Mechanoenergetic Effects of Pimobendan in Canine Left Ventricles Comparison With Dobutamine

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Background. We hypothesized that the effect of pimobendan (UD-CG 115 BS) to increase calcium sensitivity of contractile protein might result in less myocardial oxygen consumption (Vo2) in comparison with dobutamine when they enhance ventricular contractility to the same extent. To examine this hypothesis, we compared the effects of pimobendan and dobutamine on left ventricular contractility and energetics using the frameworks of E\text{max} (contractility index) and the relation between Vo2 and PVA (systolic pressure–volume area, a measure of left ventricular total mechanical energy).

Methods and Results. We measured Vo2, E\text{max}, PVA, and force–time integral (FTI) in excised, cross-circulated, nonfailing dog hearts. The slope of the Vo2–PVA relation reciprocally indicates the efficiency from PVA-dependent Vo2 to the total mechanical energy (contractile efficiency). The Vo2 intercept of the Vo2–PVA relation, i.e., PVA-independent Vo2, reflects energy utilization for excitation–contraction coupling. The ratio of FTI to PVA-dependent Vo2 can be called contractile economy. Both drugs comparably enhanced E\text{max}. Although the contractile economy was greater by 14±19% (p<0.05) for pimobendan than for dobutamine, the contractile efficiency was similar between the two drugs. Oxygen cost of contractility, defined as the slope of the relation between the PVA-independent Vo2 and E\text{max}, was the same between the two drugs. Other mechanoenergetic effects of both drugs were similar except for a greater coronary vasodilating effect of pimobendan.

Conclusions. Pimobendan has almost the same mechanoenergetic effects as dobutamine but slightly greater contractile economy and coronary vasodilation. The calcium-sensitizing effect of pimobendan did not save the oxygen cost of contractility. (Circulation 1992;86:1291–1301)

Key Words • pressure–volume relation • contractility, oxygen cost • myofilament, Ca\textsuperscript{2+} sensitivity

For treatment of chronic heart failure, many types of positive inotropic agents, especially cyclic adenosine monophosphate (cAMP)–phosphodiesterase (PDE) inhibitors such as amrinone and milrinone, have been developed as alternative drugs to cardiac glycosides and catecholamines.1 However, recent studies have shown that these PDE inhibitors are accompanied by similar increases in cardiac oxygen consumption (Vo2) to catecholamines when they enhance contractility to the same extent.2,3 In these energetic aspects, PDE inhibitors do not seem to be superior to catecholamines.

Pimobendan (UD-CG 115 BS), a pyridazinone benzimidazole derivative, inhibits cAMP-PDE,4 prolongs the action potential duration of twitching myocardium,5 and increases the calcium sensitivity of contractile proteins in skinned guinea pig, dog, and failing human cardiac muscle fibers.6–7 These results suggest that pimobendan could produce higher contractility than other positive inotropic agents that have no Ca\textsuperscript{2+}-sensitizing effect at any levels of intracellular Ca\textsuperscript{2+} concentration; through an increase in Ca\textsuperscript{2+} sensitivity, pimobendan might result in less Vo2 for increased contractility compared with other positive inotropic agents without Ca\textsuperscript{2+}-sensitizing effect.

Holubarsch et al8 found that pimobendan increased myocardial oxygen consumption less than isoproterenol for a given increase in force–time integral in guinea pig papillary muscles and human idiopathic dilated cardiomyopathy hearts.9,10 However, they did not find that pimobendan had an advantage over cardiac glycosides in contractile economy.8 Because they only studied the effect of pimobendan on contractile economy and not on contractile efficiency and oxygen cost of contractility, the cardiac energetic advantage of pimobendan has not been fully studied.

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The purpose of this study was to assess the effects of pimobendan on left ventricular (LV) contractility and energetics in comparison with dobutamine by using our concept of $E_{\text{max}}$ (ventricular contractility index) and pressure-volume area (PVA, a measure of the total mechanical energy yielded by the LV). Dobutamine does not alter the contractile efficiency.\textsuperscript{11} We examined whether pimobendan alters the contractile efficiency of the LV and whether the oxygen cost of $E_{\text{max}}$ with pimobendan is smaller than with dobutamine in the blood-perfused dog heart.

**Methods**

**Surgical Preparation**

We used the excised, cross-circulated dog heart preparation as previously described in detail.\textsuperscript{2,11-13} Briefly, two mongrel dogs (weight, 12–22 kg) were anesthetized with sodium pentobarbital (30 mg/kg i.v.) after premedication with ketamine hydrochloride (5 mg/kg i.m.). Both dogs were heparinized (1,000 units/kg). The left subclavian artery and the right ventricle (RV) of the donor dog heart were cannulated and connected to the common carotid arteries and the external jugular vein of the support dog, respectively. The heart of the donor dog was excised from the chest after cross-circulation was started so that there was no interruption of coronary circulation. Systemic hypotension under cross-circulation was prevented with diphenhydramine hydrochloride (30–60 mg i.m.) and indomethacin (0.5–1.0 mg/kg i.v.).

A thin, latex balloon (unstressed volume, 60 ml) with a miniature pressure transducer (model P-7, Konigsberg Instruments, Pasadena, Calif.) was placed in the LV. The balloon was connected to a servo pump (International Servo Data, Tokyo), with which we were able to precisely control and accurately measure instantaneous LV volume. The LV epicardial ECG was recorded to trigger the volume command signal of the servo pump.

Systemic arterial blood pressure of the support dog served as coronary perfusion pressure (CPP) of the excised heart. The mean level of this pressure was reasonably stable (120±18 mm Hg) throughout each experiment. To keep this pressure above 80 mm Hg, either fresh blood obtained from the donor dog heart or dextran solution was infused as needed. Arterial pH, $P_{O_2}$, and $P_{CO_2}$ of the support dog were maintained within their physiological ranges by using supplemental oxygen and intravenous sodium bicarbonate as needed. Temperature of the heart was monitored and maintained at 35–37°C.

Total coronary blood flow (CBF) was continuously measured with an electromagnetic flowmeter (MFV-2100, NIHON Koden, Tokyo) in the coronary venous drainage tube from the RV. We neglected LV thebesian venous flow because of its very small fraction in the total coronary flow.\textsuperscript{14} Coronary vascular resistance (CVR) was calculated as CPP divided by CBF. Coronary arteriovenous $O_2$ content difference was measured continuously with our custom-made oximeter (PWA-200A, Erma Inc., Tokyo).\textsuperscript{15} The oximeter was calibrated against an IL-282 CO-oximeter (Instrumentation Laboratory Inc.) at the beginning of each experiment.

**Experimental Protocol**

Experiments were performed on 15 isolated hearts. To eliminate the difference of the chronotropic effect of pimobendan and dobutamine, the heart rate was kept constant at 147±7 beats per minute by left atrial pacing.

Sequential measurements were made either at various LV volumes in a stable contractile state (volume run) or at a fixed LV volume in variously altered contractile states with dobutamine, pimobendan, or propranolol and pimobendan (inotropism run).

**Control volume run (12 hearts).** First, in a stable control contractile state, we produced steady-state isovolumic contractions at six to eight different LV volumes including $V_0$ to obtain a control relation between $V_0$ and PVA. We waited 2–3 minutes after each change in LV volume until the cardiac variables were stabilized after transient changes. Because we used isovolumic contractions, the obtained $V_0$–PVA relation should be independent of confounding effects of the mode of contraction.\textsuperscript{13,14}

**Dobutamine and pimobendan inotropism runs (12 hearts).** We fixed LV volume at 22.6±4.9 ml, a level of moderate peak isovolumic pressure (about 80 mm Hg). Dobutamine and pimobendan inotropism runs were performed once after the other in each heart to enhance LV contractility. The dobutamine inotropism run preceded the pimobendan inotropism run in six experiments (dobutamine first). The pimobendan inotropism run preceded the dobutamine inotropism run in the other six experiments (pimobendan first).

In the dobutamine inotropism run, dobutamine was continuously infused into the coronary arterial perfusion tube with an infusion pump (STC-521, Terumo, Tokyo) at a starting rate of 1.0 mg/min. The infusion rate was increased in steps every 5 minutes until $E_{\text{max}}$ was nearly doubled to obtain six to eight sets of $E_{\text{max}}$, $V_0$, PVA, and other data at the preset volume. The maximal infusion rate of dobutamine averaged 7.5±6.2 μg/min in all 12 hearts.

In the pimobendan inotropism run, pimobendan was continuously infused into the coronary arterial tube to give a constant plasma concentration of about 1 μM. This dose of pimobendan gradually increased LV contractility and resulted in a nearly doubled $E_{\text{max}}$ in about 30 minutes. In each of the six to eight stably enhanced contractile states, measurements were repeated. Moreover, in a stably enhanced contractile state with pimobendan, we obtained another $V_0$–PVA relation in a similar manner to the control volume run (pimobendan volume run). We arbitrarily selected the contractile state for this run in the middle of the pimobendan inotropism run.

The dobutamine and pimobendan runs were intervened by a 20-minute (dobutamine first) or a 45-minute (pimobendan first) period of recovery. The contractility in the dobutamine-first group returned to pre–dobutamine infusion level after the 20-minute recovery period. However, the contractility in the pimobendan-first group after the 45-minute recovery period was significantly greater than the prepimobendan level because of the long-lasting action of pimobendan ($E_{\text{max}}$ was 32.9±24.4% greater than the prepimobendan level). Therefore, the subsequent comparisons of the two drugs were performed in two ways: for the data in the
dobutamine-first group (n=6) and for pooled data of both drugs (n=12).

Propanolol–pimobendan inotropism run (three hearts).
In the remaining three hearts, effects of pimobendan were assessed under β-adrenergic blockade. The LV volume was fixed at a moderate level of 25.6±7.9 ml where peak isovolumic pressure was about 80 mm Hg. β-Adrenergic receptors were blocked with propranolol (initial bolus dose of 0.3 mg followed by continuous infusion rate of 0.01 mg/min into the coronary arterial tube). Measurements were repeated in the same way as in the pimobendan inotropism run.

Circulating catecholamines (12 hearts). To examine whether any circulating catecholamines released from the support dog modified the positive inotropic effect of pimobendan, epinephrine and norepinephrine concentrations were measured in coronary arterial blood before and after the pimobendan inotropism run. The analysis was performed at the Otsuka Assay Laboratories of Otsuka Pharmaceuticals.

KCl arrest. Nine hearts (five hearts in the dobutamine-first group and four hearts in the pimobendan-first group) were arrested at V0 by injecting KCl (5–6 ml bolus dose of 0.75 eq/l) into the coronary arterial tube. When both coronary flow and arteriovenous O2 content difference were stabilized, V0 was determined as basal metabolic VO2 under KCl arrest.

Drugs. Dobutamine and propranolol were dissolved in physiological saline. Because pimobendan is almost insoluble in water, we dissolved it in a solvent composed of mannitol 500 mg and methane sulfonic acid 30 mg in 10 ml distilled water before each experiment. We confirmed that intracoronary infusion of the solvent at a rate (30–100 ml/hr) equivalent to that used for the administration of pimobendan did not affect LV mechanical energetics in a preliminary study in three hearts (Emax, 5.0±0.3 mm Hg·ml−1·100 g to 4.9±0.3 mm Hg·ml−1·100 g; VO2 intercept of the VO2-PVA regression line, 0.027±0.008 ml O2/beat/100 g to 0.027±0.009 ml O2/beat/100 g).

Plasma level of pimobendan and its metabolite (UD-CG 212 CL) in support dogs. In nine support dogs, plasma levels of pimobendan and its metabolite (UD-CG 212 CL) were measured in arterial blood before the pimobendan run and every 15 minutes thereafter. The Nippon Boehringer Ingelheim Company measured the levels.

Data Analysis
All data were sampled at 2-msec intervals, and the digital data were analyzed with a signal processor (7T18, NEC San-Ei, Tokyo).

Contractility. The LV contractility was assessed by two indexes, mainly by Emax and auxiliarly by dP/dtmax.

The Emax sensitively reflects ventricular contractility for the most part independent of ventricular loading condition (Figure 1A). The Emax was computed as the maximum value of the instantaneous ratio P(t)/[V(t)−V0] where P(t) and V(t) are LV instantaneous pressure and volume, respectively, in each contraction. The V0 was determined as the volume at which peak isovolumic pressure was zero. The Emax was normalized for LV weight and presented as mm Hg·ml−1·100 g. The Tmax was determined as the time to Emax from the rising phase of R wave of ECG (end diastole). The dP/dtmax was determined as the positive maximum value of the first derivative of P(t).

Relaxation. The time course of LV relaxation was assessed by three indexes: −dP/dtmax, time to −dP/dtmax, and τ. The −dP/dtmax (mm Hg/sec) is the peak rate of LV pressure decay, determined as the negative maximum of the first derivative of P(t). Time to −dP/dtmax (msec) is the time to −dP/dtmax from end diastole. τ is the time constant of LV pressure decay during the relaxation phase. τ was calculated as the reciprocal of the negative slope of the ln P(t) versus time relation, using the digitized data from the time of −dP/dtmax until LV pressure fell to 5 mm Hg above the end-diastolic pressure.

Pressure–volume area (PVA). The PVA is the area circumscribed by the end-systolic pressure–volume (P-V) relation line, the end-diastolic P-V curve, and the systolic P-V trajectory (Figure 1A). The PVA represents the total mechanical energy generated by each contraction of the LV. We calculated PVA of each beat from the digitized P(t) and V(t) data in the same way as before. The PVA was normalized for 100 g LV, and its dimensions are mm Hg·ml·beat−1·100 g−1.

Oxygen consumption. The VO2 was obtained as the product of CBF and arteriovenous oxygen content difference. It was divided by heart rate to obtain VO2 per beat in steady state. To minimize the contribution of RV VO2 to the measured total VO2, we kept the RV collapsed by continuous hydrostatic drainage of the coronary venous blood. The collapsed RV was assumed to have virtually zero PVA and hence no PVA-dependent VO2.

The RV component of PVA-dependent VO2 was calculated by multiplying biventricular PVA-independent VO2 in each contractile state with (RV weight)/(LV+RV weight). The RV-PVA-independent VO2 was subtracted from the total VO2 to yield LV VO2. At the end of each experiment, the LV including the septum and the RV free wall were weighed. They were 80.2±11.6 g (SD) and 24.4±6.3 g, respectively.

VO2-PVA relationship. The VO2-PVA relationship was obtained because the relation between VO2 and PVA is linear in a stable, contractile state (Figure 1B). The VO2 and PVA data in the control volume run and pimobendan volume run were subjected to linear regression analysis. The slope a in the regression line, 1/a is the contractile efficiency, and b is the VO2 intercept. The aPVA value represents the PVA-dependent VO2, and b represents the PVA-independent VO2, which reflects the VO2 component of both excitation–contraction (E-C) coupling and basal metabolism (Figure 1B).

PVA-independent VO2. We calculated PVA-independent VO2 for each enhanced Emax during the dobutamine and pimobendan inotropism runs (Figure 2A). The VO2 of a contraction at an enhanced Emax consists of three components: from the top down, an increase in PVA-independent VO2, PVA-dependent VO2, and the same PVA-independent VO2 as the control value (=b). The sum of the first and the last VO2 terms is the PVA-independent VO2 of this contraction at the enhanced Emax. The second VO2 term, PVA-dependent VO2, was calculated as the product of the slope a and PVA of this contraction. The PVA-independent VO2 at each enhanced Emax was calculated as LV VO2 minus aPVA. In this calculation, we assumed that slope a remained the same at all Emax levels, based on the parallelism of the
VO2-PVA relation.11,14 The parallelism was also confirmed in this study.

Oxygen cost of \( E_{\text{max}} \). Enhancement of \( E_{\text{max}} \) elevates the VO2-PVA relation in a parallel manner (Figure 1C).14 Therefore, the relation between the PVA-independent VO2 values and the corresponding \( E_{\text{max}} \) values in each of the dobutamine and pimobendan inotropism runs in individual hearts was obtained by linear regression analysis, as schematically shown in Figure 1D. The slope (c) of this regression line, or the ratio of an increase in PVA-independent VO2 to an increase in \( E_{\text{max}} \), was obtained as oxygen cost of \( E_{\text{max}} \).2,12

Pressure–time integral (PTI) and force–time integral (FTI). The PTI and FTI are the time integral of LV pressure and total ventricular wall force through one cardiac cycle. Total ventricular wall force (F) (g) was calculated as 1.36 (g · cm⁻² · mm Hg⁻¹) times the product of ventricular pressure (P) (mm Hg) and lumen cross-sectional area (A) (cm²) based on the force–equilibrium equation for a sphere.16 We assumed that lumen area A was constant throughout one cardiac cycle of isovolumic contractions. Thus, \( F=1.36 \cdot P \cdot A = 1.64 \cdot P \cdot V^{2/3} \). To obtain FTI, this total wall force was integrated throughout one cardiac cycle according to the previously reported methods.17,18

Contractile economy. Contractile economy was determined as the ratio of FTI to the FTI-dependent VO2 (g · sec/ml O₂) at control and a matched maximally enhanced \( E_{\text{max}} \) in the dobutamine and pimobendan inotropism runs at the same LV volume (Figure 2B). Because
comparisons of the regression lines of PVA-independent $V_O_2$ on $E_{max}$ between the dobutamine and pimobendan inotropic runs were performed by analysis of covariance (ANCOVA). The statistical significance of these differences was determined by $F$ test. Comparisons of mean values between the dobutamine and pimobendan runs in six hearts in dobutamine-first run and in all 12 hearts (Table 1) were performed by paired $t$ test. A value of $p<0.05$ was considered statistically significant. Data are presented as mean±SD. ANOVA was applied to compare mean values of the contractile economy before and after infusion of dobutamine and pimobendan. When ANOVA showed statistical significance by $F$ test, the mean values were compared by the least significant difference method.

**Results**

**Control Volume Run**

The control $E_{max}$ value was $4.0±1.6$ mm Hg·ml$^{-1}$·100 g in all pooled 12 hearts. In every tested heart, $V_O_2$ increased linearly with $PVA$ with a correlation coefficient close to unity ($0.987±0.008$) in the control contractile state. The slope ($a$) and $V_O_2$ intercept ($b$) of the control $V_O_2$-$PVA$ relation were $1.665±0.330×10^{-5}$ ml $O_2$·mm Hg$^{-1}$·ml$^{-1}$ and $0.0218±0.0029$ ml $O_2$·beat$^{-1}$·100 g$^{-1}$, respectively. There was no significant difference between the dobutamine-first and pimobendan-first groups in $E_{max}$, correlation coefficient, or $a$ or $b$.

**Dobutamine and Pimobendan Runs**

Figure 3 shows representative tracings of LV isovolumic pressure, volume, ECG, and coronary arteriogenous oxygen content difference during the pimobendan inotropic run in a representative heart in the dobutamine-first group. Pimobendan infusion increased $E_{max}$ from 6.1 to 10.5 mm Hg·ml$^{-1}$·beat$^{-1}$·100 g, PVA from 706 to 1,202 mm Hg·ml·100 g, and $V_O_2$ from 0.037 to 0.052 ml·$O_2$·beat$^{-1}$·100 g$^{-1}$. All other hearts showed similar results to this heart.

Table 1 summarizes mechanoenergetic variables before and after the comparable enhancement of $E_{max}$ with dobutamine and pimobendan. The left half of this table lists the data of the dobutamine-first group to assess the pure effect of dobutamine on mechanoenergetics because the long-lasting positive inotropic effect of pimobendan might have modified the effect of dobutamine in the pimobendan-first group. The right half of this table lists pooled (dobutamine-first and pimobendan-first groups) data in all 12 hearts.

At the comparably enhanced contractility, dobutamine and pimobendan had almost the same effect on relaxation parameters ($-dP/dt_{max}$ and $\tau$). However, pimobendan showed greater FTI and FTI than dobutamine despite the similar increases in $V_O_2$. Moreover, pimobendan increased CBF (by $62.6±55.8$% in the dobutamine-first data and by $84.3±50.6$% in the pooled data) and decreased CVR (by $46.9±19.2$% in the dobutamine-first data and by $48.6±14.4$% in the pooled data). In contrast, the changes with dobutamine were less marked; CBF increased by $39.6±18.9$% in the dobutamine-first data and by $35.1±18.4$% in the pooled data, and CVR decreased by $29.4±15.6$% in the dobutamine-first data and by $25.7±14.3$% in the pooled data.

**Statistics**

Comparisons of the $V_O_2$-$PVA$ regression lines between the control and pimobendan volume runs and
### Table 1. Summary of Changes in Cardiac Mechanoenergetics

<table>
<thead>
<tr>
<th></th>
<th>Dobutamine first (n=6)</th>
<th>Dobutamine Pooled (n=12)</th>
<th>Pimobendan</th>
<th>Pimobendan</th>
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<tr>
<td><strong>Run</strong></td>
<td><strong>Predrug</strong></td>
<td><strong>Maximum Em</strong></td>
<td><strong>Predrug</strong></td>
<td><strong>Maximum Em</strong></td>
</tr>
<tr>
<td>E_max (mm Hg/ml·100 g)</td>
<td>3.5±1.9</td>
<td>7.2±3.2</td>
<td>3.4±1.7</td>
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<td>ESP (mm Hg)</td>
<td>63.3±17.3</td>
<td>136.9±25.0</td>
<td>62.2±17.9</td>
<td>137.3±20.3</td>
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<td>EDP (mm Hg)</td>
<td>0.7±5.6</td>
<td>0.2±5.6</td>
<td>1.1±5.3</td>
<td>0.5±5.1</td>
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<td>T_max (msec)</td>
<td>179±12</td>
<td>162±13</td>
<td>182±21</td>
<td>164±10</td>
</tr>
<tr>
<td>dP/dt_max (mm Hg/sec)</td>
<td>683±173</td>
<td>1,560±352</td>
<td>687±223</td>
<td>1,619±275</td>
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<td>–dP/dt_max (mm Hg/sec)</td>
<td>565±137</td>
<td>1,477±339</td>
<td>607±205</td>
<td>1,567±262</td>
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<tr>
<td>Time to –dP/dt_max (msec)</td>
<td>293±25</td>
<td>250±24</td>
<td>288±34</td>
<td>249±14</td>
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<td>τ (msec)</td>
<td>40.5±15.1</td>
<td>30.0±9.1</td>
<td>42.0±15.6</td>
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<td>PTI (mm Hg/sec)</td>
<td>12.5±3.7</td>
<td>21.7±3.6</td>
<td>12.4±3.5</td>
<td>22.7±3.5*</td>
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<tr>
<td>FTI (g/sec)</td>
<td>164±38</td>
<td>287±40</td>
<td>163±46</td>
<td>299±39*</td>
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<td>CBF (ml/min)</td>
<td>59±31</td>
<td>79±33</td>
<td>81±79</td>
<td>122±62*</td>
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<tr>
<td>CPP (mm Hg)</td>
<td>137±23</td>
<td>132±20</td>
<td>128±17</td>
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<tr>
<td>CVR (mm Hg/ml/min)</td>
<td>2.84±1.39</td>
<td>1.92±0.80</td>
<td>2.43±1.12</td>
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<tr>
<td>VO₂ (mlO₂/beat/100 g)</td>
<td>0.034±0.005</td>
<td>0.057±0.009</td>
<td>0.035±0.005</td>
<td>0.060±0.006</td>
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</table>

E_max, slope of the end-systolic pressure–volume relation; ESP, end-systolic pressure; EDP, end-diastolic pressure; T_max, time from onset of R wave of left ventricular (LV) epicardial ECG to E_max; dP/dt_max, maximum positive value of time derivative of LV pressure; –dP/dt_max, maximum negative value of time derivative of LV pressure; Time to –dP/dt_max, time from onset of R wave to –dP/dt_max; τ, time constant of LV pressure decay during the relaxation phase; PTI, time integral of LV pressure through one cardiac cycle; FTI, time integral of total ventricular wall force through one cardiac cycle; CBF, coronary blood flow; CPP, coronary perfusion pressure; CVR, coronary vascular resistance; VO₂, myocardial oxygen consumption.

*Significantly smaller or larger than maximum E_max in dobutamine run at p<0.05 by paired t test.
†Significantly smaller than predrug in dobutamine run at p<0.05 by paired t test.

Figures 4A and 4B show the VO₂–PVA data points in the dobutamine and pimobendan inotropism runs in a representative heart in the dobutamine-first group. With sequentially enhanced E_max in either inotropism run, the VO₂–PVA data points (open and solid circles) diverged right-upward from the control VO₂–PVA regression line (thick dotted lines). The resultant regressive lines for the two drugs (solid lines) appear to be superimposable.

Figure 4B also shows the VO₂–PVA relations in the control and pimobendan volume runs. The upper VO₂–PVA regression line obtained in the pimobendan volume run (thin dotted line) appears parallel to the control VO₂–PVA regression line. On the average, pimobendan elevated the VO₂–PVA regression line in a parallel manner and increased the VO₂- intercept from 0.0218±0.0029 ml O₂·beat⁻¹·100 g⁻¹ to 0.0295±0.0048 ml O₂·beat⁻¹·100 g⁻¹ (p<0.05). The slope of the VO₂–PVA line was (1.67±0.33)×10⁻⁵ ml O₂/(mm Hg·ml) in the control volume run and (1.64±0.38)×10⁻⁵ ml O₂/(mm Hg·ml) in the pimobendan volume run. No heart showed significant difference in the slope of the VO₂–PVA lines between the control and pimobendan volume runs (ANCOVA).

Figure 5A plots PVA-independent VO₂–E_max relations to Figure 5A were obtained for dobutamine and pimobendan in all other 11 hearts. Correlation coefficients of the relation were close to unity (median, 0.957 for dobutamine and 0.964 for pimobendan). ANCOVA showed no significant difference in their slopes (oxygen cost of E_max) between dobutamine and pimobendan in eight of 12 hearts. Three of the other four hearts had about 1.4 times greater oxygen cost of E_max for pimobendan than for dobutamine, whereas the remaining one heart had a twice-greater oxygen cost of E_max for dobutamine than for pimobendan.

Figure 5B compares mean values for oxygen cost of E_max with dobutamine and pimobendan in all 12 hearts. There was no significant difference in oxygen cost of E_max between the two drugs (0.00209±0.00068 ml O₂·ml·mm Hg⁻¹·beat⁻¹·100 g⁻² versus 0.00207±0.00094 ml O₂·ml·mm Hg⁻¹·beat⁻¹·100 g⁻²).

Figure 6A compares mean values of the contractile efficiency (the reciprocal of the slope of the VO₂–PVA relation) in the control and pimobendan volume runs in all 12 hearts. There was no significant difference between the two mean values (41.6±9.3% in the control volume run and 42.5±10.0% in the pimobendan volume run), indicating that pimobendan elevates the VO₂–PVA relation in a parallel manner, as does dobutamine.

Figure 6B compares mean values of the contractile economy before and after infusion of dobutamine and pimobendan in all 12 hearts. Dobutamine significantly decreased the contractile economy from the predobu-
The E_max decreased from 6.6±4.4 mm Hg · ml⁻¹ · 100 g at control to 3.3±1.6 mm Hg · ml⁻¹ · 100 g by β-adrenergic receptor blockade with propranolol. Under β-blockade, pimobendan infusion at a constant concentration of about 1 μM enhanced E_max to 6.1±3.3 mm Hg · ml⁻¹ · 100 g, which was similar to the prepropranolol control level. This result indicates that the positive inotropic effect of pimobendan is not due to a stimulation of the β-adrenergic receptor system. In the pimobendan inotropism run, CBF was greater (74±21 ml/min for control versus 113±43 ml/min for pimobendan), and CPP (125±12 mm Hg versus 95±7 mm Hg) and CVR (1.78±0.62 mm Hg/ml/min versus 0.97±0.53 mm Hg/ml/min) were smaller than the control values at a matched E_max in all three hearts.

Circulating Catecholamines

Epinephrine and norepinephrine concentrations in the support dog's arterial blood did not significantly change before and after the pimobendan inotropism run (epinephrine, from 0.74±0.69 ng/ml to 1.00±0.87 ng/ml; norepinephrine, from 0.86±0.51 ng/ml to 1.23±0.73 ng/ml). This result indicates that the mechanoenergetic effects of pimobendan were not contaminated by catecholamines released from the support dog.

KCl Arrest

Basal metabolic VO₂ during KCl arrest was 1.30±0.36 ml O₂ · min⁻¹ · 100 g⁻¹ for pooled data (nine hearts) and 1.32±0.31 ml O₂ · min⁻¹ · 100 g⁻¹ for the dobutamine-first group, in which pimobendan was continuously infused into the hearts (five hearts). These basal metabolism values were close to our previously reported value in normal canine left ventricles. Thus, pimobendan did not influence VO₂ for basal metabolism. This result indicates that the elevation of the PVA-independent VO₂ with pimobendan was primarily due to an increase in VO₂ for E-C coupling.
Plasma Level E, in squares and solid bendan line. Regression during eto oxygen cost getic heart. The dotted diagonal lines through controls and squares in both panels indicate the V02-PVA regression line obtained in the control volume run (thick lines) and pimobendan volume run (thin line). With sequentially enhanced Emax in either inotropism run, the V02-PVA data points (open and solid circles) diverged right-upward from each V02-PVA regression line.

**FIGURE 4.** V02-PVA (pressure–volume area) data points obtained during sequential enhancing of contractility in the dobutamine (open circles) (panel A) and pimobendan inotropic runs (solid circles) (panel B) in a representative heart. The dotted diagonal lines through crosses and open squares in both panels indicate the V02-PVA regression line obtained in the control volume run (thick lines) and pimobendan volume run (thin line). With sequentially enhanced Emax in either inotropism run, the V02-PVA data points (open and solid circles) diverged right-upward from each V02-PVA regression line.

**Plasma Level of Pimobendan and UD-CG 212 CL in the Support Dog**

Although pimobendan had been continuously infused at about 1 μM into the coronary arterial tube, the mean plasma levels of pimobendan and UD-CG 212 CL at 45 minutes after starting pimobendan infusion were 0.07±0.03 μM and 0.06±0.05 μM, respectively.

**Discussion**

In the present study, we have shown that pimobendan and dobutamine have almost the same mechanoeenergetic effects in terms of both contractile efficiency and oxygen cost of Emax in the canine left ventricle. Although the contractile economy of pimobendan is statistically significantly greater than catecholamines, as Holubarsch et al619 pointed out, the difference is small. Contractile efficiency and economy, the two major myocardial energetic indexes, do not necessarily change in a parallel manner.

Because pimobendan is known to increase the Ca2+ sensitivity of contractile proteins and dobutamine is known to decrease it, we expected that the oxygen cost of Emax of pimobendan would be smaller than that of dobutamine; pimobendan would consume less Vo2 than dobutamine in increasing contractility to the same level. However, we found almost the same oxygen cost of Emax between pimobendan and dobutamine. Why did we find the same energy cost of contractility for the two drugs?

In the pimobendan inotropism run, the cumulative concentration of pimobendan in the support dog was only 0.07 μM on average in the middle of the pimobendan run, although we continuously infused pimobendan into the coronary arterial tube at a level of about 1 μM. This indicates that the concentration of pimobendan entering into the excised heart was virtually constant throughout the run regardless of recirculation of pimobendan from the support dog. Even with pimobendan at about 1 μM, we were able to enhance LV contractility to the same level as dobutamine. The calcium-sensitizing effect of pimobendan is reported to appear at 50–100 μM in skinned cardiac muscles.6-20 However, such a high concentration of pimobendan is far out of
the physiological range for a blood-perfused whole heart. In fact, we found in our preliminary experiments that even 3 μM pimobendan markedly increased LV contractility by over 300%, resulting in LV peak isovolumic pressure above 250 mm Hg even at a moderate LV volume. Thus, the reason for the same oxygen cost of $E_{\text{max}}$ between pimobendan and dobutamine in our preparation might be attributable to the different effective concentrations of pimobendan as a Ca\textsuperscript{2+} sensitizer between the skinned myocardium and our more physiological heart preparation.

The enhancement of Ca\textsuperscript{2+} sensitivity with pimobendan in nonfailing myocardium has been rather controversial. One study in isolated ferret papillary muscles has indicated no change\cite{3} and another an increase\cite{22} in Ca\textsuperscript{2+} sensitivity. An increase in cAMP with inhibition of cAMP-PDE by pimobendan will lead to a decrease in Ca\textsuperscript{2+} sensitivity caused by phosphorylation of troponin I.\cite{23} Thus, in our study, the Ca\textsuperscript{2+}-sensitizing effect of pimobendan might have been masked by an increase in cAMP; hence, pimobendan might have had no influence on the oxygen cost of $E_{\text{max}}$.

The situation could be different in failing myocardium. Recently, Böhm et al\cite{15} have reported that only 0.01 μM of pimobendan increases Ca\textsuperscript{2+} sensitivity of human failing, skinned myocardium. This suggests that the effect of calcium sensitization on the oxygen cost of $E_{\text{max}}$ is manifest in failing heart but not in normal hearts. We must consider UD-CG 212 CL, a metabolite of pimobendan. The mean concentration of UD-CG 212 CL in the middle of the pimobendan run was only 0.06 μM. However, UD-CG 212 CL was more than 500 times as potent as pimobendan in increasing the force of myocardial contraction.\cite{24} This concentration of UD-CG 212 CL seems close to its clinically measured level in patients with congestive heart failure when a maximum increase in cardiac output was achieved after a single, 10-mg oral dose of pimobendan.\cite{25} In addition, UD-CG 212 CL has been shown to be a more potent PDE inhibitor\cite{3} and to have little Ca\textsuperscript{2+}-sensitizing effect.\cite{5} Therefore, even such a low concentration (0.06 μM) of UD-CG 212 CL might have modified the positive inotropic effect of pimobendan with no influence on the Ca\textsuperscript{2+}-sensitizing effect in our study. This problem would not occur in a preparation without recirculation of the perfusate but may be inevitable in a more physiological preparation such as ours or in a clinical setting.

The difference between the contractile efficiency and contractile economy should be discussed. There is a possibility that the Ca\textsuperscript{2+}-sensitizing effect of pimobendan might be reflected to increase contractile economy but not contractile efficiency. A previous study has suggested that increased Ca\textsuperscript{2+} sensitivity prolongs the time course of tension development and increases resting tension.\cite{26} In contrast, catecholamines in general shorten the contraction duration while increasing contractility.\cite{9,23} The dimensions of contractile economy are J · g\textsuperscript{-1} · sec\textsuperscript{-1} or cm/sec (i.e., the unit of velocity), which differ from the unit of contractile efficiency (J/sec or dimensionless).\cite{14} Therefore, when the Ca\textsuperscript{2+}-sensitizing effect of pimobendan exerts a positive inotropic effect, the contractile economy with pimobendan could be greater than that with dobutamine despite the same contractile efficiency. In fact, Goto et al\cite{18} have demonstrated that isoproterenol increases the slope of the $V_O_2$–FRI relation, i.e., decreases contractile economy without affecting contractile efficiency.

**FIGURE 6.** Panel A: Bar graph compares mean values of contractile efficiency between the control and pimobendan volume runs. Panel B: Comparison of mean values of contractile economy before (Cont.-1 or Cont.-2) and after dobutamine or pimobendan infusion. NS, not significant. *Statistically significant.

**FIGURE 7.** Plot shows relation between pressure–time integral (PTI) versus $E_{\text{max}}$ before and after the enhancement of $E_{\text{max}}$ in the dobutamine inotropism run (open circles) and the pimobendan inotropism run (solid circles). Slopes of the solid and dashed lines indicate $\Delta PTI/\Delta E_{\text{max}}$. Note that pimobendan had a greater $\Delta PTI/\Delta E_{\text{max}}$. The data are from the human heart. The slope of the solid line is 0.4; the slope of the dashed line is 0.2. **A:** The effect of pimobendan on human heart function is shown in *Panel A*. The open circles indicate the control condition, and the solid circles indicate the pimobendan condition. The slope of the solid line is 0.4; the slope of the dashed line is 0.2. **B:** The effect of pimobendan on human heart function is shown in *Panel B*. The open circles indicate the control condition, and the solid circles indicate the pimobendan condition. The slope of the solid line is 0.4; the slope of the dashed line is 0.2.
We also calculated the ratio of the increase in PTI to the increase in $E_{\text{max}}$ ($\Delta PTI/\Delta E_{\text{max}}$) in the dobutamine and pimobendan inotropism runs at a matched end-diastolic volume. Figure 7 shows mean PTI and $E_{\text{max}}$ values before and after dobutamine and pimobendan. The $\Delta PTI/\Delta E_{\text{max}}$ was slightly but significantly greater with pimobendan (2.35±0.82 sec⋅ml·100 g LV) than with dobutamine (2.12±0.85 sec⋅ml·100 g LV, $p<0.05$). This finding, together with slightly longer $T_{\text{max}}$ value of pimobendan than dobutamine (Table 1), indicates that pimobendan facilitates maintenance of tension more than dobutamine for a given positive inotropic effect. This effect might reflect the Ca$^{2+}$-sensitizing effect of pimobendan.

The other difference between pimobendan and dobutamine is the greater coronary vasodilating effect of pimobendan than that of dobutamine at the same level of enhanced contractility. Furthermore, in the propranolol–pimobendan inotropism run, the restoration of the control contractility level with pimobendan was accompanied by increased coronary flow above control. Recently, Goto et al.$^{27}$ have shown that a doubled coronary flow with adenosine at a constant perfusion pressure increases $E_{\text{max}}$ by 18% (Gregg's phenomenon$^{27}$) and produces a parallel upward shift of the $V_{O_2}$–PVA relation. Therefore, it seems that the enhanced contractility with pimobendan was attributable at least in part to the increased coronary flow, or Gregg's phenomenon, besides inhibition of cAMP-PDE. In the study by Goto et al.$^{27}$ coronary hyperemia did not abbreviate the duration of contraction (personal communication), which is in contrast to catecholamines. Thus, the potent coronary vasodilating effect of pimobendan could be another factor that produced larger PTI and FTI than dobutamine at matched $E_{\text{max}}$ and $V_{O_2}$ without changing the contractile efficiency.

The results of this study may have an important pathophysiological significance. Most diseased hearts accompany contractile dysfunction with not only insufficient tension development but also relaxation disturbance. In the present study, pimobendan maintained tension longer than dobutamine for a given positive inotropic effect without impairing relaxation. This tension-maintaining effect would enable pimobendan to be a positive inotropic drug superior to catecholamines in treating heart failure. A result from a recent clinical study that has indicated the beneficial effect of pimobendan on exercise tolerance and quality of life in patients with heart failure$^{28}$ might have come from the tension-maintaining effect of pimobendan. However, the actual difference in PTI between pimobendan and dobutamine at the comparably enhanced $E_{\text{max}}$, was only 6.2±9.8% in the present study in the normal heart. Further studies in failing hearts will be needed to determine whether the surplus in tension maintenance with pimobendan is pathophysiologically meaningful or clinically beneficial.

We have recently found that the oxygen cost of contractility with $2.0 \times 10^{-5}$ M CaCl$_2$ was 1.5 times$^{29}$ and 2.2 times$^{12}$ higher, respectively, in acidic and stunned hearts than in normal hearts. Both acidic and stunned hearts are known to have decreased Ca$^{2+}$ sensitivity.$^{30,31}$ In addition, Lee and Allen$^{32}$ have recently reported that EMD 53998, a new Ca$^{2+}$ sensitizer, has more potent Ca$^{2+}$-sensitizing effect than pimobendan. Studies using these failing heart models and purer Ca$^{2+}$ sensitizers may clarify the energetic significance of increasing Ca$^{2+}$ sensitivity on oxygen cost of contractility in the future.

**Summary**

We assessed the effects of pimobendan and dobutamine on LV mechanoenergetics using the frameworks of $E_{\text{max}}$ and the $V_{O_2}$–PVA relation in the excised, cross-circulated dog heart. Pimobendan exerts a positive inotropic effect even under $\beta$-blockade and has a greater coronary vasodilating effect than dobutamine, reflecting a different positive inotropic mechanism from catecholamines. However, both drugs have the same contractile efficiency, although pimobendan has a slightly greater contractile economy. In addition, the oxygen costs of contractility of the two drugs are the same. Therefore, the energetic effects of the two drugs are similar regardless of the different positive inotropic mechanisms, i.e., despite the known Ca$^{2+}$-sensitizing effect of pimobendan in blood-perfused, nonfailing dog hearts.

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