Amrinone Enhances Myocardial Contractility and Improves Left Ventricular Diastolic Function in Conscious and Anesthetized Chronically Instrumented Dogs

Paul S. Pagel, M.D.,* Douglas A. Hetrick, M.S.,† David C. Wartlir, M.D., Ph.D.‡

Background: Volatile anesthetics depress left ventricular mechanical performance in vivo by altering intracellular calcium regulation. Although amrinone has been shown to reverse the negative inotropic effects of volatile anesthetics, the actions of amrinone on anesthetic-induced diastolic dysfunction are unknown. This investigation examined and compared the direct effects of amrinone on left ventricular systolic and diastolic function in conscious and anesthetized dogs.

Methods: Experiments were conducted in the presence of pharmacologic blockade of the autonomic nervous system, because autonomic activity may influence the hemodynamic actions of volatile anesthetics and amrinone in vivo. Three groups, comprising a total of 27 experiments, were conducted using 9 dogs chronically instrumented for measurement of aortic and left ventricular pressure, left ventricular dP/dt, subendocardial segment length, diastolic coronary blood flow velocity, and cardiac output. Myocardial contractility was evaluated using the preload recruitable stroke work relationship slope (Mw). Diastolic function was characterized by a time constant of isovolumic relaxation (τ), a regional chamber stiffness constant (Ks), and maximum segment lengthening velocity during rapid ventricular filling (dL/dtmax). On three separate days, an amrinone bolus of 1 mg/kg, followed by an infusion at 10, 20, 40, or 80 μg·kg⁻¹·min⁻¹, was administered. Hemodynamics and ventricular pressure-segment length loops and waveforms were recorded after a 15-min equilibration at each dose in the conscious state or during isoflurane or halothane anesthesia (1.25 MAC).

Results: In conscious dogs, amrinone significantly increased myocardial contractility in a dose-dependent manner (Mw of 65 ± 8 to 108 ± 10 mmHg at the high dose). Amrinone also shortened isovolumic relaxation (τ of 32.7 ± 2.1 to 24.8 ± 0.9 ms at the high dose) and enhanced rapid ventricular filling (dL/dtmax of 34.8 ± 1.2 to 45.1 ± 2.3 mm/s at the high dose) in a dose-related fashion. In addition, amrinone reduced regional chamber stiffness (Ks of 0.49 ± 0.09 to 0.31 ± 0.08 mmHg at the high dose) in conscious dogs. Amrinone also enhanced left ventricular systolic (increase in Mw) and diastolic function (decreases in τ and Ks) in isolated myocardium (dL/dtmax) when this drug was administered to dogs anesthetized with isoflurane or halothane.

Conclusions: Amrinone produced positive inotropic and lusitropic effects in both conscious and anesthetized dogs with autonomic nervous system blockade. These results indicate that amrinone-induced improvement of left ventricular performance are related to actions in diastole, as well as systole. (Key words: Anesthetics, volatile; halothane; isoflurane. Heart, diastoles; diastolic left ventricular function; isovolumic relaxation; ventricular compliance. Heart, myocardial performance: left ventricular function; myocardial contractility; preload recruitable stroke work. Pharmacology, inotropic agents: amrinone.)

INHIBITORS of myocardial phosphodiesterase fraction III (PDE III), including amrinone, improve cardiac performance by reducing degradation of cyclic adenosine monophosphate (cAMP), an action that promotes a cascade of intracellular events leading to enhanced calcium (Ca²⁺) availability for contractile activation. In addition to positive inotropic and chronotropic effects, PDE III inhibitors produce pulmonary and systemic vasodilation, and may also improve left ventricular diastolic performance, making this class of drug useful for the treatment of compromised ventricular function associated with congestive heart failure. PDE III inhibitors have also been shown to qualitatively reverse the negative ino-
tropic effects of potent inhalational anesthetics in vitro\textsuperscript{11,12} and in vivo.\textsuperscript{13-16}

Volatile anesthetics, including isoflurane and halothane, depress myocardial contractility to varying degrees\textsuperscript{17-19} by interfering with normal intracellular Ca\textsuperscript{2+} regulation through a variety of mechanisms,\textsuperscript{20} including partial inhibition of voltage-dependent Ca\textsuperscript{2+} channels in the sarcolemmal membrane;\textsuperscript{21-25} disruption of Ca\textsuperscript{2+} storage and mobilization functions of the sarcoplasmic reticulum;\textsuperscript{26-29} and alteration of contractile protein affinity for, and responsiveness to, activator Ca\textsuperscript{2+}.\textsuperscript{28-31} Isoflurane and halothane also affect left ventricular function during diastole, producing dose-related prolongation of isovolumic relaxation\textsuperscript{32-34} and decreases in rapid ventricular filling.\textsuperscript{35} In addition, halothane may contribute to decreases in ventricular chamber compliance, although this action remains controversial.\textsuperscript{32,55-57} Amrinone improves volatile anesthetic-induced depression of systolic myocardial dysfunction, but the effects of amrinone on diastolic dysfunction produced by potent inhalational agents are uncharacterized.

The current investigation examined and compared the effects of multiple doses of amrinone, a clinically used PDE III inhibitor, on left ventricular systolic and diastolic function in the conscious and anesthetized chronically instrumented dog. Myocardial contractility was evaluated using the preload recruitable stroke work (PRSW) relationship, an easily quantified and relatively heart rate- and load-insensitive index of contractile state in canine myocardium in vivo.\textsuperscript{19,35,58} The PRSW relationship was determined from a series of left ventricular pressure-segment length loops generated by abrupt preload reduction. Ventricular function during various phases of diastole was determined using several indices: a time constant of isovolumic relaxation (\(\tau\)); the maximum segment lengthening velocity during rapid ventricular filling (\(dL/dt_{\text{max}}\)); and a regional chamber stiffness constant (\(K_p\)). Experiments were conducted in the presence of pharmacologic blockade of the autonomic nervous system, to avoid amrinone- and volatile anesthetic-induced alterations in systemic hemodynamics mediated \textit{via} intact autonomic nervous system function. Therefore, effects of amrinone on left ventricular systolic and diastolic function in conscious and anesthetized dogs were examined independent of autonomic nervous system reflexes.

\textbf{Materials and Methods}

All experimental procedures and protocols used in this investigation were reviewed and approved by the Animal Care Committee of the Medical College of Wisconsin. Furthermore, all conformed to the \textit{Guiding Principles in the Care and Use of Animals of the American Physiologic Society}, and were in accordance with the \textit{Guide for the Care and Use of Laboratory Animals}.\textsuperscript{§}

\textbf{General Preparation}

Surgical implantation of instruments has been previously described in detail.\textsuperscript{19,32,35} Under general anesthesia and aseptic conditions, a left thoracotomy was performed and catheters were placed in the descending thoracic aorta and the right atrium for measurement of arterial pressure and drug administration, respectively. An ultrasonic flow probe (Transonic, Ithaca, NY) was positioned around the ascending thoracic aorta for measurement of cardiac output. A pair of ultrasonic segment length transducers (5 MHz) for measurement of changes in regional contractile function (percent segment shortening; \%SS) were implanted within the subendocardium of the anterior wall of the left ventricle. A high-fidelity micromanometer (P7; Konigsberg Instruments, Pasadena, CA) was positioned in the left ventricular apex for measurement of continuous left ventricular pressure and the maximum rate of increase of left ventricular pressure (\(dP/dt_{\text{max}}\)). A catheter was inserted in the left atrial appendage, and the left ventricular micromanometer was cross calibrated \textit{in vivo} against pressures measured \textit{via} arterial and left atrial catheters (Gould P23, pressure transducer, Oxnard, CA). A single-port 16-G catheter was placed in the apex of the thoracic cavity between the left lung and chest wall through the thoracotomy incision for subsequent measurement of continuous intrathoracic pressure. A 1.5–2-cm segment of the proximal left anterior descending coronary artery was isolated, and a precalibrated Doppler ultrasonic flow transducer was placed around this vessel for determination of diastolic coronary blood flow velocity. A hydraulic vascular occluder (In Vivo Metric, Healdsburg, CA) was placed around the inferior vena cava for abrupt alteration of left ventricular preload. All instrumentation was secured, tunneled between the scapulae, and exteriorized \textit{via} several small incisions. The pericardium was left widely open, the chest wall closed in layers, and the pneumothorax evacuated by a chest tube. Each dog was fitted with a

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jacket (Alice King Chatham, Los Angeles, CA) to prevent damage to the instruments and catheters, which were housed in an aluminum box within the jacket pocket.

After surgery, each dog (N = 9) was treated with analgesics as needed (buprenorphine, 0.02 mg/kg). Antibiotic prophylaxis consisted of cephalothin (40 mg/kg) and gentamicin (4.5 mg/kg). Dogs were allowed to recover for a minimum of 7 days before experimentation and were trained to stand quietly in a sling during monitoring of hemodynamics. Segment length and coronary blood flow velocity signals were driven and monitored by ultrasonic amplifiers (Crystal Biotech, Hopkinton, MA). End-systolic segment length (ESL) was determined at maximum negative left ventricular dP/dt, and end-diastolic segment length (EDL) was determined just before the onset of left ventricular isovolumic contraction. The lengths were normalized according to the method described by Theroux et al. Percent segment shortening (%SS) was calculated by use of the equation: %SS = (EDL − ESL) · 100 · EDL⁻¹. Relative diastolic coronary vascular resistance was calculated as the quotient of diastolic arterial pressure and diastolic coronary blood flow velocity (Hz × 10²). An estimate of myocardial oxygen consumption, the pressure-work index, was determined using a formula developed by Rooke and Feigl. All hemodynamic data were continuously recorded on a polygraph (model 7758A; Hewlett Packard, San Francisco, CA) and digitized via a computer interfaced with an analog-to-digital converter. Ventricular pressure and segment length data were also transmitted to a digital storage oscilloscope (model 4094; Nicolet, Madison, WI) for recording of left ventricular pressure-segment length waveforms and loops.

Experimental Protocol

All dogs (weighing 26.0 ± 0.8 kg, mean ± SEM) were assigned to receive amrinone in the conscious state or during isoflurane or halothane anesthesia in a random fashion on separate days. Dogs were fasted overnight, and fluid deficits were replaced before experimentation with crystalloid (500 ml 0.9% normal saline). Maintenance fluids were continued at 3 ml · kg⁻¹ · h⁻¹ for the duration of each experiment. After instrumentation was calibrated and baseline hemodynamic data were recorded, the autonomic nervous system was pharmacologically blocked with intravenous propranolol (2 mg/kg), atropine methyl nitrate (3 mg/kg), and hexamethonium (20 mg/kg). Adequacy of autonomic blockade was demonstrated by lack of reflex changes in heart rate during an abrupt decrease in venous return via inflation of the inferior vena caval hydraulic occluder before and after completion of each experiment.

Continuous left ventricular pressure, intrathoracic pressure, and segment length waveforms were recorded on the digital oscilloscope for later off-line analysis of diastolic function. Left ventricular preload was altered to generate a series of left ventricular pressure-segment length loops used to evaluate myocardial contractility in the conscious and anesthetized states. The inferior vena cava was abruptly occluded to reduce left ventricular systolic pressure approximately 30 mmHg over 10–20 cardiac cycles. Respiratory variation in ventricular pressure in the conscious state was later eliminated off-line by electronic subtraction of the continuous intrathoracic pressure waveform from the left ventricular pressure waveform using the digital oscilloscope, as previously described. The resultant left ventricular pressure-segment length loops were used to evaluate myocardial contractility in the conscious state. No changes in heart rate were observed in response to occlusion of the inferior vena cava in any experiment. The occlusion of the inferior vena cava was released immediately after the left ventricular pressure-segment length loops were recorded.

In one group of experiments, amrinone was administered during the conscious state after hemodynamics and left ventricular pressure-segment length loops had been recorded. Continuous intravenous infusions of amrinone at 10, 20, 40, or 80 μg · kg⁻¹ · min⁻¹ were administered in a random fashion immediately after an intravenous bolus of 1 mg/kg amrinone on the same experimental day. Hemodynamics were recorded and ventricular pressure-segment length waveforms and loops were obtained, using the techniques described above, after 15 min of equilibration at each dose of amrinone. The infusion rate of amrinone was then changed, and measurements were repeated after a similar period of equilibration.

In two other groups of experiments, amrinone was administered after each dog had been anesthetized with isoflurane or halothane. After inhalation induction and tracheal intubation, anesthesia was maintained with 1.25 MAC (end-tidal concentration) isoflurane or halothane in a nitrogen (79%) and oxygen (21%) mixture. 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

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spectrometer was calibrated using known standards before and during experimentation. Systemic hemodynamics were recorded and ventricular pressure-segment length waveforms and loops were generated and stored on the digital oscilloscope after 30 min of equilibration in the anesthetized state. Intravenous infusions of amrinone at 10, 20, 40, or 80 \( \mu \)g \( \cdot \) kg\(^{-1}\) \( \cdot \) min\(^{-1}\) were administered in a random fashion after an intravenous bolus of 1 mg/kg amrinone. Hemodynamics were recorded and ventricular pressure-segment length loops were obtained at each dose of amrinone, as described above. Arterial blood gases were maintained at conscious levels by adjustment of nitrogen and oxygen concentrations and respiratory rate throughout the experiment. Anesthesia was discontinued and emergence allowed to occur at the completion of each experiment. Each dog was allowed to recover from anesthesia and autonomic nervous system blockade for 3 days before subsequent experimentation. A total of 27 experiments in 3 separate groups (amrinone administered in the conscious state and during isoflurane or halothane anesthesia) were completed, in which the same 9 dogs were used.

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

Myocardial contractility was evaluated using the slope (M\(_{\infty}\)) of the PRSW relationship, as previously described.\(^{35,38}\) Briefly, a series of ventricular pressure-segment length loops were obtained by transient occlusion of the inferior vena cava in the conscious or anesthetized states and during each dose of amrinone. The area of each loop, corresponding to segmental stroke work (SW), was plotted against the corresponding EDL for each loop, and a linear regression analysis was used to describe the PRSW relationship slope (M\(_{\infty}\)) and length intercept (L\(_{\infty}\)). The time constant of isovolumic relaxation (\(r\)) was determined assuming a nonzero asymptote of left ventricular pressure decay, using the method of Raff and Glantz.\(^{41}\) Left ventricular negative dP/dt was plotted against the corresponding left ventricular pressure in 2-ms intervals between peak negative dP/dt and 5 mmHg above end-diastolic pressure. The time constant was calculated as the negative inverse of the slope of the negative dP/dt—left ventricular pressure relationship.\(^{41}\) The maximum segment lengthening velocity during rapid ventricular filling (dL/dt\(_{\text{max}}\)) was determined by differentiation of the continuous segment length waveform, as previously characterized.\(^{35}\) The regional chamber stiffness constant (K\(_{\infty}\)) was derived from ventricular pressure-segment length data between minimum ventricular pressure and the onset of atrial systole using a simple monoeponential relationship assuming an elastic model.\(^{32}\)

**Statistical Analysis**

Statistical analysis of data within and between groups in the conscious state with and without autonomic nervous system blockade, and during anesthetic interventions or amrinone infusions, was performed by multiple analysis of variance (MANOVA) with repeated measures, followed by application of the Student's t test with Bonferroni's correction. Changes within and between groups were considered statistically significant when the P value was < 0.05. The relationships between \(-dP/dt\) and ventricular pressure used to calculate \(r\), and between SW and EDL used to calculate M\(_{\infty}\) and L\(_{\infty}\), were described by use of linear regression analysis. Least-squares regression analysis was used to characterize the exponential relationship between ventricular pressure and segment length (calculation of K\(_{\infty}\)). All data were expressed as mean ± SEM.

**Results**

Autonomic nervous system blockade produced significant (P < 0.05) increases in heart rate and decreases in mean arterial pressure, left ventricular systolic pressure, systemic vascular resistance, stroke volume, and diastolic coronary vascular resistance. 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 between groups.

Administration of amrinone in the conscious state produced a significant increase in heart rate (table 1). Increases in cardiac output, pressure work index, rate pressure product, and stroke volume, and decreases in left ventricular end diastolic pressure, were also observed. No changes in mean arterial pressure, left ventricular systolic pressure, systemic vascular resistance, and diastolic coronary blood flow velocity occurred. Administration of amrinone to conscious dogs produced a dose-dependent increase in M\(_{\infty}\) (65 ± 8 during control to 108 ± 10 mmHg during 80 \( \mu \)g \( \cdot \) kg\(^{-1}\) \( \cdot \) min\(^{-1}\) amrinone), indicating a direct increase in myocardial contractility (fig. 1). Concomitant and dose-related in-
Table 1. Hemodynamic Effects of Amrinone in Conscious Dogs

<table>
<thead>
<tr>
<th>n</th>
<th>Conscious Control</th>
<th>ANS Blockade</th>
<th>Amrinone Infusion (μg·kg⁻¹·min⁻¹)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>10</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td>9</td>
<td>83 ± 5*</td>
<td>121 ± 4</td>
</tr>
<tr>
<td>MBP (mmHg)</td>
<td>9</td>
<td>99 ± 4*</td>
<td>74 ± 5</td>
</tr>
<tr>
<td>RPP (mmHg·beats·min⁻¹·10⁶)</td>
<td>9</td>
<td>10.1 ± 0.6</td>
<td>11.0 ± 1.0</td>
</tr>
<tr>
<td>LVSP (mmHg)</td>
<td>9</td>
<td>122 ± 4*</td>
<td>92 ± 5</td>
</tr>
<tr>
<td>LVEDP (mmHg)</td>
<td>9</td>
<td>9 ± 2</td>
<td>7 ± 1</td>
</tr>
<tr>
<td>DCBF (Hz·10⁶)</td>
<td>8</td>
<td>46 ± 3</td>
<td>53 ± 4</td>
</tr>
<tr>
<td>DCVR (μl)</td>
<td>8</td>
<td>2.02 ± 0.19*</td>
<td>1.28 ± 0.10</td>
</tr>
<tr>
<td>dP/dtmax (mmHg/s)</td>
<td>8</td>
<td>2.39 ± 131*</td>
<td>1.707 ± 56</td>
</tr>
<tr>
<td>EDL (mm)</td>
<td>8</td>
<td>17.0 ± 0.7</td>
<td>16.4 ± 0.8</td>
</tr>
<tr>
<td>ESL (mm)</td>
<td>9</td>
<td>14.3 ± 0.7</td>
<td>14.1 ± 0.8</td>
</tr>
<tr>
<td>SS (%)</td>
<td>9</td>
<td>15.7 ± 1.4*</td>
<td>14.5 ± 1.1</td>
</tr>
<tr>
<td>CO (l/min)</td>
<td>8</td>
<td>2.5 ± 0.2</td>
<td>2.8 ± 0.2</td>
</tr>
<tr>
<td>SVR (dynes·s·cm⁻⁵)</td>
<td>8</td>
<td>3,440 ± 250*</td>
<td>2,220 ± 160</td>
</tr>
<tr>
<td>SV (ml)</td>
<td>8</td>
<td>30 ± 2*</td>
<td>24 ± 2</td>
</tr>
<tr>
<td>PWI (ml·min⁻¹·100 g⁻¹)</td>
<td>8</td>
<td>9.1 ± 0.6</td>
<td>9.1 ± 0.7</td>
</tr>
</tbody>
</table>

Data are mean ± SEM. ANS = autonomic nervous system; 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; dP/dtmax = maximum rate of increase of left ventricular pressure; EDL and ESL = end-diastolic and end-systolic segment length, respectively; SS = segment shortening; CO = cardiac output; SVR = systemic vascular resistance; SV = stroke volume; PWI = pressure work index.

* Significantly (P < 0.05) different from ANS blockade.
† Significantly (P < 0.05) different from 10 μg·kg⁻¹·min⁻¹ amrinone.
‡ Significantly (P < 0.05) different from 20 μg·kg⁻¹·min⁻¹ amrinone.

Increases in peak positive left ventricular dP/dt (1,706 ± 56 during control to 2,127 ± 78 mmHg/s during 80 μg·kg⁻¹·min⁻¹ amrinone) and %SS were also observed (table 1). Dose-related decreases in the time constant of isovolumic relaxation (τ) were produced during administration of amrinone to conscious dogs (32.7 ± 2.1 during control to 24.8 ± 0.9 during 80 μg·kg⁻¹·min⁻¹ amrinone) consistent with shortened ventricular relaxation (fig. 2). Concomitant increases in segment lengthening velocity (dL/dtmax; 34.8 ± 1.2 during control to 45.1 ± 2.3 mm/s during 80 μg·kg⁻¹·min⁻¹ amrinone) occurred, indicating enhanced rapid ventricular filling (fig. 3). Regional chamber stiffness (Kp) also decreased significantly when amrinone infusions were administered at the three highest doses, indicating that amrinone caused improvement in regional chamber distensibility (fig. 4).

Isoflurane anesthesia (1.25 MAC) produced significant decreases in heart rate, mean arterial pressure, left ventricular systolic pressure, cardiac output, stroke volume, rate pressure product, pressure work index, and diastolic coronary vascular resistance (table 2). No changes in left ventricular end-diastolic pressure, diastolic coronary blood flow velocity, or systemic vascular resistance were observed during administration of isoflurane to autonomically blocked dogs. In the presence of isoflurane, amrinone produced a dose-related increase in cardiac output and concomitant declines in systemic vascular resistance. Heart rate was increased by amrinone only at the 80-μg·kg⁻¹·min⁻¹ infusing rate. No changes in mean arterial pressure or left ventricular systolic pressure were produced by amrinone during isoflurane anesthesia. Estimated myocardial oxygen consumption, as calculated by the rate pressure product and the pressure work index, increased during administration of amrinone to isoflurane anesthetized dogs (table 2).

Isoflurane caused decreases in Maw, dP/dtmax, and %SS with a direct negative inotropic effect (table 2; fig. 1). Increases in τ (30.8 ± 1.4 during control to 42.9 ± 2.7 ms at 1.25 MAC) and Kp (0.49 ± 0.08 during control to 0.61 ± 0.14 mm⁻¹ during 1.25 MAC), and a decrease in dL/dtmax (33.2 ± 2.5 during control to 22.7 ± 2.5 mm/s during 1.25 MAC), were also observed, consistent with impairment of diastolic function. Thus, isoflurane caused depression of contractile state, as well as negative lusitropic effects assessed in multiple phases of diastole. The PRSW slope (Maw) was increased by amrinone in a dose-dependent fashion (40...
Table 2. Hemodynamic Effects of Amrinone in Isoflurane-anesthetized Dogs

<table>
<thead>
<tr>
<th></th>
<th>HR (beats/min)</th>
<th>MBBP (mmHg)</th>
<th>RPP (mmHg-beats·min⁻¹·10⁻⁸)</th>
<th>LVSP (mmHg)</th>
<th>LVEDP (mmHg)</th>
<th>DCOBFV (Hz·10⁻¹)</th>
<th>DCFR (µL·s⁻¹)</th>
<th>dP/dtmax (mmHg/s)</th>
<th>EDL (mm)</th>
<th>ESL (mm)</th>
<th>SS (%)</th>
<th>CO (l/mm²)</th>
<th>SVR (dynes·s·cm⁻¹)</th>
<th>SV (ml)</th>
<th>PWI (ml·min⁻¹·100 g⁻¹)</th>
<th>ET (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>10</td>
<td>20</td>
<td>40</td>
<td>80</td>
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<td></td>
</tr>
<tr>
<td>Control</td>
<td>9</td>
<td>78 ± 4*</td>
<td>126 ± 5</td>
<td>106 ± 4*</td>
<td>111 ± 4*</td>
<td>113 ± 3*</td>
<td>114 ± 3*</td>
<td>116 ± 3*</td>
<td>89 ± 4*</td>
<td>95 ± 4*</td>
<td>77 ± 3*</td>
<td>9 ± 1</td>
<td>50 ± 5</td>
<td>5 ± 1</td>
<td>5 ± 1</td>
<td>6 ± 1</td>
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<tr>
<td>Blockade</td>
<td>9</td>
<td>78 ± 4*</td>
<td>126 ± 5</td>
<td>106 ± 4*</td>
<td>111 ± 4*</td>
<td>113 ± 3*</td>
<td>114 ± 3*</td>
<td>116 ± 3*</td>
<td>89 ± 4*</td>
<td>95 ± 4*</td>
<td>77 ± 3*</td>
<td>9 ± 1</td>
<td>50 ± 5</td>
<td>5 ± 1</td>
<td>5 ± 1</td>
<td>6 ± 1</td>
</tr>
</tbody>
</table>

Data are mean ± SEM.

ANS = autonomic nervous system; HR = heart rate; MBBP = mean aortic blood pressure; RPP = rate pressure product; LVSP and LVEDP = left ventricular systolic and end-diastolic pressure, respectively; DCOBFV = diastolic coronary blood flow velocity; DCFR = diastolic coronary vascular resistance; dP/dtmax = maximum rate of increase of left ventricular pressure; EDL and ESL = end-diastolic and end-systolic segment length, respectively; SS = segment shortening; CO = cardiac output; SVR = systemic vascular resistance; SV = stroke volume; PWI = pressure work index; ET = end-tidal anesthetic concentration.

* Significantly (P < 0.05) different from ANS blockade.
† Significantly (P < 0.05) different from isoflurane.
‡ Significantly (P < 0.05) different from 10 µg·kg⁻¹·min⁻¹ amrinone.
§ Significantly (P < 0.05) different from 20 µg·kg⁻¹·min⁻¹ amrinone.

± 5 during isoflurane alone to 71 ± 5 mmHg during 80 µg·kg⁻¹·min⁻¹) in the presence of isoflurane (fig. 1). Concomitant changes in dP/dtmax and %SS were also observed (table 2). Amrinone also improved the alterations in left ventricular diastolic function produced by isoflurane. Amrinone decreased τ in a dose-related fashion (42.9 ± 2.7 during isoflurane alone to 33.8 ± 2.2 ms during 80 µg·kg⁻¹·min⁻¹), indicating an enhancement of isovolumic relaxation (fig. 2). Similarly, improvement in rapid ventricular filling, as indicated by dL/dtmax (22.7 ± 2.5 during isoflurane alone to 28.9 ± 2.9 mm/s during 80 µg·kg⁻¹·min⁻¹), was also observed (fig. 3). Regional chamber stiffness (Kp) was decreased toward preanesthetic control levels by amrinone during isoflurane anesthesia (fig. 4), indicating that a possible improvement in regional wall compliance had occurred.

In the presence of autonomic nervous system blockade, halothane produced systemic and coronary hemodynamics that were similar to those produced by isoflurane. In contrast to isoflurane, however, halothane caused a significant increase in systemic vascular resistance and left ventricular end-diastolic pressure (table 3). Halothane also caused a significant and dose-dependent depression of left ventricular systolic (decreases in Maw, dP/dtmax, and %SS) and diastolic function (increases in τ and Kp) and decreases in dL/dtmax. Although halothane caused significantly greater negative inotropic actions than did isoflurane at 1.25 MAC as evaluated by Maw when these agents were administered alone and during amrinone infusions (fig. 1), no significant differences between isoflurane and halothane were noted when parameters describing diastolic function were compared. Administration of amrinone to dogs anesthetized with halothane produced changes in systemic hemodynamics that were similar to those produced during isoflurane anesthesia. In the presence of halothane, however, amrinone caused significant decreases in left ventricular end-diastolic pressure that were not dose dependent, and no changes in diastolic

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Table 3. Hemodynamic Effects of Amrinone in Halothane-anesthetized Dogs

<table>
<thead>
<tr>
<th>n</th>
<th>Conscious</th>
<th>Control</th>
<th>ANS</th>
<th>Blockade</th>
<th>Halothane (1.25 MAC)</th>
<th>Amrinone Infusion (µg·kg⁻¹·min⁻¹)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
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<td></td>
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<td></td>
<td>10</td>
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<tr>
<td>HR (beats/min)</td>
<td>9</td>
<td>84 ± 4*</td>
<td>121 ± 5</td>
<td>100 ± 4*</td>
<td>106 ± 4*</td>
<td>108 ± 4*</td>
</tr>
<tr>
<td>MBP (mmHg)</td>
<td>9</td>
<td>94 ± 3*</td>
<td>70 ± 5</td>
<td>59 ± 5*</td>
<td>59 ± 6*</td>
<td>59 ± 6*</td>
</tr>
<tr>
<td>RPP (mmHg·beats· min⁻¹·10⁵)</td>
<td>9</td>
<td>10.1 ± 0.3</td>
<td>10.4 ± 0.7</td>
<td>7.2 ± 0.5†</td>
<td>7.5 ± 0.7†</td>
<td>7.7 ± 0.7†</td>
</tr>
<tr>
<td>LVSP (mmHg)</td>
<td>9</td>
<td>125 ± 4*</td>
<td>92 ± 4</td>
<td>77 ± 3*</td>
<td>78 ± 5*</td>
<td>78 ± 5*</td>
</tr>
<tr>
<td>LVEDP (mmHg)</td>
<td>9</td>
<td>9 ± 1</td>
<td>8 ± 1</td>
<td>11 ± 1*</td>
<td>8 ± 1†</td>
<td>8 ± 1†</td>
</tr>
<tr>
<td>DCBFV (Hz· 10⁵)</td>
<td>8</td>
<td>42 ± 6</td>
<td>41 ± 6</td>
<td>35 ± 4*</td>
<td>35 ± 4*</td>
<td>35 ± 4*</td>
</tr>
<tr>
<td>DCVR (µl)</td>
<td>8</td>
<td>2.15 ± 0.29*</td>
<td>1.67 ± 0.22</td>
<td>1.66 ± 0.24</td>
<td>1.62 ± 0.18</td>
<td>1.59 ± 0.17</td>
</tr>
<tr>
<td>dP/dtmax (mmHg/s)</td>
<td>9</td>
<td>2,368 ± 143*</td>
<td>1,589 ± 120</td>
<td>896 ± 47*</td>
<td>986 ± 40*</td>
<td>1,047 ± 46*</td>
</tr>
<tr>
<td>EDL (mm)</td>
<td>9</td>
<td>17.4 ± 0.8</td>
<td>17.2 ± 0.7</td>
<td>17.1 ± 0.8</td>
<td>16.5 ± 0.9</td>
<td>16.4 ± 1.0</td>
</tr>
<tr>
<td>ESL (mm)</td>
<td>9</td>
<td>14.8 ± 0.7</td>
<td>14.8 ± 0.6</td>
<td>15.8 ± 0.7*</td>
<td>14.8 ± 0.8†</td>
<td>14.8 ± 0.8†</td>
</tr>
<tr>
<td>SS (%)</td>
<td>9</td>
<td>14.9 ± 1.0</td>
<td>13.9 ± 1.0</td>
<td>7.3 ± 1.1*</td>
<td>9.9 ± 1.2†</td>
<td>10.0 ± 1.4†</td>
</tr>
<tr>
<td>CO (l/min)</td>
<td>8</td>
<td>2.6 ± 0.2*</td>
<td>3.0 ± 0.3</td>
<td>1.8 ± 0.1*</td>
<td>2.0 ± 0.2*</td>
<td>2.1 ± 0.2†</td>
</tr>
<tr>
<td>SVR (dynes·s·cm⁻⁵)</td>
<td>8</td>
<td>3,050 ± 280*</td>
<td>1,920 ± 130</td>
<td>2,830 ± 340*</td>
<td>2,600 ± 420*</td>
<td>2,530 ± 410*</td>
</tr>
<tr>
<td>SV (ml)</td>
<td>8</td>
<td>31 ± 2*</td>
<td>25 ± 2</td>
<td>17 ± 1*</td>
<td>19 ± 2*</td>
<td>19 ± 2*</td>
</tr>
<tr>
<td>PWI (mi·min⁻¹·100 g⁻¹)</td>
<td>9</td>
<td>9.3 ± 0.5</td>
<td>9.0 ± 0.6</td>
<td>6.0 ± 0.4‡</td>
<td>6.4 ± 0.4*</td>
<td>6.6 ± 0.5*</td>
</tr>
<tr>
<td>ET (%)</td>
<td>9</td>
<td>—</td>
<td>—</td>
<td>1.07 ± 0.03</td>
<td>1.07 ± 0.03</td>
<td>1.07 ± 0.03</td>
</tr>
</tbody>
</table>

Data are mean ± SEM. 

ANS = autonomic nervous system; HR = heart rate; MBP = mean aortic blood pressure; RPP = rate pressure product; LVSP and LVEDP = left ventricular systolic and end-diastolic pressure, respectively; DCBFVV = diastolic coronary blood flow velocity; DCVR = diastolic coronary vascular resistance; dP/dtmax = maximum rate of increase of left ventricular pressure; EDL and ESL = end-diastolic and end-systolic segment length, respectively; SS = stroke index; SV = stroke volume; PWI = pressure work index; ET = end-tidal anesthetic concentration.

* Significantly (p < 0.05) different from control.
† Significantly (p < 0.05) different from halothane.
‡ Significantly (p < 0.05) different from ANS blockade.

 Coronary vascular resistance (table 3). Amrinone produced progressive and dose-related increases in contractile state (fig. 1) and improvement of halothane-induced diastolic dysfunction (figs. 2–4). No significant differences in indices of left ventricular systolic or diastolic function were present between isoflurane- and halothane-anesthetized dogs during administration of amrinone.

Discussion

Potent inhalational anesthetics, including isoflurane and halothane, produce cardiac depression characterized by abnormal left ventricular mechanics during systole17–19 and diastole32–35 that can be attributed to alterations in intracellular Ca²⁺ homeostasis at several sites within the cardiac myocyte.26 Volatile anesthetics have been shown to reversibly depress myocardial contractility, prolong isovolumic relaxation, and decrease rapid ventricular filling in a dose-related fashion.32–35

Halothane may also decrease left ventricular chamber distensibility, although this contention remains somewhat controversial.32,34–37 Isoflurane and halothane interfere with the inward Ca²⁺ current across the sarcolemmal membrane produced by depolarization by reducing the number, or partially inhibiting the function, of voltage-dependent Ca²⁺ channels.21–25 This blunting of the Ca²⁺ influx responsible for the initiation of mechanical systole has several important consequences, including decreases in the availability of Ca²⁺ for contractile activation, depression of Ca²⁺-dependent Ca²⁺ release from the sarcoplasmic reticulum (SR), and reduction of the amount of Ca²⁺ that can be subsequently stored in the SR.20 Isoflurane and halothane also depress the peak concentration of intracellular Ca²⁺ during systole by directly affecting the SR, as well. Partial inhibition of Ca²⁺ uptake and enhanced Ca²⁺ leak from this organelle lead to decreases in accumulation of intracellular Ca²⁺ during systole, and may also contribute to delays in the removal of Ca²⁺ from the contractile apparatus during diastole.20,26–29 These actions of volatile anesthetics cause direct declines in contractile force, and may also result in concomitant delays in isovolumic relaxation and impairment of rapid ventricular filling in vivo.

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Cyclic adenosine monophosphate (cAMP) plays an important role in the regulation of Ca\(^{2+}\) transients in the cardiac myocyte. Increases in intracellular concentrations of this cyclic nucleotide lead to activation of protein kinases responsible for several intracellular events that enhance systolic and diastolic performance.

Amrinone inhibits phosphodiesterase fraction III, the enzyme responsible for degradation of cAMP in the myocyte, increasing the concentration of cAMP. Amrinone-induced increases in Ca\(^{2+}\) influx resulting from cAMP-induced phosphorylation of the voltage-dependent Ca\(^{2+}\) channel represents a major mechanism by which amrinone exerts positive inotropic and chronotropic effects. Amrinone also augments function of the Ca\(^{2+}\)-ATPase in the SR via protein kinase-mo-

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Fig. 3. Maximum segment lengthening velocity during rapid ventricular filling (dL/dtmax) in conscious (top panel) and isoflurane- (iso; middle panel) or halothane-anesthetized (hal; bottom panel) dogs in the presence of pharmacologic blockade of the autonomic nervous system (ANS block). *Significantly \( P < 0.05 \) different from ANS block; **significantly \( P < 0.05 \) different from 1.25 MAC isoflurane or halothane; ***significantly \( P < 0.05 \) different from 10 \( \mu \text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1} \) amrinone.

Fig. 4. Regional chamber stiffness \( (K_r) \) in conscious (top panel) and isoflurane- (iso; middle panel) or halothane-anesthetized (bottom panel) dogs in the presence of pharmacologic blockade of the autonomic nervous system (ANS block). *Significantly \( P < 0.05 \) different from ANS block; **significantly \( P < 0.05 \) different from 1.25 MAC isoflurane or halothane.

diated phosphorylation of the regulatory protein phospholamban, causing increases in \( \text{Ca}^{2+} \) storage ability of this organelle and promoting greater concentrations of \( \text{Ca}^{2+} \) to be released during the next systole, while simultaneously enhancing the rate of uptake of \( \text{Ca}^{2+} \) from the sarcoplasm during diastole.\(^{42,43}\) In addition, amrinone-induced cAMP-stimulated phosphorylation of the troponin I subunit of the troponin-tropomyosin complex decreases the affinity of troponin C for \( \text{Ca}^{2+} \), enhancing dissociation of \( \text{Ca}^{2+} \) from this regulatory protein during diastole.\(^{44}\) Amrinone may also alter \( \text{Ca}^{2+} \) regulation by affecting Na⁺-Ca²⁺ exchange through the sarcolemmal membrane, a mechanism that is independent of phosphodiesterase inhibition.\(^1\)

Amrinone increases myocardial contractility in a variety of clinical and experimental settings of depressed contractile performance, including cardiac depression produced by volatile anesthetics.\(^{11-15}\) Presumably, this occurs through increasing intracellular \( \text{Ca}^{2+} \) availability during contraction by overcoming anesthetic-induced decreases in the concentration of activator \( \text{Ca}^{2+} \) resulting from depressed \( \text{Ca}^{2+} \) channel and SR function. Komai and Ruÿ\(^1\) demonstrated that amrinone could partially reverse the negative inotropic effects of halo-
thane in isolated rabbit papillary muscle. Makela and Kapur\textsuperscript{14} demonstrated that amrinone blunted the cardiovascular depression caused by isoflurane or enflurane in acutely instrumented dogs. These investigators\textsuperscript{15} subsequently demonstrated that amrinone also improved myocardial contractility in the presence of β-adrenergic and Ca\textsuperscript{2+} channel blockade during isoflurane anesthesia. Rooney \textit{et al.}\textsuperscript{12} also showed that amrinone reversed isoflurane-induced depression of contractile state and augmented coronary vasodilation produced by isoflurane when administered to isolated hearts. These effects occurred with concomitant increases in myocardial oxygen consumption. Although such investigations indicate that amrinone produces positive inotropic effects in the presence of volatile anesthetics, the results of these studies require qualification, because the indices of myocardial contractility used \textit{e.g.}, cardiac index and left ventricular peak positive dP/dt only indirectly indicate alteration in contractile state or are significantly dependent on ventricular loading conditions,\textsuperscript{15,46} which are known to occur because of amrinone-induced changes in systemic and pulmonary hemodynamics.\textsuperscript{7,8}

The results of the current investigation confirm and extend the findings of previous studies \textit{in vitro}\textsuperscript{11,12} and \textit{in vivo},\textsuperscript{13–15} Amrinone produced a dose-dependent increase in myocardial contractility measured by a relatively heart rate- and load-independent index, the PRSW slope (M\textsubscript{sw}), in conscious (67% increase from control at 80 μg·kg\textsuperscript{-1}·min\textsuperscript{-1}) and anesthetized dogs (78 and 89% increase from control at 80 μg·kg\textsuperscript{-1}·min\textsuperscript{-1} during 1.25 MAC isoflurane and halothane anesthesia, respectively). Elevation of the pressure work index also occurred, indicating that the augmentation of contractile state produced by amrinone in the conscious and anesthetized states was accompanied by modest but significant increases in myocardial oxygen consumption. This coupling of enhanced contractile state and myocardial oxygen consumption during amrinone administration has been documented previously by Rooney \textit{et al.}\textsuperscript{12} and other investigators.\textsuperscript{47,48}

Phosphodiesterase III inhibitors have also been shown to enhance indices of left ventricular diastolic performance in severe congestive heart failure.\textsuperscript{9,10} This clinical syndrome is associated with markedly abnormal intracellular Ca\textsuperscript{2+} homeostasis, which may represent a final common pathway in chronic myocardial ischemia and ventricular hypertrophy.\textsuperscript{49,50} Monrad \textit{et al.}\textsuperscript{9} and Piscione \textit{et al.}\textsuperscript{10} demonstrated improvement in isovolumic relaxation, peak ventricular filling rate, and left ventricular diastolic pressure-volume relations when milrinone was administered intravenously or orally to patients with advanced congestive heart failure, respectively. In contrast, Herrmann \textit{et al.}\textsuperscript{51} and Kraus \textit{et al.}\textsuperscript{52} attributed improvement in measures of diastolic function associated with enoximone or milrinone to the vasodilator actions, but not the positive inotropic effects, of these agents. The conflicting nature of these findings may be attributed, at least in part, to alterations in systemic and pulmonary hemodynamics\textsuperscript{7,8} produced either directly by phosphodiesterase inhibition or indirectly \textit{via} intact autonomic nervous system reflexes. The mechanisms responsible for volatile anesthetic-induced negative lusitropic effects have yet to be completely described, but may involve acute alteration of similar subcellular targets, as are chronically affected in congestive heart failure. Although amrinone reverses isoflurane-induced depression of global myocardial contractility and partially improves halothane-induced negative inotropic actions, the effects of amrinone on diastolic dysfunction caused by volatile anesthetics have yet to be described. The results of this investigation indicate that intravenous administration of amrinone causes equivalent improvement of indices of diastolic function in conscious and anesthetized chronically instrumented dogs with pharmacologic blockade of the autonomic nervous system. Improvement of diastolic function is manifested by enhancement of isovolumic relaxation (decreases in the time constant, \textit{r}), rapid ventricular filling (increases in \textit{dL/dt\textsubscript{max}}), and regional chamber stiffness (decreases in \textit{K\textsubscript{p}}) concomitant with increased myocardial contractility. Amrinone-induced increases in the reuptake of Ca\textsuperscript{2+} into the sarcoplasmic reticulum (SR), and enhanced dissociation of Ca\textsuperscript{2+} from the contractile apparatus during diastole \textit{via} increased CAMP concentrations, may explain the positive lusitropic effects of amrinone in the conscious and anesthetized states.

The results of this investigation must be interpreted within the constraints of several limitations. Amrinone causes changes in systemic hemodynamics in autonomically blocked dogs, which may have influenced the current interpretation of alterations in measured indices of left ventricular diastolic performance. Amrinone produced an increase in heart rate and a decrease in preload (as indicated by left ventricular end-diastolic pressure and end-diastolic segment length) which was not dose related. Positive chronotropic actions and venodilatory actions of amrinone have been previously described,\textsuperscript{1,2,6,12} although tachycardia usually occurs.
in response to peripheral vasodilation. The time constant of isovolumic relaxation (τ) is dependent on heart rate, and may be dependent on preload,\textsuperscript{53,54} and amrinone-induced changes in these variables may have contributed to enhanced isovolumic relaxation. Amrinone caused decreases in end-systolic segment length (ESL) in the presence of isoflurane or halothane that was not dose-related. Decreased affinity of the contractile apparatus for Ca\textsuperscript{2+} at shorter muscle lengths has been previously demonstrated,\textsuperscript{55-57} and it is possible that enhanced myocardial contractility or declines in afterload (as indirectly indicated by systemic vascular resistance) produced by amrinone in anesthetized dogs also resulted in simultaneous decreases in ESL (consistent with changes in cellular myofibrillar length). These observations indicate decreased myofibrillar affinity for, and enhanced release of, Ca\textsuperscript{2+} during this period of increased contractile state or decreased impedance to left ventricular outflow, leading to shortened isovolumic relaxation and augmented early ventricular filling.\textsuperscript{58} However, no changes in ESL were observed in the conscious state, and decreases in ESL observed in anesthetized dogs were not dose related, indicating that the enhancement in isovolumic relaxation and early ventricular filling caused by amrinone were not purely myocyte length-dependent events associated with increased myocardial contractility. The rate of rapid ventricular filling (as evaluated by dL/dt\textsubscript{max}) is partially dependent on the gradient between left atrial and left ventricular pressure during this period of the cardiac cycle, which was not specifically measured in the current study. Alterations in ventricular loading conditions (most notably, declines in left ventricular end-diastolic pressure observed in conscious and halothane-anesthetized dogs) and increases in myocardial contractility may have also influenced passive ventricular elastic properties and subsequent interpretation of decreases in regional chamber stiffness.

The doses of amrinone used in this investigation were chosen to produce increases in left ventricular peak positive dP/dt in the conscious and anesthetized states following the methods described by Makela and Kapur.\textsuperscript{13-15} The amrinone bolus dose and infusion rates (1 mg/kg bolus followed by 10, 20, 40, or 80 μg·kg\textsuperscript{-1}·min\textsuperscript{-1} infusions) represent a "mid-range" dose in dogs. Makela and Kapur\textsuperscript{13-15} used bolus doses and infusion rates between 1 mg/kg plus 5 μg·kg\textsuperscript{-1}·min\textsuperscript{-1} and 4 mg/kg plus 100 μg·kg\textsuperscript{-1}·min\textsuperscript{-1}, resulting in plasma concentrations ranging between 0.7 ± 0.1 to 14.1 ± 0.7 μg/ml, respectively. The bolus dose and infusion rates of amrinone used in the current investigation would be expected to produce plasma concentrations bounded within the limits described by Makela and Kapur.\textsuperscript{13-15} Similar amrinone plasma concentrations also produced dose-related increases in cardiac index in patients with congestive heart failure.\textsuperscript{59} Nevertheless, plasma concentrations of amrinone were not obtained in this investigation and, therefore, direct comparison of the effects of amrinone between the chronically instrumented canine model and humans should be approached with caution.

In summary, the results of this investigation indicate that amrinone causes dose-dependent positive inotropic effects, as evaluated using M\textsubscript{ae} in both conscious and anesthetized chronically instrumented dogs with autonomic nervous system blockade. In addition, amrinone produces positive lusitropic actions in the conscious state and improves isoflurane- or halothane-induced diastolic dysfunction, as indicated by shortened isovolumic relaxation, increased early ventricular filling, and reduced regional chamber stiffness. These results were accompanied by concomitant increases in myocardial oxygen consumption, as indicated by the pressure work index. The findings are consistent with amrinone-induced increases in cAMP that lead to enhanced Ca\textsuperscript{2+} availability during systole, and simultaneously improved Ca\textsuperscript{2+} sequestration during diastole. Thus, amrinone augments left ventricular performance in the conscious and anesthetized states, which may be related not only to positive inotropic actions, but also to positive lusitropic effects.

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