.1–4 Chromosomal DNA fragmentation is the biological hallmark of apoptosis.5,6 The apoptotic process is regulated by a number of genes including Fas and ICE/CED-3 (Interleukin-1β-converting enzyme),8 p53,9 and Bcl-2.10 Recognition of the factors responsible for the initiation or prevention of this programmed cell death may eventually lead to specific therapeutic interventions. In hypertension, several studies in experimental animals and in patients have demonstrated the relationship between vascular structural alteration11–13 and apoptosis.14–18 At the level of the smaller coronary arteries, vascular hypertrophy accompanied by smooth muscular cell (SMC) hypertrophy or hyperplasia act as amplifiers for elevated vascular resistance and blood pressure. Cardiovascular remodeling, including vascular hypertrophy, is now considered a key factor responsible for the progression of disease and its increased morbidity and mortality.

We have previously reported that, in the spontaneously hypertensive rat (SHR) kidney, prolonged (three week) nitric oxide synthase (NOS) blockade produced marked proteinuria and severe hypertensive nephrosclerosis manifested by intense afferent and efferent arteriolar constriction, glomerular hypertension and hyperfiltration, and fibrinoid necrosis that could be prevented or reversed by angiotensin-converting enzyme inhibition or certain calcium antagonists, but were exacerbated by a diuretic.19–22 The present study was designed to investigate the regulation of endothelial cell or SMC apoptosis during the reversal of cardiovascular medial hypertrophy and improved cardiac hemodynamics induced by prolonged NOS blockade in SHR.

Methods

Thirty-one male 17-week-old SHR (purchased from Charles River Laboratories, Wilmington, Massachusetts), having body weights in the range 290 to 330 g (mean±1 SEM, 306±5 g) were used. This study had been approved by our institutional animal care committee. Ten SHR were administered only tap water for 3 weeks and served as untreated controls (Group 1). Groups 2 and 3 included 21 SHR given the nitric oxide synthase inhibitor L-NAME (Sigma Chemical Co, St. Louis, Missouri, USA) in their drinking water (either 50 mg/L; dose of 9.0±0.4 mg/kg per day for 3 weeks; 12 rats or 80 mg/L; dose of 14.3±0.7 mg/kg per day 9 SHRs for 3 weeks). The
drinking water containing L-NAME was changed daily to ensure that the precise dose of L-NAME and fluid volume and food intake could be quantified.

### Hemodynamics
All rats were deprived of food overnight prior to the systemic hemodynamic study although they were allowed free access to water. They were anesthetized with inactin (100 mg/kg body weight) and were then placed on a heating pad in order to maintain their rectal temperature at 37°C throughout the study. After a tracheotomy, a polyethylene catheter (PE-50) was inserted into the abdominal aorta through the right femoral artery to permit blood sampling and direct measurement of mean arterial pressure (MAP) and heart rate. The right carotid artery and right jugular vein were cannulated with PE-50 tubing for determination of cardiac output using a thermocouple microprobe connected to a thermilution device (Carditherm 500: Columbus Instrument, Columbus, Ohio, USA) as reported previously.19–22 Cardiac output, expressed in mL/min, was normalized with respect to body weight and expressed as cardiac index (mL/min per kg). Pressures were measured using Gould-Statham transducers (Model P23 Db; Statham Instruments, Oxnard, California, USA) connected to a multichannel polygraph (Sensor Medics R612, Beckman Instruments Inc, Dayton, Ohio, USA).

### Histological Studies
Light microscopic examinations were performed after each systemic hemodynamic study. Heart and thoracic aorta were fixed in fresh 10% paraformaldehyde, after which the organs were removed and weighed. The fixation pressure was the mean arterial pressure of the rats or 230 mm Hg for the L-NAME/SHR and 186 mm Hg for the control SHR. Sections (3 µm thick) were stained with hematoxylin and cosin (HE), periodic acid-Schiff (PAS), modified Verhoffs van Gieson (EVG), Elastica Masson periodic acid-methenamine-silver (PAM), and phosphotungstic acid-methenamine-silver (PTAH) for assessment of myocyte injuries, extracellular matrix, fibrinogen, and collagen.

Grading of coronary arteriolar injury (inside diameter, less than 100 µm) was performed on a scale from 0 to 3+, in which: 0 denoted no injury; 1+ denoted hyalinosis of the arteriolar wall up to 50% of its circumference; 2+ denoted 50% to 100% hyalinosis of the wall circumference but without luminal narrowing; and 3+ denoted complete hyalinosis of the wall with luminal encroachment. Thus, a coronary injury score (CIS) was calculated by examining 40 to 50 coronary arterioles in the epicardial and endocardial areas. These scores were obtained as a result of independent study by two co-authors of this report who scored all tissues in a blinded manner.

Percentage of irregular fibrosis in the interstitial space was assessed with EVG stain using an image analysis computer system (Optimas; Bioscan, Edmonds, WA, USA). Except for measurement of coronary arterial circumference and area of myocardial infarction, five fields were randomly selected from each specimen. The percentage of interstitial fibrosis (red area by EVG stain) was determined for all three rat groups as the percentage of fibrosis in all fields.

### Immunohistochemical and Apoptosis Studies
Five cardiac specimens were placed immediately in fresh 10% paraformaldehyde for two hours, and then transferred to a 30% sucrose-phosphate buffer saline (PBS) solution before being embedded in paraffin. For each specimen, several 5-µm sections were obtained for classic histological analysis, in situ determination of apoptosis, and immunohistochemical studies. In situ detection of apoptotic cells (terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling of fragmented DNA, TUNEL) was performed according to Gavieli et al.23 Paraffin sections were first deparaffinized, transferred to xylenes, and dehydrated in ascending alcohol series (100%, 95%, 50%, and 0%). After dehydration, the slides were incubated with 70 µg/mL proteinase K in PBS, then endogenous peroxidase was inactivated by treating the slides with hydrogen peroxide. Tissue sections were also stained with dioxigenin nucleotide using the in situ Apoptosis Detection Kit (Oncor, Gaithersburg, MD) with which cellular internucleosomal DNA fragmentation are detected.

 Immunostaining of the ventricular sections of each group was carried out with the streptavidin/biotin immunoperoxidase method (LSAB kit, DAKO) after deparaffinization. Antibodies used were: anti-α-smooth muscle actin (α-SMA) was detected with murine monoclonal antibody 1A4(DAKO); fibronectin with mouse monoclonal antibody NCL-FIB (Novocasta, Vector); and anti-proliferative cell nuclear antigen (PCNA) was used to detect nonquiescent cells.

### Morphometrical Analysis of Coronary Arteries
Wall thickness was assessed as the ratio of media thickness to the outer radius of the small vessels of coronary arterioles, according to our previous report.24 Inner and outer circumferences were measured by computer analyzer system (ImageQuest; Hamamatsu Photonics K.K., Hamamatsu, Japan). Values were corrected to the radius of the coronary arteries, which were assumed to be circular, by the following calculation: Wall thickness ratio=(R-r)/R, where R is the radius of the outer circumference, and r represents medial thickening.

In the same coronary arteries, the labeling index of TUNEL and PCNA positive cells were determined by evaluating the number of positive cells in the same areas of serial sections using computer-assisted image analysis (ImageQuest).25–27

### Electron Microscopy
Electron microscopy was performed to evaluate the structure of apoptotic bodies and cells. For this purpose, tissues were fixed at room temperature in 2.5% glutaraldehyde (TAAB, UK) in 0.1 mol/L sodium cacodylate. They were stained en bloc in uranyl maleate for one hour, post-fixed in 1% osmium tetroxide, dehydrated, and embedded in Epok 812 (Shell Chemical). Ultra thin sections were stained with manyletate and lead citrate and examined in an JEM-1200EX electron microscope (JEOL Ltd. Tokyo, Japan).

### Statistical Analysis
All data are presented as mean ± SEM. The statistical analyses for hemodynamic and histological studies were performed with one-way ANOVA, followed by Duncan’s multiple range test. Unpaired Student’s t test was used for comparison of apoptosis between control and L-NAME treated rats.28 A probability level of less than 0.05 was considered to be statistically significant.

### Results

#### Body and Organ Weight and Cardiac Hemodynamics
Left ventricular mass and thoracic aortic weight were significantly greater in Group 3 rats; but this was associated with a decreased body weight especially in Group 3. Right ventricular weight was not significantly different among the three groups (Table 1). MAP was elevated in SHR receiving prolonged L-NAME, but this pressure increase was not dose-dependent. High-dose L-NAME (80 mg/L) (Group 3) impaired cardiac functions (eg, reduced HR, CI) as compared with the low-dose L-NAME (50 mg/L) in (Group 2) (Table 2).

#### Cardiac Morphological Findings
The morphological appearance of the cardiac myocytes and the interstitium remained entirely normal and displayed only minor alterations of coronary arterial endothelium, perivascular fibrosis and mild hypertrophy of medial SMC in the control 20-week-old SHR. However, the myocytes of L-NAME–treated SHR (Groups 2 and 3) demonstrated marked hypertrophy and severe vascular remodeling. The
TABLE 1. Wet Organ Weights

<table>
<thead>
<tr>
<th>Indices</th>
<th>SHR Groups</th>
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<tbody>
<tr>
<td></td>
<td>Group 1, Control</td>
</tr>
<tr>
<td>No. of rats</td>
<td>10</td>
</tr>
<tr>
<td>Body weight, g</td>
<td>333±10</td>
</tr>
<tr>
<td>LV weight, mg</td>
<td>1043±50</td>
</tr>
<tr>
<td>LVW/BW, mg/g</td>
<td>3.11±0.09</td>
</tr>
<tr>
<td>RV weight, mg</td>
<td>232±24</td>
</tr>
<tr>
<td>RVW/BW, mg/g</td>
<td>0.69±0.07</td>
</tr>
<tr>
<td>Ao weight, mg/mm²/kg</td>
<td>0.362±0.029</td>
</tr>
<tr>
<td>AoW/BW, mg/mm²/kg</td>
<td>1.077±0.075</td>
</tr>
</tbody>
</table>

Data are mean±1 SEM, P value of 1-way ANOVA. LV indicates left ventricle; RV, right ventricle; and Ao, thoracic descending aorta (20 mm-long segment superior to the diaphragm).

†P<0.01, †P<0.05 vs Control group.
‡P<0.01, §<0.05 vs L-NAME (50 mg/L) Group.

Expression of PCNA and Apoptotic Cells

Apoptotic cells and PCNA-positive cells demonstrated strong staining of oval-shaped muscle of subendocardial coronary endothelial smooth muscle and left ventricular endocardial cells. There was also expression of PCNA and apoptotic cells in small subepicardial coronary arteries (Figure 1). There was no difference between the control and L-NAME–treated rats with regard to the frequency of SMCs expressing PCNA (Table 4). Nevertheless, the frequency of apoptosis in SMCs was greater in L-NAME than control (15.3±6 versus 1.9±6×10³/mm²; P<0.05). The PCNA/apoptosis ratio was significantly reduced in L-NAME with respect to (control) Group 1 (0.3±0.1 versus 2.4±1; P<0.001). However, in endothelial cells of coronary arteries in Group 2, the PCNA/apoptosis ratio was not different from that of control rats (0.20±0.06 versus 0.11±0.04). The ratio was not different in left ventricular endocardial endothelial cells because of an increase in both PCNA (3.7±0.8 versus 1.2±0.4/mm²; P<0.01) and apoptosis (29±2 versus 12±1/mm², P<0.0001) expression. Electron microscopy also demonstrated apoptosis of coronary arterial SMC (Figure 2).

A large number of PCNA positive cells in hearts of Group 3 corresponded to fibroblasts within the area of myocardial infarction. Moreover, apoptotic cells were present in focal areas of fibroblast proliferation. These lesions were associated with α-SMA and fibronectin expression (Figure 3); they do not include the inflammatory cells in the small areas of myocardial infarction. In this study, we assessed the alteration of epicardial coronary arteries with respect to medial wall thickness and smooth muscular cell activity using the ratio of apoptosis and PCNA positive cells in low-dose L-NAME rats without myocardial infarction. In the high dose L-NAME (Group 3), the lesions of the small coronary arterioles surrounding myocardial infarction were observed as neointimal thickening, medial hypertrophy, and vascular thrombosis.

**Discussion**

The renal hemodynamic and histological responses to prolonged NOS blockade in 20-week-old SHR have been the subject of an earlier study from our laboratory, but cardiac hemodynamics and histopathologic responses to increasing doses of NOS inhibition remain unclear. We, therefore, designed the present study to determine whether: (1) the impaired systemic hemodynamics reported earlier as a result of prolonged treatment with L-NAME were related to ischemia and interstitial fibrosis that had been previously demonstrated from our laboratory in the kidney; and (2) NOS blockade affects apoptosis of cardiovascular SMC and endothelial cells. The data presented herein demonstrate that SHR, given L-NAME for three weeks, developed severe hypertensive heart disease associated with severe interstitial fibrosis and myocardial infarction as well as impaired systemic hemodynamics. Histologically, the increased LV mass was also associated with interstitial fibrosis, myocardial infarction, coronary arterial medial hypertrophy, and increased SMC apoptosis and severe arteriolar injury.

Apoptosis of the coronary arteries (by TUNEL) occurred in SMCs, but not endothelial cells, without evidence of cellular proliferation (PCNA) as a consequence of L-NAME. The decreased PCNA/apoptosis ratio in SMC is suggestive of reduced proliferative activity of SMC. Despite apoptotic changes in SMC and endothelium, L-NAME induced no changes in the endothelial cells of coronary arteries. Han et al reported an analysis of apoptosis (TUNEL) and cellular

**TABLE 2. Systemic and Cardiac Hemodynamic Measurements in SHR After 3-Week Treatment With Chronic L-NAME Administration**

<table>
<thead>
<tr>
<th>Indices</th>
<th>SHR GROUPS</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Group 1, Control</td>
</tr>
<tr>
<td>MAP, mm Hg</td>
<td>185±4</td>
</tr>
<tr>
<td>HR, bpm</td>
<td>352±10</td>
</tr>
<tr>
<td>Hct, %</td>
<td>52.3±0.5</td>
</tr>
<tr>
<td>CI, mL/min/kg</td>
<td>323±29</td>
</tr>
<tr>
<td>SI, mL/kg</td>
<td>0.93±0.07</td>
</tr>
<tr>
<td>TPRI, PRU</td>
<td>0.61±0.05</td>
</tr>
</tbody>
</table>

Data are presented as mean±1 SEM. P value of 1-way ANOVA. MAP indicates mean arterial pressure; HR, heart rate; Hct, hematocrit; CI, cardiac index; SI, stroke volume index; TPRI, total peripheral-resistance index; Control, 20-week-old SHR; L-NAME (50), SHR treated with L-NAME (50 mg/L in drinking water); L-NAME (80), SHR treated with L-NAME (80 mg/L in drinking water).

*P<0.01, †P<0.05 vs Control group. ‡P<0.01, §<0.05 vs L-NAME (50 mg/L) group.
proliferation (PCNA) in balloon-injured rat arteries that suggested the vessel wall presented a profound apoptotic response in neointimal SMCs that was associated with SMC proliferation. However, the number of SMC in the media demonstrating apoptosis and positivity for PCNA was very low. In this balloon vascular injury model in the rat, apoptosis primarily affected neointimal SMCs from 7 to 28 days. In contrast, Perlman et al reported (in rat carotid arteries at 30 minutes after balloon injury) extensive apoptosis of medial SMCs manifested by 70% TUNEL-positive cells, suggesting marked downregulation of bcl-x expression. The difference in time-course between neointimal and medial SMCs apoptosis suggests that balloon vascular injury may directly induce apoptosis in medial SMC. By contrast, the results of our studies of medial SMC apoptosis induced by NOS inhibition, also suggest that other direct factors could have affected the medial SMC. Exacerbation of hypertension may be directly involved in vascular SMC proliferation through induction of c-myc proto-oncogenes since c-myc expression by SMC has been shown to induce cell proliferation and apoptosis.

LV and aortic mass indexes were increased in the high dose L-NAME rats. These changes were not associated with an

<table>
<thead>
<tr>
<th>Indices</th>
<th>Group 1, Control</th>
<th>Group 2, L-NAME (50 mg/L)</th>
<th>Group 3, L-NAME (80 mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interstitial fibrosis, %</td>
<td>0.45±0.1</td>
<td>3.96±1.2</td>
<td>14.0±2.1*</td>
</tr>
<tr>
<td>Coronary injury score</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sub epicardial arterioles</td>
<td>15.9±5.6</td>
<td>77.9±16.7†</td>
<td>118.9±20.5†</td>
</tr>
<tr>
<td>Sub endocardial arterioles</td>
<td>11.9±8.8</td>
<td>24.0±7.2</td>
<td>101.8±20.0†</td>
</tr>
<tr>
<td>Total</td>
<td>14.7±5.7</td>
<td>52.8±8.7†</td>
<td>112.0±19.1†</td>
</tr>
<tr>
<td>Wall:lumen ratio</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Epicardial arteries</td>
<td>0.16±0.02</td>
<td>0.23±0.02†</td>
<td>0.23±0.03†</td>
</tr>
<tr>
<td>Endocardial arteries</td>
<td>0.26±0.02</td>
<td>0.29±0.02</td>
<td>0.35±0.04†</td>
</tr>
</tbody>
</table>

Values are mean±SEM. *P<0.01; †P<0.05 vs Control; ‡P<0.01 vs L-NAME (50).

Figure 1. Detection of apoptosis in hypertensive vascular remodeling. Serial sections from coronary arteries in control (A-C) and L-NAME (D-F) treated SHR. The epicardial coronary artery in L-NAME (D-F) shows wall-thickening, hypertrophic SMCs, and perivascular fibrosis compared with that in control. This vascular wall hypertrophy was associated with marked increase in TUNEL positive cells with (F) unchanged PCNA (E). Microphotographs were stained by Elastica-Masson (A, D), PCNA (B, D), and TUNEL (C, F). Original magnification, ×120.
increased MAP as compared with the rats receiving a lower dose rats of L-NAME with respect to the relationship between LV hypertrophy and myocyte apoptosis. Hamet et al.16,32–34 reported that cardiac hypertrophy was initiated by a wave of apoptosis of cardiomyocytes. Thus, after aortic banding in rats, apoptosis of myocytes could be identified in tissue sections, further emphasizing the potential role of hemodynamic factors. Peak cell loss through apoptosis was observed four days after aortic banding whereas cardiac growth continued for 30 days. The cardiac hypertrophy was associated with the narrow apoptosis window of cardiomyocyte in early stage. Furthermore, in the present study, the apoptosis labeling index of arteriolar SMCs with the lower dose of L-NAME was much greater than in control SHR. This phenomenon might explain the increased wall:lumen ratio of the epicardial arterioles in the higher dose of L-NAME rats, despite no effect on the apoptosis of arteriolar endothelial cells.

### Table 4. Frequency of Apoptotic and PCNA Positive Nuclei in Left Ventricular Endocardial Cells and Smooth Muscle Cells of SHR Treated With or Without L-NAME

<table>
<thead>
<tr>
<th>Indices</th>
<th>SHR GROUPS</th>
<th>P&lt;</th>
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<tbody>
<tr>
<td></td>
<td>Group 1, Control</td>
<td>Group 2, L-NAME (50 mg/L)</td>
</tr>
<tr>
<td>Coronary arteries:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endothelial cells (no.±SEM of PCNA positive and apoptotic nuclei/min of tissue section):</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PCNA</td>
<td>7.7±2</td>
<td>5.1±1 NS</td>
</tr>
<tr>
<td>Apoptosis</td>
<td>41±5</td>
<td>49±4 NS</td>
</tr>
<tr>
<td>PCNA/Apoptosis ratio</td>
<td>0.20±0.06</td>
<td>0.11±0.04 NS</td>
</tr>
<tr>
<td>Smooth muscle cells (no.±SEM of PCNA positive and apoptotic nuclei×10²/mm² of tissue section):</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PCNA</td>
<td>4.8±1</td>
<td>4.6±1 NS</td>
</tr>
<tr>
<td>Apoptosis</td>
<td>1.9±1</td>
<td>15.3±6 0.05</td>
</tr>
<tr>
<td>PCNA/Apoptosis ratio</td>
<td>2.4±1</td>
<td>0.3±0.1 0.001</td>
</tr>
<tr>
<td>Left ventricular endocardial cells (no.±SEM of PCNA positive and apoptotic nuclei/mm of tissue section):</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PCNA</td>
<td>1.2±0.4</td>
<td>3.7±0.8 0.01</td>
</tr>
<tr>
<td>Apoptosis</td>
<td>12±1</td>
<td>29±2 0.0001</td>
</tr>
<tr>
<td>PCNA/Apoptosis ratio</td>
<td>0.12±0.04</td>
<td>0.13±0.03 NS</td>
</tr>
</tbody>
</table>

Each value represents the mean±SEM of the data for all rats of each group. For each animals, labels nuclei were counted in 20 microscopic arteries at a magnification of ×120. P value shown by unpaired t test; ns, not significant.
cells. Taken together, SMC apoptosis could explain the apoptosis window associated with arteriolar hypertrophy. It is of interest that, despite the intense apoptosis and proliferative changes of endocardial cells, as compared with those of PCNA and TUNEL staining in the ventricular endocardium of L-NAME treated rats. This might be explained by the severe shear stress of the ventricular wall endothelial cells as compared with those of the coronary arteriolar wall, as demonstrated by the greater PCNA and TUNEL staining by L-NAME in the ventricular endocardium.

Treatment with the high dose of L-NAME–induced myocardial infarction (MI) associated with severe interstitial fibrosis, severe arteriolar wall injury, and medial smooth muscle cell hypertrophy. These myocardial infarctions were associated with strong fibronectin and slight α-SMA expression, indicating a healing process following MI.35,36 Moreover, these pathological findings may provide an explanation for the reduction in stroke and cardiac indices since, in earlier studies, these systemic hemodynamic changes could not be explained by contracted plasma volume or hemoconcentration. This reduction in cardiac output provides an explanation for the reduced RBF and other pathophysiological changes associated with renal ischemia.19 It follows that the hemodynamic changes associated with coincidental structural changes of microvascular remodeling. These structural changes have been explained heretofore on the basis of three possibilities: (1) adaptive responses to severe arterial hypertension57,58; (2) increased production of mitogen- or growth-promoting factors resulting from decreased NO synthesis39,40; or (3) upregulation of systemic or local renin-angiotensin system due to impaired NO synthesis.41–43 Fabris et al44 demonstrated in a uremic rat model with renal ablation that NOS blockade may induce coronary arteriolar SMC apoptosis without apoptosis in vascular endothelial cells, progressing to MI with more intense NOS inhibition.

Prolonged NOS inhibition has produced cardiovascular damage such as clinically microvascular angina pectoris.52–54 Moreover, in this study, the wall:lumen ratio of L-NAME–treated SHR was increased in a dose-dependent manner. Deng et al55,56 have evaluated, in similar L-NAME–treated SHR, the prepro-endothelin (ET)-1 mRNA expression using in situ hybridization methods. Prepro ET-1 mRNA expression increased in small coronary arterial endothelial cells with L-NAME/SHR, suggesting that vascular hypertrophy, caused by endothelial ET-1, progresses. Sventek et al have reported that blood pressure was elevated by prolonged L-NAME administration; and this was associated with increased plasma renin activity and plasma immunoreactive ET, although the wall:lumen ratio was not significantly increased in small coronary arteries. Their morphometric analysis (using media-cross-sectional area) averaged 9867 ± 461 μm² in L-NAME treated SHR; and these values correspond to our findings of vascular wall area of more than 40 μm of outside diameter (Table 4). Coronary arteriolar wall thickness ratio up to 40 μm was significantly greater in L-NAME treated SHR as compared with the control SHR. Other hypertensive experimental models,57,58 including our L-NAME–treated rats and those with puromycin aminonucleoside nephrosis,60 have demonstrated an increased wall:lumen ratio with increased ET-1 concentration, suggesting vascular growth resulting from an abnormality in endothelial function.

Thus, these findings in young SHR with exacerbated hypertensive cardiovascular changes produced by L-NAME provide an innovative experimental model for the microvascular angina that has been reported to occur in patients with essential hypertension.52–54 These changes have been associated clinically with interstitial ventricular fibrosis and myocardial infarction in the absence of atherosclerotic occlusive epicardial disease,61 as found in our model of SHR treated with L-NAME.

Acknowledgments

We thank Mr T. Serizawa for excellent electron microscopic technical assistance and Miss Y. Kawamura for help in image analysis.

References


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