Amlodipine Monotherapy, Angiotensin-Converting Enzyme Inhibition, and Combination Therapy With Pacing-Induced Heart Failure

Scott B. Kribbs, William M. Merritt, Mark J. Clair, R. Stephen Krombach, Ward V. Houck, Michael G. Dodd, Rupak Mukherjee, Francis G. Spinale

Abstract—In patients with congestive heart failure (CHF) receiving therapy with angiotensin-converting enzyme (ACE) inhibition, institution of calcium channel antagonism with amlodipine provided favorable effects. The goal of the present study was to define potential mechanisms for these effects by measuring left ventricular function, hemodynamics, and neurohormonal system activity in a model of CHF in which amlodipine treatment had been instituted either as a monotherapy or in combination with ACE inhibition. Thirty-two pigs were instrumented to allow measurement of cardiac index, total systemic resistance index, and neurohormonal activity in the conscious state and assigned to one of four groups: (1) rapid atrial pacing (240 bpm) for 3 weeks (n=8), (2) amlodipine (1.5 mg·kg⁻¹·d⁻¹) and pacing (n=8), (3) ACE inhibition (fosinopril 1.0 mg/kg BID) and pacing (n=8), and (4) amlodipine and ACE inhibition (1.0 mg·kg⁻¹·d⁻¹ and 1.0 mg/kg BID, respectively) and pacing (n=8). Measurements were obtained in the normal control state and after the completion of the treatment protocols. With rapid pacing, basal resting cardiac index was reduced compared with control values (2.7±0.2 versus 4.7±0.1 L·min⁻¹·m⁻², respectively, P<.05) and increased from rapid pacing–only values with either amlodipine or combination therapy (3.7±0.3 and 4.4±0.5 L·min⁻¹·m⁻², respectively, P<.05). Basal resting total systemic resistance index was higher in the rapid pacing–only group compared with control values (273±263 versus 1721±53 dyne·s·cm⁻⁵·m², respectively, P<.05), was reduced with either amlodipine treatment or ACE inhibition (2125±226 and 2379±222 dyne·s·cm⁻⁵·m², respectively, P<.05), and was normalized with combination therapy. Plasma catecholamines, renin activity, and endothelin levels were increased threefold with rapid pacing. Amlodipine, either as a monotherapy or in combination with ACE inhibition, did not result in increased plasma catecholamines and renin activity compared with the rapid pacing–only group. Furthermore, combination therapy reduced steady state norepinephrine and normalized epinephrine levels. The results of the present study demonstrated that monotherapy with either amlodipine or ACE inhibition provides beneficial effects in this pacing model of CHF. Combined amlodipine and ACE inhibition provided greater benefit with respect to vascular resistance properties and neurohormonal system activity compared with either monotherapy. (Hypertension. 1998;31:755-765.)

Key Words: angiotensin-converting enzyme inhibition ■ amlodipine ■ heart failure ■ exercise ■ ventricular function, left

The development and progression of CHF is associated with significant morbidity and mortality. Current strategies in the treatment of CHF include LV afterload reduction by vasodilation and/or neurohormonal modulation. For example, ACE inhibition has been clearly demonstrated to have beneficial effects on LV function and survival in patients with developing CHF. However, clinical trials with short-acting calcium channel antagonists, which also effectively reduce systemic vascular resistance and thereby LV afterload, have reported deleterious effects in patients with CHF. Specifically, treatment with the calcium channel antagonist nifedipine in patients with CHF was associated with a worsening of symptoms and heightened neurohormonal system activation. However, longer-acting compounds of the dihydropyridine subclass of calcium channel antagonists, such as amlodipine, have been shown to significantly reduce vascular resistance properties without apparent negative effects on LV myocardial contractility and neurohormonal activity. In a recent clinical trial, chronic amlodipine therapy was associated with no adverse effects on morbidity and mortality in patients with severe CHF and appeared to provide favorable effects in patients with nonischemic etiologies. In this past report, however, patients were concomitantly treated with ACE inhibition therapy, and therefore the potential mechanism for the effects of amlodipine therapy in CHF remains poorly understood. Past studies have demonstrated the synergistic
Amlodipine and ACE Inhibition in Heart Failure

Selected Abbreviations and Acronyms

ACE = angiotensin-converting enzyme
Ang I = angiotensin I
Ang II = angiotensin II
CHF = congestive heart failure
CI = cardiac index
HPLC = high-performance liquid chromatography
LV = left ventricular, ventricle
TSR = total systemic resistance index

Effects of combined amlodipine and ACE inhibition therapy in the treatment of hypertension.21–24 However, whether and to what extent combined amlodipine treatment and ACE inhibition provides interactive effects with the development of CHF remain unexplored. Accordingly, the overall goal of the present study was to determine the effects of amlodipine treatment, ACE inhibition, or combination therapy on LV function, hemodynamics, and regional blood flow patterns in the normal state and after the development of CHF.

Methods

Rationale

Past reports from this laboratory and others have demonstrated that chronic pacing tachycardia in animals causes progressive changes in LV geometry and pump function and neurohormonal system activation consistent with the clinical spectrum of CHF.25–32 Specifically, the development of pacing-induced CHF is associated with severe LV pump failure, heightened catecholamine levels, and increased vascular resistance in several circulatory beds.25–32 In addition, the pacing model of CHF has been successfully used to examine specific systemic and neurohormonal changes that occur during exercise with the development of CHF.27,29 Accordingly, in the present study, we used a pacing-induced model of chronic pacing tachycardia in animals causes progressive changes in LV pump function, hemodynamics, and neurohormonal system activation which is consistent with the clinical spectrum of CHF.25–32 Specifically, the development of pacing-induced CHF is associated with severe LV pump failure, heightened catecholamine levels, and increased vascular resistance in several circulatory beds.25–32 In addition, the pacing model of CHF has been successfully used to examine specific systemic and neurohormonal changes that occur during exercise with the development of CHF.27,29 Accordingly, in the present study, we used a pacing-induced model of CHF to examine the potentially differential effects of amlodipine treatment, ACE inhibition, and combination treatment on LV pump function, systemic hemodynamics, and regional blood flow patterns both at rest and with treadmill-induced exercise.

The first objective of this study was to select an appropriate dosing strategy for amlodipine treatment, ACE inhibition, and combined amlodipine therapy and ACE inhibition. The amlodipine dosage in both the monotherapy and combination therapy groups was selected to produce plasma drug levels that have been previously reported to have pharmacological activity against the vascular smooth muscle L-type calcium channel.25,33,34 For the ACE inhibition dose-determination studies, the criterion for dose selection was to obtain a relative differential effects on resting blood pressure. After the identification of suitable dosing regimens, the second objective of the present study was to institute concomitant amlodipine treatment, ACE inhibition, or combination therapy with chronic rapid pacing. After 21 days of concomitant treatment and pacing tachycardia, terminal studies were performed in which LV function and geometry, neurohormonal profiles, and regional blood flow patterns were examined. For comparison purposes, age- and weight-matched pigs were used that underwent chronic pacing without treatment.

Dose-Selection Studies

Nine Yorkshire pigs (20–kg males; Hambone Farms, Orangeburg, SC) were chronically instrumented to measure arterial blood pressure in the conscious state. The pigs were anesthetized with isoflurane (3%/1.5 L per minute) and nitrous oxide/oxygen (50:50), intubated with a cuffed endotracheal tube, and ventilated at a flow rate of 22 mL/kg·min and a respiratory rate of 15 min⁻¹. A left thoracotomy was performed, and the thoracic aorta at the location of the hemiazygous crossover was exposed. A catheter connected to a vascular access port (model GPV, 9F; Access Technologies) was placed in the aorta and sutured in place. The access port was buried in a subcutaneous pocket over the thoracolumbar fascia. After a recovery period of 7 to 10 days, the animal was returned to the laboratory for an initial Ang I and Ang II pressor response study. For these studies, the animals were sedated with diazepam (20 mg PO [Valium]; Hoffmann-LaRoche) and placed in a custom-designed sling that allowed the animal to rest comfortably. All studies were performed with the animals in the conscious state without the additional use of sedation. The vascular access port was entered with the use of a 12-gauge Huber needle (Access Technologies), and basal, resting arterial pressure, and heart rate were recorded. Pressures from the fluid-filled aortic catheter were obtained using an externally calibrated transducer (Statham P23ID; Gould). The ECG and pressure wave forms were recorded with a multichannel recorder (Hewlett Packard), as well as digitized on computer for subsequent analysis at a sampling frequency of 100 Hz/channel (80486 processor; Zenith Data Systems). After these baseline measurements, an infusion of Ang I (10 μg·kg⁻¹·min⁻¹) was started. The Ang I pressor response for 48 hours and then entered into the dose-determination protocols. Pigs were randomly assigned to receive either the calcium channel antagonist amlodipine (1.5 mg·kg⁻¹·d⁻¹), the ACE inhibitor fosinopril (1.0 mg/kg BID), or combination therapy (1.5 mg·kg⁻¹·d⁻¹ amlodipine and 1.0 mg/kg fosinopril BID) for 3 days, after which the Ang I and Ang II pressor response studies were repeated. A 3-day drug treatment interval was selected to achieve steady-state plasma drug levels. With amlodipine, basal resting blood pressure was reduced compared with control animals (85 ± 4 versus 103 ± 3 mm Hg, respectively, P < .05). The Ang I (P = .07) and Ang II pressor response was reduced with amlodipine (Fig 1). The decreased pressor response with amlodipine likely reflected the decrease in vascular smooth muscle tone and responsiveness.33 This dose of amlodipine resulted in plasma drug levels of ~18 to 30 ng/mL. With ACE inhibition, minimal effects were observed on resting blood pressure (95 ± 3 versus 103 ± 3 mm Hg, P = .16) and the Ang II pressor response (Fig 1). However, the response after Ang I pressor challenge was significantly reduced (Fig 1). In preliminary dual-therapy dose-determination studies, combined treatment using both monotherapy doses resulted in significant hypotension and reflex tachycardia. Accordingly, the dose

Figure 1. Percent change in mean arterial pressure after a separate infusion (10 μg) of Ang I or Ang II in conscious pigs. Amlodipine (1.5 mg·kg⁻¹·d⁻¹) resulted in a decreased Ang I (P = .07) and Ang II (P < .05) pressor response compared with control values. ACE inhibition with fosinopril (1.0 mg/kg BID) caused a significant blunting of the Ang I pressor response (P < .05), with no effect on the Ang II pressor response. Combined amlodipine and ACE inhibition (1.0 mg·kg⁻¹·d⁻¹ and 1.0 mg/kg BID, respectively) reduced the Ang I pressor response (P < .05), whereas the Ang II pressor response remained unchanged. These monotherapy and combination therapy dosing regimens were then administered concomitantly with chronic rapid pacing. P < .05 vs corresponding control values.
of amiodipine was reduced to 1.0 mg · kg\(^{-1}\) · d\(^{-1}\), and this new combined dose (1.0 mg · kg\(^{-1}\) · d\(^{-1}\) amiodipine and 1.0 mg/kg fosinopril BID) was used in further studies. With combined treatment, basal resting blood pressure was reduced from control values (74±2 versus 103±3 mm Hg, respectively, \(P<.05\)). Although the Ang II pressor response remained unchanged, the response after Ang I pressor challenge was significantly reduced (Fig 1). After the dose-selection studies, the effects of these monotherapy and combination therapy protocols with chronic rapid pacing were examined.

Instrumentation and Experimental Design

Thirty-two pigs were chronically instrumented to allow measurement of systemic hemodynamics and neurohormonal profiles in the conscious state. The pigs were anesthetized and intubated as described. After a left thoracotomy was performed, a catheter connected to a vascular access port was placed in the aorta as previously described. Additional catheters connected to vascular access ports were placed in the pulmonary artery and left atrium in a similar fashion. The access ports were buried in a subcutaneous pocket over the thoracolumbar fascia. A 20-mm-flow probe (Transonic) was placed around the pulmonary artery immediately distal to the pulmonary artery catheter, and the electrical connection was exteriorized through the thoracolumbar fascia. A shielded stimulating electrode was sutured onto the left atrium, connected to a modified programmable pacemaker (8329; Medtronic), and buried in a subcutaneous pocket. The thoracotomy was closed in layers, and the pleural space was evacuated of air. After a 14- to 21-day recovery from the surgical procedure, the animals were returned to the laboratory for baseline studies.

After measurements under normal resting conditions and with exercise, the pacemakers were activated to 240 bpm for a period of 21 days. It has been demonstrated in this laboratory that this rate and duration of chronic rapid atrial pacing reliably cause LV dilation and pump dysfunction.\(^{25,26,28,32}\) In the present study, the pigs were randomly assigned to one of four groups: (1) rapid atrial pacing (240 bpm) for 3 weeks (\(n=8\)), (2) concomitant amiodipine and rapid pacing (\(n=8\)), (3) concomitant ACE inhibition and rapid pacing (\(n=8\)), and (4) concomitant ACE inhibition and amiodipine and rapid pacing (\(n=8\)). The drug treatment protocols were begun at the initiation of pacing and continued for the entire 21-day pacing protocol. Cardiac auscultation and electrocardiograms were performed frequently during the pacing protocol to ensure proper operation of the pacemaker and the presence of 1:1 conduction. At the completion of the pacing protocol, the animals were returned to the laboratory, and the pacemaker was deactivated. After a 1-hour stabilization period, resting and exercise data were collected again. After the final set of measurements, the animals were killed with an overdose of pentobarbital (1000 mg), and tissue was harvested. All animals were treated and cared for in accordance with the National Institutes of Health “Guide for the Care and Use of Laboratory Animals” (National Research Council, Washington, 1996).

Measurements at Rest and With Exercise

On the day of study, the animals were sedated with diazepam (20 mg PO) and placed in a custom-designed sling that allowed the animals to rest comfortably. All experiments were performed with the animals in the conscious state without additional use of sedation. An ECG was established, and the pacemaker was deactivated (pacing groups only). After a 60-minute stabilization period, two-dimensional and M-mode echocardiographic studies (ATL Ultramark VI 2.25-MHz transducer) were used to image the LV from the right parasternal approach.\(^{25,26,28,32}\) Echocardiographic studies (ATL Ultramark VI 2.25-MHz transducer) were used to image the LV from the right parasternal approach.\(^{25,26,28,32}\) After collection of the hemodynamic data and blood samples, fluorescein microspheres (3×10\(^{10}\); Molecular Probes) of specific emission spectra were injected into the left atrium. A reference aortic sample was withdrawn at a rate of 7 mL/min, which was initiated 5 seconds before injection and continued for 120 seconds after injection. The pigs were then placed in custom-designed vests that protected all connections. The animals were positioned in a modified treadmill containing balanced and
TABLE 1. Systemic Hemodynamics and LV Function With Pacing-Induced Heart Failure: Effects of Amlodipine, ACE Inhibition, or Combined Amlodipine and ACE Inhibition During the Progression of Heart Failure

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Rest</th>
<th>Exercise</th>
<th>Rest</th>
<th>Exercise</th>
<th>Rest</th>
<th>Exercise</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate, bpm</td>
<td>118±3</td>
<td>263±5</td>
<td></td>
<td>153±7*</td>
<td>214±9</td>
<td></td>
<td>139±6*</td>
</tr>
<tr>
<td>Respiratory rate, min⁻¹</td>
<td>33±2</td>
<td>64±2</td>
<td></td>
<td>53±4*</td>
<td>87±6</td>
<td></td>
<td>51±6*</td>
</tr>
<tr>
<td>Pump function</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stroke index, mL/m²</td>
<td>41.3±1.7</td>
<td>39.5±1.4</td>
<td></td>
<td>18.0±1.5*</td>
<td>31.6±1.9</td>
<td></td>
<td>28.1±2.3*</td>
</tr>
<tr>
<td>Cardiac index, L·min⁻¹·m⁻²</td>
<td>4.7±0.1</td>
<td>10.4±0.4</td>
<td></td>
<td>2.7±0.2*</td>
<td>6.9±0.4</td>
<td></td>
<td>3.7±0.3*</td>
</tr>
<tr>
<td>Pressure, mm Hg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aorta</td>
<td>99±2</td>
<td>108±2</td>
<td></td>
<td>90±2*</td>
<td>87±2</td>
<td></td>
<td>94±4</td>
</tr>
<tr>
<td>Pulmonary artery</td>
<td>18±1</td>
<td>27±1</td>
<td></td>
<td>35±4*</td>
<td>38±3*</td>
<td>33±1*</td>
<td>36±2*</td>
</tr>
<tr>
<td>Left atrium</td>
<td>11±1</td>
<td>10±1</td>
<td></td>
<td>28±3*</td>
<td>26±2*</td>
<td>22±2†</td>
<td>18±3†</td>
</tr>
<tr>
<td>Resistance index, dyne·s·cm⁻⁵·m²</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pulmonary</td>
<td>126±13</td>
<td>134±15</td>
<td></td>
<td>235±73*</td>
<td>147±32</td>
<td></td>
<td>251±43*</td>
</tr>
<tr>
<td>Total systemic</td>
<td>1721±55</td>
<td>883±39</td>
<td></td>
<td>2731±263*</td>
<td>1031±84</td>
<td></td>
<td>2125±226†</td>
</tr>
<tr>
<td>Neurohormone</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Norepinephrine, pg/mL</td>
<td>136±11</td>
<td>881±98</td>
<td></td>
<td>698±171*</td>
<td>3377±1406</td>
<td></td>
<td>427±89*</td>
</tr>
<tr>
<td>Epinephrine, pg/mL</td>
<td>82±19</td>
<td>226±27</td>
<td></td>
<td>252±47*</td>
<td>619±242</td>
<td></td>
<td>259±41*</td>
</tr>
<tr>
<td>Renin activity, ng·mL⁻¹·h⁻¹</td>
<td>4.3±0.4</td>
<td>9.2±1.1</td>
<td></td>
<td>32.4±10.1*</td>
<td>30.2±7.6*</td>
<td>13.5±3.5†</td>
<td>22.6±4.9*</td>
</tr>
<tr>
<td>Endothelin, fmol/mL</td>
<td>3.1±0.2</td>
<td>3.7±0.2</td>
<td></td>
<td>10.2±1.0*</td>
<td>11.4±1.9*</td>
<td>6.8±1.0†</td>
<td>6.6±1.1†</td>
</tr>
<tr>
<td>Sample size, n</td>
<td>32</td>
<td>32</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td></td>
</tr>
</tbody>
</table>

Values are mean±SEM.
*P<0.05 vs control.
†P<0.05 vs rapid pacing only.
‡P<0.05 vs rapid pacing and amlodipine.
§P<0.05 vs rapid pacing and ACE inhibition.
||P<0.05 vs resting state.
¶Rapid pacing: 21 days of chronic rapid pacing at 240 bpm.
**Rapid pacing and amlodipine: 1.5 mg/kg amlodipine SID.
††Rapid pacing and ACE inhibition: 1 mg/kg fosinopril PID.
‡‡Rapid pacing and amlodipine/ACE inhibition: 1 mg/kg amlodipine SID and 1 mg/kg fosinopril PID.

Regional Blood Flow Measurements

All of the tissues samples were fixed in 10% formaldehyde to facilitate sectioning. The midregion of the LV free wall was separated into endocardial and epicardial layers weighing ~3 g each. Samples of ~3 to 5 g were also collected and prepared from the basal regions of the lung, kidney, lattissimus dorsi, and gluteus maximus. The tissue samples were carefully weighed and then digested with the use of a potassium hydroxide solution as described previously.30,31 The aortic reference samples were extracted using an identical digest solution. The fluorescence of the extracted samples were then determined with spectrophotometry (Giford Fluoro IV). The fluorescent microspheres used in this study with respect to excitation/emission characteristics were blue-green, 428/457 nm; orange, 534/550 nm; red, 580/594 nm; and scarlet, 650/674 nm. These fluorescent microspheres were chosen because a spectral scan that conformed to a gaussian distribution could be obtained uniformly, minimal spectral crosstalk occurred, and an equivalent sensitivity was provided.39 Regional blood flow computations were determined using the standard formula Qm=(A m·A s)/Q r, where Q m is the blood flow (mL/min), A m is the fluorescence of the aortic reference samples, A s is the fluorescence of the tissue sample, and Q r is the withdrawal rate of the reference sample. Final blood flow values were normalized to sample weight and expressed as mL·min⁻¹·g⁻¹. Coronary vascular resistance was determined as the mean aortic pressure divided by LV myocardial blood flow and expressed as dyne·s·cm⁻⁵·g⁻¹.
Table 1. Continued

<table>
<thead>
<tr>
<th>Rapid Pacing and ACE Inhibition†‡</th>
<th>Rapid Pacing and Amlodipine/ACE Inhibition†‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rest</td>
<td>Exercise</td>
</tr>
<tr>
<td>146±10*</td>
<td>227±6</td>
</tr>
<tr>
<td>39±3†‡</td>
<td>64±4</td>
</tr>
<tr>
<td>20.0±2.1†‡</td>
<td>29.2±1.5†‡</td>
</tr>
<tr>
<td>2.8±0.2†‡</td>
<td>6.6±0.4†‡</td>
</tr>
<tr>
<td>80±5†‡</td>
<td>74±4†‡</td>
</tr>
<tr>
<td>29±3††</td>
<td>29±2†‡</td>
</tr>
<tr>
<td>21±3†</td>
<td>12±2†‡</td>
</tr>
<tr>
<td>282±56*</td>
<td>226±46*</td>
</tr>
<tr>
<td>2379±222†</td>
<td>911±59</td>
</tr>
<tr>
<td>599±138*</td>
<td>1796±277†§</td>
</tr>
<tr>
<td>247±55*</td>
<td>240±49†‡</td>
</tr>
<tr>
<td>14.9±2.5†‡</td>
<td>20.3±4.3*‡</td>
</tr>
<tr>
<td>7.6±1.7†‡</td>
<td>7.7±1.3†‡</td>
</tr>
</tbody>
</table>

Data Analysis

Indexes of LV function, systemic hemodynamics, and regional blood flow were compared among the treatment groups using ANOVA for repeated measures. If the ANOVA revealed significant differences, pairwise tests of individual group mean values were compared using two-tailed Bonferroni probabilities. For comparisons of neurohormonal profiles, the Student-Newman-Keuls test was used. All statistical procedures were performed using BMDP statistical software. Results are presented as mean±SEM, and values of P<.05 were considered to be statistically significant.

Results

After 3 weeks of chronic rapid pacing, all pigs in the untreated group demonstrated clinical symptoms consistent with CHF, including tachypnea and the development of ascites and peripheral edema during the last week of rapid pacing. In the pigs that underwent either amlodipine monotherapy, ACE inhibition monotherapy, or combination therapy, these clinical symptoms of CHF were not as readily apparent. In the concomitant amlodipine and rapid pacing group, plasma amlodipine levels were 25±6 ng/mL, and in the combination amlodipine and ACE inhibition group, plasma amlodipine levels were 22±3 ng/mL.

LV Function With Chronic Rapid Pacing: Effects of Monotherapy and Combination Therapy

Resting State

LV size and function were assessed by echocardiography under basal resting conditions in the rapid pacing–only group and all three treatment groups (Fig 2). In the rapid pacing–only group, LV end-diastolic dimension increased, fractional shortening decreased, and LV peak wall stress increased compared with the control state. In the amlodipine monotherapy group, LV end-diastolic dimension decreased, fractional shortening increased, and LV peak wall stress decreased, compared with rapid pacing only values. In the ACE inhibition group, LV end-diastolic dimension and LV peak wall stress decreased from rapid pacing only values. In the combination therapy group, LV end-diastolic dimension decreased, fractional shortening increased, and LV peak wall stress decreased from rapid pacing only values. In the ACE inhibition group, LV end-diastolic dimension was reduced from either amlodipine monotherapy or combination therapy values. In the ACE inhibition and combination therapy groups, LV peak wall stress was reduced from amlodipine monotherapy values.

Figure 3. The absolute change from resting values for plasma norepinephrine, epinephrine, renin activity, and endothelin with treadmill exercise. The relative rise in plasma norepinephrine was increased from the control state in the rapid pacing–only, amlodipine monotherapy, and combination therapy groups. With either amlodipine or ACE inhibition monotherapy, the relative rise in plasma norepinephrine was reduced from rapid pacing–only. In the rapid pacing–only group, the relative rise in plasma epinephrine was increased from the control state. In all treatment groups, the relative change in plasma epinephrine was reduced from the rapid pacing–only group. With ACE inhibition, the relative change in plasma epinephrine was blunted. In the rapid pacing–only group, the relative change in plasma renin activity was increased. In all treatment groups, the relative rise in plasma renin activity was increased above that of the rapid pacing–only group. With monotherapy treatment, the relative change in plasma endothelin was reduced from control, rapid pacing–only, and ACE inhibition groups. *P<.05 vs control. †P<.05 vs rapid pacing Amlodipine. ‡P<.05 vs rapid pacing ACE.
TABLE 2. Blood Flow With Pacing-Induced Heart Failure: Effects of Amlodipine, ACE Inhibition, or Combined Amlodipine and ACE Inhibition During the Progression of Heart Failure

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Rapid Pacing</th>
<th>Rapid Pacing and Amlodipine**</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rest</td>
<td>Exercise</td>
<td>Rest</td>
</tr>
<tr>
<td>LV myocardium, mL · min⁻¹ · g⁻¹</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endocardial flow</td>
<td>2.06±1</td>
<td>8.65±0.51</td>
<td>1.52±0.16*</td>
</tr>
<tr>
<td>Epicardial flow</td>
<td>1.66±1</td>
<td>7.46±4.11</td>
<td>1.21±0.11*</td>
</tr>
<tr>
<td>Average myocardial flow</td>
<td>1.86±0.12</td>
<td>8.05±0.45</td>
<td>1.37±0.13*</td>
</tr>
<tr>
<td>Coronary vascular resistance (×10⁶ dyne · s · cm⁻² · g)</td>
<td>4650±243</td>
<td>1191±80</td>
<td>5656±748*</td>
</tr>
<tr>
<td>Blood flow to regional beds, mL · min⁻¹ · g⁻¹</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lung</td>
<td>0.9±0.1</td>
<td>2.9±4</td>
<td>0.2±0.1*</td>
</tr>
<tr>
<td>Kidney</td>
<td>4.9±0.3</td>
<td>7.8±0.5</td>
<td>2.6±0.5*</td>
</tr>
<tr>
<td>Lattisimus dorsi</td>
<td>0.37±0.02</td>
<td>1.74±0.17</td>
<td>0.41±0.17</td>
</tr>
<tr>
<td>Gluteus maximus</td>
<td>0.37±0.02</td>
<td>1.77±0.17</td>
<td>0.39±0.15</td>
</tr>
<tr>
<td>Sample size, n</td>
<td>32</td>
<td>32</td>
<td>8</td>
</tr>
</tbody>
</table>

Values are mean±SEM.
*P<.05 vs control.
†P<.05 vs rapid pacing only.
‡P<.05 vs rapid pacing and ACE inhibition.
§P<.05 vs rapid pacing and ACE inhibition.†P<.05 vs resting state.
¶Rapid pacing: 21 days of chronic rapid pacing at 240 bpm.
**Rapid pacing and amlodipine: 1 mg/kg amlodipine per day.
††Rapid pacing and ACE inhibition: 1 mg/kg BID fosinopril.
†††Rapid pacing and amlodipine/ACE Inhibition: 1 mg/kg per day amlodipine and 1 mg/kg BID fosinopril.

 Hemodynamic indices measured in the resting awake state for the rapid pacing-only group and all three treatment groups are summarized in Table 1. Ambient resting heart rate was increased by ~30% in all rapid pacing groups compared with the control state. Stroke volume index was reduced from control values in the rapid pacing-only group and in all treatment groups. In the amlodipine monotherapy and combination therapy groups, stroke volume index was increased from rapid pacing-only values. Cardiac index was reduced from the control state in the rapid pacing-only and monotherapy treatment groups. With either amlodipine monotherapy or combination therapy, cardiac index was increased from rapid pacing-only values. Moreover, with combination therapy, cardiac index was not significantly different from control values (P=.11). Resting aortic pressure was decreased from control values in the rapid pacing-only group. In the ACE inhibition and combination therapy groups, aortic pressure was reduced from control, rapid pacing-only, and amlodipine monotherapy values. Pulmonary vascular resistance index was increased in all rapid pacing groups compared with the control state. In the monotherapy and combination therapy groups, total systemic resistance index was reduced from rapid pacing-only values. Further, total systemic resistance index was reduced in the combination therapy group to a greater degree than monotherapy values.

Treadmill Exercise
Changes in LV pump function and hemodynamics with treadmill-induced exercise are summarized in Table 1. In all groups, respiratory rate significantly increased with treadmill exercise compared with resting values. In the normal control state, heart rate increased by more than twofold from resting values. In all rapid pacing groups, heart rate increased significantly with exercise from resting values but remained lower than that achieved in the normal control state. Stroke volume index remained unchanged from resting values with treadmill exercise in the normal control state, but cardiac index increased by more than twofold. In the rapid pacing-only group and both monotherapy treatment groups, treadmill exercise resulted in a significant increase in stroke volume index and cardiac index from resting values. In the combination therapy group, cardiac index significantly increased with exercise and were higher than corresponding rapid pacing-only and ACE inhibition values. Pulmonary vascular resistance index fell in the rapid pacing-only group with treadmill exercise but remained elevated from control values in all the treatment groups. Total systemic resistance index decreased in all groups with treadmill exercise. Total systemic resistance index was reduced in the combination therapy group compared with rapid pacing-only values. In the normal control state, systemic oxygen consumption (V̇O₂) increased by more than fourfold with treadmill-induced exercise (199±29 versus 862±55 mL of O₂ · min⁻¹ · m⁻², P<.05). In the rapid pacing group, V̇O₂ was reduced from control values both at rest and with treadmill exercise (165±11 versus 602±34 mL of O₂ · min⁻¹ · m⁻², P<.05). In all three treatment groups, basal resting V̇O₂ was similar to the normal control state.
TABLE 2. Continued

<table>
<thead>
<tr>
<th>Rapid Pacing and ACE Inhibition††</th>
<th>Rapid Pacing and Amlodipine/ACE Inhibition‡‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rest</td>
<td>Exercise</td>
</tr>
<tr>
<td>1.69±0.24</td>
<td>4.48±0.48*</td>
</tr>
<tr>
<td>1.20±0.09*‡</td>
<td>3.52±0.39*</td>
</tr>
<tr>
<td>1.44±0.16*</td>
<td>4.00±0.41*</td>
</tr>
<tr>
<td>4745±496</td>
<td>1576±144*</td>
</tr>
<tr>
<td>0.6±0.1†</td>
<td>1.4±0.2*</td>
</tr>
<tr>
<td>2.9±0.4*</td>
<td>4.7±0.8*</td>
</tr>
<tr>
<td>0.45±0.1†</td>
<td>1.00±0.14*</td>
</tr>
<tr>
<td>0.46±0.11</td>
<td>0.89±0.12*</td>
</tr>
<tr>
<td>8</td>
<td>8</td>
</tr>
</tbody>
</table>

However, with treadmill exercise, $\dot{V}_O_2$ was equivalent to rapid pacing–only values.

Neurohormonal Activity: Effects of Monotherapy and Combination Therapy

Resting State

Plasma neurohormonal profiles in the normal control state and in the rapid pacing groups are summarized in Table 1. Under basal resting conditions, plasma norepinephrine increased from control values in all rapid pacing groups. With combination therapy, plasma norepinephrine was reduced from rapid pacing–only and ACE inhibition values. In the rapid pacing–only and monotherapy treatment groups, plasma epinephrine was elevated from the control state. In contrast, with combination therapy, plasma epinephrine was similar to control values ($P=.14$). In all rapid pacing groups, plasma renin activity and endothelin levels were increased from control values. Plasma renin activity and endothelin levels were reduced from rapid pacing–only values in all treatment groups.

Treadmill Exercise

The effects of treadmill exercise on plasma neurohormones are summarized in Table 1. In general, with treadmill exercise, plasma catecholamines were increased from the resting state in all groups. In the monotherapy treatment groups, plasma norepinephrine was reduced from rapid pacing–only values. In all treatment groups, plasma epinephrine was reduced from rapid pacing–only values. With treadmill exercise, plasma renin activity and endothelin levels remained elevated from normal control values in all rapid pacing groups. The relative change in neurohormonal activity from resting values with treadmill exercise is summarized in Fig 3. With either amlodipine monotherapy or ACE inhibition, the relative rise in plasma norepinephrine was reduced from rapid pacing–only values. In all treatment groups, the relative change in plasma epinephrine was reduced from the rapid pacing–only group, whereas the relative rise in plasma renin activity was increased above rapid pacing values. With combination therapy, the relative change in endothelin was reduced from control, rapid pacing–only, and ACE inhibition values.

Regional Blood Flow: Effects of Monotherapy and Combination Therapy

Resting State

Regional blood flow values to specific circulatory beds in the normal control state and in all rapid pacing groups are summarized in Table 2. Under ambient resting conditions, LV myocardial blood flow was reduced in the rapid pacing–only group compared with normal control values. With amlodipine monotherapy, LV myocardial blood flow was normalized, whereas in the ACE inhibition and combination therapy groups, LV myocardial blood flow values remained reduced from the normal control state. Coronary vascular resistance was increased in the rapid pacing–only group compared with control values, but in all treatment groups, coronary vascular resistance was normalized. Pulmonary parenchymal flow was reduced by >50% from normal control values in the rapid pacing group. In all treatment groups, pulmonary parenchymal blood flow was increased from rapid pacing–only values. Renal blood flow was reduced from the normal control state in all rapid pacing groups and was not influenced by drug treatment. Representative resting skeletal muscle blood flow, as determined by blood flow to the latissimus dorsi and gluteus maximus, was unchanged from normal control values in all rapid pacing groups.

Treadmill Exercise

Changes in regional blood flow distribution after treadmill exercise in the normal control state and in all rapid pacing groups are summarized in Table 2. LV myocardial blood flow increased by more than fourfold in the normal control state with treadmill exercise. In the rapid pacing group, LV myocardial blood flow increased with exercise but was 37% lower than normal control values. LV myocardial blood flow remained reduced from control values in all treatment groups. With amlodipine monotherapy, LV myocardial flow was increased from rapid pacing–only values. Coronary vascular resistance was reduced with treadmill exercise but remained increased from control values in either the ACE inhibition or combination therapy groups. With amlodipine monotherapy, coronary vascular resistance was reduced from rapid pacing–only values. Pulmonary parenchymal flow increased by threefold in the normal control state with treadmill exercise and was significantly blunted in the rapid pacing group. In the amlodipine monotherapy and combination therapy groups, pulmonary parenchymal flow was normalized to normal control state values. Renal blood flow increased by >50% in the normal control state with exercise. In all rapid pacing groups, renal blood flow remained reduced from normal control values. Skeletal muscle blood flow increased by more than fivefold in the control state and was significantly reduced in all of the rapid pacing groups.

Discussion

ACE inhibition has been demonstrated to provide beneficial effects on LV function and survival in patients with developing CHF.4,5 However, clinical trials with short-acting calcium
channel antagonists in patients with CHF have reported deleterious effects, including heightened neurohormonal system activity. Therefore, the therapeutic potential of calcium channel antagonists in the setting of developing CHF remains controversial. In a recent clinical trial, the longer-acting calcium channel antagonist amlodipine was associated with no adverse effects on morbidity and mortality in patients with severe CHF and appeared to provide beneficial effects in patients with nonischemic etiologies. In this past report, however, amlodipine was administered in the background of ACE inhibition therapy. Therefore, combination therapy with amlodipine and ACE inhibition may provide additive and/or interactive effects with developing CHF. This issue was addressed in the present study, which examined LV pump function, hemodynamics, neurohormonal profiles, and regional blood flow distribution in an animal model of pacing-induced CHF. The unique and significant findings from the present study were twofold. First, in the resting state, indexes of LV function were improved from rapid pacing-only values with either amlodipine monotherapy or combination therapy. Furthermore, in the resting state, combination therapy normalized cardiac index and reduced total systemic resistance index to a greater degree than either amlodipine monotherapy or ACE inhibition alone. However, with treadmill exercise, LV pump performance was not improved with any treatment modality. Second, in the resting state, combination therapy reduced plasma norepinephrine by 50% from rapid pacing-only and ACE inhibition groups and epinephrine by ~40% from rapid pacing-only and amlodipine monotherapy values and significantly blunted the relative rise in plasma epinephrine and endothelin with treadmill exercise. These results suggest that combination therapy with amlodipine and ACE inhibition with developing CHF reduced vascular resistive properties without an exacerbation of neurohormonal system activity.

LV Function and Systemic Hemodynamics

Past studies have demonstrated that monotherapy with either amlodipine or ACE inhibition during the development of CHF provided beneficial effects on LV function and hemodynamics. Specifically, ACE inhibition has been shown to improve LV function and survival in patients with developing CHF. Furthermore, Weinberg et al demonstrated that chronic administration of amlodipine normalized LV end-diastolic pressure in a model of LV hypertrophy. Consistent with past reports, chronic rapid pacing caused LV dilation and pump dysfunction. In the present study, concomitant amlodipine monotherapy, ACE inhibition, or combination therapy instituted during chronic rapid pacing reduced the degree of LV dilation. With either amlodipine monotherapy or combination therapy, indexes of LV pump function were improved in the basal resting state compared with pacing CHF values. To our knowledge, this was the first study to examine the direct effects of combined amlodipine and ACE inhibition therapy with the development of a CHF process. With combination therapy instituted during chronic rapid pacing, cardiac index was normalized. Under resting conditions, mean aortic pressure was not different from control values in the amlodipine monotherapy group, whereas in the ACE inhibition and combination treatment groups, mean aortic pressure was reduced from both control and rapid pacing-only values. In the combination treatment group, mean aortic pressure was similar to ACE inhibition values, but total systemic resistance index was reduced to a greater degree compared with either monotherapy value. Thus, likely contributory mechanisms for the improved cardiac index with combination therapy in this model of CHF include decreased LV afterload as demonstrated by decreased peak wall stress and reduced total systemic resistance index.

Neurohormonal System Activity

In the present study, the development of pacing-induced CHF was associated with an approximately threefold increase in plasma catecholamines, renin activity, and endothelin levels. Past studies have demonstrated that the use of short-acting calcium channel antagonists in the setting of CHF was associated with heightened neurohormonal activity as demonstrated by increased plasma catecholamines and renin activity. In the present study and in contrast to these past reports, chronic amlodipine as either a monotherapy or in combination with ACE inhibition did not result in increased plasma catecholamines and renin activity. Furthermore, in the resting state, combination therapy reduced plasma norepinephrine from rapid pacing-only values and normalized plasma epinephrine levels. With either amlodipine monotherapy, ACE inhibition, or combination therapy plasma renin activity was decreased from rapid pacing-only values. However, with treadmill exercise, the relative change in plasma renin activity was increased from rapid pacing-only values; likely reflecting a global reduction in renal perfusion pressure. With treadmill exercise, the relative change in plasma norepinephrine was higher in the combination therapy group that may have been secondary to sympathetic activation due to a reduction in the total systemic resistance index. However, this issue remains speculative and warrants further investigation. With the development of pacing-induced CHF, plasma levels of the potent vasoactive peptide endothelin were significantly elevated. With either amlodipine monotherapy, ACE inhibition, or combination therapy, plasma endothelin levels were reduced from pacing CHF values; both at rest and with treadmill exercise. Interestingly, with treadmill exercise, the relative rise in plasma endothelin appeared to be blunted to a greater degree with combination therapy compared with either monotherapy treatment. In patients with CHF, increased levels of plasma endothelin have been correlated with the degree of LV dysfunction and have been demonstrated to influence pulmonary and systemic vascular resistance. Kiowski et al reported that acute administration of an endothelin receptor antagonist reduced systemic and pulmonary vascular resistance in patients with CHF. Therefore, the relative reduction in plasma endothelin levels with either amlodipine monotherapy or combination therapy likely contributed to the reduced total systemic resistive properties in this model of CHF. The direct mechanistic relationship between endothelin levels, vascular resistance, and amlodipine therapy with the development of CHF warrants further investigation. Nevertheless, an important finding of the present study was that amlodipine treatment, either as a monotherapy or in combination with ACE
inhibition, was not associated with significant neurohormonal activation in this model of CHF.

Regional Blood Flow Distribution

Consistent with past reports,\textsuperscript{28,45} the present results demonstrated that the development of pacing-induced CHF was associated with a significant reduction in myocardial blood flow at rest. This reduction in myocardial blood flow occurred in the absence of a physical obstruction to flow and therefore was likely due to changes in vascular resistive properties in the coronary vasculature. It has been reported previously that in patients with nonischemic cardiomyopathy, abnormalities in myocardial oxygen delivery/demand exist.\textsuperscript{46} Thus, although remaining speculative, the global reduction in LV myocardial blood flow may be a contributory factor toward the diminished LV performance with pacing-induced CHF. In the present study, amlodipine monotherapy normalized resting myocardial blood flow. With either ACE inhibition or combination therapy, LV myocardial blood flow was similar to pacing CHF values. However, in the ACE inhibition and combination therapy groups, aortic pressure was reduced despite normalized coronary vascular resistance. These results suggest that the persistent reduction in LV myocardial blood flow with either ACE inhibition or combination therapy was likely due to a significant reduction in coronary driving pressure. To more carefully examine LV myocardial blood flow under a physiological stress, measurements were also performed during treadmill exercise. In the rapid pacing–only and concomitant treatment groups, relative LV myocardial blood flow was increased with treadmill exercise but remained lower than control values. A blunted response to endothelium-mediated vasodilation as well as to adenosine has been reported with the development of CHF.\textsuperscript{28,47} In addition, it has been suggested that the diminished coronary flow reserve with pacing-induced CHF may be due to increased LV myocardial wall stress during diastole.\textsuperscript{45} Thus, in the present study, the persistent reduction in LV myocardial blood flow, regardless of treatment modality, was likely due to additional vasoconstrictive and hemodynamic influences.

With the development of pacing-induced CHF, pulmonary parenchymal flow was significantly reduced both at rest and with treadmill-induced exercise. In all treatment groups, resting pulmonary parenchymal flow was increased from pacing CHF values. Tsutamoto et al\textsuperscript{48} demonstrated that in patients with severe CHF, endothelin spillover in the pulmonary circuit occurred and correlated to the degree of pulmonary vascular resistance. Thus, with either amlodipine monotherapy, ACE inhibition, or combination therapy, a likely contributory mechanism for increased resting pulmonary parenchymal flow was decreased circulating endothelin levels. With treadmill exercise, pulmonary parenchymal flow was normalized with either amlodipine monotherapy or combination therapy but not with ACE inhibition. Roy et al\textsuperscript{49} demonstrated that Ang II–mediated vasoconstriction was decreased in the pulmonary vasculature with pacing CHF. Thus, the effectiveness of ACE inhibition in reducing pulmonary vascular resistance may be diminished with the development of CHF. Although increased from rapid pacing values, resting pulmonary parenchymal blood flow remained reduced from control values in all treatment groups. Thus, in the present study, inherent defects in the vasodilatory properties of the bronchial smooth muscle may have occurred with pacing CHF, which in turn contributed to persistent defects in bronchial flow regardless of treatment modality. With the development of pacing CHF, renal blood flow was reduced from normal control values; both at rest and with exercise. Concomitant treatment with either amlodipine monotherapy, ACE inhibition, or combination therapy did not improve renal blood flow at rest or with exercise. Nevertheless, these treatment regimens did not compromise renal blood flow. With treadmill exercise, skeletal muscle blood flow was reduced with pacing CHF. This observation is consistent with past experimental and clinical reports in which abnormalities in skeletal muscle perfusion were noted during exercise.\textsuperscript{47,48,50} Chronic treatment with amlodipine monotherapy, ACE inhibition, or combination therapy did not increase skeletal muscle blood flow from CHF values during exercise. With exercise, significant vascular smooth muscle vasodilation occurs primarily due to the local release of a number of metabolites,\textsuperscript{51} and the vascular response to these local metabolites has been reported to be abnormal with CHF.\textsuperscript{52,53} These abnormalities in local vasodilatory responses at the level of the muscle vasculature likely superseded any potential beneficial effects of concomitant amlodipine monotherapy, ACE inhibition, or combination treatment.

Study Limitations and Summary

The present project used a model of chronic rapid pacing that produced changes in LV functional and neurohormonal characteristics that appeared similar to that of the clinical spectrum of CHF.\textsuperscript{28–32} This model of CHF provided an opportunity to examine the effects of pharmacological interventions in the absence of confounding influences that may be encountered in clinical studies. However, it must be recognized that any animal model will not fully represent the complex clinical spectrum of CHF. Specifically, the changes in LV myocardial structure that occur with pacing-induced CHF are not similar to clinical forms of CHF due to chronic ischemia or hypertensive disease. Thus, extrapolation of the findings of this project to clinical forms of CHF should be done with caution. In the present study, the ACE-inhibition dose was selected based on attenuating the Ang I pressor response while not producing a significant hypotensive effect. Thus, whether higher doses of ACE inhibition and amlodipine, either as a monotherapy or in combination, may influence hemodynamic and neurohormonal profiles with the development of CHF was not addressed with the present experimental design. These limitations notwithstanding, the present study demonstrated that with CHF, combined amlodipine and ACE inhibition effectively improved indexes of LV pump function and vascular resistive properties without an exacerbation of neurohormonal system activity. In a past clinical study of patients with severe CHF undergoing ACE inhibition treatment, the institution of concomitant amlodipine therapy was not associated with increased hemodynamic compromise or mortality but rather may have provided favorable effects in a subset of patients.\textsuperscript{19} The results of the present study demonstrated that monotherapy with either amlodipine or ACE inhibition pro-
vides beneficial effects in this pacing model of CHF. Combined amlodipine and ACE inhibition provided greater benefit with respect to vascular resistance properties and neurohormonal system activity compared with either monotherapy.

Acknowledgments

This work was supported by National Institutes of Health grant R01-HL-56603, a Grant-in-Aid from the American Heart Association, and a Basic Research Grant from Pfizer Inc. Dr Kribbs is a Medical Student Research Fellow of the American Heart Association. Dr Spinale is an Established Investigator of the American Heart Association. The authors wish to express their appreciation to Jennifer Hendrick, Mark Little, and Douglas Kugley for their excellent technical and administrative assistance in this project.

References

Amlodipine Monotherapy, Angiotensin-Converting Enzyme Inhibition, and Combination Therapy With Pacing-Induced Heart Failure
Scott B. Kribbs, William M. Merritt, Mark J. Clair, R. Stephen Krombach, Ward V. Houck, Michael G. Dodd, Rupak Mukherjee and Francis G. Spinale

Hypertension. 1998;31:755-765
doi: 10.1161/01.HYP.31.3.755

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1998 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/31/3/755