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ORIGINAL CONTRIBUTION

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Comparison of the effects of levosimendan, pimobendan, and milrinone on canine left ventricular-arterial coupling and mechanical efficiency

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Abstract We examined and compared the effects of levosimendan, a new myofilament calcium sensitizer with phosphodiesterase inhibiting activity, pimobendan, and milrinone on left ventricular-arterial coupling and mechanical efficiency in 21 experiments performed in open-chest, barbiturate-anesthetized dogs instrumented for measurement of aortic and left ventricular (LV) pressure (micromanometer-tipped catheter), +dP/dt, and LV volume (conductance catheter). Myocardial contractility was assessed with the end-systolic pressure-volume relation (E_{es}) and preload recruitable stroke work (M_{sw}) generated from a series of differentially loaded LV pressure-volume diagrams. LV-arterial coupling and mechanical efficiency were determined by the ratio of E_{es} to effective arterial elastance (E_a; the ratio of end-systolic arterial pressure to stroke volume) and the ratio of stroke work (SW) to pressure-volume area (PVA), respectively. Levosimendan (0.75, 1.5, and 3.0 $\mu g \cdot kg^{-1} \cdot min^{-1}$) significantly ($p < 0.05$) increased heart rate, +dP/dt, and ejection fraction (EF) and decreased mean arterial pressure (MAP), pressure-work index (PWI; an estimate of myocardial oxygen consumption), and LV systolic and end-diastolic pressures (LVSP and LVEDP) and volumes (EDV and ESV). Levosimendan-induced augmentation of myocardial contractility (E_{es}, M_{sw}, and +dP/dt) and reductions in LV afterload (E_a) caused increases in the E_{es}/E_a ratio (0.61 ± 0.10 during control to 3.3 ± 0.7 during the high dose) consistent with enhancement of LV-arterial coupling. Levosimendan increased SW/PVA (0.48 ± 0.05 during control to 0.84 ± 0.04 during the high dose), indicating this drug improves the transfer of myocardial potential energy to external work. Levosimendan also increased the ratio of SW to PWI (109 ± 18 during control to 255 ± 50 mmHg·min·100 g during the high dose), suggesting that myocardial metabolic efficiency was improved as well. Like levosimendan, pimobendan and milrinone (10, 20, and 40 and 1.0, 2.0, and 4.0 $\mu g \cdot kg^{-1} \cdot min^{-1}$, respectively) increased HR, +dP/dt, EF, E_{es}, and M_{sw} and decreased MAP, LVSP, LVEDP, EDV, ESV, and E_a. In contrast to levosimendan, neither agent reduced PWI. Pimobendan and milrinone caused dose-related increases in E_{es}/E_a, SW/PVA, and SW/PWI. The results indicate that levosimendan, pimobendan, and milrinone augment myocardial contractility, produce venous and arteriolar vasodilation, and enhance LV-arterial coupling and mechanical efficiency in open-chest, barbiturate-anesthetized dogs.

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Key words Heart: myocardial contractility – mechanical efficiency – end-systolic pressure-volume relation – ventricular-arterial coupling – pressure-volume area. Myofilaments: calcium sensitizer – troponin C. Pharmacology: inotropes – levosimendan, pimobendan, milrinone

Introduction

Levosimendan [(*R*)-[[4-(1,4,5,6-tetrahydro-4-methyl-6-oxo-3-pyridazinyl)phenyl]hydra-zono]propanedinitrile] is a new drug in the myofilament Ca^{2+} sensitizer class of positive inotropic compounds (19). Levosimendan has been shown to augment myocardial contractility by binding to troponin C and stabilizing the Ca^{2+}-bound conformation of this regulatory protein without directly affecting actin-myosin interaction (11, 18, 38), mechanisms of action that are similar to those of pimobendan and sulmazole (6, 13, 22, 41, 55). Unlike other myofilament Ca^{2+} sensitizers, however, levosimendan-induced increases in the binding affinity of Ca^{2+} to troponin C are dependent on intracellular Ca^{2+} concentration. Myofilament Ca^{2+} sensitivity is enhanced in the presence of higher Ca^{2+} concentrations found during systole but is relatively unchanged at low intracellular Ca^{2+} concentrations in diastole (16, 17). Similar to other myofilament Ca^{2+} sensitizers (6, 13, 53), levosimendan partially inhibits cardiac and vascular smooth muscle phosphodiesterase (PDE) isoforms, actions that contribute to the positive inotropic and lusitropic effects of higher doses of this drug (11). Previous studies have demonstrated that levosimendan enhances myocardial contractility, improves indices of diastolic function, and causes venous and arterial dilation in conscious and anesthetized dogs (20, 35, 36) and produces favorable hemodynamic alterations in humans with normal (27, 50) and abnormal left ventricular function (28).

Optimal transfer of stroke volume from the left ventricle to the arterial circulation requires appropriate matching of these mechanical systems. Left ventricular-arterial coupling has been shown to be conveniently studied in pressure-volume phase space by characterizing the elastances of the left ventricle (E_{es}) and the arterial vasculature (E_a) using left ventricular end-systolic pressure-volume and end-systolic arterial pressure-stroke volume relations, respectively (9, 48, 49). The ratio of E_{es} to E_a defines mechanical coupling between the left ventricle and the arterial circulation (48, 49) and provides a useful technique for assessment of the actions of pharmacological agents, including vasoactive drugs, on overall cardiovascular performance *in vivo* (24, 29, 43). In addition, the analysis of the pressure-volume plane creates a framework for the study of left ventricular mechanical efficiency defined by the ratio of stroke work (SW) to pressure-volume area (PVA) (45). This investigation was designed to compare the actions of levosimendan, pimobendan, and milrinone, a PDE inhibitor without myofilament Ca^{2+} sensitizing activity, on left ventricular-arterial coupling and mechanical efficiency in barbiturate-anesthetized dogs.

Materials and methods

All experimental procedures and protocols used in this investigation were reviewed and approved by the Animal Care and Use Committee of the Medical College of Wisconsin. All procedures conformed to the Guiding Principles in the Care and Use of Animals of the American Physiological Society and were performed in accordance with the Guide for the Care and Use of Laboratory Animals [DHEW(DHHS) publication (NIH) no. 85–23, revised 1985].

Implantation of instruments

Conditioned mongrel dogs (n = 23) of either sex weighing between 25 and 30 kg were fasted overnight and anesthetized with sodium pentobarbital (25 $mg \cdot kg^{-1}$) and sodium barbital (200 $mg \cdot kg^{-1}$). Fluid deficits were replaced prior to experimentation with 0.9 % saline (500 ml), which was continued at 3 $ml \cdot kg^{-1} \cdot hr^{-1}$ for the duration of each experiment. After tracheal intubation, the dogs were ventilated *via* positive pressure with a mixture (1 $L \cdot min^{-1}$) of oxygen (90 %) and air (10 %). Respiratory rate and tidal volume were adjusted to maintain acid-base status (pH = 7.35 – 7.40) and carbon dioxide partial pressure (PCO_2 = 30 – 35 mmHg) within physiologic limits. The right femoral vein was isolated through a small incision and a catheter was placed in this vessel for fluid and drug administration. A 7F, dual micromanometer-tipped catheter (Millar Instruments, Houston, TX) was inserted through the left carotid artery and positioned across the aortic valve with the distal transducer in the left ventricle and the proximal transducer in the ascending thoracic aorta for measurement of continuous left ventricular and arterial pressures, respectively. The peak rate

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of increase of left ventricular pressure ($+dP/dt_{max}$) was determined by electronic differentiation of the left ventricular pressure waveform. A thoracotomy was performed in the left fifth intercostal space, and the lung was gently retracted. The pericardium was incised, and the heart was temporarily elevated from the thoracic cavity to allow access to the left ventricular apex. A 7F eight-electrode conductance catheter with a fluid-filled lumen (Webster Labs, Baldwin Park, CA) was inserted into the left ventricular cavity through a small incision in the apex. Using a fluid-filled pressure transducer system, the conductance catheter was positioned so that the distal tip was located in the ascending thoracic aorta just beyond the aortic valve. The conductance catheter was secured firmly with a purse string suture. A hydraulic vascular occluder was positioned around the inferior vena cava for abrupt alteration of left ventricular preload. Lastly, a fluid-filled catheter was placed in the left atrial appendage for administration of hypertonic saline (20 %; 5 ml) used to determine parallel conductance volume (V_p). The experimental preparation was allowed to stabilize for at least 30 min after instrumentation had been completed.

Measurement of left ventricular volume

The conductance technique was used to measure left ventricular volume (3). This method has been shown to accurately determine beat-to-beat changes in stroke volume (SV) and end-diastolic volume (EDV) under a variety of experimental conditions *in vivo* (1, 2, 4, 25). The multielectrode catheter was interfaced to a conductance module designed and constructed in our laboratory that drove a constant current (20 μA at 5 kHz) between the two outermost electrodes and measured the resultant voltage difference between each adjacent remaining electrode. Counterclockwise development of each left ventricular pressure-segmental volume diagram identified electrode pair signals that were located within the left ventricle. Measured time-dependent left ventricular volume [V(t)] was determined using the equation: $V(t) = G(t) \cdot L^2 \cdot (\alpha \cdot \sigma)^{-1} - V_p$, where G(t) = the sum of time-dependent conductances from each intraventricular electrode pair, L = the intraelectrode distance (1.0 cm), α = a slope correction factor relating the measured conductance volume to actual left ventricular volume (assumed to equal 1), and σ = the blood conductivity.

Parallel conductance (offset) volume was determined using the hypertonic saline technique (3, 4) and subtracted from measured volume to obtain absolute left ventricular volume during each experimental intervention. No changes in parallel conductance volume were observed during drug infusions. Blood conductivity (σ) was determined at each intervention from a 5 ml blood sample using a cuvette that was precalibrated with solutions of known conductivity. No changes in σ were observed during each experiment. Previous investigations have shown that α is approximately equal to one (range = 0.78 to 1.2) and remains relatively constant during a variety of physiological or pharmacological interventions (1, 3, 7, 52). End-systolic volume (ESV) and EDV were measured at maximum left ventricular elastance (47) and immediately prior to the onset of left ventricular isovolumic contraction, respectively. Typical hemodynamic waveforms and left ventricular pressure-volume diagrams obtained during abrupt occlusion of the inferior vena cava are depicted in Fig. 1. Ejection fraction (EF) was determined using the equation: $EF = (EDV\text{-}ESV) \cdot EDV^{-1}$. The pressure-work index (PWI), a calculated estimate of global myocardial oxygen consumption, was determined using a formula validated previously in anesthetized dogs (39). Hemodynamic data were continuously recorded on a polygraph (model 7, Grass Instruments, Quincy, MA) and simultaneously digitized by a computer interfaced with an analog to digital converter for recording and subsequent analysis of left ventricular pressure-volume diagrams.

Experimental protocol

After instrumentation had been completed, left ventricular pressure-volume diagrams used to assess myocardial contractility were obtained at end-expiration by abruptly decreasing left ventricular preload *via* inflation of the inferior vena caval balloon cuff occluder, resulting in an approximately 25 mmHg decline in left ventricular systolic pressure over 10 to 15 cardiac cycles (Fig. 1). Using a linear regression analysis, the end-systolic pressure (P_{es}) and volume (V_{es}) of each left ventricular pressure-volume diagram during the inferior caval occlusion were fit to the equation: $P_{es} = E_{es} \cdot (V_{es}\text{-}V_o)$, where E_{es} = left ventricular end-systolic elastance and V_o = the extrapolated volume intercept of the relation. Myocardial contractility was also evaluated with the preload recruitable stroke work (PRSW) relation derived from the same left ventricular pressure-volume diagrams using linear regression analysis: $SW = M_{sw} \cdot (EDV\text{-}V_{sw})$, where SW = stroke work (calculated as the integral of the pressure-volume diagram for each cardiac cycle) and M_{sw} and V_{sw} = the slope and volume intercept of the PRSW relation (14). Effective arterial elastance (E_a) was calculated as the ratio of end-systolic arterial pressure and stroke volume under steady-state hemodynamic conditions immediately before the vena caval occlusion (48, 49). Left ventricular-arterial coupling was described as the ratio of E_{es} and E_a (48). The pressure-volume area (PVA; total mechanical energy)

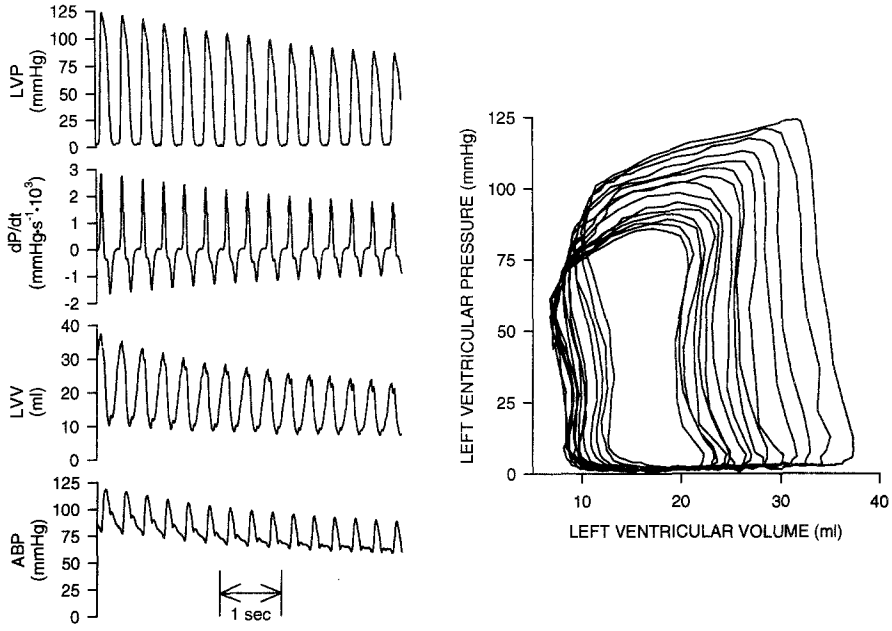

Fig. 1 Continuous left ventricular pressure (LVP), dP/dt, left ventricular volume (LVV), and arterial blood pressure (ABP) waveforms (left panel) and left ventricular pressure-volume diagrams (right panel) during inferior vena caval occlusion observed in a typical experiment.

was determined at each intervention as the sum of SW and potential energy (PE), where $PE = 0.5 \cdot P_{es} \cdot (V_{es}-V_o)$ (46). The ratio of SW to PVA was used to determine the mechanical efficiency of energy transfer of PVA to externally performed stroke work (34). Stroke work was corrected for alterations in EDV produced by levosimendan, pimobendan, and milrinone by use of PRSW (14, 29). The average EDV obtained under control conditions and during drug infusions in each dog was used to calculate SW at constant EDV during each intervention (14, 29). Lastly, the ratio of SW to PWI was determined at each intervention as an index of myocardial metabolic efficiency.

Dogs were assigned to receive levosimendan, pimobendan, or milrinone in a random manner in three separate groups of experiments. Baseline systemic hemodynamics and left ventricular pressure-volume diagrams were recorded during control conditions 30 min after the instrumentation was completed. In one group of experiments, infusions of levosimendan at 0.75, 1.5, and 3.0 $\mu g \cdot kg^{-1} \cdot min^{-1}$ for 15 min were administered in a sequential fashion. Hemodynamics were recorded, and left ventricular pressure-volume diagrams were obtained using the techniques described above immediately after 15 min of each dose (cumulative doses = 11.25, 33.75, and 78.75 $\mu g \cdot kg^{-1}$). In two other groups of experiments, dogs received infusions of pimobendan at 10, 20, and 40 $\mu g \cdot kg^{-1} \cdot min^{-1}$ or milrinone at 1.0, 2.0, and 4.0 $\mu g \cdot kg^{-1} \cdot min^{-1}$ for 15 min in a sequential manner. Hemodynamics and left ventricular pressure-volume diagrams were recorded at the time intervals described above (cumulative doses of pimobendan and milrinone = 150, 450, 1050 and 15, 45, and 105 $\mu g \cdot kg^{-1}$, respectively). At the end of each experiment, the heart was electrically fibrillated, and the positions of the fluid-filled and conductance catheters and the micromanometer-tipped catheter were confirmed.

Drugs

Levosimendan, pimobendan, and milrinone were generously donated by Orion-Farmos Pharmaceuticals, Espoo, Finland. The drug vehicle for all agents consisted of 25 % ethanol (95 %), 25 % polyethylene glycol (5 %), and 50 % normal saline. No hemodynamic effects were produced by the drug vehicle.

Statistical analysis

Statistical analysis of the data within and between groups before and during the administration of levosimendan, pimobendan, and milrinone was performed by multiple analysis of variance (MANOVA) with repeated measures, followed by use of Student's *t*-test with Duncan's adjustment for multiplicity. Changes were considered to be statistically significant when the probability (p) value was < 0.05. All data are expressed as mean ± SEM.

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Results

Twenty-three dogs were used to provide 21 complete experiments. Two dogs were excluded from analysis because of instrument failure. Levosimendan caused significant ($p < 0.05$) and dose-related decreases in mean arterial pressure, left ventricular systolic and end-diastolic pressures, EDV, ESV, and PWI (Table 1). An increase in heart rate and stroke volume was observed during administration of the high dose of levosimendan. Dose-related increases in E_{es} (2.8 ± 0.5 during control to 8.3 ± 1.7 mmHg·ml^{-1} during the 3.0 μg·kg^{-1}·min^{-1} dose), M_{sw} (44 ± 5 during control to 91 ± 9 mmHg during the 3.0 μg·kg^{-1}·min^{-1} dose), $+dP/dt_{max}$, and EF occurred, consistent with a positive inotropic effect. Levosimendan (3.0 μg·kg^{-1}·min^{-1}) reduced E_a (4.8 ± 0.7 during control to 2.7 ± 0.3 mmHg·ml^{-1}) and increased the ratio of E_{es} to E_a (0.61 ± 0.10 during control to 3.3 ± 0.7; Fig. 2). Levosimendan also caused dose-related increases in SW and decreases in cardiac potential energy (PE). PVA remained unchanged. Levosimendan increased the ratio of SW to PVA (0.48 ± 0.05 during control to 0.84 ±0.03 during the 3.0 μg·kg^{-1}·min^{-1} dose; Fig. 2), demonstrating that this myofilament Ca^{2+} sensitizer increases the mechanical efficiency of left ventricular energy transfer to external stroke work. Levosimendan caused dose-related increases in the ratio of SW to PWI (109 ± 18 during control to 255 ± 50 mmHg·min·100 g during the 3.0 μg·kg^{-1}·min^{-1} dose; Fig. 2), suggesting that this drug also enhances intrinsic myocardial efficiency.

Pimobendan caused hemodynamic effects that were similar to those produced by levosimendan. Pimobendan decreased mean arterial pressure, left ventricular enddiastolic pressure, EDV and ESV (Table 2). In contrast to the findings with levosimendan, however, pimobendan caused dose-related increases in heart rate. A reduction in left ventricular systolic pressure occurred at the 40 μg·kg^{-1}·min^{-1} dose. PWI was unchanged. Pimobendan increased E_{es}, M_{sw}, $+dP/dt_{max}$, and EF and decreased E_a in a dose-related manner, indicating that inotropic state was augmented and effective arterial elastance was reduced, respectively, by this drug. Pimobendan increased SW and reduced PE. In contrast to levosimendan, however, pimobendan caused a reduction in PVA at the 10 μg·kg^{-1}·

Table 1 Hemodynamic effects of levosimendan

	Control	Levosimendan (μg·kg^{-1}·min^{-1}) 0.75	1.5	3.0
HR (beats·min^{-1})	126 ± 6	129 ± 8	131 ± 9	136 ± 8[1]
MAP (mmHg)	110 ± 8	99 ± 7	82 ± 4[1,2]	73 ± 3[1,2]
PWI (ml·min^{-1}·100 g^{-1})	15.1 ± 1.4	13.5 ± 1.5	12.1 ± 1.2[1]	11.5 ± 1.0[1,2]
LVSP (mmHg)	120 ± 5	109 ± 6[1]	100 ± 2[1,2]	97 ± 2[1,2]
LVEDP (mmHg)	8.1 ± 1.1	5.4 ± 1.5[1]	4.0 ± 1.7[1,2]	3.8 ± 1.6[1,2]
$+dP/dt_{max}$ (mmHg·s^{-1})	1604 ± 57	1733 ± 74	1954 ± 69[1,2]	2013 ± 70[1,2]
EDV (ml)	54 ± 5	46 ± 6[1]	42 ± 6[1,2]	42 ± 6[1,2]
ESV (ml)	29 ± 6	23 ± 6[1]	16 ± 6[1,2]	14 ± 5[1,2]
SV (ml)	25 ± 2	23 ± 3	26 ± 3	28 ± 3[1]
EF	0.50 ± 0.06	0.54 ± 0.08	0.68 ± 0.09[1,2]	0.72 ± 0.08[1,2]
M_{sw} (mmHg)	44 ± 5	51 ± 6	76 ± 7[1,2]	91 ± 9[1,2]
V_{sw} (ml)	8.3 ± 5.6	12.8 ± 5.0	14.5 ± 5.5	15.1 ± 5.8
E_{es} (mmHg·ml^{-1})	2.8 ± 0.5	4.0 ± 0.7	5.4 ± 1.0[1]	8.3 ± 1.7[1,2,3]
V_o (ml)	–15.1 ± 4.7	–8.1 ± 3.1[1]	–6.4 ± 3.0[1]	–3.4 ± 3.4[1]
SW (mmHg·ml)	1722 ± 412	1852 ± 502	2447 ± 469[1]	2875 ± 616[1,2]
PE (mmHg·ml)	1809 ± 379	1338 ± 240[1]	763 ± 157[1,2]	551 ± 138[1,2]
PVA (mmHg·ml)	3531 ± 723	3190 ± 686	3210 ± 581	3425 ± 716
E_a (mmHg·ml^{-1})	4.8 ± 0.7	4.7 ± 0.8	3.3 ± 0.4[1,2]	2.7 ± 0.3[1,2]

Data are mean ± SEM; n = 7

1 Significantly ($p < 0.05$) different from control.
2 Significantly ($p < 0.05$) different from 0.75 μg·kg^{-1}·min^{-1} levosimendan.
3 Significantly ($p < 0.05$) different from 1.5 μg·kg^{-1}·min^{-1} levosimendan.

Abbreviations: HR = heart rate; MAP = mean arterial pressure; PWI = pressure-work index; LVSP = left ventricular systolic pressure; LVEDP = left ventricular end-diastolic pressure; EDV and ESV = end-diastolic and end-systolic volume, respectively; SV = stroke volume; EF = ejection fraction; M_{sw} and V_{sw} = preload recruitable stroke work slope and volume intercept respectively; E_{es} = end-systolic elastance; V_o = volume intercept; SW = stroke work; PE = potential energy; PVA = pressure-volume area; E_a = effective arterial elastance.

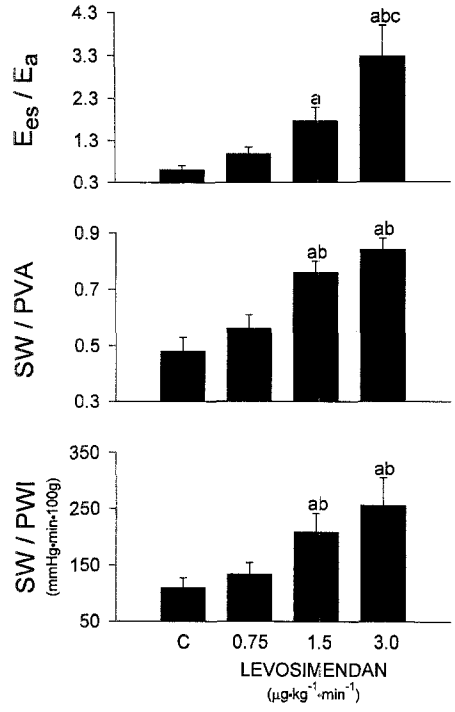

Fig. 2 Histograms depicting left ventricular-arterial coupling (E_{es}/E_a; top panel), left ventricular mechanical efficiency (SW/PVA; middle panel), and myocardial efficiency (SW/PWI; bottom panel) under control conditions (C) and during administration of levosimendan (0.75, 1.5, and 3.0 $\mu g \cdot kg^{-1} \cdot min^{-1}$). [a]Significantly ($p < 0.05$) different from control (C); [b]Significantly ($p < 0.05$) different from 0.75 $\mu g \cdot kg^{-1} \cdot min^{-1}$ levosimendan; [c]Significantly ($p < 0.05$) different from 1.5 $\mu g \cdot kg^{-1} \cdot min^{-1}$ levosimendan.

min^{-1} dose. Pimobendan (40 $\mu g \cdot kg^{-1} \cdot min^{-1}$), like levosimendan, enhanced left ventricular-arterial coupling (E_{es}/E_a: 0.60 ± 0.11 during control to 3.3 ± 0.4), energy transfer efficiency (SW/PVA: 0.48 ± 0.04 during control to 0.90 ± 0.02), and calculated myocardial efficiency (SW/PWI: 151 ± 20 during control to 278 ± 47 mmHg·min·100 g; Fig. 3) as a result of these inotropic and vasodilating effects.

Milrinone caused hemodynamic actions (Table 3) that shared many similarities with those produced by the myofilament Ca^{2+} sensitizers. Milrinone increased heart rate and decreased left ventricular end-diastolic pressure, EDV and ESV in a dose-related fashion. In contrast to the findings with levosimendan and pimobendan, however, mean arterial pressure was decreased only at the 4.0 $\mu g \cdot kg^{-1} \cdot min^{-1}$ dose of milrinone. In addition, left ventricular systolic pressure and PWI were unchanged. Dose-related increases in E_{es}, M_{sw}, $+dP/dt_{max}$, and EF and decreases in E_a occurred with milrinone. Alterations in these indices of myocardial contractility and left ventricular afterload were similar to those observed with the myofilament Ca^{2+} sensitizers. Milrinone caused dose-related increases in SW and decreases in PE. Like levosimendan and pimobendan, milrinone (4.0 $\mu g \cdot kg^{-1} \cdot min^{-1}$) also increased E_{es}/E_a (0.58 ± 0.12 during control to 2.9 ± 0.9), SW/PVA (0.46 ± 0.06 during control to 0.82 ± 0.05), SW/PWI (160 ± 23 during control to 245 ± 46 mmHg·min·100g; Fig. 4), indicating that this positive inotrope with veno- and vasodilating properties enhanced left ventricular-arterial coupling and improved mechanical and myocardial efficiency.

Discussion

The present investigation is the first to examine the effects of myofilament Ca^{2+} sensitizers on left ventricular-arterial coupling and mechanical efficiency. The results indicate

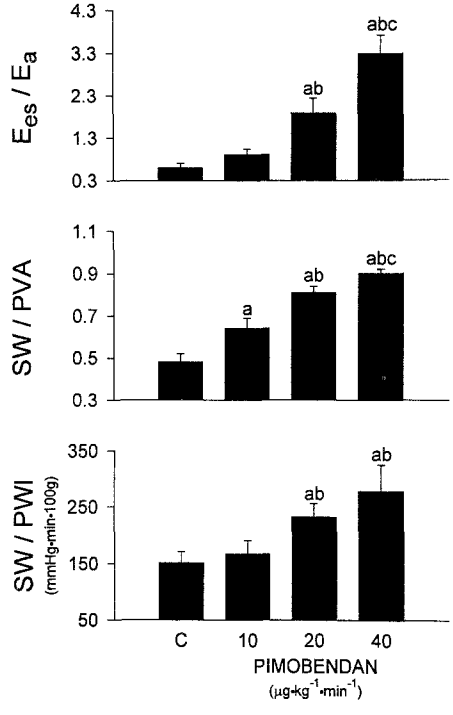

Fig. 3 Histograms depicting left ventricular-arterial coupling (E_{es}/E_a; top panel), left ventricular mechanical efficiency (SW/PVA; middle panel), and myocardial efficiency (SW/PWI: bottom panel) under control conditions (C) and during administration of pimobendan (10, 20, and 40 $\mu g \cdot kg^{-1} \cdot min^{-1}$). [a]Significantly ($p < 0.05$) different from control (C); [b]Significantly ($p < 0.05$) different from 10 $\mu g \cdot kg^{-1} \cdot min^{-1}$ pimobendan; [c]Significantly ($p < 0.05$) different from 20 $\mu g \cdot kg^{-1} \cdot min^{-1}$ pimobendan.

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that levosimendan and pimobendan enhance myocardial contractility and cause venous and arterial vasodilation leading to declines in left ventricular preload and afterload. These findings support the observations of several previous investigations from this (20, 35, 36) and other laboratories (5, 15, 26, 56) and presumably occurred as a result of the combined effects of myofilament Ca^{2+} sensitization (13, 16, 18, 41) and PDE inhibition in cardiac and vascular smooth muscle (6, 11). As a result of increases in intrinsic inotropic state and reductions in left ventricular afterload, levosimendan and pimobendan caused similar dose-related increases in E_{es}/E_a, indicating that these drugs enhance left ventricular-arterial coupling. In addition, levosimendan and pimobendan increased SW/PVA, indicating that these myofilament Ca^{2+} sensitizers increase the mechanical efficiency of left ventricular energy transfer to external stroke work. Similar increases in E_{es}/E_a and SW/PVA were observed with milrinone, a phosphodiesterase inhibitor without myofilament Ca^{2+} sensitizing properties. The nonlinear correlation between E_{es}/E_a and SW/PVA observed with these vasodilating positive inotropes was closely related to the theoretical model predicted by Burkhoff and Sagawa (Fig. 5) (9). These findings agree with the results of previous studies in anesthetized (34) and conscious dogs (29) and indicate that the conversion of PVA to external mechanical work increases with contractile state.

The actions of the myofilament Ca^{2+} sensitizers and milrinone on E_{es}/E_a and SW/PVA observed in the present investigation are similar to those previously reported with other positive inotropes including amrinone (24) and dobutamine (29, 34, 43). Enhanced left ventricular-arterial coupling resulting from increases in myocardial contractility and reductions in left ventricular afterload produced by myofilament Ca^{2+} sensitizers and milrinone may be especially important in the failing heart because these agents not only improve "forward flow" but also enhance the conversion of total myocardial energy to external work. The increases in the transfer of left ventricular energy to the arterial circulation observed with levosimendan, pimobendan, and milrinone may also cause reductions in maximum left ventricular stroke work (SW_{max}) because optimal stroke work occurs when $E_{es}/E_a = 1$ (9, 49). Little and Cheng (29) recently demonstrated

Table 2 Hemodynamic effects of pimobendan

	Control	Pimobendan ($\mu g \cdot kg^{-1} \cdot min^{-1}$) 10	20	40
HR (beats·min^{-1})	131 ± 6	138 ± 9	149 ± 11[1,2]	158 ± 12[1,2]
MAP (mmHg)	98 ± 4	93 ± 5	83 ± 4[1,2]	74 ± 4[1,2,3]
PWI (ml·min^{-1}·100 g^{-1})	13.7 ± 0.9	13.1 ± 0.8	13.7 ± 1.4	13.3 ± 1.4
LVSP (mmHg)	116 ± 4	114 ± 4	112 ± 4	109 ± 4[1]
LVEDP (mmHg)	8.8 ± 1.5	5.0 ± 1.2[1]	2.8 ± 1.3[1,2]	1.5 ± 1.3[1,2]
$+dP/dt_{max}$ (mmHg·s^{-1})	1777 ± 101	2040 ± 92[1]	2422 ± 116[1,2]	2611 ± 78[1,2,3]
EDV (ml)	50 ± 4	40 ± 4[1]	37 ± 4[1]	38 ± 4[1]
ESV (ml)	25 ± 3	17 ± 3[1]	12 ± 2[1,2]	10 ± 1[1,2]
SV (ml)	24 ± 4	23 ± 4	25 ± 4	28 ± 4[1]
EF	0.49 ± 0.05	0.56 ± 0.06[1]	0.68 ± 0.05[1,2]	0.72 ± 0.04[1,2]
M_{sw} (mmHg)	55 ± 4	64 ± 8	98 ± 10[1,2]	120 ± 8[1,2,3]
V_{sw} (ml)	2.5 ± 4.5	5.8 ± 2.5	8.0 ± 1.7	11.4 ± 1.9[1]
E_{es} (mmHg·ml^{-1})	2.4 ± 0.3	3.9 ± 0.4	6.5 ± 1.1[1,2]	9.2 ± 1.3[1,2,3]
V_o (ml)	–23.1 ± 5.6	–8.7 ± 1.9[1]	–5.1 ± 2.0[1]	–1.2 ± 2.1[1]
SW (mmHg·ml)	2131 ± 368	2228 ± 406	3139 ± 362[1,2]	3535 ± 485[1,2]
PE (mmHg·ml)	2395 ± 583	1225 ± 217[1,2]	731 ± 117[1]	379 ± 73[1,2]
PVA (mmHg·ml)	4526 ± 860	3453 ± 454[1]	3870 ± 422	3914 ± 511
E_a (mmHg·ml^{-1})	4.7 ± 0.7	4.8 ± 0.7	3.6 ± 0.4[1,2]	2.9 ± 0.4[1,2]

Data are mean ± SEM; n = 7

1 Significantly ($p < 0.05$) different from control.
2 Significantly ($p < 0.05$) different from 10 $\mu g \cdot kg^{-1} \cdot min^{-1}$ pimobendan.
3 Significantly ($p < 0.05$) different from 20 $\mu g \cdot kg^{-1} \cdot min^{-1}$ pimobendan.

Abbreviations: HR = heart rate; MAP = mean arterial pressure; PWI = pressure-work index; LVSP = left ventricular systolic pressure; LVEDP = left ventricular end-diastolic pressure; EDV and ESV = end-diastolic and end-systolic volume; respectively; SV = stroke volume; EF = ejection fraction; M_{sw} and V_{sw} = preload recruitable stroke work slope and volume intercept respectively; E_{es} = end-systolic elastance; V_o = volume intercept; SW = stroke work; PE = potential energy; PVA = pressure-volume area; E_a = effective arterial elastance.

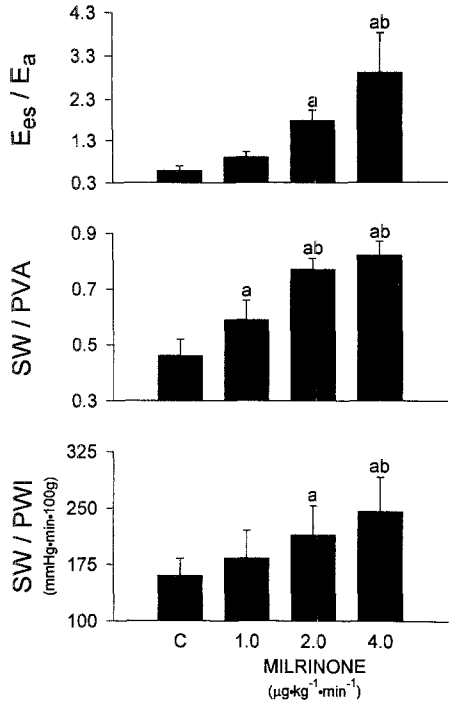

Fig. 4 Histograms depicting left ventricular-arterial coupling (E_{es}/E_a; top panel), left ventricular mechanical efficiency (SW/PVA; middle panel), and myocardial efficiency (SW/PWI; bottom panel) under control conditions (C) and during administration of milrinone (1.0, 2.0, and 4.0 $\mu g \cdot kg^{-1} \cdot min^{-1}$).[a] Significantly ($p < 0.05$) different from control (C); [b]Significantly ($p < 0.05$) different from 1.0 $\mu g \cdot kg^{-1} \cdot min^{-1}$ milrinone.

that SW_{max} remains relatively high (> 80 %) when $2 < E_{es}/E_a < 3$. These observations confirmed theoretical predictions (9) and indicated that the left ventricle continues to operate on the relatively flat plateau of the SW-E_{es}/E_a relationship (29). Thus, although increases in E_{es}/E_a observed with vasodilating positive inotropes in the present investigation may contribute to reductions in SW_{max}, such decreases in SW_{max} are probably relatively small. Left ventricular mechanical efficiency has been shown to be optimized when $E_{es}/E_a = 2$ (9). Thus, levosimendan, pimobendan, and milrinone shift the left ventricle toward optimal efficiency without inappropriately compromising SW_{max}.

Pressure-work index was decreased by levosimendan, suggesting that myocardial oxygen consumption was reduced by this drug in open-chest, barbiturate-anesthetized dogs. These levosimendan-induced decreases in PWI probably occurred as a result of relatively constant heart rate and declines in left ventricular preload and afterload despite simultaneous increases in myocardial contractility. In contrast, PWI was unchanged by pimobendan and milrinone. As a result of simultaneous increases in SW at constant EDV, increases in the SW/PWI ratio also occurred. These findings suggest that levosimendan, pimobendan, and milrinone cause increases in overall myocardial efficiency (defined as the ratio of external left ventricular work (SW) to myocardial oxygen consumption (9)). Simultaneous increases in SW/PVA also support the hypothesis that these drugs increase myocardial efficiency. Although PVA has been shown to be directly correlated with myocardial oxygen consumption, this variable does not account for alterations in basal myocardial metabolism produced by changes in contractile state (49). PWI may be an accurate estimate of myocardial oxygen consumption over a wide range of hemodynamic conditions and contractile states *in vivo* (40). However, the profound hemodynamic changes observed with levosimendan, pimobendan, and milrinone in the present investigation may have exceeded the limitations of PWI to predict alterations in myocardial oxygen consumption. Thus, the conclusion that myofilament Ca^{2+} sensitizers and milrinone enhance myocardial efficiency requires qualification not only because coronary sinus oxygen content was not measured and actual myocardial oxygen consumption was not calculated, but also because PWI may have degenerated as an accurate predictor of myocardial oxygen consumption under the present experimental conditions. Because myocardial oxygen consumption is maintained or even reduced with pimobendan (21, 26) and milrinone (21, 26, 33, 37) in heart failure, increases in SW/PVA observed with these vasoactive drugs may also be associated with

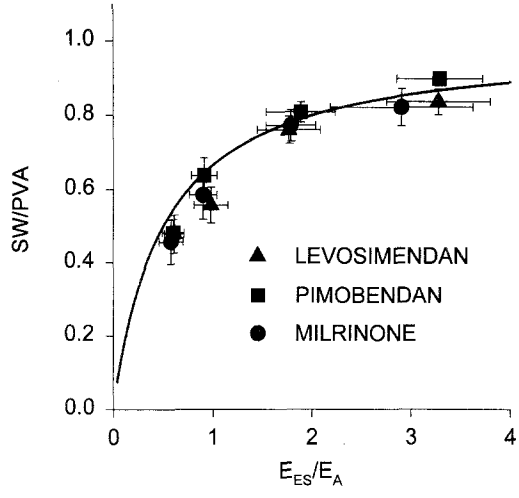

Fig. 5 Nonlinear relationship between E_{es}/E_a and SW/PVA for levosimendan (triangles), pimobendan (squares), and milrinone (circles) compared to the theoretical model (solid line) predicted by the equation: (9) $SW/PVA = (1+0.5 \cdot E_a/E_{es})^{-1}$.

increases in overall myocardial efficiency in this setting as well. This hypothesis remains to be tested, however.

The present results should be interpreted within the constraints of several potential limitations. Previous studies have demonstrated that E_{es} (12, 54), but not M_{sw} (14, 30), may be sensitive to acute alterations in left ventricular afterload. Thus, reductions in E_a produced by levosimendan, pimobendan, and milrinone may have contributed to relatively greater increases in E_{es}. However, increases in the magnitude of E_{es} and M_{sw} in response to these drugs were appropriately matched, indicating that reductions in afterload did not adversely affect evaluation of contractility using E_{es}. The slope of the end-systolic pressure-volume relation has also been reported to be curvilinear over a wide range of pressures (10, 23, 31). However, over the relatively narrow range of pressures observed in the present investigation, the end-systolic pressure-volume relation has been shown to be essentially linear (31).

A previous study (1) has demonstrated that E_{es} and V_o were consistently underestimated with the conductance catheter measurement of left ventricular volume. However, alterations E_{es} in response to interventions which alter contractile state (e.g., autonomic nervous system blockade and dobutamine) were appropriately detected and quantified with the conductance technique as compared to three-dimensional sonomicrometry (1). Thus, it is very likely that increases in myocardial contractility produced by levosimendan, pimobendan, and milrinone were accurately quantified with E_{es} calculated from conductance catheter-derived left ventricular volume. Effective arterial elastance cannot be used to strictly quantify alterations in left ventricular afterload because this parameter fails to account for the frequency-dependence of arterial input impedance (32). Nevertheless, E_a provides a useful framework for the analysis of left ventricular-arterial coupling relations *in vivo* (29). The determination of stroke volume required for the determination of E_a using the conductance technique has been validated previously under a variety of experimental conditions (3, 4). The values of E_{es}/E_a under control conditions and during administration of positive inotropic agents in the present investigation were similar to those reported by Nozawa et al. (34) and Kass et al. (24) in acutely instrumented dogs.

Table 3 Hemodynamic effects of milrinone

	Control	Milrinone ($\mu g \cdot kg^{-1} \cdot min^{-1}$) 1.0	2.0	4.0
HR (beats·min^{-1})	120 ± 6	127 ± 5	134 ± 5[1]	137 ± 7[1,2]
MAP (mmHg)	102 ± 6	104 ± 6	97 ± 5	86 ± 4[1,2]
PWI (ml·min^{-1}·100 g^{-1})	14.4 ± 2.2	15.5 ± 2.1	15.5 ± 1.7	14.1 ± 1.5
LVSP (mmHg)	115 ± 6	115 ± 5	112 ± 4	106 ± 3
LVEDP (mmHg)	8.8 ± 2.0	6.2 ± 1.6[1]	5.0 ± 1.1[1]	4.4 ± 1.3[1]
$+dP/dt_{max}$ (mmHg·s^{-1})	1540 ± 180	1837 ± 211[1]	2024 ± 192[1]	2060 ± 180[1]
EDV (ml)	54 ± 6	50 ± 6	47 ± 6[1]	44 ± 4[1]
ESV (ml)	28 ± 4	24 ± 3	17 ± 3[1,2]	15 ± 3[1,2]
SV (ml)	26 ± 4	27 ± 4	29 ± 5	30 ± 4
EF	0.48 ± 0.05	0.53 ± 0.02	0.62 ± 0.04[1,2]	0.67 ± 0.05[1,2]
M_{sw} (mmHg)	52 ± 5	67 ± 7	97 ± 7[1,2]	109 ± 13[1,2]
V_{sw} (ml)	4.7 ± 4.3	7.0 ± 4.7	14.2 ± 3.6[1,2]	14.9 ± 3.9[1,2]
E_{es} (mmHg·ml^{-1})	2.2 ± 0.3	3.6 ± 0.7	6.3 ± 1.1[1,2]	7.4 ± 1.3[1,2]
V_o (ml)	−19.0 ± 5.0	−7.7 ± 4.6[1]	2.0 ± 2.8[1,2]	3.2 ± 3.3[1,2]
SW (mmHg·ml)	2144 ± 344	2618 ± 400	3232 ± 560[1]	3393 ± 666[1]
PE (mmHg·ml)	2776 ± 567	1957 ± 468[1]	875 ± 138[1,2]	671 ± 163[1,2]
PVA (mmHg·ml)	4920 ± 686	4575 ± 649	4106 ± 627[1]	4064 ± 710[1]
E_a (mmHg·ml^{-1})	4.7 ± 0.9	4.3 ± 0.7	3.8 ± 0.6	3.2 ± 0.6[1,2]

Data are mean ± SEM; n = 7

1 Significantly ($p < 0.05$) different from control.

2 Significantly ($p < 0.05$) different from 1.0 $\mu g \cdot kg^{-1} \cdot min^{-1}$ milrinone.

Abbreviations: HR = heart rate; MAP = mean arterial pressure; PWI = pressure-work index; LVSP = left ventricular systolic pressure; LVEDP = left ventricular end-diastolic pressure; EDV and ESV = end-diastolic and end-systolic volume, respectively; SV = stroke volume; EF = ejection fraction; M_{sw} and V_{sw} = preload recruitable stroke work slope and volume intercept respectively; E_{es} = end-systolic elastance; V_o = volume intercept; SW = stroke work; PE = potential energy; PVA = pressure-volume area; E_a = effective arterial elastance.

The present results must also be interpreted within the possible constraints of the conductance technique for measurement of left ventricular volume. We assumed that the slope correction factor (α) was equal to 1 (8, 24). The value of α may vary to some degree between dogs because of differences in left ventricular geometry. However, this potential source of bias was probably eliminated because dogs were assigned to receive levosimendan, pimobendan, and milrinone in a random manner. Nevertheless, it is conceivable that alterations in α occurred because of differential effects of levosimendan, pimobendan, and milrinone on left ventricular geometry. Recent evidence suggests that α may also vary with the changes in left ventricular volume that occur during the cardiac cycle or as a result of rapid changes in ventricular volume (42, 44, 51). Such volume-dependent alterations in α may theoretically contribute to a relative underestimation of stroke volume and end-diastolic volume (51). In addition, the value of V_p may change during large alterations in left ventricular volume (1, 7), again introducing possible error in the measurement of absolute left ventricular volume during experimental interventions or abrupt alteration of preload. However, potential errors in α and V_p were probably minimized because left ventricular arterial coupling and mechanical efficiency were described using ratios (E_{es}/E_a and SW/PVA) of volume-derived variables. Despite these potential limitations, the conductance method used to determine left ventricular volume in the present study has been widely established as a valid technique for determining beat-to-beat changes in stroke volume and end-diastolic volume under a variety of experimental conditions *in vivo* (1, 2, 4, 25).

In summary, the present results demonstrate that levosimendan, a novel myofilament Ca^{2+} sensitizer with PDE-inhibiting properties, increases myocardial contractility and produces arterial and venous dilation while reducing a calculated estimate of myocardial oxygen consumption. As a result of these positive inotropic effects and hemodynamic actions, levosimendan enhanced left ventricular-arterial coupling as evaluated by the ratio of E_{es} to E_a. Levosimendan also improved left ventricular mechanical efficiency (SW/PVA) and a calculated index of intrinsic myocardial metabolic efficiency (SW/PWI). The effects of levosimendan on coupling and myocardial energetics were similar to those produced by pimobendan and milrinone, indicating that these vasodilating positive inotropes cause beneficial effects on the conversion of total left ventricular energy to external stroke work in open-chest, barbiturate-anesthetized dogs.

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References

1. Applegate RJ, Cheng C-P, Little WC (1990) Simultaneous conductance catheter and dimension assessment of left ventricle volume in the intact animal. Circulation 81: 638–648
2. Applegate RJ, Little WC (1994) Alteration of autonomic influence on left ventricular contractility by epicardial superfusion with hexamethonium and procaine. Cardiovasc Res 28: 1042–1048
3. Baan J, Jong TT, Kerkhof PLM, Moene RJ, Van Dijk AD, Van der Velde ET, Koops J (1981) Continuous stroke volume and cardiac output from intraventricular dimensions obtained with impedance catheter. Cardiovasc Res 15: 328–334
4. Baan J, Van der Velde ET, De Bruin HG, Smeenk GJ, Koops J, van Dijk AD, Temmerman D, Senden J, Buis B (1984) Continuous measurement of left ventricular volume in animals and humans by conductance catheter. Circulation 70: 812–823
5. Baumann G, Ningel K, Permanetter B (1989) Cardiovascular profile of UDCG 115 BS-pimobendane and reversibility of catecholamine subsensitivity in severe congestive heart failure secondary to idiopathic dilated cardiomyopathy. J Cardiovasc Pharmacol 13: 730–738
6. Bohm M, Morano I, Pieske B, Ruegg JC, Wankerl M, Zimmermann R, Erdmann E (1991) Contribution of cAMP-phosphodiesterase inhibition and sensitization of the contractile proteins for calcium to the inotropic effect of pimobendan in the failing human myocardium. Circ Res 68: 689–701
7. Boltwood Jr CM, Appleyard RF, Glantz SA (1989) Left ventricular volume measurement by conductance catheter in intact dogs. Parallel conductance volume depends on left ventricular size. Circulation 80: 1360–1377
8. Burkhoff D (1990) The conductance method of left ventricular volume estimation. Methodologic limitations put into perspective. Circulation 81: 703–706
9. Burkhoff D, Sagawa K (1986) Ventricular efficiency predicted by an analytical model. Am J Physiol 250: R1021–R1027
10. Burkhoff D, Sugiura S, Yue DT, Sagawa K (1987) Contractility-dependent curvilinearity of end-systolic pressure-volume relations. Am J Physiol 252: H1218–H1227

11. Edes I, Kiss E, Kitada Y, Powers FM, Papp JG, Kranias EG, Solaro RJ (1995) Effects of levosimendan, a cardiotonic agent targeted to troponin C, on cardiac function and on phosphorylation and Ca^{2+} sensitivity of cardiac myofibrils and sarcoplasmic reticulum in guinea pig heart. Circ Res 77: 107–113
12. Freeman GL, Little WC, O'Rourke RA (1986) The effect of vasoactive agents on the left ventricular end-systolic pressure-volume relation in closed chest dogs. Circulation 74: 1107–1113
13. Fujino K, Sperelakis N, Solaro RJ (1988) Sensitization of dog and guinea pig heart myofilaments to Ca^{2+} activation and the inotropic effect of pimobendan: Comparison with milrinone. Circ Res 63: 911–922
14. Glower DD, Spratt JA, Snow ND, Kabas JS, Davis JW, Olsen CO, Tyson GS, Sabiston Jr DC, Rankin JS (1985) Linearity of the Frank-Starling relationship in the intact heart: the concept of preload recruitable stroke work. Circulation 71: 994–1009
15. Hagemeijer F, Brand HJ, van Mechelen R (1989) Hemodynamic effects of pimobendan given orally in congestive heart failure secondary to ischemic or idiopathic dilated cardiomyopathy. Am J Cardiol 63: 571–576
16. Haikala H, Kaivola J, Nissinen E, Wall P, Levijoki J, Linden I-B (1995) Cardiac troponin C as a target for a novel calcium sensitizing drug, levosimendan. J Mol Cell Cardiol 27: 1859–1866
17. Haikala H, Linden I-B (1995) Mechanism of action of calcium-sensitizing drugs. J Cardiovasc Pharmacol 26 (Suppl 1): S20–S31
18. Haikala H, Nissinen E, Etemadzadeh E, Levijoki J, Linden I-B (1995) Troponin C-mediated calcium sensitization induced by levosimendan does not impair relaxation. J Cardiovasc Parmacol 25: 794–801
19. Hajjar RJ, Gwathmey JK (1991) Calcium-sensitizing inotropic agents in the treatment of heart failure: A critical view. Cardiovasc Drugs Ther 5: 961–965
20. Harkin CP, Pagel PS, Tessmer JP, Warltier DC (1995) Systemic and coronary hemodynamic actions and left ventricular functional effects of levosimendan in conscious dogs. J Cardiovasc Pharmacol 26: 179–188
21. Holubarsch C, Hasenfuss G, Heiss WH, Just H (1988) Influence of enoximone and UDCG-115 on coronary hemodynamics in idiopathic dilated cardiomyopathy. Am J Cardiol 62: 104E–107E
22. Jaquet K, Heilmeyer Jr LMG (1987) Influence of association and of positive inotropic drugs on calcium binding to cardiac troponin C. Biochem Biophys Res Comm 145: 1390–1396
23. Kass DA, Beyar R, Lankford E, Heard M, Maughan WL, Sagawa K (1989) Influence of contractile state on curvilinearity of in situ end-systolic pressure-volume relations. Circulation 79: 167–178
24. Kass DA, Grayson R, Marino P (1990) Pressure-volume analysis as a method for quantifying simultaneous drug (amrinone) effects on arterial load and contractile state in vivo. J Am Coll Cardiol 16: 726–732
25. Kass DA, Yamazaki T, Burkhoff D, Maughan WL, Sagawa K (1986) Determination of left ventricular end-systolic pressure-volume relationships by the conductance (volume) catheter technique. Circulation 73: 586–595
26. Katz SD, Kubo SH, Jessup M, Brozena S, Troha JM, Wahl J, Cohn JN, Sonnenblick EH, LeJentel TH (1992) A multicenter, randomized, double-blind, placebo-controlled trial of pimobendan, a new cardiotonic and vasodilator agent, in patients with severe congestive heart failure. Am Heart J 123: 95–103
27. Lilleberg J, Sundberg S, Hayha M, Akkila J, Nieminen MS (1994) Haemodynamic dose-efficacy of levosimendan in healthy volunteers. Eur J Clin Pharmacol 47: 267–274
28. Lilleberg J, Sundberg S, Nieminen MS (1995) Dose-ranging study of a new calcium sensitizer, levosimendan, in patients with left ventricular dysfunction. J Cardiovasc Pharmacol 26 (Suppl 1): S63–S69
29. Little WC, Cheng C-P (1991) Left ventricular-arterial coupling in conscious dogs. Am J Physiol 261: H70–H76
30. Little WC, Cheng CP, Mumma M, Igarashi Y, Vinten-Johansen J, Johnston WE (1989) Comparison of measures of left ventricular contractile performance derived from pressure-volume loops in conscious dogs. Circulation 80: 1378–1387
31. Little WC, Cheng CP, Peterson T, Vinten-Johansen J (1988) Response of the left ventricular end-systolic pressure-volume relation in conscious dogs to a wide range of contractile states. Circulation 78: 736–745
32. Milnor WR (1975) Arterial impedance as ventricular afterload. Circ Res 36: 565–570
33. Monrad ES, McKay RG, Baim DS, Colucci WS, Fifer MA, Heller GV, Royal HD, Grossman W (1984) Improvement in indexes of diastolic performance in patients with congestive heart failure treated with milrinone. Circulation 70: 1030–1037
34. Nozawa T, Yasumura Y, Futaki S, Tanaka N, Venishi M, Suga H (1988) Efficiency of energy transfer from pressure-volume area to external mechanical work increases with contractile state and decreases with afterload in the left ventricle of the anesthetized closed-chest dog. Circulation 77: 1116–1124
35. Pagel PS, Harkin CP, Hettrick DA, Warltier DC (1994) Levosimendan (OR-1259), a myofilament calcium sensitizer, enhances myocardial contractility but does not alter isovolumic relaxation in conscious and anesthetized dogs. Anesthesiology 81: 974–987
36. Pagel PS, Harkin CP, Hettrick DA, Warltier DC (1995) Zatebradine, a specific bradycardic agent, alters the hemodynamic and left ventricular mechanical actions of levosimendan, a new myofilament calcium sensitizer, in conscious dogs. J Pharmacol Exp Ther 275: 127–135
37. Piscione F, Jaski BE, Wenting GJ, Serruys PW (1987) Effect of a single oral dose of milrinone on left ventricular diastolic performance in the failing human heart. J Am Coll Cardiol 10: 1294–1302
38. Pollesello P, Ovaska M, Kaivola J, Tilgmann C, Lundstrom K, Kalkkinen N, Ulmanen I, Nissinen E, Taskinen J (1994) Binding of a new Ca^{2+} sensitizer, levosimendan, to recombinant human cardiac troponin C. A molecular modelling, fluorescence probe, and proton nuclear magnetic resonance study. J Biol Chem 269: 28584–28590
39. Rooke GA, Feigl EO (1982) Work as a correlate of canine left ventricular oxygen consumption, and the problem of catecholamine oxygen wasting. Circ Res 50: 273–286
40. Schipke JD, Burkhoff D, Kass DA, Alexander Jr J, Schaefer J, Sagawa K (1990) Hemodynamic dependence of myocardial oxygen consumption indexes. Am J Physiol 258: H1281–H1291

41. Solaro RJ, Fujino K, Sperelakis N (1989) The positive inotropic effect of pimobendan involves stereospecific increases in the calcium sensitivity of cardiac myofilaments. J Cardiovasc Pharmacol 14 (Suppl 2): S7–S12
42. Stamato TM, Szwarc RS, Benson LN (1995) Measurement of right ventricular volume by conductance catheter in close-chest pigs. Am J Physiol 269: H869–H876
43. Starling MR (1993) Left ventricular-arterial coupling relations in the normal human heart. Am Heart J 125: 1659–1666
44. SteendijkP, Van der Velde ET, Baan J (1993) Left ventricular stroke volume by single and dual excitation of conductance catheter in dogs. Am J Physiol 264: H2198–H2207
45. Suga H (1990) Ventricular Energetics. Physiol Rev 70: 247–277
46. Suga H, Hayashi T, Shirahata M (1981) Ventricular systolic pressure-volume area as a predictor of cardiac oxygen consumption. Am J Physiol 240: H39–H44
47. Suga H, Sagawa K (1974) Instantaneous pressure-volume relationships and their ratio in the excised, supported canine left ventricle. Circ Res 35: 117–126
48. Sunagawa K, Maughan WL, Burkhoff D, Sagawa K (1983) Left ventricular interaction with arterial load studied in isolated canine ventricle. Am J Physiol 245: H773–H780
49. Sunagawa K, Maughan WL, Sagawa K (1985) Optimal arterial resistance for the maximal stroke work studied in isolated canine left ventricle. Circ Res 56: 586–595
50. Sundberg S, Lilleberg J, Nieminen MS, Lehtonen L (1995) Hemodynamic and neurohumoral effects of levosimendan, a new calcium sensitizer, at rest and during exercise in healthy men. Am J Cardiol 75: 1061–1066
51. Szwarc RS, Laurent D, Allegrini PR, Ball HA (1995) Conductance catheter measurement of left ventricular volume: evidence for nonlinearity within the cardiac cycle. Am J Physiol 268: H1490–H1498
52. Szwarc RS, Mickleborough LL, Mizuno S, Wilson GJ, Liu P, Mohamed S (1994) Conductance catheter measurements of left ventricular volume in the intact dog: parallel conductance is independent of left ventricular size. Cardiovasc Res 28: 252–258
53. Thormann J, Kramer W, Schlepper M (1982) Hemodynamic and myocardial energetic changes induced by the new cardiotonic agent, AR-L 115, in patients with coronary artery disease. Am Heart J 104: 1294–1302
54. Van de Velde ET, Burkhoff D, Steendijk P, Karsdon J, Sagawa K, Baan J (1991) Nonlinearity and load sensitivity of end-systolic pressure-volume relation of canine left ventricle in vivo. Circulation 83: 315–327
55. van Meel JCA, Zimmermann R, Diederen W, Erdman E, Mrwa U (1988) Increase in calcium sensitivity of cardiac myofibrils contributes to the cardiotonic action of sulmazole. Biochem Pharmacol 37: 213–220
56. Walter M, Liebens I, Goethals H, Renard M, Dresse A, Bernard R (1988) Pimobendane (UD-CG 115 BS) in the treatment of severe congestive heart failure. An acute haemodynamic cross-over and double-blind study with two different doses. Br J Clin Pharmacol 25: 323–329