Effects of losartan treatment on cardiac autonomic control during volume loading in patients with DCM

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1Department of Internal Medicine, Cardiology and Heart Surgery, University of Naples “Federico II,” 80131 Naples; 2Institute of Cybernetics, National Research Council, 80072 Naples; 3Department of Experimental Medicine and Pathology, University of Rome “La Sapienza,” 00161 Roma; and 4Istituto di Ricovero e Cura a Carattere Scientifico, Istituto Neurologico Mediterraneo, 86077 Pozzilli, Italy

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Petretta, M., L. Spinelli, F. Marciano, C. Apicella, M. L. E. Vicario, G. Testa, M. Volpe, and D. Bonaduce. Effects of losartan treatment on cardiac autonomic control during volume loading in patients with DCM. Am J Physiol Heart Circ Physiol 279: H86–H92, 2000.—This study evaluated the effect of angiotensin II receptor blockade on cardiac autonomic control adaptation and urine output in response to acute isotonic volume load in patients with idiopathic dilated cardiomyopathy (DCM) and asymptomatic or mildly symptomatic heart failure. Left ventricular volumes and heart rate variability measurements were assessed at baseline and during intravenous saline load in 14 patients before and after 2 mo of losartan treatment. After losartan treatment, blood pressure values were lower, whereas left ventricular ejection fraction was higher (F = 79, P < 0.001), than before treatment. During saline load, ejection fraction decreased before losartan treatment (F = 5.6, P < 0.05) but did not change after treatment. Urinary volume, unchanged during saline load in untreated patients, increased after losartan (F = 9.38, P < 0.001). Time-domain measurements that represent vagal modulation of heart rate (root-mean-square successive differences and percentage of differences between successive R-R intervals >50 ms) decreased during saline load in untreated patients (F = 3.1, P < 0.05 and F = 6.5, P < 0.01, respectively), but not after losartan. Similarly, a decrease in very low frequency (F = 3.2, P < 0.05), low-frequency (F = 2.9, P < 0.05), and high-frequency power (F = 6.1, P < 0.01) after saline load was observed only in untreated patients. In patients with DCM, losartan treatment improves the cardiac autonomic adaptation and increases urine output in response to volume overload.

heart rate variability; isotonic volume expansion; left ventricular dysfunction; angiotensin II receptor blockade

A SIGNIFICANT IMPAIRMENT of cardiac dynamics in response to acute blood volume expansion has been demonstrated in patients with idiopathic dilated cardiomyopathy (DCM) who are asymptomatic or have mildly symptomatic heart failure (29). In particular, it has been reported that in DCM patients, volume expansion is followed by a reduction in left ventricular ejection fraction (LVEF) with a paradoxical increase in forearm vascular resistance even in patients with basal hemodynamic, hormonal, and renal profiles still in the normal range (29). It is noteworthy that in these patients, the pretreatment with an angiotensin-converting enzyme (ACE) inhibitor significantly improves the compromised cardiac response to volume overload (30). Recently, Spinelli et al. (25) reported that DCM patients also show impairment in cardiac autonomic response to acute volume overload. In fact, during volume loading a parasympathetic activation was detectable in normal subjects, whereas a parasympathetic withdrawal occurred in DCM patients. These abnormalities are responsible for peripheral vasoconstriction and consequent reduced blood flow to peripheral tissue and may contribute to water and salt retention in chronic heart failure. Angiotensin II receptor blockade is being utilized in the treatment of patients with heart failure (20). Long-term therapy with angiotensin II receptor blockers, in fact, is associated with favorable effects on hemodynamics and improvement of clinical course in patients with congestive heart failure. Although the beneficial influence of angiotensin II blockers on systemic hemodynamics at rest is well documented, very little is known about the effects of these compounds on cardiovascular adaptation to volume overload. Moreover, no study has evaluated the effects of these drugs on cardiac autonomic response during volume loading. The aim of this study was to establish whether losartan treatment modifies hemodynamic responses to volume load in patients with DCM and mild heart failure. Furthermore, we evaluated the effects of losartan treatment on cardiac autonomic nervous system activity utilizing beat-to-beat variation of heart rate during daily life and during acute volume expansion.

METHODS

Study patients. The overall study population included 14 patients with idiopathic DCM and chronic, stable, mild heart

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failure. The investigation conforms with the recommendations of the Declaration of Helsinki, and the study protocol was approved by the ethical committee of our institution. All subjects gave written, informed consent before entering the study. The patients, 9 men and 5 women, ranging in age from 38 to 66 yr (mean ± SE: 52 ± 2 yr), were selected from consecutive patients in the outpatient clinic for cardiovascular diseases of our institution. Exclusion criteria were any other major disease, ischemic heart disease, hypertension, diabetes, atrial fibrillation or severe ventricular arrhythmia, renal failure, recent acute cardiac decompensation as defined by the sudden accumulation of pulmonary congestion or peripheral edema, valvular disease or significant mitral regurgitation, and cardiothoracic anatomy not allowing for satisfactory and reproducible echocardiographic recordings. The diagnosis of DCM was based on the exclusion of any obvious underlying cause of heart failure during routine evaluation. In particular, no patient had a history of angina or myocardial infarction, and all patients had undergone coronary angiography showing normal coronary arteries. The definition of mild heart failure was based on the following criteria: no reduction (4 patients) or mild reduction (10 patients) in functional capacity according to the New York Heart Association classification (class I or II); mild to moderate limitation of exercise capacity as determined by cardio-pulmonary exercise testing using a standard protocol (upright bicycling with a stepwise increase of 10 W/min) (mean exercise duration in our patients was 9.5 ± 0.6 min, and peak oxygen consumption averaged 16.9 ± 0.6 ml · kg⁻¹ · min⁻¹); echocardiographic end-diastolic left ventricular diameter ≥56 mm; and LVEF, as determined by equilibrium radionuclide angiography, <45% on at least one measurement within 3 mo before the study. At the time of their first visit to the outpatient clinic, 10 patients were undergoing treatment with ACE inhibitors, whereas digitalis and diuretics were being given to 7 and 11 patients, respectively.

Study protocol. All drug therapy was discontinued at least 1 wk, and ACE inhibitor at least 2 wk, before the study. Alcohol, caffeine, cigarettes, and physical exercise were all prohibited 24 h before the study. After being admitted to the clinic ward, all subjects were maintained on a daily diet containing 100 mg of sodium, 50 mg of potassium, and 1,500 ml of water. Daily 24-h urine collections were analyzed for sodium, potassium, and creatinine excretion. When a satisfactory equilibrium between sodium and water excretion was achieved, the patients underwent the study protocol on two consecutive days, as previously described (29). Twenty-four-hour Holter recording started between 8:00 and 10:00 AM. Patients were asked to record the time they went to sleep and the time they awakened, and all reported sleeping normally during the nights they were monitored. After this monitoring was terminated, a second Holter recording started with the patient in a comfortable lying position after voiding, and an intravenous line was inserted into a superficial forearm vein. The temperature (22°C) and lighting of the study room were maintained constant. The patient was asked to drink 500 ml of water to ensure sufficient urinary flow. After 60 min, an intravenous isotonic saline load (0.9% NaCl, 0.25 ml · kg⁻¹ · min⁻¹) was started and maintained at a constant rate for 2 h. Arterial blood pressure was measured at 10-min intervals by standard sphygmomanometric technique. Heart rate was continuously monitored by electrocardiogram (ECG) lead II. M- and B-mode ECGs were recorded for measurements of atrial and ventricular dimensions, calculation of LVEF, and estimation of stroke volume and cardiac output before the isotonic saline load was started, at 60-min intervals during the saline load, and 60 min after its terminaion. At the same time, urinary output was measured. The Holter recording was then interrupted and the study session terminated. Thereafter, patients started treatment with losartan (Merck, Whitehouse Station, NJ), a type 1 angiotensin II (AT₁) receptor blocking agent, at a dosage of 50 mg/day orally every day at 8:00 AM, and after 2 mo of treatment, 24-h Holter recording and the saline load were repeated as described above.

Echocardiographic measurements. Wide-angle, two-dimensional echoes were recorded with a phased-array sector scanner (77020 AC, Hewlett-Packard, Andover, MA). All studies were videotaped on ¼-in. tape with the use of videocassette recorders equipped with a backscatter search module, which allows frame-by-frame bidirectional playback. The video frame rate of the system was ~60 frames/s. All patients were studied while in a comfortable lying position with multiple views through the apical window. Two views were selected for measurements: an apical four-chamber view and an apical two-chamber view. The left ventricular long axis (L_max) was measured at end diastole as the longest major axis in either of the two apical views. The measurements of L_max were rounded off to the closest whole number to ensure reproducibility. Left ventricular end-diastolic area was measured using the largest of all the left ventricular minor axes measured. Left ventricular end-diastolic volume (in ml) was calculated according to the single-plane ellipse method as EDV = 8/3(EDA²)/(π · L_max), where EDA is end-diastolic area (13). The same measurements were undertaken at end systole to calculate end-systolic volume. LVEF was measured using the averages of all the end-diastolic and end-systolic volumes (13). All studies were performed by the same investigator and read independently by two experts unaware of the protocol. The readings obtained showed correlation for both L_max (r = 0.97, P < 0.001) and end-diastolic area (r = 0.96, P < 0.001). Excellent correlations were also obtained for the measurements of end-diastolic (r = 0.97, P < 0.001) and end-systolic volume (r = 0.95, P < 0.001) between the two observers. The variability of multiple measurements of volumes over a period of 2 h did not exceed 3.5%. Stroke volume was derived as the difference between end-diastolic and end-systolic volume, and cardiac output and total peripheral resistance were estimated using standard formulas. In our laboratory, the echocardiographic measurements of cardiac output were significantly correlated with the measurements obtained using the thermodilution technique (r = 0.89, P < 0.01).

Processing 24-h Holter recordings. All 24-h Holter recordings were analyzed at the National Research Council cybernetics laboratory as previously described (5). The two electrocardiographic analogic channels were read via a modified Teac-Tascam 234 Syncapect tape deck (Teac, Tokyo, Japan) and digitized at 330 samples/s. In addition to evaluation of the usual electrocardiographic parameters, including identification of QRS widths and shapes and R-R interval abnormalities, all R-R interval sequences were stored, and each was labeled with a code number identifying its normality or its class of abnormality. Premature complexes and their adjacent R-R intervals, used only for timekeeping purposes, were rejected by the software, as were electrical noise and other aberrant electrocardiographic signals. To be eligible for the present study, data losses per tape due to persistent rhythm anomalies and artifacts could not exceed 10% of the entire recording or of daytime (7:30 AM to 9:30 PM) or nighttime recordings (midnight to 5:00 AM). The sequence of normal R-R (NN) intervals was analyzed to compute time- and frequency-domain measurements of HRV (26).

Time-domain measurements of HRV. The standard deviation of the NN intervals (SDNN) calculated over a 24-h...
period encompasses short-term as well as long-term NN interval variations. The standard deviation of the average NN intervals for all 5-min segments of the entire 24-h ECG recording (SDANN index) evaluates long-term R-R variations, whereas the mean of the standard deviations of NN intervals for all 5-min segments of the entire 24-h ECG recording (SDNN index) depends on short-term R-R variations. On the other hand, differences between successive NN intervals provide an index of cardiac vagal modulation that is firmly related to short-term variations in heart rate. Accordingly, we calculated the root-mean-square successive difference (r-MSSD) of all NN intervals and the percentage of differences between adjacent NN intervals exceeding 50 ms (pNN50) for the entire 24-h recordings.

**Frequency-domain measurements of HRV.** The 24-h heart rate power spectrum was computed by means of the fast Fourier transform algorithm, as previously described (5). A smooth shape for fast Fourier transform estimates, reducing side-lobe leakage, was obtained by cosine tapering the original time series at each end over one-tenth of the window (1). An R-R interval duration of time was obtained from the sequence of R-R intervals as follows: from the sequence of NN values, the sequence $\Delta NN = NN_{i-1} - NN = f(NN_{i}, NN_{i-1})$ was evaluated, and from this the temporal sequence $\Delta NN = f(i)$, where $i = 100, 200, 300$ ms... was computed by linear interpolation with a time step of 100 ms; this is a low-pass filtering operation that attenuates any variability above the chosen value of the sampling frequency. The final average spectrum was expressed for each band in square milliseconds. The bands explored were very low frequency power (energy in the heart rate power spectrum $<0.04$ Hz), low-frequency power (between 0.04 and 0.15 Hz), and high-frequency power (between 0.15 and 0.40 Hz). The ratio of low- to high-frequency power was calculated from the absolute values of these two components. An epoch of 300 s and a sampling period of 293 ms (1,024 samples/epoch) were chosen to cover the entire range with a sufficient number of frequency samples in each band as follows: very low frequency, 12 samples; low frequency, 34 samples; high frequency, 75 samples.

To evaluate the effect of saline loading on cardiac autonomic modulation before and after losartan treatment, four sets of HRV measurements were obtained by averaging three consecutive 5-min segments. For this purpose, time-domain measurements of long-term HRV (SDNN and SDANN indexes) were not calculated. The first set of measurements was obtained at steady state, just before the infusion started; the other three sets were centered at 60 and 120 min during the infusion and at 60 min after the end of the infusion.

**Statistical analysis.** Statistical analysis was performed using the SPSS statistical package (19). Categorical variables were expressed as percentages; continuous data were expressed as means $\pm$ SD. Because the distribution of the frequency-domain measurements of HRV was skewed, the log transformation (ln) of each measure, which produces nearly normal distributions, was applied before statistical analysis was performed. Twenty-four-hour data of HRV of DCM patients before and after losartan treatment were compared by paired t-test. A $P$ value $<0.05$ was considered significant. Repeated-measures ANOVA followed by post hoc multiple comparisons with the Bonferroni correction was performed to detect changes over time during saline load before and after losartan treatment. Moreover, to evaluate the effect of losartan treatment on cardiac and autonomic nervous system responses to saline load, two-factor repeated-measures ANOVA was performed considering the main effects of the independent variables “time” and “treatment” as well as the “time-by-treatment” interaction.

**RESULTS**

**HRV analysis of 24-h Holter recording.** Twenty-four-hour time- and frequency-domain measurements of HRV at baseline and after losartan treatment are reported in Table 1. No difference in average NN interval was detectable before and after losartan treatment. Losartan induced a significant increase in time- and frequency-domain measurements of HRV, but the ratio of low- to high-frequency power remained unchanged.

**Circulatory response to volume expansion.** Figure 1 shows the responses of systemic and cardiac hemodynamics and of urine volume to saline load in DCM patients before and after losartan treatment. Neither systolic nor diastolic arterial pressure changed during saline load before and after losartan treatment. However, blood pressure values were lower after losartan at baseline and at each time during saline load. Therefore, by two-factor ANOVA, the treatment effect was significant for systolic ($F = 56.5, P < 0.001$) and diastolic blood pressure ($F = 67.7, P < 0.001$), without time-by-treatment interaction. Left ventricular end-systolic volumes, comparable at baseline, showed a similar increase during saline load before ($F = 10.6, P < 0.001$) and after losartan treatment ($F = 17.4, P < 0.001$); however, left ventricular end-systolic volumes were lower in treated than in untreated patients. Thus a significant ($F = 50.5, P < 0.001$) treatment effect was detectable by two-factor ANOVA. LVEF showed higher values in treated than in untreated patients ($F = 79$; $P = 0.001$).

### Table 1. Heart rate variability measures by 24-h Holter recording in patients with idiopathic dilated cardiomyopathy before and after losartan treatment

<table>
<thead>
<tr>
<th>Measure</th>
<th>Before Losartan</th>
<th>After Losartan</th>
<th>$P$ Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$n$</td>
<td>14</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td><strong>Time-domain measures</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average NN, ms</td>
<td>810 ± 78</td>
<td>847 ± 114</td>
<td>NS</td>
</tr>
<tr>
<td>SDNN, ms</td>
<td>115 ± 21</td>
<td>130 ± 26</td>
<td>0.002</td>
</tr>
<tr>
<td>SDANN index, ms</td>
<td>98 ± 27</td>
<td>108 ± 25</td>
<td>0.03</td>
</tr>
<tr>
<td>SDNN index, ms</td>
<td>53 ± 16</td>
<td>65 ± 14</td>
<td>0.02</td>
</tr>
<tr>
<td>r-MSSD, ms</td>
<td>39 ± 12</td>
<td>52 ± 14</td>
<td>0.002</td>
</tr>
<tr>
<td>pNN50, %</td>
<td>9 ± 7</td>
<td>14 ± 8</td>
<td>0.03</td>
</tr>
<tr>
<td><strong>Frequency-domain measures</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total power, ms$^2$</td>
<td>2,106 ± 631</td>
<td>2,596 ± 771</td>
<td>0.006</td>
</tr>
<tr>
<td>ln total power</td>
<td>7.62 ± 0.28</td>
<td>7.81 ± 0.30</td>
<td></td>
</tr>
<tr>
<td>VLF power, ms$^2$</td>
<td>1,361 ± 368</td>
<td>1,754 ± 440</td>
<td></td>
</tr>
<tr>
<td>ln VLF power</td>
<td>7.18 ± 0.26</td>
<td>7.44 ± 0.24</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>LF power, ms$^2$</td>
<td>453 ± 121</td>
<td>623 ± 138</td>
<td></td>
</tr>
<tr>
<td>ln LF power</td>
<td>6.08 ± 0.25</td>
<td>6.41 ± 0.23</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>HP power, ms$^2$</td>
<td>283 ± 149</td>
<td>341 ± 89</td>
<td></td>
</tr>
<tr>
<td>ln HP power</td>
<td>5.56 ± 0.38</td>
<td>5.79 ± 0.29</td>
<td>0.014</td>
</tr>
<tr>
<td>LF/HF</td>
<td>1.74 ± 0.39</td>
<td>1.88 ± 0.38</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are means $\pm$ SD; $n =$ no. of patients, NN, normal of R-R intervals; SDNN, standard deviation of all NN intervals; SDANN index, standard deviation of the average NN intervals for all 5-min segments; SDNN index, mean of the standard deviations of NN intervals for all 5-min segments; r-MSSD, root-mean-square successive difference; pNN50, percentage of differences between successive NN intervals $>50$ ms; ln, logarithmic units; VLF, very low frequency; LF, low frequency; HF, high frequency; NS, not significant.
P < 0.001). Furthermore, during saline load, ejection fraction decreased slightly in untreated patients (F = 5.6, P < 0.05) but did not change significantly after losartan treatment. Also, cardiac output was higher in treated (F = 17.8; P < 0.01) than in untreated patients and increased during saline load only after losartan treatment (F = 4.9, P < 0.01).

With regard to urinary volume, no change was detectable during saline load before losartan treatment, but after losartan treatment, urinary volume increased during saline load (F = 9.38, P < 0.001), and by two-factor ANOVA the treatment effect (F = 36.2, P < 0.001) and the time-by-treatment interaction (F = 5.5, P < 0.005) were significant.

HRV and volume expansion. Figure 2 shows the responses of heart rate and time-domain measures of HRV during volume expansion before and after losartan treatment. In untreated patients, average NN decreased (F = 8.1, P < 0.001) during saline load, as did r-MSSD (F = 3.1, P < 0.05) and pNN50 (F = 6.5, P < 0.01). In contrast, after losartan treatment average NN decreased only slightly during saline load (F = 2.9, P < 0.05), and r-MSSD and pNN50 remained unchanged. Two-factor repeated-measures ANOVA showed a significant effect of losartan treatment for average NN (F = 4.6, P < 0.05), r-MSSD (F = 10.9, P < 0.01), pNN50 (F = 15.6, P < 0.01) and SDNN index (F = 7.6, P < 0.05). The effect of saline load on frequency-domain measurements is shown in Fig. 3. In untreated patients, saline load induced a decrease in very low frequency (F = 3.2, P < 0.05), low-frequency (F = 2.9, P < 0.05), and high-frequency power (F = 6.1, P < 0.01), and the ratio of low- to high-frequency power increased (F = 5.3, P < 0.01); after losartan treatment, frequency-domain measurements and the ratio of low-to high-frequency power remained unchanged during saline load. When the changes in frequency-domain measurements were analyzed by two-factor ANOVA, losartan treatment showed a significant effect for very low frequency (F = 11.1, P < 0.01), low-frequency (F = 12.9, P < 0.01), and high-frequency power (F = 23.8, P < 0.01), and the time-by-treatment interaction was significant for high-frequency power (F = 4.8, P < 0.01).

DISCUSSION

The results of the present study demonstrate that treatment with AT1 angiotensin receptor blockade induces several favorable changes in the cardiac and autonomic nervous system responses to volume overload in patients with DCM who are asymptomatic or have mildly symptomatic heart failure.

Chronic heart failure and 24-h HRV measurements. HRV measurements obtained in DCM patients by 24-h Holter recording before losartan treatment were slightly reduced and comparable to those commonly observed in patients with mild heart failure (25). Studies utilizing HRV analysis on patients with chronic heart failure have demonstrated a reduction in high-frequency power and in time-domain measurements considered to reflect cardiac vagal modulation of heart rate (3). Low-frequency power, considered a measure of sympathetic modulation, but also influenced by parasympathetic activity, is also reduced in heart failure patients, despite the increase in sympathetic activity (2, 11). Desensitization of β-adrenoceptor, impairment of post-receptor signal transduction, and reduced responsiveness of sinus node to neural inputs in conditions characterized by a marked and unopposed persistent sympathetic activation are the mechanisms hypothesized to explain these discrepancies. Recently, van de Borne et al. (28) found that in patients with chronic heart failure, the reduction of low-frequency R-R interval power was associated with a reduction in the low-frequency component of muscle sympathetic nerve activity, and they hypothesized that the distur-
bances of rhythmic oscillation of autonomic sympathetic outflow are due to the presence of a central autonomic regulatory impairment. Interestingly, these disturbances of rhythmic oscillations in autonomic outflow are reversible after heart transplantation (27).

Cardiac adaptation to volume overload. In our untreated DCM patients, volume overload induced an increase in left ventricular end-diastolic and end-systolic volumes with a slight reduction of ejection fraction. It is well known that chronically dilated ventricles operate on the flat part of the Starling curve and, when subjected to additional volume load, are unable to utilize the Frank-Starling mechanism to a significant degree (23). Moreover, the increase in heart size, caused by volume loading, induces, according to Laplace’s law, an increase in intramural wall stress and thus in afterload. The increase in afterload, unbuffered by a Starling-mediated increase in isometric force, causes ejection fraction and cardiac output to fall (afterload mismatch) (24). These hemodynamic alterations induce reflex tachycardia and peripheral vasoconstriction via parasympathetic withdrawal and sympathetic excitation.

Autonomic nervous system responses to volume overload. In our patients, saline load induced a decrease in average R-R interval and in time- and frequency-domain measurements of HRV, considered to reflect cardiac vagal modulation. Spinelli et al. (25) observed that in normal subjects, volume expansion activates mechanoreceptors with vagal afferent endings located in the heart, lungs, and vessels (7, 12). Consequently, these mechanoreceptors increase their inhibitor influence on sympathetic efferent outflow and their excitatory influence on parasympathetic outflow from the medullary cardiovascular centers. In DCM patients, a reduced sensitivity of mechanoreceptors with vagal afferent endings is probably responsible for the lack in parasympathetic activation during isotonic volume expansion (8).

Losartan treatment and 24-h Holter recording. After 2 mo of losartan treatment, all HRV measurements obtained by 24-h Holter monitoring increased significantly. Binkley et al. (4) found that treatment of congestive heart failure patients with an ACE inhibitor is associated with a restoration of autonomic balance that derives mostly from a significant increase in parasympathetic tone. Krum et al. (16) subsequently reported that digoxin therapy acts to ameliorate the autonomic dysfunction in patients with mild to moderate chronic heart failure. In that study, both low- and high-frequency power increased after digoxin therapy, whereas plasma norepinephrine levels decreased. Considering that the improvement in cardiac vagal modulation was obtained utilizing drugs with different mechanisms of action, it is conceivable that the improvement in left ventricular function contributes to the restoration of cardiac vagal modulation.

Losartan treatment and cardiac adaptation to volume overload. Our results show that losartan treatment resulted in a significant improvement of the car-
diac adaptation to volume overload. In fact, a significant increase in echocardiographically assessed cardiac output was observed after losartan. Moreover, losartan prevented the reduction of ejection fraction observed in untreated patients during saline load. Interestingly, Volpe et al. (30) found that pretreatment with an ACE inhibitor significantly improved compromised cardiac and hormonal response to acute isotonic volume overload. Therefore, the favorable influence of losartan on cardiovascular responses to volume expansion seems to be related to cardiac unloading. Also, a reduction in resting blood volume may contribute to decrease cardiac loading after losartan treatment. However, this hypothesis seems unlikely, because in our study patients, at baseline, left ventricular end-diastolic volume remained unchanged after losartan treatment. Furthermore, Raya et al. (21) demonstrated that angiotensin II receptor blockade does not modify total blood volume in a rat model of heart failure.

**Losartan treatment and autonomic nervous system responses to volume overload.** The favorable hemodynamic effect induced by losartan was associated with notable changes in cardiac autonomic response to volume loading. In fact, after losartan treatment, r-MSSD, pNN50, and low- and high-frequency powers remained unchanged in contrast to the significant reduction uniformly observed in untreated patients. Improvement of cardiac autonomic modulation in these patients may reflect the improvement of cardiac function.

It is well known that the circulating concentration of angiotensin II is increased in heart failure and may be involved in the enhanced sympathetic outflow commonly observed in this condition (14). Angiotensin II receptors have been identified in many sites of the brain system, both within and outside the blood-brain barrier (6, 22), and studies performed in dogs (18) demonstrated that angiotensin II plays an important role, in modulating, at central level, sympathetic and parasympathetic outflow.

Because losartan is known to cross the blood-brain barrier (17), it is conceivable that in our patients, some of the favorable changes observed in the autonomic responses to volume loading after losartan may also be due to the attenuation of the gain of sympathetic reflexes at central level. However, studies performed in animal models must be considered cautiously, considering that dogs, but not humans, possess a well-developed Bainbridge reflex, resulting in mechanoreceptors-mediated Bainbridge reflex, during altered baroreceptor activity that fails to decrease normally during acute intravenous saline loading (10). The extent of sympathoinhibition greatly contributes to the associ-
ated natriuretic response. In fact, greater absolute change in renal sympathetic nerve activity during volume expansion is associated with more marked natriuretic response (9). Losartan blocks AT1 angiotensin receptors and increases the magnitude of renal sympathoinhibition that occurs during volume expansion. Therefore, we hypothesize that the effect of losartan of enhancing the renal sympathoinhibitory response to volume expansion in DCM patients could be associated with an enhanced ability to excrete acute sodium load. Furthermore, the beneficial effect of losartan may be a result of the opposition to antinatriuretic action of angiotensin II on the kidney (9).

In conclusion, the results of this study demonstrate that in DCM patients who are asymptomatic or have mildly symptomatic heart failure, AT1 angiotensin receptor antagonists may improve the abnormal cardio-renal and cardiac autonomic adaptation induced by an acute increase in blood volume, which may occur frequently during daily activities in these patients. Such an effect could greatly add to the beneficial activity of AT1 antagonists in heart failure and extend their use to the early stage of the disease.

REFERENCES