The Link Among Nitric Oxide Synthase Activity, Endothelial Function, and Aortic and Ventricular Hypertrophy in Hypertension

Hiroshi Hayakawa, Leopoldo Raj

Abstract The adaptive changes that occur in the left ventricle (LV) and vessels in response to hypertension, namely, muscle hypertrophy/hyperplasia, endothelial dysfunction, and extracellular matrix increase, do not depend solely on blood pressure elevation. These changes are, in fact, maladaptive since they are forerunners of cardiac failure, stroke, and renal failure. Nitric oxide, an endogenous vasodilator and inhibitor of vascular smooth muscle cell growth, is synthesized in the endothelium by constitutive nitric oxide synthase (cNOS). We investigated the relationships among LV and aortic cNOS activity (conversion of [14C] L-arginine to [14C] L-citrulline), with LV hypertrophy (LV weight/body weight), and (2) aortic hypertension (aortic weight/length) in spontaneously hypertensive rats (SHR) and Dahl salt-sensitive (DS) rats matched for blood pressure (219 ± 12 versus 211 ± 7 mm Hg, P = NS) and age. Compared with their normotensive counterparts, aortic cNOS activity was increased 106% in SHR but reduced by 73% in DS rats. The correlation between blood pressure and aortic cNOS activity was positive (r = 0.74, P < 0.01) in SHR and negative (r = −0.82, P < 0.01) in DS rats. LV cNOS activity was increased 73% in SHR compared with normotensive Wistar-Kyoto rats (P < 0.01). On the other hand, LV cNOS activity was not increased in hypertensive DS rats compared with normotensive DS rats. In SHR, aortic hypertension did not increase significantly and LV hypertrophy increased only 15%, whereas in hypertensive DS rats the aorta and LV hypertrophied 36% and 88%, respectively (both P < 0.01). Moreover, in DS rats there was a negative correlation between cNOS activity and aortic hypertension (r = −0.70, P < 0.01). In DS rats, antihypertensive therapy consisting of an angiotensin-converting enzyme inhibitor, perindopril, and a diuretic, indapamide, normalized blood pressure, aortic cNOS activity, and LV hypertrophy and reduced aortic hypertension. Our studies imply that upregulation of vascular cNOS activity has a protective cardiovascular homeostatic role in hypertension. Clinically, the variable end-organ disease observed in individuals with similar severity of hypertension may be explained, at least in part, by genetically conditioned differences in vascular cNOS activity in response to hypertension (Hypertension. 1997;29[part 2]:235-241.)

Key Words • endothelium • nitric oxide synthase • rats, inbred, SHR • rats, Dahl • muscle, smooth, vascular • hypertrophy, left ventricular • hypertension

The homeostatic balance of local vascular tone mediated by vasodilators (such as NO) and prostaglandins and vasoconstrictors (such as angiotensin II, endothelin, and products of cyclooxygenase) is maintained by the endothelium. NO is synthesized by a constitutively expressed Ca2+-dependent endothelial cNOS that releases NO tonically and when activated by a receptor-dependent agonist such as ACh. In addition, shear stress and cyclic strain, which are increased during hypertensive states, induce through mechanisms still poorly understood a parallel increase in cNOS expression, cNOS protein, and cNOS activity.

EDRelax mediated by NO has been reported to be impaired in the forearm as well as the coronary vessels of hypertensive patients. However, the results of these studies have not been uniform. Although a recent clinical study found that EDRelax in hypertensive patients is normal, other studies have found that most patients have attenuated EDRelax. It is at present unclear whether these discrepancies are due to variations in the selection of patients regarding severity of hypertension or due to either genotypic or phenotypic differences among the populations studied. Impaired EDRelax in hypertensive animals has been attributed to decreased NO production and/or the concomitant synthesis/release of endothelium-derived contracting factors such as superoxide anion, which inactivates NO, or vasoconstrictors such as endothelins and products of cyclooxygenase.

In the heart, cNOS is constitutively expressed in endothelial cells of coronary vessels, in the endocardium, and in myocytes. However, most cardiac NO originates in the coronary vessels and the endocardium. At present the role of cardiac NO in hypertension has not been established.

In hypertension, an increase in pressure-workload fosters adaptive hypertrophic changes in the heart and vessels that, in fact, are maladaptive because they are the forerunners of cardiac failure, coronary disease, stroke, and renal failure. In vitro and in vivo studies suggest that NO is an endogenous inhibitor of vascular smooth muscle growth that may modulate vascular remodeling in response to injury.

We investigated the relationship between vascular cNOS activity and LV and aortic hypertension in two strains of rats genetically prone to hypertension, SHR and the DS rat and their normotensive counterparts. In DS rats, we also evaluated the effects of antihypertensive therapy consisting of an ACE inhibitor, perindopril, and a diuretic, indapamide, on cNOS activity and LV and aortic hypertension. We found that in age-matched rats,
hypertension of similar severity resulted in important quantitative differences in LV and aortic hypertrophy between the two strains, these differences may be linked to striking variations in vascular cNOS activity in response to hypertension Clinically, heretofore unrecognized differences in cNOS, as demonstrated in this study between DS rats and SHR, may explain the heterogeneity of results reported in studies of EDRelax and cardiovascular remodeling in hypertensive humans and provide a basis for the development of therapeutic strategies.

Methods

Materials

Male DS and DR rats from the Brookhaven strain were purchased from Harlan Sprague Dawley Inc, Indianapolis, Ind SHR and WKY rats were purchased from Taconic Farms (Germantown, NY) (14C) L-arginine was purchased from Amersham International. Dopex resin (AG50XW-8, H+ form) was purchased from Bio-Rad Laboratories. Other chemicals used were purchased from Sigma Chemical Co.

Experimental Animals

Seven-week-old DS and DR rats were fed standard rat chow that contained either 4% NaCl (DS-4%, n = 13, DR-4%, n = 7) or 0.5% NaCl (DS-0.5%, n = 12, DR 0.5%, n = 7) for 8 weeks. DS and DR groups were used for the experiments at the age of 15 weeks SHR (n = 12) with similar hypertension to DS-4% and age-matched WKY rats (n = 14) were used for experiments at 15 weeks of age.

All rats had free access to water and were housed five per cage in facilities accredited by the American Association for Accreditation of Laboratory Animal Care. SBP of all rats was measured by a tail-cuff method. The animal studies were approved by the Institutional Animal Care and Use Committee.

Determination of NOS Activity in Aorta and in Heart

cNOS activity was determined in the aorta and LV of each rat. Thoracic aortas and hearts were excised from rats and frozen in liquid nitrogen and stored at −80°C until use. The LV was carefully dissected before freezing. Tissues were homogenized in a 3:5 volume of ice-cold buffer solution containing 50 mMol/L Tris-HCl (pH 7.4), 0.1% mercaptoethanol, 0.1 mMol/L EDTA, 0.1 mMol/L EGTA, 2 μMol/L leupeptin, 1 μMol/L pepstatin A, and 1 mMol/L phenylmethylsulfonyl fluoride, using an Omni TH homogenizer (Omni International). The homogenates were centrifuged at 20,000 g for 45 minutes. The supernatants were used for measuring NOS activity and protein concentration.

The conversion of [14C] L-citrulline from [14C] L-arginine by NOS was measured in the supernatants from tissues, as described previously. Briefly, 40 μL of the supernatant was added to 100 μL of assay buffer containing 50 mMol/L KH2PO4, 1 mMol/L MgCl2, 1 mMol/L CaCl2, 50 mMol/L valine, 1 mMol/L L-citrulline, 20 mMol/L L-arginine, 2 mMol/L DTT, 2 mMol/L NADPH, 3 mMol/L tetrahydrobiopterin, 3 mMol/L flavin adenine dinucleotide, 3 μMol/L flavin mononucleotide, and 0.5 μCi/mL [14C] L-arginine. The mixture of supernatant and assay buffer solution was incubated for 20 minutes at 37°C in the presence or absence of either 1 mMol/L EGTA or 1 mMol/L EGTA + 1 mMol/L L-arginine to determine the level of the Ca2+-dependent (cNOS) and Ca2+-independent (iNOS) activities. After the incubation, the reaction was stopped by adding 500 μL of ice-cold solution containing 20 mMol/L HEPES, 2 mMol/L EDTA, and 2 mMol/L EGTA. The incubated mixture was loaded onto 1-mL columns of Dowex resin (Na+ form) to remove [14C] L-arginine. Columns were then eluted with 500 μL of distilled water. The amount of [14C] L-citrulline was determined with a liquid scintillation counter.

Organ Chamber Experiments

Vascular responses in thoracic aorta from all groups of rats (DS, WKY, and SHR) were tested in organ chambers according to techniques previously described. Briefly, rats were anesthetized by injection of sodium pentobarbital (30 mg/kg body weight, IP) and then exsanguinated from the abdominal aorta. The chest was opened through a median sternotomy, and the thoracic aorta was excised and immediately placed in a cold (4°C) modified Krebs-Ringer bicarbonate solution. The thoracic aorta was then dissected free from connective tissue under magnifying glasses and cut into rings (4 mm in length).

Aortic rings were mounted horizontally between two styrups in organ chambers filled with 25 mL of oxygenated modified Krebs-Ringer bicarbonate solution ([NaCl 118.6, KCl 2.5, MgSO4 1.2, K2HPO4 1.2, NaHCO3 25.1, glucose 10.1, and EDTA 0.026] at 37°C. One styrup was connected to an anchor and the other to a force transducer (Gould Statham UTC2) for recording of isometric tension. EDRelax to ACh (10−4 to 10−4 mol/L) in the presence or absence of indomethacin (10−5 mol/L) was studied in rings precontracted 70% of maximal contraction to L-norepinephrine obtained in each individual ring. Endothelium-independent relaxation was also tested with SNP (10−9 to 10−4 mol/L).

Effects of Antihypertensive Therapy in DS Rats Fed 4% NaCl Diet

In pilot studies, we administered perindopril, an ACE inhibitor, in various doses to DS rats receiving high amounts of dietary salt and failed to reduce blood pressure. Therefore, similar to the clinical situation, we added a diuretic, indapamide, and succeeded in normalizing blood pressure in DS rats. Thus, a separate group of DS rats (n = 13) fed a 4% NaCl diet received antihypertensive therapy consisting of perindopril (4.5 mg/kg body wt/d) and indapamide (1.44 mg/kg body wt/d) administered daily by gavage throughout the 8 weeks of the study. These rats also determined SBP, response to ACh, and NOS activity in aorta and LV.

Calculations and Statistical Analysis

Relaxations in aortic rings were expressed as percent decrease in tension. As an index of LV hypertrophy, the ratio of LV
TABLE 1. SBP, Body Weight (BW), and LV Weight in Experimental Groups of Dahl Rats

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>SBP, mm Hg</th>
<th>BW, g</th>
<th>LVW/BW, g/100 g</th>
</tr>
</thead>
<tbody>
<tr>
<td>DS-0 5%</td>
<td>12</td>
<td>133 ± 3</td>
<td>400 ± 10</td>
<td>0.182 ± 0.005</td>
</tr>
<tr>
<td>DS-4 0%</td>
<td>13</td>
<td>211 ± 7</td>
<td>388 ± 21</td>
<td>0.305 ± 0.015</td>
</tr>
<tr>
<td>DS-Rx</td>
<td>14</td>
<td>146 ± 3</td>
<td>416 ± 18</td>
<td>0.186 ± 0.006</td>
</tr>
<tr>
<td>DR-0 5%</td>
<td>7</td>
<td>107 ± 2</td>
<td>359 ± 12</td>
<td>0.152 ± 0.005</td>
</tr>
<tr>
<td>DR-4 0%</td>
<td>7</td>
<td>129 ± 3</td>
<td>397 ± 6</td>
<td>0.170 ± 0.005</td>
</tr>
</tbody>
</table>

Values are mean ± SE

*P < 0.1 vs DS-4 0%

NOS Activity in Aortas

cNOS activity was decreased approximately 75% in hypertensive DS-4.0% rats compared with normotensive DS-0.5%, DR-0.5%, or DR-4.0% rats (Fig 2A). cNOS activity in DR-4.0% and DR-0.5% rats was similar. On the other hand, SHR had 106% higher cNOS activity than normotensive WKY rats (Fig 2B). No significant activity of Ca²⁺-independent iNOS was found in the aortas studied.

A significantly positive correlation was found between aortic cNOS activity and SBP in SHR and WKY rats (Fig 3B, r = 0.74, P < 0.01), however, in Dahl rats, we found a negative correlation between cNOS activity and SBP (Fig 3A, r = −0.82, P < 0.01).

Vascular Relaxation

EDRelax to ACh in aortas from hypertensive DS-4.0% was significantly impaired compared with normotensive DS-0.5% rats (P < 0.01, Fig 4A). Responses to ACh were impaired in SHR compared with WKY rats (Fig 4B). Indomethacin (10⁻⁵ mol/L) normalized the responses to ACh in SHR (Fig 4B) but not in DS-4.0% rats (Fig 4A). Vascular relaxations to SNP were similar in all groups (data not shown).

NOS Activity in LV

cNOS activity in LV from all groups of rats was determined. Independent of blood pressure levels, LV cNOS activity was similar in DS-0.5%, DS-4.0%, DR-0.5%, and DR-4.0% rats (Fig 5A). cNOS activity was significantly higher in LV of SHR than that in WKY rats (2.58 ± 0.195 versus 1.48 ± 0.147 nmol mm⁻¹·g⁻¹ protein, P < 0.01, Fig 5B). Furthermore, LV cNOS activity was positively correlated with SBP in SHR and WKY rats (r = 0.64, P < 0.01) but not in Dahl rats.
Effects of Antihypertensive Therapy on SBP, cNOS Activity, and Responses to ACh in DS-Rx Rats

Antihypertensive therapy with perindopril plus indapamide prevented hypertension in DS-Rx rats (Table 1). This combination therapy normalized the response to ACh (Fig 4A) and aortic cNOS activity in hypertensive DS-4.0% rats (Fig 2A) and reduced LV (0.186±0.006 g/100 g, P<.01 versus DS-4.0%) and aortic hypertrophy (21.3±0.5 mg/10 mm, P<.05 versus DS-4.0%). The therapy had no effect on cNOS activity in the LV (Fig 5A).

Discussion

We investigated the relationship between hypertension, vascular cNOS activity, and LV and aortic hypertrophy in age-matched SHR and DS rats with hypertension of similar severity. Compared with their normotensive counterparts, aortic cNOS activity was increased by 106% in SHR but was reduced by 73% in DS rats. In fact there was a striking positive correlation between blood pressure and aortic cNOS in SHR (Fig 3B) and a negative correlation in DS rats (Fig 3A). Moreover, aortic cNOS activity was also significantly increased in SHR compared with hypertensive DS-4.0% rats.

EDRelax was impaired in SHR and hypertensive DS rats. Indomethacin normalized EDRelax in SHR but not in DS rats, thus supporting findings of previous studies that suggested that cyclooxygenase-derived constricting factors contribute to the abnormal regulation of local vascular tone in SHR. Moreover, in vivo administration of a thromboxane A2 blocker to SHR was reported to restore impaired EDRelax despite having no effect on systemic blood pressure.

It has been shown that hemodynamic forces such as shear stress and cyclic strain increase NO production in vivo and in vitro. Indeed, Awolesi et al demonstrated that cyclic strain induces NO production by increasing endothelial cNOS expression, cNOS protein, and cNOS activity. Our results are commensurate with the observation that SHR are able to maintain high levels of vascular cNOS in response to hypertension. Our studies also imply that the endothelium of the DS rat, in contrast to SHR, succumbs to the injurious effects of hypertension and fails to increase cNOS activity in either LVs or aortas.
In addition, in hypertensive DS rats, aortic cNOS not only failed to increase but fell to levels below those observed in normotensive DS and DR rats. The reasons why LV cNOS activity in hypertensive DS rats did not fall, similar to that in aorta, are at present unclear but may reflect differences in vascular bed sensitivity to the injurious effects of hypertension. The fact that antihypertensive therapy concomitantly prevented hypertension, abnormal EDRelax, and the fall in aortic cNOS activity in DS-Rx rats further supports the notion that the observed changes in vascular cNOS activity are a consequence of hypertension. Similar to DS rats and SHR, antihypertensive therapy normalizes EDRelax in some hypertensive subjects, whereas inhibitors of cyclooxygenase improve abnormal EDRelax in others without affecting systemic blood pressure levels. In the aggregate, these clinical and experimental studies suggest that a decrease in vascular NO production may not participate in the initiation of hypertension. In fact, heightened vascular cNOS activity appears to be part of the physiological adaptation to hemodynamic forces, i.e., cyclic strain, which are increased in hypertensive states. This contention is supported by several lines of evidence: (1) Serum levels of NO2/N03 (NOx), which are stable metabolites of NO, increase in Sprague-Dawley rats made hypertensive by placement of a clip in one of the renal arteries. Similarly, levels of NOx are increased in the serum of hypertensive patients with mild to moderate hypertension and with no evidence of end-organ damage. (2) Levels of cGMP, the second messenger of NO, are increased in the carotid artery of SHR, suggesting increased basal cNOS activity. In our study, aortic cNOS activity was similar in normotensive DS-0.5% and DR rats. Thus, the fall in aortic cNOS activity in DS-4.0% rats after development of hypertension may reflect a selective, genetically conditioned endothelial susceptibility to hypertensive injury in this strain, particularly since endothelium-independent relaxations to SNP were similar in hypertensive and normotensive DS rats. High dietary salt, although necessary to bring about hypertension in this strain, cannot by itself be incriminated in the reduction of aortic cNOS activity, because cNOS activity remained unchanged in normotensive DR rats fed a diet with high salt content.

Cardiac myocytes are endowed with a constitutive, Ca2+-dependent NOS. However, quantitatively, most cardiac NO originates in the endocardium and coronary endothelium. Cardiac NO has been reported to be increased in SHR. Our study showed that while LV cNOS activity was similar in hypertensive DS-4.0% and normotensive DS-0.5% rats (Fig 5A), LV cNOS activity was increased 73% in SHR compared with normotensive WKY rats (Fig 5B). As demonstrated by in vivo and in vitro
studies, vascular smooth muscle hypertrophy/hyperplasia is promoted by various growth factors in response to injury and, conversely, is mitigated by NO. Hence, the dramatic differences in eNOS activity that we observed between SHR and DS-4.0% rats may explain, at least in part, why aortic hypertrophy did not occur and LV hypertrophy increased only 15% in SHR, whereas in hypertensive DS-4.0% rats the aorta and LV hypertrophied 36% and 88%, respectively. In fact we found in DS rats a significant negative correlation ($r = -0.70$, $P < 0.01$) between eNOS activity and aorta weight/length ratio, an index of vascular hypertrophy.

In previous studies, we reported that hypertension of similar severity resulted in remarkably more severe proteinuria and renal injury in DS rats than in SHR. Moreover, Hayakawa et al. found in preparations of isolated perfused kidneys increased NO in the venous effluent of kidneys from SHR but decreased NO in the kidney effluent from hypertensive DS rats. Taken together, the aforementioned observations and the results of our current study strongly support the conclusion that in hypertension, increased vascular eNOS activity has a protective homeostatic role. Extrapolations from experimental studies to clinical situations must always remain speculative. Nevertheless, our findings imply that in hypertension, a physiological adaptation to the increased hemodynamic workload is to upregulate vascular eNOS activity.

Several clinical studies have suggested that in hypertensive humans, impairment of EDRelax may not be a universal finding. Moreover, the prevalence of LV hypertrophy, renal failure, and stroke, which are major causes of morbidity and mortality, varies in different populations of hypertensives. We surmise that a genetically conditioned heterogeneity in vascular eNOS activity in response to hypertension may explain, at least in part, the differences in end-organ disease observed in individuals with hypertension of similar severity. However, the findings reported herein, although currently confined to genetic rat models of hypertension, may provide important insights into the pathogenesis and therapy of cardiovascular disease in humans.

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References


21 Kato T, Iwama Y, Okumura K, Hashimoto H, Toco T, Satake T. Pros-taglandin H2 may be the endothelium-derived contracting factor released by acetylcholine in the aorta of the rat hyperton. 1990,15:475-481.


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