Mechanorenergetics of the Negative Inotropism of Isoflurane in the Canine Left Ventricle

No O₂ Wasting Effect

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**Background:** The mechanisms underlying the negative inotropic effects of isoflurane are incompletely understood. One suggested mechanism is that isoflurane may decrease Ca²⁺ sensitivity of contractile proteins. If so, more free calcium would be needed to activate contractile proteins to the same degree, which would impose a greater requirement for myocardial oxygen consumption used in the cycling of calcium. In this study, the authors use the excised, cross-circulated, canine heart model and the volume servopump technique to measure the effects of isoflurane on Emmax (a contractile index) and on the relationship between pressure—volume area (PVA, a measure of total mechanical energy) and myocardial oxygen consumption per beat (VO₂).

**Methods:** Effects of intracoronary isoflurane infused via a precorony oxygenator on myocardial mechanorenergics were studied during isovolumic contractions. The authors measured left ventricular (LV) pressure, LV volume, coronary flow, and arteriovenous oxygen content difference and computed Emmax, VO₂, and PVA at 0, 0.5, 1.0, 1.5, and 2.0% isoflurane. From these data, the authors obtained oxygen costs of PVA and Emmax in control subjects and in those receiving 2.0% isoflurane.

**Results:** Emmax, PVA, and VO₂, dose-dependently decreased by similar degrees (P < 0.05). Isoflurane did not change the oxygen costs at 1.5% and 2.0% concentration (P < 0.05).

**Conclusions:** These mechanorenergetic findings suggest that the primary method by which isoflurane decreases contractility is not by decreasing Ca²⁺ sensitivity of contractile proteins but mainly by decreasing Ca²⁺ handling in the excitation—contraction coupling without myocardial oxygen wasting effect. (Key words: Anesthetics, volatile: isoflurane. Heart, contractile protein: Ca²⁺ sensitivity. Contractility: Emmax, Myocardial oxygen consumption: pressure—volume area.)

ALTHOUGH volatile anesthetics depress myocardial contractility in a dose-dependent manner,¹,² multiple mechanisms contribute to this negative inotropism, although the relative contributions are not completely defined. Further, the effects of this negative inotropism on myocardial energetics remain unknown.

Isoflurane has less effect on contractility than halothane or enflurane at equipotent anesthetic concentrations.¹⁻³ It is well known that changes in Ca²⁺ sensitivity of contractile proteins cause marked effects on the cardiac energetics.⁴⁻⁶ A decreased Ca²⁺ sensitivity of contractile proteins by isoflurane has been proposed as a mechanism of its negative inotropism.⁷,⁸ A decreased Ca²⁺ sensitivity means that more free calcium is needed to activate the contractile protein to the same contractility level. Therefore, a decreased Ca²⁺ sensitivity may waste myocardial oxygen for Ca²⁺ handling during excitation—contraction (E-C) coupling, although to the best of our knowledge, little is known about the effect of isoflurane on cardiac energetics.
MECHANOENERGETICS OF ISOFLURANE ON LEFT VENTRICLE

The relation between left ventricular (LV) pressure-volume area (PVA, a measure of total mechanical energy) and myocardial oxygen consumption per beat (\(\text{VO}_2\)) has been used to analyze myocardial mechanoenergetics in the canine heart.\(^9\) Using this relation, a decreased Ca\(^{2+}\) sensitivity of contractile proteins would be detected as a higher \(\text{VO}_2\) at the same left ventricular contractile level. This phenomenon is seen in stunned and acidotic hearts.\(^9,10\)

The purpose of the present study was to assess the effect of isoflurane and its underlying mechanisms in excised, cross-circulated canine hearts using the framework of E\(_{\text{max}}\) (a contractile index) - PVA - \(\text{VO}_2\) relationship.

Materials and Methods

Surgical Preparation

All procedures in this study conformed to institutional and National Institutes of Health animal care guidelines. Experiments were performed on the excised, cross-circulated canine heart preparation, which has consistently been used in our laboratory.\(^11-15\) Surgical procedures were described in detail elsewhere.\(^14-16\) Briefly, two mongrel dogs (body weight, 6-23 kg) were anesthetized with pentobarbital sodium (25 mg/kg, intravenous) after premedication with ketamine hydrochloride (10 mg/kg, intramuscular) and intubated for artificial ventilation in each experiment. Anesthesia was maintained by fentanyl (10 \(\mu\)g·kg\(^{-1}\)·h\(^{-1}\)) and pentobarbital sodium (1 mg·kg\(^{-1}\)·h\(^{-1}\)). Both dogs were heparinized (10,000 units per dog). The larger dog was used as the metabolic supporter; common carotid arteries and right external jugular vein were cannulated and connected to arterial and venous cross-circulation tubes, respectively.

The chest of the smaller dog, as the heart donor, was opened midsternally. The arterial and venous cross-circulation tubes from the support dog were inserted into the left subclavian artery and the right ventricle (RV) via the right atrial appendage, respectively, of the donor dog. The heart-lung unit was isolated from the systemic and pulmonary circulation by ligating the descending aorta, brachiocephalic artery, inferior and superior vena cava, azygos vein, and finally bilateral pulmonary hili. The beating heart, supported by cross-circulation, was excised from the chest. Coronary perfusion of the excised heart was never interrupted during the preparation. Systemic hypotension during cross-circulation was prevented with indomethacin (5 mg/dog, intravenous).\(^11-15\)

The left atrium was opened, and all the LV chordae tendineae were cut. A thin latex balloon (unstressed volume, ~50 ml) mounted on a rigid connector was fitted into the LV, and the connector was secured at the mitral annulus. LV pressure was measured with a miniature pressure gauge (model P-7, Konigsberg Instruments, Pasadena, CA) placed inside the apical end of the balloon. The balloon, filled with water, was connected to a custom-made volume servopump (Air-Brown, Tokyo, Japan). LV volume was controlled and measured with the servopump. The LV epicardial electrocardiogram (ECG) was recorded with a pair of screw-in electrodes and was used to trigger the volume control and data acquisition systems.

Temperature of the heart was monitored and maintained near 36°C (35-37.0°C) throughout the experiment with heaters. The left atrium was electrically paced at 129 ± 8 (mean ± SD) beats/min, approximately 20% above a spontaneous heart rate to avoid arrhythmias. Systemic mean arterial blood pressure of the support dog, which was 100 ± 8 mmHg, served as coronary perfusion pressure of the excised heart. It was maintained stable in each experiment by slowly transfusing whole blood or dextran solution into the support animal. Arterial pH, \(\text{PO}_2\), and \(\text{PCO}_2\) of the support dog were repeatedly measured and maintained within their physiologic ranges with supplemental oxygen and intravenous sodium bicarbonate.

Contraction Mode

We used isovolumic contractions throughout this study. We considered that the contraction mode did not affect the present results because the \(\text{VO}_2\) - PVA relation is largely independent of the mode of contractions within physiologic loading conditions.\(^17\)

Oxygen Consumption

Total coronary blood flow was continuously measured with an electromagnetic flowmeter (MFJ-3200, Nihon Kohden, Tokyo, Japan) by placing an in-line flow probe (FF-040T, Nihon Kohden) in the coronary venous drainage tube from the RV. We neglected LV thebesian flow because of its small fraction (~3%) in the coronary flow.\(^4,18\) Coronary arteriovenous \(\text{O}_2\) content difference (AVO\(_{2}\)D) was continuously measured with a custom-made in-line
oximeter (PWA-2008, Sho Techinnica Inc., Chiba, Japan). The oximeter was calibrated against a blood O₂ content analyzer (IL-382 CO-oximeter, Instrumentation Laboratory Inc., Lexington, MA, USA) in each experiment.

Cardiac oxygen consumption per min was obtained as the product of coronary flow and AVO₂D. It was divided by heart rate to obtain VO₂ in steady state. The computation was performed on-line with a signal processor (T718, NEC San-ei, Tokyo, Japan). RV VO₂ was minimized by collapsing the RV by continuous hydrostatic drainage of the coronary venous return. The collapsed RV was assumed to have virtually zero PVA and no PVA-dependent VO₂. We assumed that isoflurane affected the RV and LV homogeneously. Hence, RV PVA-independent VO₂ was calculated by multiplying biventricular PVA-independent VO₂ in each contractile state with (RV weight/LV + RV weight). This RV PVA-independent VO₂ was subtracted from the total VO₂ to yield LV VO₂. At the end of each experiment, the LV, including the septum and the RV free wall, were separately weighed. They were 57 ± 10 g and 20 ± 5 g, respectively.

**Contractility (Emax)**

Left ventricular contractility was assessed by Emax. LV pressure P(t) and LV volume V(t) data were sampled at 2 ms intervals and processed with the signal processor. Emax of the LV was determined as the maximum ratio of P(t)/[V(t) - V₀]. V₉ was determined as the volume at which peak isovolumic pressure and hence PVA were zero (fig. 1A). Emax was normalized for LV weight and presented as mmHg·ml⁻¹·100 g. Tmax was determined as the time to Emax from the onset of the R wave of ECG. Tmax served as a measure of the duration of systole.

**Pressure-volume Area**

Pressure-volume area of each beat was calculated from the digitized P(t) and V(t) data with the signal processor using the algorithm described previously.

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Figure 1. Schematic illustration of the framework of the Emax–LV systolic pressure-volume area (PVA)–myocardial oxygen consumption per beat (VO₂) relation fully used in the present study. Emax, the slope of the end-systolic pressure-volume relation (ESPVR), sensitively reflects ventricular contractility, practically independent of ventricular loading conditions (A). PVA consists of potential energy (PE) alone in an isovolumic contraction which we used exclusively in the present study. LV PVA correlates linearly with LV VO₂ in a load-independent manner in a stable contractile state (B). The slope (a) of the VO₂–PVA relation at a constant Emax means the “oxygen cost of PVA” (C). In this relation, the PVA-independent VO₂ can be divided at the VO₂ intercept (b) of the VO₂–PVA relation into the PVA-independent and PVA-dependent VO₂ components (D). The PVA-dependent VO₂ component is considered to be related to crossbridge cycling (E). The PVA-independent VO₂ is considered to be primarily related to total Ca²⁺ handling in the excitation–contraction (E-C) coupling and basal metabolism. (F) indicates that the VO₂–PVA relationship shifts in a parallel manner with changes in Emax without a slope change. An upward or downward deviation of a VO₂–PVA data point from a data point on the baseline VO₂–PVA relation traverses multiple volume-loaded VO₂–PVA relations for different contractility levels (D). We called this steeper relation “the composite VO₂–PVA relation.” In this relation, the PVA-independent VO₂ of a data point increases or decreases in proportion to an increase or decrease in Emax, respectively.
Figure 2. Schematic illustration of a cross-circulated dog heart preparation. LV, left ventricle. LVP, LV pressure. ECG, electrocardiogram. AVo2D, arteriovenous O2 content difference. CF, coronary flow.

Briefly, PVA was calculated as the area in the P-V diagram surrounded by the end-systolic, end-diastolic P-V relations, and the systolic P-V trajectory, as schematically shown in figure 1A. PVA was normalized for 100 g LV and its dimensions are mmHg · ml · beat⁻¹ · 100 g⁻¹.

Coronary Vascular Resistance

Systemic arterial blood pressure (BP) of the support dog, which corresponded to coronary perfusion pressure of the heart, was measured in the left common carotid artery. Mean BP was divided by mean coronary flow to obtain coronary vascular resistance.

Infusion and Exhaust of Isoflurane

We placed membrane oxygenators (CAPIOX® II 08, Terumo Ashitaka Factory, Fujinomiya, Japan) into the arterial and venous cross-circulating circuits. One was used to add isoflurane into the coronary arterial blood, and the other was used to exhaust it from the venous blood (fig. 2). The arterial oxygenator was aerated by a 20% O2/75% N2/5% CO2 gas mixture at 3 l/min. Isoflurane, provided by a calibrated vaporizer, was added to the mixture gas supplying the arterial oxygenator. To minimize the anesthetic effect of isoflurane on the support dog, the coronary venous blood from the RV was exposed to room air, and the venous oxygenator was aerated by 100% oxygen at 6 l/min.

Measurement of Blood Isoflurane Concentration

Isoflurane concentrations of blood sampled from the inflow and outflow of the arterial oxygenator and from the outflow of venous oxygenator were determined in six dogs by gas chromatography established by Yamada et al.²⁰ Briefly, a 1-ml sample of arterial or venous blood was obtained from the cross-circulation tubes using an air-tight glass syringe. Five microliters of this blood was injected into a gas chromatograph equipped with a flame ionization detector, and the area under the curve was measured. Isoflurane blood concentration was determined by means of a calibration curve derived from appropriate standards. All analyses were performed in triplicate, and the mean values were calculated.

Experimental Protocol

Experiments were performed in 11 isolated hearts. After LV pressure, LV volume, coronary flow, and AVo2D had stabilized, experimental measurements were started.

The experimental protocol consisted of six runs: control volume run, isoflurane inotropism run, control-calcium inotropism run, isoflurane volume run, isoflurane-calcium inotropism run, and KCl arrest run.

1. Control volume run: We obtained a volume-loaded Vo2-PVA relationship (fig. 1B) of steady-state isovolumic contractions produced at 4–7 different LV volumes between 6–28 ml. We waited for 2 or 3 min after each change in LV volume until the cardiac variables reached a new steady state.

2. Isoflurane and control-calcium inotropism runs: After the control volume run, we performed these two inotropism runs to obtain different types of Vo2-PVA relationships at a single, fixed LV volume. Either isoflurane or calcium (1% CaCl2) was infused into the coronary arterial blood to obtain varied Emax, Vo2, and PVA at a preset constant LV volume (25.7 ± 3.9 ml/100 g). The infusion rate of each drug was increased in steps every 3–5 min. This interval was long enough for those variables to reach a new steady state. In 6 of the 11 hearts (isoflurane-first group), we first depressed Emax to a value equal to approximately half of baseline by increasing the isoflurane infusion concentration in steps from 0 to 1.0, 1.5, and 2.0%. We then waited for 10–15 min after the end of isoflurane inotropism run until Emax recovered to the baseline level. We next increased
Emax to a value approximately double that of baseline in steps by increasing the calcium infusion rate. In the other five hearts, order was reversed (calcium-first group). The maximum infusion rate of calcium was 0.095 ± 0.026 mmol/min. This corresponded to an increase in blood calcium concentration of 1.1 ± 0.4 mM at a coronary blood flow of 94 ± 24 ml/min. We previously measured serum calcium concentration in the three support dogs and found it to be 1.29 ± 0.09 mM. The blood calcium concentration resulting from this infusion thus reached a value of up to twice normal. We used calcium rather than a catecholamine as a positive inotropic agent because calcium increases contractility without any effect on complex phosphorylation processes of contractile proteins.

3. Isoflurane volume run: LV pressure, LV volume, coronary flow and AVO2,D were all stabilized after 5 min of 2.0% isoflurane infusion. Then, we obtained another VO2-PVA relation (fig. 1C) by varying LV volume in a similar manner to the control volume run during the isoflurane infusion.

4. Isoflurane-calcium inotropism run: After the isoflurane volume run, we fixed LV volume at the same preset volume as in the control-calcium run. Then, we repeated calcium infusion during 2.0% isoflurane in a similar manner to the control-calcium run until Emax increased to the preisoflurane control level.

5. KCl arrest run: Finally, in 6 of the 11 hearts, the KCl arrest run was performed in a new steady state 15–20 min after stopping any drug infusion and during 2.0% isoflurane infusion. The heart was arrested at Vo by a continuous infusion of 0.3 mM KCl solution at 1–2.5 ml/min into the coronary arterial tube. When coronary flow and AVO2,D reached steady state during KCl arrest, VO2 was measured as basal metabolic VO2.

Data Analyses

VO2–PVA relations. VO2 and PVA data in either control or isoflurane volume run were subjected to linear regression analysis to obtain a volume-loaded VO2–PVA relation (fig. 1B): VO2 = aPVA + b, where a is the slope of the regression line, and b is the VO2 intercept. aPVA represents PVA-dependent VO2, and b represents PVA-independent VO2. Coefficient a was called oxygen cost of PVA. We consider that the PVA-independent VO2 is a constant independent of LV end-diastolic volume at a given contractility.

VO2 and PVA data in each inotropism run were also subjected to linear regression analysis to obtain a composite VO2–PVA relation (fig. 1D).

PVA-independent VO2. The PVA-independent VO2 for each Emax level during either isoflurane-calcium or control-calcium run was calculated as the VO2 minus PVA-dependent VO2 for the respective PVA. This PVA-independent VO2 was calculated as the product of the same slope value a as the baseline a and PVA of this contraction. The PVA-independent VO2 at each Emax level was calculated as LV VO2 minus aPVA. In this calculation, we assumed that slope a remained constant at all Emax levels, based on the parallelism of the VO2–PVA relation. The parallelism was confirmed in this study (fig. 3).

Oxygen cost of Emax. The relation between PVA-independent VO2 values and the corresponding Emax values in either the isoflurane-calcium or control-calcium inotropism run was obtained by regression analysis in each heart (fig. 1E). The slope c of the regression line was identified as the oxygen cost of Emax in each run. Its dimensions are ml O2·ml·mmHg−1·beat−1·100 g−2. The y intercept d of this regression line is the PVA-independent VO2 extrapolated to zero Emax.
MECHANOENERGETICS OF ISOFLURANE ON LEFT VENTRICLE

Table 1. Abbreviations and Corresponding Definitions

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tr>
<td>RV</td>
<td>Right ventricular</td>
</tr>
<tr>
<td>LV</td>
<td>Left ventricular</td>
</tr>
<tr>
<td>LVP</td>
<td>LV pressure</td>
</tr>
<tr>
<td>E&lt;sub&gt;max&lt;/sub&gt;</td>
<td>A contractility index; maximal elastance</td>
</tr>
<tr>
<td>PVA</td>
<td>Systolic pressure-volume area</td>
</tr>
<tr>
<td>V&lt;sub&gt;O2&lt;/sub&gt;</td>
<td>Myocardial oxygen consumption per beat</td>
</tr>
<tr>
<td>AVO&lt;sub&gt;O2&lt;/sub&gt;D</td>
<td>Coronary arteriovenous oxygen content difference</td>
</tr>
<tr>
<td>CVR</td>
<td>Coronary vascular resistance</td>
</tr>
<tr>
<td>T&lt;sub&gt;max&lt;/sub&gt;</td>
<td>Time from the onset of contraction to E&lt;sub&gt;max&lt;/sub&gt;</td>
</tr>
<tr>
<td>SL</td>
<td>Cardiac sarcolemma</td>
</tr>
<tr>
<td>SR</td>
<td>Cardiac sarcoplasmic reticulum</td>
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</table>

Statistics

The V<sub>O2</sub>-PVA regression lines were compared between isoflurane-calcium and control-calcium inotropic runs and between control and isoflurane volume runs in each heart by analysis of covariance (ANCOVA). The significance of the differences in their slopes and elevations was tested by the F test. ANCOVA also was used to compare the regression lines of PVA-independent V<sub>O2</sub> on Emax between isoflurane-calcium and control-calcium inotropic runs. Comparison of paired mean values was performed by Student's paired t test. Statistical analysis of hemodynamic parameters in table 1 was performed with multiple analysis of variance (ANCOVA) for repeated measures and followed by application of Schefte's F test. A value of P < 0.05 was considered statistically significant. All data are expressed as mean ± SD.

Results

Blood Isoflurane Concentration

The isoflurane arterial coronary blood concentration was proportional to the percent value of isoflurane provided by the vaporizer. The concentrations were 289 ± 34, 412 ± 40, and 589 ± 92 μM during infusion of 1.0, 1.5, and 2.0% isoflurane, respectively. These concentrations corresponded to the blood concentrations reported in previous paper. The concentration of isoflurane in blood sampled from the outflow of the venous oxygenator was below the limit of measurement of our gas chromatography (54 μM). No isoflurane was detected from the blood sampled from the inflow of the arterial oxygenator.

Control Volume Run

Control Emax values were 4.8 ± 0.94 mmHg · ml<sup>-1</sup> · 100 g (n = 11 hearts). In every heart tested, V<sub>O2</sub> increased linearly with increases in PVA in control volume run. Their correlation coefficients were close to unity (0.989 ± 0.010) in the control contractile state. The slope a and V<sub>O2</sub> intercept b of the control V<sub>O2</sub>-PVA relation were (1.69 ± 0.26) × 10<sup>−3</sup> ml O<sub>2</sub> · mmHg<sup>−1</sup> · ml<sup>−1</sup> and 0.0231 ± 0.0077 ml O<sub>2</sub> · beat<sup>−1</sup> · 100 g<sup>−1</sup>, respectively.

Effects of Isoflurane on Mechanoenergetics and Other Parameters

Figure 3 shows a representative set of tracings of LV isovolumic pressure (LVP), LV volume (LVV), ECG, coronary flow, and AVO<sub>O2</sub>D during isoflurane inotropic run at a constant LV volume in one heart of the isoflurane-first group. The concentration of isoflurane infused into the coronary artery was increased in steps from 0 up to 2.0% (figure 4). Isoflurane at the maximal concentration decreased Emax from 4.1 to 2.1 mmHg · ml<sup>−1</sup> · beat<sup>−1</sup> · 100 g, PVA from 731 to 360 mmHg · ml · 100 g<sup>−1</sup>, and V<sub>O2</sub> from 0.0394 to 0.0293 ml O<sub>2</sub> · beat<sup>−1</sup> · 100 g<sup>−1</sup>. All other hearts showed similar results.

Table 2 compares Emax, PVA, V<sub>O2</sub>, and other cardiohemodynamic parameters obtained at a constant LV volume (25.4 ± 3.8 ml/100 g) before (isoflurane 0%) and during isoflurane inotropic run in the six hearts of the “isoflurane-first group.” At a constant LV volume, Emax, PVA, and V<sub>O2</sub> decreased dose-dependently by similar degrees. However, coronary flow rather increased at 1.5 and 2.0% concentration (P < 0.05) by different degrees among individual hearts despite linear and uniform decreases in Emax (P < 0.05; fig. 5). AVO<sub>O2</sub>D was significantly decreased by 1.0, 1.5, and 2.0% concentration of isoflurane. Tmax was also significantly but slightly increased. LV enddiastolic pressure did not exceed 18 mmHg in any volume runs. In the five hearts of the “calcium-first group,” we also obtained similar results.

Comparison of the Effects on Oxygen Cost of PVA Between Control and Isoflurane Volume Runs

Figure 3 shows the V<sub>O2</sub>-PVA relations obtained in control and isoflurane volume runs in a heart. The slope was not significantly different between the two.
volume runs by ANCOVA. We compared the slopes of the $\text{VO}_{2}$-PVA relations between control and isoflurane volume runs in 11 hearts. There were no significant differences in slopes between the two $\text{VO}_{2}$-PVA relations in any of the 11 hearts (ANCOVA). The mean values of the slopes were virtually the same in the two runs (control volume run: $(1.69 \pm 0.26)$ × 10$^{-5}$; isoflurane volume run: $(1.70 \pm 0.24)$ × 10$^{-5}$ ml O$_2$·mmHg$^{-1}$·ml$^{-1}$] (not significant by paired t test). These results indicate that isoflurane did not affect the oxygen cost of PVA. The mean $\text{VO}_{2}$ intercept value significantly decreased in isoflurane volume run from $0.0231 \pm 0.0077$ to $0.0135 \pm 0.0059$ ml O$_2$·beats$^{-1}$·100 g$^{-1}$ (paired t test).

Table 2. Effects of Isoflurane on Left Ventricular Mechanoenergetics and Coronary Circulation in Six Canine Hearts of the
Isoflurane first Group

<table>
<thead>
<tr>
<th>Mechanoenergetic and Coronary Parameters</th>
<th>0%</th>
<th>1.0%</th>
<th>1.5%</th>
<th>2.0%</th>
</tr>
</thead>
<tbody>
<tr>
<td>LVP (mmHg)</td>
<td>72 (13)</td>
<td>61 (11)*</td>
<td>48 (8.1)*†</td>
<td>42 (7.4)*†</td>
</tr>
<tr>
<td>$E_{max}$ (mmHg·ml$^{-1}$·100 g)</td>
<td>6.1 (2.3)</td>
<td>5.0 (2.1)*</td>
<td>4.1 (1.8)*</td>
<td>3.5 (1.5)*†</td>
</tr>
<tr>
<td>PVA (mmHg ml·beat$^{-1}$·100 g$^{-1}$)</td>
<td>507 (176)</td>
<td>437 (153)*</td>
<td>338 (99)*†</td>
<td>294 (90)*†</td>
</tr>
<tr>
<td>$\text{VO}_{2}$ (ml O$_2$·beat$^{-1}$·100 g$^{-1}$)</td>
<td>0.0419 (0.0142)</td>
<td>0.0349 (0.0129)*</td>
<td>0.0303 (0.0111)*</td>
<td>0.0234 (0.0089)*†</td>
</tr>
<tr>
<td>CF (ml·min$^{-1}$·100 g$^{-1}$)</td>
<td>128 (76)</td>
<td>141 (71)†</td>
<td>170 (83)*</td>
<td>210 (83)*†</td>
</tr>
<tr>
<td>AVO$_2$D (vol%)</td>
<td>5.1 (2.0)</td>
<td>4.1 (1.9)*</td>
<td>2.4 (1.2)*†</td>
<td>1.4 (0.6)*‡‡</td>
</tr>
<tr>
<td>CVR (mmHg·ml$^{-1}$·min$^{-1}$·100 g)</td>
<td>1.13 (0.67)</td>
<td>0.88 (0.50)</td>
<td>0.66 (0.35)*</td>
<td>0.52 (0.26)*†</td>
</tr>
<tr>
<td>$T_{max}$ (ms)</td>
<td>179 (26)</td>
<td>184 (30)</td>
<td>188 (30)*</td>
<td>193 (32)*†</td>
</tr>
</tbody>
</table>

LV = left ventricular; LVP = LV pressure; $E_{max}$ = an LV contractility index; PVA = LV systolic pressure-volume area; $\text{VO}_{2}$ = LV myocardial oxygen consumption per beat; CF = coronary flow; AVO$_2$D = coronary arteriovenous oxygen content difference; CVR = coronary vascular resistance, $T_{max}$ = time from the onset of contraction to $E_{max}$. All these parameters were measured at a constant LV volume of 25.4 (3.8) ml·100 g$^{-1}$. Values are mean (±SD).

*Significantly different ($P < 0.05$) from 0% isoflurane.
†Significantly different ($P < 0.05$) from 1.0% isoflurane.
‡‡Significantly different ($P < 0.05$) from 1.5% isoflurane.
Figure 5. Individual values of coronary flow (CF) and Emax in six hearts of isoflurane first group are plotted as percent of control (0% isoflurane) during isoflurane infusion. Mean values of CF and Emax during 0, 1.0, 1.5, and 2.0% isoflurane are connected by lines. * Significantly different ($P < 0.05$) from the control.

**Comparison of Composite $V_{O_2}$–PVA Relations Between Control-calcium and Isoflurane-calcium Inotropism Runs**

Figure 6 compares the composite $V_{O_2}$–PVA relations in control-calcium (fig. 6A) and isoflurane-calcium inotropism runs (fig. 6B) obtained in the same heart. As Emax gradually increased during both calcium runs, the $V_{O_2}$–PVA data points deviated right upward at a constant LV volume (23.6 ml/100 g) from the corresponding volume-loaded $V_{O_2}$–PVA relations of control and isoflurane volume runs. The control-calcium and the isoflurane-calcium inotropism runs yielded nearly superimposable linear regression lines. ANCOVA showed differences in neither the slope nor elevation between the lines. The mean slope values in the 11 hearts were not significantly different between the two calcium inotropism runs (paired $t$ test). The mean extrapolated $V_{O_2}$ intercept values also showed no difference between the two calcium inotropism runs (paired $t$ test).

**PVA-independent $V_{O_2}$–Emax Relations**

Figure 7A plots PVA-independent $V_{O_2}$ values against corresponding Emax values during control-calcium and isoflurane-calcium inotropism runs in the same heart as shown in figure 6. PVA-independent $V_{O_2}$ values were calculated for corresponding Emax values as explained in the Data Analyses section. In this heart, PVA-independent $V_{O_2}$ increased linearly with increases in Emax by calcium, with correlation coefficients close to unity.

Figure 6. $V_{O_2}$–PVA relations in the control volume run (crosses and dotted line; $V_{O_2} = 1.85 \times 10^{-3}$ PVA + 0.0228, $r = 0.993$) and the control-calcium inotropism run (open circles and solid line; $V_{O_2} = 3.10 \times 10^{-3}$ PVA + 0.0124, $r = 0.985$), and the isoflurane volume run (open squares and dotted line; $V_{O_2} = 1.76 \times 10^{-3}$ PVA + 0.0191, $r = 0.999$) and the isoflurane-calcium inotropism run (closed circles and solid line; $V_{O_2} = 3.10 \times 10^{-3}$ PVA + 0.0129, $r = 0.999$; B) in the same heart as figure 4. The $V_{O_2}$–PVA composite relations during the calcium inotropism runs during control and 2.0% isoflurane were steeper than the control and isoflurane volume-loaded $V_{O_2}$–PVA relations. The composite relation in the isoflurane-calcium inotropism run is nearly superimposable on the composite relation in the control-calcium inotropism run.
(0.965 for control-calcium and 0.992 for isoflurane-calcium inotropism run). No significant difference was found in either the slope or elevation between these two PVA-independent VO₂-Emax relations (ANCOVA). Therefore, the oxygen cost of Emax was virtually the same between the two calcium inotropism runs in control situations and during isoflurane administration.

Similar linear PVA-independent VO₂-Emax relations to figure 7A were obtained for the two calcium inotropism runs in all the other 10 hearts. Correlation coefficients of the relations were close to unity (mean, 0.979 for control-calcium and 0.977 for isoflurane-calcium inotropism run). ANCOVA showed no significant differences in their slopes (oxygen cost of Emax) between the control-calcium and isoflurane-calcium inotropism runs in the 11 hearts.

Figure 7B compares mean values of oxygen costs of Emax in the control-calcium and isoflurane-calcium inotropism runs in the 11 hearts. There was no significant difference in oxygen costs of Emax between the two calcium runs in control and during isoflurane by paired t test.

**Comparison of Basal Metabolism by KCl Arrest Runs**

Basal metabolic VO₂ during KCl arrest was $1.08 \pm 0.33$ ml O₂·min⁻¹·100 g⁻¹ in control and $1.07 \pm 0.30$ ml O₂·min⁻¹·100 g⁻¹ during 2.0% isoflurane infusion. Thus, isoflurane did not affect VO₂ for basal metabolism (paired t test).

**Discussion**

**Contractility**

Myocardial contractility (Emax) decreased in a linear fashion to approximately 60% of control at an isoflurane concentration of 2.0%, accompanied by decreased PVA and VO₂. Isoflurane increased coronary flow in a dose-dependent manner up to nearly twice control. Goto et al.⁵⁷ reported that approximately 200% increase in coronary flow by adenosine caused 18% increase in Emax (Gregg’s phenomenon; increase in LV contractility as a result of increase in coronary flow) using the Emax-PVA-VO₂ framework in rabbit hearts. In the present study, in canine hearts, as shown in figure 5, Emax linearly and uniformly decreased in a dose-depen-
dent manner, even though Gregg’s phenomenon substantially affects the negative inotropism of isoflurane.

**Oxygen Cost of PVA**

The oxygen cost of PVA was not affected by isoflurane. It has been reported that the oxygen cost of PVA remained unchanged during negative and positive inotropic interventions with propranolol,17 catecholamines,14,28 calcium,14,28 capsaicin,12 or pentobarbital sodium13 in normal canine hearts. The oxygen cost of PVA was increased in the hyperthyroid rabbit heart27 (accompanied with a marked increase in the myosin isofrom V1 to V3 ratio) and decreased in postischemic stunned4 and acidicotic canine hearts.10 The oxygen cost of PVA has been considered to reflect the product of the ATP-to-Vo2 coupling ratio in mitochondria (mitochondrial oxidative phosphorylation) and PVA-to-ATP coupling ratio in the contractile machinery (crossbridge cycling). Therefore, the unchanged oxygen cost of PVA indicates that the overall myocardial efficiency of energy use from oxygen to total mechanical energy (i.e., PVA via ATP is not changed by isoflurane.

**PVA-independent Vo2**

In the present study, PVA-independent Vo2 proportionally and significantly decreased with decreased Emax by isoflurane. The PVA-independent Vo2 reflects the Vo2 fraction for nonmechanical activities, primarily consisting of basal metabolism and E-C coupling. Because KCl-arrest basal metabolic Vo2 was not decreased by isoflurane, the decreased PVA-independent Vo2 by isoflurane is mainly a result of a decrease in the E-C coupling energy.4,22,23,25 The energy use of E-C coupling occurs primarily at the sarcoplasmic reticulum (SR) Ca2+-ATPase and secondarily at the sarcolemmal (SL) Na+, K+-ATPase mediated via Na+–Ca2+ exchange to decrease cytosolic-free Ca2+ concentration and to relax the myocardium after contraction in each cardiac cycle.5,5

**Oxygen Cost of Emax; Ca2+ Sensitivity of the Contractile Protein during Isoflurane**

Oxygen cost of Emax is based on the following relations.4,22,23,25 First, the Ca2+-ATP coupling ratios of SR Ca2+-ATPase (2 Ca2+:1 ATP) and SL Na+, K+-ATPase mediated via Na+–Ca2+ exchange (1 Ca2+:1 ATP) are constant. Second, the amount of calcium involved in the E-C coupling is proportional to myocardial contractility unless the sensitivity of the contractile machinery to Ca2+ changes. If isoflurane decreases Ca2+ sensitivity of the contractile proteins, more free calcium should be needed to activate the contractile protein to the same contractility level. For the SR to sequester the additional calcium, more ATP should be hydrolyzed by the SR Ca2+-ATPase and SL Na+, K+-ATPase, leading to a disproportionate increase in PVA-independent Vo2 (oxygen waste) at a given myocardial contractility.1–6

The present results showing almost the same oxygen cost of Emax between the control-calcium and isoflurane-calcium inotropism runs suggest that isoflurane decreased neither Ca2+ sensitivity of the contractile protein nor the Ca2+ handling ATP ratio in the SR and SL. Therefore, isoflurane would have an advantage of no waste of myocardial oxygen consumption.

Because isoflurane did not change the Ca2+ sensitivity, the decreased Emax by isoflurane is probably a result of a decreased amount of Ca2+ handled in the E-C coupling. Some studies showed that isoflurane decreased the amount of Ca2+ handled in E-C coupling by inhibiting Ca2+ influx through the SL calcium channels5,50 and decreasing the total capacity of SR for Ca2+ by altering calcium uptake and release.31,32 However, the mechanisms of the decreased amount of Ca2+ handled in E-C coupling are beyond the goal of the present study. Therefore, we only consider the two possible sites of the myocyte, the SL and SR, as the main targets of isoflurane.

**Coronary Effect of Isoflurane**

The dilatation of the coronary artery induced by isoflurane is well known. However, the magnitude of this dilatation effect is inconsistent among many studies.26,53,54 In our study, total coronary flow was measured, and 1.5 and 2.0% isoflurane significantly increased coronary flow and hence decreased coronary vascular resistance (table 2) despite a reduced myocardial oxygen demand. AVO2,D decreased reciprocally to coronary flow.

**No Influence via the Support Dog**

In the present study, fentanyl and pentobarbital were used as the basal anesthesia for the support dogs. We have already reported that neither pentobarbital13 nor fentanyl15 qualitatively affected cardiac mechanoenergetics. Further, the anesthetic level of the support dog was maintained constant throughout the experiment to avoid any inconsistent influences of fentanyl and pento-
barbital on the mechanoenergetic effects of intracoronal isoflurane in the excised heart.

Isoflurane in the cross-circulated coronary venous blood from the RV was exhausted first by exposure to room air at the blood reservoir, next by the venous oxygenator, and finally by the lung of the support dog. No isoflurane was detected in the systemic arterial blood sampled from the inflow of the arterial oxygenator. Consequently, intracoronal isoflurane had no chance to affect any hemodynamics such as systemic blood pressure in the support dog. Therefore, we were able to detect the direct effects of intracoronal isoflurane on LV mehanoenergetics without any indirect effects via the support dog.

**Conclusion**

We investigated mechanoenergetic effects of isoflurane using the framework of the Emax-PVA-VO$_2$ relation in the excised, cross-circulated (blood-perfused) canine left ventricle. Isoflurane depressed Emax, PVA, and VO$_2$ (PVA-dependent and PVA-independent) dose-dependently but did not change the oxygen costs of PVA and Emax. These findings indicate that the negative inotropic of isoflurane resembles that of other negative inotropic agents such as capsaicin, pentobarbital, and $\beta$-blockers in terms of cardiac mehanoenergetics.

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