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L-Arginine attenuates cardiovascular impairment in DOCA-salt hypertensive rats

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Nitric oxide (NO), essential for the proper functioning of the cardiovascular system, is derived from L-arginine by NO synthase in endothelial cells (35). NO synthase inhibition produces hypertension, endothelial damage, cardiac hypertrophy, inflammation, atherosclerosis, ventricular contractile dysfunction, and fibrosis (20, 24, 31, 35). Many of these pathophysiological responses are also characteristic of rat models of mineralocorticoid hypertension such as the deoxycorticosterone acetate (DOCA)-salt hypertensive rat (15), suggesting that decreased NO availability may be a mechanism in these models. This possibility is supported by the important role of decreased NO synthase activity in inducing salt-sensitive hypertension in the Dahl rat (37, 42). Furthermore, increased production of superoxide by NADPH oxidase, which has been reported in aortic rings from DOCA-salt hypertensive rats (11, 29, 41), would also decrease NO availability, because superoxide reacts rapidly with NO, producing peroxynitrite, possibly inducing further cellular damage. Administration of L-arginine as a source of NO has been shown to prevent severe nephrosclerosis, hypertension, hyper trophy, and collagen increases in aging spontaneously hypertensive rats (SHR) (32, 40, 44), whereas L-arginine prevented the development of hypertension in DOCA-salt hypertensive rats (27).

The aim of this project was to determine whether oral administration of L-arginine to DOCA-salt hypertensive rats prevents or attenuates the development of structural and functional changes in the heart and blood vessels. Structural changes were characterized by histology and echocardiography, whereas heart function was measured in vivo using echocardiography and ex vivo in isolated perfused hearts. Single-cell microelectrode recordings from left ventricular papillary muscles were used to determine changes in cardiac action potentials. Isolated thoracic aortic rings were used to measure vascular reactivity.

METHODS

DOCA-Salt Hypertensive Rats

Male Wistar rats (8–10 wk old) were obtained from the Central Animal Breeding House of The University of Queensland. All experimental protocols were approved by the Animal Experimentation Ethics Committee of The University of Queensland under the guidelines of the National Medical and Health Research Council of Australia, which conform to the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85-23, revised 1996). All treated rats were uninephrectomized. The rats were anesthetized with an intraperitoneal injection of tiletamine (25 mg/kg) and zolazepam (25 mg/kg, Zoletil) together with xylazine (10 mg/kg, Rompun); a lateral abdominal incision provided access to the kidney, and the left renal vessels and ureter were ligated. The left kidney was removed and weighed, and the incision site was sutured. Uninephrectomized rats were given no further treatment or were given 1% NaCl in the drinking water with subcutaneous injections of DOCA (25 mg in 0.4 ml of dimethylformamide every 4th day, DOCA-salt rats) (13). L-Arginine was administered as a 5% mixture in powdered rat food, which was available ad libitum for 28 days. Experiments were performed 28 days after surgery, as in previous studies (2, 9, 13, 27, 33).

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Assessment of Physiological Parameters

Food and water intake and body weights of all rats were measured daily. Systolic blood pressure and heart rate were measured in selected rats during light anesthesia [tiletamine (15 mg/kg ip) with zolazepam (15 mg/kg ip)] using a tail pulse transducer (model MLT1010) and an inflatable tail cuff with a Capto SP844 physiological pressure transducer (model MLT844/D) connected to a PowerLab data acquisition unit (ADInstruments, Sydney, Australia). Rats were killed with an injection of pentobarbital sodium (200 mg/kg ip). Blood was taken from the abdominal vena cava and centrifuged, and the plasma was frozen. After the plasma was thawed, glucose was measured by Precision Plus blood glucose electrodes (Medisense, Abbott Laboratories); plasma sodium and potassium concentrations were determined by flame photometry; plasma malondialdehyde levels were determined by HPLC (43).

Echocardiography

Serial, in vivo left parasternal and left apical echocardiographic images of rats were obtained with a 12-MHz-frequency fetal transducer (Hewlett-Packard Sonos 5500) at an image depth of 3 cm using two focal zones (10). Rats were anesthetized as described for uninephrectomy. Left ventricular M-mode measurements at the level of the papillary muscles included left ventricular end-diastolic dimensions, left ventricular end-systolic dimensions, interventricular septum, and posterior wall thicknesses and fractional shortening. Cardiac output, ejection fraction, and left ventricular mass were derived from these values (30). Pulsed-wave Doppler analyses of mitral valve inflows were used as estimates of diastolic function.

Isolated Heart Preparations

The nonrecirculating heart preparation was used for isolated myocardial experiments (9, 33). Briefly, after anesthesia with pentobarbital sodium (100 mg/kg ip) and administration of heparin (1,000 IU iv) into the femoral vein, hearts were rapidly excised and placed in ice-cold modified Krebs-Henseleit buffer containing (in mM) 119.1 NaCl, 4.75 KCl, 1.19 MgSO4, 1.19 KH2PO4, 25.0 NaHCO3, 11.0 glucose, and 2.16 CaCl2. Retrograde perfusion was initiated at constant pressure (100 cmH2O) with modified Krebs-Henseleit buffer maintained at 37°C and bubbled with 95% O2-5% CO2. A latex balloon catheter was inserted into the left ventricle for measurement of isovolumic function via connection to a Capto SP844 physiological pressure transducer (model MLT844/D) linked to a PowerLab recording system.

Table 1. Physiological parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>UNX</th>
<th>UNX + l-Arginine</th>
<th>DOCA-Salt (4 wk)</th>
<th>DOCA-Salt + l-Arginine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic blood pressure, mmHg</td>
<td>126±6 (14)</td>
<td>131±6 (10)</td>
<td>184±5*(14)</td>
<td>139±2† (30)</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>390±11 (14)</td>
<td>378±8 (10)</td>
<td>406±18 (14)</td>
<td>365±14† (30)</td>
</tr>
<tr>
<td>LV wt, mg/g body wt</td>
<td>2.06±0.04 (14)</td>
<td>2.11±0.03 (10)</td>
<td>3.35±0.05† (14)</td>
<td>2.79±0.04† (30)</td>
</tr>
<tr>
<td>RV wt, mg/g body wt</td>
<td>0.58±0.02 (14)</td>
<td>0.60±0.03 (10)</td>
<td>0.65±0.03† (14)</td>
<td>0.59±0.02† (30)</td>
</tr>
<tr>
<td>Kidney wt, mg/g body wt</td>
<td>4.96±0.14 (14)</td>
<td>5.03±0.31 (10)</td>
<td>11.03±0.29† (14)</td>
<td>9.49±0.41† (30)</td>
</tr>
<tr>
<td>TAR wall thickness, µm</td>
<td>192.3±6.6 (6)</td>
<td>184.2±8.6 (6)</td>
<td>311.6±5.1* (6)</td>
<td>256.1±5.9* (6)</td>
</tr>
<tr>
<td>LV perivascular collagen fraction, %area</td>
<td>27.9±4.4 (6)</td>
<td>24.3±5.6 (6)</td>
<td>38.9±2.8* (6)</td>
<td>31.4±2.1† (6)</td>
</tr>
<tr>
<td>LV interstitial collagen fraction, %area</td>
<td>2.7±0.3 (6)</td>
<td>3.1±0.8 (6)</td>
<td>11.7±1.3* (6)</td>
<td>7.9±1.2† (6)</td>
</tr>
<tr>
<td>Diastolic stiffness</td>
<td>20.3±0.8 (10)</td>
<td>21.6±1.2 (12)</td>
<td>32.3±1.7* (10)</td>
<td>20.5±0.9† (12)</td>
</tr>
<tr>
<td>Maximum +dP/dt, mmHg/s</td>
<td>1.760±180 (10)</td>
<td>1.810±90 (12)</td>
<td>1.380±170* (10)</td>
<td>1.450±160 (12)</td>
</tr>
<tr>
<td>Minimum −dP/dt, mmHg/s</td>
<td>1.500±80 (10)</td>
<td>1.550±80 (12)</td>
<td>1.370±170 (10)</td>
<td>1.320±160 (12)</td>
</tr>
<tr>
<td>Blood glucose, mmol/l</td>
<td>10.3±0.9 (14)</td>
<td>10.5±1.4 (10)</td>
<td>11.3±0.7 (14)</td>
<td>11.0±0.3 (30)</td>
</tr>
<tr>
<td>Plasma Na+, mmol/l</td>
<td>142.2±1.2 (14)</td>
<td>145.3±3.2 (10)</td>
<td>150.5±6.3 (14)</td>
<td>152.6±7.0 (30)</td>
</tr>
<tr>
<td>Plasma K+, mmol/l</td>
<td>4.4±0.2 (14)</td>
<td>4.5±0.4 (10)</td>
<td>2.1±0.3* (14)</td>
<td>2.0±0.3* (30)</td>
</tr>
<tr>
<td>Plasma MDA, µM</td>
<td>23.2±1.5 (9)</td>
<td>25.4±1.1 (10)</td>
<td>30.8±1.3 (10)</td>
<td>25.7±1.2† (10)</td>
</tr>
</tbody>
</table>

Values are means ± SE; number of experiments in parentheses. DOCA, deoxycorticosterone acetate; +dP/dt, time derivative of pressure; LV, left ventricle; RV, right ventricle; TAR, thoracic aortic ring; MDA, malondialdehyde. *P < 0.05 vs. UNX. †P < 0.05 vs. DOCA-salt.
small stainless steel pin embedded in a rubber base. The hook was attached to a modified sensor element (SensoNor AE801) connected to an amplifier (model TBM-4, World Precision Instruments). The muscle was slowly (over 1 min) stretched to optimal preload. Contraction were induced by field stimulation (Grass SD-9) via electrodes on either side of the muscle (stimulation frequency = 1 Hz, pulse width = 0.5 ms, stimulus strength = 20% above threshold).

After maximum preload was attained, the muscle was allowed to equilibrate for a further 45 min before impalement with a filamented borosilicate glass microelectrode (1.5 mm OD; World Precision Instruments) with a tip resistance of 5–15 MΩ when filled with 3 M KCl. The reference electrode was an Ag-AgCl electrode. An electrometer (Cyto 721, World Precision Instruments) was used to record bioelectrical activity. All signals were recorded via a PowerLab 4S data acquisition unit (ADInstruments). All preparations with a stable resting potential more negative than –60 mV were accepted. Continual impalement throughout an experiment was not always possible; however, if displacement occurred, the results of a subsequent impalement were accepted, provided the data fitted the criteria described above.

After a 20-min control period with microelectrode impalement, the drug-free Tyrode solution perfusing the chamber was changed to a solution containing 4-aminopyridine, and perfusion continued for a further 25 min. Previous data obtained with Wistar rats indicated that a maximum effect of 4-aminopyridine occurred after 20 min. Action potential duration (APD) at 20%, 50%, and 90% of repolarization, a maximum effect of 4-aminopyridine occurred after 20 min. Action potential duration (APD) at 20%, 50%, and 90% of repolarization, action potential amplitude, and action potential voltage over time (dV/dtmax) were measured. Contracile parameters measured were force of contraction and force of contraction over time (dF/dt).

Isolated Thoracic Aortic Rings

Thoracic aortic rings (~4 mm long) were suspended in Tyrode solution at 35°C with a resting tension of 10 mN (8). Cumulative concentration-response curves were performed for norepinephrine and either acetylcholine or sodium nitroprusside in the presence of a submaximal contraction to norepinephrine. Maximal contraction was recorded as that produced by addition of a modified isotonic Tyrode solution containing 100 mM KCl.

Collagen Distribution by Picrosirius Red Staining and Laser Confocal Microscopy

The major organs were removed from all experimental animals and then weighed. The left ventricle and septum underwent histological analysis. Tissues were initially fixed for 3 days in Telly’s fixative (100 ml of 70% ethanol, 5 ml of glacial acetic acid, and 10 ml of 40% formaldehyde) and then transferred into a prestain/fixative (modified Bouin’s fluid: 85 ml of saturated picric acid, 5 ml of glacial acetic acid, and 10 ml of 40% formaldehyde) for 2 days. The samples were then dehydrated and embedded in paraffin wax. Thick sections (15 µm) were cut and placed on glass slides coated with Mayer’s albumin solution (1 g of powdered egg albumin, 50 ml of glycerol, and 50 ml of distilled water), left to air dry for 2 days, and then heated in an oven at 56°C for 1 h. Phosphomolybdic acid (0.2% in distilled water, 5 min) was applied to reduce nonspecific binding of the stain to the section, and the slides were washed in distilled water. The slides were stained with collagen-selective picrosirius red (0.1% sirius red F3BA in saturated picric acid) and allowed to incubate for 90 min. The sections were washed, dehydrated, and mounted in Depex, and a coverslip was applied. The stained sections were analyzed on a laser scanning confocal microscope (model MRC-1024, Bio-Rad) with a rhodamine-Texas red filter (emission at 568 nm and green excitation at 609 nm, DF 32). Randomly assigned slides and sections representing the perivascular areas of the left ventricle were scanned. The images were taken with a ×40 objective lens and analyzed for pixel intensity in a specified area of the section. The data were compiled by a software image-rendering program (IA-IP-Lab, Scanalitics, Australia).

Inflammatory cells were observed after hematoxylin and eosin staining of 5-µm-thick sections of left ventricle. Slides were visualized under a standard light microscope initially at ×450 magnification, and the tissue was scanned to gain an overall indication of inflammatory cell infiltration. Once areas of inflammation were identified, ×1,000 magnification was used to confirm and identify inflammatory cells.
Hypertension significantly reduced plasma potassium concentrations but did not alter plasma glucose and sodium concentrations (Table 1). Treatment with L-arginine failed to alter the increased ejection fraction and cardiac output observed in DOCA-salt rats (Table 1). 

**Table 2. Cardiac function assessed by echocardiography**

<table>
<thead>
<tr>
<th></th>
<th>UNX (n = 10)</th>
<th>UNX + L-Arginine (n = 8)</th>
<th>DOCA-Salt (4 wk) (n = 10)</th>
<th>DOCA-Salt + L-Arginine (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LVIDd, mm</td>
<td>6.69 ± 0.18</td>
<td>7.24 ± 0.12</td>
<td>5.51 ± 0.37*</td>
<td>6.41 ± 0.17†</td>
</tr>
<tr>
<td>LVDPd, mm</td>
<td>1.74 ± 0.17</td>
<td>1.84 ± 0.04</td>
<td>1.98 ± 0.09</td>
<td>1.80 ± 0.12</td>
</tr>
<tr>
<td>E/A</td>
<td>1.92 ± 0.02</td>
<td>1.83 ± 0.07</td>
<td>1.64 ± 0.10*</td>
<td>1.81 ± 0.14†</td>
</tr>
<tr>
<td>Ejection fraction, %</td>
<td>83.1 ± 4.4</td>
<td>85.6 ± 1.8</td>
<td>92.7 ± 1.2*</td>
<td>92.9 ± 2.1*</td>
</tr>
<tr>
<td>Cardiac output, ml/min</td>
<td>75.3 ± 3.6</td>
<td>82.4 ± 3.8</td>
<td>53.7 ± 6.7*</td>
<td>51.9 ± 3.1*</td>
</tr>
<tr>
<td>Ascending aorta, m/s</td>
<td>0.75 ± 0.08</td>
<td>0.81 ± 0.03</td>
<td>1.23 ± 0.05*</td>
<td>0.92 ± 0.07†</td>
</tr>
<tr>
<td>LV mass, g</td>
<td>0.76 ± 0.04</td>
<td>0.77 ± 0.02</td>
<td>1.36 ± 0.09*</td>
<td>0.79 ± 0.06†</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, number of experiments. LVIDd, LV internal diameter in diastole; LVDPd, LV posterior wall thickness in diastole; E/A, mitral valve flow rate ratio. *P < 0.05 vs. UNX. †P < 0.05 vs. DOCA-salt.

Data Analysis

Values are means ± SE. The results were analyzed by one-way ANOVA with post hoc comparisons of group means via unpaired or paired t-tests as appropriate. P < 0.05 was considered significant.

**Drugs**

DOCA, L-arginine, heparin, norepinephrine, acetylcholine, and sodium nitroprusside were purchased from Sigma Chemical (St. Louis, MO). L-Arginine was thoroughly mixed with ground rat food pellets to a final concentration of 5%. Norepinephrine, acetylcholine, and sodium nitroprusside were dissolved in distilled water; 4-aminopyridine was dissolved in 0.01 M HCl; DOCA was dissolved in dimethylformamide with mild heating.

**RESULTS**

DOCA-salt hypertensive uninephrectomized rats developed hypertension (Table 1) and an increased water intake (Fig. 1) but failed to significantly gain weight (Fig. 2). L-Arginine significantly prevented the increase in systolic blood pressure in DOCA-salt hypertensive rats (Table 1) but failed to alter body weight or water intake (Figs. 1 and 2). DOCA-salt hypertension significantly reduced plasma potassium concentrations but did not alter plasma glucose and sodium concentrations; oral L-arginine treatment did not alter these parameters (Table 1). Plasma malondialdehyde concentrations, as a measure of oxidative stress, were increased after 4 wk of DOCA-salt hypertension; this increase was prevented by L-arginine treatment (Table 1). Intake of L-arginine, calculated from the daily food intake, was not significantly different between the treated groups: 3.6 ± 0.1 and 3.4 ± 0.3 g·kg body wt·day−1 in uninephrectomized rats treated with L-arginine and DOCA-salt rats treated with L-arginine, respectively (Fig. 3).

Hearts from DOCA-salt rats showed marked cardiac hypertrophy, as evidenced by left ventricular wet weight relative to body weight and left ventricular mass derived from echocardiography (Tables 1 and 2). This was associated with a 21% decrease in left ventricular internal diameter, indicating concentric cardiac hypertrophy (Table 2). Additionally, the relative wet weights of the right ventricle and the remnant kidney from DOCA-salt rats were increased (Table 1). Treatment with L-arginine for 28 days attenuated the increased organ weights of both ventricles and kidney and reduced the decrease in left ventricular chamber diameter (Tables 1 and 2).

The left ventricle of DOCA-salt rats showed marked inflammatory cell infiltration perivascularly, in scar tissue, and throughout the interstitium compared with uninephrectomized rats or uninephrectomized rats treated with L-arginine. Scar tissue also contained many inflammatory cells in L-arginine-treated DOCA-salt rats, but there were fewer sites of scarring in the L-arginine-treated rats. In addition, interstitial tissue showed fewer inflammatory cells in DOCA-salt rats treated with L-arginine. The left ventricles from DOCA-salt rats showed a significant increase in collagen, particularly interstitial collagen content, which was partially prevented by administration of L-arginine (Table 1, Fig. 4). Functionally, diastolic stiffness obtained from the isolated Langendorff-perfused heart preparation in DOCA-salt rats was significantly increased (Table 1). This increase was prevented by oral L-arginine treatment (Table 1). Furthermore, thoracic aortic wall thicknesses and aortic blood flow velocities were increased by DOCA-salt treatment; L-arginine prevented these vascular changes (Tables 1 and 2).

Echocardiographic assessment of heart function showed that L-arginine failed to alter the increased ejection fraction and decreased cardiac output observed in DOCA-salt rats (Table 2). The lack of significant improvement in systolic function by L-arginine in vivo was supported by contractility studies on isolated Langendorff-perfused hearts. Hearts from DOCA-salt

![Fig. 4](image-url) Represented photomicrographs of collagen distribution visualized by picrosirius red staining in the left ventricle of uninephrectomized rats (A), DOCA-salt hypertensive rats (B), and DOCA-salt hypertensive rats treated with L-Arg (C).
rats showed a decreased $+dP/dt$ that was unaltered by L-arginine treatment (Table 1). The decreased mitral valve flow rate ratio indicated a restrictive filling pattern that was prevented by L-arginine treatment (Table 2).

In hearts from DOCA-salt rats, APD was markedly increased at 20%, 50%, and 90% of repolarization; L-arginine prevented prolongation at 20% and 90% repolarization (Fig. 5). However, when challenged by 4-aminopyridine, L-arginine failed to maintain normalization of the resultant increases in APD from that of DOCA-salt-treated myocytes (Fig. 5). No changes in the resting membrane potential were evident between any of the four groups (data not shown).
DOCA-salt hypertension caused decreased contractile responses to norepinephrine and clear endothelial dysfunction, shown as minimal relaxation responses to acetylcholine in isolated sections of thoracic aortic rings (Fig. 6, A and B). These changes were accompanied by unaltered responses to sodium nitroprusside across all treatment groups (Fig. 6C). Treatment with oral L-arginine normalized these decreased responses (Fig. 6, A and B).

Assessment of metabolic status of hearts using 31P NMR spectroscopy in response to hypoxia-reperfusion showed that L-arginine increased metabolic status at rest (increased ATP and PCr) compared with uninephrectomized and DOCA-salt rats (Table 3). Hypoxia led to a rapid reduction in contractile function and high-energy phosphates, and there was no difference in the rate of fall of ATP, PCr, or pH between groups during hypoxia (results not shown). Normoxic reperfusion quickly restored metabolite levels in all groups; however, L-arginine-treated hearts again recovered to higher levels consistent with prehypoxic values that were mirrored by higher coronary flow rates.

Purine efflux from isolated Langendorff-perfused hearts was used as an indicator of metabolic efficiency (ATP breakdown) in response to increased workload. An increase in heart rate from 360 to 540 beats/min produced an increase in purine efflux in uninephrectomized hearts from 0.76 ± 0.29 to 1.3 ± 0.34 nmol·min⁻¹·g tissue⁻¹, an increase of 76 ± 9%. In contrast, hearts from DOCA-salt rats displayed a much smaller increase in purine release in response to increased workload (from 0.35 ± 0.18 to 0.45 ± 0.12 nmol·min⁻¹·g tissue⁻¹, an increase of 17 ± 13%), suggesting an inability to produce or utilize ATP. L-Arginine increased the purine release in hearts from DOCA-salt rats (from 0.41 ± 0.15 to 1.04 ± 0.25 nmol·min⁻¹·g tissue⁻¹, an increase of 74 ± 23%), results similar to uninephrectomized hearts, suggesting improved functional and metabolic capacity in response to an increased workload.

DISCUSSION

Cardiovascular damage and impairment of function in humans as a result of hypertension can be mimicked by rat models of hypertension such as the DOCA-salt hypertensive rat (14, 15). In this study, the structural and functional changes in DOCA-salt hypertensive rats included left ventricular hypertrophy with an increased left ventricular wall thickness and decreased ventricular internal diameter, increased inflammatory cell infiltration, increased ventricular interstitial and perivascular collagen deposition, increased passive diastolic stiffness, prolonged APD, increased oxidative stress, and inability to increase purine efflux in response to an increased workload. Administration of L-arginine markedly attenuated or prevented these structural and functional changes. L-Arginine also normalized the reduced efficacy of norepinephrine and acetylcholine in isolated thoracic aortic rings of DOCA-salt hypertensive rats.

L-Arginine, as substrate for NO synthase, is the precursor of the short-acting paracrine vasodilator NO (35). Selective inhibition of NO synthase by compounds such as nitro-L-arginine methyl ester induces hypertension (3, 28, 35). Salt-sensitive hypertension may result from dysregulation of the L-arginine-NO system (37, 42). In Dahl salt-sensitive rats, where a high-salt diet induces hypertension within 2–8 wk, a marked downregulation of inducible NO synthase was shown in animals fed a regular diet with further reductions in animals fed a high-salt diet (37). L-Arginine prevented the increase in blood pressure in salt-sensitive Dahl rats fed a high-salt diet (42). L-Arginine administration up to 3 g·kg⁻¹·day⁻¹ also prevented the development of hypertension in DOCA-salt hypertensive rats (1, 27). Our results showed that a similar dose of
\[31P \text{NMR-derived metabolite concentrations during hypoxia-reperfusion in isolated hearts}\]

<table>
<thead>
<tr>
<th></th>
<th>Prehypoxia</th>
<th>15 min Hypoxia</th>
<th>10 min</th>
<th>30 min</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ATP, mmol/l</td>
<td>PCr, mmol/l</td>
<td>ATP, mmol/l</td>
<td>PCr, mmol/l</td>
</tr>
<tr>
<td>UNX (4 wk)</td>
<td>9.6±0.3</td>
<td>16.0±2.8</td>
<td>6.6±1.8</td>
<td>12.1±3.4</td>
</tr>
<tr>
<td>DOCA-salt (4 wk)</td>
<td>9.2±0.5</td>
<td>16.5±1.0</td>
<td>9.0±1.3</td>
<td>7.4±1.6</td>
</tr>
<tr>
<td>DOCA-salt + L-Arg (4 wk)</td>
<td>10.9±0.6*</td>
<td>20.4±1.5*</td>
<td>5.7±0.7</td>
<td>9.1±2.6</td>
</tr>
</tbody>
</table>

Values are means ± SE. PCr, phosphocreatine. *P < 0.05 vs. DOCA-salt.

1-arginine markedly attenuated the increase in blood pressure in DOCA-salt rats, strongly indicating that a decreased availability of NO plays a key role in the development of hypertension in the DOCA-salt model, as in the Dahl salt-sensitive rat.

NO is rapidly removed by reaction with superoxide to form the very reactive free radical peroxynitrite. Aortic rings from DOCA-salt hypertensive rats showed an increased production of superoxide by NADPH oxidase (11, 29, 41). This was confirmed in our study by the increased plasma malondialdehyde concentrations that were reduced in L-arginine-treated rats. Thus the decreased NO availability in DOCA-salt rats could also be a result of this increased oxidative stress in this model of hypertension; 1-arginine, by increasing NO production, reduced this stress and any subsequent damage. This finding is consistent with results showing that a reduction in aortic superoxide production in DOCA-salt hypertensive rats by administration of sesamin, a lignan from sesame oil, was associated with a lowered systolic blood pressure and reduced impairment of endothelium-dependent vascular relaxation (36).

Although treatment with 1-arginine markedly attenuated hypertension in these rats, ventricular hypertrophy was reduced to a lesser extent. In DOCA-salt hypertensive rats, endothelin acting predominantly via endothelin type A receptors is the most likely candidate as the mediator of hypertrophy, because selective antagonism of the endothelin type A receptors prevented further ventricular hypertrophy in several rat models of hypertension, including the DOCA-salt hypertensive rat (2); this mechanism is likely to be unaffected by increased NO concentrations.

The prevention of an excessive deposition of extracellular matrix proteins in the heart, termed cardiac fibrosis, has been studied largely in rat models, because fibrosis is a process that is inherently difficult to study in normal human patients. Excessive collagen deposition, principally collagens I and III in the heart, impairs the ability of the ventricle to relax (diastolic dysfunction) and also increases the risk of cardiac arrhythmias (4). Cardiac fibrosis has been defined as reactive (interstitial and perivascular fibrosis) or reparative (scarring following necrosis) (45); both types occur in the heart of the DOCA-salt hypertensive rat (6, 46). Inhibition of the renin-angiotensin-aldosterone system with the angiotensin-convert- ing enzyme inhibitor captopril, the AT1 receptor antagonist candesartan, or the aldosterone antagonist spironolactone (9) or with the anti-inflammatory compound pirfenidone (33) reversed the reactive fibrosis in DOCA-salt hypertensive rats.

Because inflammation may play an important role in cardiac fibrosis (18, 38), the decreased collagen deposition may be a consequence of the decreased inflammatory cell infiltration in the interstitium and scar tissue. Suppression of infiltration of inflammatory cells (monocyte/macrophages) and myocardial fibrosis has been shown with tranilast in DOCA-salt hypertensive rats (19). In several models of chronic renal damage, 1-arginine reversed macrophage infiltration, blunted increases in interstitial volume and collagen IV, and decreased proteinuria (23). In aged SHR rats, chronic 1-arginine administration reversed severe nephrosclerosis (40). Fibrosis may be strongly linked to a lack of NO initiating an inflammatory response. The NO-deficient nitro-L-arginine methyl ester model is characterized by a malignant inflammatory response leading to perivascular and cardiac fibrosis (26). Our results suggest that an NO deficiency may represent the key process leading to inflammatory cell infiltration and excessive collagen deposition during DOCA-salt hypertension.

The complexity of collagen synthesis and degradation provides several points of attack for pharmacological therapy to prevent deposition of collagens (7). The precise mechanisms underlying the observed reduction in collagen deposition in DOCA-salt hypertensive rats observed after 1-arginine treatment are not known. Compounds that release NO, for example, bradykinin, decreased cardiac fibroblast function to decrease collagen expression, probably by increasing intracellular cGMP concentrations (21). In addition, the NO donor diethylamine NONOate (100 µM), but not bradykinin, decreased proliferation of fibroblasts (25). In cultured rabbit vascular smooth muscle cells, NO-generating compounds such as S-nitroso-N-acetylpenicillamine and sodium nitroprusside showed reversible, hemoglobin-sensitive inhibition of collagen synthesis, implicating NO release (25). Thus NO from the endothelium may inhibit local collagen production in the heart and blood vessels. Furthermore, the formation of plasminogen activator inhibitor-1, an inhibitor of matrix degradation, is suppressed by NO (5, 17).

The observed changes after 1-arginine treatment (attenuation of blood pressure increase and fibrosis) are only relevant if cardiac function can be shown to be improved by this intervention. Echocardiographic measurement of wall thickness in 1-arginine-treated DOCA-salt rats confirmed the results of heart weight measurements, i.e., minimal improvements with 1-arginine treatment. However, 1-arginine prevented the narrowing of the internal ventricular diameter that characterizes the DOCA-salt rat. This should improve diastolic filling and allow efficient cardiac contraction and expulsion of blood, as shown by the maintained cardiac output. The metabolic status of the myocardium of DOCA-salt rats was markedly improved by 1-arginine supplementation, as shown by the improved response to workload stress imposed by pacing and hypoxia. Purine efflux during increased workloads in 1-arginine-treated...
hearts returned to the normal levels seen in hearts from uninephrectomized rats. L-Arginine increased high-energy phosphate levels at rest above that seen in uninephrectomized and DOCA-salt rats, and these elevated concentrations were evident after hypoxia aided by improved coronary flow. An increased release of NO during exercise has been proposed to provide the heart with improved blood flow and metabolite delivery (22); l-arginine treatment may be providing this increase in NO in NO-deficient hearts from DOCA-salt rats.

The increased collagen deposition in hearts from DOCA-salt rats was associated with an increased diastolic stiffness, as measured in the isolated perfused Langendorff-perfused heart preparation. The relation between collagen deposition and stiffness is very complex: in the SHR heart, stiffness is correlated with the degree of cross-linking, rather than the total collagen content (39), and the alignment of fibers is probably also relevant. Importantly, l-arginine totally prevented the increase in diastolic stiffness. This observation clearly shows that the decrease in fibrosis in l-arginine-treated DOCA-salt rats has functional significance and that the l-arginine-NO system plays an integral role in the pathogenesis of diastolic dysfunction. Our previous studies showed that the increased passive stiffness in DOCA-salt hypertensive rats can be attenuated or reversed by inhibition of the renin-angiotensin system by captopril, candesartan, or spironolactone or by other compounds such as pirfenidone or amiloride (9, 33).

Cardiac hypertrophy and excess collagen deposition are linked closely with cardiac dysfunction and ventricular arrhythmias (4). The incidence of arrhythmias in compensated cardiac hypertrophy is related to fibrosis and the changes in membrane proteins linked to cardiac hypertrophy and fibrosis (4). It has been shown in SHR rats that hypertension-induced fibrosis leads to fatal ventricular arrhythmias (4). Additionally, in DOCA-salt rats, hypertrophy directly prolonged APD as a result of a large reduction in the early transient outward potassium channel currents (34) after downregulation of K+ channel expression (12). L-Arginine treatment of DOCA-salt rats prevented the resting prolongation in APD observed in tissues from DOCA-salt rats, suggesting normalization of the early transient outward current, which should in turn increase susceptibility to inhibition by 4-aminoypyridine, an effect that was observed. Therefore, NO supplementation elicits a protective response to electrical activity in hearts from DOCA-salt rats directly by restoring the early transient outward current and/or indirectly by reducing hypertrophy and fibrosis.

In summary, many of the cardiac and vascular changes observed in the DOCA-salt rat can be prevented or attenuated by treatment with l-arginine. This suggests that a functional NO deficit in blood vessels and the heart due to decreased NO synthase activity or increased release of reactive oxygen species such as superoxide may be a key change initiating many of these cardiovascular impairments.

REFERENCES


