Angiotensin II contributes to arterial compliance in congestive heart failure

SILVIA G. LAGE,1 LILIANE KOPEL,1 CAIO C. J. MEDEIROS,1 RICARDO T. CARVALHO,1 AND MARK A. CREAGER2
1Heart Institute, School of Medicine, University of São Paulo, São Paulo 05403-000, Brazil; and 2Brigham and Women’s Hospital, Harvard Medical School, Boston, Massachusetts 02115

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THE ELASTIC BEHAVIOR of conduit arteries contributes importantly to left ventricular function and aortic flow (17, 21, 25, 39, 41, 43). Increased pulse pressure, an index of the pulsatile hemodynamic load, is a risk factor for the development of congestive heart failure (CHF) (11, 12). The increased pulsatile load that results from a decrease in arterial compliance reduces left ventricular stroke volume more so when the contractile state is depressed than in the normally functioning ventricle (31). Therefore, impaired arterial elasticity is particularly deleterious in patients with congestive heart failure.

Elasticity of conduit arteries is determined by both structural and functional factors. Both sets of factors are governed by the tunica media, which constitutes a large part of the arterial wall and is the principal determinant of the vessel’s mechanical properties (45). Structural factors that passively alter arterial elastic properties include degeneration of elastic fibers, increased collagen content, and calcium deposition. Functional factors actively reduce distensibility of arteries by constricting vascular smooth muscle. These factors, which include vasoconstrictive substances related to increased activity of sympathetic nervous and renin-angiotensin systems, may be particularly relevant to abnormal elasticity in patients with heart failure. Arterial compliance in vitro is reduced by norepinephrine and angiotensin II (6, 14). In addition, exogenous administration of norepinephrine and angiotensin II reduces arterial compliance when administered to animals and normal subjects in vivo (8, 13, 29). Endothelium-derived nitric oxide may also contribute to the regulation of arterial smooth muscle tone of the large arteries and influence the mechanical properties of conductance vessels (23, 30).

We and others (1, 10, 18, 21, 28, 33) have demonstrated previously that arterial compliance is reduced in patients with heart failure. Because angiotensin II is frequently elevated in these patients, we reasoned that it may contribute to decreased arterial distensibility. We utilized a technique that uses high-resolution ultrasonography to directly visualize the common carotid artery and determine its elastic properties by relating changes in arterial diameter to changes in pressure generated with each heart beat. Our aim was to test the hypothesis that acute administration of an angiotensin-converting enzyme inhibitor would improve arterial compliance in patients with CHF.
METHODS

Subjects. The subject population included 23 patients with CHF secondary to idiopathic dilated cardiomyopathy. Primary valvular heart disease and systemic arterial hypertension were excluded by history and physical examination. Coronary artery disease was excluded by coronary angiography, which was performed in all patients. All participants were in sinus rhythm and clinically stable for ≥3 wk before the study. Fourteen patients were in New York Heart Association class II, 6 were in class III, and 3 were in class IV. Medical treatment included digoxin in 21 patients, diuretics in 22, angiotensin-converting enzyme (ACE) inhibitors in 21, and other vasodilators in 4 patients. ACE inhibitors and vasodilators were withheld 72 h before the investigation. The protocol was approved by the Committee for the Protection of Humans from Research Risks at the Heart Institute of the University of São Paulo, and each subject gave written informed consent.

Experimental protocol. All studies were performed in a temperature-controlled (22–24°C) cardiovascular research laboratory. An indwelling venous catheter was placed in an arm vein of each patient for blood collection and drug administration. All subjects rested in the supine position for 10 min before acquisition of hemodynamic measurements and drug infusion.

Patients received either enalaprilat (1 mg) or vehicle (saline, 0.9%), each administered as a 1-ml intravenous bolus. Measurements of carotid artery elastic properties and carotid artery blood flow (described below) were acquired at baseline and at 1, 2, and 3 h after enalaprilat or saline administration. Blood samples were collected for neurohormonal assays at baseline and 3 h after the drug infusion.

Carotid artery image acquisition. An ultrasound scanner (Ultramark-8, ATL, Bothell, WA) equipped with a high-resolution transducer (7.5 MHz) was used to image the carotid artery. A longitudinal image of the cephalic portion of the common carotid artery, 1 cm below the bifurcation, was acquired with the transducer positioned at 90° to the vessel so that the near- and far-wall interface were clearly discernible. These images, as well as an electrocardiographic signal, were recorded on a super VHS videotape recorder. Videotape images were selected at time points that corresponded with systolic expansion of the carotid artery (within 60 ms of the electrocardiographic T wave) and with diastolic relaxation (concurrent with the onset of the electrocardiographic R wave). These images were digitized with a video-frame grabber (Willow Publishers VGA, Willow Peripherals; Bronx, NY) and stored on a microcomputer.

Image analysis was performed with the aid of a dedicated image workstation to determine carotid artery diameter. The operator identified and traced the posterior wall boundary (far wall), corresponding to the interface between the lumen and intima, and the anterior wall boundary (near wall), corresponding to the interface of the adventitia and media. An automated algorithm then determined the average distance between corresponding points along both arterial walls. Carotid artery diameter was then calculated in millimeters using a calibration factor derived from the real-time ultrasound image. An average of three measurements of carotid artery diameter was obtained in each patient. The repeatability of carotid artery diameter in systole and diastole is \( r = 0.95, P = 0.0001, \) and \( r = 0.96, P = 0.0001, \) respectively. The interobserver and intraobserver variability of the method is 1.5 ± 1.0% and 1.0 ± 0.8%, respectively (27).

Arterial elastic properties determination. Blood pressure was determined during carotid artery image acquisition by upper arm sphygmomanometry using an automated oscillometric method (Dinamap 1466, Critikon; Tampa, FL). The two measurements of carotid artery elastic properties described in this report include compliance (C) and stiffness index (\( \beta \)). Arterial compliance was calculated using the following equation (22)

\[
C = \frac{(D_s - D_d) / D_s}{2(P_s - P_d)} \cdot \pi D_0^2 (N^{-1} \cdot m^4)
\]

\( \beta \), a measurement of rigidity of the vessel, was calculated according to the equation (22)

\[
\beta = \frac{\log P_s / P_d}{(D_s - D_d) / D_s}
\]

where \( D_s \) is carotid artery diameter in systole, \( D_d \) is carotid artery diameter in diastole, \( P_s \) is systolic blood pressure, and \( P_d \) is diastolic blood pressure.

Carotid artery blood flow. Carotid artery blood flow velocity curves were obtained using the pulsed Doppler system (Ultramark-8, ATL) equipped with a 7.5-MHz transducer. The carotid artery blood flow velocity image acquisition uses the same computer system as described above for the carotid artery image acquisition, and the blood flow velocity curve analysis was performed with the aid of the same dedicated image workstation. The operator identified the contour of the curve, and an automated algorithm was used to calculate carotid artery blood flow velocity (m/s). The repeatability of carotid artery blood flow velocity is \( r = 0.90, P = 0.001, \) and the variability of this method is 0.2 ± 2.7%. An average of five measurements of carotid artery blood flow velocity was obtained in each patient. Carotid artery blood flow was calculated as the product of carotid artery blood flow velocity and carotid artery cross-sectional area (during systole) according to the equation

\[
CBF = \frac{CBF \cdot \pi}{BSA \cdot 4} \cdot 0.06 (1 \cdot min^{-1} \cdot m^{-2})
\]

where CBF is carotid artery blood flow, CBFV is carotid artery blood flow velocity (m/s), BSA is body surface area (m²), and 0.06 is the factor to convert units.

Carotid and brachial arteries tonometry. To ascertain that carotid and brachial artery blood pressure were comparable, carotid and brachial artery pressure waves were recorded noninvasively in five patients using a micromanometer-tipped probe (model SPR-428, Millar Instruments; Houston, TX) by the technique of applanation tonometry (26). This technique yields an analog output, which is digitized and recorded on a personal computer. Pulse recordings were performed consecutively from the right common carotid and right brachial arteries. Sphygmomanometric measurements of right brachial artery systolic and diastolic pressures were made with an oscillometric recorder (Dinamap 1466, Critikon). The brachial artery pressure wave was assigned peak and minimal amplitudes determined by the sphygmomanometric measurement of systolic and diastolic pressures. Carotid artery pressure was then calibrated by equating the carotid mean and end-diastolic pressures to the brachial artery measurements (26).

Five patients (age, 47 ± 4 y) with dilated cardiomyopathy and heart failure class III or IV were analyzed. The protocol was the same as the one employed in the primary study. Carotid and brachial artery pressure waves were recorded at...
baseline and at 1, 2, and 3 h after the infusion of vehicle or 1 mg of enalaprilat intravenously on two separate occasions.

**Neurohormonal assays.** Venous blood samples were collected in 22 patients from the indwelling catheter for determination of plasma angiotensin II, plasma ACE, and plasma norepinephrine levels. Samples were immediately placed on ice and centrifuged at 2°C. Plasma concentration of norepinephrine was quantified by high-performance liquid chromatography (6). Plasma angiotensin II was quantified by a radioimmunoassay method (5) and plasma ACE by a fluorometric assay (37).

**Statistical analysis.** All values are presented as means ± SE. The proportionality of sex distribution in each group was compared by the Fisher exact test. Between-group comparisons of age, left ventricle ejection fraction, plasma norepinephrine, plasma angiotensin II and plasma ACE concentration, basal arterial compliance, arterial stiffness, mean blood pressure, carotid artery blood flow velocity, carotid artery blood flow, and carotid artery cross-sectional area employed a t-test for unpaired data. The effect of enalaprilat and saline within and between each group on arterial compliance, arterial stiffness, carotid artery blood flow velocity, carotid artery blood flow, mean blood pressure, and carotid artery cross-sectional area was analyzed by analyses of variance for repeated measures and F-Wilks post hoc testing for statistical significance. Brachial artery pressure and tonometry-estimated carotid artery pressure were compared by using a Wilcoxon test. Univariate linear regression analysis was used to determine the relationship between selected continuous variables, including arterial compliance, mean blood pressure, plasma angiotensin II, plasma ACE concentrations, and diameter variability. Independence of association was assessed by stepwise multiple regression. Statistical significance was accepted at the 95th percentile (P < 0.05).

**RESULTS**

Clinical characteristics and baseline compliance and neurohormonal measurements of each group of subjects are shown in Table 1. There were no significant differences between the two groups for any of these baseline measurements.

**Effects of vehicle.** The hemodynamic and arterial elastic properties at baseline and 1, 2, and 3 h after vehicle are presented in Table 2. There were no statistical changes in any of the variables after saline (vehicle) administration (Fig. 1).

**Effects of enalaprilat.** The hemodynamic and arterial elastic measurements at baseline and 1, 2, and 3 h after drug administration are presented in Table 3. The changes in compliance (P < 0.01), stiffness index (P < 0.01), and blood pressure (P < 0.01) were significantly greater following enalaprilat administration compared with vehicle. Enalaprilat increased carotid artery compliance from 3.0 ± 0.4 at baseline to a maximum of 5.0 ± 0.4 × 10⁻¹⁰ N⁻¹m⁻⁴ and decreased arterial stiffness from 17.5 ± 1.8 to a nadir of 10.1 ± 0.6 three hours after drug administration (each P < 0.01) (Fig. 1). Enalaprilat significantly reduced mean blood pressure from 85 ± 3 to 78 ± 3 mmHg at the 1-h time point (P < 0.01). Blood pressure returned to baseline values by 3 h after drug administration. Thus changes in compliance and stiffness at 3 h are under isobaric conditions. After adjustment of both the compliance and stiffness index for mean blood pressure, the changes in each variable remain statistically significant.

**Carotid and brachial arteries tonometry.** There was no significant difference in systolic or diastolic blood pressure between the carotid and brachial arteries whether measured at baseline or after drug or vehicle administration (Table 4). The relationship between the carotid and brachial artery systolic blood pressure (SBP) was defined as SBP_carotid = 0.82 × SBP_brachial + 21.17 (r = 0.74, standard error of estimate = 6.81), and that between carotid and brachial diastolic blood pressure (DBP) was defined as DBP_carotid = 0.84 × DBP_brachial + 13.63 (r = 0.87, standard error of estimate = 4.30).

**Neurohormonal measurements.** Plasma angiotensin II was 11.7 ± 0.8 pg/ml at baseline and decreased to 6.6 ± 0.5 pg/ml 3 h after enalaprilat administration (P < 0.01). Plasma ACE was 53.9 ± 5.8 nmol·min⁻¹·ml⁻¹ at baseline and decreased to 21.5 ± 5.8 nmol·min⁻¹·ml⁻¹ after enalaprilat (P < 0.01). There were no statistical changes in plasma angiotensin II and plasma ACE after vehicle.

### Table 1. Clinical characteristics and baseline compliance and neurohormonal measurements in vehicle and enalaprilat groups of patients with CHF

<table>
<thead>
<tr>
<th></th>
<th>Vehicle</th>
<th>Enalaprilat</th>
</tr>
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<tbody>
<tr>
<td>n</td>
<td>9</td>
<td>14</td>
</tr>
<tr>
<td>Age, y</td>
<td>45 ± 3</td>
<td>48 ± 3</td>
</tr>
<tr>
<td>Sex (male/female)</td>
<td>9/0</td>
<td>13/1</td>
</tr>
<tr>
<td>LVEF</td>
<td>24 ± 4</td>
<td>19 ± 1</td>
</tr>
<tr>
<td>Mean blood pressure, mmHg</td>
<td>94 ± 3</td>
<td>85 ± 3</td>
</tr>
<tr>
<td>Compliance, 10⁻¹⁰ N⁻¹·m⁻⁴</td>
<td>3.9 ± 0.8</td>
<td>3.0 ± 0.4</td>
</tr>
<tr>
<td>Stiffness index</td>
<td>14.8 ± 2.4</td>
<td>17.5 ± 1.8</td>
</tr>
<tr>
<td>Plasma NE, pg/ml</td>
<td>419 ± 203</td>
<td>466 ± 95</td>
</tr>
<tr>
<td>Plasma ANG II, pg/ml</td>
<td>12.5 ± 0.4</td>
<td>11.7 ± 0.8</td>
</tr>
<tr>
<td>Plasma ACE, nmol·min⁻¹·ml⁻¹</td>
<td>40.8 ± 2.1</td>
<td>53.9 ± 5.8</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, number of patients. LVEF, left ventricular ejection fraction; NE, norepinephrine; ANG II, angiotensin II; ACE, angiotensin-converting enzyme. P values were not significant for all groups.
The relationships between baseline plasma angiotensin II, as well as plasma ACE concentration and carotid compliance, were analyzed by univariate linear regression. The concentration of plasma angiotensin II and plasma ACE correlated inversely with arterial compliance \((r = -0.52; P = 0.015\) and \(r = -0.45; P = 0.041\), respectively). Mean blood pressure was not related to plasma angiotensin II \((r = -0.26; P = 0.26)\) or plasma ACE concentrations \((r = -0.26; P = 0.27)\). A stepwise multiple regression analysis, where plasma angiotensin II, plasma ACE, and mean blood pressure were included in the model, found that only plasma angiotensin II correlated with carotid arterial compliance \((P = 0.043)\).

### DISCUSSION

The new findings from this study are that plasma angiotensin II is associated with decreased carotid artery compliance and increased carotid arterial stiffness in patients with CHF and that ACE inhibition improves these arterial elastic properties. These results implicate angiotensin II as an important functional determinant of arterial elasticity in patients with heart failure.

**Determinants of arterial compliance.** Previous studies have demonstrated an impairment of arterial elastic properties in CHF \((1, 10, 18, 21, 28, 33)\). Using methodology identical to that employed in this study, we found previously that arterial compliance is reduced 56% in patients with CHF compared with normal subjects \((28)\). The mechanism, however, responsible for modifications of the arterial elastic properties in CHF is not entirely known, and several factors may be involved. Structural factors associated with an increase in the sodium and water content of the arterial wall and a decrease in elastic:collagen ratio may participate. We previously determined that carotid artery wall thickness averaged 19.5% more in patients with CHF secondary to idiopathic dilated cardiomyopathy \((28)\) than in healthy age-matched subjects. Yet, even though carotid artery wall thickness was greater in...
patients with CHF, it does not appear to contribute to distensibility (28).

Functional factors that modify arterial smooth muscle tone may be particularly important in patients with CHF once there is an increase in neurohormonal activity in these patients. Indeed, mechanical properties of large arteries can be modified by neurohormonal mechanisms, particularly the sympathetic nervous system and renin-angiotensin system (20, 34). Dobrin and Rovick (14) and Barra et al. (4) showed that norepinephrine decreased arterial incremental elastic modulus under isobaric conditions. In addition, both norepinephrine and angiotensin II have been shown to reduce arterial compliance in vitro (16). Wilson et al. (44) demonstrated that arterial compliance of conduit arteries in pigs can decrease in response to acute infusion of norepinephrine in vivo. Further support for an active or functional component of arterial compliance comes from Bank et al. (3), who, using intravascular ultrasound in normal human subjects, demonstrated that administration of nitroglycerin and norepinephrine significantly shifted the brachial artery stress-strain curve in opposite directions. Boutouyrie et al. (7) showed that sympathetic activation induced by cold pressor test or mental stress test reduced arterial compliance in healthy humans; however, Joannides et al. (24) showed an opposite result. Previously, Creager’s laboratory (28) found a significant positive relationship between plasma norepinephrine concentration and Young’s modulus of elasticity in patients with CHF.

Effect of ACE inhibition. Activation of renin-angiotensin-aldosterone system is intrinsic to the pathophysiology of CHF (15, 19, 40). To assess the role of angiotensin II in modulating arterial elasticity, we measured the effects of ACE inhibition on carotid artery compliance in patients with CHF secondary to dilated cardiomyopathy. We observed a substantial increase in arterial compliance (59.5%) and decrease in arterial stiffness (57.8%) after administration of enalaprilat. These changes of arterial elastic properties were independent of blood pressure, which had returned to baseline levels 3 h after drug administration, at which time the effect of enalaprilat on compliance was maximal. Moreover, arterial compliance correlated inversely with pretreatment plasma angiotensin II and plasma ACE levels. Giannattasio et al. (21) found similarly that radial artery compliance improved in patients with CHF after 4–8 wk of treatment with benazepril. Important features that distinguish the two studies include the acuity of treatment, the techniques used to assess arterial compliance and stiffness, and the administration of vehicle as a control for time and conditions in our experimental procedures.

Inhibition of ACE has been shown to enhance peripheral endothelium-dependent vasodilation in patients with mild heart failure (32). Because flow stimulates release of endothelium-derived nitric oxide and vasodilation (35), we considered the possibility that enalaprilat-induced increase in carotid artery blood flow would provoke endothelium-dependent relaxation and alter compliance. However, we did not observe changes in arterial cross-sectional area or carotid blood flow. Thus flow-mediated factors cannot satisfactorily explain the improvement of arterial elastic properties after ACE inhibition.

Our findings are comparable to those observed in hypertensive patients in whom the compliance and diameter of large (brachial and carotid) arteries are increased by ACE inhibition, even at doses that do not produce a systemic hypotensive response (2, 36, 38). Therefore, the local effect of angiotensin II in conduit vessels is a potential mechanism to explain the arterial elastic properties in CHF. This can be modified by ACE inhibition.

Study limitations. The formulas used to calculate arterial compliance and stiffness assume that there is a constant linear, rather than curvilinear, relation between changes in arterial diameter and pressure. This limitation should be acceptable in clinical studies, because the changes in arterial diameter and blood pressure are usually <25% and occur over the linear portion of the curve (9). Also, the arterial elastic properties were calculated by measuring carotid artery diameter and brachial artery pressure. In a subset of five patients, we measured both carotid artery and brachial artery pressure and found them to be comparable, lending credibility to using brachial artery pressure in the calculations. Also, we did not assess the effect of ACE inhibition on arterial compliance in healthy subjects and therefore do not discount the possibility that angiotensin contributes to arterial compliance under normal physiological conditions.

In conclusion, the findings in this study enable us to conclude that angiotensin II adversely affects compliance of conduit vessels in patients with CHF. Moreover, ACE inhibition improves arterial elastic properties. This favorable effect on the pulsatile component to the afterload may contribute to the improvement in left ventricular performance that occurs in patients with heart failure treated with ACE inhibitors.

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