Levosimendan improves LV systolic and diastolic performance at rest and during exercise after heart failure

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Lemonsimendan improves LV systolic and diastolic performance at rest and during exercise after heart failure. Am J Physiol Heart Circ Physiol 288: H914–H922, 2005. First published October 14, 2004; doi:10.1152/ajpheart.00465.2004.—The new myofilament Ca\textsuperscript{2+} sensitizer lemonsimendan (LSM) is a positive inotropic and vasodilatory agent. Its beneficial effects have been demonstrated at rest in congestive heart failure (CHF). However, its effect during exercise (Ex) in CHF is unknown. We assessed the effects of LSM on left ventricular (LV) dynamics at rest and during Ex in eight conscious, instrumented dogs with pacing-induced CHF. After CHF, with dogs at rest, LSM decreased arterial elastance (E\textsubscript{a}) and increased LV contractile performance as assessed by the slope of LV pressure-volume (P-V) relation. LSM caused a >60% increase in the peak rate of mitral flow (dV/dt\textsubscript{max}) due to decreases in minimal LV pressure and the time constant of LV relaxation (\tau). LV arterial coupling, quantified as the ratio of end-systolic elastance (E\textsubscript{es}) to E\textsubscript{a}, was increased from 0.47 to 0.85%. LV mechanical efficiency, determined as the ratio of stroke work to total P-V area, was improved from 0.54 to 0.67 ± 0.07. These beneficial effects persisted during Ex after CHF. Compared with CHF Ex dogs, treatment with LSM prevented Ex-induced abnormal increases in mean left atrial pressure and end-diastolic pressure and decreased E\textsubscript{es}/E\textsubscript{a}. With LSM treatment during CHF Ex, the early diastolic portion of the LV P-V loop was shifted downward with decreased minimal LV pressure and \tau values and a further augmented dV/dt\textsubscript{max}. E\textsubscript{es}/E\textsubscript{a} improved, and mechanical efficiency further increased from 0.61 ± 0.07 to 0.67 ± 0.07, which was close to the value reached during normal Ex. After CHF, LSM produced arterial vasodilatation; improved LV relaxation and diastolic filling; increased contractility, LV arterial coupling, and mechanical efficiency; and normalized the response to Ex.

congestive heart failure; left ventricular dynamics; filling; contractility; mechanical efficiency

IMPORTANT GOALS IN THE TREATMENT OF PATIENTS WITH CONGESTIVE HEART FAILURE (CHF) are to prolong survival and improve the patient’s quality of life. The major symptom and cause of disability in CHF patients is exercise (Ex) intolerance (5, 32, 45). Observational work in the general healthy population has shown that Ex capacity is a more powerful prognostic indicator than traditional risk factors for cardiovascular disease (36, 44). However, despite enormous advances in the understanding and treatment of CHF that have taken place during the last 50 years, CHF remains a serious and, in fact, growing health problem. Present treatment of Ex intolerance is unsatisfactory in CHF patients (45, 63). The \beta-adrenergic agonist dobutamine and phosphodiesterase inhibitor milrinone were associated with worse survival and clinical outcomes and did not improve quality of life for severe CHF patients (42, 48). Angiotensin-converting enzyme inhibitor therapy and \beta-blockade treatment improve survival in patients with CHF but do not always enhance Ex tolerance (37). Recently myofilament Ca\textsuperscript{2+} sensitizers, which are a new class of nonnglycosidic, nonadrenergic, positive inotropic agents, have been studied in patients with CHF. Because impaired relaxation often accompanies systolic dysfunction (5, 18, 32, 45), enhanced Ca\textsuperscript{2+} sensitivity might have negative effects with increased sensitivity to Ca\textsuperscript{2+} during diastole, thereby further slowing relaxation and worsening diastolic dysfunction (22). Such prolonged relaxation impairing diastolic function has been observed with the Ca\textsuperscript{2+} sensitizers EMD-57033 and -53998, ORG-30029, and CGP-48506 (19, 22, 26, 64). Both EMD-57033 (without phosphodiesterase inhibition properties) and ORG-30029 (with phosphodiesterase inhibition properties) impaired relaxation to a greater extent in failing than nonfailing human hearts (22).

The limitation of Ex tolerance in CHF results from both cardiac and peripheral factors (45). The exacerbation of diastolic dysfunction during CHF Ex and the resulting increase in left atrial pressure (LAP) contribute to exertional dyspnea. Observations in our laboratory demonstrate that there is a reversal of the normal Ex-induced augmentation of left ventricular (LV) relaxation and a decrease in early diastolic LV pressure (LVP) with a resulting rise in LAP (9, 10). We found that before CHF, the effect of an Ex-induced increase in systolic load alone slows the rate of LV relaxation. However, this effect is overcome during normal Ex by sympathetic stimulation and increased heart rate, which enhance the rate of LV isovolumic pressure decrease. After CHF, the Ex-induced increase in systolic load persists, but the effects of increased heart rate and \beta-adrenergic stimulation to speed relaxation are reduced. In addition, both ANG II and endothelin-1 increase to very high levels. Thus LV relaxation slows, and LV end-systolic volume (ESV) and minimum pressure (LVP\textsubscript{min}) increase. The early diastolic portion of the LV pressure-volume (P-V) loop shifts upward and rightward during Ex after CHF. Thus any diastolic dysfunction present at rest in CHF is exacerbated during Ex (8, 11).

Levosimendan (LSM), a novel Ca\textsuperscript{2+} sensitizer, is reported to be an inotropic and vasodilator agent without adverse effects on diastolic function (24, 43, 48, 61, 62). Unlike most other Ca\textsuperscript{2+} sensitizers, LSM acts through direct binding with tropolin C and increases the affinity of troponin C for Ca\textsuperscript{2+} in a...
Ca\textsuperscript{2+}-dependent manner. A lack of Ca\textsuperscript{2+} sensitization under low Ca\textsuperscript{2+} concentrations (i.e., during diastole) may prevent worsening of diastolic dysfunction. The phosphodiesterase III inhibitory component may possibly exert a positive effect on the rate of relaxation (14, 20). In recent clinical trials, intravenous administration of LSM produced a dose-dependent favorable hemodynamic effect in the acute treatment of stable or decompensated CHF (38, 48). LSM improved the survival of patients with severe low-output CHF (15). In addition, LSM reduced the risks of worsening CHF and death in patients with CHF (35, 62). Although beneficial effects of LSM in CHF at rest have been demonstrated, its effects during Ex are not known.

The present study tests the hypothesis that LSM, a pyridazino-dinitrile derivative with phosphodiesterase III inhibitory properties, may improve both LV systolic and diastolic performance at rest and normalize Ex response after CHF. Studies were performed on a conscious, instrumented dog model with pacing-induced CHF that shares many of the structural hemodynamic and neurohormonal changes seen in human CHF (5, 9, 10, 32).

Our results provide insight into a mechanism of Ex intolerance in CHF and suggest a therapeutic strategy to enhance Ex performance in CHF patients.

**MATERIALS AND METHODS**

**Instrumentation**

This investigation was approved by the Institutional Animal Care and Use Committee. Eight healthy, adult, heartworm-negative mongrel dogs (body wt, 25-35 kg) were instrumented to measure three LV internal dimensions, LVP, and LAP. Hydraulic occluders were placed around the venae cavae using a previously described technique (9, 11).

**Data Collection**

Studies were performed after the animals had recovered fully from instrumentation (10 days after original surgery) with the dogs standing and then running on a motorized treadmill (model 1849C; Quinton; Seattle, WA) as previously described (8, 9).

**Experimental Protocol**

**Effects of normal Ex.** Steady-state measurements at rest were obtained while the dogs stood on a motorized treadmill. Variably loaded LV P-V loops were generated by sudden, transient occlusion of the venae cavae as previously described (9). The first normal Ex session was then performed with the dogs running on the treadmill. The treadmill speed was gradually increased over 1-2 min from 2.5 mph to the maximum tolerated level of steady-state Ex of 5.5-8.5 mph. The animals exercised at this level until they could no longer keep up with the treadmill. Immediately after steady-state data collection, the treadmill was suddenly stopped and caval occlusions were performed. Data were acquired during 15-s periods throughout the Ex protocol. We analyzed the data recorded during the last minutes of Ex. Because we could not perform caval occlusions during Ex, we assessed LV P-V relations by using caval occlusion data generated immediately after Ex. The total Ex time ranged from 7 to 10 min. After dogs rested for 30 min, a second Ex session was performed. We previously observed that there is no difference in the response to Ex repeated after a 30-min rest period (8, 10). The values of resting controls were also similar before and with a 30-min resting period after Ex.

**Induction of CHF.** After completion of the baseline studies as previously described (12), rapid right ventricular (RV) pacing (at 220–240 beats/min) was initiated using the pacing protocol. After we employed the pacing protocol for 4-5 wk, when the LV end-diastolic pressure (EDP) during the nonpaced period had increased by >20 mmHg over the prepping control level, we obtained CHF data.

**Effects of CHF Ex.** During the stable CHF period, we examined responses to LSM administration. Briefly, before each study, the pacemaker was turned off and the dog was allowed to equilibrate for 1 h. The animal then ran on the treadmill as the speed was increased and adjusted to the maximum tolerated steady-state level, and data were collected while the dog was running. After CHF, the maximum level of Ex was decreased to 3.5-6.0 mph. The total Ex duration was also reduced. After dogs rested for 30 min, LSM was started with loading doses of 24 μg/kg administered for 10 min followed by infusion of LSM at rates of 0.2 μg·kg \textsuperscript{-1}·min \textsuperscript{-1} for 40 min. When the arterial pressure reached a stable level, steady-state and caval occlusion data were collected and treadmill Ex was performed (10).

The LSM dosing protocol used in this study was identified from a dose-response study. The LSM dosage was chosen to produce a similar increase in LV contractility and a decline in net vascular loading, which was estimated by arterial elastance (E\textsubscript{a}) as produced by pimobendan (Pim, 2.5 mg/kg iv) in our past report (41). Briefly, five normal, conscious dogs receiving LSM loading doses of 24 μg/kg for 10 min) followed by infusion at rates of 0.4 μg·kg \textsuperscript{-1}·min \textsuperscript{-1} for 40 min. End-systolic elastance (E\textsubscript{a}) increased by 56% (6.6 ± 1.7 to 10.3 ± 2.1 mmHg/ml), E\textsubscript{es} decreased 15% (7.4 ± 1.2 to 6.3 ± 1.1 mmHg/ml), and the time constant of LV relaxation (τ) decreased 26% (27.8 ± 3.1 to 20.6 ± 3.7 ms; P < 0.05). The alterations in these parameters lasted ~2 h. The LSM doses used in the present study were similar to those used by Pagel et al. (43) in conscious dogs before and after CHF and were also similar to those used in clinical studies of CHF patients (38, 48).

**Data Processing and Analysis**

As previously described, LV volume, τ, the LV end-systolic pressure (ESP)-ESV relation and its slope (E\textsubscript{a}), and the stroke work (SW)-end-diastolic volume (EDV) relation and its slope (M\textsubscript{sw}) were analyzed (9, 10). The τ value was analyzed by determining the time constant of the isovolumic decrease of LVP. LVP from the time of peak –dP/dt until mitral valve opening was fit to the exponential equation LVP = P\textsubscript{a} exp(–t/τ) + P\textsubscript{b}, where t is time and P\textsubscript{a}, P\textsubscript{b}, and τ are constants determined by the data. Although the decrease in isovolumic pressure is not exactly exponential (58), the time constant, which is derived from the exponential approximation, provides an index of the rate of LV relaxation (17, 34). In addition, τ was also calculated by the Weiss method (monoexponential decay model to zero asymptote). As previously described, the average LV chamber stiffness value during diastole was obtained by dividing the change in pressure from the time of LVP\textsubscript{es} to EDP (ΔLVP) by the change of volume during this period. The mean end-systolic circumference stress (WS\textsubscript{es}, in g/cm\textsuperscript{2}) of the left ventricle was calculated by use of a thick-walled spherical model (51, 52)

$$WS_{es} = G \times ESP \times ESV^{2/3},$$

where G (1.36) is a conversion factor (from mmHg to g/cm\textsuperscript{2}) and ESV\textsuperscript{2/3} is the LV wall volume (in ml), which is assumed to be 5 ml/kg of body wt (51, 52). Because pacing-induced CHF was previously demonstrated (55) to not alter LV wall mass, we used the same V\textsubscript{m} value to calculate WS\textsubscript{es} both before and after CHF in each animal. LV-arterial coupling was quantitated as the ratio of E\textsubscript{es} to E\textsubscript{a} and was determined as ESP/stroke volume (SV). The LV P-V area (PVA), which represents the total mechanical energy, was determined as the area under the end-systolic P-V relation and systolic P-V trajectory above the EDP-volume curve. The efficiency of conversion of mechanical energy to external work of the heart was calculated as SW/PVA (39). Ex data were analyzed at two levels: a low speed (2.5 mph) and a high speed (maximum tolerated).
Table 1. Effects of levosimendan on steady-state hemodynamics before and after CHF

<table>
<thead>
<tr>
<th></th>
<th>Before CHF</th>
<th>Control</th>
<th>Levosimendan Treated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rest</td>
<td>Exercise</td>
<td>Rest</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>106±11</td>
<td>173±15*</td>
<td>119±18</td>
</tr>
<tr>
<td>Maximum dP/dt, mmHg/s</td>
<td>2,510±353</td>
<td>3,108±659*</td>
<td>1,582±355$</td>
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<tr>
<td>Minimum dP/dt, mmHg/s</td>
<td>−2,024±229</td>
<td>−2,485±271*</td>
<td>−1,446±244$</td>
</tr>
<tr>
<td>Stroke volume, ml</td>
<td>14.8±3.5</td>
<td>16.2±4.3*</td>
<td>11.2±3.6$</td>
</tr>
<tr>
<td>LV end-diastolic pressure, mmHg</td>
<td>10.1±1.8</td>
<td>11.0±1.7</td>
<td>32.5±3.0$</td>
</tr>
<tr>
<td>LV end-systolic pressure, mmHg</td>
<td>104±8</td>
<td>124±9*</td>
<td>93±18</td>
</tr>
<tr>
<td>Minimum LV pressure, mmHg</td>
<td>0.9±1.9</td>
<td>−2.8±2.2*</td>
<td>16.5±4.4$</td>
</tr>
<tr>
<td>Mean LA pressure, mmHg</td>
<td>7.0±1.3</td>
<td>7.6±1.2</td>
<td>27.9±4.5$</td>
</tr>
<tr>
<td>LV end-diastolic volume, ml</td>
<td>44.7±11.0</td>
<td>45.6±11.3*</td>
<td>50.9±15.0$</td>
</tr>
<tr>
<td>LV end-systolic volume, ml</td>
<td>29.9±10.1</td>
<td>29.4±9.6</td>
<td>38.9±13.9$</td>
</tr>
<tr>
<td>Maximum dV/dr, ml/s</td>
<td>158±59</td>
<td>247±49*</td>
<td>129±49$</td>
</tr>
<tr>
<td>Stroke work, mmHg/ml</td>
<td>1,539±380</td>
<td>2,009±410*</td>
<td>1,062±276</td>
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<tr>
<td>Cardiac output, ml/min</td>
<td>1,568±316</td>
<td>2,803±517*</td>
<td>1,332±264$</td>
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<tr>
<td>Arterial elastance, mmHg/ml</td>
<td>6.8±1.4</td>
<td>7.7±2.7*</td>
<td>7.7±1.3$</td>
</tr>
<tr>
<td>Time constant of relaxation, ms</td>
<td>27.8±2.4</td>
<td>21.5±2.1*</td>
<td>35.9±2.9$</td>
</tr>
<tr>
<td>LV chamber stiffness, mmHg/ml</td>
<td>0.67±0.27</td>
<td>0.82±0.32</td>
<td>1.48±0.28$</td>
</tr>
<tr>
<td>Wsae, g/cm²</td>
<td>65.4±17.8</td>
<td>78.1±20.2*</td>
<td>72.4±19.1$</td>
</tr>
<tr>
<td>Mwae, mmHg</td>
<td>80.4±7.8</td>
<td>114.9±12.2*</td>
<td>50.1±4.5$</td>
</tr>
</tbody>
</table>

Values are means ± SD; n = 8 dogs. CHF, congestive heart failure; dP/dt, rate of rise of left ventricular (LV) pressure; LA, left atrial; maximum dV/dr, peak rate of mitral flow; Wsae, mean end-systolic circumference stress of left ventricle; Mwae, slope of stroke work-end-diastolic volume relation. *P < 0.05, exercise vs. control rest; †P < 0.05, levosimendan rest vs. control rest; ‡P < 0.05, CHF levosimendan exercise vs. CHF control exercise; §P < 0.05, CHF rest vs. control rest.

Statistical Analysis

Statistical comparisons were made with Student’s t-test for paired observations and ANOVA with the Bonferroni method of multiple-paired comparisons as appropriate. Significance was established as P < 0.05. Data for steady state are expressed as means ± SD; values for LV P-V relations are expressed as means ± SE.

Postmortem Evaluation

At the conclusion of the studies, the animals were killed by lethal injection of pentobarbital sodium (100 mg/kg iv), and the hearts were examined to confirm that instrumentation was properly positioned.

RESULTS

Effects of Pacing-Induced CHF at Rest and During Ex

At rest. As summarized in Table 1 and displayed in Figs. 1 and 2, consistent with our past reports (9, 10), chronic ventricular rapid pacing in a canine model produced progressive LV systolic and diastolic dysfunction. After 4–5 wk of rapid pacing, LV EDP progressively elevated. The ESV and EDV also increased, whereas cardiac output decreased due to a marked reduction of SV (11.2 ± 3.6 vs. 14.8 ± 3.5 ml). Although mean LAP (27.9 ± 4.5 vs. 7.0 ± 1.3 mmHg) was significantly increased, dV/dr max decreased due to a marked increase in LVP min (16.5 ± 4.4 vs. 9.1 ± 1.9 mmHg). The τ value (35.9 ± 2.9 vs. 27.8 ± 2.4 ms), total systemic resistance, Ea, and LV chamber stiffness values all increased. Mean Wsae increased from 65.4 ± 17.8 to 72.4 ± 19.1 g/cm² (P < 0.05). LV contractility significantly decreased as indicated by significant reductions in the slopes of ESP-ESV (Ees: 47%; 6.7 ± 1.8 vs. 3.5 ± 0.4 mmHg/ml), dP/dt max-EDV (dEdV/dt max: 45%; 71.0 ± 11.2 vs. 38.6 ± 6.4 mmHg·s⁻¹·ml⁻¹), and SW-EDV (Mwae: 40%; 80.4 ± 7.8 vs. 50.1 ± 4.5 mmHg) relations (Table 2). The Ees/Ea ratio decreased from 0.89 ± 0.31 to 0.47 ± 0.05, and LV mechanical efficiency, measured as SW/PVA, decreased from 0.63 ± 0.07 to 0.54 ± 0.09 (Table 3).

Fig. 1. Left ventricular (LV) pressure-volume (P-V) loops obtained from one animal at rest (A) and during exercise (Ex; B) after and before congestive heart failure (CHF). Each loop was generated by averaging the data obtained during a 15-s recording period that spanned several respiratory cycles. During normal Ex, the early diastolic portion of the LV P-V loop was shifted downward so that the early diastolic LV pressure (LVP) was lower during Ex than at rest. However, after CHF, the early diastolic portion of the LV P-V loop was shifted upward during Ex so that early diastolic LVP was much higher.
During Ex. After CHF, the duration of Ex was reduced to 4.5 ± 1.9 min, and the speed of Ex decreased to 3.5–5.3 mph. As shown in Fig. 1 and Table 1, consistent with our previous observations (7, 8), normal Ex caused an improvement in LV diastolic performance with a decreased τ (21.5 ± 2.1 vs. 27.8 ± 2.4 ms), a lower LVPmin, and an increased dV/dtmax (247 ± 49 vs. 184 ± 59 ml/s) without an increase in LAP (7.6 ± 1.2 vs. 7.0 ± 1.3 mmHg). The early diastolic portion of the LV P-V loop was shifted downward so that LVP was lower throughout early and mid-diastole during Ex than at rest (Fig. 1A). In contrast with the findings during Ex before CHF, τ (40.4 ± 5.3 vs. 35.9 ± 2.9 ms), LV EDP, LVPmin (21.9 ± 3.1 vs. 16.5 ± 4.4 mmHg), and mean LAP (33.7 ± 3.9 vs. 27.9 ± 4.5 mmHg) all increased during Ex after CHF. As shown in Fig. 2. LV P-V loops and P-V relations determined from a conscious dog after CHF and before and after administration of levosimendan (LSM). Treatment with LSM produced leftward shifts of the LV end-systolic pressure (ESP)-end-systolic volume (ESV; A), dP/dtmax-end-diastolic volume (VEd; B), and stroke work-end-diastolic volume (C) relations with increased slopes. This indicates that LSM increased LV contractility after CHF.

Table 2. Effects of levosimendan on LV pressure-volume relations after CHF

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Exercise</th>
<th>Levosimendan Treated</th>
<th>Exercise</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rest</td>
<td>Exercise</td>
<td>Rest</td>
<td>Exercise</td>
</tr>
<tr>
<td>ESP-ESV relation</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Ees, mmHg/ml</td>
<td>3.5 ± 0.4</td>
<td>3.1 ± 0.9*</td>
<td>5.7 ± 0.6†</td>
<td>7.4 ± 0.8*‡</td>
</tr>
<tr>
<td>V0,es, ml</td>
<td>8.4 ± 3.4</td>
<td>6.6 ± 2.8</td>
<td>15.4 ± 3.9†</td>
<td>17.2 ± 1.3†‡</td>
</tr>
<tr>
<td>V100,es, ml</td>
<td>38.3 ± 5.5</td>
<td>41.5 ± 5.4</td>
<td>33.9 ± 4.6†</td>
<td>30.0 ± 1.3†‡</td>
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<tr>
<td>dP/dtmax-EDV relation</td>
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</tr>
<tr>
<td>dE/dtmax, mmHg·s⁻¹·ml⁻¹</td>
<td>38.6 ± 6.4</td>
<td>34.3 ± 7.8*</td>
<td>59.6 ± 7.1†</td>
<td>65.7 ± 6.5†‡</td>
</tr>
<tr>
<td>V0_dP/dtmax, ml</td>
<td>10.1 ± 3.8</td>
<td>8.3 ± 3.5</td>
<td>8.8 ± 6.4</td>
<td>9.8 ± 5.1</td>
</tr>
<tr>
<td>V1000_dP/dtmax, ml</td>
<td>41.3 ± 6.0</td>
<td>43.9 ± 6.2</td>
<td>27.1 ± 6.5†</td>
<td>25.6 ± 5.3‡</td>
</tr>
<tr>
<td>SW-EDV relation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Msw, mmHg</td>
<td>50.1 ± 4.5</td>
<td>44.5 ± 2.6*</td>
<td>69.9 ± 5.1†</td>
<td>76.8 ± 3.9*‡</td>
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<tr>
<td>V0Sw, ml</td>
<td>29.9 ± 4.9</td>
<td>28.1 ± 3.5</td>
<td>27.1 ± 6.5†</td>
<td>29.9 ± 3.1‡</td>
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<tr>
<td>V1000Sw, ml</td>
<td>49.1 ± 4.9</td>
<td>51.0 ± 2.4</td>
<td>44.7 ± 4.4†</td>
<td>42.8 ± 3.9*‡</td>
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</tbody>
</table>

Values are means ± SE; n = 8 dogs. ESP, end-systolic pressure; ESV, end-systolic volume; Ees, slope of linear ESP-ESV relation; V0,es, intercept with volume axis; V100,es, volume associated with ESP of 100 mmHg; dP/dtmax rate of rise of LV pressure; EDV, end-diastolic volume; dE/dtmax, slope of dP/dtmax-EDV relation; V0_dP/dtmax, intercept with volume axis; V1000_dP/dtmax, volume associated with dP/dt of 1,000 mmHg/s; SW, stroke work; V0Sw, intercept with volume axis; V1000Sw, volume associated with SW of 1,000 mmHg/ml. *P < 0.05, exercise vs. rest; †P < 0.05, levosimendan rest vs. control rest; ‡P < 0.05, CHF levosimendan exercise vs. CHF control exercise.
Figs. 1B and 2, these changes were accompanied by a consistent rightward and upward shift of the early diastolic portion of the LV P-V loop. During early diastole, at an equivalent LV volume, LVP was significantly higher during Ex than at rest after CHF. Thus any diastolic dysfunction present at rest in CHF is exacerbated during Ex (8, 11). As summarized in Table 3, before CHF, the \( E_a/E_s \) ratio was 0.89 ± 0.31 and increased to 1.27 ± 0.21 during Ex. Thus SW/PVA was improved from 0.63 ± 0.07 to 0.69 ± 0.09 during normal Ex. In contrast, after severe CHF, the \( E_a/E_s \) ratio significantly decreased to 0.47 ± 0.05 and further decreased to 0.41 ± 0.03 during CHF Ex, which led to a significant reduction in SW/PVA (0.51 ± 0.06 vs. 0.54 ± 0.09).

**Effects of LSM After CHF at Rest and During Ex**

At rest. As summarized in Table 1 and displayed in Figs. 2 and 3, compared with CHF at rest, LSM produced no changes in heart rate and rhythm. LSM significantly reduced resting LV \( E_a \) (6.1 ± 1.2 vs. 7.7 ± 1.3 mmHg/ml), EDP (26.7 ± 3.2 vs. 32.5 ± 3.0 mmHg), and mean LAP (22.7 ± 3.9 vs. 27.9 ± 4.5 mmHg; \( P < 0.05 \)). The peak mitral flow (dV/dt_max) (217 ± 59 vs. 129 ± 49 mmHg/s) was increased due to decreased LVP_min (11.4 ± 4.8 vs. 16.5 ± 4.4 mmHg) and \( \tau \) (30.8 ± 2.2 vs. 35.9 ± 2.9 ms). SV (14.7 ± 3.6 vs. 11.2 ± 3.6 ml) and cardiac output increased dramatically. Both LV chamber stiffness (1.08 ± 0.22 vs. 1.48 ± 0.28 mmHg/ml) and WS_{es} were significantly reduced. The slopes of LV P-V relations were increased (\( E_{es}\), 62%; dE/dt_{max}, 54%; M_{Sw}, 39%; see Table 2 and Fig. 2). \( E_a/E_s \) was increased from 0.47 ± 0.05 to 0.85 ± 0.07. Mechanical efficiency, measured as SW/PVA, was also significantly improved (0.61 ± 0.07 vs. 0.54 ± 0.09; see Table 3).

During Ex. Compared with CHF Ex without treatment, LSM significantly attenuated the Ex-induced increase in LV ESP and reversed Ex-induced abnormal increases in LVP_{min}, \( \tau \), and the upward shift of the early diastolic portion of the LV P-V loop (see Figs. 2, 4, and 5 and Table 1). As demonstrated in Fig. 4, during CHF Ex without LSM treatment, increased levels of Ex revealed adverse actions with a further decreased rate of LV relaxation and reduced LV contractility as measured by M_{sw}. LSM treatment completely reversed these abnormal responses. Treatment with LSM and increased levels of Ex caused a further augmented rate of LV relaxation and M_{sw}. Compared with CHF controls, after LSM treatment, CHF Ex caused a downward shift of the diastolic portion of LV P-V loops. The dV/dt_{max} (\( \Delta = 83 \pm 26 \text{ ml/s} \)) value was additionally augmented due to significant decreases in \( \Delta \) (\( \Delta = 4.5 \pm 2.1 \text{ ms} \)) and LVP_{min} (\( \Delta = 5.8 \pm 1.6 \text{ mmHg} \)) without an abnormal increase in mean LAP. In addition, during CHF Ex after treatment with LSM, \( E_a/E_s \) increased, which resulted from a significantly increased \( E_{es} \) but relatively unchanged \( E_a \). Thus with LSM treatment, CHF Ex further improved the SW/PVA value to 0.67 ± 0.07, which is close to the values of normal controls before CHF at rest and during Ex (see Fig. 5 and Table 3). With LSM treatment, the duration of Ex was also significantly increased (4.5 ± 1.9 vs. 7.5 ± 1.5 min; \( P < 0.05 \)).

**DISCUSSION**

We investigated the effects of LSM, a new Ca\(^{2+}\) sensitizer, on LV systolic and diastolic function at rest and during Ex in an animal model of CHF that mimics many of the functional and neurohormonal changes of clinical CHF (5, 10, 23, 50). We found that after CHF, at rest, a clinically relevant dose of LSM produces arterial vasodilatation, improves LV relaxation and diastolic filling, and increases contractility, LV arterial coupling, and mechanical efficiency. Importantly, the effects of treatment with LSM persisted during Ex after CHF. Treatment with LSM prevents CHF Ex-induced abnormal increases in early diastolic LVP and mean LAP and restores the normal Ex response.

CHF is associated with progressive sympathetic nervous system activation but diminished inotropic response to β-ad-
favorable reductions in LV EDP, total systemic resistance, at rest and during Ex. Treatment with LSM also caused increase in LV contractility and improved LV relaxation both dogs with pacing-induced CHF, LSM produced a marked edly an inotropic and vasodilator agent without adverse effects dilator response to Ex.

frequency relation (40), and a blunted peripheral arterial vaso-
dilator response with clinical consequences of a decline in myocardial reserve (4, 5, 46) and an impairment in Ex response (8, 11). Consistently, in the present study, the conscious dogs with pacing-induced CHF exhibited progressive LV systolic and diastolic dysfunction and abnormal LV function during Ex. We found that in CHF, the impaired LV arterial coupling and diastolic dysfunction present at rest are exacerbated during Ex. During CHF Ex, there is a reversal of the normal Ex-induced augmentation of LV relaxation and a decrease in early diastolic LVP with a resulting increase in LAP. We and others reported earlier (9, 45) that abnormal Ex response is attributable to several factors such as impaired intrinsic contractility, blunted inotropic responses to β-adrenoceptor agonists, enhanced sen-
sitivity of LV relaxation to Ex-induced increased systolic load, high levels of ANG II and endothelin-1 (10), altered force-
frequency relation (40), and a blunted peripheral arterial vasodila-
tor response to Ex.

LSM, which is a pyridazinone-dinitrile derivative, is report-
edly an inotropic and vasodilator agent without adverse effects on diastolic function. In this study, we found that in conscious dogs with pacing-induced CHF, LSM produced a marked increase in LV contractility and improved LV relaxation both at rest and during Ex. Treatment with LSM also caused favorable reductions in LV EDP, total systemic resistance, $E_a$, and LV stiffness without changing heart rate. Moreover, after CHF, LSM treatment prevented Ex-induced increases in EDP and mean LAP. With LSM, there was a normal enhancement of LV relaxation and a decrease in early LV diastolic pressure that occurred during Ex.

These hemodynamic beneficial effects result from the posi-
tive inotropic action of LSM and a reduction in systolic load. The mechanism of the positive inotropic action of LSM is not entirely clear. Unlike most other myofilament $Ca^{2+}$ sensitizers, the inotropic effect of LSM is partly mediated by $Ca^{2+}$ con-
centration-dependent conformational changes in troponin C that lead to sensitization of the contractile apparatus to $Ca^{2+}$ ions during systole (20, 21). At high LSM concentrations, phosphodiesterase III inhibition may play some role in the positive inotropic effects (13). However, LSM was reported to be a cardiotonic agent with $Ca^{2+}$-sensitizing and -mobilizing actions over identical concentration ranges (14, 47). Increases in the activity of the Na+/Ca2+-exchanger with LSM may also contribute to improved relaxation (25). LSM-induced vasodi-
lator lability is likely mediated by the opening of ATP-dependent K+ channels in vascular smooth muscle (29, 59, 60).

LSM improved systolic function without impairing relaxation and also improved relaxation during Ex. Previously, we found an impaired LV systolic and diastolic force-frequency relation and an adverse effect of tachycardia on LV arterial coupling and mechanical efficiency in conscious dogs with pacing-induced CHF (40). Treatment with LSM has been shown to improve frequency-induced force generation in human failing myocardium (25) and convert a negative force-
frequency relationship into a positive relationship (6, 27). Thus an LSM reversal of the negative force-frequency relationship may have contributed to the beneficial effects of LSM that we observed during CHF Ex.

Previous observations in our laboratory have demonstrated that normally functioning LV and arterial systems are nearly optimally coupled to produce SW both at rest and during Ex (30). In the present study, we found that during the development of CHF, the $E_{cd}/E_a$ ratio was reduced, which resulted in less than maximal SW. Furthermore, this coupling ratio was additionally depressed during Ex, thus contributing to Ex intolerance in CHF. Treatment with LSM significantly reduced LV wall stress and caused an increase in the $E_{cd}/E_a$ ratio with resulting nearly maximum SW both at rest and during Ex after CHF. Thus with LSM, LV mechanical efficiency was signifi-
cantly augmented in CHF both at rest and during Ex. This finding is consistent with recent clinical observations that LSM induces enhanced contractility without increasing myocardial oxygen demand (54, 57) and induction of arrhythmias (15, 38).
The upward shift in the early diastolic portion of the LV P-V loop that we observed during CHF Ex is similar to that reported by Miyazaki et al. (34) in exercising dogs with coronary stenosis as well as that found in clinical studies of Ex-induced ischemia (33, 53). In these studies, the decrease in LV distensibility during Ex was due to the effect of myocardial ischemia. Although our animals did not have coronary stenosis, Ex-induced ischemia may have contributed to our findings.

Thus LSM caused coronary vasodilatation (29) and may have contributed to improved LV relaxation and filling during Ex after CHF.

LSM appears to have some advantages over other positive inotropic agents. Although β-adrenergic stimulation improves contraction and relaxation, its effects are reduced after CHF (11, 49, 56). Furthermore, we found that compared with CHF at rest, LSM was more effective than Pim in producing decreases in LVPmin, ESV, and τ during CHF Ex. Ex duration in CHF was increased only by LSM. Treatment with LSM also reversed the CHF Ex-induced decrease in cardiac efficiency. These findings support a previous report that Pim has only weak Ca²⁺-sensitizing effects (2, 3, 16, 41). It has been shown that as a Ca²⁺-sensitizing agent, treatment with LSM is 100 times more potent than Pim (21).

In recent clinical trials, intravenous administration of LSM produced dose-dependent favorable hemodynamic effects in the acute treatment of stable or decompensated CHF (38, 48). Furthermore, LSM improved the survival of patients with severe low-output CHF compared with dobutamine (15). In addition, LSM reduced worsening CHF and death in patients with CHF complicating acute myocardial infarction (35). The present findings and clinical observations are consistent with the known pharmacological actions of the drug as a Ca²⁺-sensitizer and direct vasodilator (14, 20, 21, 25, 48).

Several methodological issues should be considered in the interpretation of our data. First, although we studied an animal model of CHF (pacing tachycardia) that reproduces many of the functional and neurohormonal features of clinical CHF, this experimental model demonstrated biventricular chamber dilatation with increased LV and RV filling pressures and striking abnormalities in systolic and diastolic function similar to those
found in patients with dilated congestive cardiomyopathy (1, 5, 8, 9, 28, 32, 43, 45, 49). We cannot be certain that our results apply to CHF of other causes such as hypertrophic cardiomyopathy.

Second, we studied the acute effects of LSM treatment. We do not know the effects of prolonged treatment with LSM.

Third, the use of a simple exponential to characterize relaxation is an approximation, because LV isovolumic pressure does not decay exactly exponentially. Yellin et al. (58) evaluated the validity of a monoexponential characterization of relaxation and revealed that late relaxation is more rapid than predicted by a monoexponential relation. However, when $\tau$ is calculated from a filling cycle by assuming a zero-pressure asymptote (i.e., the conventional way), there is no significant difference with the true value based on the nonfilling cycle (17, 58). The monoexponential $\tau$ remains a useful index of relaxation and correlates well with other temporal indexes (isovolumic relaxation time and relaxation half-time (17, 58).

In conclusion, after CHF, a clinically relevant dose of LSM produces arterial vasodilation; improves LV relaxation and diastolic filling; increases contractility, LV arterial coupling, and mechanical efficiency; and restores normal Ex response. The present study supports the view that LSM may be the first inotropic agent that is both safe and effective in altering clinical outcomes such as mortality and poor Ex tolerance for patients with CHF.

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