Effects of Cariporide and Losartan on Hypertrophy, Calcium Transients, Contractility, and Gene Expression in Congestive Heart Failure

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Background—The purpose of this study was to compare long-term effects of cariporide with those of losartan in postinfarction heart failure.

Methods and Results—Female Sprague-Dawley rats with large myocardial infarctions and sham controls were randomized to losartan, cariporide, or placebo after 7 days and treated for 49 days. Cardiac function was assessed by echocardiography and measurement of left ventricular pressures, and gene expression was assessed by competitive reverse transcription–polymerase chain reaction. Cell dimensions, shortening, and relaxation were determined by videomicroscopy and calcium transients by fura 2. Losartan reduced postinfarction systolic and diastolic left ventricular dilation (by 24% and 31%, respectively), left and right ventricular weight (by 22% and 26%, respectively), and cardiomyocyte hypertrophy length and width (by 62% and 54%, respectively). Induction of myocardial atrial natriuretic peptide decreased 66%. Cariporide did not affect postinfarction hypertrophy or atrial natriuretic peptide. Losartan and cariporide respectively improved reduced cellular contractility (55% and 30%) and reduced elevated systolic (86% and 27%) and diastolic (49% and 43%) calcium. Losartan and cariporide respectively reduced prolonged time to 50% relaxation (66% and 25%) and time to 50% calcium reduction (55% and 53%).

Conclusions—Losartan and cariporide improve cardiomyocyte contractility and calcium regulation in chronic heart failure. Losartan has salutary effects on postinfarction remodeling and gene expression, whereas cariporide is neutral.

Key Words: myocardial infarction ■ echocardiography ■ myocytes ■ calcium ■ genes

Na+/H+ exchange (NHE) inhibition prevents myocardial ischemic and reperfusion injury in experimental studies, and the NHE inhibitor cariporide protects the human heart during percutaneous interventions in acute myocardial infarction. Salutary effects of NHE inhibition in heart failure have been suggested on the basis of cellular signaling mechanisms and experimental studies. Of 5 known isoforms, only NHE-1 is normally expressed in mammalian cardiomyocytes. Yamazaki and colleagues demonstrated that the NHE-1 inhibitor Hoe694 reduced stretch-induced protein synthesis and activation of Raf-1 and mitogen-activated protein (MAP) kinase and suggested that NHE acts as a mechanosensitive ion exchanger in stress-induced cardiomyocyte hypertrophy. Intracellular alkalization and calcium elevation may be the subcellular mechanism of NHE-mediated MAP kinase activation. Cingolani and colleagues demonstrated NHE activation mediated by angiotensin II and endothelin-1 (ET-1) release in stretched papillary muscles. However, the role of NHE in vivo is uncertain. Selective NHE-1 inhibition by cariporide reduced right ventricular (RV) and cardiomyocyte hypertrophy and improved cardiomyocyte responsiveness to isoproterenol. Because NHE inhibition was initiated before or during coronary artery occlusion, the effects of cariporide on infarct size might be difficult to distinguish from its effects on hypertrophy and progression of heart failure. The first aim of the present study was to evaluate cariporide effects on late remodeling and heart failure by measuring cardiac hypertrophy, left ventricular (LV) dilation, gene expression, cellular contractility, and calcium handling. Cariporide treatment was compared with angiotensin II type 1 (AT1) antagonism by losartan, which has been proven efficient in the treatment of heart failure, with an effect equivalent to that of ACE inhibition. Losartan reduces LV dilation and hypertrophy and improves hemodynamics after myocardial infarction, probably by reducing mechanical load and humoral and paracrine stimuli. Impaired cardiomyocyte contraction and relaxation, calcium handling, and normal function have been demonstrated in congestive heart failure in humans and rats. Losartan inhibits hypertrophy in stretched cardiomyocytes and in spontaneously hypertensive rats, but the effects of angiotensin antagonism on myo-

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cytotoxic function in heart failure are largely unknown. The second aim of the present study was therefore to determine the effects of chronic losartan treatment on cardiomyocyte hypertrophy, contractility, and calcium handling in postinfarction heart failure.

Methods

Study Design

Left coronary artery ligation or sham operation was performed in female Sprague-Dawley rats (weight 240 ± 15 g; Mollegaards Breeding Center Ltd, Denmark) during isoflurane anesthesia 1% in 70% O2/30% N2O. Buprenorphine (0.05 mg) was given subcutaneously immediately and 10 hours after surgery. Echocardiography (2D, M-mode) was performed after 5 to 6 days to exclude animals with small infarcts. After 7 days, animals were randomized to cariporide in chow (0.3%), losartan in drinking water (2 g/L), or placebo chow and water. All animals were examined by echocardiography after 55 ± 1 days. LV pressures, cardiac weights, and myocardial gene expression were measured after 56 ± 1 days in all animals not randomized to myocyte isolation. Cardiomyocytes were isolated from 5 to 6 animals in each infarction group and in the sham placebo group after 59 ± 1 days for measurement of cellular dimensions, shortening, and calcium transients. The investigation conformed to the “Guide for the Care and Use of Laboratory Animals” (National Institutes of Health publication No. 85-23, revised 1996).

Echocardiography

Echocardiography was performed after intraperitoneal sedation with ketamine hydrochloride (40 mg/kg) and xylazine (8 mg/kg). LV dimensions were measured in M-mode long-axis recordings. Mitral inflow deceleration time, peak velocity of early and late component of mitral inflow, and isovolumetric relaxation time were calculated as the mean of 5 consecutive cycles of pulsed-wave Doppler spectra recordings.

LV Pressure, Infarct Size, and Tissue Sampling

LV pressures were measured during ventilation with 0.5% isoflurane in 70% O2/30% N2O. The heart was sectioned into atria and ventricles, including the interventricular septum in the LV. Infarcted and total LV areas were determined by photographic recordings. The LV was divided into infarcted, border-zone (0 to 2 mm outside the infarct), and remote areas (>2 mm outside the infarct).

mRNA Isolation and Competitive Reverse Transcription–Polymerase Chain Reaction

Myocardial mRNA was isolated with Dynabeads Oligo (d(T))20 (Dynal AS). Competitive reverse transcription–polymerase chain reaction (RT-PCR) was performed in a Perkin Elmer GeneAmp 2400 PCR system with rTth DNA polymerase (Perkin Elmer/Roche Molecular Systems Inc) as described previously. Specific sense and antisense primers were as follows: for NHE-1, 5'-TGGTTTGCGCGGCTGTGTC-3' (bp 1690 to 1706, accession M85299) and 5'-GGCAGTAGCCGAGAAGAC-3' (bp 2045 to 2028, accession M85299); for atrial natriuretic peptide (ANP), 5'-GGGGCTCTTCTCCATGACC-3' (bp 67 to 85, accession M27498) and 5'-CTCCAACTGCTGCACCTCACC-3' (bp 479 to 458, accession M27498); and for ET-1, 5'-TGCTCTACCAAAAAGACAAG-3' (bp 519 to 538, accession M64711) and 5'-CGATGTCTGATAGATACCTC-3' (bp 667 to 646, accession M64711). Expected amplified fragment lengths for NHE-1, ANP, and ET-1 were 356, 413, and 149 bp, respectively. Coefficients of variation for NHE, ANP, and ET-1 were 0.07, 0.18, and 0.11, respectively. ET-1 and ANP analyses demonstrated close correlation between added and measured amounts of mRNA (R² = 0.99), and similar results were found for NHE-1 (R² = 0.94).

Cardiomyocyte Isolation

Cardiomyocytes were isolated with collagenase. LV tissue was cut into infarcted, border-zone, and remote areas. Only data from remote-area cardiomyocytes are presented. The number of myocytes isolated from LVs of sham-operated animals was 3.4 ± 0.6 × 10⁶ cells, and yields of rod-shaped cells were 77 ± 2% after primary isolation and >95% of cells on laminin-coated coverslips.

Cellular Calcium and Shortening

Isolated myocytes attached to laminin-coated coverslips were loaded with fura 2, placed in a chamber on an inverted microscope (Diaphot-TMD, Nikon), and stimulated electrically by bipolar pulses (5-ms duration, 2 to 10 Hz). During stimulation, the cells were superfused at 2 mL/min with HEPES buffer at 37°C with 1.8 mmol/L Ca²⁺. Only cells that remained rod shaped, without blebs or other visible morphological alterations, and that responded adequately to stimulation at 2 Hz were included in the protocol. The amplitude of cell shortening and velocity of contraction and relaxation were analyzed with a video/edge monitor detector. Fura 2 fluorescence was measured with a photomultiplier tube at a sampling rate of 500 Hz. In vivo calcium calibration was performed on 30 myocytes from 6 sham-operated hearts and 30 myocytes from 4 infarcted hearts. Minimum and maximum fluorescence ratios (Rmin and Rmax) were determined as described previously. Rmin and Rmax were not significantly different among groups. Intracellular calcium concentration was calculated assuming a dissociation constant of 200 mmol/L. Contractility and calcium data were calculated from 10 consecutive contractions after stabilization at each stimulation frequency. Only data from cells that completed the entire protocol were included. Approximately 5% of the rod-shaped cells did not respond properly to stimulation at 2 Hz, 14% of cells were excluded because edge detection failed, and 3% of the remaining cells did not complete the protocol. Myocyte length and midpoint width were calculated in 166 ± 6 cells from each animal.

Statistical Analysis

Differences among groups were analyzed with Friedman test for related observations and Kruskal-Wallis test for unrelated observations, with appropriate procedures applied for multiple comparisons. Repeated variables were analyzed with a repeated-measures ANOVA and Bonferroni post hoc test. P < 0.05 was considered statistically significant. Results are presented as mean ± SEM.

Results

Hypertrophy

Myocardial infarction induced cardiomyocyte hypertrophy, with a 29% (P < 0.001) increase in cell length and 45% (P < 0.001) increase in cell width (Table 1). Losartan attenuated the increase by 62% and 54% (P < 0.001), whereas cariporide treatment had no effect. Total LV endocardial surface was 12% smaller with losartan than with placebo, demonstrating reduced postinfarction LV dilation (Table 2). Cariporide did not affect total LV area. Absolute infarct area was equivalent in all groups, which suggests that losartan and cariporide did not affect infarct remodeling when started after 7 days. However, infarct area as percent of total LV area tended to be higher in losartan-treated animals owing to less dilation of noninfarcted LV. Losartan reduced LV and RV hypertrophy (Table 2), whereas there were no effects of cariporide. Myocardial infarction increased diastolic and systolic LV diameters markedly (by 40% and 82%, respectively; P < 0.001; Table 3). Losartan reduced dilation by 31% and 24% (P < 0.01), respectively. Cariporide moderately attenuated the increase in diastolic LV diameter (15%; P < 0.05) but did not affect systolic LV diameter. LV dimensions were
similar in infarction groups before treatment (Table 4). Losartan markedly reduced systolic pressures in sham-operated animals and LV systolic and end-diastolic pressures after myocardial infarction, whereas cariporide reduced LV end-diastolic pressure after myocardial infarction (Table 3). The restrictive mitral inflow pattern after myocardial infarction (higher E-wave peak velocity, lower A-wave peak velocity, and shorter isovolumetric relaxation time) was significantly attenuated by cariporide but not by losartan (Table 3). Plasma levels of cariporide were 2124±320 ng/mL (n=6) after 1 week and 2795±946 after 6 weeks (n=6), which are 3 to 8 times the levels in previous studies demonstrating effective attenuation of hypertrophy and heart failure when cariporide was given before coronary ligation.3-4 A mean concentration of 100 ng/mL is sufficient to inhibit NHE.4 Because rats eat very regularly, circadian variation of cariporide plasma levels were probably low.

**Contractility**

Cardiomyocyte contractility measured as cellular fractional shortening was substantially reduced after myocardial infarction (Figure 1). Time to 50% relaxation increased by 35% (P<0.001) at 7 Hz, which suggests reduced diastolic function, and there was a trend toward increased time to 50% shortening (9%, P=0.11; Table 1). Losartan markedly increased cellular shortening amplitude at all stimulation frequencies (23%, P<0.001, 7 Hz; Figure 1) and reduced time to 50% relaxation (17%, P<0.001, 7 Hz; Table 1). Although cariporide had no effect on cardiomyocyte hypertrophy, contractility and relaxation were improved (13% and 6%, respectively; P<0.01; 7 Hz). As demon-

### TABLE 1. Myocyte Dimensions and Contractile Characteristics of Myocytes Stimulated at 7 Hz

<table>
<thead>
<tr>
<th></th>
<th>Sham Placebo</th>
<th>Myocardial Infarction Placebo</th>
<th>Losartan</th>
<th>Cariporide</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myocyte length, µm</td>
<td>119±0.7</td>
<td>153±0.9*</td>
<td>132±1.1†</td>
<td>152±0.8*</td>
</tr>
<tr>
<td>Myocyte width, µm</td>
<td>25±0.5</td>
<td>37±0.5*</td>
<td>31±1.0†</td>
<td>36±0.8*</td>
</tr>
<tr>
<td>Number of cells measured</td>
<td>1160</td>
<td>1011</td>
<td>786</td>
<td>919</td>
</tr>
<tr>
<td>Time to peak shortening, ms</td>
<td>41.6±0.7</td>
<td>42.2±0.6</td>
<td>43.1±0.9</td>
<td>41.4±0.2</td>
</tr>
<tr>
<td>Time to 50% shortening, ms</td>
<td>17.9±0.2</td>
<td>19.5±0.7</td>
<td>18.4±0.6</td>
<td>20.1±0.8</td>
</tr>
<tr>
<td>Time to 50% relaxation, ms</td>
<td>35.1±0.4</td>
<td>47.3±0.1*</td>
<td>39.3±0.4†</td>
<td>44.3±0.3†</td>
</tr>
<tr>
<td>Time to peak [Ca²⁺], ms</td>
<td>30.1±0.8</td>
<td>31.2±0.7</td>
<td>29.4±0.8</td>
<td>30.0±0.9</td>
</tr>
<tr>
<td>Time to 50% peak [Ca²⁺], ms</td>
<td>13.2±0.5</td>
<td>13.7±0.5</td>
<td>13.6±0.6</td>
<td>13.9±0.7</td>
</tr>
<tr>
<td>Number of cells stimulated</td>
<td>38</td>
<td>36</td>
<td>28</td>
<td>29</td>
</tr>
<tr>
<td>Number of animals</td>
<td>6</td>
<td>6</td>
<td>5</td>
<td>5</td>
</tr>
</tbody>
</table>

*P<0.001 vs sham placebo, †P<0.01 vs myocardial infarction placebo, ‡P<0.001 vs myocardial infarction cariporide. Values are mean±SEM.

### TABLE 2. Myocyte Dimensions, Weight and Area of Ventricles, and Body Weight

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>Losartan</th>
<th>Cariporide</th>
<th>Placebo</th>
<th>Losartan</th>
<th>Cariporide</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infarct area, cm²</td>
<td>1.7±0.1</td>
<td>1.7±0.2</td>
<td>1.7±0.1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infarct area of LV, %</td>
<td>42±3</td>
<td>49±2</td>
<td>43±3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Noninfarcted LV area, cm²</td>
<td>2.4±0.1</td>
<td>1.9±0.1†</td>
<td>2.3±0.1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total LV area, cm²</td>
<td>4.1±0.2</td>
<td>3.6±0.1‡</td>
<td>4.0±0.1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RV, mg</td>
<td>397±38*</td>
<td>243±13†</td>
<td>339±31*</td>
<td>211±4</td>
<td>188±5§</td>
<td>207±4</td>
</tr>
<tr>
<td>Noninfarcted LV, mg</td>
<td>603±17*</td>
<td>400±14†</td>
<td>547±28*</td>
<td>676±50</td>
<td>529±13*</td>
<td>655±12</td>
</tr>
<tr>
<td>Infarct, mg</td>
<td>99±11</td>
<td>104±5</td>
<td>105±5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RV/BW, mg/g</td>
<td>1.29±0.14*</td>
<td>0.95±0.06*</td>
<td>1.16±0.13*</td>
<td>0.72±0.01</td>
<td>0.67±0.01§</td>
<td>0.72±0.01</td>
</tr>
<tr>
<td>Noninfarcted LV/BW, mg/g</td>
<td>1.98±0.08*</td>
<td>1.55±0.04†</td>
<td>1.87±0.08*</td>
<td>2.32±0.01</td>
<td>1.88±0.04*</td>
<td>2.28±0.03</td>
</tr>
<tr>
<td>Number of animals measured</td>
<td>9</td>
<td>11</td>
<td>9</td>
<td></td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>BW preoperative, g</td>
<td>255±2</td>
<td>252±3</td>
<td>253±2</td>
<td>258±3</td>
<td>255±4</td>
<td>254±3</td>
</tr>
<tr>
<td>BW at end of study, g</td>
<td>301±4</td>
<td>264±7‡</td>
<td>290±2‡</td>
<td>298±4</td>
<td>283±6§</td>
<td>288±4</td>
</tr>
<tr>
<td>Deaths/total number of animals</td>
<td>1/16</td>
<td>1/17</td>
<td>4/16</td>
<td>0/15</td>
<td>0/15</td>
<td>0/15</td>
</tr>
</tbody>
</table>

LV indicates left ventricle; RV, right ventricle; and BW, body weight.

*P<0.01 and †P<0.05 vs sham-operated controls placebo; ‡P<0.01 and §P<0.05 vs myocardial infarction placebo.

Two animals died during anesthesia for pressure measurements (weights are included). Values are mean±SEM.
TABLE 3. LV Echocardiographic and Pressure Measurements

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>Losartan</th>
<th>Cariporide</th>
<th>Placebo</th>
<th>Losartan</th>
<th>Cariporide</th>
</tr>
</thead>
<tbody>
<tr>
<td>LV diastolic diameter, mm</td>
<td>11.5±0.2*</td>
<td>10.5±0.1†</td>
<td>11.0±0.1‡</td>
<td>8.2±0.1</td>
<td>8.1±0.1</td>
<td>7.9±0.1</td>
</tr>
<tr>
<td>LV systolic diameter, mm</td>
<td>10.3±0.2*</td>
<td>9.2±0.2‡</td>
<td>9.9±0.2*</td>
<td>5.7±0.1</td>
<td>5.7±0.1</td>
<td>5.3±0.1</td>
</tr>
<tr>
<td>Fractional shortening, %</td>
<td>10±1*</td>
<td>12±1*</td>
<td>10±1*</td>
<td>31±1</td>
<td>30±1</td>
<td>33±1</td>
</tr>
<tr>
<td>LV posterior wall diastole, mm</td>
<td>2.2±0.1</td>
<td>1.9±0.1</td>
<td>2.3±0.1*</td>
<td>1.9±0.1</td>
<td>1.7±0.1</td>
<td>1.9±0.1</td>
</tr>
<tr>
<td>LV posterior wall systole, mm</td>
<td>2.9±0.1</td>
<td>2.6±0.1</td>
<td>2.8±0.1</td>
<td>2.6±0.1</td>
<td>2.4±0.1</td>
<td>2.8±0.1</td>
</tr>
<tr>
<td>E-wave peak velocity, cm/s</td>
<td>94±4*</td>
<td>88±5*</td>
<td>83±5‡</td>
<td>72±2</td>
<td>72±3</td>
<td>71±2</td>
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<tr>
<td>A-wave peak velocity, cm/s</td>
<td>14±2*</td>
<td>19±2*</td>
<td>23±4‡</td>
<td>25±2</td>
<td>21±1</td>
<td>37±2*</td>
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<tr>
<td>E-wave deceleration time, ms</td>
<td>27±2*</td>
<td>28±2*</td>
<td>33±2‡</td>
<td>40±2</td>
<td>44±3</td>
<td>38±2</td>
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<td>Isovolumetric relaxation time, ms</td>
<td>27±2*</td>
<td>26±2*</td>
<td>33±2</td>
<td>35±1</td>
<td>31±1</td>
<td>37±1</td>
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<tr>
<td>Number of animals echo</td>
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<td>48±4</td>
</tr>
<tr>
<td>LV end diastolic pressure, mm Hg</td>
<td>80±5</td>
<td>52±3‡</td>
<td>85±4</td>
<td>80±5</td>
<td>67±4*</td>
<td>94±3*</td>
</tr>
<tr>
<td>LV systolic pressure, mm Hg</td>
<td>10±6</td>
<td>75±4‡</td>
<td>108±5</td>
<td>113±6</td>
<td>94±4</td>
<td>125±3*</td>
</tr>
<tr>
<td>Diastolic aortic pressure, mm Hg</td>
<td>12±3</td>
<td>10±2‡</td>
<td>13±3‡</td>
<td>13±4</td>
<td>10±2‡</td>
<td>13±3‡</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>380±23</td>
<td>352±8*</td>
<td>365±12</td>
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<td>Number of animals pressure</td>
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<td>39±4</td>
<td>39±4</td>
<td>39±4</td>
<td>39±4</td>
</tr>
</tbody>
</table>

LV indicates left ventricular; E, early LV filling; and A, LV filling after atrial contraction. Values are mean±SEM.

*P<0.01 vs sham-operated controls placebo; †P<0.001 and ‡P<0.05 vs myocardial infarction placebo.

Calcium Handling

Diastolic and systolic cytosolic calcium was increased in cardiomyocytes from infarcted LVs at all frequencies (62% and 30%, respectively; P<0.001; 7 Hz, Figures 2A and 2B). In all cell groups, calcium concentrations increased at higher frequencies. Losartan markedly reduced systolic and diastolic calcium (19% and 18%, respectively; P<0.01; 7 Hz), whereas cariporide caused a moderate reduction (7% and 16%, respectively; P<0.03; 7 Hz). Cytosolic calcium amplitude was similar in cardiomyocytes from all groups at low frequencies (2 to 5 Hz) but increased at high frequencies (7 to 10 Hz) after myocardial infarction (Figure 2C). This increase disappeared after losartan but remained unchanged by cariporide. Time to 50% decay in cytosolic calcium was prolonged (31%; 7 Hz, P<0.001; Table 1), which indicates impaired diastolic calcium handling. Losartan and cariporide reduced the increase in time to 50% decay in cytosolic calcium (55% and 53%, respectively; 7 Hz, P<0.001). Time to 50% of peak calcium remained unchanged after myocardial infarction and drugs. A calcium sensitivity index (cellular shortening/calcium transient amplitude) indicated a markedly reduced responsiveness to calcium during heart failure (Figure 2D), a substantial increase after losartan, and a moderate increase after cariporide.

Border-Zone Cardiomyocytes

In the border zone, there was a trend toward longer myocytes, reduced cell shortening, higher calcium concentrations, longer time to 50% relaxation time, and more impaired calcium handling compared with remote area cells. However,
the mean differences were small (2% to 10%) and did not reach statistical significance (data not shown).

Gene Expression

Regional myocardial expression of ANP, ET-1, and NHE-1 was measured by competitive RT-PCR (Figure 3). ANP expression, an established indicator of hypertrophy and heart failure, was markedly increased in remote area and border zone (30- and 34-fold, respectively; *P*, 0.001) after myocardial infarction. Losartan reduced ANP expression in remote area and border zone (46% and 38%, respectively; †*P*, 0.05), consistent with the changes in cardiac weights, LV areas, cellular dimensions, and echocardiographic findings. Cariporide tended to increase ANP expression in remote area after myocardial infarction (23%, *P*, 0.09), whereas there was no effect on expression in remote area (P=0.76) or in sham-operated animals (P=0.76). Expression of ET-1, which correlates with impaired cardiac function and high mortality after myocardial infarction, increased 3-fold in the remote area (P<0.001) and 4-fold in the border zone (P<0.001). There was no effect of losartan or cariporide on ET-1 expression in remote area (P=0.24), border zone (P=0.91), or LV of sham-operated animals (P=0.39). NHE-1 mRNA increased moderately in remote area and border zone (55%, P<0.05) after myocardial infarction. After losartan, there was a trend toward reduced expression in remote area and border zone (28% for remote area, *P*=0.21; 33% for border zone, *P*=0.08) but no effect after cariporide.

Discussion

This study is the first to demonstrate improved cardiomyocyte contractile function and calcium handling by losartan and cariporide in postinfarction failure. Improved cardiomyocyte diastolic function was associated with enhanced cytosolic calcium clearance, which might be due to increased expression and function of SR-Ca\(^{2+}\)-ATPase during heart failure. Angiotensin antagonism improves SR-Ca\(^{2+}\)-ATPase expression and function in hypertensive cardiomyopathy.\(^{20}\)

Whereas cariporide and losartan changed diastolic calcium decay similarly, myocyte relaxation was substantially more improved by losartan, which suggests that other mechanisms in addition to calcium handling might be involved. Recent studies indicate that systolic function is reduced by impaired calcium sensitivity in heart failure.\(^{21–23}\) The present study suggests that losartan and, to a lesser degree, cariporide improve cardiomyocyte systolic function by increasing calcium sensitivity.

Whereas increased diastolic calcium concentrations in heart failure cardiomyocytes were in line with results of previous studies,\(^{21,23,24}\) the increased peak systolic calcium...
after myocardial infarction is in agreement with some studies but in contrast to others. Calcium concentrations increased markedly at 7 and 10 Hz, especially in failing myocytes, probably because of reduced SR uptake at high frequencies. Interestingly, the in vivo rat heart rate is 6 to 11 Hz. The positive force-frequency relationship contrasts some previous reports. The discrepancy probably results from higher stimulation frequency in the present study; Borzak et al found a negative force-frequency relationship up to 1 Hz and a positive relationship from 2 Hz to 6 Hz. In the present study, the difference in myocyte shortening between normal and failing cardiomyocytes increased with higher frequencies, but even in heart failure, there was no negative force-frequency relationship.

To minimize confounding of body growth on myocardial hypertrophy, we used female animals. Sex differences in cardiomyocyte size, contractility, and calcium handling might account for some of the differences between the present study and other studies. After infarction, cardiomyocyte width increased more than reported previously. This was probably not due to sex differences, because myocyte width increases less in females than in males. Differences were probably due to infarct size and time after infarction; myocyte width changes little after small infarcts, and the increase in width occurs later than the increase in length. Reduced RV weight and LV dilation after losartan concurred with previous findings whereas reduced cardiomyocyte hypertrophy has been demonstrated in spontaneously hypertensive rats but not in heart failure. There were trends of reduced RV and LV weight/body weight after cariporide (10% [P = 0.32] and 6% [P = 0.16], respectively). However, cardiomyocyte dimensions were unchanged and were the most sensitive indicators of hypertrophy (estimated probability of 80% to detect a 2.5% difference in cell length, P < 0.05). The missed effect of cariporide is in contrast to previous studies demonstrating reduced myocyte and RV hypertrophy and attenuation of postinfarction LV dilation and hypertrophy. In those studies, treatment was begun before or immediately after coronary occlusion; thus, NHE inhibition might have affected infarct size or survival of border-zone cardiomyocytes. Reduced infarct size and cardiac hypertrophy occurred when cariporide was given within 24 hours of coronary ligation but not when it was started after 7 days.

Whereas losartan had an effect on myocyte contractility and hypertrophy (Figure 4), cariporide only changed contractility, which suggests that postinfarction hypertrophy and impaired cellular function are not similarly regulated and that losartan and cariporide effects might be mechanistically different. Most likely, attenuated hypertrophy by losartan resulted from reduced systolic mechanical loading (indicated by markedly lower systolic pressure and LV diameter) and from inhibition of local trophic effects mediated by AT1 receptors. Diastolic loading appeared to be less important, because cariporide had no effect on hypertrophy despite a significant reduction in diastolic pressure and LV diameter.

Figure 3. ANP, NHE-1, and ET-1 mRNA measured by competitive RT-PCR in LV myocardium adjacent to (border, 0 to 2 mm) and remote from (remote, >2 mm) large myocardial infarcts in rats and from sham-operated controls (sham) treated for 49 days with losartan, cariporide, or placebo. "P < 0.05 vs sham, †P < 0.05 vs placebo, ‡P < 0.05 vs cariporide. For ANP and ET-1, all border and remote values were greater than sham values (P < 0.001). For ET-1, all border values were greater than remote values (P < 0.01).

Figure 4. Resting length and relative shortening during 7-Hz stimulation of cardiomyocytes isolated from border zone (open symbols) or remote area (closed symbols) from LV with large myocardial infarction or sham. Abbreviations as in Figure 1. Note associated effect on hypertrophy and contractile function for losartan, whereas cariporide only affects contractility.
Reduced myocardial ANP expression by losartan in infarcted hearts is in agreement with reduced hypertrophy and previous findings. The trend toward increased ANP after cariporide indicates failure to affect hypertrophy. Even though angiotensin II induces ET-1 expression in isolated cardiomyocytes, losartan did not affect infarct-induced myocardial ET-1 expression. Because mRNA was isolated from myocardial tissue, the expression in myocytes versus nonmyocytes could not be identified. However, ANP is not expressed in cardiac nonmyocytes, and ET-1 expression in myocytes followed a similar pattern as in myocardium in a previous study. The reduced NHE expression after losartan might have contributed to reduced hypertrophy due to less stretch-induced alkalization. However, if NHE-1 activation were an important myocardial growth signal in vivo, reduced hypertrophy by cariporide would be expected.

In conclusion, our results suggest that the cardiac sodium-hydrogen exchange contributes to the deterioration of cardiomyocyte contractility and calcium homeostasis and exerts neutral effects on hypertrophy in postinfarction heart failure. In contrast, angiotensin II appears to affect both myocyte function and growth either by direct or indirect effects.

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References
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