Ryanodine Wastes Oxygen Consumption for Ca\(^{2+}\) Handling in the Dog Heart
A New Pathological Heart Model

Toshiyuki Takasago, Yoichi Goto, Osamu Kawaguchi, Katsuya Hata, Akio Saeki, Takehiko Nishioka, and Hiroyuki Suga
Departments of Cardiovascular Dynamics and Internal Medicine, National Cardiovascular Center, Osaka, 565 Japan; and Second Department of Physiology, Okayama University Medical School, Okayama, 700 Japan

Abstract
Ryanodine (RYA) at a low concentration (several tens of nM) is known to selectively bind to Ca\(^{2+}\) release channels in sarcoplasmic reticulum (SR) and to fix them open. The present study was designed to investigate the effects of the selective change in Ca\(^{2+}\) release channel activity on cardiac mechanoenergetics as a model of Ca\(^{2+}\)-leaky SR observed in pathological hearts. We analyzed the negative inotropic effect of RYA at a low concentration (up to 30±13 nM) on left ventricular (LV) mechanoeenergetics using frameworks of LV \(E_{\text{max}}\) (a contractility index) and the myocardial oxygen consumption (LV \(V_{02}\))—systolic pressure-volume area (PVA) (a measure of total mechanical energy) relation in 11 isolated, blood-perfused dog hearts. RYA significantly decreased \(E_{\text{max}}\) by 42%, whereas PVA-independent \(V_{02}\) remained disproportionately high (93% of control). This oxygen-wasting effect of RYA was quite different from ordinary inotropic drugs, which alter \(E_{\text{max}}\) and PVA-independent \(V_{02}\) proportionally. The present result suggests that RYA suppresses force generation of cardiac muscle for a given amount of total sequestered Ca\(^{2+}\) by SR in a similar way to myocardial ischemia and stunning. We speculate about the underlying mechanism that RYA makes SR leaky for Ca\(^{2+}\) and thereby wastes energy for Ca\(^{2+}\) handling by SR. (J. Clin. Invest. 1993. 92:823-830.) Key words: cardiac energetics • \(E_{\text{max}}\) • calcium transient • myocardial oxygen consumption • pressure-volume area

Introduction
Recent studies have indicated that dysfunction in the Ca\(^{2+}\) transport system of the sarcoplasmic reticulum (SR)\(^1\) plays an important role in pathophysiological states such as ischemic, acidotic, and stunned hearts (1-7). It has been proposed that dysfunction of not only Ca\(^{2+}\) uptake (2, 5) but also Ca\(^{2+}\) release (2-4) and Ca\(^{2+}\) permeability of the SR (1) contribute to this SR dysfunction. The SR Ca\(^{2+}\) release channel has been shown to have a single Ca\(^{2+}\) release channel activity, which is regulated by the cellular components such as Ca\(^{2+}\), Mg\(^{2+}\), and ATP (8-11). Although SR Ca\(^{2+}\) release has been assumed to play a central role in the regulation of cardiac contractility in both physiological and pathophysiological states, the relationship between the change of the Ca\(^{2+}\) release channel activity and cardiac contractility is still unclear.

On the other hand, our studies on cardiac energetics have revealed a unique relationship between cardiac contractility and energy utilization for excitation-contraction-relaxation coupling (13-20). Namely, increases in ventricular contractility by CaCl\(_2\), catecholamines, or other cardiotonic agents (OPC-8212 [15], ouabain [16]), denopamine [17], and Amrinone [18]) linearly correlate with changes in the fraction of oxygen consumption that we consider primarily related to the total intracellular Ca\(^{2+}\) handling sequesetered by Ca\(^{2+}\)-pump ATPase and independent of mechanical contraction. Therefore, we consider that the determination of the nonmechanical energy consumption enables us to indirectly but quantitatively analyze changes in the total amount of calcium cycling with each beat (total Ca\(^{2+}\) handling) in myocardium under various inotropic interventions.

The present study was designed to investigate the effects of the selective change in Ca\(^{2+}\) release channel activity on cardiac mechanoenergetics in a ryanodine (RYA)-treated heart as a pathological heart model with Ca\(^{2+}\)-leaky SR as observed in ischemic, acidotic, and stunned hearts (1-7). RYA specifically binds to the open state Ca\(^{2+}\) release channel in cardiac SR, fixing it open at a low concentration (several tens of nM), and makes SR leaky for Ca\(^{2+}\) (9-11). We fully used the relationship between ventricular contractility and nonmechanical energy consumption in the excised, cross-circulated (blood-perfused) dog heart. We obtained results suggesting that the negative inotropicism of RYA primarily relates to suppression of the force generation of cardiac muscle for a given amount of total sequestered Ca\(^{2+}\) by SR.

Theoretical considerations
Left ventricular contractile index (LV \(E_{\text{max}}\)) and the myocardial oxygen consumption-systolic pressure-volume area (LV \(V_{02}\)-PVA) relationship have the following physiological significance: \(E_{\text{max}}\) • the slope of the end-systolic pressure-volume (P-V) relation, sensitively reflects ventricular contractility practically independent of ventricular loading conditions except for a situation with large changes in ejection fraction (Fig. 1 A) (12, 14, 21, 22). PVA is a measure of the total mechanical energy generated by a ventricular contraction. PVA is quantified by the area in the P-V diagram that is bounded by the end-systolic P-V relation line, end-diastolic P-V relation curve, and systolic P-V trajectory (Fig. 1 A) (12-14). In a stable contractile state, PVA linearly correlates with LV \(V_{02}\) in a load-independent manner in a stable contractile state (Fig. 1 B). The reciprocal of the slope of the \(V_{02}\)-PVA relation at a constant \(E_{\text{max}}\) means the “contractile efficiency” (14). \(V_{02}\) can be di-
Figure 1. Schematic illustration of LV systolic pressure-volume area (PVA, A). LV VO2-PVA relation in the volume-loading run (B), volume-loaded V02-PVA relations at different E_max levels (C), VO2-PVA relation in the inotropism run (D), and the relation between PVA-independent VO2 and E_max to determine oxygen cost of E_max (E). A shows LV systolic PVA in the P-V diagram. PVA consists of both potential energy (PE) and external work (EW) in an ejecting contraction and PE alone in an isovolumic contraction (see Fig. 3). PE and EW are energetically equivalent. B shows the volume-loaded VO2-PVA relation in a baseline contractile state (thick dashed line) and VO2 components. C shows the volume-loaded VO2-PVA relations in the baseline contractile state and in altered contractile states (thick dashed diagonal lines). D shows an upward or downward deviation of a VO2-PVA data point (solid circle) from a baseline VO2-PVA relation (open circle) with an increase or a decrease in E_max, respectively, at a constant LV volume during each inotropism run. We called this steeper VO2-PVA relation the “composite relation” (solid line). VO2 of this point can be divided into two components: PVA-dependent VO2 corresponding to the PVA of the contraction and PVA-independent VO2, which is the sum of the same basic PVA-independent VO2 (equal to b in B) and the change in PVA-independent VO2. E shows the relation between PVA-independent VO2 and E_max. The slope (c) of this relation is the oxygen cost of contractility in terms of E_max, and the y-intercept (d) of this relation indicates the PVA-independent VO2 extrapolated to zero E_max (see text for more details).

The VO2-PVA relation is elevated in a parallel manner with an enhancement of E_max (Fig. 1 C) (14). When E_max is increased or decreased by a positive or negative inotropic intervention, respectively, at a constant LV volume, a VO2-PVA point deviates upward or downward from a baseline VO2-PVA relation and forms a new, steeper VO2-PVA relation, which traverses multiple volume-loaded VO2-PVA relations for different contractility levels (Fig. 1 D). We call such a steeper VO2-PVA relation obtained during changing inotropism “the composite relation” (19, 20). In the inotropism run, PVA-independent VO2 increases or decreases with an increase or decrease in E_max, respectively (Fig. 1 E) (13, 14, 19). The slope of the relation between the PVA-independent VO2 and E_max means the “oxygen cost of contractility” (Fig. 1 E) (20). These features of the VO2-PVA-E_max relation have been thoroughly reviewed by Suga (14).

From the results of previous studies, it has been considered that the contractility-dependent changes in the PVA-independent VO2 quantitatively reflect changes in energy expenditure for the total Ca2+ handling in myocardium (13-20). This relation depends on the assumption of the stoichiometry that 1 mol of ATP is hydrolyzed for 2 mol of Ca2+ taken up by the Ca2+ pump ATPase (14, 16).

We hypothesized the effects of RYA on the composite relation as follows (Fig. 2): If RYA changes contractility in a similar way to ordinary inotropic drugs by simply decreasing the total amount of Ca2+ handling, the RYA-composite relation (shown by the diagonal solid arrow) will be superimposed on the same line as the CaCl2-composite relation (shown by the diagonal dashed arrow) (Fig. 2 A). In contrast, if the negative inotropism of RYA is mainly due to the open-fix effect on the Ca2+ release channel of SR (9-11), the PVA-independent VO2 will decrease very little whereas only the PVA-dependent VO2 will decrease with reduced PVA. In this manner, VO2 for each point on the composite relation will be higher than the originally expected value and, hence, the RYA-composite relation will be flatter than the CaCl2-composite relation (Fig. 2 B).

**Methods**

**Surgical preparation**

Experiments were performed on the excised cross-circulated dog heart preparation as previously described in detail (13). Briefly, two mongrel dogs (11-20 kg) were anesthetized with sodium pentobarbital (30 mg/kg, i.v.) after premedication with ketamine hydrochloride (7 mg/kg, i.m.). Both dogs were heparinized (1,000 U/kg body wt). The heart donor dog (body wt 13.0±1.1 [SD] kg) was thoracotomized under artificial ventilation. The left subclavian artery and the right ventricle were cannulated and connected to the bilateral common carotid arteries and external jugular vein of the metabolic support dog (16.3±2.0 kg), respectively, with the cross-circulation tubes. All systemic and pulmonary vascular connections to the heart were ligated. The heart was excised from the chest after cross-circulation was started without an interruption of coronary perfusion.

The left atrium was opened and the chordae tendineae were cut. A rubber balloon with an unstressed volume of 60 ml was fitted into the LV chamber. The balloon was connected to our custom-made volume servo pump (International Servo Data, Tokyo, Japan) and primed with water. A miniature pressure gauge (P-7; Konigsberg Instruments, Inc., Pasadena, CA) was placed inside the apical end of the balloon to measure LV pressure.

The temperature of the heart was kept at 35-37°C with heaters. Heart rate was fixed constant in each heart at 145±9 (135-165) beats per min by left atrial pacing. Coronary arterial pH, PO2, and PCO2 were repeatedly measured and corrected to normal as needed.

---

![Figure 2. Schematic illustrations of alternatively possible effects of RYA on the composite VO2-PVA relation in inotropism run (see text for more details).](image-url)
**Contraction mode**

We used isovolumic contractions in all of these runs. We consider that the contraction mode does not essentially affect the present results, as described in the "Theoretical considerations" section (14, 21, 24).

**Oxygen consumption**

Total coronary blood flow was measured with an electromagnetic flowmeter in the coronary venous cross-circulation tube. Coronary arteriovenous oxygen content difference (AVo2D) was measured continuously with our custom-made oxygen content difference analyzer (model PWA-200S; Erma, Inc, Tokyo, Japan) (14). The oximeter was calibrated against an oxygen content analyzer (model IL-282 CO-Oximeter; Instrumentation Laboratory, Lexington, MA). Cardiac oxygen consumption was obtained as the product of the total coronary blood flow and AVo2D. It was divided by heart rate to obtain VO2 per beat in steady state. These computations were performed on-line with a signal processor (model 771R; NEC San-ei, Tokyo, Japan).

Right ventricular (RV) VO2 was minimized by keeping the right ventricle collapsed with continuous hydrostatic drainage of the coronary venous return. The collapsed right ventricle was assumed to have virtually zero PVA and hence no PVA-dependent VO2. The RV component of the PVA-independent VO2 was calculated by multiplying biventricular PVA-independent VO2 in each contractile state by (RV weight)/(LV + RV weight). PVA-independent LV VO2 was then obtained by subtracting the RV component from biventricular PVA-independent VO2 in each contractile state. Postmortem LV weight (the LV free wall plus the septum) was 72.6±9.8 g. RV weight (the RV free wall only) was 21.7±5.1 g. RV/(LV + RV) weight ratio was 0.23±0.04.

**Contractility index (Emax)**

LV pressure (P(t)) and volume (V(t)) data were sampled at 2-ms intervals and processed with the signal processor. Emax of the LV was determined as the ratio of P(t)/[V(t) - V0] (25). V0 is the volume at which peak isovolumic pressure and PVA are zero. The peak positive and negative values for the first derivative of LV pressure (max dp/dt and -max dp/dt, respectively) were determined. Time to Emax and time to -max dp/dt from the rising phase of R wave of epicardial electrocardiogram (ECG) were also determined. The time constant of left ventricular pressure decay during the isovolumic relaxation phase was determined by the method of Weiss et al. (26).

**PVA**

As shown in Fig. 3, we calculated PVA of each isovolumic beat from the digitized P(t) and V(t) data in the same way as before (13). PVA is the area surrounded by the end-systolic PV relation line, the end-diastolic PV relation curve, and the vertical isovolumic pressure trajectory at the isovolumic volume. PVA was normalized for 100 g LV. Its dimensions are mmHg·ml·beat-1·100 g-1.

**Blood pH and catecholamine measurements**

We measured pH of the arterial blood in the coronary arterial perfusion tube before and during RYA infusion in five hearts. We also measured the concentration of catecholamines in the arterial blood before and during RYA in six hearts to eliminate the possibility that circulating catecholamines released from the support dog modified the inotropic effect of RYA. The analysis of catecholamines was performed in Otsuka Assay Laboratories of Otsuka Pharmaceuticals (Tokushima, Japan).

**Experimental protocol**

The experimental protocol consisted of three categories: The first category was "volume-loading runs" to obtain the volume-loaded VO2-PVA relations. Steady state isovolumic contractions were produced at five to nine different LV volumes to cover a wide range of PVA in both the baseline contractility and depressed contractility level with RYA. We called these runs the Baseline-VOL run and RYA-VOL run, respectively.

The second category was "inotropism runs" to obtain composite VO2-PVA relations. CaCl2 and RYA were infused into the coronary arterial perfusion tube to obtain Emax and VO2-PVA data at increasing or decreasing, respectively, contractile levels at a preset constant LV volume (22.4±2.4 ml) as shown in Fig. 1 D. The infusion rate of CaCl2 was increased in steps every 5 min until Emax was nearly doubled. In contrast, RYA was continuously infused at one or two constant infusion rates because the time course of RYA binding to Ca2+ release channel is very slow (8-11). We called these runs CaCl2-INO run and RYA-INO run, respectively.

The third category was a "KCl-attack run" in which the heart was arrested with KCl at V0 to obtain VO2 for basal metabolism (13, 14, 23).

Experiments were performed in a total of 11 hearts. First, the Baseline-VOL run without any inotropic intervention was performed in all 11 hearts. Then, inotropic runs were performed to compare the effects of CaCl2 and RYA on Emax and the composite relation in the absence of propranolol in 8 of the 11 hearts. The CaCl2-INO run preceded the RYA-INO run in all eight hearts (Table 1) because the effect of RYA on the Ca2+ release channel is almost irreversible (8-11). The RYA-INO run was performed 15-30 min after CaCl2 infusion was discontinued when Emax and VO2 returned to the baseline levels.

Propranolol was used in the other three hearts to compare the effects of CaCl2 and RYA in the absence of effects of circulating catecholamines released from the support dog. During continuous infusion of propranolol (1 mg/h), complete β-blockade of the isolated heart was confirmed by a bolus injection of 1 μg isoproterenol. Then, the CaCl2-INO run was performed to enhance Emax to the prepropranolol level. Finally, the RYA-INO run was performed by continuous infusion of both propranolol (1 mg/h) and CaCl2 at the constant infusion rates.

The maximum dose of CaCl2 was 0.18±0.06 mmol/min. The maximum dose of RYA was 1.36±0.53 nmol/min. This dose corresponded to a blood concentration of 29.0±12.5 nM at a coronary blood flow of 50.5±21.0 ml/min.

The RYA-VOL run was performed under the condition of steady state contractility at the end of the RYA-INO run in all 11 hearts. Both RYA-INO and RYA-VOL runs were performed without a significant elevation of the end-diastolic pressure relation by RYA. LV end-diastolic pressure did not exceed 18 mmHg in any volume runs.

![Figure 3. Simultaneous tracings of left ventricular isovolumic pressure (LVP), isovolumic volume (LVV), epicardial electrocardiogram (ECG), coronary flow (CF), and arteriovenous oxygen content difference (AVo2D). Heart rate and LV volume were fixed constant. Intracoronary dose of RYA was increased from 0 (A) to 0.67 nmol/min (B) and 1.33 nmol/min (C). Bottom panels show isovolumic pressure-volume trajectories (vertical solid line) and end-systolic pressure-volume relations or Emax lines (diagonal dashed line) under each condition. The triangular areas under Emax lines are the PVAs of the individual contractions.](image-url)
Table I. Summary of Negative Inotropic Effects by RYA

<table>
<thead>
<tr>
<th></th>
<th>Run</th>
<th>Baseline</th>
<th>RYA</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>11</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>Emax (mmHg·ml⁻¹·100 g⁻¹)</td>
<td>4.2±1.6</td>
<td>*</td>
<td>2.3±0.8</td>
</tr>
<tr>
<td>PVA (mmHg·ml⁻¹·beat⁻¹·100 g⁻¹)</td>
<td>1,036±321</td>
<td>*</td>
<td>572±227</td>
</tr>
<tr>
<td>VO₂ (ml O₂·beat⁻¹·100 g⁻¹)</td>
<td>0.0246±0.0046</td>
<td>NS</td>
<td>0.0226±0.0038</td>
</tr>
<tr>
<td>CF (ml·min⁻¹·100 g⁻¹)</td>
<td>79±34</td>
<td>NS</td>
<td>68±24</td>
</tr>
<tr>
<td>AVO₂D (vol %)</td>
<td>10.2±3.8</td>
<td>*</td>
<td>9.4±3.9</td>
</tr>
<tr>
<td>+max (dp/dt)</td>
<td>996±289</td>
<td>*</td>
<td>508±127</td>
</tr>
<tr>
<td>-max (dp/dt)</td>
<td>-907±235</td>
<td>*</td>
<td>-420±142</td>
</tr>
<tr>
<td>Tmax (ms)</td>
<td>181±12</td>
<td>*</td>
<td>192±17</td>
</tr>
<tr>
<td>τ (ms)</td>
<td>36±7</td>
<td>*</td>
<td>61±28</td>
</tr>
<tr>
<td>time -max (dp/dt)</td>
<td>277±18</td>
<td>*</td>
<td>309±34</td>
</tr>
</tbody>
</table>

Each parameter was compared between baseline contractile state and the most depressed contractile state by RYA at the same LV volume. n, number of hearts subjected to analysis; CF, coronary blood flow; AVO₂D, coronary arterio-venous oxygen content difference; +max (dp/dt), maximum positive value of time-derivative of left ventricular pressure; -max (dp/dt), maximum negative value of time-derivative of left ventricular pressure; time to -max (dp/dt) time from onset of R wave of ECG to -max (dp/dt). * P < 0.05 by paired t test.

Finally, the KCl-arrest run was performed when a new steady state was reached 20–30 min after all drug infusions were discontinued in 10 of the 11 hearts.

Data analysis
VO₂-PVA relation in VOL and INO runs. VO₂ and PVA data in Baseline-VOL runs were subjected to linear-regression analysis to obtain a volume-loaded VO₂-PVA regression equation (Fig. 1 B): VO₂ = aPVA + b, where a is the slope of the regression line and b is the VO₂ intercept. The reciprocal of the slope (1/a) means the contractile efficiency (13, 14).

VO₂ and PVA data in each inotropic run were also subjected to linear-regression analysis to obtain a regression equation of the composite relation (Fig. 1 D) (20).

PVA-independent VO₂. The PVA-independent VO₂ of a VO₂-PVA data point during the inotropic run was calculated as total VO₂ minus PVA-dependent VO₂. The PVA-dependent VO₂ was calculated as the product of the same slope value a as the baseline a and PVA of this contraction. Thus, the PVA-independent VO₂ at each altered contractility level was calculated as LV VO₂ minus aPVA.

Oxygen cost of Eₘₐₓ. The relation between PVA-independent VO₂ and corresponding Eₘₐₓ in each of the CaCl₂-INO and RYA-INO runs was plotted in each heart (Fig. 1 E). The slope (c) of the regression line was obtained in each run (20). Its dimensions are ml O₂·ml·mmHg⁻¹·beat⁻¹·100 g⁻¹. The y-intercept (d) of this regression line was obtained as the PVA-independent VO₂ extrapolated to zero Eₘₐₓ (20).

Statistics
The VO₂-PVA regression lines were compared between CaCl₂-INO and RYA-INO runs and between Baseline-VOL and RYA-VOL runs in each heart by analysis of covariance (ANCOVA). Significance of the differences in their slopes and elevations was tested by F test. ANCOVA was also applied to compare the regression lines of PVA-independent VO₂ on Eₘₐₓ between CaCl₂-INO and RYA-INO runs.

Comparisons of paired mean values were performed by paired t test. Comparisons of mean values among three runs were performed by analysis of variance followed by the least significance difference method. A P value < 0.05 was considered statistically significant. Data are presented as means±SD.

Results

Effect of RYA on energetics and other parameters. Fig. 3 shows tracings of LV isovolumic pressure, volume, ECG, coronary flow, and AVO₂D during the RYA-INO run at a constant LV volume in a representative heart, in which intracoronary RYA infusion rate was increased from 0 (Fig. 3 A) to 0.67 nmol/min (Fig. 3 B) and to a maximum dose of 1.33 nmol/min (Fig. 3 C). This maximal dose was calculated to correspond to a blood concentration of ~ 40 nM of RYA. RYA gradually depressed Eₘₐₓ from 4.7 to 2.2 mmHg·ml⁻¹·100 g in 45 min. In this heart, the CaCl₂-INO run preceding the RYA-INO run increased Eₘₐₓ from 4.6 to 7.6 mmHg·ml⁻¹·100 g.

Table I compares variables before and during the RYA-INO run in all 11 hearts. The data during the RYA-INO run was obtained at maximally depressed Eₘₐₓ with RYA. At a constant LV volume, RYA significantly depressed Eₘₐₓ by 42.1±14.8% (P < 0.001) and PVA by 44.4±15.3% (P < 0.001). However, RYA depressed VO₂ only by 7.4±12.1%; this decrease in VO₂ was not statistically significant. Coronary blood flow was unchanged during RYA whereas AVO₂D slightly decreased (P < 0.05). RYA significantly depressed both max dp/dt and -max dp/dt (P < 0.001) and increased Tmax (P < 0.01), time to -max dp/dt (P < 0.01), and τ (P < 0.05). Thus, RYA decreased the contraction speed and retarded the relaxation.

Comparison of the effects of CaCl₂ and RYA on energetics. Fig. 4 compares composite relations in CaCl₂-INO and RYA-INO runs in the same heart as in Fig. 3 (Table II, No. 1). LV VO₂ increased linearly with increases in PVA in the CaCl₂-INO run (r = 0.984). With decreases in Eₘₐₓ by RYA, VO₂ decreased markedly from a pre-RYA level of 906 to 408 mmHg·ml·beat⁻¹·100 g⁻¹, whereas VO₂ decreased only moderately from the pre-RYA level of 0.0402 to 0.0298 ml O₂·beat⁻¹·100 g⁻¹. As a result, the RYA-composite relation (r = 0.985) rotated clockwise with a smaller slope and a greater VO₂ intercept compared with the CaCl₂-composite relation.

Figure 4. Plots of the composite VO₂-PVA relations in the CaCl₂-inotropic run (CaCl₂-INO, open circle) and the RYA-inotropic run (RYA-INO, closed circle). The RYA-composite relation had a gentler slope than the CaCl₂-composite relation.
Table II. Comparison of the Effect of CaCl₂ and RYA on Ventricular Mechanics and Energetics

| No. | Drug | HR  | \( r \) | Slope \( \text{beat} \cdot \text{min}^{-1} \) | \( 10^{-1} \text{ml O}_2 \cdot \text{min}^{-1} \cdot \text{mg}^{-1} \) | \( \text{ml O}_2 \cdot \text{beats}^{-1} \cdot 100 \text{ g}^{-1} \) | ANCOVA | \( r \) | Slope* | \( \text{VO}_2\text{INT} \) \( \text{ml} \cdot \text{min}^{-1} \cdot \text{mg}^{-1} \) | ANCOVA | \( r \) | Slope* | \( \text{VO}_2\text{INT} \) \( \text{ml} \cdot \text{min}^{-1} \cdot \text{mg}^{-1} \) |
|-----|------|-----|-------|-----------------|-----------------|-----------------|--------|-------|----------|--------|-------|-----------------|--------|-------|-----------------|
| 1   | CaCl₂ | 150 | 0.984 | 3.84 | 0.0049 | 0.952 | 4.32 | 0.0048 |
|     | RYA   | 150 | 0.985 | 2.22 | 0.0208 | ** * | 0.834 | 1.17 | 0.0208 | ** * |
| 2   | CaCl₂ | 165 | 0.998 | 2.72 | 0.0174 | 0.960 | 1.34 | 0.0173 |
|     | RYA   | 165 | 0.970 | 1.75 | 0.0234 | ** NS | -0.563 | -0.58 | 0.0234 | ** NS |
| 3   | CaCl₂ | 140 | 0.997 | 3.21 | 0.0128 | 0.981 | 4.13 | 0.0132 |
|     | RYA   | 140 | 0.672 | 0.85 | 0.0382 | ** NS | -0.771 | -3.62 | 0.0382 | ** NS |
| 4   | CaCl₂ | 140 | 0.998 | 2.58 | 0.0144 | ** NS | 0.974 | 2.22 | 0.0144 |
|     | RYA   | 140 | 0.968 | 2.15 | 0.0191 | * NS | 0.396 | 0.79 | 0.0191 | * NS |
| 5   | CaCl₂ | 140 | 0.992 | 2.33 | 0.0144 | ** NS | 0.960 | 3.38 | 0.0112 |
|     | RYA   | 140 | 0.989 | 1.88 | 0.0150 | * NS | 0.931 | 2.05 | 0.0150 | * NS |
| 6   | CaCl₂ | 140 | 0.987 | 2.51 | 0.0161 | 0.878 | 3.03 | 0.0170 |
|     | RYA   | 140 | 0.981 | 2.27 | 0.0208 | NS NS | 0.741 | 2.12 | 0.0209 | NS NS |
| 7   | CaCl₂ | 140 | 0.997 | 2.77 | 0.0117 | 0.988 | 1.87 | 0.0108 |
|     | RYA   | 140 | 0.979 | 2.40 | 0.0135 | NS * | 0.905 | 1.32 | 0.0135 | NS * |
| 8   | CaCl₂ | 150 | 0.997 | 2.77 | 0.0117 | 0.974 | 4.02 | 0.0054 |
|     | RYA   | 150 | 0.894 | 1.58 | 0.0219 | ** NS | -0.037 | -0.01 | 0.0215 | ** NS |
| 9   | CaCl₂ | 160 | 0.918 | 3.90 | 0.0089 | 0.776 | 3.67 | 0.0083 |
|     | RYA   | 160 | 0.995 | 2.54 | 0.0237 | ** ** | 0.924 | 1.22 | 0.0231 | ** ** |
| 10  | CaCl₂ | 140 | 0.992 | 2.45 | 0.0089 | 0.942 | 3.48 | 0.0083 |
|     | RYA   | 140 | 0.997 | 1.97 | 0.0160 | ** ** | 0.928 | 1.63 | 0.0155 | ** ** |
| 11  | CaCl₂ | 133 | 0.997 | 3.29 | 0.0039 | 0.991 | 4.77 | 0.0033 |
|     | RYA   | 133 | 0.996 | 2.50 | 0.0176 | ** ** | 0.976 | 2.79 | 0.0170 | ** ** |

CaCl₂ Mean±SD 145±9 0.987±0.023 2.94±0.54 0.0111±0.0042 0.943±0.063 3.29±0.081 0.0104±0.0048
RYA Mean±SD 145±9 0.956±0.094 2.01±0.49 0.0209±0.0066 0.410±0.064 2.81±0.175 0.0407±0.0067
Paired t test *** *** *** *** *** *** ***

RYA and CaCl₂, RYA-INO and CaCl₂-INO runs, respectively. Three hearts (Nos. 9–11) were studied in the presence of propranolol. HR, constant heart rate. \( r \), correlation coefficient of the VO₂-PVA relation. Slope, the slope of the VO₂-PVA regression line. VO₂INT, PVA-independent VO₂. *Slope = the slope of the PVA-independent VO₂-E₂max regression line. \( \text{VO}_2\text{INT} \) = PVA-independent VO₂ at 0 E₂max. Difference of the slope (SL) and the elevation (EL) of the regression lines were tested by \( F \) test (* \( P < 0.05 \), ** \( P < 0.01 \), ANCOVA). Similar results were obtained in all other hearts regardless of β-blockade.

The slope of the linear-regression line of the RYA-composite relation (2.22 × 10⁻³ \( \text{ml O}_2 \cdot \text{mmHg}^{-1} \cdot \text{min}^{-1} \)) was significantly smaller than that of the CaCl₂-composite relation (3.84 × 10⁻³, \( P < 0.01 \), ANCOVA). Similar results were obtained in all other hearts regardless of β-blockade.

Table II (left) summarizes the data for the composite relations during CaCl₂-INO and RYA-INO runs in all 11 hearts. Numbers 9–11 corresponded to β-blockade hearts. The slope of the RYA composite relation was significantly smaller than that of the CaCl₂ composite relation in 9 of the 11 hearts (ANCOVA). The other two hearts also showed smaller slope values, although the difference was not significant. The mean slope value was significantly smaller in the RYA-INO run (paired \( t \) test; \( P < 0.001 \)). The mean VO₂-intercept value was significantly greater in the RYA-INO run than in the CaCl₂-INO run and was also significantly greater than KCl-arrest VO₂ of 0.0104±0.0022 \( \text{ml O}_2 \cdot \text{beats}^{-1} \cdot 100 \text{ g}^{-1} \) (analysis of variance, \( P < 0.001 \)). The mean VO₂-intercept value in the CaCl₂-INO run was not significantly different from KCl-arrest VO₂ (paired \( t \) test).

Comparison of the contractile efficiency between Baseline-VOL and RYA-VOL runs. Fig. 5 shows the VO₂-PVA relations obtained in Baseline-VOL (open squares) and RYA-VOL (closed circles) runs in a representative heart. Their slope values were not significantly different by ANCOVA.

![Figure 5](image-url)
In all 11 hearts, the slope of the VO₂-PVA regression line was not significantly different between Baseline-VOL and RYA-VOL runs by ANCOVA (1.65±0.23 x 10⁻⁵ ml O₂·mmHg⁻¹·ml⁻¹ for Baseline-VOL run versus 1.5±0.22 x 10⁻⁵ ml O₂·mmHg⁻¹·ml⁻¹ for RYA-VOL run). The elevation difference between the two regression lines was statistically significant in 8 of 11 hearts (ANCOVA). Paired t test also showed a significantly smaller PVA-independent VO₂ in RYA-VOL run on the average (0.025±0.0040 ml O₂·beat⁻¹·100 g⁻¹ for Baseline-VOL run vs. 0.022±0.0033 ml O₂·beat⁻¹·100 g⁻¹ for RYA-VOL run, P < 0.01). Thus, the downward shift of the VO₂-PVA relation in the RYA-VOL run compared with the Baseline-VOL run was mainly a parallel shift. These results indicate that the contractile efficiency was not affected by RYA.

Fig. 6 plots PVA-independent VO₂ against corresponding E_max during CaCl₂-INO and RYA-INO runs in the same heart as in Fig. 4. In this heart, PVA-independent VO₂ increased linearly with decreases in E_max with CaCl₂ and decreased linearly with decreases in E_max with RYA. The slope of the regression line was significantly smaller in RYA-INO runs (ANCOVA). These results indicate that in the RYA-INO run, PVA-independent VO₂ remained disproportionately high despite the progressively decreased E_max. Similar results were obtained in all the other 10 hearts (Table II, right). ANCOVA showed significance difference in the slope between CaCl₂-INO and RYA-INO runs in 9 of the 11 hearts. The mean slope value in the RYA-INO run was significantly lower than that in the CaCl₂-INO run by 75.3±50.1% (P < 0.01). The PVA-independent VO₂ value in RYA-INO run was significantly greater by 150±143% (P < 0.001).

**Discussion**

Using RYA, we have obtained quite different results in cardiac energetics from those of ordinary inotropic drugs (13–20). The major findings of the present study are as follows: (a) RYA at a low concentration lowered both VO₂ and PVA linearly with decreases in ventricular contractility (Fig. 4). (b) However, the magnitude of the change in VO₂ for a unit change in PVA was significantly smaller with RYA than with CaCl₂ (Fig. 4). (c) The downward shift of the volume-loaded VO₂-PVA relation with RYA was a parallel shift (Fig. 5). (d) PVA-independent VO₂ remained disproportionately high despite the significantly decreased E_max with RYA (Fig. 6). These findings indicate that RYA does not proportionately decrease the nonmechanical VO₂ despite its potent negative inotropic effect and that RYA does not affect the contractile efficiency per se. In other words, the negative inotropic effect of RYA is accompanied by an oxygen-wasting effect in the nonmechanical energy utilization process of myocardium.

**RYA and cardiac contractility.** RYA at a low concentration (several tens of nM) has been shown to selectively bind the Ca²⁺ release channels and fix them in a long-term open state with a reduced unit conductance (8–11), although RYA at a high concentration (above µM) fixes the channel in a closed state. This feature of RYA is quite different from that of other Ca²⁺ release channel regulators such as Ca²⁺, Mg²⁺, and ATP (8–11).

In the present study, we used RYA at a relatively low concentration (calculated value of 29±13 nM) and observed that RYA significantly depressed cardiac contractility. This finding is consistent with previous observations in isolated canine and cat cardiac muscles (27, 28). In addition, RYA significantly slowed relaxation speed in terms of r and time to −max dp/dt (Table 1). From these findings, the conventional view that RYA increases the leak of Ca²⁺ from SR seems to hold in our present blood-perfused dog heart preparation.

**RYA and PVA-independent VO₂.** PVA-independent VO₂ reflects the VO₂ fraction for nonmechanical activities, i.e., basal metabolism and excitation-contraction-relaxation coupling (14). We consider that KCl-arrest VO₂ is a reasonable estimate of the energy utilization for basal metabolism (14). Although KCl-arrest VO₂ was measured under a condition in which RYA remained in the cross-circulating blood, the value was comparable to those obtained in our previous studies (13, 14). This suggests that RYA does not significantly affect energy utilization for basal metabolism and that the decreased PVA-independent VO₂ in RYA-INO run is mainly due to a decrease in the excitation-contraction-relaxation coupling energy.

In the present study, PVA-independent VO₂ gradually but significantly decreased with decreases in E_max by RYA (Fig. 6). This result seems to reflect that RYA makes SR leaky for Ca²⁺ and decreases the Ca²⁺ store in SR (8–11, 29–31). However, PVA-independent VO₂ remained disproportionately high despite the significantly decreased E_max by RYA (Fig. 6). This indicates that total Ca²⁺ handling is not suppressed in proportion to the negative inotropism in the presence of RYA on the basis of the 2:1 stoichiometry of sequestered Ca²⁺ to hydrolyzed ATP by Ca²⁺ pump in SR (14, 32). This finding is in striking contrast to that with ordinary inotropic drugs, which show proportional changes in both E_max and PVA-independent VO₂ (OPC-8212 [15], ouabain [16], denopamine [17], and...
Amrinone [18]) (14, 20). To explain the discrepancy of the findings between RYA and ordinary inotropic drugs, we raise the following possible subcellular mechanisms of the abnormal relation between the amount of total Ca^{2+} handling and cardiac contractility with RYA.

The gradually decreasing contractility with RYA at a low concentration can be explained by the view that SR gradually became leaky for Ca^{2+} and, hence, the Ca^{2+} accumulating activity of SR decreases despite the continuous Ca^{2+} uptake into SR (29–31). When Ca^{2+} leaks from SR, it would be taken up into SR by Ca^{2+} pump ATPase, which is an energy consuming process. We consider that this Ca^{2+} futile cycle was detected energetically as a disproportionate increase in PVA-independent VO_{2} for a given contractility in the present study.

**RYA and contractile efficiency.** The contractile efficiency in terms of the inverse value of the slope of the VO_{2}-PVA relation was not changed by RYA (Fig. 5). This result means that energy using efficiency of the myoflament for force generation is not changed despite the changed Ca^{2+} handling by RYA. This result seems to reflect the previous finding that RYA does not affect Ca^{2+} sensitivity of the myoflament from the relation between the steady state force and Ca^{2+} transient (33).

**Implications of the selective change in SR Ca^{2+} release for cardiac contractility.** Recent studies have indicated that dysfunction in Ca^{2+} transport system of SR has an important role in pathophysiological states such as ischemic, acidicotic, and stunned hearts (1–7). It has been proposed that dysfunction of not only Ca^{2+} uptake (2, 5) but also Ca^{2+} release (2–4) and Ca^{2+} permeability of the SR (1) contribute to this SR dysfunction. Feher et al. (4) have shown that the decrease in SR Ca^{2+} uptake caused by ischemia is not due to a defect in the SR Ca^{2+} pumping capability but is due to an increased efflux through the SR Ca^{2+} release channel.

However, in such pathological states, there are other subcellular mechanisms altering cardiac contractile function at the same time (3, 7, 34, 35). For example, ischemia decreases the adenine nucleotide pool (34) and damages contractile protein and cell membranes (7) and acidosis changes Ca^{2+} sensitivity of the myoflament (3, 35). It is difficult to clarify the magnitude of contribution of each of these subcellular mechanisms on contractile dysfunction in such pathological preparations.

In contrast, we were able to characterize the energetic role of the selective change in SR Ca^{2+} release on cardiac mechanoenergetics when the Ca^{2+} release channel activity was selectively modified without changes in other subcellular mechanisms, including changes in Ca^{2+} uptake activity of Ca^{2+} pump ATPase (2, 5). In conclusion, we have indicated the importance of the effect of a change in SR Ca^{2+} release on the contractile dysfunction as observed in pathological hearts.

**Acknowledgments**

Dr. Takasago greatly appreciated the continuous encouragement throughout the period of this study by Professor Goro Kajiyama of the First Department of Internal Medicine, Hiroshima University School of Medicine, from which T. Takasago was on leave as a postdoctoral research fellow. We thank Otsuka Pharmaceuticals for assays of catecholamines free of charge.

This work was supported in part by Grants-in-Aid for Scientific Research (02670418, 04237219, 04454267, and 04557041) from the Ministry of Education, Science, and Culture; Research Grants for Cardiovascular Diseases (1A-1, 3A-2, and 4C-4) from the Ministry of Health and Welfare; and grants from Suzuki Memorial Foundation, the Japan Cardiovascular Research Foundation, and Nakatani Electronics Measuring Technology Association of Japan.

**References**


