Evoked Changes in Cardiovascular Function in Rats by Infusion of Levosimendan, OR-1896 ([R]-N-(4-(4-Methyl-6-oxo-1,4,5,6-tetrahydropyridazin-3-yl)phenyl)acetamide], OR-1855 ([R]-6-(4-Aminophenyl)-5-methyl-4,5-dihydropyridazin-3(2H)-one], Dobutamine, and Milrinone: Comparative Effects on Peripheral Resistance, Cardiac Output, dP/dt, Pulse Rate, and Blood Pressure

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Abstract

Levosimendan enhances cardiac contractility primarily via Ca\(^{2+}\)-sensitization, and it induces vasodilation through the activation of ATP-sensitive potassium channels and large conductance Ca\(^{2+}\)-activated K\(^+\) channels. However, the concentration-dependent hemodynamic effects of levosimendan and its metabolites ([R]-N-(4-(4-methyl-6-oxo-1,4,5,6-tetrahydropyridazin-3-yl)phenyl)acetamide (OR-1896) and (R)-6-(4-aminophenyl)-5-methyl-4,5-dihydropyridazin-3(2H)-one (OR-1855) have not been well defined. Thus, levosimendan (0.03, 0.10, 0.30, and 1.0 \(\mu\)mol/kg/30 min; \(n = 6\)) was infused as four escalating 30-min i.v. doses targeting therapeutic to supratherapeutic concentrations of levosimendan (C\(_{\text{max}}\), \sim 62.6 ng/ml); metabolites were infused at one-half log-unit lower doses and responses compared to dobutamine (\(\beta_1\)-agonist) and milrinone (phosphodiesterase 3 inhibitor). Peak concentrations of levosimendan, OR-1896, and OR-1856 at the end of the high dose were 323 \(\pm\) 14, 83 \(\pm\) 2, and 6 \(\pm\) 2 ng/ml, respectively (OR-1855 rapidly metabolized to OR-1896; peak = 82 \(\pm\) 3 ng/ml). Levosimendan and OR-1896 produced dose-dependent reductions in blood pressure and peripheral resistance with a rank potency, based on ED\(_{50}\) values, of OR-1896 (0.03 \(\mu\)mol/kg) > OR-1855 > levosimendan > milrinone (0.24 \(\mu\)mol/kg); an ED\(_{50}\) for dobutamine could not be defined. Only dobutamine produced increases in pulse pressure (30 \(\pm\) 5\%) and rate-pressure product (34 \(\pm\) 4\%). All of the compounds, with the exception of OR-1855, elicited dose-dependent increases in dP/dt with a rank potency, based on ED\(_{50}\) values, of dobutamine (0.03 \(\mu\)mol/kg) > levosimendan > OR-1896 > milrinone (0.08 \(\mu\)mol/kg), although only levosimendan produced sustained increases in cardiac output (9 \(\pm\) 4\%). Thus, levosimendan and OR-1896 are hemodynamically active at sub- to supratherapeutic concentrations (whereas the effects of OR-1855 in the rat are thought to be predominantly mediated by conversion to OR-1896) and produce direct inotropic effects and also direct relaxation of the peripheral vasculature, which clearly differentiates them from dobutamine, which does not elicit K\(^+\) channel activation, suggesting a more balanced effect on the cardiac-contractile state and K\(^+\) channel-mediated changes in vascular resistance.

The treatment of congestive heart failure is based on both increasing the contractile force of the heart and unloading of the heart by reducing preload and afterload (Chatterjee, 1987). Indeed, an increase in contractile function by increasing intracellular calcium has traditionally been the common paradigm of many inotropic drugs, including digoxin, adrenergic-\(\beta_1\)-agonists, and phosphodiesterase inhibitors. However, their clinical value is limited by the narrow therapeutic index of these agents, associated toxicity including arrhythmias, and only modest efficacy in chronic therapy (Lehmann et al., 2003). In contrast, levosimendan and other calcium-sensitizing agents are able to enhance the contractile status of the heart (mechanism recently reviewed in Antoniades et al., 2007) without concomitant elevations in calcium, and thus, they represent a potentially valuable alternative to...
increasing left ventricular function in the treatment of cardiac dysfunction and heart failure without the cardiovascular risks associated with increased intracellular-free calcium (Lehmann et al., 2003). However, it should be noted that some studies have suggested that levosimendan might actually increase Ca$^{2+}$ transients (Takahashi and Endoh, 2002), and it has also been suggested that phosphodiesterase 3 (PDE3) inhibitory activity might also contribute to the positive inotropic effects of the compound (Sato et al., 1998), suggesting a complex mechanism of action that might result in clinical benefit versus classic inotropic agents (Endoh and Hori, 2006). Reducing load on the heart is achieved by many drugs used today through the targeted relaxation of the vasculature and the subsequent reduction in peripheral resistance through various mechanisms, including activation of K$^+$ channels ($K_{ATP}$ and BK$_{Ca}$) (Chatterjee, 1987), an effect also ascribed to levosimendan.

Traditionally, polypharmacology in patients was necessary to directly target both mechanisms (increasing ventricular contractile status and unloading the heart). However, levosimendan, (R)-[4-(1,4,5,6-tetrahydro-4-methyl-6-oxo-3-pyridazinyl)phenyl]hydrazonolpropane nitrite, is a calcium sensitizer with potent vasodilatory properties (De Luca et al., 2006). Levosimendan increases myocardial contractility (Lilleberg et al., 1995) by increasing the affinity of troponin-C for Ca$^{2+}$ (Pollesello et al., 1994; Sorsa et al., 2001), reduces filling pressure (Lilleberg et al., 1995), and dilates both the peripheral and coronary vessels (Pataricza et al., 2000; Kaheinen et al., 2001) through activation of $K_{ATP}$ channels in vascular smooth muscle cells (Yokoshiki et al., 1997a; Sorsa et al., 2001). In addition, levosimendan may elicit activation of mitochondrial $K_{ATP}$ channels (Kopustinskiene et al., 2001, 2004) that have been implicated in cardioprotection (Gross and Fryer, 1999, 2000; Fryer et al., 2001).

Levosimendan can be administered to patients intravenously. Indeed, the i.v. formulation of levosimendan has been investigated in several clinical trials in subjects with decompensated heart failure (Kivikko and Lehtonen, 2005), whereby both efficacy and tolerability have been demonstrated in patients with heart failure resulting from either an ischemic or nonischemic etiology (Follath et al., 2002; Moiseyev et al., 2002). Efficacious plasma concentrations of levosimendan were assessed in an open-label, nonrandomized, Phase II study in patients diagnosed with heart failure (New York Health Association III–IV); a 24-h continuous infusion of levosimendan produced peak plasma concentrations of 62.6 ng/ml, and peak concentrations of OR-1896 and OR-1855, the two primary circulating metabolites of levosimendan, reached 5.5 and 6.8 ng/ml, respectively (Kivikko et al., 2002).

Because of the reduction of levosimendan to OR-1855 in humans and subsequent acetylation to OR-1896 (Antilla et al., 2004, 2007), the contribution of the parent versus each metabolite to the hemodynamic and cardiovascular effects observed in patients can not be definitively described. However, in the rat, levosimendan is not readily metabolized to any relevant circulating metabolite (K. C. Marsh, unpublished observation). 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 the rat. Thus, the present study sought to characterize the effects of levosimendan, OR-1896, and OR-1855 on myocardial and hemodynamic function in the rat at plasma concentrations deemed therapeutic to supratherapeutic. Results were compared to two other agents routinely prescribed in the treatment of heart failure: the $\beta_1$ agonist dobutamine and the PDE3 inhibitor milrinone (Endoh and Hori, 2006; Shin et al., 2007).

Materials and Methods

**Instrumentation.** Male Sprague-Dawley rats (325–400 g) were anesthetized with the long-acting barbiturate inactin (100 mg/kg i.p.). Subsequently, rats were used as instruments to record hemodynamic and cardiovascular function as described previously (Liu et al., 2007). In brief, polyethylene tubing (PE240) was placed in the trachea to keep the airway patent, and rats continued to breathe spontaneously. Vascular catheters (PE50) were placed in the femoral arteries to measure mean arterial pressure (MAP) and heart rate (HR) and to collect blood samples; rate-pressure product, an index of myocardial oxygen consumption, was calculated as (MAP $\times$ HR) $\times$ 100$^{-1}$, and pulse pressure was calculated as systolic pressure – diastolic pressure. Femoral vein catheters (PE50) were used for compound administration and saline infusion to maintain hydration. A specialized transducer tip catheter was placed in the right carotid artery and advanced into the left ventricle of the heart for measurement of left ventricular pressure; dP/dt at 50 mm Hg, an index of cardiac contractility, was derived from the left ventricular pressure trace. Through a laparotomy, a pulse-doppler cuff-type flow probe was placed around the upper abdominal aorta just below the diaphragm but above the renal arteries for measurement of peripheral cardiac output (PCO) defined as full cardiac output – cardiac output.

Fig. 1. In the anesthetized rat, levosimendan was infused as four escalating i.v. doses (0.03, 0.10, 0.30, 1.0 $\mu$mol/kg/30 min; n = 6/group) targeting therapeutic to supratherapeutic concentrations of total levosimendan ($C_{max} = 62$ ng/ml); the levosimendan metabolites OR-1896 and OR-1855 were infused at one-half log-unit lower doses. Blood samples were collected at 30-min intervals throughout the experimental protocol. The same protocol was carried out in separate groups of rats that received vehicle alone and in rats infused with either dobutamine (0.01, 0.03, 0.1, and 0.3 $\mu$mol/kg) or milrinone (0.14, 0.45, 1.4, and 4.3 $\mu$mol/kg).
to the carotid, coronary, brachiocephalic, and subclavian vessels. Post-hoc vascular resistance was calculated as MAP/PCO. Body temperature was monitored throughout the experiment and maintained between 37 and 37.5°C by using a heating pad. The primary hemodynamic variables were computed using commercial software and a signal processing workstation (Ponemah; Gould Instrument Systems, Inc., Cleveland, OH).

Animals were randomly divided into one of six treatment or vehicle (5% dextrose water) groups. After 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 as a series of four escalating doses dissolved in a 5% dextrose water vehicle (Abbott Laboratories, Abbott Park, IL); after termination of the high-dose infusion, animals were observed for 30 min (see Fig. 1). Levosimendan was infused at 0.03, 0.10, 0.30, and 1.0 μmol/kg/30 min, and OR-1896 and OR-1855 were infused at one-half log-unit lower doses (synthesized at Orion Pharmaceuticals, Espoo, Finland). Blood samples were withdrawn at 15-min intervals for determination of plasma concentrations of levosimendan and each metabolite by high-performance liquid chromatography-mass spectrometry; all three compounds were assayed for presence in the plasma of each dosing group. Dobutamine (Sigma-Aldrich, St. Louis, MO) was infused at the same doses as the metabolites, and milrinone (Sigma-Aldrich) was infused at 0.14, 0.43, 1.4, and 4.3 μmol/kg/30 min; doses for dobutamine and milrinone were chosen to achieve similar reductions in peripheral resistance and blood pressure as those produced by infusion of levosimendan.

**Statistical Analysis.** Data are expressed as the group mean ± S.E.M. Results were analyzed with repeated measures one-way analysis of variance (ANOVA) (GraphPad Prism version 4.03; GraphPad Software, Inc., San Diego, CA) and Dunnett’s post-test as change from baseline during drug treatment versus change from baseline in vehicle controls. Statistical significance was determined at $p < 0.05$.

![Fig. 2. Plasma concentrations of levosimendan (○), OR-1896 (□), and OR-1855 (△) assayed at 30-min intervals throughout the experimental protocol. Peak plasma concentrations of levosimendan (323 ± 15 ng/ml) represent approximately 5-fold above the concentration expected in humans after a continuous infusion of 0.2 μg/kg/min for 24 h [62.6 ± 29.2 ng/ml (Kivikko et al., 2002); the dotted line represents the mean, and the shaded area represents 1 S.D. of the mean]. Peak concentrations of levosimendan and each metabolite by high-performance liquid chromatography-mass spectrometry; all three metabolites, and milrinone (Sigma-Aldrich)](image)

![Fig. 3. A, peripheral vascular resistance (percentage change from baseline) in anesthetized vehicle-treated rats and in the presence of levosimendan (○), OR-1896 (□), OR-1855 (△), dobutamine (□), and milrinone (□); statistical significance is indicated by filled symbols ($p < 0.05$). B, the efficacious dose for each compound to produce a 15% reduction in peripheral resistance (ED$_{15}$) was calculated, and values are shown in the figure as increase in peripheral resistance as a function of dose (log transformed from micromoles per kilogram); based on potency for reductions in blood pressure, compounds can be rank-ordered as OR-1896 > OR-1855 > levosimendan > milrinone; dobutamine elicited no significant change in peripheral resistance, and thus an ED$_{15}$ value could not be accurately calculated.](image)

### TABLE 1
Baseline hemodynamic values

<table>
<thead>
<tr>
<th>n</th>
<th>MAP (mm Hg)</th>
<th>Pulse Pressure</th>
<th>Heart Rate (beats/min)</th>
<th>RPP</th>
<th>VR (mm Hg/kHz)</th>
<th>dP/dt (mm Hg/s)</th>
<th>PCO (kHz)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>6</td>
<td>102 ± 3</td>
<td>58 ± 2</td>
<td>377 ± 7</td>
<td>38 ± 2</td>
<td>3.6 ± 0.2</td>
<td>7035 ± 405</td>
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<td>Levosimendan</td>
<td>6</td>
<td>97 ± 4</td>
<td>63 ± 2</td>
<td>386 ± 10</td>
<td>38 ± 2</td>
<td>3.7 ± 0.3</td>
<td>6085 ± 250</td>
</tr>
<tr>
<td>OR-1896</td>
<td>6</td>
<td>105 ± 3</td>
<td>61 ± 2</td>
<td>380 ± 10</td>
<td>40 ± 2</td>
<td>3.1 ± 0.3</td>
<td>6433 ± 117</td>
</tr>
<tr>
<td>OR-1855</td>
<td>6</td>
<td>104 ± 4</td>
<td>60 ± 1</td>
<td>372 ± 12</td>
<td>39 ± 2</td>
<td>3.7 ± 0.3</td>
<td>6863 ± 240</td>
</tr>
<tr>
<td>Dobutamine</td>
<td>6</td>
<td>98 ± 3</td>
<td>67 ± 1</td>
<td>378 ± 8</td>
<td>37 ± 2</td>
<td>3.3 ± 0.2</td>
<td>6997 ± 241</td>
</tr>
<tr>
<td>Milrinone</td>
<td>6</td>
<td>99 ± 4</td>
<td>63 ± 2</td>
<td>389 ± 5</td>
<td>39 ± 2</td>
<td>3.7 ± 0.6</td>
<td>6465 ± 228</td>
</tr>
</tbody>
</table>

VR, vascular resistance.

* $p < 0.05$ vs. vehicle, one-way ANOVA Dunnett’s t test.
Results

In the anesthetized rat, escalating i.v. infusion of levosimendan produced peak plasma concentrations of 323 ± 14 ng/ml (Fig. 2), corresponding to approximately 5-fold above the concentration expected in humans after a continuous infusion of 0.2 μg/kg/min for 24 h (62 ng/ml) (Kivikko et al., 2002) (represented by the dotted line in Fig. 2); infusion of levosimendan produced no detectable concentrations of OR-1896 or OR-1855. Peak concentrations of OR-1896 and OR-1855 were 84 ± 2 and 6 ± 2 ng/ml, respectively. In rats administered OR-1855, the majority of the compound was rapidly metabolized to OR-1896 (peak concentration = 82 ± 3 ng/ml); the hemodynamic effects of OR-1855 alone could not be clearly delineated in the present study due to conversion to the OR-1896 active metabolite. However, for completeness, the hemodynamic effects upon OR-1855 are shown in all subsequent figures.

Baseline mean arterial pressure, pulse pressure, HR, rate-pressure product, vascular resistance, dP/dt, and peripheral cardiac output were not different among the six treatment groups (Table 1; one-way ANOVA, Dunnett’s t test versus vehicle).

Infusion of levosimendan, OR-1896, OR-1855, and milrinone produced dose-dependent reductions in peripheral vascular resistance, whereas dobutamine produced no consistent changes in vascular tone, although resistance did trend down during the high-dose infusion. Statistical analysis was based on the change from baseline within each treatment group with repeated measures one-way ANOVA and Dunnett’s post-test (Fig. 3A). The efficacious dose to elicit a 15% reduction in peripheral resistance was as follows: levosimendan, 0.17 μmol/kg; OR-1896, 0.03 μmol/kg; OR-1855, 0.06 μmol/kg; and milrinone, 0.24 μmol/kg (Fig. 3B). An EC₁₅ for dobutamine could not be calculated because the compound elicited no significant effect on resistance.
Reductions in systemic vascular resistance were paralleled by dose-dependent decreases in mean arterial pressure for all compounds tested, with the exception of dobutamine (Fig. 4, A–E). The efficacious dose to elicit a 10% reduction in blood pressure was as follows: levosimendan, 0.10 μmol/kg; OR-1896, 0.02 μmol/kg; OR-1855, 0.02 μmol/kg; and milrinone, 0.09 μmol/kg (Fig. 4F). An ED_{10} value for dobutamine could not be calculated.

Relative to vehicle controls, levosimendan, OR-1896, OR-1855, and milrinone all produced dose-dependent reductions in pulse pressure (systolic pressure – diastolic pressure); peak reductions in pulse pressure were $-41 \pm 6$, $-35 \pm 12$, $-41 \pm 12$, and $-30 \pm 5\%$ below baseline, respectively (Fig. 5, A–C and E). In contrast, dobutamine produced marked increases in pulse pressure at doses between 0.03 and 0.3 μmol/kg (maximal increase = $30 \pm 5\%$ above baseline) (Fig. 5D).

All of the compounds tested produced some degree of increase in HR in anesthetized rats; the most pronounced effects were observed with dobutamine whereby heart rate increased to $49 \pm 2\%$ above baseline at the end of the high-dose infusion. Increases in heart rate produced by levosimendan and OR-1896 reached $18 \pm 3$ and $18 \pm 2\%$ above baseline, respectively, whereas heart rate increased to only $10 \pm 3$ and $12 \pm 3\%$ above baseline in the presence of OR-1855 and milrinone (Fig. 6). Despite increases in heart rate produced by all of the compounds tested in the present study, only dobutamine elicited significant increases in myocardial oxygen consumption as indicated by large increases in rate-pressure product (Fig. 6).

Levosimendan produced dose-dependent increases in dP/dt at 50 mm Hg (dP/dt_{50}; Fig. 7A). At the end of each dosing period, dP/dt_{50} increased to $10 \pm 4$, $25 \pm 5$, $43 \pm 4$, and $72 \pm 8\%$ above baseline, respectively. Increases in dP/dt_{50} produced by OR-1896 were less pronounced than those of levosimendan, and changes in dP/dt_{50} produced by OR-1855 were not statistically different from vehicle-treated rats. In contrast, dobutamine produced large and dose-dependent increases in dP/dt_{50} at all of the doses tested (to $13 \pm 4$, $55 \pm 3$, $101 \pm 5$, and $108 \pm 8\%$ above baseline, respectively). The

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**Fig. 5.** Pulse pressure (percentage of change from baseline) in anesthetized vehicle-treated rats and in the presence levosimendan (A), OR-1896 (B), OR-1855 (C), dobutamine (D), and milrinone (E). *, p < 0.05, change from baseline relative to vehicle controls at each post-treatment time point, one-way ANOVA with repeated measures, and Dunnett’s post-test.
Fig. 6. Heart rate (percentage of change from baseline; left panels) and rate-pressure product (percentage of change from baseline; right panels) in rats infused with levosimendan (A), OR-1896 (B), OR-1855 (C), dobutamine (D), and milrinone (E). \( * \), \( p < 0.05 \), change from baseline relative to vehicle controls at each post-treatment time point, one-way ANOVA with repeated measures, and Dunnett’s post-test.
Efficacy to elicit changes in dP/dt50 and peripheral vascular resistance was plotted versus plasma concentrations achieved for levosimendan and its OR-1896 metabolite (from direct infusion or via infusion of OR-1855; Fig. 9); the slope of the linear regression line from animals infused with OR-1896 was greater than that of levosimendan. However, when analyzed at clinical Cmax concentrations (62.6 ng/ml for levosimendan, 5.5 ng/ml for OR-1896; Kivikko et al., 2002), levosimendan seems to be responsible for the majority of the inotropic and vasodilatory effects (levosimendan = 15% increase in dP/dt and 8.5% fall in peripheral resistance at 62.6 ng/ml versus 4% increase in dP/dt and 2% fall in peripheral resistance for OR-1896 at 5.5 ng/ml; Fig. 9, inset). In addition, when analyzed as change in dP/dt50 versus change in peripheral vascular resistance, dobutamine delineates itself as a more potent positive inotropic agent, whereas the regression profiles of levosimendan, OR-1896, and OR-1855 are more in line with that of milrinone, suggesting a more balanced effect on the cardiac-contractile state and K̄ channel-mediated changes in vascular resistance (Fig. 10).

Discussion

Acute decompensated congestive heart failure can be treated with i.v. levosimendan to elicit systemic vasodilation through K̄ channel activation and to enhance ventricular contractility (Pataricza et al., 2000; Kivikko et al., 2002; Ng and Akhter, 2005). Thus, an i.v. infusion dosing regimen was used in the present study in rats to effectively capture the hemodynamic and cardiovascular effects of levosimendan as well as its metabolites, infused separately, over a large, dynamic range of plasma concentrations deemed therapeutic to supratherapeutic. We demonstrate in anesthetized rats that levosimendan and its circulating metabolite OR-1896 produce dose-dependent reductions in peripheral vascular resistance and mean arterial pressure without affecting pulse pressure, effects paralleled by increases in left ventricular contractility and consistent with K̄ channel activation and increased Ca2+ sensitization/transients, respectively. The sole hemodynamic effects of OR-1895 could not be delineated in the present study due to the rapid and almost complete conversion to its metabolite OR-1896, which is hemodynamically active in the rat when infused alone.

We demonstrate in the present study that infusion of OR-1896, OR-1855, and levosimendan are more potent to produce decreases in peripheral resistance (ED15 = 0.03, 0.06, and 0.17 μmol/kg, respectively) than milrinone (ED15 = 0.24 μmol/kg; no ED15 value could be calculated for dobutamine); similar potencies were noted for reductions in blood pressure by the compounds tested in the present study. It is noteworthy that reductions in both peripheral resistance and blood pressure produced by levosimendan and OR-1896 occurred at concentrations within those expected clinically in patients with heart failure and are consistent with activation of K̄ channel-BKCa channels (Erdei et al., 2006).

Levosimendan and OR-1896, as well as the positive inotropic agents dobutamine and milrinone, produced dose-dependent increases in left ventricular contractility in the present study. Compared on a dose-to-dose basis, dobutamine is...
markedly more potent than levosimendan, OR-1896, and milrinone (ED₅₀ values = 0.03, 0.38, 0.14, and 0.09 μmol/kg, respectively) to elicit increases in dP/dt₅₀. Although maximal increases in dP/dt₅₀ were not different between levosimendan and OR-1896 (72 ± 8 and 52 ± 11% above baseline, respectively; p > 0.05), increases in dP/dt produced by infusion of OR-1855 (up to 18 ± 10% above baseline) were markedly attenuated versus direct infusion of OR-1896 (p < 0.05, t test) despite virtually identical plasma concentrations of OR-1896 in both groups (84 ± 2 versus 82 ± 3 ng/ml, respectively), possibly suggesting that the small amount of parent OR-1855 present in the OR-1855-infusion studies (6 ± 2 ng/ml) countered OR-1896-induced increases in dP/dt₅₀ through an undefined mechanism. However, we are unaware of any studies reporting a negative inotropic effect of OR-1855 in preclinical models or in patients. In fact, in similar infusion studies in anesthetized dogs, OR-1855 elicited no effect on dP/dt at concentrations well above those achieved in the present study (up to 136 ± 6 ng/ml) (Banfor et al., 2007).

Whether levosimendan elicits increases in contractility through or independent of PDE3 inhibition is controversial and may be species-dependent, but it may be important when it comes to clinical efficacy (Endoh, 2001, 2002). In guinea pig heart preparations, Szilágyi et al. (2004) demonstrated that levosimendan and OR-1896 elicit increases in contractility independent of PDE inhibitory activity. However, in the rabbit, Sato et al. (1998) suggested that, in addition to the increase in sensitivity of contractile proteins to Ca²⁺, the
accumulation of cAMP subsequent to PDE3 inhibition may contribute to the inotropic effects of the drug. Moreover, Ajiro et al. (2002) have demonstrated a differential effect of levosimendan on $I_{\text{Ca,L}}$ current, which is regulated by cAMP-dependent phosphorylation in human versus rabbit and rat atrial cells and suggest that levosimendan may function as a PDE3 inhibitor in man. Thus, whether levosimendan elicits inotropic effects solely via Ca$^{2+}$ sensitization or in combination with PDE3 inhibitory activity is unclear and warrants further investigation.

Although in the present study, the ED$_{50}$ for OR-1896 to elicit increase in dP/dt is lower than that calculated for levosimendan, in vitro OR-1896 is less potent than levosimendan to elicit increases in force of contraction. Indeed, in permeabilized guinea pig left ventricular cardiomyocytes, Szilágyi et al. (2004) demonstrated that levosimendan increased isometric force production by 51 ± 7% with an EC$_{50}$ of 8 ± 1 nM, whereas the EC$_{50}$ for OR-1896 to elicit a similar effect on force production was 36 ± 7 nM, suggesting four to five times less potency than levosimendan. It is speculated that the apparent increased potency of OR-1896 versus 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 blood pressure. In contrast, levosimendan did not elicit significant decreases in blood pressure until 30-fold higher doses (0.3 μmol/kg). In addition, it is important to note that when the present results were analyzed as change in dP/dt$_{50}$ and peripheral vascular resistance versus plasma concentrations was achieved, OR-1896 seemed to be more potent than levosimendan to elicit both increases in dP/dt$_{50}$ and reductions in peripheral vascular resistance on a concentration-for-concentration basis. However, when taking into account the clinical C$_{\text{max}}$ values for each compound (62.6 ng/ml for levosimendan versus 5.5 ng/ml for OR-1896), it can be argued that levosimendan, rather than OR-1896 accounts for the majority of the inotropic and vasodilatory effects of the compound at therapeutic levels in patients.

It is important to note that Haikala et al. (1995) demonstrated in guinea pig heart myofibrils that levosimendan elicits no effect on myosin ATPase activity, 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 increases myocardial oxygen consumption. However, in profound contrast to the effects of levosimendan and OR-1896, rate-pressure product was substantially and dose-dependently increased in the present study by the $\beta_1$-agonist dobutamine (to 34 ± 4% above baseline during the 0.10 μmol/kg dose; vehicle = −3 ± 2% below baseline), results consistent with those previously demonstrated in patients (Akosah et al., 1999), which may explain the increase in cardiac failure in patients treated with dobutamine versus levosimendan (Mohazza et al., 2007).

Interestingly, in this study only levosimendan elicited consistent and significant increases in peripheral cardiac output (maximal effect = 20 ± 7% above baseline), whereas neither direct infusion of OR-1896 nor OR-1896 produced through metabolic conversion from OR-1855 elicited any significant effect. These results suggest that, similar to increases in dP/dt$_{50}$ produced by levosimendan, increases in cardiac output in patients administered levosimendan may be mediated by the parent rather than the OR-1896 metabolite. It is noteworthy that dobutamine, despite marked increases in dP/dt, elicited no effect on peripheral cardiac output in the present study. However, because technical limitations restricted cardiac output measurement to only the periphery without measurement of output to some vessels, it is possible that in the presence of dobutamine full cardiac output was actually enhanced despite no clear increase in this model.

In the present study, heart rate increased in response to all of the groups, with the exception of rats administered OR-1855 despite rapid conversion to OR-1896; again, why OR-1896 generated from OR-1855 elicited little to no effect on heart rate (similar to a lack of effect on dP/dt$_{50}$ in the OR-1855-infusion studies) is unclear but may suggest an already unknown property of OR-1855 whereby small concentrations of the parent can mitigate the effects of the OR-1896 metabolite. Nevertheless, increases in heart rate produced by levosimendan and OR-1896 infusion probably represent a direct effect on the sinoatrial node (Haikala et al., 1997).

The same group also demonstrated in isolated rat hearts that both levosimendan and milrinone produced direct and dose-dependent increases in blood flow (Haikala et al., 1997). Indeed, the effects of levosimendan to dilate the vasculature directly has been well described; patch-clamp studies in rat cardiomyocytes have shown that levosimendan opens K$_{\text{ATP}}$ channels, increases K$^+$ current, and elicits hyperpolarization of the cell (Yokoshiki et al., 1997b). Levosimendan also increases K$_{\text{ATP}}$ current in rat (Yokoshiki et al., 1997a) and human (Pataricza et al., 2000) vascular smooth muscles, an effect that would be expected to result in vasodilation as observed in the present study. The vasodilatory effects of levosimendan seem to be mediated by selective activation of K$_{\text{ATP}}$ current because levosimendan does not activate K$_{\text{Ca}}$ at therapeutic concentrations (Pataricza et al., 2003) (although OR-1896 has been reported to elicit activation of the BK$_{\text{Ca}}$ in rat coronary arterioles; Erdei et al., 2006), or they seem 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 Virág et al. (1996) using whole-cell patch clamp in rabbit ventricular cardiomyocytes. Moreover, levosimendan-induced vasodilation is
endothelium-independent, an effect that may have clinical significance because coronary artery disease is often an underlying pathology in heart failure patients.

Thus, levosimendan and OR-1896 produce direct inotropic effects in the heart and also direct relaxation of the peripheral vasculature, resulting in dose-dependent vasodilatation. Moreover, results from the present study demonstrate that both parent and the OR-1896 metabolite clearly differentiate themselves from dobutamine, which does not cause K⁺ channel activation, suggesting a more balanced effect on the cardiac-contraction state and K⁺ channel-mediated reductions in vascular resistance. Moreover, in the anesthetized rat, both levosimendan and OR-1896 are hemodynamically active at concentrations at and above those observed clinically. The effects of OR-1855 in the rat are thought to be predominantly mediated by rapid and almost complete conversion to OR-1896.

References

Ajiro Y, Hagiwara N, Katsube Y, Sperelakis N, and Kasanuki H (2002) Levosimendan and OR-1896 produce direct inotropic effects in the heart and also direct relaxation of the peripheral vasculature, resulting in dose-dependent vasodilatation. Moreover, results from the present study demonstrate that both parent and the OR-1896 metabolite clearly differentiate themselves from dobutamine, which does not cause K⁺ channel activation, suggesting a more balanced effect on the cardiac-contraction state and K⁺ channel-mediated reductions in vascular resistance. Moreover, in the anesthetized rat, both levosimendan and OR-1896 are hemodynamically active at concentrations at and above those observed clinically. The effects of OR-1855 in the rat are thought to be predominantly mediated by rapid and almost complete conversion to OR-1896.