Ryanodine Wastes Oxygen Consumption for Ca^{2+} Handling in the Dog Heart

A New Pathological Heart Model

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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) mechanoenergetics using frameworks of LV E_{max} (a contractility index) and the myocardial oxygen consumption (LV VO_2)–systolic pressure-volume area (PVA) (a measure of total mechanical energy) relation in 11 isolated, blood-perfused dog hearts. RYA significantly decreased E_{max} by 42%, whereas PVA-independent VO_2 remained disproportionately high (93% of control). This oxygen-wasting effect of RYA was quite different from ordinary inotropic drugs, which alter E_{max} and PVA-independent VO_2 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_{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 sequestered 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, fixes 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 inotropism 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_{max}) and the myocardial oxygen consumption-systolic pressure-volume area (LV VO_2-PVA) relationship have the following physiological significance: E_{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 VO_2 in a load-independent manner in a stable contractile state (Fig. 1 *B*). The reciprocal of the slope of the VO_2-PVA relation at a constant E_{max} means the "contractile efficiency" (14). VO_2 can be di-

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Received for publication 8 September 1992 and in revised form 4 March 1993.

1. *Abbreviations used in this paper:* AVO_2D, arteriovenous oxygen content difference; E_{max}, contractile index; LV, left ventricular; P-V, pressure volume; PVA, pressure-volume area; RV, Right ventricle; RYA, ryanodine; SR, sarcoplasmic reticulum; VO_2, oxygen consumption.

J. Clin. Invest.

0021-9738/93/08/0823/08 $2.00
Volume 92, August 1993, 823–830

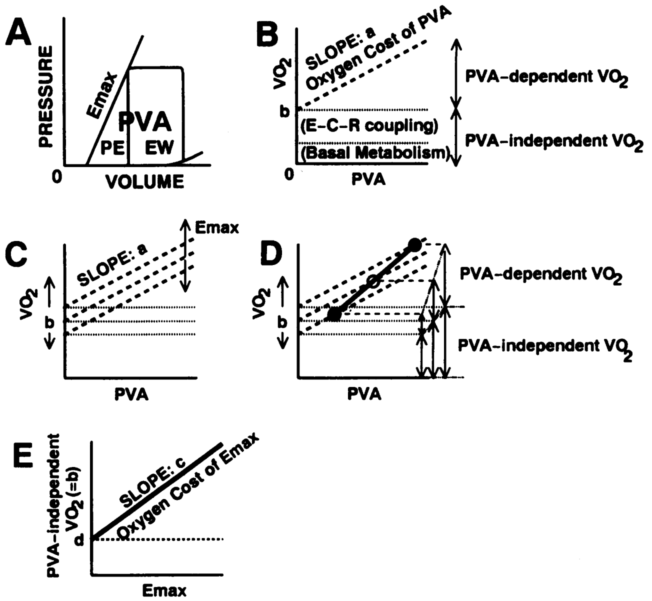

Figure 1. Schematic illustration of LV systolic pressure-volume area (*PVA, A*), LV VO_2-PVA relation in the volume-loading run (*B*), volume-loaded VO_2-PVA relations at different E_{max} levels (*C*), VO_2-PVA relation in the inotropism run (*D*), and the relation between PVA-independent VO_2 and E_{max} 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 VO_2-PVA relation in a baseline contractile state (*thick dashed line*) and VO_2 components. *C* shows the volume-loaded VO_2-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 VO_2-PVA data point (*solid circle*) from a baseline VO_2-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 VO_2-PVA relation "the composite relation" (*solid line*). VO_2 of this point can be divided into two components: PVA-dependent VO_2 corresponding to the PVA of the contraction and PVA-independent VO_2, which is the sum of the same baseline PVA-independent VO_2 (equal to *b* in *B*) and the change in PVA-independent VO_2. *E* shows the relation between PVA-independent VO_2 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 VO_2 extrapolated to zero E_{max} (see text for more details).

vided at the VO_2 intercept of the VO_2-PVA relation into PVA-dependent and -independent VO_2 components (Fig. 1 *B*). PVA-independent VO_2 is considered to be primarily related to the total sequestered Ca^{2+} by SR and basal metabolism (14, 23).

The VO_2-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 VO_2-PVA point deviates upward or downward from a baseline VO_2-PVA relation and forms a new, steeper VO_2-PVA relation, which traverses multiple volume-loaded VO_2-PVA relations for different contractility levels (Fig. 1 *D*). We call such a steeper VO_2-PVA relation obtained during changing inotropism "the composite relation" (19, 20). In the inotropism run, PVA-independent VO_2 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 VO_2 and E_{max} means the "oxygen cost of contractility" (Fig. 1 *E*) (20). These features of the VO_2-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 VO_2 quantitatively reflect changes in energy expenditure for the total Ca^{2+} 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 Ca^{2+} taken up by the Ca^{2+} 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 Ca^{2+} handling, the RYA-composite relation (shown by the diagonal solid arrow) will be superimposed on the same line as the $CaCl_2$-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 Ca^{2+} release channel of SR (9–11), the PVA-independent VO_2 will decrease very little whereas only the PVA-dependent VO_2 will decrease with reduced PVA. In this manner, VO_2 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 $CaCl_2$-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, Po_2, and Pco_2 were repeatedly measured and corrected to normal as needed.

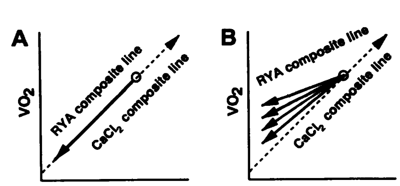

Figure 2. Schematic illustrations of alternatively possible effects of RYA on the composite VO_2-PVA relation in inotropism run (see text for more details).

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 (AVO_2D) 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; Instrumention Laboratory, Lexington, MA). Cardiac oxygen consumption was obtained as the product of the total coronary blood flow and AVO_2D. It was divided by heart rate to obtain VO_2 per beat in steady state. These computations were performed on-line with a signal processor (model 7T18; NEC San-ei, Tokyo, Japan).

Right ventricular (RV) VO_2 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 VO_2. The RV component of the PVA-independent VO_2 was calculated by multiplying biventricular PVA-independent VO_2 in each contractile state by (RV weight)/(LV + RV weight). PVA-independent LV VO_2 was then obtained by subtracting the RV component from biventricular PVA-independent VO_2 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 (E_{max})

LV pressure (P(t)) and volume (V(t)) data were sampled at 2-ms intervals and processed with the signal processor. E_{max} of the LV was determined as the ratio of $P(t)/[V(t) - V_0]$ (25). V_0 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 E_{max} 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 $mmHg \cdot ml \cdot beat^{-1} \cdot 100\ g^{-1}$.

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 VO_2-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.

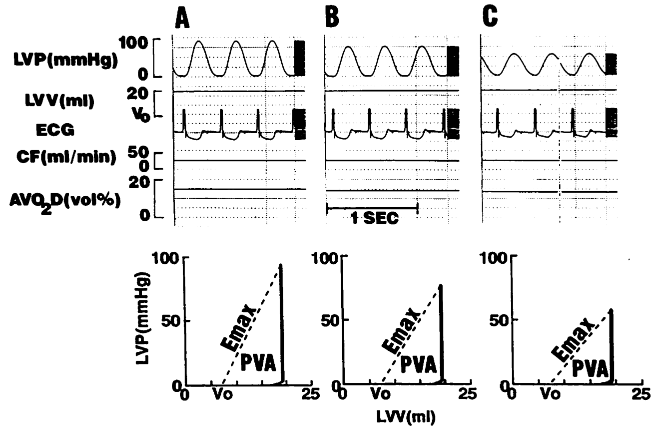

Figure 3. Simultaneous tracings of left ventricular isovolumic pressure (*LVP*), isovolumic volume (*LVV*), epicardial electrocardiogram (*ECG*), coronary flow (*CF*), and arteriovenous oxygen content difference (*AVO_2D*). Heart rate and LV volume were fixed constant. Intracoronary dose of RYA was increased 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 E_{max} lines (*diagonal dashed line*) under each condition. The triangular areas under E_{max} lines are the PVAs of the individual contractions.

The second category was "inotropism runs" to obtain composite VO_2-PVA relations. $CaCl_2$ and RYA were infused into the coronary arterial perfusion tube to obtain E_{max} and VO_2-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 $CaCl_2$ was increased in steps every 5 min until E_{max} was nearly doubled. In contrast, RYA was continuously infused at one or two constant infusion rates because the time course of RYA binding to Ca^{2+} release channel is very slow (8–11). We called these runs $CaCl_2$-INO run and RYA-INO run, respectively.

The third category was a "KCl-arrest run" in which the heart was arrested with KCl at V_0 to obtain VO_2 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, inotropism runs were performed to compare the effects of $CaCl_2$ and RYA on E_{max} and the composite relation in the absence of propranolol in 8 of the 11 hearts. The $CaCl_2$-INO run preceded the RYA-INO run in all eight hearts (Table I) because the effect of RYA on the Ca^{2+} release channel is almost irreversible (8–11). The RYA-INO run was performed 15–30 min after $CaCl_2$ infusion was discontinuated when E_{max} and VO_2 returned to the baseline levels.

Propranolol was used in the other three hearts to compare the effects of $CaCl_2$ 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 $CaCl_2$-INO run was performed to enhance E_{max} to the prepropranolol level. Finally, the RYA-INO run was performed by continuous infusion of both propranolol (1 mg/h) and $CaCl_2$ at the constant infusion rates.

The maximum dose of $CaCl_2$ was 0.18±0.06 meq/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 P-V relation by RYA. LV end-diastolic pressure did not exceed 18 mmHg in any volume runs.

Table I. Summary of Negative Inotropic Effects by RYA

Run	Baseline	P^*	RYA
n	11		11
Emax ($mmHg \cdot ml^{-1} \cdot$ 100 g)	4.2±1.6	*	2.3±0.8
PVA ($mmHg \cdot ml \cdot beat^{-1} \cdot 100\ g^{-1}$)	1,036±321	*	572±227
VO_2 ($ml\ O_2 \cdot beat^{-1} \cdot 100\ g^{-1}$)	0.0246±0.0046	NS	0.0226±0.0038
CF ($ml \cdot min^{-1} \cdot 100\ g^{-1}$)	79±34	NS	68±24
AVO_2D (vol %)	10.2±3.8	*	9.4±3.9
+max (dp/dt) ($mmHg \cdot s^{-1}$)	996±289	*	508±127
−max (dp/dt) ($mmHg \cdot s^{-1}$)	−907±235	*	−420±142
Tmax (ms)	181±12	*	192±17
τ (ms)	36±7	*	61±28
time −max (dp/dt) (ms)	277±18	*	309±34

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; AVO_2D, 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

VO_2-PVA relation in VOL and INO runs. VO_2 and PVA data in Baseline-VOL run were subjected to linear-regression analysis to obtain a volume-loaded VO_2-PVA regression equation (Fig. 1 *B*): $VO_2 = a\text{PVA} + b$, where a is the slope of the regression line and b is the VO_2 intercept. The reciprocal of the slope ($1/a$) means the contractile efficiency (13, 14).

VO_2 and PVA data in each inotropism run were also subjected to linear-regression analysis to obtain a regression equation of the composite relation (Fig. 1 *D*) (20).

PVA-independent VO_2. The PVA-independent VO_2 of a VO_2-PVA data point during the inotropism run was calculated as total VO_2 minus PVA-dependent VO_2. The PVA-dependent VO_2 was calculated as the product of the same slope value a as the baseline a and PVA of this contraction. Thus, the PVA-independent VO_2 at each altered contractility level was calculated as LV VO_2 minus aPVA.

Oxygen cost of E_{max}. The relation between PVA-independent VO_2 and corresponding E_{max} in each of the $CaCl_2$-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 $ml\ O_2 \cdot ml \cdot mmHg^{-1} \cdot beat^{-1} \cdot 100\ g^{-2}$. The y-intercept (d) of this regression line was obtained as the PVA-independent VO_2 extrapolated to zero E_{max} (20).

Statistics

The VO_2-PVA regression lines were compared between $CaCl_2$-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 VO_2 on E_{max} between $CaCl_2$-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 mean±SD.

Results

Effect of RYA on energetics and other parameters. Fig. 3 shows tracings of LV isovolumic pressure, volume, ECG, coronary flow, and AVO_2D 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 E_{max} from 4.7 to 2.2 $mmHg \cdot ml^{-1} \cdot 100$ g in 45 min. In this heart, the $CaCl_2$-INO run preceding the RYA-INO run increased E_{max} from 4.6 to 7.6 $mmHg \cdot ml^{-1} \cdot 100$ 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 E_{max} with RYA. At a constant LV volume, RYA significantly depressed E_{max} by 42.1±14.8% ($P < 0.001$) and PVA by 44.4±15.3% ($P < 0.001$). However, RYA depressed VO_2 only by 7.4±12.1%; this decrease in VO_2 was not statistically significant. Coronary blood flow was unchanged during RYA whereas AVO_2D slightly decreased ($P < 0.05$). RYA significantly depressed both max dP/dt and −max dP/dt ($P < 0.001$) and increased T_{max} ($P < 0.01$), 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 $CaCl_2$ and RYA on energetics. Fig. 4 compares composite relations in $CaCl_2$-INO and RYA-INO runs in the same heart as in Fig. 3 (Table II, No. 1). LV VO_2 increased linearly with increases in PVA in the $CaCl_2$-INO run ($r = 0.984$). With decreases in E_{max} by RYA, PVA decreased markedly from a pre-RYA level of 906 to 408 $mmHg \cdot ml \cdot beat^{-1} \cdot 100\ g^{-1}$, whereas VO_2 decreased only moderately from the pre-RYA level of 0.0402 to 0.0298 $ml\ O_2 \cdot beat^{-1} \cdot 100\ g^{-1}$. As a result, the RYA-composite relation ($r = 0.985$) rotated clockwise with a smaller slope and a greater VO_2 intercept compared with the $CaCl_2$-composite relation.

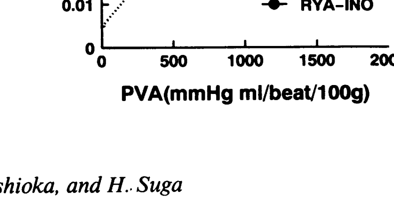

Figure 4. Plots of the composite VO_2-PVA relations in the $CaCl_2$-inotropism run (*$CaCl_2$-INO, open circle*) and the RYA-inotropism run (*RYA-INO, closed circle*). The RYA-composite relation had a gentler slope than the $CaCl_2$-composite relation.

Table II. Comparison of the Effect of $CaCl_2$ and RYA on Ventricular Mechanics and Energetics

			Composite VO_2 PVA relation			ANCOVA		PVA-independent VO_2-E_{max} relation			ANCOVA	
No.	Drug	HR	r	Slope	VO_2INT	SL	EL	r	Slope*	VO_2INT‡	SL	EL
		beat · min^{-1}		*10^{-5} ml O_2 · mmHg^{-1} · ml^{-1}*	*ml O_2 beat^{-1} · 100 g^{-1}*				*10^{-3} ml O_2 · ml · mmHg^{-1} · beat^{-1} · 100 g^{-2}*	*ml O_2 · beat^{-1} · 100 g^{-1}*		
1	$CaCl_2$	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	$CaCl_2$	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	$CaCl_2$	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	$CaCl_2$	140	0.998	2.58	0.0144			0.974	2.22	0.0144		
	RYA	140	0.968	2.15	0.0191	*	NS	0.396	0.79	0.0191	*	NS
5	$CaCl_2$	140	0.992	2.33	0.0144			0.960	3.38	0.0112		
	RYA	140	0.989	1.88	0.0150	*	NS	0.931	2.05	0.0150	*	NS
6	$CaCl_2$	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	$CaCl_2$	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	$CaCl_2$	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	$CaCl_2$	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	$CaCl_2$	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	$CaCl_2$	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	**	**
$CaCl_2$	Mean±SD	145±9	0.987±0.023	2.94±0.54	0.0111±0.0042			0.943±0.063	3.29±1.08	0.0104±0.0048		
RYA	Mean±SD	145±9	0.956±0.094	2.01±0.49	0.0209±0.0066			0.410±0.642	0.81±1.75	0.0207±0.0067		
Paired *t* test				***	***				***	***		

RYA and $CaCl_2$, RYA-INO and $CaCl_2$-INO runs, respectively. Three hearts (Nos. 9–11) were studied in the presence of propranolol. HR, constant heart rate. r, correlation coefficient of the VO_2-PVA relation. Slope, the slope of the VO_2-PVA regression line. VO_2INT, PVA-independent VO_2. *Slope = the slope of the PVA-independent VO_2-E_{max} regression line. ‡ VO_2INT = PVA-independent VO_2 at 0 E_{max}. Difference of the slope (SL) and the elevation (EL) of the regression lines were tested by *F* test (* $P < 0.05$, ** $P < 0.01$). *** *P* values <0.001 by paired *t* test.

The slope of the linear-regression line of the RYA-composite relation (2.22×10^{-5} ml O_2 · mmHg^{-1} · ml^{-1}) was significantly smaller than that of the $CaCl_2$-composite relation (3.84×10^{-5}, $P < 0.01$, ANCOVA). Similar results were obtained in all other hearts regardless of β-blockade.

Table II (*left*) summarizes the data for the composite relations during $CaCl_2$-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 $CaCl_2$ 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 VO_2-intercept value was significantly greater in the RYA-INO run than in the $CaCl_2$-INO run and was also significantly greater than KCl-arrest VO_2 of 0.0104 ± 0.0022 ml O_2 · beat^{-1} · 100 g^{-1} (analysis of variance, $P < 0.001$). The mean VO_2-intercept value in the $CaCl_2$-INO run was not significantly different from KCl-arrest VO_2 (paired *t* test).

Comparison of the contractile efficiency between Baseline-VOL and RYA-VOL runs. Fig. 5 shows the VO_2-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.

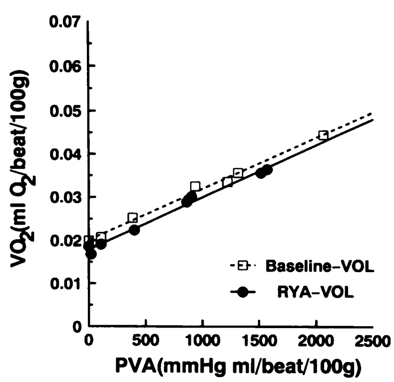

Figure 5. Plots of the VO_2-PVA relation in the baseline volume-loading run (*Baseline-VOL, open square*) and the RYA volume-loading run (*RYA-VOL, closed circle*). RYA shifted the volume-loaded VO_2-PVA relation slightly downward in a parallel manner.

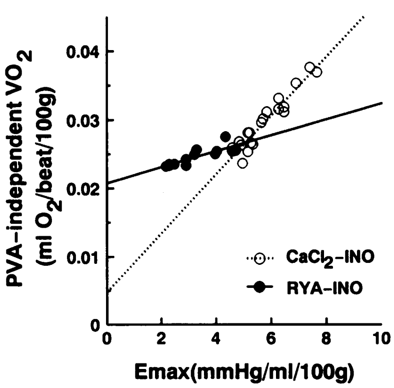

Figure 6. Plots of the PVA-independent VO_2-E_{max} relations during $CaCl_2$-inotropism run (*$CaCl_2$-INO, open circle*) and RYA-inotropism (*RYA-INO, closed circle*). PVA-independent VO_2 remained dispropotionately high despite significantly decreased E_{max} in the RYA-INO run compared with the proportional increases in PVA-independent VO_2 and E_{max} in the $CaCl_2$-INO run.

In all 11 hearts, the slope of the VO_2-PVA regression line was not significantly different between Baseline-VOL and RYA-VOL runs by ANCOVA ($1.65\pm0.23 \times 10^{-5}$ ml $O_2 \cdot mmHg^{-1} \cdot ml^{-1}$ for Baseline-VOL run versus $1.54\pm0.22 \times 10^{-5}$ ml $O_2 \cdot mmHg^{-1} \cdot ml^{-1}$ 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 VO_2 in RYA-VOL run on the average (0.0255 ± 0.0040 ml $O_2 \cdot beat^{-1} \cdot 100\ g^{-1}$ for Baseline-VOL run vs. 0.0229 ± 0.0033 ml $O_2 \cdot beat^{-1} \cdot 100\ g^{-1}$ for RYA-VOL run, $P < 0.01$). Thus, the downward shift of the VO_2-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 VO_2 against corresponding E_{max} during $CaCl_2$-INO and RYA-INO runs in the same heart as in Fig. 4. In this heart, PVA-independent VO_2 increased linearly with increases in E_{max} with $CaCl_2$ 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 VO_2 remained dispropotionately high despite the progressively decreased E_{max}. Similar results were obtained in all the other 10 hearts (Table II, *right*). ANCOVA showed significant difference in the slope between $CaCl_2$-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 $CaCl_2$-INO run by $75.3\pm50.1\%$ ($P < 0.01$). The PVA-independent VO_2 value in RYA-INO run was significantly greater by $150\pm143\%$ ($P < 0.001$).

pH and catecholamine measurements. pH of the arterial blood in the coronary arterial perfusion tube was 7.42 ± 0.08 before RYA and 7.40 ± 0.08 during 26 ± 10 nM RYA. The difference was not significant (paired *t* test).

The concentration of epinephrine in the arterial blood was 2.5 ± 1.8 ng/ml before RYA and 2.8 ± 1.8 ng/ml during RYA of 26 ± 13 nM; norepinephrine was 0.74 ± 0.65 ng/ml before RYA and 0.77 ± 0.40 ng/ml during RYA. Thus, catecholamines in the arterial blood were not significantly changed by RYA. This result means the effect of RYA was not modified by effects of circulating catecholamines without complete β-blockade in our present study.

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 VO_2 and PVA linearly with decreases in ventricular contractility (Fig. 4). (*b*) However, the magnitude of the change in VO_2 for a unit change in PVA was significantly smaller with RYA than with $CaCl_2$ (Fig. 4). (*c*) The downward shift of the volume-loaded VO_2-PVA relation with RYA was a parallel shift (Fig. 5). (*d*) PVA-independent VO_2 remained dispropotionately high despite the significantly decreased E_{max} with RYA (Fig. 6). These findings indicate that RYA does not proportionately decrease the nonmechanical VO_2 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 Ca^{2+} 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 close state. This feature of RYA is quite different from that of other Ca^{2+} release channel regulators such as Ca^{2+}, Mg^{2+}, 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 τ and time to $-$max dP/dt (Table I). From these findings, the conventional view that RYA increases the leak of Ca^{2+} from SR seems to hold in our present blood-perfused dog heart preparation.

RYA and PVA-independent VO_2. PVA-independent VO_2 reflects the VO_2 fraction for nonmechanical activities, i.e., basal metabolism and excitation-contraction-relaxation coupling (14). We consider that KCl-arrest VO_2 is a reasonable estimate of the energy utilization for basal metabolism (14). Although KCl-arrest VO_2 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 VO_2 in RYA-INO run is mainly due to a decrease in the excitation-contraction-relaxation coupling energy.

In the present study, PVA-independent VO_2 gradually but significantly decreased with decreases in E_{max} by RYA (Fig. 6). This result seems to reflect that RYA makes SR leaky for Ca^{2+} and decreases the Ca^{2+} store in SR (8–11, 29–31). However, PVA-independent VO_2 remained dispropotionately high despite the significantly decreased E_{max} by RYA (Fig. 6). This indicates that total Ca^{2+} 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 Ca^{2+} to hydrolyzed ATP by Ca^{2+} 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 VO_2 (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 myofilament 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 myofilament 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, 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. 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 myofilament (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 Suzuken Memorial Foundation, the Japan Cardiovascular Research Foundation, and Nakatani Electronic Measuring Technology Association of Japan.

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