Improved Mechanoenergetics and Cardiac Rest and Reserve Function of In Vivo Failing Heart by Calcium Sensitizer EMD-57033

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Background—Myofilament Ca$^{2+}$ sensitizers enhance contractility but can adversely alter diastolic function through sensitization to low intracellular Ca$^{2+}$ concentration. Concomitant phosphodiesterase III inhibition (PDE3I) may offset diastolic changes but limit the mechanoenergetic benefits. We tested whether selective Ca$^{2+}$ sensitization in vivo with the use of EMD-57033 enhances both systolic and diastolic function in failing hearts at minimal energetic cost.

Methods and Results—Pressure-dimension data were measured with sonomicrometry/micromanometry in conscious dogs before (CON, n=9) and after tachycardia-induced heart failure (HF, n=11). In contrast to blunted dobutamine (DOB) responses in HF, low-dose EMD-57033 (0.4 mg · kg$^{-1}$ · min$^{-1}$ for 20 minutes) markedly enhanced contractility, doubling end-systolic elastance and raising fractional shortening similarly in CON-treated and HF hearts. EMD-57033 effects were achieved at a reduced heart rate, without vasodilation. EMD-57033 augmented blunted heart rate-dependent contractility responses in HF at a rate of twice that of DOB, despite matched basal inotropic responses. EMD-57033 also improved diastolic function, lowering left ventricular end-diastolic pressure and increasing the filling rate. At equipotent inotropic doses and matched preload, EMD-57033 lowered the oxygen cost of contractility by $-11.4 \pm 5.8\%$, whereas it rose 64$\%$ with DOB ($P=0.001$) and 28$\%$ with milrinone. Doubling EMD-57033 dose further augmented positive inotropy in CON and HF, accompanied by vasodilation, increased heart rate, and other changes consistent with PDE3I coactivity, but the oxygen cost of contractility remained improved compared with the use of DOB.

Conclusions—Selective Ca$^{2+}$ sensitization with minimal PDE3I in vivo is achieved with the use of EMD-57033, improving basal and rate-stimulated contractility and mechanoenergetics of HF without compromising diastolic function. Despite PDE3I activity at higher doses, energetic benefits persist. (Circulation. 2000;101:1040-1048.)

Key Words: mechanics ■ calcium ■ diastole ■ contractility ■ heart failure

Adrenoceptor agonists and phosphodiesterase III (PDE3I) inhibitors enhance inotropy by raising intracellular Ca$^{2+}$ concentration ([Ca$^{2+}]_{i}$) while simultaneously reducing myofilament Ca$^{2+}$ sensitivity and increasing sarcoplasmic reticulum (SR) Ca$^{2+}$ influx. These changes in Ca$^{2+}$ handling increase net energy demand and may elevate arrhythmia risk, limiting their efficacy for chronic heart failure (HF) treatment. Myofilament Ca$^{2+}$ sensitizers, in contrast, improve contractility without changing [Ca$^{2+}]_{i}$ and should thereby improve systolic function at a lower energetic cost. However, when administered in vivo, most Ca$^{2+}$ sensitizers demonstrate inhibition of phosphodiesterase III (PDE3I). Accordingly, many such agents display far less than the anticipated benefit on in vivo mechanoenergetics.

Agents highly selective for Ca$^{2+}$ sensitization, on the other hand, can negatively affect diastole by facilitating cross-bridge activation at diastolic [Ca$^{2+}]_{i}$ levels. This could impede their use in HF, which is typified by reduced basal distensibility and delayed Ca$^{2+}$ cycling kinetics. EMD-57033 is one such agent that potently augments the force developed by actin-myosin cross-bridges with relatively low PDE3I activity. In vitro studies have reported adverse effects on relaxation and chamber stiffening and although this is less pronounced in normal in vivo hearts, responses in failing hearts remain unknown. Furthermore, no study has tested the in vivo efficacy of EMD-57033 in altering mechanoenergetics in failure or enhancing contractile reserve.

The present investigation was designed to test whether EMD-57033 can act principally as a Ca$^{2+}$ sensitizer in the intact failing heart of conscious animals to augment rest and reserve systolic function and improve mechanoenergetics without compromising diastolic function. Studies were performed in dogs, with HF induced through long-term tachycardia pacing. The results support selective Ca$^{2+}$ sensitization in vivo, with potent positive...
inotropic effects, marked oxygen sparing effects, and enhanced diastolic chamber performance.

Methods

Preparation

Twenty mongrel dogs of either sex (45 to 65 lb) were anesthetized with 1% to 2% halothane after induction with sodium thiopental (3 to 5 mg/kg). The chest was opened via lateral thoracotomy. A micro-manometer (P22; Konigsberg Instruments) was placed in the left ventricle (LV) at the apex. Right atrial and descending aortic catheters were placed, the latter for LV manometer calibration. Endocardial sonomicrometers were used to measure anteroposterior short-axis dimension, and a pneumatic occluder around the inferior vena cava enabled load reduction for LV pressure–dimension relation analysis. Epicardial pacing leads were sutured to the LV free wall and connected to a programmable stimulator (Spectravx; Medtronics) inserted within a subcutaneous pocket. Leads were secured to the left atrium for pacing during hemodynamic recordings. The chest was closed, and catheters and leads were externalized to the midscapulae. Morphine (10 mg SC) was administered postoperatively as required. Dogs were provided 10 days for recovery before the study. The surgical and experimental animal protocol was approved by the Johns Hopkins University Animal and Care Use Committee.

Conscious Protocol

Studies were performed in control (CON, n=9) and HF (n=11) animals, with the latter induced by tachypacing (210 bpm × 3 weeks, 240 bpm × 1 week). Data were measured in conscious animals standing quietly in a sling, with pacing suspended at least 30 minutes before the study in HF dogs. Four principal interventions were studied in each animal. First, a dose response to 5 to 15 µg·kg⁻¹·min⁻¹, min⁻¹ dobutamine (DOB) was determined, allowing 5- to 10-minute stabilization at each dose. To assess the interaction between DOB and heart rate (HR)-dependent contractile reserve, a constant dosage of 10 µg·kg⁻¹·min⁻¹ was administered, and the atrial rate increased from sinus to 240 bpm with pacing. DOB was then stopped, and the baseline was reestablished. There were no significant differences in systolic or diastolic function between initial and second baseline levels (Table 1). EMD-57033 (dissolved in 1,2-propanediol, infused over 20 minutes) was then administered at 0.4 or 0.8 mg·kg⁻¹·min⁻¹, min⁻¹. After the recording of hemodynamics, data were again obtained during incremental HR while EMD-57033 was continued. EMD-57033–related responses were all referenced to the second (post-DOB) baseline. In 4 separate studies, we administered 1,2-propanediol alone at the same infusion rate, and no significant effects on baseline hemodynamics were observed (Table 1).

To further probe the mechanism of action of EMD-57033 in vivo (ie, Ca²⁺ sensitization versus PDE3I activity), the decay rate of postrest contractile potentiation was assessed. After several minutes of atrial tachycardia, pacing was stopped and HR returned to sinus rhythm. Contractile potentiation observed after pacing gradually declined over sequential cycles, and the rate of decay (recirculation fraction [RF]) reflected intracellular Ca²⁺ recycling by the SR. Because both PDE3I and β-adrenergic stimulation enhance SR Ca²⁺ uptake due to phospholamban phosphorylation, this increases the RF. In contrast, pure Ca²⁺ sensitization has minimal influence on the RF. All of the preceding protocols were performed in every CON animal and 7 HF dogs.

Mechanoenergetic Studies

In 9 HF dogs, we contrasted the energetic costs of improving contractility with DOB (n=5, 5 µg·kg⁻¹·min⁻¹); EMD-57033 (0.4 mg·kg⁻¹·min⁻¹, n=3), EMD-57033 (0.8 to 1.0 mg·kg⁻¹·min⁻¹, n=4), or a PDE3I (milrinone, 10 µg·kg⁻¹·min⁻¹). Five studies were performed in anesthetized animals (n=5; 50 µg/kg fentanyl plus 1% to 2% isoflurane), with coronary flow velocity measured in the proximal circumflex coronary artery with intravascular Doppler probe (0.014 in; Cardiometrics), and contrast imaging of the coronary was used to determine cross-sectional diameter (CathView v1.36; Image Comm Systems). A coronary sinus catheter provided blood sampling for oxygen measurement. The remaining studies were conducted in conscious animals preinstrumented with a coronary flow probe (Transonic) and a coronary sinus catheter. There were no significant differences in mechanoenergetic responses between conscious and sedated studies. Hemodynamics, coronary flow, and arteriovenous oxygen difference were measured under basal conditions and at steady state with each drug and dose.

Hemodynamic and Energetic Analysis

Pressure-dimension data were digitized at 250 Hz. Rest parameters were determined from data averaged from 10 to 20 consecutive beats, whereas data measured during transient inferior vena cava occlusion were used to determine pressure–dimension relations. Systolic function was indexed on the basis of stroke dimension, fractional shortening (stroke dimension/end-diastolic dimension [EDD]), peak rate of pressure rise (dP/dtmax), end-systolic elastance (Ees, slope of end-systolic pressure–dimension relation [ESPDR]), and the slope of the relation between dimension work (pressure–dimension loop area) and EDD. The latter 2 indexes provide relatively load-insensitive measures of contractility. dP/dtmax was calculated from a running 5-point weighted slope, and ESPDR was derived through perpendicular regression of end-systolic points, with the use of an iterative method.

The use of pressure-dimension relations included the assumption that they correspond to pressure-volume data. Although previously reported in normal hearts, we confirmed this correlation in several animals after the induction of HF. Figures 1A and 1B displays representative simultaneously derived pressure-dimension and pressure-volume relations (volume assessed with the use of a conductance catheter). Volume-dimension plots from these same data (Figure 1C) revealed an excellent correlation (r=0.96), with an average r value of 0.92±0.04 from 22 such comparisons. Figure 1D shows a strong correlation between Ees change assessed with either method for the same inotropic changes.

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TABLE 1. Reproducibility of Baseline Condition Before and After DOB Dose-Response Testing and Minimal Effect of Propanediol (Vehicle) on Baseline Ventricular Function

<table>
<thead>
<tr>
<th>FS, %</th>
<th>dP/dtmax, mm Hg/s</th>
<th>Ees, mm Hg/mm</th>
<th>τ, ms</th>
<th>Msw, mm Hg</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Before and after DOB</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline 1</td>
<td>19.1±3.5</td>
<td>2338.4±199.1</td>
<td>15.7±3.0</td>
<td>22.3±2.8</td>
</tr>
<tr>
<td>Baseline 2</td>
<td>18.2±3.2</td>
<td>2303.7±249.0</td>
<td>16.2±2.3</td>
<td>23.4±2.5</td>
</tr>
<tr>
<td><strong>Effect of propanediol</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>22.4±1.6</td>
<td>3169.0±513.3</td>
<td>10.4±2.3</td>
<td>23.5±4.8</td>
</tr>
<tr>
<td>Propanediol (vehicle)</td>
<td>22.6±1.3</td>
<td>2793.6±624.0</td>
<td>9.4±1.3</td>
<td>22.1±4.2</td>
</tr>
</tbody>
</table>

FS indicates fractional shortening; Msw, preload-recruitable stroke work.
Diastolic function was indexed on the basis of end-diastolic pressure (EDP), time constant of relaxation (τ) with a logistic fit, peak filling rate normalized by EDD, and the EDP-dimension relation measured during caval occlusion. The logistic τ was used because it provides a more stable parameter in failing hearts than that derived from monoeXponential models. Peak filling rate was calculated from a smoothed first derivative of the dimension signal. Arterial afterload was assessed by effective arterial elastance, equal to the ratio of end-systolic pressure to stroke dimension.

Recirculation fraction was measured from the linear regression slope of relations between dp/dt max(n) and dp/dt max(n+1), where n represents data from the nth beat after the cessation of rapid pacing and return to sinus rhythm. dp/dt max served as a reliable index for contractile function in this setting because preload is nearly identical to the ratio of end-systolic pressure to stroke dimension.

In the animals that underwent energetic analysis, myocardial oxygen consumption (MVO2) was indexed from the product of coronary flow and arterial-venous oxygen difference. Relative changes in oxygen consumption were determined and compared with relative changes in dp/dt max and stroke work to yield the oxygen cost of contractile enhancement and changes in efficiency.

Statistical Analysis
Data are presented as mean±SD unless otherwise stated. Dose-dependent drug effects were tested by repeated measures ANOVA, with a multiple comparisons test (Tukey). Comparisons of DOB- and EMD-57033-induced changes were tested with a paired t test. Comparisons for baseline data and drug responses between CON and HF were analyzed with an unpaired t test. Differences in the force-frequency response between CON and HF animals with and without DOB or EMD-57033 were performed with the use of 2-way repeated measures ANOVA.

Results
Inotropic and Loading Effects of EMD-57033
Figure 2A shows typical pressure-dimension data before and after 0.4 or 0.8 mg·kg⁻¹·min⁻¹ EMD-57033, along with responses to 15 μg·kg⁻¹·min⁻¹ DOB. EMD-57033 substantially enhanced contractility at both doses, with similar changes in CON and HF hearts. This contrasts with DOB, which enhanced contractility far more in CON than in HF. Table 2 summarizes group systolic data, which support these findings with a variety of indexes. Importantly, in HF dogs, low-dose EMD-57033 yielded comparable contractile changes to high-dose DOB, whereas higher doses greatly exceeded a DOB response.

At the lower dose, EMD-57033 had minimal or slowing effects on HR and did not significantly alter arterial load or preload (Table 3). At higher doses, EMD-57033 increased HR in CON and HF hearts (similar to DOB) and reduced arterial load and preload. These dose-dependent loading and HR differences are consistent with concomitant PDE3I effects at the higher dose.

EMD-57033 Effects on Diastolic Function
We next tested whether potent inotropic effects from the use of EMD-57033 were accompanied by compromised diastolic function as reported in vitro. EMD-57033 had little effect on relaxation time constants at a low dose, yet significantly improved early filling rate (comparable to DOB), with even greater effects at the higher dose. Unlike DOB, these changes were similarly induced in HF. Similar diastolic changes were observed at a constant HR (140 bpm; data not shown). EMD-57033 reduced EDP, although this did not reach a statistical significance at a low dose in HF due to a slower HR and enhanced filling in 2 animals. At a constant HR (140 bpm), EDP declined in these animals (−5.9±3.6, P<0.05). EMD-57033 did not alter chamber compliance (Figure 3); rather, the EDP reduction was due to a slight fall in net chamber filling and right heart load (Table 4).

Force-Frequency Potentiation and Recirculation Fraction
To test whether EMD-57033 augmented inotropic reserve, we studied its interaction with incremental HR. Both Ees and dp/dt max rose with HR in CON and HF animals, but this rate response was markedly attenuated with HF (P<0.05) (Figure 4). DOB (10 μg·kg⁻¹·min⁻¹) and EMD-57033 (0.4 mg·kg⁻¹·min⁻¹) amplified the rate dependence in CON, but the synergistic effect with EMD-57033 far exceeded that with DOB (maximal Ees rise 17.6±0.5 versus 7.8±0.0 mm Hg/mm, P<0.01) in HF. Importantly, both drugs were administered at doses yielding matched basal contractility before change in HR, so this discrepancy could not be ascribed to downregulation of β-receptor signaling.

To further probe mechanisms by which varying doses of EMD-57033 influence in vivo contractility, we measured effects on beat-to-beat potentiation decay after the abrupt cessation of rapid pacing. Figure 5A plots dp/dt max of the nth beat versus the n+1th beat after return to normal sinus rhythm after atrial pacing. The slope of each relation (RF) reflects the relative percentage of Ca²⁺ recycled by the SR. DOB increased the slope at both doses, which is consistent
with phospholamban phosphorylation, whereas it was unchanged by low-dose EMD-57033. At higher-dose EMD-57033, the slope rose similar to that for DOB. Summary data support these responses (Figure 5B) and confirm lower RFs in HF. These results also suggest that low-dose EMD-57033 acts primarily as a Ca\(^{2+}\) sensitizer.

### Mechanoenergetics

Figure 6 summarizes the mechanoenergetics results. Contractile augmentation ($\Delta \text{dP/dt}_{\text{max}}$) in response to 5 $\mu$g $\cdot$ kg\(^{-1}\) $\cdot$ min\(^{-1}\) DOB (low dose) was compared with that for low- and higher-dose EMD-57033, with HR maintained during the interventions. Percent changes in dP/dt\(_{\text{max}}\) were matched for

#### TABLE 2. Effects of EMD-57033 Versus DOB on Systolic Function

<table>
<thead>
<tr>
<th>Drug</th>
<th>Normal Baseline</th>
<th>Normal $\Delta$ Lower Dose</th>
<th>Normal $\Delta$ Higher Dose</th>
<th>Heart Failure Baseline</th>
<th>Heart Failure $\Delta$ Lower Dose</th>
<th>Heart Failure $\Delta$ Higher Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>DOB</td>
<td>7.2±0.5</td>
<td>3.4±0.2*</td>
<td>4.0±0.3*</td>
<td>5.2±1.3</td>
<td>1.1±0.5§</td>
<td>1.0±0.8§</td>
</tr>
<tr>
<td>EMD</td>
<td>7.9±0.24</td>
<td>0.9±0.9†</td>
<td>1.1±1.0†</td>
<td>4.4±0.9§</td>
<td>1.2±0.5</td>
<td>4.0±0.6*†§</td>
</tr>
<tr>
<td>DOB</td>
<td>17.2±1.6</td>
<td>7.6±0.5*</td>
<td>9.2±0.6†</td>
<td>13.1±3.4</td>
<td>3.1±1.1§</td>
<td>3.9±2.0*§</td>
</tr>
<tr>
<td>EMD</td>
<td>18.9±0.9</td>
<td>3.4±2.0†</td>
<td>6.0±2.5*</td>
<td>12.1±2.2§</td>
<td>4.1±1.0†</td>
<td>16.9±4.8*†§</td>
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<tr>
<td>DOB</td>
<td>2517±133</td>
<td>2007±299*</td>
<td>3167±373‡</td>
<td>1630±183§</td>
<td>526±96§</td>
<td>900±201*§</td>
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<tr>
<td>EMD</td>
<td>2767±149</td>
<td>1027±311*†</td>
<td>2788±521‡</td>
<td>1406±122§</td>
<td>608±106†</td>
<td>2400±449†‡</td>
</tr>
<tr>
<td>DOB</td>
<td>10.0±1.0</td>
<td>7.2±1.0*</td>
<td>9.0±1.8*</td>
<td>7.9±0.8</td>
<td>0.9±0.5†</td>
<td>3.6±1.9†</td>
</tr>
<tr>
<td>EMD</td>
<td>10.9±0.8</td>
<td>7.0±1.2†</td>
<td>30.0±7.0‡</td>
<td>7.3±1.0§</td>
<td>6.5±3.5†</td>
<td>18.8±5.7†‡</td>
</tr>
<tr>
<td>DOB</td>
<td>66.7±3.3</td>
<td>37.9±4.6*</td>
<td>47.0±4.1*</td>
<td>53.5±3.8§</td>
<td>7.8±3.2§</td>
<td>13.4±5.6†</td>
</tr>
<tr>
<td>EMD</td>
<td>73.5±2.3</td>
<td>23.6±7.6*</td>
<td>50.7±1.2‡</td>
<td>46.9±12.6§</td>
<td>19.3±4.6†</td>
<td>44.2±8.4†‡</td>
</tr>
</tbody>
</table>

SD indicates stroke dimension; FS, fractional shortening; and $M_{\text{sw}}$, preload-recruitable stroke work.

*P<0.05 vs baseline; †P<0.05 vs DOB; ‡P<0.05 vs lower dose; §P<0.05 vs normal.
milrinone was accompanied by reduced MV˙ O 2, lowering the oxygen cost of dP/dt max by 6 SEM with DOB versus 21.2 47% (mean with intravenous fluids during milrinone infusion. Al-

rable conditions, end-diastolic volume was restored to base-

DOB and low-dose EMD-57033, yet MV˙ O 2 rose by 144±47% (mean±SEM) with DOB versus 21.2±7% with EMD-57033 (P<0.001). Thus, the oxygen cost of contractil-

ity declined with EMD, whereas it increased by >50% with DOB. Mechanical efficiency, estimated as the ratio of regional stroke work to MV˙ O 2, improved 64±32% with EMD-57033 but declined −29±6% with DOB (P<0.01). Importantly, these energetic benefits persisted at the higher dose of EMD-57033, with a −7.8±19.5% change in oxygen cost of dP/dt max (P=0.007 versus DOB) and improved efficiency.

Experiments were also conducted with the selective PDE3 inhibitor milrinone (Figure 6), with dP/dt max matched to that of DOB and low-dose EMD-57033. Increased systolic function with milrinone was accompanied by reduced MV˙ O 2, lowering the oxygen cost of dP/dt max by −29±3.8% and improving efficiency. However, mechanisms for this response differed from those with EMD-57033. As shown in Figure 7, milrinone had concomitant potent arterial and venous dilator effects, with a −30±5% decline in afterload and a −12±2% decline in preload (both P<0.001). These changes could themselves reduce MV˙ O 2 and improve efficiency. To test more comparable conditions, end-diastolic volume was restored to baseline with intravenous fluids during milrinone infusion. Although the inotropic response was similar, MV˙ O 2 rose by 75±20%, yielding an increased oxygen cost of dP/dt max and no significant change in efficiency.

Discussion

The primary new findings of the present study were that in vivo, low-dose EMD-57033 acting principally via Ca 2+

sensitization with minimal demonstrable PDE3I action markedly improves inotropy in failing hearts at a substantially reduced energetic cost compared with DOB or with milrinone (with preload restored to baseline). Importantly, EMD-57033 generated these changes with net beneficial effects on diastolic function. These data counter the results of previous studies performed in vitro with this agent that reported adverse changes in diastolic function. They also contrast with studies of other Ca 2+

sensitizers with combined PDE3I activity that show much less mechanoenergetic benefit.

Mechanism of Inotropic Effect

A common feature of most agents that enhance Ca 2+

sensitization of the myofilaments is PDE3I coactivity. This holds for EMD-57033 as well, although in vitro data suggest higher selectivity for Ca 2+

sensitization, at least at certain doses. The identification of these effects in vivo is important, because primary PDE3 inhibitors have resulted in increased mortality rates in chronic HF clinical trials. PDE3I elevates cAMP, increasing contractility and HR, enhancing relaxation, inducing vasodilation, and accelerating postrest potentiation decay. The current data support minimal PDE3I at a dosage of 0.4 mg · kg⁻¹ · min⁻¹ dose but some activity at the 2-fold higher dose. Even at the higher dose, however, EMD-57033 remained equally effective in CON and HF hearts, which is not expected from the use of pure PDE3I and supports primarily Ca 2+

sensitization effects occurring at this dose as well. The dose dependency of PDE3I effects is consistent with prior data obtained in conscious swine and dogs.

The capacity of EMD-57033 to induce quantitatively similar inotropic responses in CON and HF hearts and to restore force-frequency reserve in HF further supports a Ca 2+

sensitization mechanism. This contrasts with DOB, which had a blunted basal and rate-enhanced activity in HF. The disparity between DOB and EMD-57033 responses differs.
from that with levosimendan, another Ca$^{2+}$ sensitizer and PDE3I that is being tested in clinical trials. In the latter case, contractile responses to isoproterenol and levosimendan were similarly depressed by HF.4

The mechanism of Ca$^{2+}$ sensitization by EMD 57033 has been postulated to involve direct modification of the actin-myosin interface.6 This prevents inhibition of the actin-myosin interaction by troponin-tropomyosin, promoting cross-bridge formation from weak to strong force-generating states. As a consequence, cross-bridges stay attached longer while generating greater force, leading to more prolonged contraction with less effect on the rising phase of contraction. The present data revealed such disparities at the lower dose. Basal myocardial Ca$^{2+}$ sensitivity is reportedly enhanced in the pacing-tachycardia model, 22 perhaps related to reduced troponin I phosphorylation. Despite this, Ca$^{2+}$ sensitization effects due to EMD-57033 were similar in HF and CON hearts.

Contractile Energetics

The oxygen cost of contractility enhancement in HF was considerably lower with EMD-57033 than with DOB. The selective use of PDE3I with milrinone also yielded favorable energetic effects, but this was found principally due to concomitant loading changes. However, even after preload restoration, the energetic cost of contractile improvement with milrinone was still lower than that with DOB. These results support data from Holubarsch et al,23 who found similar declines in isometric contraction economy from adrenergic stimulation versus PDE3I but less tension-independent heat (ie, Ca$^{2+}$ turnover) with PDE3I. Our study is the first to demonstrate energetic effects of EMD-57033 in conscious animals with HF and supports data obtained from studies on isolated rat cardiomyocytes24 and rabbit and guinea pig papillary muscles.25 An oxygen-sparing effect is predicted from Ca$^{2+}$ sensitization because less ATP hydrolysis per unit force is required if the cross-bridge cycle favors longer

### Table 4. Effects of EMD-57033 Versus DOB on Diastolic Function

<table>
<thead>
<tr>
<th>Drug</th>
<th>Normal</th>
<th>Heart Failure</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Δ Lower Dose</td>
</tr>
<tr>
<td>Δt (ms)</td>
<td>DOB</td>
<td>18.0±0.9</td>
</tr>
<tr>
<td></td>
<td>EMD</td>
<td>18.4±1.1</td>
</tr>
<tr>
<td>ΔPFR/EDD (s)</td>
<td>DOB</td>
<td>1.49±0.12</td>
</tr>
<tr>
<td></td>
<td>EMD</td>
<td>1.53±0.10</td>
</tr>
<tr>
<td>EDP (mm Hg)</td>
<td>DOB</td>
<td>10.1±1.5</td>
</tr>
<tr>
<td></td>
<td>EMD</td>
<td>11.5±1.0</td>
</tr>
</tbody>
</table>

*P<0.05 vs baseline; †P<0.05 vs lower dose; ‡P<0.05 vs normal; §P<0.05 vs DOB.

**Figure 4.** Amplification of HR contractility dependence by EMD-57033 or DOB. Ees increased with HR in CON (●), but rate sensitivity was greatly reduced in HF (▲). EMD-57033 restored rate sensitivity to control levels in HF (●), markedly enhanced it in CON (○), and, in both conditions, exceeded responses with DOB.
periods in the force-generating state. However, the combined PDE3I effects of many sensitizers may limit improved efficiency in vivo. For example, Tokada et al[^4] found that the oxygen cost of enhancing contractility by levosimendan was similar to that obtained with isoproterenol. Other compounds with combined sensitization and PDE3I activity have similarly yielded minimal oxygen-sparing effects[^5]. The net energetic effect likely depends on the relative balance between Ca\(^{2+}\) sensitization and PDE3I. In the case of EMD-57033, the persistence of favorable energetics and inotropic responses in HF even at the higher dose supports a balance favoring sensitization.

**Effects on Diastolic Function**

In isolated muscle, EMD-57033 shifts leftward the force-pCa\(^{2+}\) relation, reflecting enhanced filament sensitization to Ca\(^{2+}\) in both systolic and lower diastolic ranges[^7]. This could prolong the time required for strongly bound cross-bridges to detach, lengthening the duration of tension decay and increasing diastolic stiffness. Prior studies of isolated ferret trabeculae or rabbit hearts reported longer relaxation times and higher diastolic pressure or resting force after EMD-57033 administration[^8]–[^10] and shorter diastolic cell length[^6], all of which support myofilament activation. This has led to suggestions that concurrent

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**Figure 5.** Effects of EMD-57033 and DOB on postrest potentiation decay. Top, Maximal dP/dt from sequential beats after abrupt cessation of rapid pacing, with data for each successive beat (y axis) versus data for the prior beat (x axis). An increased slope of this relation reflects enhanced Ca\(^{2+}\) recycling by the SR. DOB increased the slope at both doses, whereas low-dose EMD-57033 had no effect and high-dose EMD-57033 increased it like the response to DOB. Bottom, Summary data for recirculation fraction results (see text for details).

**Figure 6.** Energetic cost of enhanced contractility and efficiency change from low-dose (EMD-L) to higher-dose (EMD-H) EMD-57033. Data are contrasted to those for DOB, milrinone (MIL), and milrinone with preload volume restoration (MIL+V). *P*<0.05 versus DOB by repeated measures ANOVA. †P<0.01 versus MIL+V. P values on top right of each panel are for ANOVA with all 5 groups. Top left, Percent change in dP/dt\(_{\text{max}}\). Bottom left, Percent change in MVO\(_2\). Top right, Percent change in the oxygen cost of increasing dP/dt\(_{\text{max}}\). Bottom right, Percent change in efficiency. Data are mean±SEM. (See text for details.)
PDE3I may be necessary to obviate detrimental diastolic effects from selective Ca\(^{2+}\) sensitization. However, we did not observe adverse diastolic influences even at the lower EMD-57033 dose. At both doses, EMD 57033 improved peak filling rates, and EDP declined. Shortening of relaxation at the higher dose likely reflected concurrent PDE3I.

The reduced EDP and improved filling despite little change in relaxation with low-dose EMD-57033 are intriguing and could reflect increased elastic restoring forces due to ejection to lower systolic volumes. This mechanism would be relevant to ejecting but not isolated isovolumic or isometric hearts or tissues and could explain the discrepancy with prior studies. This is not the only explanation; Slinker et al. reported negative lusitropic effects from EMD-57033 in both isovolumic and ejecting animals versus isolated, crystalloid-perfused hearts.

**Force-Frequency Potentiation**

The dependence of cardiac contractility on HR is principally due to an increased Ca\(^{2+}\) transient associated with enhanced SR Ca\(^{2+}\) loading from a calmodulin II–dependent pathway. The force-frequency response is blunted in human and experimental heart failure and can contribute to impaired cardiac reserve during stress or exercise. The force-frequency dependence can be enhanced by β-adrenergceptor stimulation and adenylate cyclase activation, although phosphorylation of phospholamban is not needed for the rate-dependent acceleration of SR Ca\(^{2+}\) uptake. Conversely, blunted adrenergic signaling or diminished adenylate cyclase with HF impairs this amplification, as found in the present study. EMD-57033, in contrast, markedly increased the force-frequency response in both CON and HF. This is an important feature of Ca\(^{2+}\) sensitizers, which not only enhance basal function but also can improve the contractile response to chronotropic reserve.

**Conclusions**

Despite the disappointing results of trials with inotropic agents, HF with depressed systolic function remains a disorder in which cardiac pumping capacity is often inadequate to meet the requirements of the organism. Ca\(^{2+}\) sensitizers potentially offer an important avenue for the enhancement of function, particularly if they simultaneously provide an energy-sparing effect. The present data are important in that they provide the first demonstration that considerable improvement in systolic performance can be achieved with Ca\(^{2+}\) sensitization in intact failing hearts at substantial energetic savings and without compromise of diastolic function. Such agents have the potential to improve the treatment of HF.

**References**


7. Tobias HA, Slinker BK, Kirkpatrick RD, Campbell KB. Functional effects of EMD-57033 in isovolumically beating isolated rabbit hearts, which highlights the differences between the performance of such compounds in conscious animals versus isolated, crystalloid-perfused hearts.

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