

## Levosimendan (OR-1259), a Myofilament Calcium Sensitizer, Enhances Myocardial Contractility but Does Not Alter Isovolumic Relaxation in Conscious and Anesthetized Dogs

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**Background:** Levosimendan is a myofilament calcium sensitizer with phosphodiesterase III inhibiting properties which increases contractile state *in vitro* by stabilizing calcium-induced changes in troponin C. This latter effect may produce positive inotropic actions but may also cause deleterious negative lusitropic effects. This investigation examined the effects of levosimendan on systemic and coronary hemodynamics and left ventricular systolic and diastolic function in conscious and anesthetized dogs.

**Methods:** Because autonomic nervous system activity may influence the actions of levosimendan and volatile anesthetics *in vivo*, experiments were conducted in the presence of pharmacologic blockade of the autonomic nervous system. A total of 24 experiments were performed in eight dogs chronically instrumented for measurement of aortic and left ventricular pressure, the peak rate of increase and decrease of left ventricular pressure, subendocardial segment length, diastolic coronary blood flow velocity, and cardiac output. The slope of the regional preload recruitable stroke work relation was used to assess myocardial contractility. Diastolic function was evaluated by the peak rate of decrease of left ventricular pressure, a time constant of isovolumic relaxation, maximum segment lengthening velocity during rapid ventricular filling, and a regional chamber stiffness constant. Systemic and coronary hemodynamics and left ventricular pressure-segment length diagrams and waveforms were recorded after 10 min equil-

ibration at each dose of levosimendan (0.5, 1.0, 2.0, and 4.0  $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ) in the conscious state or during isoflurane or halothane anesthesia (1.0 MAC) on 3 days of experimentation.

**Results:** In conscious dogs, levosimendan increased heart rate, cardiac output, diastolic coronary blood flow velocity, and segment shortening and decreased left ventricular end-diastolic pressure, systemic vascular resistance, and diastolic coronary vascular resistance. Levosimendan caused dose-dependent increases in the slope of the regional preload recruitable stroke work relation ( $65 \pm 6$  during control to  $139 \pm 9$  mmHg during the high dose), consistent with a direct positive inotropic effect. No changes in the peak rate of decrease of left ventricular pressure or in the time constant of isovolumic relaxation were produced by with levosimendan in conscious dogs, indicating that isovolumic relaxation was unaffected. In contrast, increases in rapid ventricular filling were observed (maximum segment lengthening velocity  $34 \pm 3$  during control to  $47 \pm 5$  mm·s<sup>-1</sup> at the high dose). In the presence of isoflurane and halothane, levosimendan caused cardiovascular actions which were similar to those observed in the conscious state. Levosimendan increased, in a dose-related manner, the slope of the regional preload recruitable stroke work relation and in the maximum segment lengthening velocity during rapid ventricular filling in anesthetized dogs. However, there were no changes in the time constant of isovolumic relaxation or in the peak rate of decrease of left ventricular pressure.

**Conclusions:** The results indicate that levosimendan causes systemic and coronary vasodilatation in conscious and anesthetized dogs during blockade of the autonomic nervous system. Levosimendan caused direct positive inotropic effects and improved rapid ventricular filling but did not alter indices of isovolumic relaxation, suggesting that levosimendan may selectively enhance systolic performance and diastolic filling without affecting left ventricular relaxation. (Key words: Anesthetics, volatile: halothane; isoflurane. Heart, diastole: diastolic left ventricular function; isovolumic relaxation; ventricular filling; ventricular compliance. Heart, myocardial performance: left ventricular function; myocardial contractility; preload recruitable stroke work. Myofilaments: calcium sensitizer; troponin C. Pharmacology, inotropes: amrinone; levosimendan; pimobendan; sulmazole.)

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A new class of positive inotropic agents, the myofilament calcium ( $\text{Ca}^{2+}$ ) sensitizers, have been the subject

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of intense experimental and clinical research in recent years. Myofilament  $\text{Ca}^{2+}$  sensitizers, including pimobendan and sulmazole, are imidazopyridine derivatives which enhance myocardial contractility *via* direct actions on the contractile apparatus.<sup>1,2</sup> This class of compounds increases the sensitivity of the myofilaments to  $\text{Ca}^{2+}$  by augmenting  $\text{Ca}^{2+}$  binding to the  $\text{Ca}^{2+}$ -specific regulatory site of cardiac troponin C, thereby stabilizing  $\text{Ca}^{2+}$ -induced conformational changes in this protein in a highly stereospecific manner.<sup>3-6</sup> These agents also produce partial inhibition of cardiac phosphodiesterase III (PDE-III),<sup>1,3,5</sup> an effect which may serve to enhance dissociation of  $\text{Ca}^{2+}$  from the contractile apparatus during diastole and lead to shortened isovolumic relaxation *in vivo* despite concomitant augmentation of  $\text{Ca}^{2+}$  binding during systole. Myofilament  $\text{Ca}^{2+}$  sensitizers have been shown to improve many of the hemodynamic derangements associated with congestive heart failure in humans<sup>7-17</sup> and may represent another clinically important therapeutic modality in the acute and chronic management of end-stage heart failure resulting from ischemic heart disease, left ventricular hypertrophy, or idiopathic dilated cardiomyopathy.<sup>1,18</sup>

The functional interaction between drugs of the myofilament  $\text{Ca}^{2+}$ -sensitizer class and volatile anesthetics have yet to be described. Levosimendan [(*R*)-[4-(1,4,5,6-tetrahydro-4-methyl-6-oxo-3-pyridazinyl)phenyl]hydrazono]propanedinitrile; OR-1259] is a novel myofilament  $\text{Ca}^{2+}$  sensitizer with cardiac PDE-III-inhibiting properties (fig. 1). Preliminary studies have suggested that levosimendan causes cardiovascular effects<sup>19-23,</sup> which are similar to those produced by pimobendan<sup>7-15</sup> and sulmazole.<sup>16,17</sup>

The current investigation examined and compared the effects of levosimendan on systemic and coronary hemodynamics and left ventricular systolic and diastolic function in conscious and anesthetized, chronically instrumented dogs. These experiments tested the following hypotheses. (1) Levosimendan augments myocardial contractility and improves several indices of diastolic function, including assays of isovolumic relaxation, in conscious dogs in a dose-dependent manner *via* enhancement of  $\text{Ca}^{2+}$  affinity for and PDE-III-induced  $\text{Ca}^{2+}$  release from the contractile apparatus, respectively. (2) Levosimendan reverses depression of contractile function caused by isoflurane and halothane by overcoming the functional impairments of the voltage-dependent  $\text{Ca}^{2+}$  channel<sup>24-27</sup> and the sarcoplasmic reticulum<sup>28</sup> induced by volatile anesthetics. (3) The PDE-III inhibitory activity and positive inotropic effects of levosimendan reverse abnormalities in diastolic mechanics caused by volatile anesthetics despite direct increases in  $\text{Ca}^{2+}$  affinity for the myofilaments. Experiments were conducted in the presence of pharmacological blockade of the autonomic nervous system to avoid changes in systemic and coronary hemodynamics mediated by autonomic nervous system reflexes during administration of levosimendan or volatile anesthetics.<sup>29</sup> Thus, the effects of levosimendan on left ventricular systolic and diastolic function were studied in conscious and anesthetized dogs independent of changes in autonomic nervous system tone.

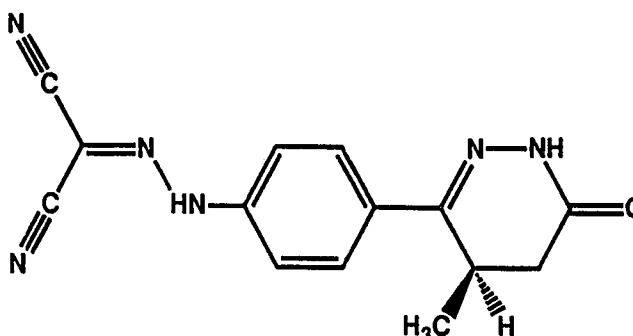


Fig. 1. Chemical structure of levosimendan.

tus, respectively. (2) Levosimendan reverses depression of contractile function caused by isoflurane and halothane by overcoming the functional impairments of the voltage-dependent  $\text{Ca}^{2+}$  channel<sup>24-27</sup> and the sarcoplasmic reticulum<sup>28</sup> induced by volatile anesthetics. (3) The PDE-III inhibitory activity and positive inotropic effects of levosimendan reverse abnormalities in diastolic mechanics caused by volatile anesthetics despite direct increases in  $\text{Ca}^{2+}$  affinity for the myofilaments. Experiments were conducted in the presence of pharmacological blockade of the autonomic nervous system to avoid changes in systemic and coronary hemodynamics mediated by autonomic nervous system reflexes during administration of levosimendan or volatile anesthetics.<sup>29</sup> Thus, the effects of levosimendan on left ventricular systolic and diastolic function were studied in conscious and anesthetized dogs independent of changes in autonomic nervous system tone.

## Materials and Methods

All experimental procedures and protocols used in this investigation were reviewed and approved by the Animal Care and Use Committee of the Medical College of Wisconsin. All procedures conformed to the *Guiding Principles in the Care and Use of Animals* of the American Physiologic Society and were performed in accordance with the *Guide for the Care and Use of Laboratory Animals*.#

### General Preparation

The implantation of instruments has been previously described in detail.<sup>30,31</sup> In brief, during general anesthesia and in sterile surgical conditions, mongrel dogs ( $n = 8$ ;  $25.3 \pm 0.6$  kg, mean  $\pm$  SEM) underwent a left thoracotomy for placement of instruments for mea-

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surement of aortic and left ventricular pressure, peak rates of positive and negative change in left ventricular pressure ( $+dP/dt_{\max}$  and  $-dP/dt_{\min}$ , respectively), subendocardial segment length, intrathoracic pressure, diastolic coronary blood flow velocity, and cardiac output (fig. 2). A hydraulic vascular occluder was positioned around the inferior vena cava for control of left ventricular preload. All instrumentation was firmly secured, tunneled between the scapulae, and exteriorized *via* several small incisions. The pericardium was left open, the chest wall closed in layers, and the pneumothorax evacuated by a chest tube.

All dogs received systemic analgesics [Innovar-Vet (fentanyl-droperidol); Pitman-Moore, Mundelein, IL] as needed after surgery. Dogs were allowed to recover a minimum of 7 days prior to experimentation during which time all were treated with intramuscular antibiotics [cephalothin (40 mg/kg) and gentamicin (4.5 mg/kg)] and trained to stand quietly in an animal sling during hemodynamic monitoring. Coronary blood flow velocity and segment length signals were monitored by ultrasonic amplifiers (Crystal Biotech, Hopkinton, MA). End-systolic (ESL) and end-diastolic segment length (EDL) were measured at  $-dP/dt_{\min}$  and imme-

diately prior to the onset of left ventricular isovolumic contraction, respectively. The lengths were normalized according to the method of Theroux *et al.*<sup>32</sup> Percent segment shortening (%SS) was calculated as  $\%SS = (EDL - ESL) \times 100 \times EDL^{-1}$ . Relative diastolic coronary vascular resistance was calculated as the quotient of diastolic arterial pressure and peak diastolic coronary blood flow velocity. The pressure work index, an estimate of myocardial oxygen consumption, was determined using the formula of Rooke and Feigl.<sup>33</sup> The hemodynamic data were continuously recorded on a polygraph (7758A, Hewlett-Packard, San Francisco, CA) and digitized by a computer interfaced with an analog to digital converter. Left ventricular pressure and segment length data were also transmitted to a digital storage oscilloscope (4094, Nicolet, Madison, WI) for recording of left ventricular pressure-segment length waveforms and diagrams.

#### Experimental Protocol

Dogs were randomly assigned to receive levosimendan in the conscious or anesthetized state on separate experimental days. Each dog was fasted overnight, and fluid deficits were replaced prior to experimentation

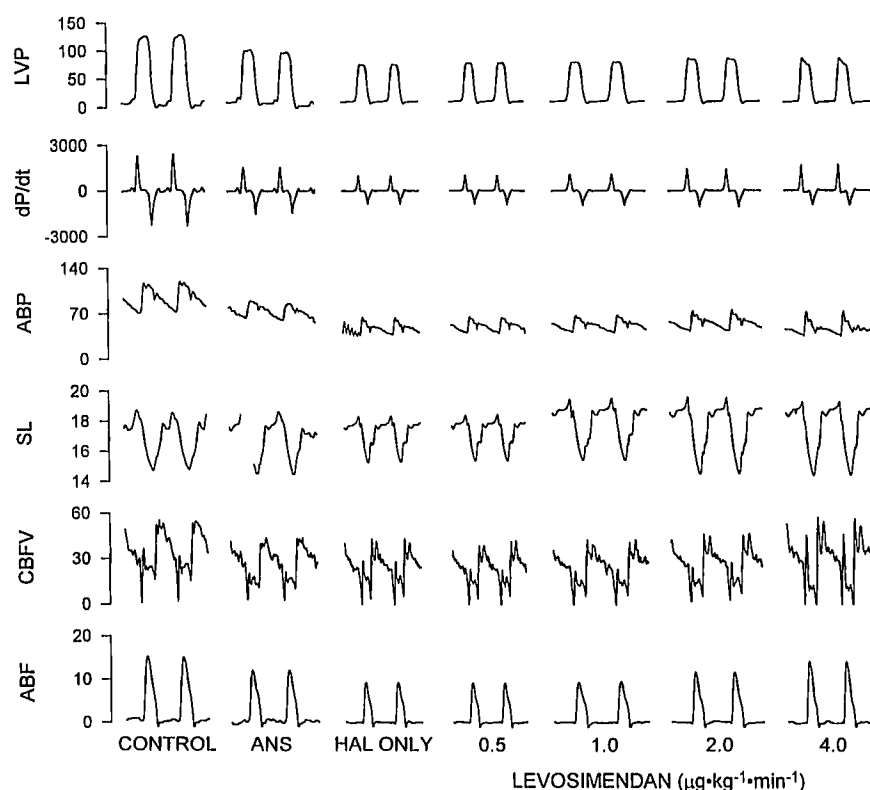


Fig. 2. Continuous left ventricular pressure (LVP), rate of change of left ventricular pressure ( $dP/dt$ ), aortic blood pressure (ABP), segment length (SL), coronary blood flow velocity (CBFV), and aortic blood flow (ABF) waveforms during control conditions, autonomic nervous system (ANS) blockade, halothane (HAL) anesthesia (1 MAC), and levosimendan infusions (0.5, 1.0, 2.0, and 4.0  $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ) in a typical experiment.

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with crystalloid (500 ml 0.9% saline) which was continued at  $3 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$  for the duration of each experiment. Pharmacological blockade of the autonomic nervous system consisted of intravenous propranolol hydrochloride (2 mg/kg), atropine methylnitrate (3 mg/kg), and hexamethonium bromide (20 mg/kg). Adequacy of autonomic nervous system blockade was established by lack of reflex change in heart rate during a rapid decline of venous return caused by inflation of the inferior vena caval hydraulic occluder before, during and after each experiment. Left ventricular pressure, intrathoracic pressure, and segment length waveforms were recorded continuously on the digital oscilloscope for later off-line analysis of diastolic function. Left ventricular pressure-segment length diagrams used to assess contractile state were generated by abruptly decreasing left ventricular preload. This was accomplished by constricting the inferior vena cava resulting in an approximately 30 mmHg decline in left ventricular systolic pressure over 10 to 15 cardiac cycles. Respiratory variation in ventricular pressure in the conscious state was later reduced off-line by electronic subtraction of the continuous intrathoracic pressure waveform from the left ventricular pressure waveform *via* the digital oscilloscope as detailed previously.<sup>31</sup> During anesthesia, waveforms were recorded at end expiration. Inferior vena caval occlusion was released immediately after recording of the waveforms. Abrupt alteration of preload did not cause a change in heart rate in any experiment.

In one group of experiments, levosimendan was administered in the conscious state after pharmacologic blockade of the autonomic nervous system had been completed. Control systemic and coronary hemodynamics and left ventricular pressure-segment length waveforms and diagrams were recorded. Intravenous infusions of levosimendan at 0.5, 1.0, 2.0, or 4.0  $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  (1.8, 3.6, 7.2, or 14.3  $\text{nmol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ) were then administered in a random fashion. Hemodynamics were recorded, and left ventricular pressure-segment length waveforms and diagrams were obtained using the techniques described above after 10 min of equilibration at each dose of levosimendan. The infusion rate of levosimendan was then changed, and measurements were repeated after a similar period of equilibration.

Levosimendan was also administered to autonomically blocked dogs anesthetized with isoflurane or halothane in two other groups of experiments on separate days. After autonomic nervous system blockade,

inhalation induction, and tracheal intubation, anesthesia was maintained with 1.0 MAC (end-tidal concentration) isoflurane or halothane in a nitrogen (75%) and oxygen (25%) mixture *via* positive pressure ventilation. The canine MAC values for isoflurane and halothane used in this investigation were 1.28% and 0.86%, respectively. End-tidal concentrations of isoflurane and halothane were measured using a mass spectrometer (Advantage 2000, Marquette, St. Louis, MO). The mass spectrometer was calibrated using known standards before and during experimentation. Systemic and coronary hemodynamics were recorded, and left ventricular pressure-segment length waveforms and diagrams were generated and stored on the digital oscilloscope after a 30-min equilibration period in the anesthetized state. Intravenous infusions of levosimendan (0.5, 1.0, 2.0, or 4.0  $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ) were administered in a random fashion, and data was recorded as described above. Each dog was allowed to recover from anesthesia and autonomic nervous system blockade for 3 days prior to subsequent experimentation. A total of 24 experiments in three groups (levosimendan administered in the conscious state and during isoflurane or halothane anesthesia) were completed in which the same eight dogs were used.

### Drugs

Propranolol hydrochloride, atropine methyl nitrate, and hexamethonium bromide were purchased from Sigma Chemical Company, St Louis, MO and were dissolved in 0.9% normal saline. Levosimendan was generously donated by Orion-Farmos Pharmaceuticals, Espoo, Finland. The drug vehicle for levosimendan consisted of 25% ethanol (95%), 25% polyethylene glycol (5%), and 50% normal saline. No hemodynamic effects were produced the drug vehicle as previously described.<sup>34</sup>

### Calculation of Indices of Systolic and Diastolic Left Ventricular Function

The slope of the regional preload recruitable stroke work relation ( $M_w$ ) was used to determine myocardial contractility.<sup>35</sup> A series of left ventricular pressure-segment length diagrams were obtained by transient constriction of the inferior vena cava in the conscious or anesthetized state and during each dose of levosimendan. The area of each diagram, corresponding to segmental stroke work, was plotted against the corresponding EDL for each loop, and linear regression analysis was used to determine  $M_w$  and the length intercept

**Table 1. Hemodynamic Effects of Levosimendan in Conscious Dogs**

|   | N | Conscious Control | ANS Blockade    | Levosimendan Infusion ( $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ) |                 |                   |                   |
|---|---|-------------------|-----------------|--|-----------------|-------------------|-------------------|
|   |   |                   |                 | 0.5  | 1.0             | 2.0               | 4.0               |
| HR (bpm)  | 8 | 74 $\pm$ 6*       | 116 $\pm$ 4     | 120 $\pm$ 4  | 126 $\pm$ 5     | 135 $\pm$ 5*†     | 145 $\pm$ 5*†§    |
| MBP (mmHg)  | 8 | 97 $\pm$ 4*       | 79 $\pm$ 5      | 77 $\pm$ 5   | 78 $\pm$ 4      | 74 $\pm$ 5        | 71 $\pm$ 3        |
| RPP (mmHg $\cdot$ bpm $\cdot 10^3$ )                            | 8 | 9.8 $\pm$ 0.6     | 10.8 $\pm$ 0.4  | 11.1 $\pm$ 0.8   | 11.6 $\pm$ 0.5  | 12.1 $\pm$ 1.0    | 12.8 $\pm$ 0.8*   |
| LVSP (mmHg)   | 8 | 126 $\pm$ 5*      | 96 $\pm$ 4      | 94 $\pm$ 5   | 97 $\pm$ 5      | 89 $\pm$ 4        | 86 $\pm$ 5*       |
| LVEDP (mmHg)  | 8 | 10 $\pm$ 2        | 9 $\pm$ 1       | 9 $\pm$ 1  | 9 $\pm$ 1       | 7 $\pm$ 1         | 6 $\pm$ 1*†       |
| DCBFV (Hz $\cdot 10^2$ )  | 7 | 54 $\pm$ 5        | 56 $\pm$ 5      | 61 $\pm$ 6   | 63 $\pm$ 6      | 70 $\pm$ 6*       | 83 $\pm$ 9*†§     |
| DCVR (mmHg $\cdot$ Hz <sup>-1</sup> $\cdot 10^{-2}$ )           | 7 | 1.61 $\pm$ 0.19*  | 1.33 $\pm$ 0.18 | 1.16 $\pm$ 0.14  | 1.19 $\pm$ 0.19 | 1.03 $\pm$ 0.17*  | 0.83 $\pm$ 0.15*† |
| EDL (mm)  | 8 | 14.8 $\pm$ 0.7    | 14.6 $\pm$ 0.7  | 14.4 $\pm$ 0.6   | 14.2 $\pm$ 0.7  | 13.6 $\pm$ 0.7*†  | 13.1 $\pm$ 0.7*†§ |
| ESL (mm)  | 8 | 11.7 $\pm$ 0.5    | 11.7 $\pm$ 0.6  | 11.5 $\pm$ 0.5   | 11.3 $\pm$ 0.5  | 10.6 $\pm$ 0.6*†§ | 10.0 $\pm$ 0.6*†§ |
| SS (%)  | 8 | 20.9 $\pm$ 2.0    | 20.0 $\pm$ 1.7  | 19.9 $\pm$ 1.6   | 20.0 $\pm$ 1.5  | 21.9 $\pm$ 1.7    | 23.5 $\pm$ 2.0*†§ |
| CO (l $\cdot$ min <sup>-1</sup> )                               | 7 | 2.5 $\pm$ 0.2     | 2.9 $\pm$ 0.2   | 2.9 $\pm$ 0.2  | 3.1 $\pm$ 0.2   | 3.2 $\pm$ 0.3     | 3.7 $\pm$ 0.4*†§  |
| SVR (dyne $\cdot$ s $\cdot$ cm <sup>5</sup> )                   | 7 | 3,150 $\pm$ 220*  | 2,160 $\pm$ 130 | 2,080 $\pm$ 170  | 1,970 $\pm$ 120 | 1,950 $\pm$ 220   | 1,600 $\pm$ 120*† |
| SV (ml)   | 7 | 35 $\pm$ 4*       | 25 $\pm$ 2      | 24 $\pm$ 2   | 25 $\pm$ 2      | 24 $\pm$ 3        | 25 $\pm$ 3        |
| PWI (ml $\cdot$ min <sup>-1</sup> $\cdot$ 100 g <sup>-1</sup> ) | 7 | 8.5 $\pm$ 0.6     | 9.2 $\pm$ 0.5   | 9.2 $\pm$ 0.7  | 9.7 $\pm$ 0.6   | 10.2 $\pm$ 0.7    | 10.9 $\pm$ 0.9*†  |

Data are mean  $\pm$  SEM.

HR = heart rate; MBP = mean aortic blood pressure; RPP = rate pressure product; LVSP and LVEDP = left ventricular systolic and end-diastolic pressure, respectively; DCBFV = diastolic coronary blood flow velocity; DCVR = diastolic coronary vascular resistance; EDL and ESL = end-diastolic and end-systolic segment length, respectively; SS = segment shortening; CO = cardiac output; SV = stroke volume; SVR = systemic vascular resistance; ANS = autonomic nervous system; PWI = pressure work index.

\* Significantly ( $P < 0.05$ ) different from ANS blockade.

† Significantly ( $P < 0.05$ ) different from 0.5  $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  levosimendan infusion.

§ Significantly ( $P < 0.05$ ) different from 1.0  $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  levosimendan infusion.

‡ Significantly ( $P < 0.05$ ) different from 2.0  $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  levosimendan infusion.

of the preload recruitable stroke work relation ( $L_w$ ): segmental stroke work =  $M_w \cdot (\text{EDL} - L_w)$ . The time constant of isovolumic relaxation ( $\tau$ ) was determined assuming a nonzero asymptote of left ventricular pressure decay.<sup>36</sup> The maximum segment lengthening velocity during rapid ventricular filling ( $dL/dt_{\max}$ ) was determined by differentiation of the continuous segment length waveform.<sup>31</sup> The regional chamber stiffness constant ( $K_p$ ) was derived from left ventricular pressure-segment length data between minimum ventricular pressure and the beginning of atrial systole using a monoexponential relation assuming a simple elastic model.<sup>37</sup>

### Statistical Analysis

Statistical analysis of the data within and between groups in the conscious state with and without blockade of the autonomic nervous system, during anesthetic interventions, and during multiple doses of levosimendan was performed by multiple analysis of variance (MANOVA) with repeated measures, followed by use of the student's  $t$  test with Bonferroni's correction.<sup>38</sup> Changes were considered to be statistically significant

when the probability ( $P$ ) value was  $< 0.05$ . All data are expressed as mean  $\pm$  SEM.

### Results

Autonomic nervous system blockade caused significant ( $P < 0.05$ ) increases in heart rate and decreases in mean arterial pressure, left ventricular systolic pressure, systemic vascular resistance, diastolic coronary vascular resistance and stroke volume. No changes in left ventricular end-diastolic pressure, cardiac output, diastolic coronary blood flow velocity, rate pressure product, or pressure-work index were observed (tables 1–3). There were no differences in baseline systemic or coronary hemodynamics with or without autonomic nervous system blockade among groups.

Administration of levosimendan to conscious dogs produced significant and dose-related increases in heart rate and diastolic coronary blood flow velocity and decreases in diastolic coronary vascular resistance, ESL, and EDL (table 1). Significant increases in cardiac output, rate-pressure product, pressure-

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Table 2. Hemodynamic Effects of Levosimendan in Isoflurane-Anesthetized Dogs

|   | N | Conscious Control | ANS Blockade    | Isoflurane (1.0 MAC) | Levosimendan Infusion ( $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ) |                  |                  |                   |
|---|---|-------------------|-----------------|----------------------|--|------------------|------------------|-------------------|
|   |   |                   |                 |                      | 0.5  | 1.0              | 2.0              | 4.0               |
| HR (bpm)  | 8 | 82 $\pm$ 5*       | 108 $\pm$ 4     | 90 $\pm$ 5*          | 89 $\pm$ 4*  | 93 $\pm$ 4*      | 98 $\pm$ 4*†     | 102 $\pm$ 5*†§    |
| MBP (mmHg)  | 8 | 100 $\pm$ 5*      | 77 $\pm$ 2      | 63 $\pm$ 3*          | 65 $\pm$ 3*  | 62 $\pm$ 3*      | 60 $\pm$ 4*      | 55 $\pm$ 3*†§     |
| RPP (mmHg $\cdot$ bpm $\cdot 10^3$ )              | 8 | 10.3 $\pm$ 0.8    | 9.9 $\pm$ 0.4   | 6.8 $\pm$ 0.5*       | 6.9 $\pm$ 0.5*   | 6.8 $\pm$ 0.5*   | 7.0 $\pm$ 0.6*   | 6.9 $\pm$ 0.6*    |
| LVSP (mmHg)                                       | 8 | 127 $\pm$ 5*      | 93 $\pm$ 3      | 77 $\pm$ 3*          | 78 $\pm$ 3*  | 74 $\pm$ 3*      | 70 $\pm$ 4*      | 65 $\pm$ 3*†§     |
| LVEDP (mmHg)                                      | 8 | 10 $\pm$ 1        | 9 $\pm$ 1       | 8 $\pm$ 1            | 7 $\pm$ 1  | 7 $\pm$ 1        | 6 $\pm$ 1*       | 5 $\pm$ 1*†       |
| DCBFV (Hz $\cdot 10^3$ )                          | 7 | 53 $\pm$ 3        | 52 $\pm$ 4      | 56 $\pm$ 4           | 56 $\pm$ 4   | 60 $\pm$ 4       | 64 $\pm$ 5*†     | 74 $\pm$ 5*†§     |
| DCVR (mmHg $\cdot$ Hz $^{-1} \cdot 10^{-2}$ )     | 7 | 1.65 $\pm$ 0.15*  | 1.37 $\pm$ 0.11 | 1.01 $\pm$ 0.07*     | 1.04 $\pm$ 0.06*   | 0.95 $\pm$ 0.08* | 0.87 $\pm$ 0.09* | 0.67 $\pm$ 0.03†§ |
| EDL (mm)  | 8 | 13.9 $\pm$ 0.4    | 13.5 $\pm$ 0.4  | 13.0 $\pm$ 0.4       | 12.8 $\pm$ 0.4   | 12.6 $\pm$ 0.4*  | 12.2 $\pm$ 0.5*† | 11.9 $\pm$ 0.5*†  |
| ESL (mm)  | 8 | 11.1 $\pm$ 0.4    | 11.0 $\pm$ 0.5  | 10.6 $\pm$ 0.4       | 10.4 $\pm$ 0.4   | 10.0 $\pm$ 0.5*  | 9.6 $\pm$ 0.4*†§ | 9.2 $\pm$ 0.5*†§  |
| SS (%)  | 8 | 20.1 $\pm$ 2.0    | 19.0 $\pm$ 1.9  | 18.1 $\pm$ 1.7       | 18.8 $\pm$ 2.1   | 20.4 $\pm$ 2.2   | 21.3 $\pm$ 2.2†  | 22.8 $\pm$ 2.6*†§ |
| CO (l $\cdot$ min $^{-1}$ )                       | 7 | 2.8 $\pm$ 0.2     | 2.6 $\pm$ 0.2   | 2.0 $\pm$ 0.2*       | 2.0 $\pm$ 0.2*   | 2.1 $\pm$ 0.2*   | 2.2 $\pm$ 0.3    | 2.3 $\pm$ 0.3     |
| SVR (dyne $\cdot$ s $\cdot$ cm $^{-5}$ )          | 7 | 2,900 $\pm$ 120*  | 2,410 $\pm$ 170 | 2,680 $\pm$ 260      | 2,750 $\pm$ 200  | 2,490 $\pm$ 170  | 2,320 $\pm$ 180  | 2,100 $\pm$ 170†§ |
| SV (ml)   | 7 | 32 $\pm$ 2*       | 24 $\pm$ 2      | 22 $\pm$ 2           | 22 $\pm$ 2   | 22 $\pm$ 2       | 22 $\pm$ 3       | 22 $\pm$ 3        |
| PWI (ml $\cdot$ min $^{-1} \cdot 100$ g $^{-1}$ ) | 7 | 9.1 $\pm$ 0.8     | 8.5 $\pm$ 0.3   | 6.2 $\pm$ 0.4*       | 6.2 $\pm$ 0.4*   | 6.2 $\pm$ 0.4*   | 6.5 $\pm$ 0.5*   | 6.4 $\pm$ 0.4*    |
| ET (%)  | 8 | —                 | —               | 1.30 $\pm$ 0.01      | 1.30 $\pm$ 0.01  | 1.29 $\pm$ 0.01  | 1.30 $\pm$ 0.01  | 1.29 $\pm$ 0.01   |

Data are mean  $\pm$  SEM.

HR = heart rate; MBP = mean aortic blood pressure; RPP = rate pressure product; LVSP and LVEDP = left ventricular systolic and end-diastolic pressure, respectively; DCBFV = diastolic coronary blood flow velocity; DCVR = diastolic coronary vascular resistance; EDL and ESL = end-diastolic and end-systolic segment length, respectively; SS = segment shortening; CO = cardiac output; SV = stroke volume; SVR = systemic vascular resistance; ANS = autonomic nervous system; PWI = pressure work index; ET = end-tidal isoflurane concentration.

\* Significantly ( $P < 0.05$ ) different from ANS blockade.

† Significantly ( $P < 0.05$ ) different from isoflurane alone.

§ Significantly ( $P < 0.05$ ) different from 0.5  $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  levosimendan infusion.

‡ Significantly ( $P < 0.05$ ) different from 1.0  $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  levosimendan infusion.

work index, and %SS and decreases in left ventricular systolic pressure, left ventricular end-diastolic pressure, and systemic vascular resistance were observed at the 4.0  $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  dose of levosimendan. No changes in mean arterial pressure or stroke volume were observed. Levosimendan increased myocardial contractility in a dose-dependent manner ( $M_w$  65  $\pm$  6 during control to 139  $\pm$  9 mmHg at 4.0  $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ) in conscious dogs (table 4). No significant change in  $L_w$  occurred. Concomitant increases in  $+dP/dt_{max}$  were also observed (1929  $\pm$  84 during control to 2865  $\pm$  205 mmHg  $\cdot$  s $^{-1}$  at 4.0  $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ). No alterations in  $\tau$  (38  $\pm$  1 during control to 35  $\pm$  2 ms at 4.0  $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ) and  $-dP/dt_{min}$  ( $-1838 \pm 90$  during control to  $-1667 \pm 76$  mmHg  $\cdot$  s $^{-1}$  at 4.0  $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ) were observed, indicating that this phase of diastole was unaltered by administration of levosimendan. Dose-related increases in  $dL/dt_{max}$  (34  $\pm$  3 during control to 47  $\pm$  5 mm  $\cdot$  s $^{-1}$  at 4.0  $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ) occurred, consistent with enhanced early ventricular filling.  $K_p$  was unchanged by levosimendan, suggesting that chamber compliance was not affected by the myofilament  $\text{Ca}^{2+}$  sensitizer in conscious dogs (table 4).

Isoflurane anesthesia (1.0 MAC) caused decreases in heart rate, mean arterial pressure, left ventricular systolic pressure, diastolic coronary vascular resistance, cardiac output, rate-pressure product, and pressure-work index in the presence of autonomic nervous system blockade. No changes in left ventricular end-diastolic pressure, diastolic coronary blood flow velocity, systemic vascular resistance, %SS, or stroke volume were observed (table 2). The hemodynamic effects of halothane were similar to those produced by isoflurane (table 3). In contrast to isoflurane, however, 1.0 MAC halothane caused a significant decrease in %SS. Isoflurane and halothane depressed myocardial contractility (decreases in  $M_w$  and  $+dP/dt_{max}$ ), prolonged isovolumic relaxation (increase in  $\tau$  and decrease in the magnitude of  $-dP/dt_{min}$ ), and impaired rapid ventricular filling (decrease in  $dL/dt_{max}$ ) without alteration in  $K_p$  (tables 5 and 6, respectively). No changes in  $L_w$  were observed during anesthesia. Halothane caused significantly greater negative inotropic effects than isoflurane at 1.0 MAC ( $M_w$  of 38  $\pm$  3 for halothane compared to 57  $\pm$  4 mmHg for isoflurane); however, no differences in indices of diastolic function were noted during halothane and isoflurane anesthesia.

**Table 3. Hemodynamic Effects of Levosimendan in Halothane-Anesthetized Dogs**

|  | N | Conscious Control | ANS Blockade    | Halothane (1.0 MAC) | Levosimendan Infusion ( $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ) |                 |                   |                    |
|--|---|-------------------|-----------------|---------------------|--|-----------------|-------------------|--------------------|
|  |   |                   |                 |                     | 0.5  | 1.0             | 2.0               | 4.0                |
| HR (bpm)   | 8 | 83 $\pm$ 5*       | 107 $\pm$ 5     | 89 $\pm$ 5*         | 88 $\pm$ 5*  | 90 $\pm$ 5*     | 94 $\pm$ 5*       | 98 $\pm$ 5         |
| MBP (mmHg)   | 8 | 102 $\pm$ 4*      | 77 $\pm$ 3      | 63 $\pm$ 4*         | 64 $\pm$ 4*  | 63 $\pm$ 4*     | 61 $\pm$ 4*       | 56 $\pm$ 4*†§      |
| RPP (mmHg $\cdot$ bpm $\cdot 10^3$ )                       | 8 | 10.6 $\pm$ 0.7    | 9.7 $\pm$ 0.5   | 6.7 $\pm$ 0.6*      | 6.8 $\pm$ 0.6*   | 6.8 $\pm$ 0.7*  | 6.9 $\pm$ 0.7*    | 6.7 $\pm$ 0.8*     |
| LVSP (mmHg)  | 8 | 128 $\pm$ 5*      | 94 $\pm$ 5      | 76 $\pm$ 4*         | 77 $\pm$ 4*  | 76 $\pm$ 4*     | 74 $\pm$ 4*       | 68 $\pm$ 5*†§      |
| LVEDP (mmHg)   | 8 | 9 $\pm$ 1         | 9 $\pm$ 1       | 8 $\pm$ 1           | 8 $\pm$ 1  | 7 $\pm$ 1       | 5 $\pm$ 1*        | 5 $\pm$ 1*†        |
| DCBFV (Hz $\cdot 10^2$ )                                   | 7 | 53 $\pm$ 5        | 54 $\pm$ 5      | 51 $\pm$ 5          | 52 $\pm$ 6   | 53 $\pm$ 6      | 56 $\pm$ 6        | 63 $\pm$ 7†§       |
| DCVR (mmHg $\cdot \text{H}^{-1} \cdot 10^{-2}$ )           | 7 | 1.67 $\pm$ 0.15*  | 1.31 $\pm$ 0.13 | 1.16 $\pm$ 0.13     | 1.18 $\pm$ 0.14  | 1.13 $\pm$ 0.14 | 1.06 $\pm$ 0.14   | 0.86 $\pm$ 0.12*†§ |
| EDL (mm)   | 8 | 14.6 $\pm$ 0.7    | 14.4 $\pm$ 0.6  | 14.0 $\pm$ 0.7      | 14.1 $\pm$ 0.6   | 13.9 $\pm$ 0.7* | 13.5 $\pm$ 0.7*§  | 13.1 $\pm$ 0.7*†§  |
| ESL (mm)   | 8 | 11.5 $\pm$ 0.7    | 11.7 $\pm$ 0.5  | 11.8 $\pm$ 0.6      | 11.8 $\pm$ 0.6   | 11.4 $\pm$ 0.6  | 10.8 $\pm$ 0.6*†§ | 10.1 $\pm$ 0.6*†§¶ |
| SS(%)  | 8 | 20.7 $\pm$ 1.3    | 18.7 $\pm$ 1.1  | 15.7 $\pm$ 1.0*     | 16.3 $\pm$ 0.9*  | 17.8 $\pm$ 0.9† | 19.7 $\pm$ 0.7†§  | 23.0 $\pm$ 1.4*†§  |
| CO (l $\cdot \text{min}^{-1}$ )                            | 7 | 2.7 $\pm$ 0.3     | 2.9 $\pm$ 0.3   | 2.0 $\pm$ 0.3*      | 2.1 $\pm$ 0.3*   | 2.1 $\pm$ 0.3*  | 2.2 $\pm$ 0.3*    | 2.3 $\pm$ 0.4      |
| SVR (dyne $\cdot \text{s} \cdot \text{cm}^{-5}$ )          | 7 | 3,020 $\pm$ 240*  | 2,180 $\pm$ 220 | 2,680 $\pm$ 330     | 2,680 $\pm$ 310  | 2,560 $\pm$ 270 | 2,460 $\pm$ 260   | 2,210 $\pm$ 290†§  |
| SV (ml)  | 7 | 35 $\pm$ 5*       | 27 $\pm$ 3      | 22 $\pm$ 2          | 23 $\pm$ 2   | 23 $\pm$ 3      | 23 $\pm$ 3        | 23 $\pm$ 3         |
| PWI (ml $\cdot \text{min}^{-1} \cdot 100 \text{ g}^{-1}$ ) | 7 | 9.6 $\pm$ 0.5     | 8.5 $\pm$ 0.5   | 6.0 $\pm$ 0.6*      | 6.2 $\pm$ 0.6*   | 6.2 $\pm$ 0.7*  | 6.3 $\pm$ 0.7*    | 6.2 $\pm$ 0.7*     |
| ET (%)   | 8 | —                 | —               | 0.89 $\pm$ 0.01     | 0.89 $\pm$ 0.01  | 0.88 $\pm$ 0.01 | 0.88 $\pm$ 0.01   | 0.89 $\pm$ 0.01    |

Data are mean  $\pm$  SEM.

HR = heart rate; MBP = mean aortic blood pressure; RPP = rate pressure product; LVSP and LVEDP = left ventricular systolic and end-diastolic pressure, respectively; DCBFV = diastolic coronary blood flow velocity; DCVR = diastolic coronary vascular resistance; EDL and ESL = end-diastolic and end-systolic segment length, respectively; SS = segment shortening; CO = cardiac output; SV = stroke volume; SVR = systemic vascular resistance; ANS = autonomic nervous system; PWI = pressure work index; ET = end-tidal halothane concentration.

\* Significantly ( $P < 0.05$ ) different from ANS blockade.

† Significantly ( $P < 0.05$ ) different from halothane alone.

§ Significantly ( $P < 0.05$ ) different from 0.5  $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  levosimendan infusion.

‡ Significantly ( $P < 0.05$ ) different from 1.0  $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  levosimendan infusion.

¶ Significantly ( $P < 0.05$ ) different from 2.0  $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  levosimendan infusion.

Levosimendan caused changes in systemic and coronary hemodynamics during isoflurane and halothane anesthesia which were qualitatively similar to those observed in the conscious state (tables 2 and 3, re-

spectively). Levosimendan decreased diastolic coronary and systemic vascular resistances, left ventricular end-diastolic pressure, EDL, and ESL and increased diastolic coronary blood flow velocity and %SS in isoflurane- and

**Table 4. Effects of Levosimendan on Indices of Left Ventricular Function in Conscious Dogs**

|   | ANS Blockade    | Levosimendan Infusion ( $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ) |                  |                    |                     |
|---|-----------------|--|------------------|--------------------|---------------------|
|   |                 | 0.5  | 1.0              | 2.0                | 4.0                 |
| $M_w$ (mmHg)  | 65 $\pm$ 6      | 67 $\pm$ 3   | 80 $\pm$ 5*      | 102 $\pm$ 9*†§     | 139 $\pm$ 9*††§     |
| $L_w$ (mm)  | 10.4 $\pm$ 0.6  | 10.3 $\pm$ 0.6   | 10.8 $\pm$ 0.6   | 10.7 $\pm$ 0.6     | 10.9 $\pm$ 0.8      |
| $+dP/dt_{\text{max}}$ (mmHg $\cdot \text{s}^{-1}$ ) | 1,929 $\pm$ 84  | 1,933 $\pm$ 88   | 2,059 $\pm$ 106  | 2,432 $\pm$ 146*†§ | 2,865 $\pm$ 205*††§ |
| $-dP/dt_{\text{min}}$ (mmHg $\cdot \text{s}^{-1}$ ) | -1,838 $\pm$ 90 | -1,810 $\pm$ 112   | -1,912 $\pm$ 114 | -1,825 $\pm$ 139   | -1,667 $\pm$ 76     |
| $\tau$ (ms)   | 38 $\pm$ 1      | 37 $\pm$ 1   | 36 $\pm$ 1       | 35 $\pm$ 2         | 35 $\pm$ 2          |
| $K_p$ (mm $^{-1}$ )                                 | 0.36 $\pm$ 0.03 | 0.32 $\pm$ 0.03  | 0.32 $\pm$ 0.03  | 0.31 $\pm$ 0.04    | 0.32 $\pm$ 0.03     |
| $dL/dt_{\text{max}}$ (mm $\cdot \text{s}^{-1}$ )    | 34 $\pm$ 3      | 37 $\pm$ 5   | 38 $\pm$ 4       | 44 $\pm$ 5*        | 47 $\pm$ 5*†        |

Data are mean  $\pm$  SEM; n = 8.

$M_w$  and  $L_w$  = preload recruitable stroke work slope and length intercept, respectively; SS = segment shortening;  $\tau$  = time constant of isovolumic relaxation;  $k_p$  = regional chamber stiffness; ANS = autonomic nervous system.

\* Significantly ( $P < 0.05$ ) different from ANS blockade.

† Significantly ( $P < 0.05$ ) different from 0.5  $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  levosimendan infusion.

§ Significantly ( $P < 0.05$ ) different from 1.0  $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  levosimendan infusion.

‡ Significantly ( $P < 0.05$ ) different from 2.0  $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  levosimendan infusion.

## LEVOSIMENDAN AND LEFT VENTRICULAR FUNCTION

**Table 5. Effects of Levosimendan on Indices of Left Ventricular Function in Isoflurane-anesthetized Dogs**

|   | ANS Blockade    | Isoflurane<br>(1.0 MAC) | Levosimendan Infusion ( $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ) |                    |                          |                            |
|---|-----------------|-------------------------|--|--------------------|--------------------------|----------------------------|
|   |                 |                         | 0.5  | 1.0                | 2.0                      | 4.0                        |
| $M_w$ (mmHg)                              | $72 \pm 5$      | $57 \pm 4^*$            | $66 \pm 5$   | $77 \pm 6^\dagger$ | $96 \pm 7^*\dagger\$$    | $116 \pm 12^*\dagger\$$    |
| $L_w$ (mm)                                | $10.2 \pm 0.6$  | $10.1 \pm 0.5$          | $8.9 \pm 1.2$  | $10.2 \pm 0.5$     | $10.1 \pm 0.5$           | $10.1 \pm 0.6$             |
| $+dP/dt_{\max}$ (mmHg $\cdot$ s $^{-1}$ ) | $1,935 \pm 67$  | $1,637 \pm 76^*$        | $1,671 \pm 55^*$   | $1,770 \pm 54$     | $2,040 \pm 94^\dagger\$$ | $2,317 \pm 160^*\dagger\$$ |
| $-dP/dt_{\min}$ (mmHg $\cdot$ s $^{-1}$ ) | $-1,837 \pm 62$ | $-1,317 \pm 80^*$       | $-1,327 \pm 63^*$  | $-1,278 \pm 80^*$  | $-1,254 \pm 91^*$        | $-1,206 \pm 81^*$          |
| $\tau$ (ms)                               | $38 \pm 1$      | $44 \pm 2^*$            | $45 \pm 1^*$   | $44 \pm 2^*$       | $43 \pm 2^*$             | $42 \pm 1$                 |
| $K_p$ (mm $^{-1}$ )                       | $0.45 \pm 0.06$ | $0.41 \pm 0.07$         | $0.39 \pm 0.05$  | $0.37 \pm 0.06$    | $0.30 \pm 0.05$          | $0.29 \pm 0.05$            |
| $dL/dt_{\max}$ (mm $\cdot$ s $^{-1}$ )    | $34 \pm 2$      | $27 \pm 3^*$            | $28 \pm 3^*$   | $29 \pm 3$         | $33 \pm 3$               | $34 \pm 3^\dagger\$$       |

Data are mean  $\pm$  SEM;  $n = 8$ .

$M_w$  and  $L_w$  = preload recruitable stroke work slope and length intercept, respectively; SS = segment shortening;  $\tau$  = time constant of isovolumic relaxation;  $K_p$  = regional chamber stiffness constant; ANS = autonomic nervous system.

\* Significantly ( $P < 0.05$ ) different from ANS blockade.

† Significantly ( $P < 0.05$ ) different from isoflurane.

§ Significantly ( $P < 0.05$ ) different from  $0.5 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  levosimendan infusion.

‡ Significantly ( $P < 0.05$ ) different from  $1.0 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  levosimendan infusion.

halothane-anesthetized dogs. Levosimendan significantly increased heart rate in the presence of isoflurane but not halothane. In contrast to the findings in the conscious state, levosimendan decreased mean arterial pressure and did not change calculated estimates of myocardial oxygen consumption (rate-pressure product and pressure-work index) in anesthetized dogs. Levosimendan enhanced myocardial contractility in a dose-related manner during anesthesia ( $M_w$   $57 \pm 4$  and  $38 \pm 3$  during isoflurane and halothane alone to  $116 \pm 12$  and  $92 \pm 8$  mmHg at  $4.0 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ , re-

spectively; tables 5 and 6). No changes in  $L_w$  were observed. Differences in contractile state between isoflurane and halothane groups were maintained during levosimendan infusions; however, relative increases in  $M_w$  produced by levosimendan were similar in both conscious and anesthetized dogs (fig. 3). No alterations in  $\tau$  or  $-dP/dt_{\min}$  were produced by levosimendan during isoflurane or halothane anesthesia. Despite the lack of change in indices of isovolumic relaxation, levosimendan enhanced rapid ventricular filling (e.g.,  $27 \pm 3$  during isoflurane alone to  $34 \pm 3$  mm  $\cdot$  s $^{-1}$  at  $4.0$

**Table 6. Effects of Levosimendan on Indices of Left Ventricular Function in Halothane-anesthetized Dogs**

|   | ANS Blockade    | Halothane<br>(1.0 MAC) | Levosimendan Infusion ( $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ) |                    |                           |                           |
|---|-----------------|------------------------|--|--------------------|---------------------------|---------------------------|
|   |                 |                        | 0.5  | 1.0                | 2.0                       | 4.0                       |
| $M_w$ (mmHg)                              | $66 \pm 7$      | $38 \pm 3^*$           | $47 \pm 6^*$   | $56 \pm 6^\dagger$ | $75 \pm 7^\dagger\$$      | $92 \pm 8^*\dagger\$$     |
| $L_w$ (mm)                                | $10.5 \pm 0.6$  | $10.4 \pm 0.7$         | $10.8 \pm 0.7$   | $10.7 \pm 0.7$     | $10.8 \pm 0.7$            | $10.7 \pm 0.7$            |
| $+dP/dt_{\max}$ (mmHg $\cdot$ s $^{-1}$ ) | $1,828 \pm 79$  | $1,211 \pm 76^*$       | $1,246 \pm 80^*$   | $1,312 \pm 90^*$   | $1,514 \pm 106^\dagger\$$ | $1,734 \pm 145^\dagger\$$ |
| $-dP/dt_{\min}$ (mmHg $\cdot$ s $^{-1}$ ) | $-1,758 \pm 96$ | $-1,240 \pm 97^*$      | $-1,246 \pm 92^*$  | $-1,252 \pm 98^*$  | $-1,272 \pm 105^*$        | $-1,198 \pm 107^*$        |
| $\tau$ (ms)                               | $38 \pm 1$      | $46 \pm 2^*$           | $47 \pm 2^*$   | $45 \pm 2^*$       | $43 \pm 2$                | $43 \pm 2$                |
| $K_p$ (mm $^{-1}$ )                       | $0.47 \pm 0.09$ | $0.45 \pm 0.06$        | $0.46 \pm 0.07$  | $0.40 \pm 0.05$    | $0.34 \pm 0.05$           | $0.31 \pm 0.05$           |
| $dL/dt_{\max}$ (mm $\cdot$ s $^{-1}$ )    | $32 \pm 2$      | $24 \pm 1^*$           | $25 \pm 1^*$   | $26 \pm 1^*$       | $28 \pm 2^*$              | $33 \pm 2^\dagger\$$      |

Data are mean  $\pm$  SEM;  $n = 8$ .

$M_w$  and  $L_w$  = preload recruitable stroke work slope and length intercept, respectively; SS = segment shortening;  $\tau$  = time constant of isovolumic relaxation;  $K_p$  = regional chamber stiffness constant; ANS = autonomic nervous system.

\* Significantly ( $P < 0.05$ ) different from ANS blockade.

† Significantly ( $P < 0.05$ ) different from halothane.

§ Significantly ( $P < 0.05$ ) different from  $0.5 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  levosimendan infusion.

‡ Significantly ( $P < 0.05$ ) different from  $1.0 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  levosimendan infusion.

¶ Significantly ( $P < 0.05$ ) different from  $2.0 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  levosimendan infusion.



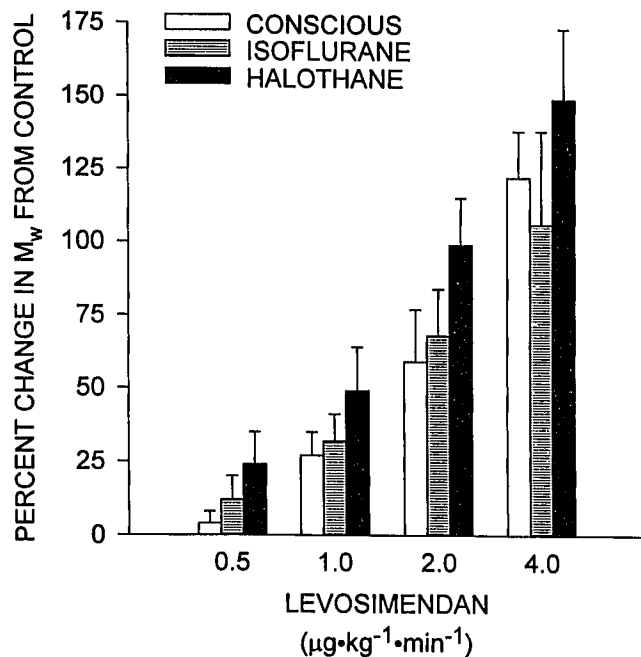


Fig. 3. Histograms depicting the percent change in the regional preload recruitable stroke work slope ( $M_w$ ) in conscious (open bar), isoflurane-anesthetized (horizontal bar), and halothane-anesthetized (filled bar) dogs during administration of levosimendan. No significant difference in the percent change in  $M_w$  was observed between conscious and anesthetized states at each dose of levosimendan.

$\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ) during anesthesia (table 5 and 6). Increases in  $dL/dt_{\text{max}}$  produced by levosimendan were similar in the conscious and anesthetized states. No changes in  $K_p$  occurred in isoflurane- or halothane-anesthetized dogs, suggesting that levosimendan did not affect this measure of regional ventricular compliance during anesthesia as well.

## Discussion

Recognition that the performance of cardiac muscle can be substantially improved by increased delivery of  $\text{Ca}^{2+}$  to the contractile apparatus has been a fundamental underlying principle in the pharmacological management of depressed contractility in the failing heart.<sup>18</sup> Traditional positive inotropic agents, including cardiac glycosides,  $\beta$ -adrenoceptor agonists, and specific PDE-III inhibitors, improve contractility by increasing the myoplasmic  $\text{Ca}^{2+}$  concentration available for contractile activation after membrane depolarization through a variety of mechanisms including partial

inhibition of sarcolemmal  $\text{Na}^+ - \text{K}^+$  adenosine triphosphatase activity, receptor-mediated stimulation of the adenylate cyclase-cyclic adenosine monophosphate (cAMP) pathway, and blockade of cAMP degradation, respectively. In contrast, drugs in the myofilament  $\text{Ca}^{2+}$ -sensitizing class directly augment contractile force by increasing the affinity of the regulatory site of troponin C for  $\text{Ca}^{2+}$  in a highly stereospecific manner.<sup>3-6,39</sup> This effect stabilizes  $\text{Ca}^{2+}$ -induced conformational changes in troponin C and allows for prolonged systolic interaction of actin and myosin filaments.<sup>5,6</sup> Thus, these cardiotonic drugs enhance contractile performance by modulating the response of the myofilament regulatory proteins<sup>1-6,39</sup> or the contractile elements<sup>40</sup> to  $\text{Ca}^{2+}$  without specifically altering the intracellular concentration of this ion.

Myofilament  $\text{Ca}^{2+}$ -sensitizing agents may also possess PDE-III-inhibiting activity which contribute to<sup>3,39</sup> but do not entirely account for<sup>3,5</sup> the positive inotropic effects of these compounds *via* cAMP-induced, protein kinase A-mediated phosphorylation of the voltage-dependent  $\text{Ca}^{2+}$  channel. Inhibition of PDE-III by myofilament  $\text{Ca}^{2+}$  sensitizers may also result in positive lusitropic (relaxation) effects through direct intracellular mechanisms, including PDE-III-mediated, protein kinase A-induced promotion of  $\text{Ca}^{2+}$  dissociation from troponin C and increased sarcoplasmic reticular  $\text{Ca}^{2+}$ -adenosine triphosphatase function *via* phosphorylation of troponin I and phospholamban, respectively. Positive lusitropic actions induced by these agents may also be mediated by indirect systemic hemodynamic effects resulting from improved left ventricular loading conditions *via* PDE-III-induced venodilation and vasodilation.<sup>1,18,41-43</sup> Thus, shortened isovolumic relaxation may occur with myofilament  $\text{Ca}^{2+}$  sensitizers with PDE-III activity despite the primary increases in  $\text{Ca}^{2+}$  binding to troponin C resulting from these agents. These positive lusitropic effects may be particularly important because impaired left ventricular relaxation frequently occurs in end-stage heart failure, resulting from delayed  $\text{Ca}^{2+}$  clearance from the sarcoplasm and, subsequently, diminished restoration of submicromolar  $\text{Ca}^{2+}$  concentrations during diastole.<sup>1,44,45</sup>

The effects of myofilament  $\text{Ca}^{2+}$  sensitizers, including pimobendan and sulmazole, on systemic and coronary hemodynamics, myocardial oxygen consumption, and left ventricular pump performance have been examined in experimental animals and humans. Several investigators have described increases in cardiac index,  $+dP/dt_{\text{max}}$ , and ejection fraction with concomitant decreases

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in left ventricular preload (end-diastolic pressure or wall stress) and afterload (calculated systemic vascular resistance or end-systolic wall stress) after administration of pimobendan to patients with severe congestive heart failure resulting from chronic ischemia or idiopathic dilated cardiomyopathy.<sup>7-15,46,47</sup> These effects were often accompanied by decreases in myocardial oxygen consumption and increases in exercise duration and capacity, peak oxygen uptake, and the ratio of myocardial oxygen supply to demand.<sup>10,11,13,47</sup> Favorable improvements in myocardial energetics presumably resulted from reductions in left ventricular preload and afterload. Decreases in circulating endogenous catecholamines and an enhanced response to  $\beta$ -adrenoceptor stimulation have also been observed with chronic pimobendan treatment,<sup>8,9,48</sup> findings which suggest that pimobendan may indirectly reverse  $\beta$ -receptor down regulation associated with congestive heart failure. Pimobendan increased the magnitude of  $-dP/dt_{min}$  in patients with left ventricular dysfunction consistent with a positive lusitropic effect.<sup>12</sup> This improvement in  $-dP/dt_{min}$  may have occurred because of the direct PDE-III-inhibiting effects of pimobendan or may have been caused by indirect actions of the drug on ventricular loading conditions, enhanced  $\beta$ -adrenoceptor responsiveness to circulating catecholamines, or reflex effects mediated by intact autonomic nervous system function, however. Another myofilament  $Ca^{2+}$  sensitizer which has been less extensively studied, sulmazole, has been reported to cause systemic hemodynamic effects and left ventricular mechanical actions in patients with coronary artery disease which are similar to those produced by pimobendan.<sup>16,17</sup>

The current investigation is the first to examine the interactions between a drug in the myofilament  $Ca^{2+}$ -sensitizing class of positive inotropic agents and volatile anesthetics. The results indicate that levosimendan causes systemic and coronary hemodynamic effects in the absence of autonomic nervous system tone which are qualitatively similar to those produced by pimobendan and sulmazole in patients with congestive heart failure. A dose-related increase in heart rate was observed during administration of levosimendan to conscious dogs with blockade of the autonomic nervous system, indicating that levosimendan may cause direct increases in heart rate independent of autonomic reflexes. Kitzen *et al.* observed increases in heart rate with pimobendan which resulted from direct increases in atrioventricular nodal conduction and decreases in effective ventricular refractory period in barbiturate-

anesthetized, acutely instrumented dogs.<sup>49</sup> Pimobendan-induced tachycardia has also been reported in open-chest pigs with and without pretreatment with propranolol, excluding activity at the  $\beta$ -adrenoceptor as a potential mechanism for the increases in heart rate.<sup>48</sup> Levosimendan-induced increases in heart rate were also observed during isoflurane but not halothane anesthesia in the current study, suggesting that the direct negative chronotropic effects of halothane<sup>50</sup> may differentially antagonize the positive chronotropic actions of levosimendan.

Levosimendan caused decreases in left ventricular systolic and end-diastolic pressures in conscious and anesthetized dogs. Concomitant declines in ESL and calculated systemic vascular resistance, and decreases in EDL also occurred, suggesting that the myofilament  $Ca^{2+}$  sensitizer causes a reduction in left ventricular preload and afterload, respectively, *via* dilatation of the venous and arterial vasculature. The current findings in autonomically blocked dogs are similar to the results of several previous studies which reported decreases in left ventricular end-systolic and end-diastolic pressure, volume and wall stress with administration of pimobendan<sup>7-12,14,46,47</sup> or sulmazole<sup>16,17</sup> to patients with congestive heart failure, observations which have been attributed to the PDE-III-inhibiting activity of these agents.

The rate-pressure product and pressure-work index increased only at the highest dose of levosimendan in conscious dogs and did not change during isoflurane and halothane anesthesia, indicating that levosimendan has little, if any, direct effect on calculated estimates of myocardial oxygen consumption. These results probably occurred because levosimendan-induced increases in heart rate and myocardial contractility were offset by concomitant declines in left ventricular preload and afterload. The current findings in autonomically blocked dogs are consistent with the relative maintenance or modest reduction of myocardial oxygen consumption observed in response to pimobendan in humans with compromised left ventricular function.<sup>10,11,13,47</sup> Levosimendan caused declines in diastolic coronary vascular resistance and concomitant increases in peak diastolic coronary blood flow velocity at higher doses in conscious and anesthetized dogs. These alterations in coronary hemodynamics occurred without parallel changes in calculated myocardial oxygen consumption, suggesting that levosimendan may also produce direct coronary vasodilation. Although the pressure-work index has been shown to accurately reflect

alterations in measured myocardial oxygen consumption over a wide range of heart rates, ventricular loading conditions, and contractile states,<sup>51</sup> coronary sinus oxygen tension and myocardial oxygen consumption were not specifically measured in the current investigation. Similar increases in coronary blood flow, measured using an electromagnetic flow probe or with the radioactive microsphere technique, without changes in myocardial oxygen consumption have been previously reported for pimobendan in acutely instrumented dogs<sup>49</sup> and pigs,<sup>48</sup> respectively.

The results of the current investigation indicate that levosimendan has important functional actions on left ventricular systolic and diastolic mechanical performance. Levosimendan caused dose-dependent increases in myocardial contractility in the conscious and anesthetized states as assessed using  $M_w$  derived from a series of left ventricular pressure-segment length diagrams, an easily quantified and relatively heart rate- and load-independent index of contractility *in vivo*.<sup>35</sup> No changes in  $L_w$  occurred with any intervention, indicating that changes in myocardial contractility were reflected solely by changes in  $M_w$ . Levosimendan did not alter measures of isovolumic relaxation including  $\tau$  and  $-dP/dt_{min}$ , indicating that levosimendan did not affect this phase of diastole. Increases in the  $dL/dt_{max}$  were observed during administration of levosimendan to conscious and anesthetized dogs, demonstrating an improvement in rapid ventricular filling. No changes in  $K_p$  were observed during the conscious or anesthetized states, suggesting that levosimendan does not alter regional ventricular compliance.

The current results contrast with those observed with amrinone, a specific cardiac PDE-III inhibitor without myofilament  $Ca^{2+}$ -sensitizing activity, was administered in a previous investigation from this laboratory using the identical canine model.<sup>31</sup> Amrinone produced dose-related increases in  $M_w$  and decreases in  $\tau$  in conscious and anesthetized dogs.<sup>31</sup> The positive inotropic effects of amrinone, resulting from increases in total cytosolic  $Ca^{2+}$  during systole *via* increased voltage-dependent  $Ca^{2+}$  channel conductance, did not lead to simultaneous alterations in  $Ca^{2+}$  clearance during diastole because amrinone also stimulated  $Ca^{2+}$  dissociation from the contractile apparatus and facilitated  $Ca^{2+}$  uptake into the sarcoplasmic reticulum *via* cAMP-mediated mechanisms. In contrast, increases in  $M_w$  caused by levosimendan were not accompanied by similar reductions in  $\tau$  in either the conscious or anesthetized states. A lack of change in  $\tau$  during the administration of le-

vosimendan was also present despite concomitant declines in left ventricular afterload (as indirectly assessed by ESL and calculated systemic vascular resistance) and increases in heart rate, hemodynamic effects which were qualitatively similar to those observed during amrinone infusions and usually contribute to shortening of the isovolumic relaxation phase of diastole.<sup>52-54</sup> No changes in the absolute value of  $-dP/dt_{min}$  were also observed, supporting the contention that relaxation was not improved by levosimendan. However,  $-dP/dt_{min}$  has been shown to be a less reliable indicator of relaxation behavior than  $\tau$  because the value of  $-dP/dt_{min}$  is directly dependent on developed left ventricular pressure.<sup>53</sup> The current results suggest that levosimendan, an agent which improves contractile function by enhancing the affinity of troponin C for  $Ca^{2+}$ , caused partial delays in  $Ca^{2+}$  dissociation and sequestration which were of sufficient magnitude to blunt the anticipated improvement in indices of isovolumic relaxation which are normally observed with PDE-III inhibitors alone. The results further indicate that levosimendan-induced alterations in  $Ca^{2+}$  dissociation from the contractile apparatus were balanced by but could not be overcome by the PDE-III-inhibiting properties of the drug. No differences in the inotropic and lusitropic responses to levosimendan were observed between the conscious and isoflurane- or halothane-anesthetized states, suggesting that levosimendan does not differentially affect myofilament  $Ca^{2+}$  sensitivity in the presence of volatile anesthetics. This observation also lends indirect support to recent evidence that inhalational anesthetics do not substantially alter the affinity of troponin C for  $Ca^{2+}$ .<sup>55</sup>

Levosimendan caused increases in  $dL/dt_{max}$ , indicating that the myofilament  $Ca^{2+}$  sensitizer enhanced early ventricular filling in conscious and anesthetized dogs. The rate of rapid ventricular filling has been shown to be inversely related to the rate and extent of isovolumic relaxation and directly proportional to inotropic state, left ventricular preload, and the pressure gradient between the left atrium and left ventricle during this period of the cardiac cycle.<sup>54,56,57</sup> Thus, the increases in  $dL/dt_{max}$  observed after administration of levosimendan may have occurred as a direct consequence of increases in myocardial contractility despite concomitant declines in left ventricular end-diastolic pressure and the lack of change in indices of isovolumic relaxation. In addition, the left atrial-ventricular pressure gradient during rapid ventricular filling was not specifically measured in the current investigation and alterations

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in this gradient caused by levosimendan or volatile anesthetics may have influenced the observed changes in  $dL/dt_{max}$ . Passive ventricular elastic properties may have also been influenced by alterations in ventricular loading conditions and contractile state produced by levosimendan or anesthetics and may have contributed to the lack of changes in  $K_p$  observed during the administration of these agents.<sup>54</sup>

In summary, the results of this investigation indicate that levosimendan, a new myofilament  $Ca^{2+}$  sensitizer, produces direct positive inotropic effects as evaluated using regional  $M_w$  in conscious and anesthetized, chronically instrumented dogs. Levosimendan also improved rapid ventricular filling in a dose-related fashion as indicated by  $dL/dt_{max}$ . Despite causing increases in myocardial contractility and rapid ventricular filling, however, levosimendan did not alter indices of left ventricular isovolumic relaxation. This finding indicates that levosimendan selectivity enhances contractile state and filling without affecting diastolic relaxation (lusitropic state). The current results are in contrast to the findings obtained with amrinone,<sup>31</sup> an inhibitor of cardiac PDE-III without myofilament  $Ca^{2+}$ -sensitization characteristics, suggesting that the enhanced affinity of troponin C for  $Ca^{2+}$  caused by levosimendan was contributing to a relative delay in  $Ca^{2+}$  dissociation from the contractile apparatus during early diastole that could not be completely overcome by the PDE-III-inhibiting activity of the drug.

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