Comparative effects of levosimendan, OR-1896, OR-1855, dobutamine, and milrinone on vascular resistance, indexes of cardiac function, and \( \text{O}_2 \) consumption in dogs

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The intravenous formulation of levosimendan has been studied in several randomized, comparative studies in patients with decompensated heart failure (23) and efficacy, and tolerability has been demonstrated in heart failure patients of both ischemic and nonischemic etiology (10, 31). Plasma concentrations associated with levosimendan efficacy were assessed in an open-label, nonrandomized phase II study in patients diagnosed with heart failure (New York Heart Association III-IV), whereby a 24-h continuous infusion of levosimendan produced peak plasma concentrations of 62.6 ng/ml; in the same patients, peak concentrations of OR-1896 and OR-1855, the two primary circulating metabolites of levosimendan, reached 5.5 and 6.8 ng/ml, respectively (22).

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Due to the reduction of levosimendan to OR-1855 in humans and subsequent acetylation to OR-1896 (1, 2), the contribution of the parent vs. each metabolite to the hemodynamic and cardiovascular effects observed in patients cannot be definitively described. However, in the dog, neither levosimendan nor OR-1855 is metabolized to OR-1896. Moreover, a comprehensive assessment of the effects of levosimendan and its metabolites (in relation to plasma concentrations achieved) on cardiovascular function has not been fully described in dog. Thus the present study sought to characterize the effects of levosimendan, OR-1896, and OR-1855 on myocardial and hemodynamic function in the comprehensively instrumented dog at plasma concentrations deemed therapeutic to supratherapeutic concentrations.

RESULTS

Plasma concentrations of levosimendan (323 ± 15 ng/ml) represent approximately fivefold above the concentration expected in humans after a 0.2 mg·kg⁻¹·min⁻¹ continuous infusion for 24 h (62.6 ± 29.2 ng/ml; mean represented by dotted line, 1 SD of mean represented by shaded area). Peak concentrations of levosimendan were 126 ± 30 min⁻¹, n = 6 – 7/group) targeting therapeutic to supratherapeutic concentrations of total levosimendan (Cmax = 62.6 ng/ml). Blood samples were collected at 15-min intervals throughout the experimental protocol. The same protocol was carried out in separate groups of dogs that received vehicle alone and in dogs infused with either dobutamine or milrinone at 1-log unit higher doses.

METHODS

All procedures were approved by Abbott Laboratories’ Institutional Animal Care and Use Committee and carried out in American Association for Accreditation of Laboratory Animal Care accredited facilities. Male beagle dogs (9–12 kg) were anesthetized with pentobarbital (35.0 mg/kg iv), immediately placed on a constant intravenous infusion (6.0 mg·kg⁻¹·h⁻¹), and subsequently comprehensively instrumented, as previously described (11, 12). Dogs were intubated and ventilated with room air. Expiratory CO₂ was monitored with an end-tidal CO₂ monitor and maintained at 4–5% CO₂. A Swan-Ganz catheter (5.5 F) was advanced into the pulmonary artery via the right jugular vein for measurement of cardiac output; central venous and pulmonary artery pressures were measured through the proximal and distal ports of the catheter, respectively. A dual-tip micromanometer catheter (Millar, model SPC-770, 7F) was advanced into the left ventricle of the heart via the right carotid artery for measurement of left ventricular and aortic blood pressure. Polyethylene catheters were inserted into the right femoral vein and artery for infusion of test agents and collection of blood samples, respectively. Systemic vascular resistance (SVR) was calculated as [mean arterial pressure (MAP) – mean central venous pressure]/cardiac output. Pulmonary vascular resistance was calculated as (pulmonary arterial pressure – central venous pressure)/cardiac output. Body temperature was monitored throughout the experiment and kept constant through the use of a heating pad. The primary hemodynamic variables were computed using commercial software and a signal processing workstation (Ponemah, Gould Instrument Systems).

Animals were randomly divided into one of six treatment or vehicle (5% dextrose in water) groups. Following the completion of the surgical protocol, animals were allowed to stabilize for 1 h, and baseline data were collected at 5-min intervals 30 min before treatment. Each dose of active drug was administered as a 30-min infusion (0.02 ml·kg⁻¹·min⁻¹) as a series of four escalating doses dissolved in a 5% dextrose in water vehicle (Abbott Laboratories); following termination of the high-dose infusion, animals were observed for 30 min. Levosimendan, OR-1896, and OR-1855 (synthesized at Orion Pharmaceuticals, Espoo, Finland) were infused at 0.01, 0.03, 0.10, and 0.33 μmol·kg⁻¹·30 min⁻¹; blood samples were withdrawn at 15-min intervals for determination of plasma concentrations of levosimendan and each metabolite by HPLC-mass spectrometry. Dobutamine (Sigma) and milrinone (Sigma) were infused at 1-log unit higher doses to achieve similar reductions in systemic resistance as that produced by levosimendan and OR-1896 (Fig. 1). For additional depth and perspective, results from the present study were also compared with those of three K⁺ channel openers (KCOs) previously profiled in the anesthetized dog cardiovascular model, A-278637 (11), ZD-6169 (11), WAY-133537 (11), A-325100 (12), and the dihydropyridine Ca²⁺ channel blocker nifedipine (11).

Statistical analysis of the hemodynamic and cardiovascular data were performed as described in Figs. 1–11 legends for each cardiovascular parameter measured in the present study. Briefly, two-sided unpaired t-tests were used to assess change from baseline in drug-treated dogs relative to change from baseline in vehicle controls when a single drug group was compared with vehicle. Comparison of multiple groups within a single hemodynamic variable were computed with one-way ANOVA, Dunnett’s t-test vs. vehicle (Prism 4.01 software).

To further characterize potential differences in the hemodynamic response to infusion of levosimendan, OR-1896, and OR-1855 (synthesized at Orion Pharmaceuticals, Espoo, Finland) were infused at 0.01, 0.03, 0.10, and 0.33 μmol·kg⁻¹·30 min⁻¹; blood samples were withdrawn at 15-min intervals for determination of plasma concentrations of levosimendan and each metabolite by HPLC-mass spectrometry. Dobutamine (Sigma) and milrinone (Sigma) were infused at 1-log unit higher doses to achieve similar reductions in systemic resistance as that produced by levosimendan and OR-1896 (Fig. 1). For additional depth and perspective, results from the present study were also compared with those of three K⁺ channel openers (KCOs) previously profiled in the anesthetized dog cardiovascular model, A-278637 (11), ZD-6169 (11), WAY-133537 (11), A-325100 (12), and the dihydropyridine Ca²⁺ channel blocker nifedipine (11).

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RESULTS

Peak plasma concentrations of levosimendan at the end of the 0.01, 0.03, 0.10, and 0.33 μmol/kg infusion period were 12.9 ± 0.7, 47.6 ± 2.1, 158 ± 6, and 455 ± 21 ng/ml, respectively (Fig. 2). These concentrations are 0.2-, 0.8-, 2.5-, and 7.2-fold above the therapeutic concentrations of total levosimendan (C_{max}) observed in patients (62.6 ng/ml) (22) administered a 0.2 mg·kg^{-1}·min^{-1} continuous infusion for 24 h. The same doses of OR-1896 produced peak concentrations of 2.6 ± 0.2, 10.9 ± 0.6, 39.7 ± 2.6, and 126 ± 6 ng/ml.

Fig. 3. Mean arterial pressure (MAP; ●, mmHg change from baseline) and systemic vascular resistance (SVR; ○, percent change from baseline) in anesthetized dogs in the presence of vehicle (A), levosimendan (B), OR-1896 (C), OR-1855 (D), dobutamine (E), and milrinone (F). Mean MAP and mean SVR in vehicle-treated animals are depicted in B–F by the dotted and dashed lines, respectively. Statistical significance (change from baseline relative to vehicle controls; t-test at each posttreatment time point) was determined at P < 0.05 for *MAP and +SVR.

Fig. 4. Effect of each compound tested on pulse pressure [PP; mmHg, defined as (systolic arterial pressure − diastolic arterial pressure); equivalent scale used for all panels in figure]. A: levosimendan; B: OR-1896; C: OR-1855; D: dobutamine; E: milrinone. Relative to vehicle controls, only dobutamine produced significant increases in PP, an effect observed at all doses tested; maximal increase in PP during each infusion period were 6 ± 2, 14 ± 1, 23 ± 2, and 19 ± 3 mmHg, respectively. *P < 0.05, change from baseline in drug relative to vehicle controls at each posttreatment time point, t-test.
Fig. 5. Effect of levosimendan (○), OR-1896 (□), dobutamine (△), and milrinone (●) on time to peak systolic arterial pressure (time, in ms, defined as time from rise of systolic arterial pressure pulse to the time of peak systolic blood pressure) (A), and time to systolic arterial pressure recovery (time, in ms, defined as time from rise of systolic arterial pressure pulse to time of 70% recovery from peak systolic blood pressure) (B). The efficacious dose for each compound to produce a 35% reduction in time to peak pressure (ED$_{35}$; ~38 ms) or a 35% reduction in systolic pressure recovery time (ED$_{35}$; ~35 ms) was calculated, and values are shown within the figure; relevant ED$_{35}$ values could not be calculated for OR-1855, since the compound produced no effect on either parameter. Based on potency for both reductions in time to peak pressure and time to systolic pressure recovery, compounds can be ranked as OR-1896 > levosimendan > milrinone > dobutamine. Maximal reductions in both time to peak pressure and time to systolic pressure recovery are, respectively, as follows: levosimendan: 84 and 123 ms; OR-1896: 85 and 132 ms; dobutamine: 104 and 145 ms; milrinone: 82 and 134 ms.

Fig. 6. Time constant of isovolumic relaxation ($\tau$, ms) and an index of diastolic performance in dogs infused with levosimendan (A), OR-1896 (B), OR-1855 (C), dobutamine (D), and milrinone (E). *$P < 0.05$, change from baseline in drug relative to vehicle controls at each posttreatment time point, $t$-test.
(0.5-, 2.0-, 7.2-, and 23-fold, respectively, above the $C_{\text{max}}$ for OR-1896; 5.5 ng/ml) (22), and OR-1855 produced concentrations of $3.1 \pm 0.1$, $12.6 \pm 0.5$, $42.5 \pm 1.4$, and $136 \pm 6$ ng/ml (0.5-, 1.9-, 6.3-, and 20-fold, respectively, above the $C_{\text{max}}$ for OR-1855; 6.8 ng/ml) (22), respectively. In dogs infused with OR-1896, a small proportion of the compound was metabolized to OR-1855 (peak concentration = $22.4 \pm 2.3$ ng/ml at the end of the experimental protocol).

Relative to vehicle controls, levosimendan produced dose-dependent reductions in MAP and SVR beginning at 0.1 $\mu$mol/kg (to $-31 \pm 2$ mmHg and $-15 \pm 7\%$, respectively, below baseline at 0.3 $\mu$mol/kg) and OR-1896 at 0.01 $\mu$mol/kg (to $-42 \pm 3$ mmHg and $-32 \pm 4\%$, respectively, below baseline at 0.3 $\mu$mol/kg); OR-1855 produced no effect on MAP or SVR at any dose tested (Fig. 3). Dobutamine produced dose-dependent reductions in SVR beginning at 0.3 $\mu$mol/kg.

**Fig. 7.** Heart rate (beats/minute; left) and rate pressure product [(MAP × heart rate) × 1,000]; right] in dogs infused with levosimendan (A), OR-1896 (B), OR-1855 (C), dobutamine (D), and milrinone (E). *P < 0.05, change from baseline in drug relative to vehicle controls at each posttreatment time point, t-test.
without concomitant decreases in MAP. In fact, MAP increased in the presence of dobutamine to 17 ± 2 mmHg above baseline at 1.0 μmol/kg, despite a 33 ± 2% fall in SVR at the same dose. Reductions in MAP and SVR in the presence of milrinone at 3.3 μmol/kg were similar to that of levosimendan and OR-1896 at lower doses (peak decrease at 3.3 μmol/kg = −38 ± 6 mmHg and −34 ± 4% below baseline, respectively).

Since recent evidence suggests that a high pulse pressure (PP) is an important risk factor for heart disease (a 10–20% increase in PP increased the risk of major cardiovascular complications and mortality by nearly 20%) (3), the effect of each compound on PP was evaluated in the anesthetized dog. Neither levosimendan, OR-1896, OR-1855, nor milrinone produced any significant effect on PP at any dose tested (Fig. 4). However, dobutamine produced increases in PP at all doses tested (maximal effect = 23 ± 2 mmHg above vehicle).

All compounds tested, with the exception of OR-1855, produced reductions in the time to peak systolic arterial pressure (Fig. 5A) and time to systolic arterial pressure recovery (Fig. 5B). Maximal reductions in time to peak pressure and time to systolic pressure recovery (occurring at the highest dose tested in each treatment group) were, respectively, as follows: levosimendan, 84 and 123 ms; OR-1896, 85 and 132 ms; dobutamine, 104 and 145 ms; milrinone, 82 and 134 ms. The efficacious dose for each compound to produce a 35% reduction in time to peak pressure (ED35; ~38 ms) or a 35% reduction in systolic pressure recovery time (ED35; ~35 ms) were calculated using nonlinear regression, and values are shown in the table below Fig. 5; relevant ED35 values could not be calculated for OR-1855, since the compound produced no effect on either parameter. Based on potency for both reductions in time to peak pressure and time to systolic pressure recovery, compounds could be ranked as OR-1896 > levosimendan > milrinone > dobutamine.

The effect of the agents on diastolic performance was also evaluated. The time constant of isovolumic relaxation (τ), expressed in milliseconds, is shown in Fig. 6. The τ remained constant in vehicle controls (30–33 ms throughout the entire experimental protocol). Levosimendan and OR-1896 elicited reductions in τ at 0.03 and 0.01 μmol/kg, respectively, an effect sustained for the remainder of infusion, whereas OR-1855 did not affect τ. Dobutamine elicited dose-dependent reductions in τ (from 33 ± 0.3 ms at baseline to 8 ± 0.7 ms during the 3.3 μmol/kg infusion); a similar decrease was observed during milrinone infusion (from 30 ± 0.3 ms at baseline to 13 ± 2.5 ms during the 3.3 μmol/kg infusion).

Heart rate (Fig. 7, right) was dose-dependently elevated in dogs infused with levosimendan (from 117 ± 2 beats/min at baseline to 166 ± 3 beats/min at 0.3 μmol/kg), OR-1896 (from 128 ± 5 to 173 ± 5 beats/min), dobutamine (from 115 ± 8 to 217 ± 7 beats/min), and milrinone (from 135 ± 4 to 194 ± 8 beats/min); OR-1855 produced no effect on heart rate at any dose tested. Although levosimendan and milrinone produced modest, but statistically significant, increases in rate-pressure product (RPP; Fig. 7, left), an index of myocardial consumption, increases in RPP were not dose dependent. However, in dogs infused with dobutamine, RPP was dose-dependently elevated in a stepwise fashion from 15 ± 1 at baseline to 26 ± 1 mmHg·beats·min⁻¹·1,000⁻¹ at the end of the high dose.

Neither vehicle nor OR-1855 produced any effect on change in pressure over time (dP/dt) at 50 mmHg, an index of myocardial contractility (Fig. 8A). However, levosimendan, OR-1896, dobutamine, and milrinone all produced significant and dose-dependent increases in dP/dt; at the end of the 0.3 μmol/kg dose, the highest common dose tested for all of the compounds, dP/dt was increased to 113 ± 7, 132 ± 14, 57 ± 10, and 120 ± 23% above baseline, respectively. At 1 log-unit higher doses, increases in dP/dt produced by dobutamine and milrinone were exacerbated (to 297 ± 33 and 119 ± 48% above baseline, respectively). Concomitant with large increases in dP/dt, levosimendan, OR-1896, dobutamine, and milrinone all produced significant reductions in left ventricular end-diastolic pressure (Fig. 8B); vehicle and OR-1855 produced no effect on dP/dt and thus no effect on left ventricular end-diastolic pressure.

Consistent with dose-dependent increases in myocardial contractility, all compounds tested, with the exception of OR-1855, produced dose-dependent reductions in ejection time (defined as the time from the rise of the systolic pressure pulse
to maximal $-\frac{dP}{dt}$; Fig. 9). The efficacious dose for each compound to produce a 35% reduction in ejection time (ED35; $-62$ ms) was calculated, and values are shown within the figure. In order of potency, OR-1896 > levosimendan > milrinone > dobutamine > OR-1855.

The effects of levosimendan and OR-1896 were limited to the systemic circulation and produced no significant effects on either pulmonary arterial pressure or pulmonary vascular resistance (Fig. 10). Dobutamine, on the other hand, produced dose-dependent increases in pulmonary arterial pressure (to $9 \pm 2, 32 \pm 4, 61 \pm 7,$ and $74 \pm 13\%$ above baseline at the end of each dosing period, respectively), despite no relevant changes in pulmonary vascular resistance. Milrinone produced no effect on pulmonary arterial pressure but did produce a modest decrease in pulmonary vascular resistance at 0.1 and 0.3 $\mu$mol/kg (to $-8 \pm 2$ and $-12 \pm 3\%$ below baseline, respectively). With one exception, baseline pulmonary pressure and pulmonary vascular resistance values were not different between most groups relative to vehicle. Pulmonary pressure and vascular resistance were, respectively, $16.1 \pm 0.6$ mmHg and $9.6 \pm 0.2$ mmHg $\cdot$ l$^{-1}$ $\cdot$ min$^{-1}$ in vehicle-treated dogs; $12.9 \pm 0.3$ mmHg ($P < 0.05$, one-way ANOVA, Dunnett’s posttest) and $7.6 \pm 0.4$ mmHg $\cdot$ l$^{-1}$ $\cdot$ min$^{-1}$ for levosimendan; $15.7 \pm 0.06$ mmHg and $8.7 \pm 0.6$ mmHg $\cdot$ l$^{-1}$ $\cdot$ min$^{-1}$ for OR-1896; $14.8 \pm 0.7$ mmHg and $9.6 \pm 0.5$ mmHg $\cdot$ l$^{-1}$ $\cdot$ min$^{-1}$ for OR-1855; $15.0 \pm 0.9$ mmHg and $8.5 \pm 1.3$ mmHg $\cdot$ l$^{-1}$ $\cdot$ min$^{-1}$ for dobutamine; and $17.0 \pm 0.4$ mmHg and $7.5 \pm 0.3$ mmHg $\cdot$ l$^{-1}$ $\cdot$ min$^{-1}$ for milrinone.

Changes in SVR and left ventricular contractility ($\frac{dP}{dt}$) were analyzed relative to plasma concentrations achieved for levosimendan and its metabolites (although OR-1855 produced no relevant effect on either parameter). The compounds elicit systemic vasodilation with rank potency of OR-1896 > levosimendan > OR-1855, suggesting that the vasodilatory effects of levosimendan in patients are predominantly mediated by the OR-1896 metabolite (Fig. 11A). Moreover, levosimendan and its metabolites enhance cardiac contractility, with rank potency of OR-1896 > levosimendan > OR-1855, suggesting that the inotropic of levosimendan in patients is predominantly mediated by the OR-1896 metabolite and parent drug, with little to no contribution from OR-1855 (Fig. 11B). When analyzed as change in $\frac{dP}{dt}$ vs. SVR (Fig. 11C), both levosimendan and OR-1896 clearly differentiate themselves from other KCOs (WAY-133537, ZD-6169, A-325100, A-278637) and the Ca$^{2+}$ channel blocker nifedipine, suggesting that the increases in $\frac{dP}{dt}$ produced by levosimendan and OR-1896 are not wholly compensatory in response to the fall in SVR and MAP. Rather, changes in $\frac{dP}{dt}$ relative to SVR produced by levosimendan are more similar, if not more potent, than those of dobutamine and milrinone.

**DISCUSSION**

Levosimendan can be administered intravenously to patients with acute decompensated CHF, thereby enhancing cardiac contractility via Ca$^{2+}$ sensitization and eliciting vasodilation through K$^{+}$ channel activation (22, 27, 32, 34, 41). Thus a similar dosing paradigm was used in the present study (intravenous infusion) to capture the hemodynamic and cardiovascular effects of levosimendan, as well as its metabolites,
Ca^{2+}/KATP/large-conductance Ca^{2+}/H11001 sensitization, respectively.

These effects occurred at therapeutic to supratherapeutic time to systolic recovery than either milrinone or dobutamine. To produce reductions in time to peak systolic blood pressure and to effects on blood pressure, we demonstrate in the present study, in vivo, is due to baroreflex activation at low doses of OR-1896 (0.01 μmol/kg) in response to significant reductions in SVR and blood pressure. In contrast, levosimendan did not elicit significant decreases in resistance and blood pressure until higher doses (0.1 μmol/kg).

Both parent and metabolite directly produce positive inotropic responses that have been demonstrated in various test systems, including isolated preparations of heart (4, 8, 14, 18, 37), papillary muscle (16, 17, 48), skinned cardiac fibers (8), and myocytes (25, 38), via a mechanism markedly different from classical positive inotropes, which increase intracellular free Ca^{2+}. Indeed, although levosimendan increases cellular responsiveness to calcium, it does not increase the intracellular concentration of Ca^{2+} within the cardiac myocyte (4, 8). In rabbit ventricular muscle, levosimendan does not affect the amplitude of the inward Ca^{2+} current, but does increase the amplitude of the twitch tension (48). Lack of effects on intracellular Ca^{2+} concentrations have also been demonstrated for OR-1896; in the isolated guinea pig heart, OR-1896 increased contraction force without changing the peak Ca^{2+} transient, indicative of Ca^{2+} sensitization (24), and, in isolated dog papillary muscle and rabbit ventricular tissue, OR-1896 proportionally increased contraction force more than intracellular free calcium, again indicating the Ca^{2+} sensitizing effect of the compound on contractile proteins (44–46).

The mechanism of Ca^{2+} sensitization by levosimendan has been studied directly using purified recombinant human cTnC; Haikala et al. (15) demonstrated that the mechanism of Ca^{2+} sensitization by levosimendan, unlike that of the other Ca^{2+} sensitizers, such as pimobendan, MCI-154, and EMD 53998, is based on Ca^{2+}-dependent binding to the NH_{2}-terminal domain of cTnC, an effect proposed to amplify the trigger of contraction induced by cTnC in the cardiac muscle. Additional studies demonstrated that levosimendan binds to Ca^{2+}-saturated human cTnC, in the hydrophobic pocket of the regulatory N-domain (36, 41), a binding site consistent with a stabilization of the Ca^{2+} bound conformation of the N-domain, and is not altered by cardiac troponin I (42). Moreover, Haikala et al. (17) demonstrated that levosimendan elicits no effect on myosin ATPase activity in guinea pig heart myofibrils, suggesting that Ca^{2+} sensitization is mediated through a mechanism that does not increase energy consumption by contractile proteins, results consistent with the present study whereby we demonstrate that neither levosimendan nor either of its metabolites increase myocardial oxygen consumption, as evidenced by no increase in RPP.

In stark contrast to that of levosimendan and OR-1896, RPP was substantial and dose-dependently increased in the present study in the presence of the β_{1}-agonist dobutamine (to 79% above baseline at the end of the 3.3 μmol/kg dose; vehicle =

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**Fig. 10.** Percent change in pulmonary arterial pressure (PAP; A) and pulmonary vascular resistance (PVR; B) at the end of each infusion period in each treatment group. Relative to vehicle controls, neither levosimendan, OR-1896, nor OR-1855 produced significant changes in PAP or PVR. Dobutamine produced dose-dependent increases in PAP, despite no increases in PVR, and milrinone produced modest reductions in PVR at the two lowest doses tested, but no effect at higher doses and no subsequent effects on PAP. *P < 0.05; one-way ANOVA, Dunnett’s posttest vs. vehicle controls.

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Infused separately, in a well-understood cardiovascular system, the anesthetized dog.

We demonstrate in anesthetized dogs that levosimendan and its circulating metabolite OR-1896, but not OR-1855, produces dose-dependent reductions in MAP and SVR without affecting PP, effects paralleled by reductions in ejection time and increases in left ventricular contractility. Moreover, with regard to effects on blood pressure, we demonstrate in the present study that OR-1896 and levosimendan are more potent to produce reductions in time to peak systolic blood pressure and time to systolic recovery than either milrinone or dobutamine. These effects occurred at therapeutic to supratherapeutic plasma concentrations and are consistent with activation of K_{ATP}/large-conductance Ca^{2+}-dependent K^{+} channels and Ca^{2+} sensitization, respectively.

Levosimendan and OR-1896, as well as the positive inotropic agents, dobutamine and milrinone, produced dose-dependent increases in left ventricular contractility, as well as enhanced diastolic performance, as evidenced by decreases in τ (39), in the present study. Compared on a dose-to-dose basis, OR-1896 appears more potent than levosimendan in the anesthetized dog to increase contractility, although maximal increases in left ventricular maximal dP/dt were similar between the parent and metabolite (121 ± 11 and 138 ± 16% above baseline, respectively). Interestingly, in vitro OR-1896 actually appears less potent than levosimendan to elicit increases in force of contraction; in permeabilized guinea pig left ventricular cardiomyocytes, Szilagyi et al. (43) demonstrated that levosimendan increased isometric force production by 51 ± 7% with an EC_{50} of 8 ± 1 nM. Although OR-1896 elicited a similar effect on force production, the metabolite was four to five times less potent than levosimendan (EC_{50} of 36 ± 7 nM). It is speculated that the apparent increased potency of OR-1896 vs. levosimendan in the present study, in vivo, is due to baroreflex activation at low doses of OR-1896 (0.01 μmol/kg) in response to significant reductions in SVR and blood pressure. In contrast, levosimendan did not elicit significant decreases in resistance and blood pressure until higher doses (0.1 μmol/kg).

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In stark contrast to that of levosimendan and OR-1896, RPP was substantial and dose-dependently increased in the present study in the presence of the β_{1}-agonist dobutamine (to 79% above baseline at the end of the 3.3 μmol/kg dose; vehicle =
−5% below baseline), an effect that, if observed in patients, may explain the increase in cardiac failure in those treated with dobutamine vs. levosimendan, as previously reported in a randomized clinical trial (30). The effect of dobutamine on RPP is not necessarily representative of other classic inotropic agents, such as milrinone. Indeed, in the present study, the PDE3 inhibitor milrinone produced no effect on myocardial oxygen consumption, despite significant increases in contractility and reductions in systemic resistance and blood pressure. Nevertheless, previous studies have demonstrated that levosimendan and milrinone do produce differential effects on the metabolic cost of enhanced cardiac function. Indeed, Pagel et al. (33) demonstrated that levosimendan increased intrinsic metabolic efficiency (as determined by pressure-work index) in open-chest dogs more than either milrinone or pimobendan, findings similar to results obtained in isolated guinea pig hearts, whereby Kaheinen et al. (20) showed that levosimendan increases contractile force more efficiently than milrinone, since the maximum increase in oxygen consumption was 10 ± 4 and 38 ± 15% after levosimendan and milrinone, respectively. However, in the present study, only dobutamine (but not levosimendan, its metabolites, nor milrinone) produced increases in myocardial oxygen consumption, as defined by increases in RPP. Results of the present study that demonstrate marked increases in myocardial oxygen consumption and PP produced by dobutamine may help explain the higher incidence of cardiac failure in the Survival of Patients With Acute Heart Failure in Need of Intravenous Inotropic Support clinical trial.
in dobutamine-treated patients with acute decompensated heart failure vs. those administered intravenous levosimendan (30).

Heart rate increased in response to all of the groups, except dogs administered OR-1855, and likely represents a direct effect on the sinoatrial node. In isolated guinea pig hearts, Haikala et al. (14) demonstrated that both levosimendan and milrinone produced comparable increases in heart rate (27–30%), indicating a direct effect on the pacemaker system, although, as also observed in the present study, the dose-response function for milrinone on heart rate was shifted approximately 1 log unit to the right compared with levosimendan. The effects of OR-1896 to induce tachycardia in the present study are proposed to also be a direct effect on the conduction system, similar to the direct stimulatory effects on the sinoatrial node by dobutamine (29). Haikala et al. (14) also demonstrated direct inotropic effects of the two compounds on twitch tension in isolated papillary muscle, although it was demonstrated that increase in twitch tension produced by levosimendan was independent of PDE3 inhibition.

In the same study in isolated rat hearts, Haikala et al. (14) demonstrated that both levosimendan and milrinone produced direct and dose-dependent increases in coronary flow. Indeed, the effects of levosimendan to dilate the vasculature directly have been well described; patch-clamp studies in rat cardiomyocytes have shown that levosimendan opens the KATP channels, increases potassium current, and elicits hyperpolarization of the cell (50). Levosimendan also increases KATP current in rat (49) and human (34) vascular smooth muscle, an effect that would be expected to result in vasodilation as observed in the present study (reductions in systemic resistance). The vasodilatory effects of levosimendan appear to be mediated by selective activation of KATP current, since levosimendan does not activate Ca2+-activated K+ channel at therapeutic concentrations (35) or appear to regulate the open state of other K+ channel currents, including inward rectifier, transient outward, and the delayed rectifier outward K+ currents, as elucidated by Virag et al. (48) in rabbit ventricular myocytes using whole-cell patch clamp. Moreover, vasodilation in response to levosimendan is endothelium independent (13), an effect that may have clinical significance in the treatment of heart failure patients, who, in many cases, have coronary artery disease as an underlying illness (13). Although dobutamine produced similar reductions in SVR as that of levosimendan and OR-1896, this is likely a reflex mechanism, secondary to pronounced increases in contractility and heart rate to maintain a relatively constant blood pressure within the animals. The authors are aware of no data, other than a single ex vivo study in rat mesenteric arteries (19), suggesting K+ channel activation by dobutamine.

Whether levosimendan induces vasodilation outside of the systemic vasculature is controversial. De Witt et al. (7), in studies investigating the effects of levosimendan on pulmonary vasodilation under conditions of controlled pulmonary blood flow, demonstrated a vasodilatory effect of the compound in anesthetized cats, an effect that was attenuated by KATP channel blockade. The same group also demonstrated that levosimendan produces a dose-dependent decrease in lobar arterial pressure (2–7 mmHg below vehicle) upon direct arterial injection (7). However, in the present study in anesthetized dogs, we demonstrate that the vasodilatory effects of levosimendan and OR-1896 are primarily limited to the systemic circulation, with little to no effect on the pulmonary vasculature, since neither compound produced reductions in pulmonary vascular resistance that translated into significant decreases in pulmonary arterial pressure (although values did trend downward in both groups). Strikingly, the effects of levosimendan and its metabolites are in stark contrast to dose-dependent increases in pulmonary arterial pressure produced by dobutamine (to 61 ± 7% above baseline during the 1.0 μmol/kg dose) when compared at doses of levosimendan and OR-1896 (0.3 μmol/kg) that produced similar changes in SVR and dP/dt.

Thus levosimendan and OR-1896 produce direct inotropic effects in the heart and also direct relaxation of the systemic vasculature, resulting in dose-dependent vasodilation. Moreover, results from the present study demonstrate that both parent and the OR-1896 metabolite clearly differentiate themselves from traditional KCOs and the Ca2+ channel blocker nifedipine, with respect to potency to induce increases in left ventricular contractility vs. vasodilation alone (please see Fig. 10). Thus these results suggest that increases in contractility produced by levosimendan in patients are not wholly due to a compensatory response to the fall in blood pressure subsequent to KATP activation, but rather are mediated by a separate and distinct mechanism (Ca2+ sensitization), eliciting a response in line with that of traditional inotropic agents without increasing intracellular Ca2+. In summary, both levosimendan and OR-1896 are hemodynamically active in the dog at concentrations at and above those observed clinically, whereas OR-1855 is inactive with regards to hemodynamic function.

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REFERENCES

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