No Dependency of a New Index for Oxygen Cost of Left Ventricular Contractility on Heart Rates in the Blood-Perfused Excised Rat Heart

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Abstract: We have reported the linear relation of myocardial oxygen consumption per beat ($\dot{V}O_2$) and systolic pressure-volume area (PVA) in the left ventricle of the cross-circulated rat heart. The $\dot{V}O_2$ intercept (PVA-independent $\dot{V}O_2$) is primarily composed of $\dot{V}O_2$ for Ca^{2+} handling in excitation-contraction coupling and basal metabolism. Recently, we proposed a new index for oxygen cost of contractility obtainable as a slope of a linear relation between PVA-independent $\dot{V}O_2$ and left ventricular contractility. This index indicates the Ca^{2+} handling $\dot{V}O_2$ per unit contractility change. However, a dependency of this index on heart rate has not yet been investigated. The aim of the present study was to investigate the dependency of oxygen cost of contractility on heart rate. This is a critical point to compare this cost under different heart rates. At first we found no differences of $\dot{V}O_2$ -PVA relations at 240 and 300 beats/min (bpm). Therefore, after control $\dot{V}O_2$ -

PVA relation at 300 bpm, we gradually enhanced left ventricular contractility by Ca^{2+} at a midrange left ventricular volume and obtained the gradually increased PVA-independent $\dot{V}O_2$. At each contractility level, the pacing rate was alternately changed at 240 and 300 bpm. We obtained the two composite $\dot{V}O_2$ -PVA relation lines and found no significant differences between the slopes of PVA-independent $\dot{V}O_2$ and left ventricular contractility relations at 240 and 300 bpm. The present results indicated no dependency of oxygen cost of left ventricular contractility on heart rates within 240–300 bpm. Based on this fact, we concluded that even under the different pacing rates within 240–300 bpm, this oxygen cost is valid for assessing cardiac mechanoenergetics, especially the economy of total Ca^{2+} handling in E-C coupling. [Japanese Journal of Physiology, 51, 177–185, 2001]

Key words: end-systolic pressure–volume relation, excitation-contraction coupling, myocardial oxygen consumption, systolic pressure-volume area (PVA).

We have already reported that a linear myocardial oxygen consumption per beat ($\dot{V}O_2$)–systolic pressure-volume area (PVA) relation in the rat left ventricle (LV) [1–4] is obtainable from a curved end-systolic pressure–volume relation as in the adult canine LV,

which has normal contractility [5–9]. PVA is the specific area defined as a total mechanical energy per beat [8, 9]. The $\dot{V}O_2$ intercept of the $\dot{V}O_2$ -PVA relation (PVA-independent $\dot{V}O_2$) mainly consists of $\dot{V}O_2$ for calcium (Ca^{2+}) handling in the excitation-contraction

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Abbreviations: Ca^{2+} , calcium ion; E-C, excitation-contraction; eE_{max} , equivalent maximal elastance; $(eESP/ESV)_{mLVV}$, equivalent end-systolic pressure–volume ratio at a midrange left ventricular volume; E_{max} , maximal elastance; ESP, end-systolic pressure; ESP_{mLVV} , end-systolic pressure at a midrange left ventricular volume; LV, left ventricle; LVP, left ventricular pressure; LVV, left ventricular volume; mLVV, midrange left ventricular volume; PVA, pressure-volume area; PVA_{mLVV} , pressure-volume area at a midrange left ventricular volume; RV, right ventricle; $\dot{V}O_2$, myocardial oxygen consumption per beat.

(E-C) coupling and basal metabolism in rat whole heart preparations [1–4].

Recently, we proposed an “equivalent” end-systolic pressure–volume ratio at a midrange LV volume (mLVV) of changed LVV, $(eESP/ESV)_{mLVV}$, as a new index for LV contractility [4]. This index is obtained as an end-systolic pressure–volume ratio of the specific virtual triangular PVA at an mLVV (PVA_{mLVV}) that is energetically equivalent to the real PVA_{mLVV} . We newly defined it as equivalent maximal elastance (eE_{max}) for simplification. Plots of gradually increased PVA-independent $\dot{V}O_2$ and eE_{max} values by cardiotonic agents show a highly linear relation [4]. Finally we were able to obtain the oxygen cost of LV contractility as the slope of this relation: $\dot{V}O_2$ for Ca^{2+} handling in the E-C coupling per unit LV contractility change [4].

However, we have not yet investigated the heart rate dependency of a new index for oxygen cost of LV contractility as well as end-systolic pressure–volume and $\dot{V}O_2$ -PVA relations. In individual canine hearts, the $\dot{V}O_2$ -PVA relation was found to be independent of heart rates (105–230 beats/min; bpm) [10], although the slight increase (10%) in E_{max} (an LV contractility index for dog heart) was accompanied by the increase (60%) in heart rate by pacing [11].

The aim of the present study was to investigate heart rate dependency of the oxygen cost of LV contractility as well as end-systolic pressure–volume and $\dot{V}O_2$ -PVA relations. If we can find no dependency of oxygen cost of LV contractility on heart rate, we will be able to compare this index under different heart rates. This is a critical point when we evaluate the Ca^{2+} handling $\dot{V}O_2$ per unit contractility change by this index among diseased hearts, such as hypothyroid rat heart [12].

METHODS

Surgical preparation. Care and experiments on all rats used in the present study were carried out according to the Guiding Principles for the Care and Use of Animals in the Field of Physiological Sciences by the Council of the Physiological Society of Japan.

Experiments were performed on 12 excised, cross-circulated rat heart preparations. In each experiment, three retired breeder male Crj: Wistar rats, weighing 630 ± 53 g (mean $\pm$ SD) were purchased from Charles River Japan, Inc. (Yokohama, Japan). They were anesthetized with pentobarbital sodium (50 mg/kg, I.P.) and intubated. All rats were heparinized (1,000 U, I.V.). The largest rat was used as a blood supplier. The middle-sized rat was used as the metabolic supporter for the excised heart. The beating heart was excised

from the smallest rat. All surgical procedures were previously reported in detail [1,2].

In the excised beating heart maintained at 37°C, a thin latex balloon (balloon material volume 0.08 ml) was inserted into the LV and primed with water. The balloon was connected to a pressure transducer (Life Kit DX-312, Nihon Kohden, Tokyo, Japan). Balloon water volume was changed with a 0.5 ml precision glass syringe (minimum scale: 0.005 ml). LV pressure (LVP) was measured with this pressure transducer. The balloon developed no pressure and thus was unstretched as long as its volume was below approximately 0.25–0.3 ml. Thus LVV was changed and measured by adjusting the intraballoon water volume with the syringe in 0.05 ml steps between 0.08 and 0.28 ml. Systolic unstressed volume ($V_0=0.08$ ml) should be determined as the volume at which peak isovolumic pressure and thus PVA were zero.

LV epicardial electrocardiogram was recorded and heart rate was maintained constant at 300 and 240 bpm by electrical pacing of the right atrium. Systemic arterial blood pressure of the support rat served as coronary perfusion pressure (100–130 mmHg). Arterial pH, PO_2 , and PCO_2 of the support rat and thus the perfused blood were maintained within their physiological ranges with supplemental oxygen and sodium bicarbonate throughout the experiment.

Oxygen consumption. Cardiac O_2 consumption was obtained as the product of coronary flow and coronary arteriovenous O_2 content difference. As shown previously [1–3], the $\dot{V}O_2$ -PVA relation was linear in the rat LV. The $\dot{V}O_2$ intercept represents PVA-independent $\dot{V}O_2$. The right ventricle (RV) was kept collapsed by continuous hydrostatic drainage of the coronary venous return; thus the RV PVA and the PVA-dependent $\dot{V}O_2$ were assumed to be negligible. The RV component of PVA-independent $\dot{V}O_2$ was calculated by multiplying biventricular PVA-independent $\dot{V}O_2$ in each contractile state with the ratio of RV weight divided by the sum of RV and LV weights. The RV PVA-independent $\dot{V}O_2$ was subtracted from the total $\dot{V}O_2$ to yield LV $\dot{V}O_2$. At the end of the experiment, the LV, including the septum, and the RV free walls were separately weighed. They were 1.06 ± 0.07 and 0.31 ± 0.04 g ($n=12$). These LV values were also used for normalizing LVV.

Experimental protocol. LVP, $\dot{V}O_2$, and PVA data were obtained at five or six changed volumes (0.08–0.23 ml; 0.08–0.28 ml in rare cases) in each heart (volume run). LVV was increased in steps up to an end-diastolic pressure of 10 mmHg in control volume run. An mLVV corresponded to $0.16 (= [0.23 - V_0]/2 + V_0)$ ml. The value of mLVV normal-

ized by LV mass to 1 g ranged from 0.13 to 0.17 ml/g. The mean of mLVV was 0.15 (± 0.01) ml/g in 12 hearts. End-systolic pressure at this mLVV (ESP_{mLVV}) was 60 to 70% of the maximum ESP, at which the changes in LV mechanoenergetics induced by various interventions could be detected sensitively. PVA_{mLVV} was relatively small, but valid for practical use. The mean of systolic unstressed volume (V_0) normalized by LV mass to 1 g was 0.08 (± 0.01) ml/g.

Different LVV loading runs in the control (control volume run) were performed with no inotropic interventions at 240 and 300 bpm in 6 hearts. In a different group of 6 hearts, after the control volume run at 300 bpm, Ca^{2+} inotropism run at a given mLVV and Ca^{2+} volume run at the same LVVs as used in the control volume run were performed during intracoronary infusion of 1% $CaCl_2$ solution. During a Ca^{2+} inotropism run 240- and 300-bpm electrical pacings were both alternately performed at each contractile state. The infusion rate was increased in steps up to 7 or 10 ml/h until end-systolic pressure (ESP) stopped to increase. Each arterial blood Ca^{2+} concentration of the excised hearts was measured by RADIOMETER ABL SYSTEM 505 (Copenhagen, Denmark), showing 1.2 ± 0.2 mmol/l in the control and 2.8 ± 0.7 mmol/l at the maximal Ca^{2+} infusion rate. Throughout the experiment, hematocrit decreased by less than 1.4%, indicating neither hemolysis nor hemodilution.

Data sampling was started 6 min after the onset of 1% $CaCl_2$ infusion. In every volume run and/or Ca^{2+} inotropism run, a steady state was reached 2–3 min after changing LVV and/or 6 min after changing the infusion rate of 1% $CaCl_2$. In each steady state, data were sampled at 500 Hz for 2 s simultaneously, and the sampling was usually repeated 3 times at intervals of 0.5–1 min.

Data analysis. We determined PVA by the method described below because the end-systolic pressure–volume relation of the rat LV was an upward convex curve [1–3, 13–15]. We fitted the end-systolic and end-diastolic pressure–volume points by the same exponential functions as used in the previous studies [1, 2, 4, 13, 14, 16] and obtained the best-fit end-systolic and end-diastolic pressure–volume relations.

PVA is defined as the pressure–volume area circumscribed by the curvilinear end-systolic pressure–volume relation, end-diastolic pressure–volume relation, and the systolic portion of the ventricular pressure–volume trajectory [1, 2, 4, 13]. The area under the end-systolic pressure–volume relation is obtained by integration of the best-fit exponential function. The area under the end-diastolic pressure–volume relation is assumed to be zero within the same LVV range be-

cause of the end-diastolic pressure being lower than 10 mmHg [1–4, 13, 14]. Finally, PVA is normalized by LV mass to 1 g.

PVA-independent $\dot{V}O_2$. We first obtained control end-systolic pressure–volume relation from the observed end-systolic pressure–volume data at five or six different LVVs as a best-fit exponential function curve. During Ca^{2+} inotropism run at an mLVV, end-systolic pressure–volume, and $\dot{V}O_2$ data were sampled. We then obtained each end-systolic pressure–volume relation as a best-fit curve to the observed end-systolic pressure–volume data and those at V_0 . We calculated PVA_{mLVV} by integrating each end-systolic pressure–volume relation from V_0 up to the mLVV.

We then obtained the two composite $\dot{V}O_2$ -PVA relations at the mLVV during stepwise increased Ca^{2+} infusion at 240 and 300 bpm. The $\dot{V}O_2$ -PVA relation of the Ca^{2+} volume run at the maximal rate of Ca^{2+} infusion at 300 bpm shifted upward in parallel to the control $\dot{V}O_2$ -PVA relation at 300 bpm, as previously reported [1, 4]. To obtain the $\dot{V}O_2$ -intercept values (PVA-independent $\dot{V}O_2$ values), we drew the lines including data of each $\dot{V}O_2$ -PVA at the mLVV during the Ca^{2+} infusion, in parallel to the control $\dot{V}O_2$ -PVA relation line at 300 bpm. Basal metabolic $\dot{V}O_2$ was unaltered during the Ca^{2+} infusion, thus indicating that the increased PVA-independent $\dot{V}O_2$ values are due to the increased $\dot{V}O_2$ for Ca^{2+} handling in the E-C coupling.

LV contractility. As an index for LV contractility, we have proposed an “equivalent” ESP-V ratio at an mLVV, ($eESP/ESV$)_{mLVV} [4]. We newly defined it as eE_{max} (equivalent maximal elastance). This index is obtained by calculating the end-systolic pressure–volume ratio of the specific virtual triangular PVA_{mLVV} that is energetically equivalent to the real PVA_{mLVV} [4]. PVA_{mLVV} has been proposed to be a good index for evaluating rat LV contractility in terms of mechanoenergetics [1, 2, 13–15].

Oxygen cost of LV contractility. The oxygen cost of LV contractility was obtained as the slope of linear relation between PVA-independent $\dot{V}O_2$ and eE_{max} . This slope is an index quantifying $\dot{V}O_2$ for Ca^{2+} handling in the E-C coupling per unit LV contractility change.

Statistics. An analysis of covariance (ANCOVA) was applied to compare the paired regression lines of LV $\dot{V}O_2$ -PVA relation obtained by control volume runs at 240 and 300 bpm in each heart, and those by control and Ca^{2+} volume runs at 300 bpm. A comparison of paired individual values was performed by the paired *t*-test. A value of $p < 0.05$ was considered statistically significant. All data are expressed as mean $\pm$ SD.

Fig. 1. Left ventricular end-systolic pressure–volume relations (ESPVRs) and end-diastolic pressure–volume relations (EDPVRs) from experimentally obtained pressure–volume data were best-fit curves by $P_{\text{es}} = A\{1 - \exp[-B(V - V_0)]\}$ and by $P_{\text{ed}} = A'\{\exp[B'(V - V_u)] - 1\}$ at 240- (∇) and 300-bpm pacing (Δ) in one heart (A). Left ventricular oxygen consumption per beat ($\dot{V}O_2$)–PVA (systolic pressure–volume area) linear relations at 240- (∇) and 300-bpm pacing (Δ) in the same heart (B). ∇ , $\dot{V}O_2 = 0.0098 \text{ PVA} + 0.345$. Δ , $\dot{V}O_2 = 0.0100 \text{ PVA} + 0.334$.

Table 1. Variables of left ventricular mechanics at pacing rates of 240 and 300 bpm.

Exp. No.		A (mmHg)	B (1/ml)	V_0 (ml/g)	ESP _{max} (mmHg)	ESP _{mLVV} (mmHg)	PVA _{mLVV} (mmHg · ml · beat ⁻¹ · g ⁻¹)	mLVV (ml/g)
1	HR 240	220.6	10.8	0.06	163.3	110.2	3.45	0.13
	HR 300	223.8	8.8	0.06	165.0	96.4	2.77	0.13
2	HR 240	231.7	9.4	0.08	195.5	120.4	5.08	0.16
	HR 300	189.5	12.6	0.08	180.1	118.4	5.29	0.16
3	HR 240	174.2	14.4	0.09	168.2	123.5	6.85	0.17
	HR 300	162.8	16.4	0.09	156.5	123.0	6.96	0.17
4	HR 240	176.3	9.6	0.08	136.2	93.0	4.08	0.16
	HR 300	158.3	11.0	0.08	130.0	91.6	4.09	0.16
5	HR 240	223.5	11.0	0.08	196.4	125.6	5.10	0.15
	HR 300	234.1	10.7	0.08	195.0	129.5	5.20	0.15
6	HR 240	312.3	7.2	0.07	180.0	125.1	4.31	0.14
	HR 300	309.7	6.3	0.07	192.1	111.6	3.92	0.14
Mean	HR 240	223.1	10.4	0.08	173.3	116.3	4.81	0.15
SD		45.9	2.2	0.01	20.7	11.6	1.08	0.01
Mean	HR 300	213.0	11.0	0.08	169.8	111.8	4.71	0.15
SD		51.6	3.1	0.01	22.4	13.7	1.32	0.01

Exp. No., experiment number; HR, heart rate; A and B, fitting parameters in a equation $P_{\text{es}} = A\{1 - \exp[-B(V - V_0)]\}$; V_0 , volume intercept of end-systolic pressure–volume relation in control volume run; ESP_{max}, maximum end-systolic pressure in control volume run; ESP_{mLVV}, end-systolic pressure at a midrange left ventricular volume; PVA_{mLVV}, pressure–volume area at a midrange left ventricular volume; mLVV, midrange left ventricular volume; SD, standard deviation of the mean values.

RESULTS

End-systolic pressure–volume and $\dot{V}O_2$ –PVA relations at 240 and 300 bpm

Representative control end-systolic pressure–volume and end-diastolic pressure–volume relations obtained from pressure–volume data at five to six varied LVVs at pacing rates of 240 and 300 bpm are shown in Fig. 1A. Each end-systolic pressure–volume relation at 240- and 300-bpm pacing was superimposable. The mean of ESP_{mLVV} was 67.4 ± 3.5 and $66.3 \pm 7.1\%$ of the maximum ESP at 240 and 300 bpm, respectively ($n=6$). As shown in Table 1, all variables of LV

mechanics showed no differences between 240- and 300-bpm pacing.

Despite the curved end-systolic pressure–volume relation, there was a linear relation between $\dot{V}O_2$ and PVA; $\dot{V}O_2$ increased in steps with increased PVA (Fig. 1B). The mean slopes of the $\dot{V}O_2$ –PVA relations at 240- and 300-bpm pacing were $0.012 \mu\text{l O}_2 \cdot \text{mmHg}^{-1} \cdot \text{ml}^{-1}$ ($= 1.2 \times 10^{-5} \text{ ml O}_2 \cdot \text{mmHg}^{-1} \cdot \text{ml}^{-1}$) and $0.011 \mu\text{l O}_2 \cdot \text{mmHg}^{-1} \cdot \text{ml}^{-1}$ ($= 1.1 \times 10^{-5} \text{ ml O}_2 \cdot \text{mmHg}^{-1} \cdot \text{ml}^{-1}$). Its mean $\dot{V}O_2$ intercept (PVA-independent $\dot{V}O_2$) at each pacing rate was $0.30 \mu\text{l O}_2 \cdot \text{beat}^{-1} \cdot \text{g}^{-1}$ ($0.030 \text{ ml O}_2 \cdot \text{beat}^{-1} \cdot 100 \text{ g}^{-1}$) and $0.28 \mu\text{l O}_2 \cdot \text{beat}^{-1} \cdot \text{g}^{-1}$ ($0.028 \text{ ml O}_2 \cdot \text{beat}^{-1} \cdot 100 \text{ g}^{-1}$) in the 6

Table 2. Slope and $\dot{V}O_2$ -intercept of control $\dot{V}O_2$ -PVA relation at pacing rates of 240 and 300 bpm.

Exp. No.	Control $\dot{V}O_2$ -PVA relation				Observed $\dot{V}O_2$ -intercept	
	Slope ($\times 10^{-2} \mu\text{l O}_2 \cdot \text{mmHg}^{-1} \cdot \text{ml}^{-1}$)		Calculated $\dot{V}O_2$ -intercept ($\mu\text{l O}_2 \cdot \text{beat}^{-1} \cdot \text{g}^{-1}$)		$(\mu\text{l O}_2 \cdot \text{beat}^{-1} \cdot \text{g}^{-1})$	
	HR 240	HR 300	HR 240	HR 300	HR 240	HR 300
1	1.13	1.31	0.21	0.20	0.20	0.19
2	1.18	0.92	0.33	0.31	0.31	0.29
3	0.88	0.69	0.25	0.24	0.25	0.25
4	1.26	0.99	0.25	0.25	0.24	0.24
5	0.98	1.00	0.35	0.33	0.33	0.32
6	1.58	1.57	0.41	0.37	0.39	0.38
Mean	1.17	1.08	0.30	0.28*	0.29	0.28
SD	0.22	0.28	0.07	0.06	0.06	0.06

Exp. No., experiment number; HR, heart rate; $\dot{V}O_2$, myocardial oxygen consumption per beat; PVA, pressure-volume area; SD, standard deviation of the mean values. *Significantly different from the mean value at 240 bpm; $p < 0.05$.

Fig. 2. Each example of experimentally obtained left ventricular (LV) pressure-volume data at a midrange LV volume (mLVV) during calcium (Ca^{2+}) intracoronary infusion at 240-bpm pacing (A) and at 300-bpm pacing (B). Control and Ca^{2+} end-systolic pressure-volume relations (ESPVRs) and end-diastolic pressure-volume relations (EDPVRs) in A and B were obtained at 300-bpm pacing. A: Pressure-volume data at 240-bpm pacing (∇) during Ca^{2+} infusion at increased rates in steps at an mLVV. B: Pressure-volume data at 300-bpm pacing (Δ) during the same Ca^{2+} infusion. PVA (systolic pressure-volume area; shaded area) at an

mLVV during Ca^{2+} infusion was obtained by integrating each ESPVR curve from V_0 to a given mLVV ($=0.15 \text{ ml/g}$).

rat hearts (Table 2). Mean slopes showed no differences, but mean $\dot{V}O_2$ intercepts calculated showed a statistically significant difference ($p < 0.05$) between 240- and 300-bpm pacing. However, the mean $\dot{V}O_2$ intercept values actually observed were 0.29 and $0.28 \mu\text{l O}_2 \cdot \text{beat}^{-1} \cdot \text{g}^{-1}$ and showed no significant differences between 240- and 300-bpm pacing (Table 2). Therefore we judged that the difference in the mean $\dot{V}O_2$ intercepts is negligible.

Changes in end-systolic pressure-volume and $\dot{V}O_2$ -PVA data during intracoronary Ca^{2+} infusion under 240- and 300-bpm pacing

After the control volume run at 300 bpm was performed in a different group of 6 hearts, ESP at an mLVV ($=0.15 \text{ ml/g}$) increased in steps with the in-

creased rates of Ca^{2+} infusion (triangles in Fig. 2). $\dot{V}O_2$ -PVA data points deviated right-upwardly on the composite $\dot{V}O_2$ -PVA lines at 240 and 300 bpm (triangles in Fig. 3A). $\dot{V}O_2$ and PVA at the mLVV increased by 44.2 ± 18.4 and $70.9 \pm 36.9\%$ ($p < 0.001$ and $p < 0.01$) at 240 bpm, and by 41.6 ± 12.4 and $58.2 \pm 27.0\%$ ($p < 0.001$ and $p < 0.01$) at 300 bpm at the maximal Ca^{2+} infusion rate (7 or 10 ml/h). The composite $\dot{V}O_2$ -PVA relations at 240 and 300 bpm could be superimposed (Fig. 3A). The blood Ca^{2+} concentration reached 2.8 mmol/l.

Changes in end-systolic pressure-volume and $\dot{V}O_2$ -PVA relations during intracoronary Ca^{2+} infusion under 300-bpm pacing

Each ESP at the LVVs corresponding to the control

Fig. 3. **A:** Left ventricular (LV) oxygen consumption per beat ($\dot{V}O_2$)–PVA (systolic pressure–volume area) relations in the control (solid circles) and at maximally enhanced contractile state (open circles) by calcium (Ca^{2+}) intracoronary infusion in the same heart as in Fig. 2. Each composite $\dot{V}O_2$ –PVA relation during Ca^{2+} infusion at increased rates in steps at a midrange LV volume ($\text{mLVV}=0.15 \text{ ml/g}$) at 240-bpm pacing (∇) and 300-bpm pacing (Δ) could be superimposed. Dotted lines indicate each $\dot{V}O_2$ –PVA relation drawn from each $\dot{V}O_2$ –PVA data point, which was obtained during Ca^{2+} infusion. The slope of each $\dot{V}O_2$ –PVA relation corresponds to that of the $\dot{V}O_2$ –

PVA relation in the control at 300-bpm pacing. The intercept of each $\dot{V}O_2$ –PVA relation corresponds to each PVA-independent $\dot{V}O_2$. **B:** Oxygen cost of left ventricular (LV) contractility (eE_{max}) during Ca^{2+} infusion in the same heart as in A. The relations between PVA-independent $\dot{V}O_2$ and eE_{max} during Ca^{2+} infusion at 240-bpm pacing (∇) and 300-bpm pacing (Δ) are highly linear and can be superimposed. The correlation coefficients of these regression lines are 0.96 and 0.97. The slopes of these relation lines denote the oxygen costs of LV contractility ($=1.1 \times 10^{-4} \mu\text{l O}_2 \cdot \text{ml} \cdot \text{mmHg}^{-1} \cdot \text{beat}^{-1} \cdot \text{g}^{-2}$ at 240 bpm and $1.0 \times 10^{-4} \mu\text{l O}_2 \cdot \text{ml} \cdot \text{mmHg}^{-1} \cdot \text{beat}^{-1} \cdot \text{g}^{-2}$ at 300 bpm) in this heart. A midrange LV volume (mLVV) was 0.15 ml/g.

volume run increased, and the end-systolic pressure–volume relation curve became steeper and reached an LV pressure level higher than control under the Ca^{2+} infusion (7 or 10 ml/h) in the 6 hearts at 300 bpm (open circles in Fig. 2). $\dot{V}O_2$ correlated linearly with PVA in each of the control and Ca^{2+} volume run at 300 bpm. The linear $\dot{V}O_2$ –PVA relation shifted upward with the enhanced contractile state (Fig. 3A). The $\dot{V}O_2$ –PVA data points deviated right-upwardly from the control $\dot{V}O_2$ –PVA data points at each LVV (circles in Fig. 3). The slope of each $\dot{V}O_2$ –PVA relation in the Ca^{2+} volume run was not significantly different from that in the control volume run, but the $\dot{V}O_2$ intercept value of each relation significantly increased ($p < 0.01$ by ANCOVA) in all 6 hearts, as previously reported [1, 4].

Oxygen cost of LV contractility during Ca^{2+} intracoronary infusion

When the Ca^{2+} infusion rate was increased in steps, the PVA-independent $\dot{V}O_2$ increased accompanied with the gradually increased PVA (shaded area in Fig. 2) at an mLVV of 0.15 ml/g at 240 and 300 bpm. Both increases in PVA-independent $\dot{V}O_2$ at 240 and 300 bpm are due to the increased total Ca^{2+} handling in the E–C coupling. The increases in $\dot{V}O_2$ intercept (PVA-independent $\dot{V}O_2$) were proportional to those in LV contractility (Fig. 3A), indicating that the $\dot{V}O_2$ intercept reflected well the LV contractility under the

constant slope of the $\dot{V}O_2$ –PVA relation at 240 and 300 bpm.

Plots of PVA-independent $\dot{V}O_2$ and eE_{max} values calculated from PVA_{mLVV} showed the highly linear relation (Fig. 3B) ($r = 0.92 \pm 0.07$ at 240 bpm and $r = 0.95 \pm 0.03$ at 300 bpm; $n = 6$). We defined the slope of this relation as the oxygen cost of LV contractility. Mean oxygen costs of LV contractility were $(1.4 \pm 0.4) \times 10^{-4} \mu\text{l O}_2 \cdot \text{ml} \cdot \text{mmHg}^{-1} \cdot \text{beat}^{-1} \cdot \text{g}^{-2}$ ($= [1.4 \pm 0.4] \times 10^{-3} \text{ ml O}_2 \cdot \text{ml} \cdot \text{mmHg}^{-1} \cdot \text{beat}^{-1} \cdot 100 \text{ g}^{-2}$ for comparison with that in the canine LV) at 240 bpm and $(1.5 \pm 0.4) \times 10^{-4} \mu\text{l O}_2 \cdot \text{ml} \cdot \text{mmHg}^{-1} \cdot \text{beat}^{-1} \cdot \text{g}^{-2}$ ($= [1.5 \pm 0.4] \times 10^{-3} \text{ ml O}_2 \cdot \text{ml} \cdot \text{mmHg}^{-1} \cdot \text{beat}^{-1} \cdot 100 \text{ g}^{-2}$) at 300 bpm (Table 3). There was no significant difference between them at 240 and 300 bpm.

DISCUSSION

As for an index assessing for LV contractility, we have well used E_{max} (maximal elastance; end-systolic pressure–volume ratio, ESP/ESV) from the specifically linear end-systolic pressure–volume relation in canine hearts [5–9]. In contrast to the linear end-systolic pressure–volume relation in canine hearts, the end-systolic pressure–volume relation in the rat LV is, more generally, curvilinear. We were therefore unable to use E_{max} for assessing LV contractility in rat hearts. Instead we have already proposed ESP_{mLVV} for assessing LV contractility [13–15]. However, this index was

Table 3. Control $\dot{V}O_2$ -PVA relation at pacing rate of 300 bpm and oxygen cost of left ventricular contractility at pacing rates of 240 and 300 bpm.

Exp. No.	Control $\dot{V}O_2$ -PVA relation at HR 300		PVA-independent $\dot{V}O_2$ - $eE_{\max}$ relation		
			Slope: O_2 cost of LV contractility ($\times 10^{-4} \mu\text{l } O_2 \cdot \text{ml} \cdot \text{mmHg}^{-1} \cdot \text{beat}^{-1} \cdot \text{g}^{-2}$) mLVV		
	Slope ($\times 10^{-2} \mu\text{l } O_2 \cdot \text{mmHg}^{-1} \cdot \text{ml}^{-1}$)	$\dot{V}O_2$ -intercept ($\mu\text{l } O_2 \cdot \text{beat}^{-1} \cdot \text{g}^{-1}$)	HR 240	HR 300	(ml/g)
1	1.21	0.34	0.87	0.96	0.15
2	1.70	0.36	1.86	2.03	0.15
3	0.97	0.34	1.51	1.72	0.15
4	0.80	0.33	1.66	1.87	0.16
5	0.93	0.26	1.05	1.00	0.15
6	0.64	0.26	1.68	1.68	0.16
Mean	1.04	0.32	1.44	1.54	0.15
SD	0.34	0.04	0.36	0.41	0.004

Exp. No., experiment number; HR, heart rate; $\dot{V}O_2$, myocardial oxygen consumption per beat; PVA, pressure-volume area; $eE_{\max}$, equivalent maximal elastance; LV, left ventricle; mLVV, midrange left ventricular volume; SD, standard deviation of the mean values.

insufficient to compare LV contractility among LVs with different volumes. To do this, such an index should have been normalized by LVVs.

On the other hand, PVA_{mLVV} has been proposed and used to be a good index for the mechanoenergetic evaluation of rat LV contractility [1, 2, 13–15]. Our recently proposed index for assessing LV contractility, $(eESP/ESV)_{\text{mLVV}}$ [4], (newly defined $eE_{\max}$) is devised from the curved end-systolic pressure–volume relation and PVA_{mLVV} . This index depends on LVV in contrast to $E_{\max}$ which is independent of LVV. Therefore we always adopted an mLVV experimentally obtained, where ESP was about 67% of the maximum ESP as well as in our previous report [4]. This yields a reasonably constant oxygen cost of LV contractility in spite of small deviations in mLVV values in individual hearts. However, the dependency of the oxygen cost of LV contractility on heart rates has not been investigated yet.

In the present study, we compared oxygen costs of LV contractility at 240 and 300 bpm, since the range of heart rates could be varied by pacing, but not widely because of higher sinus rhythm and incomplete relaxation. The pacing rate of 240 bpm should be adopted for the heart from hypothyroid rat [12]. No dependency of the cost on heart rates is expected to compare the oxygen cost of LV contractility under different heart rate conditions.

End-systolic pressure–volume and $\dot{V}O_2$ -PVA relations at 240- and 300-bpm pacing.

All variables of LV mechanics showed no differences between 240- and 300-bpm pacing, suggesting that

LV end-systolic pressure–volume relation did not differ within 240–300 bpm. In the excised canine hearts, it has been reported that the slope of linear end-systolic pressure–volume relation did not differ within a wide range of heart rates at 105–230 bpm [10] and 100–160 bpm [11]. In contrast, in the heart of conscious dogs the linear LV end-systolic pressure–volume relation has shown the progressive increase in its slope and rightward shift at increasing heart rates from 67 to 200 bpm [17]. However, we have already confirmed that the LV end-systolic pressure–volume relation in *in situ* rat hearts did not change significantly within 250–320 bpm [14]. The present results coincide with this result [14].

The mean slopes and $\dot{V}O_2$ intercepts (PVA-independent $\dot{V}O_2$) of the $\dot{V}O_2$ -PVA relations did not differ within 240–300 bpm. These values are similar to those of our previous reports in rat hearts [1, 2], though slightly smaller, and those in canine hearts [5–7]. The present results are consistent with a previous study showing that in the excised canine heart, the $\dot{V}O_2$ -PVA relation did not significantly differ within 105–230 bpm [10]. In contrast, there is another report showing that $\dot{V}O_2$ intercept (PVA-independent $\dot{V}O_2$) of the $\dot{V}O_2$ -PVA relation decreased when the heart rate was increased from 60 to 181 bpm with no change in its slope in the excised canine heart [18]. The possible explanation for the decreased $\dot{V}O_2$ intercept with the increased heart rate was as follows. Basal metabolism includes a certain “basal level” of PVA-independent $\dot{V}O_2$ related to processes such as ion pumps, whose metabolic cost does vary with heart rate. On the other

hand, we considered that the PVA-independent $\dot{V}O_2$ corresponds to the $\dot{V}O_2$ for total Ca^{2+} handling in the E-C coupling, basal metabolism, and possibly the $\dot{V}O_2$ for residual cross-bridge cycling in rat, rabbit, and canine hearts [1, 2, 19–23]. There is a report showing no differences in the resting heat production between unstimulated and stimulated rat LV papillary muscles [24]. We observed no difference in the $\dot{V}O_2$ for basal metabolism obtained by KCl-arrest hearts under 4 Hz- to 5 Hz-stimulation ($n=7$). Therefore we conclude that neither the oxygen cost of PVA nor $\dot{V}O_2$ for Ca^{2+} handling in the E-C coupling and basal metabolism changed at all within 240–300 bpm.

Oxygen cost of LV contractility during Ca^{2+} infusion at 240 and 300 bpm. The oxygen cost of LV contractility indicates the $\dot{V}O_2$ needed for total Ca^{2+} handling in the E-C coupling per unit change in LV contractility (changes in total Ca^{2+} handling $\dot{V}O_2$ in the E-C coupling in proportion to Ca^{2+} concentration)/(changes in LV contractility in proportion to Ca^{2+} concentration). We consider that this cost would be changed by a denominator (the myofilament Ca^{2+} responsiveness) and/or a numerator (the total Ca^{2+} handling/ATP ratio in the sarcoplasmic reticulum and sarcolemma) [8, 9, 25]. The mean values of oxygen costs of LV contractility in rat hearts at 240 and 300 bpm did not differ and corresponded to our previous one [4] and to oxygen costs of E_{max} in canine hearts [5–7]. The present results suggest that neither the total Ca^{2+} handling/ATP ratio nor the myofilament Ca^{2+} responsiveness changes within 240–300 bpm and that we are able to compare oxygen costs of LV contractility in the heart paced at 240–300 bpm with no correction by heart rate. In *in situ* hearts, LV contractility and heart rate are changed physiologically by the control of the autonomic nervous system. Under these conditions, heart rate independency of the oxygen cost of LV contractility seems to be critical and indispensable for characterizing LV proper mechanoenergetics. On the basis of the above-mentioned heart rate independency of the oxygen cost of LV contractility, we conclude that even under the different pacing rates within 240–300 bpm, this index is valid for assessing cardiac mechanoenergetics, especially the economy of total Ca^{2+} handling in E-C coupling.

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