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Influence of levosimendan, pimobendan, and milrinone on the regional distribution of cardiac output in anaesthetized dogs

[1]Paul S. Pagel, Douglas A. Hettrick & David C. Warltier

Departments of Anesthesiology, Pharmacology, and Medicine (Division of Cardiology), Medical College of Wisconsin, Milwaukee, Wisconsin and the Zablocki Veterans Administration Medical Center, Milwaukee, Wisconsin

1 The distribution of cardiac output during administration of levosimendan, a new myofilament calcium sensitizer, is unknown. We examined and compared the effects of levosimendan, pimobendan, and milrinone on regional tissue perfusion by use of the radioactive microsphere technique in barbiturate-anaesthetized dogs.

2 Haemodynamics and regional blood flow were determined before and during infusions of levosimendan (0.75, 1.5, and 3.0 $\mu g\ kg^{-1}\ min^{-1}$), pimobendan (10, 20, and 40 $\mu g\ kg^{-1}\ min^{-1}$), or milrinone (1.0, 2.0, and 4.0 $\mu g\ kg^{-1}\ min^{-1}$).

3 All three drugs caused similar increases in heart rate, cardiac output, and left ventricular $+dP/dt$ and decreases in end-diastolic pressure and systemic vascular resistance. No changes in subendocardial, midmyocardial, and subepicardial blood flow occurred during administration of levosimendan. However, a redistribution of blood flow from subendocardium to subepicardium was observed. Pimobendan increased midmyocardial and subepicardial blood flow and reduced the endo/epi ratio to a greater degree than levosimendan. Milrinone did not affect myocardial perfusion.

4 Levosimendan increased blood flow to the renal medulla and decreased renal medullary and cortical vascular resistance. Levosimendan increased blood flow to the small intestine and liver and reduced vascular resistance in these organs. Pimobendan increased hepatic blood flow to a greater degree than levosimendan but did not alter small intestinal perfusion. All three drugs decreased splenic blood flow to similar degrees. Levosimendan and pimobendan reduced cerebral vascular resistance. Levosimendan and milrinone reduced skeletal muscle vascular resistance.

5 The results indicate that levosimendan, pimobendan, and milrinone cause subtlety different alterations in regional tissue perfusion while producing similar haemodynamic effects.

Keywords: Systemic and coronary blood flow; myofilament calcium sensitizer; troponin C; inotropes; levosimendan; pimobendan; milrinone

Introduction

Myofilament calcium (Ca^{2+}) sensitizers, including pimobendan and sulmazole, are non-adrenergic, non-glycosidic positive inotropic agents that increase myocardial contractility by augmenting Ca^{2+} binding to the Ca^{2+}-specific regulatory site of cardiac troponin C and stabilizing Ca^{2+}-induced conformational changes in this protein without altering the intracellular concentration of this ion (Fujino *et al.*, 1988; van Meel *et al.*, 1988; Solaro *et al.*, 1989; Bohm *et al.*, 1991). These drugs also partially inhibit phosphodiesterase (PDE) isoforms in cardiac and vascular smooth muscle (Fujino *et al.*, 1988; Bohm *et al.*, 1991) producing systemic and pulmonary vasodilatation and further augmentation of contractility (Hajjar & Gwathmey, 1991). Myofilament Ca^{2+} sensitizers have been shown to improve cardiac performance in patients with congestive heart failure (Thormann *et al.*, 1983; Baumann *et al.*, 1989; Hasenfuss *et al.*, 1989; Remme *et al.*, 1989; Katz *et al.*, 1992) and represent another important therapeutic approach in the pharmacological management of the failing heart (Hajjar & Gwathmey, 1991).

Levosimendan is a new myofilament Ca^{2+} sensitizer with PDE-inhibiting properties at higher doses. Previous studies have shown that levosimendan enhances left ventricular function and causes venous and arterial dilatation in dogs (Pagel *et al.*, 1994; 1995; Harkin *et al.*, 1995) and man (Lilleberg *et al.*, 1994; 1995; Sundberg *et al.*, 1995). The mechanisms by which levosimendan produces these effects *in vivo* share many similarities with those of other myofilament Ca^{2+} sensitizers. Levosimendan augments myocardial contractility by binding to and stabilizing the Ca^{2+}-bound conformation of troponin C without directly affecting actin-myosin interaction (Pollesello *et al.*, 1994; Edes *et al.*, 1995; Haikala *et al.*, 1995). However, unlike other myofilament Ca^{2+} sensitizers, levosimendan-induced enhancement of binding affinity of Ca^{2+} to troponin C is Ca^{2+}-dependent and occurs only in the presence of the higher intracellular Ca^{2+} concentrations observed during systole (Haikala *et al.*, 1995; Haikala & Linden, 1995). Similar to pimobendan and sulmazole, the PDE-inhibiting activity of levosimendan contributes to the veno- and vasodilatation observed with this drug but only at higher doses (Edes *et al.*, 1995). As a result of these positive inotropic and peripheral vascular effects, myofilament Ca^{2+} sensitizers may cause substantial alterations in regional tissue perfusion.

Levosimendan, pimobendan and milrinone, a PDE inhibitor without myofilament Ca^{2+} sensitizing properties, cause increases in cardiac output. The objective of this investigation was to characterize if these agents produced similar changes in the distribution of cardiac output. The doses of levosimendan, pimobendan, and milrinone used in the present study were chosen to produce similar increases in cardiac output and reductions in systemic vascular resistance. Thus, we examined and compared the actions of levosimendan, pimobendan, and milrinone on myocardial blood flow and regional perfusion in the brain, kidney, liver, pancreas, small intestine, spleen and skeletal muscle. Radionuclide-labelled microspheres were used to characterize blood flow to these organs in open chest, barbiturate-anaesthetized dogs.

[1] Author for correspondence at: Medical College of Wisconsin, MEB-Room 462C, 8701 Watertown Plank Road, Milwaukee, WI 53226, U.S.A.

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 ($n=22$) of either sex weighing between 25 and 30 kg were fasted overnight and anaesthetized with sodium pentobarbitone (25 mg kg^{-1}) and sodium barbitone (200 mg kg^{-1}). Fluid deficits were replaced before experimentation with 0.9% saline (500 ml), which was continued at 3 ml kg^{-1} h^{-1} for the duration of each experiment. After tracheal intubation, the dogs were ventilated via positive pressure with a mixture (1 min^{-1}) of oxygen (90%) and air (10%). Respiratory rate and tidal volume were adjusted to maintain acid-base status and the partial pressure of carbon dioxide within physiological limits. Fluid-filled catheters were placed in the right femoral vein and artery for fluid and drug administration and withdrawal of reference arterial blood samples during radioactive microsphere administration, respectively. A 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 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 suspended in a pericardial cradle. An ultrasonic flow probe (Transonics, Ithaca, NY) was positioned around the ascending thoracic aorta for measurement of relative cardiac output (minus coronary blood flow). A fluid-filled catheter was placed in the left atrial appendage for administration of radioactive microspheres used in the determination of regional tissue blood flow. The experimental preparation was allowed to stabilize for at least 30 min after instrumentation had been completed. Coronary perfusion pressure was determined as the difference between diastolic arterial pressure and left ventricular end-diastolic pressure. Haemodynamic data were continuously recorded on a polygraph (model 7, Grass Instruments, Quincy, MA) and simultaneously digitized by a computer interfaced with an analogue to digital converter.

Regional tissue perfusion

Carbonized plastic microspheres (New England Nuclear, Boston, MA; 15 ± 2 μM diameter) labelled with ^{141}Ce, ^{103}Ru, ^{51}Cr, ^{95}Nb were used to measure regional tissue perfusion (Domenech *et al.*, 1969). Immediately before injection, the sphere suspension was ultrasonicated (Model B-3, Branson Company, Shelton, CN) for 15 min and agitated in a vortex mixer (Model K-500-2, Scientific Instruments, Bohemia, NY) for 5 min. The microsphere injection consisted of a bolus of approximately 2 to 3 million spheres injected into the left atrium over 10 s and flushed in with 10 ml of warm (37°C) saline. A timed collection of reference arterial blood was initiated a few seconds before each microsphere injection and maintained at a constant rate of 7 ml min^{-1} for 2 min (precalibrated Harvard infusion/withdrawal pump, Model 1941, South Natick, MA). At the conclusion of each experiment, transmural myocardial tissue samples were selected from the anterior left ventricular free wall for mapping of regional myocardial blood flow. The samples were subdivided into subepicardial, midmyocardial, and subendocardial layers of approximately equal thickness. Multiple tissue samples were also obtained from the left frontal lobe of the brain, cortex and medulla of the kidney, left lower lobe of the liver, proximal third of the duodenum, left margin of the spleen, head of the pancreas and skeletal muscle of the neck. The tissue samples were weighed and placed in scintillation vials. The radioactivity of each tissue and reference blood sample was determined with a gamma counter (Minaxi 5000, Packard, Downers Grove, IL). Tissue blood flow (Q_m; ml min^{-1} g^{-1} was calculated from the equation: $Q_m = Q_r C_m C_r^{-1}$, where Q_r = rate of withdrawal of the reference blood flow sample (ml min^{-1}), C_r = activity of the reference blood flow sample (counts min^{-1}), and C_m = activity of the tissue sample (counts min^{-1} g^{-1}). Relative tissue vascular resistance for each vascular bed was calculated as the quotient of mean arterial pressure and tissue blood flow.

Experimental protocol

Dogs ($n=22$) were assigned to receive levosimendan, pimobendan, or milrinone in a random manner in separate groups of experiments. The drug vehicle for levosimendan, pimobendan, and milrinone consisted of 25% ethanol (95%), 25% polyethylene glycol (5%), and 50% normal saline. A previous investigation from our laboratory (Pelc *et al.*, 1986) has demonstrated that this drug vehicle does not cause haemodynamic effects in barbiturate-anaesthetized dogs. Baseline systemic haemodynamics were recorded and regional tissue perfusion was determined during control conditions 30 min after the instrumentation was completed. In one group of experiments, intravenous infusions of levosimendan at 0.75, 1.5 and 3.0 μg kg^{-1} min^{-1} for 15 min were administered in a sequential fashion. Haemodynamics were recorded, and regional tissue perfusion was determined by the techniques described above immediately after 15 min of each dose. In two other groups of experiments, dogs received intravenous infusions of pimobendan (10, 20, and 40 μg kg^{-1} min^{-1}) or milrinone (1.0, 2.0 and 4.0 μg kg^{-1} min^{-1}) for 15 min in a sequential manner. Haemodynamics and tissue perfusion were recorded at the time intervals described above. The doses of levosimendan, pimobendan, and milrinone used in the present investigation were chosen to produce similar dose-related increases in cardiac output and also represent clinically meaningful doses in man (Monrad *et al.*, 1984; Hasenfuss *et al.*, 1989; Pouleur *et al.*, 1989; Lilleberg *et al.*, 1994; 1995). At the end of each experiment, the heart was electrically fibrillated, the positions of the fluid-filled catheters and the micromanometer-tipped catheter were confirmed, and tissue specimens obtained for analysis of blood flow.

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 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 $\pm$ s.e.mean.

Results

Levosimendan caused significant ($P<0.05$) and dose-related decreases in mean arterial pressure, left ventricular systolic and end-diastolic pressures, coronary artery perfusion pressure, rate-pressure product, and an increase in cardiac output (Table 1). A dose-dependent increase in global myocardial contractility ($+dP/dt_{max}$) occurred with levosimendan. An increase in heart rate was observed during the 3.0 μg kg^{-1} min^{-1} dose of levosimendan. Pimobendan and milrinone caused

haemodynamic effects that were very similar but not identical to those produced by levosimendan. Pimobendan caused dose-related increases in heart rate and reduced left ventricular systolic pressure only at the 40 $\mu g\ kg^{-1}\ min^{-1}$ dose. Milrinone-induced decreases in mean arterial pressure, left ventricular systolic pressure, coronary perfusion pressure, and rate-pressure product were less pronounced than those produced by levosimendan.

Subepicardial, midmyocardial, subendocardial, and transmural blood flow were unchanged by levosimendan (Table 2). The ratio of subendocardial to subepicardial perfusion (endo/epi ratio: 0.93 ± 0.05 during control to 0.78 ± 0.04 during the high dose) decreased during administration of levosimendan. Levosimendan also reduced calculated transmural vascular resistance (61 ± 8 during control to 40 ± 6 mmHg min g ml^{-1} during the high dose). In contrast to the findings with levosimendan, pimobendan increased subepicardial, midmyocardial, and transmural blood flow at the 40 $\mu g\ kg^{-1}\ min^{-1}$ dose (Table 2). These changes in regional myocardial perfusion were again accompanied by dose-related reductions in the ratio of subendocardial to subepicardial blood flow and transmural vascular resistance. Milrinone increased subendocardial and subepicardial flow at 1.0 and 2.0 $\mu g\ kg^{-1}\ min^{-1}$ doses, respectively. Unlike levosimendan, however, milrinone did not alter regional myocardial blood flow distribution or transmural vascular resistance (Table 2).

Levosimendan increased blood flow to the renal medulla (0.34 ± 0.08 during control to 0.43 ± 0.07 ml $min^{-1}\ g^{-1}$ during the high dose) and decreased vascular resistance in both the renal medulla and cortex (21 ± 4 during control to 12 ± 1 mmHg min g ml^{-1} during the high dose) in a dose-related manner (Figure 1). Reductions in renal medullary and cortical vascular resistances were less pronounced with pimobendan and milrinone, respectively, than those observed with levosimendan. Levosimendan increased blood flow to the small intestine (0.85 ± 0.11 during control to 1.14 ± 0.18 ml $min^{-1}\ g^{-1}$ during the high dose) and reduced vascular resistance to this organ (Figure 2). In contrast, pimobendan and milrinone did not alter small bowel vascular resistance. Levosimendan, pimobendan, and milrinone caused increases in hepatic blood flow (e.g. 0.26 ± 0.10 during control to 0.46 ± 0.11 ml $min^{-1}\ g^{-1}$ during the high dose of levosimendan) and similar decreases in hepatic vascular resistance (Figure 2). No changes in pancreatic blood flow and vascular resistance were observed during administration of levosimendan. In contrast, pimobendan and milrinone reduced pancreatic blood flow (e.g. 1.13 ± 0.26 during control to 0.64 ± 0.16 ml $min^{-1}\ g^{-1}$ during the high dose of pimobendan) and increased pancreatic vascular resistance. Levosimendan, pimobendan, and milrinone caused similar decreases in blood flow to the spleen (e.g. 3.02 ± 0.53 during control to 1.99 ± 0.25 ml $min^{-1}\ g^{-1}$ during the high dose of levosimendan) concomitant with relative maintenance of splenic vascular resistance. Levosimendan and pimobendan, but not milrinone, caused similar reductions in cerebral vascular resistance (e.g. 337 ± 60 during control to 203 ± 34 mmHg min g ml^{-1} during the high dose of levosimendan) but did not significantly alter cerebral blood flow. No changes in skeletal muscle blood flow were observed with levosimendan, pimobendan, and milrinone. Decreases in skeletal muscle vascular resistance occurred with levosimendan and milrinone.

Discussion

The effects of levosimendan, pimobendan, and milrinone on systemic haemodynamics and regional tissue perfusion in the

Table 1 Haemodynamic effects of levosimendan, pimobendan and milrinone

	Control	*Low dose*	*Middle dose*	*High dose*
Levosimendan ($n=9$)				
HR (beats min^{-1})	125 ± 5	129 ± 7	131 ± 8	136 ± 7^{ab}
MAP (mmHg)	106 ± 4	97 ± 5^{a}	82 ± 3^{ab}	73 ± 3^{ab}
RPP (beats min^{-1} mmHg)	13.7 ± 1.3	12.7 ± 1.3	10.5 ± 0.8^{ab}	9.8 ± 0.6^{ab}
LVSP (mmHg)	118 ± 6	109 ± 4^{a}	99 ± 2^{ab}	96 ± 2^{ab}
LVEDP (mmHg)	8 ± 1	5 ± 1^{a}	4 ± 1^{ab}	4 ± 1^{ab}
CPP (mmHg)	85 ± 6	79 ± 6	65 ± 4^{ab}	58 ± 3^{ab}
$+dP/dt_{max}$ ($mmHg\ s^{-1}$)	1641 ± 52	1762 ± 61^{a}	1961 ± 56^{ab}	2027 ± 58^{ab}
CO (l min^{-1})	3.1 ± 0.4	3.2 ± 0.3	3.5 ± 0.4	3.9 ± 0.4^{ab}
SVR (dyn-s-cm^{-5})	2930 ± 360	2970 ± 460	2080 ± 240^{ab}	1620 ± 130^{ab}
Pimobendan ($n=6$)				
HR (beats min^{-1})	129 ± 7	136 ± 10	146 ± 12^{a}	155 ± 14^{ab}
MAP (mmHg)	94 ± 1	88 ± 3	79 ± 2^{ab}	71 ± 3^{abc}
RPP (beats min^{-1} mmHg)	12.2 ± 0.8	12.2 ± 1.2	11.6 ± 0.9	10.8 ± 1.1^{a}
LVSP (mmHg)	113 ± 3	111 ± 3	110 ± 3	106 ± 4^{a}
LVEDP (mmHg)	9 ± 2	5 ± 1^{a}	4 ± 1^{ab}	2 ± 1^{abc}
CPP (mmHg)	75 ± 4	68 ± 4	60 ± 2^{ab}	53 ± 3^{abc}
$+dP/dt_{max}$ ($mmHg\ s^{-1}$)	1753 ± 117	2018 ± 105^{a}	2355 ± 112^{ab}	2569 ± 78^{abcd}
CO (l min^{-1})	3.1 ± 0.5	3.2 ± 0.6	3.4 ± 0.4	3.7 ± 0.5^{a}
SVR (dyn-s-cm^{-5})	2620 ± 400	2460 ± 280	1900 ± 230^{a}	1500 ± 180^{ab}
Milrinone ($n=7$)				
HR (beats min^{-1})	125 ± 7	131 ± 6	140 ± 6^{ab}	144 ± 8^{ab}
MAP (mmHg)	110 ± 10	113 ± 10	104 ± 9^{d}	95 ± 9^{abd}
RPP (beats min^{-1} mmHg)	14.0 ± 1.6	14.8 ± 1.6	14.5 ± 1.6^{d}	13.5 ± 1.7^{d}
LVSP (mmHg)	125 ± 11	124 ± 10	120 ± 9^{d}	114 ± 9^{d}
LVEDP (mmHg)	9 ± 2	7 ± 2^{a}	5 ± 1^{ab}	4 ± 1^{ab}
CPP (mmHg)	86 ± 8	92 ± 9	87 ± 9	80 ± 9
$+dP/dt_{max}$ ($mmHg\ s^{-1}$)	1796 ± 242	2064 ± 224^{a}	2336 ± 232^{a}	2303 ± 228^{a}
CO (l min^{-1})	2.9 ± 0.5	3.2 ± 0.6	3.4 ± 0.5	3.7 ± 0.5^{a}
SVR (dyn-s-cm^{-5})	3430 ± 720	3090 ± 510	2550 ± 390	2150 ± 380^{ab}

Data are mean ± s.e.mean. [a] Significantly ($P<0.05$) different from control. [b] Significantly ($P<0.05$) different from low dose. [c] Significantly ($P<0.05$) different from middle dose. [d] Significantly ($P<0.05$) different from corresponding value in levosimendan group. Abbreviations: HR = heart rate; MAP = mean arterial pressure; RPP = rate-pressure product; LVSP = left ventricular systolic pressure; LVEDP = left ventricular end-diastolic pressure; CPP = coronary artery perfusion pressure; $+dP/dt_{max}$ = maximum rate of increase of left ventricular pressure; CO = cardiac output; SVR = systemic vascular resistance.

Table 2 Effects of levosimendan, pimobendan and milrinone on regional myocardial blood flow and transmural resistance

	Control	*Low dose*	*Middle dose*	*High dose*
Levosimendan ($n=9$)				
Subepicardium	2.03±0.22	2.08±0.28	2.12±0.31	2.46±0.35
Midmyocardium	1.93±0.21	1.86±0.27	1.73±0.26	2.01±0.29
Subendocardium	1.89±0.22	1.79±0.28	1.63±0.25	1.89±0.25
Transmural	1.95±0.21	1.91±0.27	1.83±0.27	2.12±0.29
Transmural resistance	61±8	60±9	53±8	40±6[abc]
Endo/epi	0.93±0.05	0.87±0.06	0.79±0.05[ab]	0.78±0.04[ab]
Pimobendan ($n=6$)				
Subepicardium	1.90±0.34	2.08±0.25	2.33±0.35	3.36±0.39[abc]
Midmyocardium	2.02±0.35	1.98±0.26	2.02±0.28	2.84±0.39[abc]
Subendocardium	2.01±0.19	2.02±0.23	1.77±0.18	2.27±0.21
Transmural	1.98±0.28	2.02±0.24	2.04±0.25	3.04±0.44[abc]
Transmural resistance	52±7	47±6	42±5	26±3[abc]
Endo/epi ratio	1.25±0.07	0.98±0.04[a]	0.82±0.12[a]	0.69±0.02[ab]
Milrinone ($n=7$)				
Subepicardium	1.60±0.19	2.13±0.35	2.28±0.45[a]	2.13±0.46
Midmyocardium	1.50±0.18	1.94±0.36	1.98±0.45	1.64±0.37
Subendocardium	1.69±0.15	2.22±0.36[a]	2.00±0.37	1.70±0.29
Transmural	1.59±0.16	2.10±0.34	2.09±0.41	1.82±0.35
Transmural resistance	72±9	60±8	64±14	61±10
Endo/epi	1.09±0.06	1.08±0.11	0.94±0.11	0.89±0.12

Data are means±s.e.mean. [a]Significantly ($P<0.05$) different from control. [b]Significantly ($P<0.05$) different from low dose. [c]Significantly ($P<0.05$) different from middle dose. Abbreviation: Endo/epi = ratio of subendocardial to subepicardial blood flow; myocardial blood flow = ml min^{-1} g^{-1}; transmural vascular resistance = mmHg min g ml^{-1}

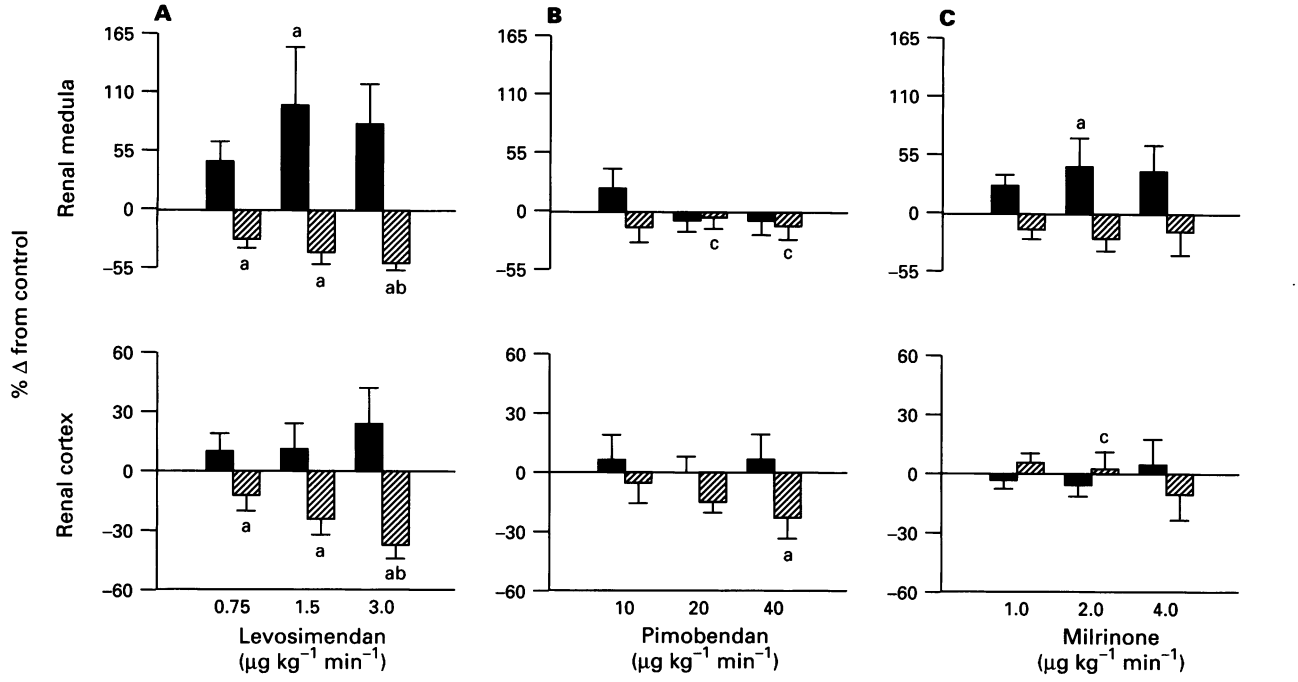

Figure 1 Histograms illustrating the effects of levosimendan (A), pimobendan (B), and milrinone (C) on blood flow (solid columns) and calculated vascular resistance (hatched columns) in the medulla (top panels) and cortex (bottom panels) of the kidney. Data are represented as % change from control. [a]Significantly ($P<0.05$) different from baseline; [b]significantly ($P<0.05$) different from the low dose; [c]significantly ($P<0.05$) different from corresponding value in levosimendan group.

heart, brain, kidney, liver, small intestine, pancreas, spleen and skeletal muscle were examined in open-chest, barbiturate-anaesthetized dogs. Levosimendan increased heart rate, decreased mean arterial pressure, enhanced cardiac output and myocardial contractility, and reduced left ventricular systolic and end-diastolic pressures. These haemodynamic effects were accompanied by reductions in rate-pressure product, suggesting that levosimendan decreases myocardial oxygen consumption via reductions in left ventricular preload and afterload despite simultaneous increases in heart rate and contractility. The cardiovascular actions produced by levosimendan in the present investigation were similar to those observed previously in conscious dogs (Pagel *et al.*, 1994; 1995; Harkin *et al.*, 1995) and man (Lilleberg *et al.*, 1994; 1995; Sundberg *et al.*, 1995). Pimobendan and milrinone also caused haemodynamic effects that were similar to those found with these vasoactive drugs by other laboratories (Monrad *et al.*, 1984; Borow *et al.*, 1985; Jaski *et al.*, 1985; Colucci *et al.*, 1986; Piscione *et al.*, 1987; Walter *et al.*, 1988; Baumann *et al.*, 1989).

Levosimendan maintained transmural myocardial perfusion

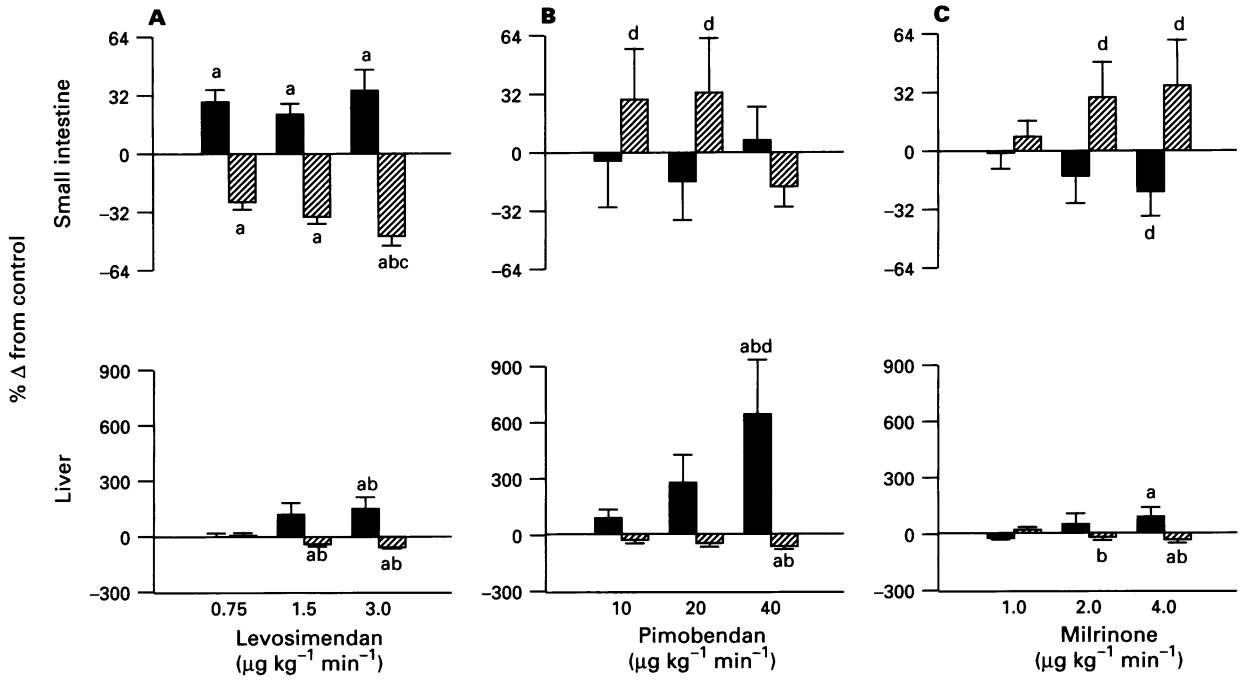

Figure 2 Histograms illustrating the effects of levosimendan (A), pimobendan (B), and milrinone (C) on blood flow (solid columns) and calculated vascular resistance (hatched columns) in the small intestine (top panels) and the liver (bottom panels). Data are represented as % change from control. [a]Significantly ($P<0.05$) different from baseline; [b]significantly ($P<0.05$) different from the low dose; [c]significantly ($P<0.05$) different from the middle dose; [d]significantly ($P<0.05$) different from corresponding value in levosimendan group.

despite dose-related reductions in coronary artery perfusion pressure and rate-pressure product. These findings were accompanied by declines in calculated transmural vascular resistance, suggesting that levosimendan may produce coronary vasodilatation. Levosimendan also decreased the endo/epi ratio, indicating that this drug causes redistribution of intramyocardial blood flow toward the subepicardium. In contrast to the findings with levosimendan, pimobendan increased subepicardial, midmyocardial, and transmural myocardial blood flow. The pimobendan-induced increases in regional myocardial blood flow occurred concomitant with reductions in coronary perfusion pressure and rate-pressure product that were similar to those produced by levosimendan. Pimobendan also caused relatively greater decreases in transmural vascular resistance and the ratio of subendocardial to subepicardial blood flow. These findings support the observations of Verdouw and Colleagues (Verdouw *et al.*, 1986) in anaesthetized pigs and suggest that pimobendan causes more profound coronary vasodilatation and redistribution of intramyocardial perfusion than levosimendan. Milrinone also increased subepicardial and subendocardial flow at the 1.0 and 2.0 $\mu g\ kg^{-1}\ min^{-1}$ doses. However, no changes in the intramyocardial blood flow distribution were observed. The present results with milrinone are partially supported by the findings of a previous study in conscious rats (Drexler *et al.*, 1987) and are qualitatively similar to those observed with another PDE inhibitor, amrinone, in anaesthetized dogs (Einzig *et al.*, 1982).

The present results also indicate that levosimendan causes several alterations in regional tissue perfusion that are somewhat different from those produced by pimobendan and milrinone. Levosimendan increased renal medullary blood flow and reduced vascular resistance in both the medullary and cortical beds, indicating that this new drug produces renal vasodilatation. Reductions in renal medullary and cortical vascular resistances were less pronounced with pimobendan and milrinone, respectively, than those observed with levosimendan, supporting the findings of two previous studies (Verdouw *et al.*, 1986; Drexler *et al.*, 1987). Levosimendan increased small intestinal blood flow and reduced vascular resistance in this organ, in contrast to the findings with pimobendan and milrinone. Maintenance of small intestinal blood flow and resistance has been demonstrated previously during administration of milrinone (Drexler *et al.*, 1987). Pimobendan has been shown to increase small intestinal blood flow and vascular conductance in anaesthetized pigs (Verdouw *et al.*, 1986). However, these findings occurred at doses of pimobendan (50 and 100 $\mu g\ kg^{-1}\ min^{-1}$) that exceeded the range used in the present investigation. Increases in hepatic blood flow and decreases in hepatic vascular resistance occurred with levosimendan, pimobendan and milrinone, results that concur with the findings of Verdouw *et al.* (1986) and Drexler *et al.* (1987). Pimobendan-induced increases in hepatic blood flow were significantly greater than those observed with levosimendan at the highest doses of these drugs.

The effects of myofilament Ca^{2+} sensitizers and milrinone on perfusion of the pancreas and spleen have not been characterized. Levosimendan, pimobendan and milrinone caused effectively similar reductions in pancreatic and splenic blood flow. Although cerebral cortical blood flow was unchanged by all three vasoactive drugs, levosimendan and pimobendan, but not milrinone, decreased vascular resistance in the frontal cerebral cortex, consistent with a modest cerebral vasodilating effect. These findings support dose-related increases in the vascular conductance of the brain that have been demonstrated with pimobendan (Verdouw *et al.*, 1986). Milrinone has also been shown to increase brain perfusion and reduce vascular resistance (Drexler *et al.*, 1987). However, these results occurred at milrinone doses ($\geqslant 6\ \mu g\ kg^{-1}\ min^{-1}$) that exceeded those used in the present investigation. Lastly, no changes in skeletal muscle perfusion were observed with levosimendan, pimobendan and milrinone in anaesthetized dogs. Pimobendan and milrinone have been shown to maintain blood flow to skeletal muscle (Verdouw *et al.*, 1986; Drexler *et al.*, 1987). Milrinone and levosimendan reduced skeletal muscle vascular resistance. Pimobendan has also been shown to cause increases in skeletal muscle vascular conductance (Verdouw *et al.*, 1986). However, this effect occurred

at doses that exceeded those used in the present investigation. In addition, maintenance of muscle vascular resistance has been previously described with milrinone in conscious rats (Drexler *et al.*, 1987). However, baseline barbiturate anaesthesia and use of an acutely instrumented experimental preparation in the present study may have influenced the effect of milrinone on skeletal muscle perfusion. In addition, although the doses of levosimendan, pimobendan and milrinone were chosen to cause similar increases in cardiac output, the haemodynamic effects of milrinone did not completely match those produced by the myofilament Ca^{2+} sensitizers. Thus, comparison of the effects of levosimendan, pimobendan and milrinone on regional tissue perfusion should be qualified because these drugs caused somewhat different alterations in systemic haemodynamics.

In summary, the present results have demonstrated that levosimendan, a new myofilament Ca^{2+} sensitizer with PDE-inhibiting activity, causes more pronounced renal vasodilatation and increases in splanchnic perfusion than pimobendan and milrinone. In contrast, pimobendan produced relatively greater increases in hepatic blood flow than levosimendan. The results indicate that levosimendan, pimobendan, and milrinone cause subtlety different alterations in regional tissue perfusion while producing similar haemodynamic effects in barbiturate-anaesthetized, acutely instrumented dogs.

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