Anesthesiology
78:141–154, 1993

Reversal of Volatile Anesthetic-induced Depression of Myocardial Contractility by Extracellular Calcium Also Enhances Left Ventricular Diastolic Function

Paul S. Pagel, M.D., John P. Kampine, M.D., Ph.D.,† William T. Schmeling, M.D., Ph.D.,‡ David C. Warltier, M.D., Ph.D.§*

Background: **Volatile anesthetics depress global left ventricular function by altering intracellular calcium (Ca^{2+}) homeostasis at several sites within the myocyte. Although extracellular Ca^{2+} partially reverses the negative inotropic effects of volatile anesthetics, the actions of extracellular Ca^{2+} on anesthetic-induced diastolic dysfunction are unexplored. This investigation examined and compared the direct effects of extracellular Ca^{2+} 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 nervous activity may significantly influence the hemodynamic actions of anesthetics and Ca^{2+} *in vivo*. Three groups comprised a total of 27 experiments conducted using nine dogs chronically instrumented for measurement of aortic and left ventricular pressure, left ventricular dP/dt, subendocardial segment length, and cardiac output. Myocardial contractility was evaluated using the preload recruitable stroke work relationship slope (M_w). Diastolic function was assessed using a time constant of isovolumic relaxation (τ), a regional chamber stiffness constant (K_p), and maximum segment lengthening velocity during rapid ventricular filling (dL/dt_E) and atrial systole (dL/dt_A). On 3 separate days, a $CaCl_2$ infusion at 1.25, 2.5, or 5 $mg \cdot kg^{-1} \cdot min^{-1}$ was administered. Hemodynamics and ventricular pressure-length loops were recorded after a 20-min equilibration at each dose in the conscious state or during halothane or isoflurane anesthesia.**

Results: **In conscious dogs, $CaCl_2$ produced a significant ($P < .05$) and dose-dependent increase in contractility as evaluated by M_w. In the presence of halothane anesthesia, $CaCl_2$ increased contractility (M_w of 26 ± 5 mmHg to 78 ± 10 mmHg during the high dose of $CaCl_2$), enhanced isovolumic relaxation (τ of 57.9 ± 4.2 ms to 41.1 ± 1.9 ms during the high dose of $CaCl_2$), improved rapid ventricular filling (dL/dt_E of 11.8 ± 1.4 mm/s to 20.2 ± 1.6 mm/s during the high dose of $CaCl_2$), and reduced regional chamber stiffness (K_p of 0.70 ± 0.18 mm^{-1} to 0.38 ± 0.04 mm^{-1} during the high dose of $CaCl_2$). Similar findings were observed when $CaCl_2$ was administered to dogs anesthetized with isoflurane.**

Conclusions: **Although $CaCl_2$ produced positive inotropic effects in both the conscious and anesthetized states, $CaCl_2$ did not alter diastolic function in conscious dogs. In contrast, $CaCl_2$ reversed halothane- and isoflurane-induced negative lusitropic actions. The results of the present investigation suggest that improvement of left ventricular performance by $CaCl_2$ during volatile anesthesia may be related to actions in diastole as well as systole. (Key words: Anesthetics, volatile: isoflurane; halothane. Heart, diastole: diastolic left ventricular function; isovolumic relaxation; ventricular compliance. Heart, myocardial performance: left ventricular function; myocardial contractility; preload recruitable stroke work. Ions: calcium.)**

POTENT inhalational anesthetics, including halothane and isoflurane, are known to significantly impair left ventricular function during systole and diastole.[1–8] On a cellular level, these negative inotropic and lusitropic (diastolic mechanical) actions can be attributed to volatile anesthetic-induced alterations in intracellular calcium homeostasis at several sites within the myocyte.[9–14] A growing body of evidence suggests that volatile anesthetics interfere with function of Ca^{2+} channels in the sarcolemmal membrane and also partially inhibit function of the sarcoplasmic reticulum as a modulator of changes in intracellular Ca^{2+} through several mechanisms.[10,13,15–22] These effects lead to diminished contractile activation during systole. Inhalational anesthetics also blunt sequestration of Ca^{2+} into

* Fellow in Anesthesiology.

† Professor and Chairman of Anesthesiology.

‡ Associate Professor of Anesthesiology and Pharmacology.

§ Professor of Anesthesiology, Pharmacology, and Medicine (Division of Cardiology); Vice Chairman for Research, Department of Anesthesiology.

Received from the Departments of Anesthesiology, Pharmacology, and Medicine, Medical College of Wisconsin, Milwaukee, Wisconsin, and the Zablocki Veterans Administration Medical Center, Milwaukee, Wisconsin. Accepted for publication August 11, 1992. Supported by US PHS grants HL 36144 and HL 32911, Anesthesiology Research Training Grant GM 08377, and VA Medical Research Funds.

Address reprint requests to Dr. Warltier: Department of Anesthesiology, Medical College of Wisconsin, MACC Fund Research Center, Room A1000, 8701 West Watertown Plank Road, Milwaukee, Wisconsin 53226.

the sarcoplasmic reticulum[11] and may interfere with sarcolemmal Ca^{2+} extrusion mechanisms during diastole. The latter alterations may result in delayed ventricular relaxation, impaired filling, or altered compliance. Although it is known that administration of extracellular Ca^{2+} can partially reverse the negative inotropic effects of volatile anesthetics *in vivo*[23,24] and *in vitro*,[25] the actions of extracellular Ca^{2+} on anesthetic-induced diastolic dysfunction remain unexplored.

The current investigation examined and compared the effects of increases in extracellular Ca^{2+} 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, a linear extension of the traditional Frank-Starling description of ventricular function.[26] This relationship has been shown to be an easily quantified and relatively load-insensitive index of inotropic state in canine myocardium *in vivo*.[3,26] Ventricular function in multiple phases of diastole was assessed by several indices: a time constant of isovolumic relaxation (τ); a regional chamber stiffness constant (K_D, a measure of passive ventricular filling); and maximal segment lengthening velocity during rapid ventricular filling (dL/dt_E) and atrial systole (dL/dt_A) obtained by differentiation of continuous segment length waveforms. Experiments were conducted in the presence of pharmacologic blockade of the autonomic nervous system because both calcium[27–29] and inhalational anesthetics[1,30] have been shown to produce significant alterations in systemic hemodynamics mediated *via* an intact autonomic nervous system. Therefore, the direct effects of increases in extracellular Ca^{2+} on left ventricular systolic and diastolic function in conscious and anesthetized dogs were examined independent of autonomic nervous system reflexes.

Materials and Methods

All experimental procedures and protocols used in this investigation were reviewed and approved by the Animal Care Committee of the authors' institution. Furthermore, all conformed to the *Guiding Principles in the Care and Use of Animals of the American Physiologic Society* and were in accordance with the *Guide for the Care and Use of Laboratory Animals* [DHEW (DHHS) publication no. (NIH) 85-23, revised 1985].

Implantation of Instruments

Surgical implantation of instruments has been described previously.[3,4] Briefly, conditioned mongrel dogs weighing between 20 and 30 kg were fasted overnight and anesthetized with sodium thiamylal (10 mg/kg). Following tracheal intubation, anesthesia was maintained with halothane (1.5–2.0%) in 100% oxygen (1 L/min) *via* positive pressure ventilation. A thoracotomy was performed under sterile conditions in the left fifth intercostal space, and heparin-filled 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 (Transonics, Ithaca, NY) was positioned around the ascending thoracic aorta for measurement of relative cardiac output (minus coronary blood flow). A pair of miniature 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, miniature micromanometer (P7, Konigsberg Instruments, Pasadena, CA) was inserted in the left ventricular apex for measurement of continuous left ventricular pressure and the maximum rate of increase of left ventricular pressure (dP/dt_{max}). A heparin-filled catheter was inserted in the left atrial appendage. The left ventricular micromanometer was cross-calibrated *in vivo* against pressures measured *via* arterial and left atrial catheters (Gould P_{50} pressure transducer, Oxnard, CA). 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 *via* several small incisions. The pericardium was left wide open, the chest wall closed in layers, and the pneumothorax evacuated by a chest tube. Each dog was fitted with a jacket (Alice King Chatham, Los Angeles, CA) to prevent damage to the instruments and catheters that were housed in an aluminum box within the jacket pocket.

After surgery, each dog was treated with analgesics as needed (buprenorphine, 0.02 mg/kg) and antibiotics [procaine penicillin G (25,000 U/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 hemodynamic monitoring. Segment length signals were driven and monitored by ultrasonic amplifiers (Hartley, Houston,

TX). End systolic segment length (ESL) was determined at maximum negative left ventricular dP/dt, and end diastolic segment length (EDL) was determined just prior to the onset of left ventricular isovolumic contraction. Percent segment shortening was calculated by use of the equation: $\%SS = (EDL - ESL)100 \cdot EDL^{-1}$. All hemodynamic data were continuously recorded on a Hewlett Packard 7758A polygraph (Hewlett Packard, San Francisco, CA) and digitized *via* a computer interfaced with an analog-to-digital converter. Ventricular pressure and segment length data also were transmitted to a digital storage oscilloscope (Nicolet 4094, Madison, WI) for recording of left ventricular pressure-segment length waveforms and loops.

Experimental Protocol

Dogs (n = 9) were assigned to receive $CaCl_2$ in the conscious state or during halothane or isoflurane anesthesia in a random fashion on alternate days. All dogs were fasted overnight, and fluid deficits were replaced before experimentation with crystalloid (500 ml lactated Ringer's solution). Maintenance fluids (lactated Ringer's solution) were continued at 3 $ml \cdot kg \cdot h^{-1}$ 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 methylnitrate (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 prior to and following completion of each experiment.

Alteration of left ventricular preload was used to generate left ventricular pressure-segment length loops in the conscious and anesthetized state. After control hemodynamics had been recorded and autonomic nervous system blockade had been completed, continuous left ventricular pressure and segment length waveforms were recorded on the digital oscilloscope for later off-line analysis of diastolic function. The inferior vena cava was then abruptly occluded to reduce left ventricular systolic pressure approximately 25–30 mmHg over 10–15 cardiac cycles. The resultant ventricular pressure-segment length loops were recorded on the digital oscilloscope. 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 ventricular pressure-segment length loops were recorded. End expiratory ventricular pressure-segment length loops were identified and used for data analysis.

In one group of experiments, $CaCl_2$ was administered to dogs in the conscious state after hemodynamics and ventricular pressure-segment length waveforms and loops had been recorded. A continuous infusion of $CaCl_2$ at 1.25, 2.5, or 5 $mg \cdot kg^{-1} \cdot min^{-1}$ (0.01125, 0.0225, or 0.045 $mM \cdot kg^{-1} \cdot min^{-1}$) was administered in a random fashion. Hemodynamics were recorded, and ventricular pressure-segment length waveforms and loops were obtained in the manner described above after 20 min of equilibration at each dose of $CaCl_2$. The dose of $CaCl_2$ was then changed, and measurements were repeated after a similar period of equilibration.

In two other groups of experiments, $CaCl_2$ was administered after each dog had been anesthetized with halothane or isoflurane. Following inhalation induction and tracheal intubation, anesthesia was maintained with 1.5 minimum alveolar concentration (MAC; end tidal concentration) halothane or isoflurane in a nitrogen (79%) and oxygen (21%) mixture. The canine 1.0-MAC values for halothane and isoflurane used in this investigation were 0.86% and 1.28%, respectively. End tidal concentrations of halothane and isoflurane were measured using a mass spectrometer (Marquette Advantage 2000, St. Louis, MO). The mass spectrometer was calibrated using known standards prior to and during experimentation. After 30 min of equilibration in the anesthetized state, systemic hemodynamics were recorded and ventricular pressure-segment length waveforms and loops were generated and stored on the digital oscilloscope. A continuous infusion of $CaCl_2$ at 1.25, 2.5, or 5.0 $mg \cdot kg^{-1} \cdot min^{-1}$ was administered in a random fashion. Hemodynamics were recorded and ventricular pressure-segment length waveforms and loops were obtained at each dose of $CaCl_2$ in the manner 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 pharmacologic blockade of the autonomic nervous system for 3 days prior to subsequent experimentation. Thus, a total of 27 experiments in three groups ($CaCl_2$ infusions administered in the conscious state and during isoflurane or halothane anes-

thesia) were completed in which the same nine dogs were used.

Determination of Indices of Systolic and Diastolic Function

Myocardial contractility was evaluated using the PRSW relationship.[3,24] A series of 10–15 ventricular pressure-segment length loops were obtained during steady-state hemodynamic conditions in the conscious or anesthetized states and during each dose of $CaCl_2$. The area of each loop, corresponding to segmental stroke work (SW), was calculated by electronic integration. The EDL of each loop was identified on the oscilloscope and converted to the appropriate units (mm) by use of a linear formula generated with voltage-segment length calibration data. The SW was then plotted against the corresponding EDL for each loop. Linear regression analysis was used to describe the PRSW relationship slope (M_w) and length intercept (L_w): $SW = M_w (EDL - L_w)$.

Offline analyses of several indices of diastolic function were completed. Isovolumic relaxation was described assuming a non-zero asymptote of ventricular pressure decline using the method of Raff and Glantz.[31] A three-constant exponential equation was used as the basis for the calculation of the time constant of isovolumic relaxation: $P = ae^{-t/\tau} + c$, where P = left ventricular pressure, c = the true asymptote to which pressure declines, a + c = ventricular pressure at peak negative dP/dt, and τ = the rate of relaxation (ms) assuming a non-zero asymptote. It can be easily shown that

$$\frac{dP}{dt} = \frac{c}{\tau} - \frac{P}{\tau}.$$

Left ventricular negative dP/dt was plotted against ventricular pressure in 2-ms intervals between peak negative dP/dt and 5 mmHg above end diastolic pressure to yield the non-zero asymptotic isovolumic relaxation time constant (τ) as the negative inverse of the slope.

Regional chamber stiffness, an indicator of ventricular compliance during passive filling, was derived from ventricular pressure-segment length data as previously described.[3] Beginning at minimum pressure, ventricular pressure was plotted against corresponding segment length at 2-ms intervals until the onset of atrial systole (incorporating both rapid and slow ventricular filling) had been reached. A least squares regression analysis was used to describe a monoexponential relationship between pressure and segment length: $P = De^{K_p \cdot L}$ where P = left ventricular pressure, L = corresponding segment length, D = a derived constant, and K_p = the regional chamber stiffness constant.

Maximum segment lengthening velocities during rapid ventricular filling (dL/dt_E) and atrial systole (dL/dt_A) were obtained by differentiation of the continuous

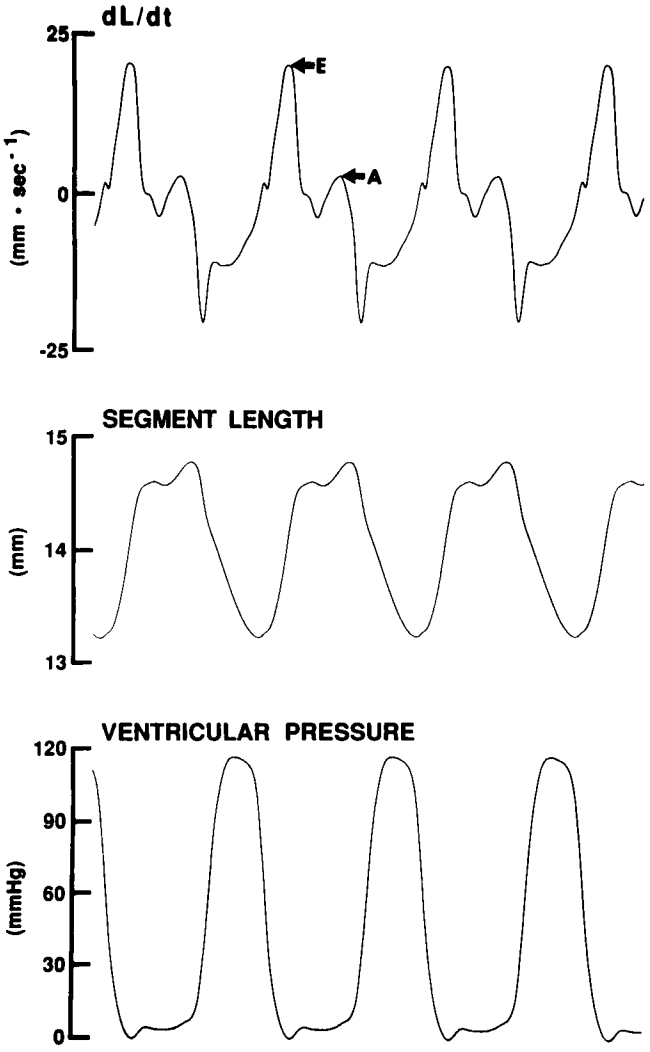

Fig. 1. Determination of maximum segment lengthening velocity during rapid ventricular (dL/dtE) and atrial filling (dL/dtA) in a typical experiment. The continuous segment length waveforms (*middle*) were differentiated to yield dL/dt waveform (*top*), and maximum segment lengthening velocity during early ventricular filling (E) and atrial systole (A) then were identified. The corresponding continuous left ventricular pressure waveform also is depicted (*bottom*).

segment length waveform[3,2] (fig. 1). Analogous to the ratio of maximum transmitral flow velocities during rapid ventricular filling (early, E) and atrial systole (late, A) defined noninvasively with Doppler echocardiography, the ratio of maximum segment lengthening velocity during rapid ventricular and atrial filling (E/A) was calculated as the quotient of dL/dt_E and dL/dt_A. Since the majority of ventricular filling occurs early in diastole under normal circumstances, a decrease in the E/A ratio may indicate diastolic dysfunction by reduction of early filling in combination with an increased dependence on atrial systole (late active filling).

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 $CaCl_2$ infusions was performed by multiple analysis of variance 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 $P < .05$. The relationships between $-dP/dt$ and ventricular pressure used to calculate τ and between PRSW and EDL used to calculate M_w and L_w were described using linear regression analysis. Least squares regression analysis was used to characterize the exponential relationship between ventricular pressure and segment length (calculation of K_p). All data were expressed as mean ± SEM.

Results

Autonomic nervous system blockade produced significant ($P < .05$) increases in heart rate and decreases in mean arterial pressure, left ventricular systolic pressure, systemic vascular resistance, and stroke volume. No changes in left ventricular end diastolic pressure or cardiac output were observed (tables 1–3). No differences in baseline hemodynamics with or without autonomic nervous system blockade were present between groups. The regression coefficients (r^2) obtained for calculation of the PRSW relationship (M_w and L_w), the time constant of isovolumic relaxation (τ), and the regional chamber stiffness constant (K_p) were ≥0.97, ≥0.99, and ≥0.94, respectively, in conscious and anesthetized dogs. End tidal concentrations of halothane and isoflurane used in this investigation were 1.33 ± 0.01% and 1.91 ± 0.01 (mean ± SEM), respectively.

Administration of $CaCl_2$ in the conscious state produced dose-related increases in mean arterial pressure, left ventricular systolic pressure, and systemic vascular resistance (table 1). Significant increases in heart rate also were observed at the high dose. No changes in left ventricular end diastolic pressure, cardiac output, or stroke volume occurred. Administration of $CaCl_2$ to conscious dogs caused dose-dependent increases in the PRSW slope (M_w; 68 ± 10 during control to 138 ± 26 mmHg during 5 $mg \cdot kg^{-1} \cdot min^{-1}$ $CaCl_2$) indicating an increase in inotropic state (table 4). No change in the PRSW length intercept (L_w) was noted. A concomitant

Table 1. Hemodynamic Effects of Calcium Chloride in Conscious Dogs

	Conscious Control	ANS Blockade	$CaCl_2$ ($mg \cdot kg^{-1} \cdot min^{-1}$)		
			1.25	2.5	5.0
HR (beats/min)	76 ± 4*	111 ± 5	113 ± 5	119 ± 6	133 ± 6*†
MBP (mmHg)	97 ± 4*	68 ± 3	84 ± 7	101 ± 7*	120 ± 9*†‡
RPP (mmHg · beats/min · 10^3)	9.6 ± 0.5	9.7 ± 0.7	11.6 ± 1.1	14.5 ± 1.3*†	19.4 ± 1.9*†‡
LVSP (mmHg)	128 ± 4*	93 ± 4	108 ± 6	122 ± 7*	145 ± 9*†‡
LVEDP (mmHg)	9 ± 1	7 ± 1	7 ± 1	7 ± 1	8 ± 1
CO ($L \cdot min^{-1}$)	2.2 ± 0.2	2.4 ± 0.3	2.6 ± 0.3	2.7 ± 0.3	2.8 ± 0.3
SVR (dyne · s · cm^{-5})	3600 ± 200*	2470 ± 190	2840 ± 360	3330 ± 380*	3840 ± 310*†
SV (ml)	30 ± 3*	21 ± 2	22 ± 2	22 ± 3	20 ± 2

Values are mean ± SEM (n = 9). HR = heart rate; MBP = mean aortic blood pressure; RPP = rate pressure product; LVSP and LVEDP = left ventricular systolic and end-diastolic pressure, respectively; CO = cardiac output; SV = stroke volume; SVR = systemic vascular resistance; ANS = autonomic nervous sytem.

* Significantly ($P < .05$) different from autonomically blocked state.

† Significantly ($P < .05$) different from 1.25 $mg \cdot kg \cdot^{-1}$ $CaCl_2$.

‡ Significantly ($P < .05$) different from 2.5 $mg \cdot kg^{-1} \cdot min^{-1}$ $CaCl_2$.

Table 2. Hemodynamic Effects of Calcium Chloride in Halothane-Anesthetized Dogs

	Conscious Control	ANS Blockade	Halothane	$CaCl_2$ (mg·kg^{-1}·min^{-1}) 1.25	2.5	5.0
HR (beats/min)	83 ± 3*	109 ± 3	86 ± 6*	84 ± 5*	84 ± 5*	88 ± 6*
MBP (mmHg)	102 ± 3*	72 ± 3	53 ± 3*	67 ± 4†	73 ± 4†	80 ± 5*†‡
RPP (mmHg·beats/min·10^3)	10.5 ± 0.5	9.9 ± 0.3	5.8 ± 0.6*	7.0 ± 0.6*	7.7 ± 0.6*†	8.8 ± 0.8†‡
LVSP (mmHg)	129 ± 4*	96 ± 2	73 ± 3*	89 ± 4†	98 ± 4†	106 ± 5†‡
LVEDP (mmHg)	8 ± 1	7 ± 1	13 ± 1*	14 ± 2*	14 ± 3*	13 ± 2*
CO (L·min^{-1})	2.3 ± 0.2	2.6 ± 0.2	1.3 ± 0.2*	1.7 ± 0.2*†	2.1 ± 0.2*†‡	2.4 ± 0.2†‡
SVR (dyne·s·cm^{-5})	3700 ± 270*	2270 ± 200	3360 ± 350*	3330 ± 210*	2960 ± 210*	2820 ± 160
SV (ml)	28 ± 2	25 ± 2	16 ± 2*	20 ± 2*	25 ± 2†	27 ± 2†‡

Values are mean ± SEM (n = 9). HR = heart rate; MBP = mean aortic blood pressure; RPP = rate pressure product; LVSP and LVEDP = left ventricular systolic and end-diastolic pressure, respectively; CO = cardiac output; SV = stroke volume; SVR = systemic vascular resistance; ANS = autonomic nervous system.

* Significantly ($P < .05$) different from autonomically blocked, conscious state.

† Significantly ($P < .05$) different from halothane.

‡ Significantly ($P < .05$) different from 1.25 mg·kg^{-1}·min^{-1} $CaCl_2$.

increase in peak positive left ventricular dP/dt (1699 ± 88 during control to 3,883 ± 244 mmHg/s during 5 mg·kg^{-1}·min^{-1} $CaCl_2$) also was observed.

No changes in the time constant of isovolumic relaxation (τ) were noted during administration of $CaCl_2$ to conscious animals. Segment lengthening velocity during atrial systole (dL/dt_A) increased during the high dose of $CaCl_2$ consistent with enhanced atrial contractility. Concomitant declines in the E/A ratio were observed, as lengthening velocity during rapid ventricular filling (dL/dt_E) remained constant during $CaCl_2$ administration (table 4). Regional chamber stiffness (K_p) decreased significantly when $CaCl_2$ was administered at the 5-mg·kg^{-1}·min^{-1} dose, but no changes in K_p occurred at lower doses (table 4).

Halothane anesthesia (table 2) caused significant decreases in heart rate, mean arterial pressure, left ventricular systolic pressure, and cardiac output. Increases in left ventricular end diastolic pressure, systemic vascular resistance, and end systolic segment length also occurred. Administration of $CaCl_2$ in the presence of halothane caused dose-related increases in mean arterial pressure, left ventricular systolic pressure, and cardiac output and decreases in end systolic length. No

Table 3. Hemodynamic Effects of Calcium Chloride in Isoflurane-anesthetized Dogs

	Conscious Control	ANS Blockade	Isoflurane	$CaCl_2$ (mg·kg^{-1}·min^{-1}) 1.25	2.5	5.0
HR (beats/min)	81 ± 3*	107 ± 4	86 ± 4*	86 ± 4*	86 ± 4*	83 ± 4*
MBP (mmHg)	99 ± 4*	75 ± 5	50 ± 2*	58 ± 2*	63 ± 2*†	71 ± 4†‡
RPP (mmHg·beats/min·10^3)	10.2 ± 0.6	10.2 ± 0.7	5.5 ± 0.3*	6.2 ± 0.4*	6.9 ± 0.5*†	7.7 ± 0.6*†‡
LVSP (mmHg)	128 ± 4*	97 ± 4	70 ± 3*	79 ± 4*†	87 ± 4*†	97 ± 6*†‡
LVEDP (mmHg)	8 ± 1	7 ± 1	11 ± 1	12 ± 1	13 ± 2*	14 ± 2*
CO (L·min^{-1})	2.1 ± 0.1	2.4 ± 0.1	1.3 ± 0.1*	1.6 ± 0.1*	2.0 ± 0.2*†	2.2 ± 0.2†‡
SVR (dyne·s·cm^{-5})	3930 ± 240*	2580 ± 170	3190 ± 300	2970 ± 250	2590 ± 150	2640 ± 190
SV (ml)	26 ± 2	22 ± 2	16 ± 1*	19 ± 2	24 ± 2†	27 ± 2*†‡

Values are mean ± SEM (n = 9). HR = heart rate; MBP = mean aortic blood pressure; RPP = rate pressure product; LVSP and LVEDP = left ventricular systolic and end-diastolic pressure, respectively; CO = cardiac output; SV = stroke volume; SVR = systemic vascular resistance; ANS = autonomic nervous system.

* Significantly ($P < .05$) different from autonomically blocked, conscious state.

† Significantly ($P < .05$) different from isoflurane.

‡ Significantly ($P < .05$) different from 1.25 mg·kg^{-1}·min^{-1} $CaCl_2$.

Table 4. Effects of Calcium Chloride on Indices of Left Ventricular Systolic and Diastolic Function in Conscious Dogs

		$CaCl_2$ (mg · kg^{-1} · min^{-1})		
	ANS Blockade	1.25	2.5	5.0
M_w (mmHg)	68 ± 10	86 ± 11*	113 ± 17*†	138 ± 26*†
L_w (mm)	15.0 ± 1.3	14.7 ± 1.3	14.8 ± 1.3	14.7 ± 1.1
dP/dt_{max} (mmHg · s^{-1})	1699 ± 88	2077 ± 81*	2843 ± 145*†	3883 ± 244*†‡
EDL (mm)	17.6 ± 0.7	17.8 ± 0.9	17.6 ± 0.8	17.1 ± 0.9
ESL (mm)	14.4 ± 0.9	14.6 ± 0.9	14 ± 0.9	13.5 ± 0.9*†
SS (%)	18.4 ± 1.7	18.3 ± 1.9	21.0 ± 2.0	21.7 ± 1.7
τ (ms)	32.6 ± 1.5	33.3 ± 1.5	31.8 ± 1.4	30.8 ± 1.8
K_p (mm^{-1})	0.38 ± 0.04	0.33 ± 0.03	0.28 ± 0.02	0.23 ± 0.03*†
dL/dt_A (mm · s^{-1})	6.0 ± 0.6	7.2 ± 0.7	8.5 ± 1.2	10.7 ± 1.4*
dL/dt_E (mm · s^{-1})	20.0 ± 1.4	21.1 ± 2.1	21.1 ± 1.7	20.9 ± 1.4
E/A ratio	3.6 ± 0.5	3.0 ± 0.4	2.8 ± 0.5	2.2 ± 0.4*

Values are mean ± SEM (n = 9). M_w and L_w = preload recruitable stroke work slope and length intercept, respectively; SS = segment shortening; EDL and ESL = end-diastolic and end-systolic segment length, respectively; τ = time constant of isovolumic relaxation; K_p = regional chamber stiffness; E/A ratio = quotient of dL/dt_E and dL/dt_A; ANS = autonomic nervous sytem.

* Significantly ($P < .05$) different from autonomically blocked state.

† Significantly ($P < .05$) different from 1.25 mg · kg^{-1} · min^{-1} $CaCl_2$.

‡ Significantly ($P < .05$) different from 2.5 mg · kg^{-1} · min^{-1} $CaCl_2$.

changes in heart rate were observed when $CaCl_2$ was administered in the presence of halothane, in contrast to findings in the conscious state.

Halothane anesthesia produced decreases in M_w, dP/dt_{max}, and %SS consistent with a negative inotropic effect (table 5). Halothane also caused significant increases in the time constant of isovolumic relaxation (τ 34.5 ± 1.4 during control to 57.9 ± 4.2 ms at 1.5 MAC) and regional chamber stiffness (K_p 0.45 ± 0.05 during control to 0.70 ± 0.18 mm^{-1} at 1.5 MAC). Furthermore, decreases in dL/dt_E (23.1 ± 2.2 during control to 11.8 ± 1.4 mm/s at 1.5 MAC) and dL/dt_A (5.9

Table 5. Effects of Calcium Chloride on Indices of Left Ventricular Systolic and Diastolic Function in Halothane-anesthetized Dogs

			$CaCl_2$ (mg · kg^{-1} min^{-1})		
	ANS Blockade	Halothane	1.25	2.5	5.0
M_w (mmHg)	77 ± 8	26 ± 5*	31 ± 8*	49 ± 10*†§	78 ± 10†‡§
L_w (mm)	14.0 ± 0.8	15.8 ± 1.2*	14.0 ± 1.3†	13.5 ± 0.6†	13.4 ± 0.3†
dP/dt_{max} (mmHg · s^{-1})	1680 ± 76	717 ± 41*	973 ± 49*	1332 ± 65*†§	1756 ± 92†‡§
EDL (mm)	18.4 ± 1.0	18.7 ± 1.0	18.9 ± 1.0	18.8 ± 0.9	18.3 ± 0.9
ESL (mm)	15.1 ± 1.1	17.3 ± 1.1*	16.9 ± 1.1*	15.9 ± 1.0*†§	14.7 ± 0.9†§
SS (%)	18.7 ± 2.3	8.1 ± 2.2*	10.8 ± 2.5*	15.7 ± 2.2†§	20.0 ± 2.2†‡§
τ (ms)	34.5 ± 1.4	57.9 ± 4.2*	54.3 ± 4.0*	45.3 ± 3.0*†§	41.1 ± 1.9†§
K_p (mm^{-1})	0.45 ± 0.05	0.70 ± 0.18*	0.70 ± 0.18*	0.59 ± 0.15	0.38 ± 0.04†§
dL/dt_E (mm · s^{-1})	23.1 ± 2.2	11.8 ± 1.4*	14.8 ± 1.5*	18.4 ± 1.3*†	20.2 ± 1.6†§
dL/dt_A (mm · s^{-1})	5.9 ± 0.6	2.5 ± 0.3*	4.7 ± 0.8*†	6.4 ± 0.7†	6.7 ± 1.0†§
E/A ratio	4.3 ± 0.8	4.8 ± 0.6	4.0 ± 0.8	3.2 ± 0.4	3.4 ± 0.4

Values are mean ± SEM (n = 9). M_w and L_w = preload recruitable stroke work slope and length intercept, respectively; SS = segment shortening; EDL and ESL = end-diastolic and end-systolic segment length, respectively; τ = time constant of isovolumic relaxation; K_p = regional chamber stiffness constant; E/A ratio = quotient of dL/dt_E and dL/dt_A; ANS = autonomic nervous system.

* Significantly ($P < .05$) different from autonomically blocked, conscious state.

† Significantly ($P < .05$) different from halothane.

‡ Significantly ($P < .05$) different from 2.5 mg · kg^{-1} · min^{-1} $CaCl_2$.

§ Significantly ($P < .05$) different from 1.25 mg · kg^{-1} · min^{-1} $CaCl_2$.

± 0.6 during control to 2.5 ± 0.3 mm/s at 1.5 MAC) occurred, demonstrating the negative lusitropic actions of this volatile anesthetic in multiple phases of diastole. No changes in the E/A ratio were observed. The PRSW slope increased in a dose-dependent fashion (26 ± 5 mmHg during halothane alone to 78 ± 10 mmHg during 5 mg·kg^{-1}·min^{-1} $CaCl_2$) when $CaCl_2$ was administered in the presence of halothane (fig. 2). Concomitant changes in dP/dt_{max} and %SS also were noted (table 5). Calcium chloride improved the alterations in left ventricular diastolic function produced by halothane. Calcium chloride decreased τ in a dose-related fashion (57.9 ± 4.2 ms during halothane alone to 41.1 ± 1.9 ms during 5 mg·kg^{-1}·min^{-1} $CaCl_2$), indicating an enhancement of isovolumic relaxation (fig. 3). Similarly, dL/dt_E (fig. 4) and dL/dt_A (table 5) increased and regional chamber stiffness (K_p) decreased (fig. 5) toward preanesthetic control levels, suggesting that improvements in early and late ventricular filling and regional wall compliance occurred during infusion of $CaCl_2$.

Isoflurane, like halothane, decreased heart rate, mean arterial pressure, left ventricular systolic pressure, and cardiac output (table 3). In contrast to the findings with halothane, however, no changes in left ventricular end diastolic pressure and systemic vascular resistance

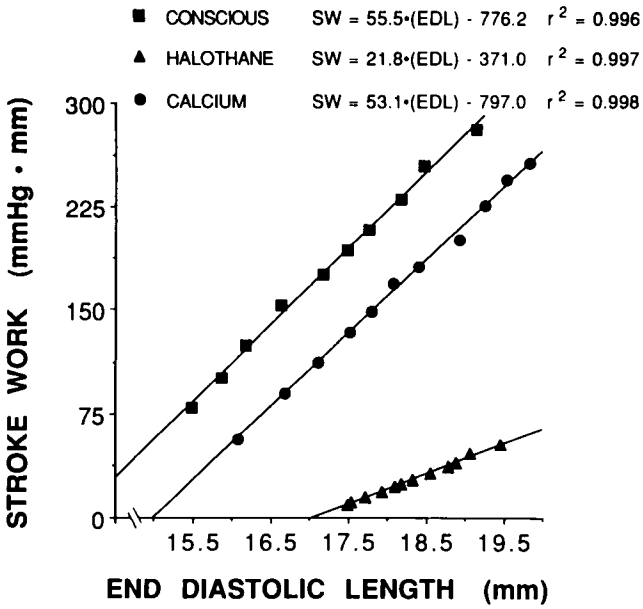

Fig. 2. Regional stroke work (SW) *versus* end diastolic segment length (EDL) relationship data in the conscious state, during 1.5 MAC halothane, and following 5 mg·kg^{-1}·min^{-1} $CaCl_2$ during halothane anesthesia in a typical experiment.

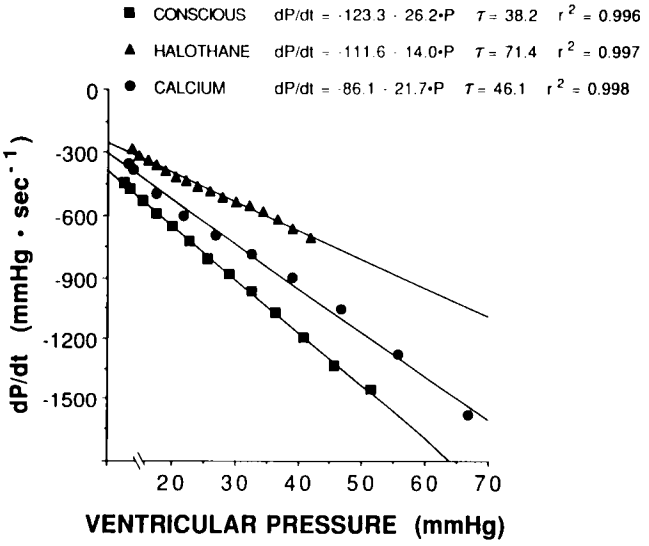

Fig. 3. Relationship between −dP/dt and left ventricular pressure used to calculate the time constant of isovolumic relaxation (τ) in the conscious state, during 1.5 MAC halothane, and following 5 mg·kg^{-1}·min^{-1} $CaCl_2$ during halothane anesthesia in a typical experiment.

were observed during isoflurane anesthesia. Isoflurane also produced significant depression of left ventricular systolic (decreases in M_w, dP/dt_{max}, and %SS) and diastolic (increases in τ and K_p and decreases in dL/dt_E and dL/dt_A function; table 6). Although halothane caused significantly greater negative inotropic actions than did isoflurane as assessed by M_w, no significant differences between isoflurane and halothane were noted when diastolic function variables (τ, K_p, dL/dt_E, dL/dt_A, and the E/A ratio) were compared.

Administration of $CaCl_2$ to dogs anesthetized with isoflurane caused changes in systemic hemodynamics that were similar to those observed during halothane

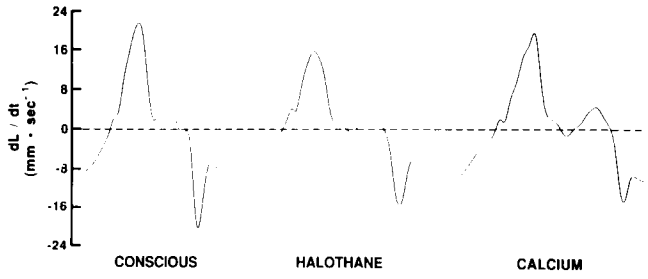

Fig. 4. Continuous rate of change of segment length (dL/dt) waveforms used to assess dL/dt_E and dL/dt_A in the conscious state, during 1.5 MAC halothane, and following 5 mg·kg^{-1}·min^{-1} $CaCl_2$ during halothane anesthesia in a typical experiment.

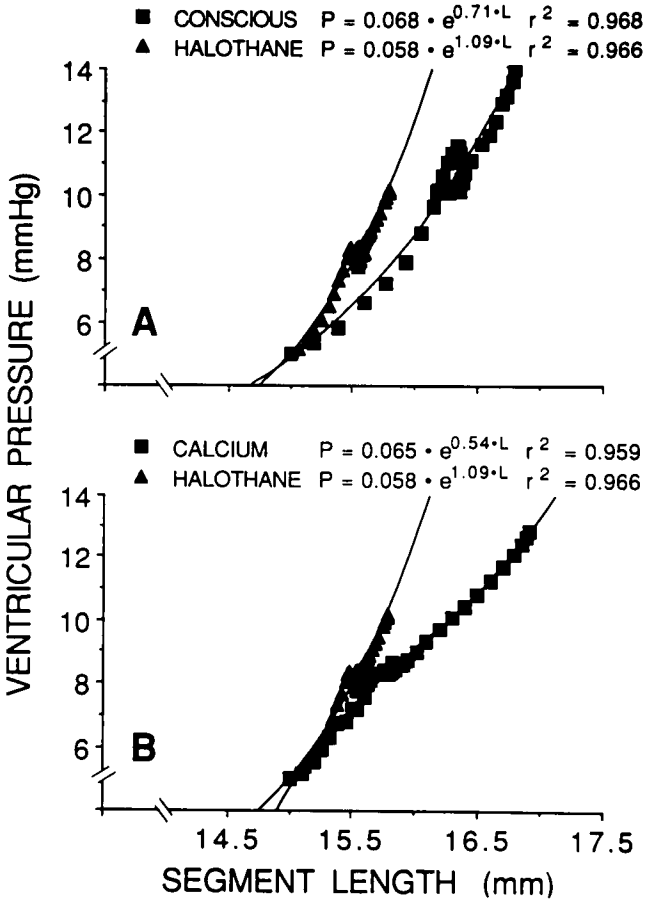

Fig. 5. Relationship between left ventricular pressure (P) and segment length (L) used to calculate the regional chamber stiffness constant (K_p) in the conscious state and under 1.5 MAC halothane (A). Also shown are data in the same experiment during halothane anesthesia and following 5 mg·kg^{-1}·min^{-1} $CaCl_2$ (B). Data are separated into two graphs for clarity.

anesthesia (table 3). In the presence of isoflurane, $CaCl_2$ caused progressive and dose-dependent increases in contractile state and improvement of isoflurane-induced diastolic dysfunction (table 6). No significant differences in indices of left ventricular systolic or diastolic function between halothane and isoflurane groups during the administration of $CaCl_2$ were present.

Discussion

Calcium was first identified as an essential component of cardiac muscle contraction by Ringer[33] in 1883 and is now uniformly considered to be the critical mediator of myocyte contractile activation.[34] It is well established that volatile anesthetics, including halothane and isoflurane, depress myocardial contractility in a dose-dependent manner *in vivo*[1-3] *via* alterations of intracellular Ca^{2+} homeostasis at several subcellular targets within the cardiac myocyte.[9] Inhalational anesthetics have been shown to depress the inward Ca^{2+} current resulting from membrane depolarization by inhibiting function of or reducing the number of voltage-dependent Ca^{2+} channels in the sarcolemmal membrane.[12,14-18] This partial inhibition of Ca^{2+} influx *via* sacrolemmal Ca^{2+} channels leads to declines in availability of Ca^{2+} for contractile activation, decreases the amount of Ca^{2+} that can be stored in sarcoplasmic reticulum, and depresses Ca^{2+}-dependent Ca^{2+} release from the sarcoplasmic reticulum.[9] Volatile anesthetics also reduce the concentration of intracellular Ca^{2+} during systole by direct alteration of sarcoplasmic reticulum function. Storage of Ca^{2+} in the sarcoplasmic reticulum declines due to anesthetic-induced decreases in intracellular Ca^{2+} concentration combined with partial inhibition of Ca^{2+} uptake and enhanced Ca^{2+} leak from this organelle.[9,10,13,19-22] Volatile anesthetics also may modify the responsiveness of contractile proteins to activator Ca^{2+}, although this hypothesis remains somewhat controversial.[10,11,35,36]

Administration of exogenous Ca^{2+} has been shown to partially reverse the negative inotropic actions of inhalational anesthetics. Presumably this occurs by enhancing intracellular Ca^{2+} availability during contraction and, therefore, overcoming anesthetic-induced depression of subcellular mechanisms responsible for regulation of activator Ca^{2+}. Price[25] first demonstrated that increased concentrations of extracellular Ca^{2+} antagonized halothane-induced depression of myocardial contractility in cat papillary muscle. Subsequently, Denlinger *et al.*[23] examined the cardiovascular responses to Ca^{2+} administered to humans anesthetized with halothane and found that Ca^{2+} produced increases in cardiac performance *in vivo*. These findings were confirmed recently and extended by Hysing *et al.*,[24] who examined the cardiovascular actions of hypo- and hypercalcemia in conscious and anesthetized chronically instrumented dogs. Those authors demonstrated that hypercalcemia (serum concentrations between 1.72 and 1.77 mM) produced direct positive inotropic effects that partially reversed the myocardial depressant actions of halothane, enflurane, and isoflurane.

In his classic experiments, Ringer[33] also suggested that $CaCl_2$ might "delay diastolic dilatation," an early

Table 6. Effects of Calcium Chloride on Indices of Left Ventricular Systolic and Diastolic Function in Isoflurane-anesthetized Dogs

			$CaCl_2$ (mg · kg^{-1} · min^{-1})		
	ANS Blockade	Isoflurane	1.25	2.5	5.0
M_w (mmHg)	73 ± 13	37 ± 7*	41 ± 9*	46 ± 8*†	87 ± 14†‡§
L_w (mm)	13.9 ± 1.0	14.7 ± 1.0	13.9 ± 1.4	13.8 ± 1.1	13.4 ± 1.2
dP/dt_{max} (mmHg · s^{-1})	1636 ± 78	784 ± 41*	1025 ± 47*†	1392 ± 59*†§	1906 ± 73*†‡§
EDL (mm)	18.4 ± 0.9	18.4 ± 0.9	18.7 ± 0.9	18.6 ± 0.9	18.5 ± 0.9
ESL (mm)	15.2 ± 1.0	16.3 ± 0.9*	16 ± 0.9	15.2 ± 0.8†	14.2 ± 0.8†§
SS (%)	18.0 ± 2.3	11.5 ± 2.2*	14.5 ± 2.0*†	18.5 ± 1.9†§	23.1 ± 2.0†‡§
τ (ms)	34.7 ± 1.5	50.2 ± 3.0*	47.5 ± 3.1*	42.2 ± 2.7*†§	39.3 ± 2.2†§
K_p (mm^{-1})	0.44 ± 0.07	0.65 ± 0.13*	0.72 ± 0.20*	0.66 ± 0.20*	0.54 ± 0.15
dL/dt_E (mm · s^{-1})	24.3 ± 1.8	12.7 ± 1.9*	16.5 ± 1.6*†	20.7 ± 2.0*†§	23.0 ± 2.6†§
dL/dt_A (mm · s^{-1})	5.4 ± 0.6	2.7 ± 0.6*	3.6 ± 0.6*	5.0 ± 0.7†§	6.7 ± 0.8*†‡§
E/A ratio	4.8 ± 0.6	5.0 ± 1.1	5.1 ± 0.6	4.8 ± 0.9	3.8 ± 0.8

Values are mean ± SEM (n = 9). M_w and L_w = preload recruitable stroke work slope and length intercept, respectively; SS = segment shortening; EDL and ESL = end-diastolic and end-systolic segment length, respectively; τ = time constant of isovolumic relaxation; K_p = regional chamber stiffness constant; E/A ratio = quotient of dL/dt_E and dL/dt_A; ANS = autonomic nervous system.

* Significantly ($P < .05$) different from autonomically blocked, conscious state.

† Significantly ($P < .05$) different from isoflurane.

‡ Significantly ($P < .05$) different from 2.5 mg · kg^{-1} · min^{-1} $CaCl_2$.

§ Significantly ($P < .05$) different from 1.25 mg · kg^{-1} · min^{-1} $CaCl_2$.

suggestion that Ca^{2+} may alter diastolic myocardial performance as well. Cardiac function during diastole has become the focus of intense experimental and clinical investigation in recent years because of increasing awareness that left ventricular performance during this phase of the cardiac cycle significantly influences the overall mechanical efficiency of the heart. Although abnormalities in diastolic function usually can be directly linked to systolic dysfunction, cardiac failure may result from primary diastolic dysfunction in the absence of or before appearance of alterations in left ventricular systolic function in a variety of disease processes including ischemic heart disease, hypertrophic or infiltrative cardiomyopathy, and hypertensive heart disease.[3-7] Left ventricular diastolic dysfunction observed in these pathologic conditions has been attributed to chronically abnormal handling of intracellular Ca^{2+} manifested by down regulation of Ca^{2+} channels in the sarcolemma and sarcoplasmic reticulum, altered structural integrity of and response to activator Ca^{2+} by contractile proteins, and impaired energy production.[3,8] Volatile anesthetics, including isoflurane and halothane, also have been shown to produce impairment of left ventricular diastolic function by delaying isovolumetric relaxation[4-6] and possibly by altering global myocardial compliance *in vivo*.[4,7,8] The mechanisms responsible for these anesthetic-induced negative lusitropic actions have yet to be completely described, but probably involve acute alteration of similar subcellular targets.

In an elegant investigation, Housmans and Murat[11] demonstrated that volatile anesthetics may inhibit sequestration of Ca^{2+} into the sarcoplasmic reticulum, an event that may lead to inadequate termination of contraction by prolonging the time course of the Ca^{2+} transient in ferret papillary muscle. This phenomenon may prolong global isovolumic relaxation and impair rapid ventricular filling *in vivo*. A subsequent investigation by Housmans,[23] however, demonstrated that indirect decreases in intracellular Ca^{2+} concentration may play a more important role in volatile anesthetic-induced contractile dysfunction than the direct effects of these agents on Ca^{2+} uptake by the sarcoplasmic reticulum. Increased leak of Ca^{2+} from the sarcoplasmic reticulum^{24} or delayed diffusion of Ca^{2+} away from the contractile apparatus also may be responsible for incomplete relaxation. Volatile anesthetic-induced depression of normal Ca^{2+} extrusion mechanisms, including the sodium-calcium exchanger and ATP-dependent Ca^{2+} pumps in the sarcolemmal membrane, may contribute to delays in removal of Ca^{2+} from myofibrils during diastole as well.[9]

Although it is clear that extracellular Ca^{2+} reverses volatile anesthetic-induced depression of global myocardial contractility, the effects of extracellular Ca^{2+} on diastolic dysfunction produced by these agents have yet to be described. The results of this investigation indicate that intravenous administration of $CaCl_2$ produces a dose-dependent increase in myocardial contractility as evaluated by M_w in conscious and anesthetized dogs, supporting the findings of Hysing *et al.*,[24] Drop and coworkers,[39] and Bristow *et al.*[40] $CaCl_2$ caused no alteration of diastolic function as assessed by multiple indices in conscious dogs with the exception of decreased regional chamber stiffness (K_p) at the highest dose of $CaCl_2$. These results are supported in part by the findings of Weiss *et al.*[41] and Drop *et al.*,[39] who demonstrated that bolus doses of $CaCl_2$ (100 mg) produced no changes in τ and sustained hypercalcemia (1.70 ± 0.01 mM) decreased left ventricular end diastolic pressure during constant SW on cardiopulmonary bypass (indirectly suggesting an improvement in left ventricular compliance) in canine hearts, respectively. In contrast, results of the current investigation demonstrate that $CaCl_2$ improves diastolic function in the presence of halothane and isoflurane as manifested by enhancement of isovolumic relaxation (decreases in τ), rapid ventricular and atrial filling (increases in dL/dt_E and dL/dt_A, respectively), and regional chamber stiffness (decreases in K_p) concomitant with enhanced myocardial contractility.

On a subcellular level, the present results may be consistent with partial reversal of volatile anesthetic-induced inhibition of Ca^{2+} sequestration and extrusion mechanisms during diastole. Administration of extracellular Ca^{2+} may have provided greater substrate for myocardial contractile activation during systole in both the conscious and the anesthetized states. Normally functioning Ca^{2+} extrusion mechanisms appeared to have adequately handled an enhanced intracellular Ca^{2+} load in the conscious state as indicated by a maintenance of several diastolic function variables. It is unlikely that intracellular Ca^{2+} overload occurred during administration of $CaCl_2$ in this investigation since prolongation of isovolumic relaxation, delayed early ventricular filling, and increased regional chamber stiffness resulting from declines in myocardial compliance probably would have been observed under these circumstances. In halothane- or isoflurane-anesthetized dogs, $CaCl_2$ demonstrated positive lusitropic effects coincident with significant decreases in end systolic segment length. Because these agents impair cardiac myocyte function by indirectly decreasing intracellular Ca^{2+} concentration,[25] increases in intracellular Ca^{2+} induced by $CaCl_2$ infusions may have improved volatile anesthetic-induced diastolic dysfunction by enhancing calcium-dependent Ca^{2+} sequestration into the sarcoplasmic reticulum[42] or by improving the function of Ca^{2+} pumps in sarcolemmal membrane or sarcoplasmic reticulum by providing additional intracellular Ca^{2+} substrate.[43]

The present findings may be explained alternatively by the muscle length dependence of Ca^{2+} affinity for troponin C. Lower myofibrillar affinity for Ca^{2+} at shorter muscle lengths has been demonstrated previously.[44–46] Calcium-induced positive inotropic actions observed in the presence of halothane and isoflurane produced dose-related decreases in end systolic segment length consistent with declines in cellular myofibrillar length. These findings may imply decreased affinity for and enhanced release of Ca^{2+} from the contractile apparatus during this period of increased contractile state, leading to shortened isovolumic relaxation and enhanced early ventricular filling during diastole. Such a conclusion must be qualified because if anesthetic-induced changes in isovolumic relaxation and early ventricular filling are purely myocyte length-dependent events associated with increased contractility, similar values of τ and dL/dt_E would be expected at identical end systolic segment lengths in the presence or absence of volatile anesthetics. However, isovolumic relaxation is prolonged and early ventricular filling remained significantly delayed in the presence of isoflurane and the $2.5\text{-mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ $CaCl_2$ infusion compared to the autonomically blocked, conscious state (τ; 42.2 ± 2.7 *vs.* 34.7 ± 1.5 ms, respectively, and dL/dt_E; 20.7 ± 2.0 *vs.* 24.3 ± 1.8 mm/s, respectively) at similar values of ESL (table 6). These findings quantitatively indicate that partial reversal of volatile anesthetic-induced diastolic dysfunction by administration of $CaCl_2$ is not an entirely length-dependent phenomenon and suggest that some impairment of diastolic function remains in the presence of anesthetics despite restoration of systolic function. This observation provides indirect evidence to suggest that sarcoplasmic reticulum function and Ca^{2+} retention in this organelle during diastole are impaired by volatile anesthetics, however, there is probably not sufficient depression of the sarcoplasmic reticulum to markedly impair relaxation and early ventricular filling when substantial

myocyte shortening does occur. This explanation also serves to emphasize that strict isolation of analysis of diastolic function independent of systolic function may be inappropriate because enhanced muscle fiber shortening observed with positive inotropes may directly affect muscle fiber affinity for Ca^{2+}, indirectly resulting in enhancement of relaxation and early filling.[37]

The results of this investigation must be interpreted within the constraints of several limitations. Although indices of diastolic function used in this study are influenced directly by active, energy-dependent forces (ventricular relaxation) and by intrinsic myocardial viscoelastic properties, extrinsic factors including right ventricular and septal interactions, myocardial blood flow and hemodynamic alterations affecting systolic function (heart rate, preload, afterload, and myocardial contractility) cannot be excluded completely from the analysis.[37] For example, the time constant of isovolumic relaxation (τ) depends on heart rate and left ventricular afterload.[41,48] Increases in calculated systemic vascular resistance (an indirect indicator of left ventricular afterload) were observed in the conscious state and during halothane anesthesia and may have influenced the measurement of isovolumic relaxation in these experimental settings. Similarly, an increase in heart rate was observed during the highest dose of $CaCl_2$ in the conscious state, which also may have influenced τ. The rate of rapid ventricular filling (as evaluated by dL/dt_E) depends on the gradient between left atrial and left ventricular pressure at this period during the cardiac cycle, which was not measured in the present study.[32] Alterations in ventricular loading conditions and myocardial contractility produced by $CaCl_2$ in the presence and absence of volatile anesthetics also may have influenced passive viscoelastic properties and interpretation of changes in diastolic regional chamber stiffness.

The doses of $CaCl_2$ used in this investigation were chosen to produce dose-related increases in left ventricular peak positive dP/dt in the conscious and anesthetized states following the method described by Bristow *et al.*[40] Although specific ionized Ca^{2+} concentrations were not measured, the $CaCl_2$ infusion rates (1.25, 2.5, and 5 $mg \cdot kg^{-1} \cdot min^{-1}$) used in the current investigation should have been expected to produce degrees of hypercalcemia bounded within the limits described by other investigators. For example, Hysing *et al.*[24] administered a 19.8-mg/kg bolus of $CaCl_2$ over 2 min followed by a 0.73-$mg \cdot kg^{-1} \cdot min^{-1}$ infusion for 10 min to yield an increase in ionized Ca^{2+} of approximately 0.35 mM. Similarly, Drop *et al.*[39] used a 12-mg/kg bolus of $CaCl^{2+}$ followed by an infusion to produce serum ionized Ca^{2+} concentrations between 1.6 and 1.7 mM. Lastly, Bristow *et al.*[40] used calcium gluconate infusions between 2 and 40 $mg \cdot kg^{-1} \cdot min^{-1}$ to produce a dose-related increase in peak positive left ventricular dP/dt. These infusion rates resulted in ionized Ca^{2+} concentrations between 4.6 and 10 $mg \cdot 100\ ml^{-1}$ (1.2–2.5 mM).

In summary, the results of this investigation indicate that the administration of $CaCl_2$ produces dose-dependent positive inotropic effects as assessed using M_w in both conscious and anesthetized chronically instrumented dogs. Although $CaCl_2$ does not significantly alter diastolic function in the conscious state, $CaCl_2$ improves halothane- or isoflurane-induced diastolic dysfunction as manifested by enhanced isovolumic relaxation, increased early and late ventricular filling, and reduced regional chamber stiffness. The results were accompanied by concomitant increases in myocardial oxygen consumption as indirectly indicated by the rate-pressure product. These findings may be consistent with subcellular activation of Ca^{2+}-dependent Ca^{2+} sequestration and extrusion mechanisms that are depressed by volatile anesthetics or may result indirectly because of enhanced systolic function and associated declines in muscle fiber length. Improvement of left ventricular performance by $CaCl_2$ during volatile anesthesia may not be related only to positive inotropic actions but also to positive lusitropic effects.

The authors extend their appreciation to John Tessmer, Doug Hettrick, and David Schwabe for technical assistance and to Mimi Mick for preparation of the manuscript.

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