Left Ventricular Mechanoenergetics in Small Animals

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Abstract: Studies on left ventricular mechanical work and energetics in rat and mouse hearts are reviewed. First, left ventricular linear end-systolic pressure-volume relation (ESPVR) and curved end-diastolic pressure-volume relation (EDPVR) in canine hearts and left ventricular curved ESPVR and curved EDPVR in rat hearts are reviewed. Second, as an index for total mechanical energy per beat in rat hearts as in canine hearts, a systolic pressure-volume area (PVA) is proposed. By the use of our original system for measuring continuous oxygen consumption for rat left ventricular mechanical work, the linear left ventricular myocardial oxygen consumption per beat (VO_2)-PVA relation is obtained as in canine hearts. The slope of VO_2 -PVA relation (oxygen cost of PVA) indicates a ratio of chemomechanical energy transduction. VO_2 intercept (PVA-independ-

ent VO_2) indicates the summation of oxygen consumption for Ca^{2+} handling in excitation-contraction coupling and for basal metabolism. An equivalent maximal elastance ($e\text{Emax}$) is proposed as a new left ventricular contractility index based on PVA at the midrange left ventricular volume. The slope of the linear relation between PVA-independent VO_2 and $e\text{Emax}$ (oxygen cost of $e\text{Emax}$) indicates changes in oxygen consumption for Ca^{2+} handling in excitation-contraction coupling per unit changes in left ventricular contractility. The key framework of VO_2 -PVA- $e\text{Emax}$ can give us a better understanding for the biology and mechanisms of physiological and various failing rat heart models in terms of mechanical work and energetics. [The Japanese Journal of Physiology 54: 175–207, 2004]

Key words: rat, left ventricle, oxygen consumption per beat (VO_2), systolic pressure-volume area (PVA), sarcoplasmic reticulum Ca^{2+} ATPase (SERCA2a), equivalent Emax ($e\text{Emax}$).

I. Introduction

We have proposed the two key mechanoenergetic indexes: “oxygen (O_2) cost of mechanical energy (PVA)” and “ O_2 cost of contractility (Emax).” These two O_2 costs are proved to be relatively constant under various physiological conditions, but they may alter under pathophysiological conditions such as myocardial stunning and acidosis. We consider that the analyses of these two costs will greatly facilitate a better understanding of cardiac mechanoenergetics of both normal and failing hearts. The heart maintains its pumping action by converting chemical energy contained in metabolic substrates into mechanical energy and work [1]. ATP is the final source of chemical energy for a mechanical contraction of the muscle [1–3]. Cardiac energy metabolism is normally aerobic, i.e., consum-

ing O_2 ; most ATP is produced by oxidative phosphorylation in mitochondria in myocardium. Therefore cardiac oxygen consumption is virtually equivalent to the total energy utilization of the heart ($1 \text{ ml } \text{O}_2 = 20 \text{ J}$) [4, 5].

ATP is used in myocardium by three major ATPases: $\text{Na}^+\text{-K}^+\text{-ATPase}$ at the sarcolemma, $\text{Ca}^{2+}\text{-ATPase}$ at the sarcoplasmic reticulum (SR), and myosin ATPase at the contractile element [5]. The $\text{Na}^+\text{-K}^+\text{-ATPase}$ is related to both Na^+ and K^+ handling in the electrical activation of the sarcolemma and to Na^+ handling coupled with $\text{Na}^+\text{-Ca}^{2+}$ and $\text{Na}^+\text{-H}^+$ exchanges. The $\text{Ca}^{2+}\text{-ATPase}$ is related to Ca^{2+} handling in the excitation-contraction (EC) coupling for the activation of contraction and its relaxation. The myosin ATPase is related to cross-bridge cycling for mechanical contraction [5]. The mechanical loading, excitation rate,

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and inotropic conditions of the heart are known to affect the rate and amount of ATP hydrolysis by these ATPase and thus myocardial oxygen consumption [3, 4]. However, their quantitative relations in normal and failing hearts would be different [6].

In this review, we summarize recent studies, including ours, especially focusing on small animal hearts analyzed mainly at the whole heart, and also at the myocardium slice, by an application of the two key indexes: the O_2 cost of mechanical energy (pressure-volume area, PVA) and the O_2 cost of equivalent $E_{\max}$ (an index of left ventricular contractility, $eE_{\max}$ for rat hearts). These two O_2 costs have been shown to be relatively constant under various physiological and pathological conditions [7–11], but to be altered under intracoronary infusion of sodium nitroprusside [12] or barium [13]. We consider that the analyses of these two costs will greatly facilitate a better understanding of cardiac mechanoenergetics of normal and failing hearts.

II. Definition and analyses of left ventricular function in canine and rabbit hearts

(1) Time-varying elastance model

When the Hill two-element model is used to calculate the energy costs of external and internal work, the energy costs of work are 1:3, respectively, in regard to external work and internal work. This leads to the general contention that internal work is three times more costly than external work [14]. However, it is not easily conceivable how the contractile element could discriminate external work from internal work and perform these two types of work with different energy costs and efficiencies. There seems to be no mechanism for cross-bridges to recognize whether the mechanical work performed by cross-bridge cycling is conveyed to the outside of the fiber or stored in the series elasticity within it.

Suga developed the idea that the time-varying elastance model of the ventricle could help solve this intriguing problem of cardiac energetics [15]. He proposed this model as a result of his own ventricular pressure and volume measurements in canine hearts [16–18]. Although this model of the ventricle is conceptually the same as the time-varying capacitance simulating cardiac pumping in computer models of the cardiovascular system [16, 19], he gave a resolute physiological basis to the time-varying elastance model of the left ventricle (LV) [16, 19, 20]. In this model, the level of ventricular contractile state was assessed in terms of a new index, $E_{\max}$ [21, 22]. $E_{\max}$ is the maximal or end-systolic value for the ratio $P(t)/[V(t) -$

$V_0]$, where $P(t)$ and $V(t)$ are LV instantaneous pressure and volume and V_0 is the ventricular dead volume at which peak isovolumic pressure is zero [16, 23]. The dimensions of $E_{\max}$ are $\text{mmHg} \cdot \text{ml}^{-1}$. Normalized $E_{\max}$ with respect to LV weight can be expressed in terms of $\text{mmHg} \cdot \text{ml}^{-1} \cdot 100 \text{ g}$. The advantages and limitations of the time-varying elastance model and the $E_{\max}$ concept have been well studied and fully reviewed [16, 24–27].

$E_{\max}$ is proposed as a slope of the linear ESPVR. However, in small animal LV the ESPVR is curvilinear, not linear. Therefore as an index for LV contractility, a new index should be proposed. Consequently, we proposed an equivalent $E_{\max}$ (a slope of a triangular area equivalent to pressure-volume area), described later [28].

(2) Total mechanical energy and ventricular pressure-volume area (PVA)

Based on the time-varying elastance model of the ventricle [21, 23–27], Suga [15] proposed a new measure of total mechanical energy of ventricular contraction. In this proposal, he used the simplest version of the time-varying elastance model that consisted of only an ideal elastance to increase and decrease as a function of time during each contraction [19–21, 29].

The energetic consequences of the time-varying elastance provide the theoretical basis for the definition and measurement of the total mechanical energy. This energy can be quantified as a specific area, the systolic pressure-volume area (PVA), in a pressure-volume diagram. Mechanical energy is generated with increases in the time-varying elastance and the counterclockwise rotation of the instantaneous pressure-volume relation curve [15, 23]. The total area swept by the instantaneous pressure-volume curve on the origin side of the working pressure-volume point during a contraction represents the total mechanical energy generated by the contraction. The total area consists of smaller areas: One is the area for external work (EW), surrounded by the P-V loop, and the other is the area for mechanical or elastic potential energy (PE) between the end-systolic and end-diastolic P-V relation curves on the origin side of the P-V loop [15]. Thus PVA is a simple sum of EW and PE. The shape of the instantaneous end-systolic and end-diastolic pressure-volume curves do not affect the definition and determination of the total mechanical energy and PVA [15, 30].

Although the amount of EW is independent of the model used, the nature and amount of PE depend on the mechanical model of the heart. Although PE is a type of internal energy, it is not equal to the previously

defined internal work [31, 32]. Therefore PE was adopted instead of internal work [15, 33].

Although PVA has been determined exclusively on the basis of the ideal time-varying elastance model, more-complex time-varying elastance models may be needed to simulate ventricular pressure-volume relations and mechanical energetics under various loading and contractile conditions [34, 35]. An output resistance may be required to simulate the flow dependence of the elastance [34, 36–38]. This series resistance, if it is a viscous one, consumes energy when ejection flow exists so that EW would be underestimated by the energy loss [15, 35]. Because PVA is determined from measured LV pressure, such a series resistance may make the actually determined PVA slightly, but variably, underestimate (by less than 10% in ordinary ejecting contractions) the true total mechanical energy generated inherently in the time-varying elastance [15, 35]. However, this series resistance has been shown to be nonviscous and not to have an important role in ventricular energetics [39].

The total mechanical energy generated by contraction could eventually be quantified by the total number of cross-bridge cycles and the thermodynamic efficiency of each cross-bridge hydrolyzing ATP [40, 41]. Although the number of cross-bridge cycles per one ATP is generally considered to be 1 and thus the number of cross-bridge cycles to be proportional to the number of ATP moles hydrolyzed [42], there is evidence that the coupling ratio between cross-bridge cycling and ATP hydrolysis deviates from unity under certain loading conditions [43]. Moreover, the cycling rate of a cross-bridge is calculated to be 1–3 cycles per beat [44]. A recent study using Spring-8 succeeded for the first time in observing myocardial cross-bridge dynamics in the beating whole heart [45].

(3) VO_2 -PVA relation

Whether and how PVA would correlate with myocardial O_2 consumption per beat (VO_2) of the LV has been studied, using the excised cross-circulated canine heart preparation [5]. The E_{max} and PVA of each contraction were determined online. LV PVA normalized for 100 g LV was expressed in $\text{mmHg} \cdot \text{ml} \cdot \text{beat}^{-1} \cdot 100 \text{ g}^{-1}$.

VO_2 was determined as the product of coronary flow and arteriovenous O_2 content difference (AVO_2D)/heart rate [46]. PVA and VO_2 were both determined in a steady state under varied loading, heart rate, and inotropic conditions.

End-diastolic LV volume (preload) was changed to obtain a volume-loaded VO_2 -PVA relation. Isovolumic and ejecting contractions in a steady state were pro-

duced at 4–5 different LV volumes including V_0 . Volume-loaded VO_2 -PVA relations were obtained before and after inotropic interventions such as Ca^{2+} or dobutamine as described later [47]. During an inotropic intervention, a different type of VO_2 -PVA relation at a constant LV volume was obtained as a composite VO_2 -PVA relation.

Important findings obtained from these studies in normal canine hearts have been previously reviewed [5, 48]. (1) VO_2 correlates closely and linearly with PVA at different LV volumes in any stable contractile state whether control, enhanced by a positive inotropic agent such as Ca^{2+} , or depressed by a negative inotropic agent such as pentobarbital sodium in any given heart. The VO_2 -axis intercept value of the VO_2 -PVA relation is the same as VO_2 obtained in unloaded contraction at V_0 . (2) The slope of the VO_2 -PVA relation is the same regardless of contraction mode, whether isovolumic or ejecting in a given heart. (3) The slope of the VO_2 -PVA relation is relatively constant in different normal hearts in a stable contractile state. (4) During a graded inotropic intervention, the slope of the composite VO_2 -PVA relation is usually steeper than the slope of the volume-loaded VO_2 -PVA relation. (5) The volume-loaded VO_2 -PVA relation shifts upward or downward usually in a parallel manner with an enhanced or depressed contractile state. (6) The magnitudes of the upward or downward shift of the VO_2 -PVA relation and thus the VO_2 intercept (PVA-independent VO_2) are usually comparable to increases or decreases in contractility assessed by E_{max} .

However, more recent studies exhibited a different type of mechanoenergetic results in rat hearts, mentioned later.

(4) Load-independence of the VO_2 -PVA relation

Isovolumic versus ejecting. In our previous studies, the VO_2 -PVA relation was compared between isovolumic and ejecting contractions in each canine ventricle [49]. In either mode of contraction, the relation was linear with a nearly unity correlation coefficient ($r = 0.96$ on average). When both modes are pooled, the two relations were superimposable and the resultant VO_2 -PVA relation was also linear with a similar r (0.96 on average).

The concept of PVA, which was originally developed for a ventricular chamber, was applied to a papillary muscle preparation [50]. The linear muscle version of PVA was called “force-length area” (FLA). The correlation coefficient, r between VO_2 and FLA, was 0.96 on average. Moreover, the VO_2 -FLA relation was common to both isometric and shortening con-

tractions. These results indicate that the linear, load-independent VO_2 versus total mechanical energy relationship holds reasonably well in myocardium in the same manner as in the ventricle. That is, the load-independent linear VO_2 -PVA relationship seems to be intrinsic to myocardium; it is not necessary to consider the complex shape of the ventricular chamber and myocardial fiber orientation within the ventricular wall in energetics. This feature of the VO_2 -PVA (or FLA) relationship seems to be closely related to the law of conservation of energy because energy is a scalar variable and the total is a simple sum of all the parts.

Energy equivalence of external work and potential energy. The load independence of the VO_2 -PVA relation was confirmed not only in the baseline contractile state without any intervention, but also in an enhanced contractile state as long as the contractile state was stable [23].

Within a constant PVA, almost all the PE can be converted effectively into EW without affecting VO_2 by appropriately unloading LV pressure-volume load during the relaxation period [51]. This result evidently indicates that the mechanical energy represented by PVA can be either PE or EW without affecting myocardial energetics, i.e., PE and EW are energetically equivalent.

In an isovolumic contraction, PE stored in the ventricle at peak contraction is given by PVA. If no external work is done, PE is released as heat, and thus the heat released in relaxation should be identical to PVA. These results confirmed the energetic prediction of the time-varying elastance model developed for the whole LV; PE (= PVA in an isovolumic contraction) is released as heat when unused.

(5) Contractility-dependence of the VO_2 -PVA relation

Cytosolic Ca^{2+} is bound to troponin C and this binding elicits cross-bridge cycling [42]. An increased concentration of extracellular Ca^{2+} increases sarcolemmal Ca^{2+} influx through voltage-dependent Ca^{2+} channels during the membrane depolarization. The resultant increase in intracellular Ca^{2+} concentration augments total released Ca^{2+} from the SR and enhances contractility.

Dobutamine binds to β_1 -adrenergic receptors (βARs) and activate adenylate cyclase by coupling through the stimulatory G-protein, $\text{G}\alpha_s$. The resulting increase in intracellular cAMP activates cAMP-dependent protein kinase (PKA). PKA has several substrates in cardiomyocytes that influence contractility in response to activated βAR signaling; these include the L-type

Ca^{2+} channel in the sarcolemma, the ryanodine receptor (RyR2), and phospholamban (PLB) in the sarcoplasmic reticulum (SR). The phosphorylation of PLB leads to the increase of SR Ca^{2+} ATPase (SERCA) and consequent accelerated Ca^{2+} accumulation in SR. Consequently, dobutamine enhances contraction and relaxation [5].

When the intracoronary arterial administration of CaCl_2 or dobutamine increased E_{max} by 50–100%, it also elevated the volume-loaded VO_2 -PVA relation in a parallel manner to the baseline-volume-loaded VO_2 -PVA relation with a 50–100% increase in the PVA-independent VO_2 [23, 47]. This is usually the standard basic protocol to examine the dependency of the VO_2 -PVA relation on E_{max} .

(6) O_2 cost of PVA

The slope of the VO_2 -PVA relation means the “ O_2 cost of PVA” or the O_2 cost of mechanical energy. When the slope is a , PVA-dependent VO_2 is expressed by $a\text{PVA}$. Then VO_2 is given as $\text{VO}_2 = a\text{PVA} + \text{PVA-independent } \text{VO}_2$. From a large number of canine LV in our previous studies, an average value of $1.4\text{--}1.6 \times 10^{-5} \text{ mlO}_2 \cdot \text{mmHg}^{-1} \cdot \text{ml}^{-1}$ was obtained for the O_2 cost of PVA [47, 52, 53]. This means that about $1.4\text{--}1.6 \times 10^{-5} \text{ mlO}_2$ is needed to increase PVA by $1 \text{ mmHg} \cdot \text{ml} \cdot \text{beat}^{-1}$. One mlO_2 is biochemically equivalent to approximately 20 J, or precisely 19.5–20.5 J, depending on the metabolic substrate used [54]. One $\text{mmHg} \cdot \text{ml}$ of PVA is physically equivalent to $1.33 \times 10^{-4} \text{ J}$. Therefore the a value of $1.5\text{--}1.8 \times 10^{-5} \text{ mlO}_2 \cdot \text{mmHg}^{-1} \cdot \text{ml}^{-1}$ is convertible to about 2.3–2.7 (dimensionless) [5]. Note that this value is independent of LV weight.

(7) O_2 cost of E_{max}

The activation component of VO_2 is considered to reflect the energy expenditure by both sarcolemmal Na^+ - K^+ -ATPase and SR Ca^{2+} -ATPases. Since the total amount of released Ca^{2+} is considered to increase in an enhanced contractile state, it is easily conceivable that a greater amount of Ca^{2+} is removed mostly into the SR by the Ca^{2+} -ATPase consuming more ATP with a stoichiometry of 2Ca^{2+} : 1ATP [55] and additionally to the outside by the sarcolemmal Na^+ - Ca^{2+} exchanger. More Ca^{2+} seems to flow into the myocardial cell through the voltage-dependent Ca^{2+} channel during membrane depolarization in an enhanced contractile state [55]. This amount of Ca^{2+} must be removed from the cell during repolarization [56]. The Na^+ - Ca^{2+} exchanger per se does not consume ATP to remove Ca^{2+} in exchange with influx Na^+ (stoichiometry = 3Na^+ : 1Ca^{2+}) [55]. However, this influx Na^+ must be pumped

out by the $\text{Na}^+\text{-K}^+\text{-ATPase}$ with a stoichiometry of $3\text{Na}^+ : 2\text{K}^+ : 1\text{ATP}$. Therefore the net stoichiometry is $1\text{Ca}^{2+} : 1\text{ATP}$, which is half that of the SR $\text{Ca}^{2+}\text{-ATPase}$. As a whole, the enhancement of contractility is associated with increases in both the amount of Ca^{2+} handled in the excitation-contraction-relaxation cycle and its energy utilization.

Therefore our finding of a PVA-independent VO_2 proportionally increased to an increase in E_{max} seems to be based on the stoichiometry between the amount of Ca^{2+} to be handled in the excitation-contraction-relaxation process and the amount of ATP consumed in the ion pumps [5].

We have introduced the O_2 cost of E_{max} to quantify the requirement of PVA-independent VO_2 per a unit increase in E_{max} . This cost is expressed by the slope of the relation between PVA-independent VO_2 and E_{max} obtained while E_{max} is being changed by an inotropic intervention [5]. When $\text{VO}_2\text{-PVA}$ relations are obtained at different levels of E_{max} , they elevate differently, but their slopes are virtually the same. The elevation increases proportionally with increased E_{max} . We fix ventricular end-diastolic volume (EDV) and change E_{max} gradually because the $\text{VO}_2\text{-PVA}$ relations at different E_{max} are parallel to each other [5].

When E_{max} and thus PVA and VO_2 are increased gradually at constant EDV, the $\text{VO}_2\text{-PVA}$ data points linearly moved right-upward, i.e., deviated upward from the baseline $\text{VO}_2\text{-PVA}$ relation line [57]. We called the regression line of these points a composite $\text{VO}_2\text{-PVA}$ relation.

From this relation, we determined the PVA-independent VO_2 for each E_{max} by drawing a line through the data point parallel to the baseline $\text{VO}_2\text{-PVA}$ relation. PVA-independent VO_2 values were plotted against the corresponding E_{max} values, and the relation was linear. This relation could be formulated by an empirical equation: PVA-independent $\text{VO}_2 = \mathbf{c}E_{\text{max}} + \mathbf{d}$. Slope coefficient $\mathbf{c}$ indicates the O_2 cost of E_{max} . Constant $\mathbf{d}$ indicates the PVA-independent VO_2 intercept of the relation. From our studies [47, 52, 53], the O_2 cost of E_{max} was approximately $0.0015\text{--}0.0030\text{ mL O}_2 \cdot \text{mmHg}^{-1} \cdot \text{beat}^{-1} \cdot 100\text{ g}^{-2}$. The reciprocal of $\mathbf{c}$ means the economy of the E-C coupling.

(8) PVA-dependent and PVA-independent VO_2

KCl-arrested VO_2 was not significantly affected by various inotropic agents when KCl was infused directly into coronary circulation at a barely minimal level to cause cardiac arrest [5]. This result indicates that the fraction of VO_2 that changed with E_{max} at V_0 , i.e., of unloaded contraction with zero PVA, is related

to myocardial activation or E-C coupling, which primarily handles intracellular Ca^{2+} transport [2, 56]. Any contraction with a positive PVA requires an extra VO_2 proportional to the PVA. Therefore the total VO_2 is composed of the three major energy expenditures, i.e., mechanical contraction, myocardial activation, and basal metabolism.

The energy expenditure of Ca^{2+} handling in the activation is assumed to be PVA-independent VO_2 . In fact, we have found that unloaded VO_2 is virtually independent of whether the unloaded contraction is either the isovolumic contraction at V_0 or completely systolic unloaded contractions from high EDVs [58].

The linear $\text{VO}_2\text{-PVA}$ relation indicates that VO_2 for mechanical contraction is proportional to PVA. Therefore it is reasonable to call the VO_2 above the unloaded VO_2 “PVA-dependent VO_2 ” and the VO_2 below the unloaded VO_2 “PVA-independent VO_2 ”.

PVA-dependent VO_2 is consumed for ATP production for myosin ATPase. PVA-independent VO_2 is consumed for ATP production for SR $\text{Ca}^{2+}\text{-ATPase}$ [59], sarcolemmal $\text{Na}^+\text{-K}^+\text{-ATPase}$, and basal metabolism. It remains unknown whether the PVA-independent VO_2 is always constant regardless of PVA, because this VO_2 component cannot explicitly be separated from the PVA-dependent VO_2 in contractions with positive PVA.

(9) E-C coupling Ca^{2+} handling energy

In cardiac myocytes, the transition from the resting relaxed state with a low intracellular concentration of Ca^{2+} to a contraction occurs because a small quantity of Ca^{2+} crosses the sarcolemma and induces a much larger release of Ca^{2+} from the SR. The initial events that couple excitation to contraction are initiated by a depolarization of the cell membrane, which causes an opening of the voltage-gated Na^+ and Ca^{2+} channels. The initial upstroke of the action potential in ventricular cardiac myocytes is caused by Na^+ influx via the Na^+ channel, whereas the subsequent slow inward current maintaining the plateau of the action potential is caused primarily by Ca^{2+} influx via the L-type Ca^{2+} channel. Although cumulative Na^+ influx via the Na^+ channel can influence intracellular Ca^{2+} concentration via the $\text{Na}^+\text{-Ca}^{2+}$ exchanger (Ca^+ influx- Na^+ efflux), it is the Ca^{2+} influx via the Ca^{2+} channel that is considered to be of greatest importance in E-C coupling [60–63].

The Ca^{2+} that enters the cell activates the Ca^{2+} release channel of the SR, releasing Ca^{2+} from internal stores within the SR: calcium-induced calcium release (CICR) [64]. The probability of opening the Ca^{2+} release channel in the SR is markedly increased by ex-

posure to μM concentrations of Ca^{2+} . The SR Ca^{2+} channel has phosphorylation, ATP-binding, and calmodulin-binding sites. The Ca^{2+} released from the SR binds to the contractile proteins and initiates contraction. In the resting state, the interaction of actin and myosin is inhibited by the troponin-tropomyosin complex, which is bound to actin. When Ca^{2+} binds to troponin C, a conformational change of the troponin-tropomyosin complex is induced, relieving this inhibition and resulting in a cross-bridge interaction and contractile element shortening [65]. Thus changes in the affinity of the contractile proteins to Ca^{2+} as well as the magnitude of the Ca^{2+} transient achieved during each beat can regulate force development by a myocyte [63].

The decay of the Ca^{2+} transient occurs primarily by a reuptake of Ca^{2+} into the SR mediated by the SR Ca^{2+} ATPase and secondarily extrusion of Ca^{2+} from the myocyte mostly by the Na^+ - Ca^{2+} exchanger: Ca^{2+} efflux- Na^+ influx "forward" mode of operation.

The contribution of the sarcolemmal Ca^{2+} -ATPase is practically negligible [69] except in failing hearts. The K_m (a K_m for Ca^{2+}) for Ca^{2+} transport of the SR Ca^{2+} ATPase is 300–400 nM. Thus the SR Ca^{2+} -ATPase is strongly activated in the range of physiological Ca^{2+} concentrations that occur during the normal contraction-relaxation cycle. The regulation of the SR Ca^{2+} ATPase occurs primarily by phosphorylation of PLB [59].

Competition between Ca^{2+} extrusion by the Na^+ - Ca^{2+} exchanger and Ca^{2+} uptake by the SR is physiologically important because Ca^{2+} that is taken up by the SR is available to the Ca^{2+} transient during a subsequent beat and thus can contribute to force development. In a steady state, the amount of Ca^{2+} entering the cell equals that extruded from the cell, and the amount released by the SR equals that sequestered by the SR.

The VO_2 for E-C coupling is used for removing SR Ca^{2+} from 1–10 μM to 0.1 μM by active transport, mainly by SR Ca^{2+} -ATPase, to relax contraction [66–68]. Changes in contractility are accompanied by proportional changes in the amount of Ca^{2+} involved in the E-C coupling unless Ca^{2+} sensitivity (or responsiveness) of the contractile proteins is altered [47, 53, 69–71]. Such Ca^{2+} changes would then change the energy required for Ca^{2+} sequestration by the following four Ca^{2+} transport systems: (1) SR Ca^{2+} -ATPase; (2) sarcolemmal Na^+ - Ca^{2+} exchanger (these two mechanisms are predominant); (3) sarcolemmal Ca^{2+} -ATPase; (4) mitochondrial Ca^{2+} uptake (the latter two mechanisms are about 50-fold slower than the former two mechanisms) [63, 72–74].

A tight coupling exists between two moles of Ca^{2+} transported and one mole of ATP hydrolyzed in myocardial SR unless the SR is leaky to Ca^{2+} [75, 76]. This tight Ca^{2+} : ATP stoichiometry suggests that VO_2 for Ca^{2+} handling by the SR is proportional to the amount of Ca^{2+} handled by the SR in the E-C coupling regardless of the Ca^{2+} release and uptake speeds [21, 75]. Uncertainty of the amount of Ca^{2+} handling via the Na^+ - Ca^{2+} exchanger makes it difficult to exactly partition the Ca^{2+} handling VO_2 into the VO_2 components of the SR Ca^{2+} pump and the Na^+ - Ca^{2+} exchanger.

Low concentrations of ryanodine open the SR Ca^{2+} release channel and thus deplete the SR Ca^{2+} stores. In the presence of a continued stimulation of myocardial cells, ryanodine does not completely deplete the SR. However, in the rested cells exposed to ryanodine, the SR becomes completely depleted [63]. Therefore in the resting myocardium, energy expenditure for sequestering leaked Ca^{2+} appears to be small [76].

The activation (or E-C coupling) energy of 0.4 J/100 g observed experimentally in a control contractile state corresponds to about 100 nmol Ca^{2+} /g tissue to be released and sequestered per beat on the basis of a Ca^{2+} pump stoichiometry of 2Ca^{2+} /ATP [77]. Then VO_2 for E-C coupling of about $0.3\text{--}0.4 \text{ J}\cdot\text{beat}^{-1}\cdot 100 \text{ g}^{-1}$ in the excised cross-circulated canine heart [23] suggests the involvement of 60–100 nmol Ca^{2+} /g tissue [3, 78]. This amount of Ca^{2+} involved in E-C coupling varies among literatures; for example, the 20 and 70% maximum force developments require Ca^{2+} of 17 and 26 nmol/g wet muscle in canine heart [79].

The parallel shifts of the VO_2 -PVA relation under varied E_{max} [21] are consistent with the contemporary concept that the primary cellular change accompanying changes in contractility is the amount of Ca^{2+} released to the myofilaments per beat. The close relation between unloaded VO_2 and E_{max} seems consistent with the linearity of the relation between Ca^{2+} transients and contractility [79]. This close relation also seems consistent with a fixed and linear stoichiometry between Ca^{2+} uptake and ATP consumption by SR independent of the contractile state [68, 75].

(10) Basal metabolism

The basal metabolism of myocardium is the energy utilization of myocardial activities other than the electrical activation and the mechanical contraction. Canine whole heart preparations can be relaxed under high K^+ (10–20 mM) perfusion, and basal metabolic VO_2 can be measured directly. The basal metabolic VO_2 is $1.24 \pm 0.59 \text{ mlO}_2$ at 5 min under KCl arrest, decreases to $1.01 \pm 0.53 \text{ ml O}_2$ within the next 5 min, and does not significantly decrease thereafter until

30 min under KCl arrest [80]. The KCl-arrested basal metabolic VO_2 significantly increases with neither increases in EDV from V_0 (about 7 ml) to 50 ml [80] nor 50–100% increases in E_{max} by dobutamine.

The basal metabolic VO_2 of myocardium seems to include ATP utilization for protein synthesis such as healing over, maintenance of cell membrane polarization by the $\text{Na}^+\text{-K}^+\text{-ATPase}$, and phosphorylation of various proteins. The $\text{Na}^+\text{-K}^+\text{-ATPase}$ contributes to only 5–20% of the basal metabolism [81]. Much remains to be elucidated regarding the components of basal metabolic energy utilization.

(11) Residual cross-bridge cycling O_2 consumption

Even the unloaded ventricle contracting with zero PVA changes its shape. The shape change suggests an existence of myocardial shortening by residual cross-bridge cycling and some mechanical energy generation. However, the VO_2 values of contractions that are mechanically unloaded throughout systole by quick release from large EDVs are close to the VO_2 of unloaded contractions at V_0 [58]. These VO_2 data may be called PVA-independent, because PVA is zero or almost zero in any of these contractions. Therefore the VO_2 attributable to the length (preload)-dependent activation seems negligible at least in the canine LV [58]. Furthermore, VO_2 did not significantly decrease even when ventricular volume was decreased from V_0 to complete collapse with a negative pressure in the ventricle [82]. The former condition greatly increased ventricular shape and size changes, and the latter condition minimized ventricular shape changes. Both these observations indicate that ventricular shape changes produced by residual cross-bridge cycling at zero PVA require no significant VO_2 .

In contrast to this contention, a group of investigators [83–85] claim that mechanical unloading cannot unequivocally distinguish mechanical from nonmechanical VO_2 because the mechanically unloaded heart undergoes shape changes with each contraction and beat-to-beat pressure fluctuations in the negative range. Thus they suspected that when the ventricle is unloaded, there is an uncertain amount of energy consumption for residual cross-bridge cycling, and it has been incorrectly assigned to the nonmechanical VO_2 . Their contention is based on the method to partition heat output in rabbit papillary muscles into tension-dependent (cross-bridge cycling) and tension-independent (E-C coupling) components by using a negative inotropic drug, 2,3-butanedione monoxime (BDM) [83]. The principle of this approach is that at relatively low

concentrations (< 5 mM) in isolated rabbit muscles, BDM inhibits cross-bridge cycling without affecting other energy consuming processes [86, 87]. They considered that extrapolation of the VO_2 -force time integral (FTI) relation at $\text{BDM} < 5$ mM to the VO_2 axis provides an estimate of nonmechanical VO_2 , which includes VO_2 for both E-C coupling and basal metabolism, but not VO_2 for residual cross-bridge cycling. Nonmechanical VO_2 was estimated to be $0.00132\text{--}0.0137 \text{ mlO}_2 \cdot \text{beat}^{-1} \cdot 100 \text{ g LV}^{-1}$ [85]. In contrast, mechanically unloaded VO_2 was estimated to be $0.0276 \text{ mlO}_2 \cdot \text{beat}^{-1} \cdot 100 \text{ g LV}^{-1}$, about twice the value of the VO_2 -axis intercept of the VO_2 -FTI relation [85]. These results suggested that during mechanically unloaded conditions, there is considerable energy utilization for “residual” cross-bridge cycling.

However, in the ferret papillary muscle, BDM at concentrations even as low as 1 mM reduced Ca^{2+} current, reduced the peak Ca^{2+} transient, and decreased the sensitivity of the myofilaments [88]. In the excised cross-circulated canine hearts, BDM 0.5–7 mM principally reduces the energy related to Ca^{2+} handling for E-C coupling [89].

Therefore the partition of mechanically unloading O_2 consumption, i.e., the ratio of O_2 consumption for the residual cross-bridge cycling has been controversial until a new approach that uses LV myocardial slices is developed [see Ref. 109].

(12) Free Ca^{2+} and total Ca^{2+} kinetics

The energy utilization for Ca^{2+} handling tends to be markedly underestimated on the basis of the popular observation that the peak Ca^{2+} transient needed to activate mechanical contraction is $0.5\text{--}2 \mu\text{M}$ [69]. However, the amount of Ca^{2+} to be related with energy utilization is the total Ca^{2+} removed by both SR $\text{Ca}^{2+}\text{-ATPase}$ and a combination of the $\text{Na}^+\text{-Ca}^{2+}$ exchanger and the $\text{Na}^+\text{-K}^+\text{-ATPase}$. Seventy-five to 95% of the total Ca^{2+} released to the contractile machinery is removed by the SR $\text{Ca}^{2+}\text{-ATPase}$ (recirculation fraction). This amount is 25–400 times greater than the free Ca^{2+} measured as the Ca^{2+} transient [69]. On the basis of the proportionality between the amount of Ca^{2+} handled in the E-C coupling and the Ca^{2+} handling energy in the SR, the O_2 cost of E_{max} indirectly indicates the total amount of Ca^{2+} handled in the E-C coupling needed to cause a unit change in E_{max} . The Ca^{2+} loading run (increasing extracellular Ca^{2+} concentration) is the standard protocol to obtain the O_2 cost of E_{max} .

It is important to recognize the difference between the Ca^{2+} transient and the total released Ca^{2+} for a better understanding of cardiac energetics. The naive

estimation of a change in the total released Ca^{2+} from an observed change in the Ca^{2+} transient may easily lead to a wrong conclusion.

III. Definition and analyses of left ventricular function in rat and mouse hearts

(1) *In situ* rat hearts

An eight-electrode conductance catheter (No. 7F, 10 cm) previously developed by Baan *et al.* [90] has been applied to big animals such as dogs and humans to measure instantaneous absolute LV volume quantitatively. For calibration they developed the formula $V(t) = (1/a) \times L^2 \times r \times G(t) - V_c$, where $V(t)$ is time-varying LV volume, a is a dimensionless constant, L is the electrode separation, r is the conductivity of blood obtained by a sampling cuvette, and $G(t)$ is the measured conductance within the LV cavity. V_c is a correction term caused by the parallel conductance of structures surrounding the cavity and is measured by transiently changing the resistivity of the blood in the LV by an injection of a small bolus of hypertonic saline (10% NaCl solution). This conductance catheter only when appropriately installed provides a reliable and simple method to measure instantaneous LV volume [90–92]. When the pressure is simultaneously recorded, we could assess the LV pump performance by displaying pressure-volume loops.

A more miniaturized conductance catheter (No. 3F, 1.2 cm) for rats was instituted by Ito *et al.* [93]. It is equipped with six equidistant (3 mm) ring electrodes. The current is delivered by a signal conditioner-processor. With this conductance catheter system and a micromanometer-tipped transducer, we obtained dynamic pressure-volume loops by changing afterload with a temporary aortic clamp. This enables us to obtain curved ESPVR and PVA at an appropriate LV volume (midrange LV volume) (PVA_{mLVV}) to assess contractile states and mechanical work, respectively, *in situ* in small experimental animals such as rats. Furthermore, this conductance catheter system is developed for mice smaller than rats. When the conductance catheter system for these small animals has been instituted, it will be easy to obtain dynamic pressure-volume loops and to estimate cardiac pump function more appropriately. For example, various types of transgenic mice such as $\text{Na}^+\text{-H}^+$ antiporter overexpression or endothelin-1 deficiency can be used for analyzing the function of a specific gene at the whole body level. Pathological model animals such as hypertrophy can also be used for analyzing pathological features at the whole body level [94].

(2) Cross-circulation with blood vs. Langendorff perfusion with crystalloid in rat *in vitro* hearts

Although canine LV mechanoenergetics has been studied mostly in the blood-perfused heart [5, 48], rat LV mechanoenergetics has not been studied in a blood-perfused whole heart preparation. Although rat LV mechanoenergetics has been well used in the crystalloid-perfused heart preparation [95], the crystalloid perfusate has only a tenth of the O_2 -carrying capacity of the normal blood. The blood-perfused rat heart preparation is more physiological than the crystalloid-perfused rat whole heart preparation and less expensive and more compliant to animal welfare than the canine preparation. Furthermore, the rat is frequently used to create genetically engineered models and pathological models of human disease such as transgenic and hypertensive rats [9, 11, 94].

(3) LV mechanoenergetics in cross-circulated rat heart preparations

The objectives for the first study in the excised cross-circulated rat whole heart preparation are to investigate ESPVR and the relation of VO_2 and PVA and to establish a new evaluation of rat LV mechanoenergetics. Although we could reasonably expect a curved ESPVR as in *in situ* rat LV [96, 97], we hypothesize that the VO_2 -PVA relation would still be linear as in the puppy LV [98]. We obtain the results to indicate linear VO_2 -PVA relations of the rat LV despite its curvilinear ESPVRs within physiological ranges of contractile and loading conditions. From these results, we propose PVA at an appropriate LV end-diastolic (= end-systolic) volume (midrange LVV) (PVA_{mLVV}) and the slope and VO_2 intercept of the VO_2 -PVA relation as good LV mechanoenergetic indexes [99].

Experimental procedure. A thin latex balloon (volume, 0.08 ml) is fitted into the LV and connected via a polyethylene tube to a pressure transducer and a 0.5 ml precision glass syringe with fine scales (minimum scale: 0.005 ml). LV pressure (LVP) is measured with this transducer. The balloon does not develop any pressure and thus is unstretched as long as its volume is below approximately 0.4 ml. LVV is therefore changed and measured by adjusting intraballoon water volume with the syringe in 0.05 ml steps and fixed constant by a three-way stopcock. Systolic unstressed volume (V_0) should be determined as the volume at which peak isovolumic pressure and thus PVA are zero. V_0 is determined as the volume-axis intercept of the pressure-volume relation by curve fitting with an equation (see below).

LV epicardial electrocardiogram (ECG) is recorded with a pair of electrodes. Heart rate is maintained constant 20% higher than the sinus rhythm by the electrical pacing of the right atrium at 300 ± 50 beats/min (mean $\pm$ SD) throughout each experiment. The temperature of the heart is maintained at 37°C by a heater throughout the experiment. Systemic arterial blood pressure of the support rat is served as coronary perfusion pressure and is maintained between 90 and 130 mmHg throughout the experiment by transfusion. Arterial pH, P_{O_2} , and P_{CO_2} of the support rat are maintained within their physiological ranges by using supplemental oxygen and changing respiratory rate as needed. We use only isovolumic contractions throughout this study.

O_2 consumption. Total coronary blood flow is continuously measured with an electromagnetic flowmeter placed in the middle of the coronary venous drainage tubing from the RV. LV thebesian flow is negligible. Coronary arteriovenous O_2 content difference (AVO_2D) is continuously measured by passing all the arterial and venous cross-circulation blood through the two cuvettes of a custom-made arteriovenous O_2 content difference analyzer.

Cardiac O_2 consumption is obtained as the product of coronary flow and AVO_2D . It is divided by heart rate (beats/min) to obtain O_2 consumption per beat (VO_2) in a steady state. As in canine hearts, the VO_2 -PVA relation is linear in the rat LV. VO_2 is known to be composed of PVA-dependent and PVA-independent VO_2 at each PVA. VO_2 intercept represents PVA-independent VO_2 . The RV is kept collapsed by con-

tinuous hydrostatic drainage of the coronary venous return; thus the RV PVA and therefore PVA-dependent VO_2 are assumed to be negligible. The RV component of PVA-independent VO_2 is calculated by multiplying biventricular PVA-independent VO_2 in each contractile state with the ratio of RV weight divided by the sum of the RV and LV weights. The RV PVA-independent VO_2 is subtracted from the total VO_2 to yield LV VO_2 . At the end of each experiment, the LV including the septum (approximately 1 g), and the RV free wall (approximately 0.2 g) are separately weighed.

Experimental protocol for volume-loading run (volume run). LV pressure, VO_2 , and PVA data are obtained at six varied volumes (mean volume range: 0.28 ml) in each heart (volume run). LV volume is increased in steps up to an end-diastolic pressure of 10 mmHg. The VO_2 of the minimally volume-loaded beating hearts at a pacing rate of 250–300 beats/min is measured by minimizing intraballoon water volume as close to zero as possible (minimally loaded VO_2). “Control volume run” is performed with no inotropic interventions. After the control volume run, “calcium volume run” at the same LV volumes used in the control volume run is performed during CaCl_2 (1%) infusion into the coronary artery. In each steady state, pressure-volume, AVO_2D , and coronary flow signals are sampled at 500 Hz for 2 sec simultaneously, and the sampling is usually repeated 2–3 times at intervals of 0.5–1 min.

Data analysis. We computed PVA by the method described below because the ESPVR of the rat LV is an upward convex curve unlike the linear ESPVR of

Fig. 1. Key framework of ESPVR- VO_2 -PVA for assessment of left ventricular mechanoenergetics. $\uparrow\downarrow$: isovolumic contraction and relaxation at mLVV. ESPVR: end-systolic pressure-volume relation. EDPVR: end-diastolic pressure-volume relation. mLVV: midrange left ventricular volume. VO_2 : myocardial oxygen consumption per beat. PVA: systolic pressure-volume area. E-C: excitation-contraction. V_0 : volume intercept of ESPVR. Detailed explanations are described in the text.

the adult canine LV [28, 99]. Figure 1A shows a representative ESPVR obtained from experimentally obtained rat LV *P-V* data. We attempted to fit the end-systolic *P-V* points by the following equation:

$$Pes = A\{1 - \exp[-B(V - V_0)]\} \quad (1)$$

where *Pes* is the end-systolic peak isovolumic pressure, *V* the isovolumic volume, *V*₀ the volume intercept of the nonlinear curve best fitted to the end-systolic *P-V* points, and *A* and *B* the fitting parameters. Equation 1 is the same as that used in the previous study on puppy LV mechanics [100]. PVA is defined as the pressure-volume area circumscribed by the curvilinear ESPVR, EDPVR, and the systolic portion of the ventricular pressure-volume trajectory (thick vertical line in Fig. 1A: an isovolumic contraction). The area under the EDPVR was reasonably assumed to be zero within the same volume range. PVA was then normalized by LV mass to 1 g. A representative PVA at midrange LVV (mLVV) is shown by the shaded area in Fig. 1A. In a representative study, mLVV was 0.15 ml/g [PVA_{0.15}] in the range of changed LVV (0.08–0.33 ml) for data sampling [99].

(4) Curved ESPVR and linear VO₂-PVA relation

Although the changes in coronary flow and AVO₂D were slightly different among individual hearts, VO₂ always increased with increased PVA in all hearts. A representative control ESPVR obtained from *P-V* data at several varied LVV is shown in Fig. 1A. The ESPVR that is the best-fit curve by Eq. 1, is an upward convex curve. On average, the best-fit parameters *A* (= mmHg) and *B* (= ml⁻¹) were approximately 160 and 16. We consider that all rat hearts physiologically have curved LV ESPVRs because there is supporting evidence. The first evidence is that in *in situ* ejecting rat LVs, we obtained similar curved ESPVRs [94, 96, 97, 101]. The second evidence is indicated by the previous literature showing that rat papillary muscles had similar convex-upward end-systolic tension-sarcomere length relations at higher Ca²⁺ concentrations [102, 103]. However, the blood Ca²⁺ concentration in rats was approximately 1 mmol/L. Therefore the upward convexity of the rat LV ESPVR may not be due to its high contractility, as in the rat papillary muscle.

Despite the curved ESPVR, there is a reasonably linear relation between VO₂ and PVA (Fig. 1B); VO₂ increases proportionally with increased PVA. For example, the slope of this VO₂-PVA relation is 0.015 μl O₂·mmHg⁻¹·ml⁻¹, and its VO₂ intercept is 0.38 μl O₂·beat⁻¹·g⁻¹.

(5) The framework for assessment of LV mechanoenergetics in rat hearts

The nonlinearity of rat LV ESPVR means that we cannot simply quantify rat LV contractility by a well-known contractility index, E_{max} [5]. Initially, we calculated AB, which had been previously used in puppies [100], but as mentioned later we propose a new contractility index, equivalent E_{max} (eE_{max}) and PVA_{mLVV} as an index to assess LV mechanoenergetics [28, 99]. If we drew a vertical (i.e., isovolumic) *P-V* line at midrange LVV, such as 0.15 ml/g, both AB and PVA_{0.15} increase with the enhanced contractile state. However, AB represents only the initial slope of the ESPVR curve at *V*₀, and PVA_{0.15} represents the total mechanical energy of an isovolumic contraction at the volume of 0.15 ml/g based on the linear VO₂-PVA relation. Thus we propose PVA_{mLVV} to quantify and compare LV mechanoenergetics.

Despite the curvilinear ESPVR, VO₂ linearly correlates with PVA in either the control or the enhanced contractile state. The parallel upward shift of the VO₂-PVA relation under the enhanced contractility by Ca²⁺ in rat hearts is comparable to that in dog hearts [48]. These results suggest that at least the VO₂-PVA relations are similar between the rat and dog hearts despite the marked differences in the shape of LV ESPVR, heart size, heart rate, and the pattern of myosin isozyme. We consider that the linear VO₂-PVA relations in both dog and rat may manifest the proportionality between actually measured energy use and our measure of total mechanical energy in different species.

(6) Mean slope and intercept of the VO₂-PVA relation

The mean slope of the VO₂-PVA relation for rats is almost the same as reported for dogs (0.013–0.021 μl O₂·mmHg⁻¹·ml⁻¹) [10, 11, 28, 48, 99, 104] and for rabbits (0.011–0.025 μl O₂·mmHg⁻¹·ml⁻¹) [105, 106]. The mean slope of this relation is also consistent with the values reported for rats under crystalloid perfusion (0.014 ± 0.005 μl O₂·mmHg⁻¹·ml⁻¹) at unphysiologically lower heart rates (150–215 beats·min⁻¹) than ours (300 beats·min⁻¹) [95, 107]. The slope of the VO₂-PVA relation for rat hearts seems to be maintained highly constant under various mechanoenergetic conditions [7–13], indicating metabolic adaptation in the whole heart level.

On the other hand, the mean VO₂ intercept of this relation for rats (0.35–0.37 μl O₂·beat⁻¹·g⁻¹) is slightly higher than the intercept values for dogs (0.25–0.35 μl O₂·beat⁻¹·g⁻¹) [6, 8, 11, 28, 48, 99], but it corresponds to those for rabbits (0.36–0.40 μl O₂·beat⁻¹·g⁻¹) [105, 106].

(7) PVA-independent VO_2

The VO_2 intercept represents PVA-independent VO_2 . The PVA-independent VO_2 seems to correspond primarily to the VO_2 for Ca^{2+} handling in the E-C coupling, basal metabolism, and possibly the VO_2 for residual cross-bridge cycling [58, 82, 85, 108]. Ca^{2+} intracoronary infusion increased the PVA-independent VO_2 , but did not increase basal metabolism [7, 10]. Increasing extracellular Ca^{2+} concentrations did not increase the corresponding value to this basal metabolism in rat myocardial slices [109]. The increase in PVA-independent VO_2 is considered to be due to the increased VO_2 for Ca^{2+} handling in the E-C coupling, though the VO_2 for residual cross-bridge cycling may exist as the minor component of PVA-independent VO_2 [82, 108, 109]. Taken together, the VO_2 for Ca^{2+} handling in the E-C coupling is the major component of the PVA-independent VO_2 except for basal metabolism in the blood-perfused rat heart preparations (Fig. 1B).

To conclude, the LV curved ESPVR and the linear VO_2 -PVA relation that coexisted in the excised cross-circulated rat whole heart preparation seem promising for mechanoenergetic assessment not only in normal rats, but also in pathological and genetically engineered model rats.

(8) Effects of thapsigargin and KCl on LV mechanoenergetics

PVA_{mLVV} and the VO_2 intercept of the VO_2 -PVA relation can be good indexes for assessing rat LV mechanoenergetics. The VO_2 intercept would mainly consist of VO_2 for Ca^{2+} handling in E-C coupling and basal metabolism. However, the fraction of the VO_2 by the SR Ca^{2+} -ATPase in the total Ca^{2+} handling VO_2 has not yet been examined. The energetic cost of SR Ca^{2+} -ATPase (involved in E-C coupling) has not been determined in rat-excised cross-circulated whole heart preparation. To evaluate this, the effects of thapsigargin, a well-known specific SR Ca^{2+} -ATPase inhibitor [110–112], and KCl on the ESPVR and VO_2 -PVA relation were examined in the rat blood-perfused whole heart preparation.

Effects of thapsigargin. The ESP at the corresponding LVVs to the control volume run decreased, and the ESPVR curve became blunter and reached a lower pressure level than control during thapsigargin infusion. These responses are opposite to those of Ca^{2+} [99]. Thapsigargin ($2.5 \mu\text{mol/L}$) significantly decreased $\text{PVA}_{0.15}$ by 65% ($p < 0.01$).

VO_2 correlated linearly with PVA in either contractile state. The linear VO_2 -PVA relation shifted downward with the decreased contractile state during

thapsigargin infusion. VO_2 -PVA data points deviated left-downward from the baseline VO_2 -PVA data points at each LVV. The slope of each VO_2 -PVA relation in the thapsigargin volume run was not significantly different from the control, but the VO_2 intercept value of each relation significantly decreased during thapsigargin infusion ($P < 0.01$ by ANCOVA). The mean VO_2 intercept of the VO_2 -PVA relations significantly decreased by 40% of control ($P < 0.01$ by paired t-test) with no changes in the mean slope.

KCl arrest. On average, the VO_2 intercept value is $92.4 \mu\text{l O}_2 \cdot \text{min}^{-1} \cdot \text{g}^{-1}$ ($30.9 \text{ mW} \cdot \text{g}^{-1}$). The basal metabolism of the heart was measured by arresting it with KCl (0.03 mM) (basal metabolic VO_2). The coronary flow did not significantly decrease, but AVO_2D significantly did. The VO_2 intercept was decreased by 75% with KCl, and basal metabolic VO_2 was found to be 25% of the VO_2 intercept. Thus the mean VO_2 intercept significantly decreased to $24.3 \mu\text{l O}_2 \cdot \text{min}^{-1} \cdot \text{g}^{-1}$ by 75% (resting VO_2 ; $8.1 \text{ mW} \cdot \text{g}^{-1}$ or $2.43 \mu\text{l O}_2 \cdot \text{min}^{-1} \cdot 100\text{g}^{-1}$), and the energy rate associated with activation processes is $22.8 \text{ mW} \cdot \text{g}^{-1}$. These data are in good agreement with results from the Alpert and Gibbs laboratories in regard to the activation or tension-independent heat [113].

To conclude, the VO_2 intercept mainly consists of VO_2 for Ca^{2+} handling in the E-C coupling (at least 40% is consumed by the SR Ca^{2+} -ATPase) and basal metabolism (25%).

Basal metabolic VO_2 measured after KCl arrest presumably includes VO_2 for Na^+ - K^+ -ATPase to maintain membrane polarization [80]. The higher basal metabolic VO_2 of the rat heart than those of dogs and rabbits is consistent with previous reports [109, 114, 115]. The higher basal metabolic VO_2 in the rat is thought to be due to the rat SR having a poor Ca^{2+} -retaining capacity [109]. Consequently, Ca^{2+} continues to cycle between the SR and the sarcoplasm even in the absence of E-C coupling activation [114]; thus the energy used at the SR is wasted. However, there is no suppression of basal metabolism by either SR Ca^{2+} -ATPase inhibitor [115] in contrast to the results by Ebuss and Stienen [116], suggesting no energy waste in SR.

IV. Advanced framework of ESPVR-PVA-eE_{max} for LV mechanoenergetics in rat hearts

(1) New index for O_2 cost of contractility (eE_{max}) from curved ESPVR

The O_2 cost of LV contractility indicates VO_2 for

Ca^{2+} handling in E-C coupling per unit of LV contractility change in the rat heart. This cost is obtainable as a slope of a linear relation between PVA-independent VO_2 and LV contractility.

(2) PVA-independent VO_2

The control curved ESPVR from the observed ESP-V data at four or five different LVVs is first obtained. During a Ca^{2+} inotropism run at mLVV in the range of changed volume, ESP-V and VO_2 data are sampled. Each best-fit ESPVR from the observed ESP-V data at the mLVV during a Ca^{2+} inotropism run, and those at V_0 are drawn. PVA is calculated by integrating each ESPVR from V_0 up to the mLVV (Fig. 2A). PVA proportionally increased to ESP under an isovolumic contraction.

Then the composite VO_2 -PVA relation at the mLVV during stepwise increased Ca^{2+} infusion is obtained. The VO_2 -PVA relation of an LVV loading run during Ca^{2+} infusion at a maximal rate shifted upward in parallel to the control VO_2 -PVA relation, as previously reported [99]. Thus the dotted lines, including every VO_2 -PVA data during the Ca^{2+} infusion in steps at the mLVV, are drawn in parallel to the control VO_2 -PVA relation line (Fig. 2B). The gradually increased VO_2 -intercept values (PVA-independent VO_2 values) (See

b1–bn in Fig. 2B) of the dotted lines proportionally to the enhanced LV contractility by Ca^{2+} are obtained by this procedure.

(3) LV contractility ($\text{eE}_{\text{max}}_{\text{mLVV}}$)

$\text{eE}_{\text{max}}_{\text{mLVV}}$ is obtained by calculating the ESP-V ratio of the specific virtual triangular PVA_{mLVV} ($1/2 \cdot \text{ESV} \cdot \text{eESP}_{\text{mLVV}}$) that is energetically equivalent to the real PVA_{mLVV} experimentally obtained (Fig. 2A-a and b). The equation for calculation is

$$\begin{aligned} \text{eE}_{\text{max}}_{\text{mLVV}} &= (\text{eESP}/\text{ESV})_{\text{mLVV}} \\ &= 2\text{PVA}_{\text{mLVV}}/\text{ESV}^2, \end{aligned}$$

where eESP is the equivalent end-systolic pressure, ESV is the end-systolic volume (mLVV – V_0), and PVA_{mLVV} is the systolic pressure-volume area at an mLVV. The values of mLVV and V_0 are normalized by LV mass to 1 g.

Our new index for quantifying LV contractility is based on PVA_{mLVV} (a total mechanical energy per beat at an mLVV) that has been proposed to be a good index for a mechanoenergetic evaluation of rat LV contractility [99, 117, 96, 97]. Therefore the value of $\text{eE}_{\text{max}}_{\text{mLVV}}$ is mechanoenergetically reasonable. Since this contractility index is an ESP value normalized by ESV at an mLVV, it is usable to compare LV contractility

Fig. 2. How to obtain oxygen cost of left ventricular contractility (eE_{max}) from the experimental data. Abbreviations are the same as in Fig. 1. Detailed explanations are described in the text.

in the hearts with different volumes. Moreover, if an ESPVR were linear, $eE_{\max_{\text{mLVV}}}$ would equal $E_{\max}$ because $eESP$ equals ESP , and ESP/ESV is constant at all LVVs. The dimensions of this new contractility index ($\text{mmHg} \cdot \text{ml}^{-1} \cdot \text{g}$ or $\text{mmHg} \cdot \text{ml}^{-1} \cdot 100 \text{ g}$) are substantially the same as those of $E_{\max}$ [5, 48]. Thus this new index is usable to compare LV contractility among hearts of different animals, such as dogs and rabbits [5, 48], regardless of the curvilinearity of ESPVR.

(4) O_2 cost of LV contractility

By plotting PVA-independent VO_2 and $eE_{\max_{\text{mLVV}}}$ data, we are able to obtain a relation between them. This relation became linear (Fig. 2C), and thus the oxygen cost of LV contractility is obtained as the slope of the relation. This slope is an index quantifying oxygen consumption for Ca^{2+} handling in the E-C coupling per unit LV contractility change. Moreover, as mentioned above, if an ESPVR is linear, $eE_{\max_{\text{mLVV}}}$ would equal $E_{\max}$. The dimensions of oxygen cost of LV contractility ($\mu\text{l } O_2 \cdot \text{ml} \cdot \text{mmHg}^{-1} \cdot \text{beat}^{-1} \cdot \text{g}^{-2}$ or $\text{ml } O_2 \cdot \text{ml} \cdot \text{mmHg}^{-1} \cdot \text{beat}^{-1} \cdot 100 \text{ g}^{-2}$) are substantially the same as those of the oxygen cost of $E_{\max}$ in canine hearts [47, 53, 118, 119]. Thus our proposed mechanoenergetic index for rat hearts, i.e., the oxygen cost of LV contractility has the generality for other animal hearts.

(5) O_2 cost of LV contractility for Ca^{2+}

The O_2 cost of LV contractility for Ca^{2+} indicates the VO_2 needed for Ca^{2+} handling in the E-C coupling per unit change in LV contractility. We would consider that this value is changed by myofilament Ca^{2+} responsiveness (the LV contractility/ Ca^{2+} handling relation) and/or the Ca^{2+} handling/ATP ratio in the sarcoplasmic reticulum [5, 48, 120]. The heart showing high cost is energetically less economical (energy wasting), and that showing low cost is energetically more economical (energy saving).

Although other inotropic agents such as a β -stimulant are also available tools to assess the oxygen cost of LV contractility, we adopted Ca^{2+} as the first tool. Ca^{2+} directly increases Ca^{2+} handling in the E-C coupling mediated neither via receptors nor via intracellular signal transduction systems. Therefore Ca^{2+} is a golden standard substance to assess the oxygen cost of LV contractility.

Limitations. Our proposed LV contractility index $eE_{\max_{\text{mLVV}}}$ is devised from the curved ESPVR and PVA_{mLVV} . In the curved ESPVR, ESP does not proportionally increase to ESV . Therefore this index somewhat depends on LVV in contrast to $E_{\max}$, which is independent of LVV. However, a midrange LVV where

ESP was 77% of the maximum ESP is constantly adopted. This yielded a reasonably constant oxygen cost of LV contractility despite small deviations in $mLVV$ values in individual hearts. In conclusion, this index is appropriate for assessing cardiac mechanoenergetics, especially the economy of total Ca^{2+} handling in E-C coupling, regardless of the curvilinearity of ESPVR. It would be meaningful to investigate how total Ca^{2+} handling changes induced by various interventions affect the oxygen cost of LV contractility.

(6) O_2 cost of LV contractility for dobutamine in normal and acute failing rat hearts

The O_2 cost of LV $eE_{\max}$ for dobutamine should be assessed instead of a golden standard substance, Ca^{2+} [28]. Dobutamine is often used clinically as a cardiotonic agent. It is known to activate the β -adrenergic receptor-adenylate cyclase-cAMP-protein kinase A (PKA) signaling pathway [121], resulting in the phosphorylation of L-type Ca^{2+} channels, ryanodine receptors, and the SR Ca^{2+} -ATPase regulator, PLB. This signal pathway increases the intracellular Ca^{2+} and LV contraction amplitude and accelerates the kinetics of the target proteins of PKA. On the other hand, Ca^{2+} directly increases the intracellular Ca^{2+} and LV contraction amplitude by increasing the Ca^{2+} influx through L-type Ca^{2+} channels. Because the mechanisms for enhancing the total Ca^{2+} handling are different, the O_2 costs of LV $eE_{\max}$ for Ca^{2+} and for dobutamine are considered to be different.

The O_2 cost of LV $eE_{\max}$ for dobutamine (a β -receptor stimulant) is assessed and compared with that for Ca^{2+} in normal rat hearts and then is assessed and compared in Ca^{2+} overload-induced failing rat hearts [10]. The mean O_2 cost of LV $eE_{\max}$ ($\times 10^{-4} \mu\text{l } O_2 \cdot \text{beat}^{-1} \cdot \text{mmHg}^{-1} \cdot \text{ml} \cdot \text{g}^{-2}$) for Ca^{2+} is 1.30 ± 0.37 , and that for dobutamine is 1.26 ± 0.30 in normal hearts. The mean O_2 cost of LV $eE_{\max}$ for dobutamine is 1.15 ± 0.27 , and that for Ca^{2+} is 0.81 ± 0.36 in the same normal hearts (paired analysis). These mean values show no significant differences between Ca^{2+} and dobutamine.

In Ca^{2+} overload-induced failing hearts, though the mean O_2 cost of LV $eE_{\max}$ for Ca^{2+} could not be assessed as mentioned later [7], the mean O_2 cost of LV $eE_{\max}$ for dobutamine is 1.04 ± 0.40 . This mean value for dobutamine does not differ significantly from those (see above 1.26 ± 0.30 or 1.15 ± 0.27) in the normal hearts. These results indicate, in terms of the coupling of mechanical work and energetics of the heart, that the total Ca^{2+} handling VO_2 in E-C coupling against unit LV contractility change for dobutamine in the contractile failing hearts does not

differ from that in the normal hearts. This suggests that in the Ca^{2+} overload-induced contractile failing hearts, there are no changes in the sensitivity of the contractile machinery for Ca^{2+} , the $\text{Ca}^{2+}/\text{ATP}$ in the total Ca^{2+} handling, and ATP/VO_2 in the mitochondria.

In normal rat hearts, 80–87% of Ca^{2+} recirculates intracellularly [122, 123], i.e., the SR Ca^{2+} -ATPase originally has a main role in the Ca^{2+} handling in E-C coupling. A previous study reveals that the O_2 cost of LV eEmax is increased when SR Ca^{2+} -ATPase is suppressed, and consequently the sarcolemmal Na^+ - Ca^{2+} exchanger and Na^+ - K^+ -ATPase are activated in rat hearts [95]. Although the basal activity of the L-type Ca^{2+} channel is considered to be impaired in the high Ca^{2+} -induced Ca^{2+} overload failing hearts [7], dobutamine could enhance the activity of L-type Ca^{2+} channels via the activation of phosphorylation. The O_2 cost of eEmax for dobutamine remained unchanged in this Ca^{2+} overload-induced failing hearts. Therefore it is possible that dobutamine could sufficiently activate L-type Ca^{2+} channels and thus activate intracellular Ca^{2+} handling without activation of sarcolemmal Na^+ - Ca^{2+} exchanger and Na^+ - K^+ -ATPase.

The high Ca^{2+} -induced Ca^{2+} overload contractile failing rat hearts with unchanged O_2 cost of PVA, decreased VO_2 intercept, and unchanged O_2 cost of eEmax for dobutamine are similar to Ca^{2+} overload-induced stunned myocardium in dog hearts with unchanged O_2 cost of PVA, decreased VO_2 intercept, and unchanged O_2 cost of Emax for Ca^{2+} [48].

On the other hand, in postischemic, hypercapnic acidotic, and postacidotic stunned myocardium in dog hearts, the O_2 costs of Emax for Ca^{2+} increase [48]. In the Ca^{2+} overload contractile failing rat hearts, a transient energy-wasting process is observed during high Ca^{2+} infusion, as previously reported [7]. This transient process may last longer in the postischemic, hypercapnic acidotic, and postacidotic stunned canine myocardium showing the high O_2 cost of Emax [48]. Furthermore, the Ca^{2+} overload contractile failing rat heart seems to induce less cellular breakdown than ischemic-reperfusion injury [7]. Therefore the increases in O_2 costs of Emax for Ca^{2+} in postischemic, hypercapnic acidotic, and postacidotic stunned myocardium in dog hearts may relate to more cellular breakdown.

No significant differences in the O_2 cost of LV contractility are found between dobutamine and Ca^{2+} in normal and high- Ca^{2+} -induced Ca^{2+} overload failing hearts. On the other hand, the O_2 cost of LV contractility for nitroprusside is found to be much higher (fivefold) than those for dobutamine and Ca^{2+} [124].

Therefore this cost could be a sensitive index to evaluate any changes in the total Ca^{2+} handling in the E-C coupling in rat hearts in terms of the coupling of cardiac mechanical work and energetics.

V. Mechanically unloaded LV myocardial slice MVO_2 and motility in rat and mouse hearts

(1) Measurements and definition

A new measuring method of the myocardial O_2 consumption of mechanically unloaded rat LV slices is established by Takaki *et al.* [109, 115]. Our recent finding is that freely shortening myocardial slices under mechanically unloaded conditions do not require a significant additional VO_2 for cross-bridge cycling [109, 125, 126]. This finding is further supported by the result showing that a specific cross-bridge cycling inhibitor, 5 mM BDM, does not affect the slice VO_2 , though 5 mM BDM markedly reduced the slice motility [109]. On the other hand, we observed the increase in VO_2 by barium as a result of the enhanced cross-bridge cycling [127]. Therefore the measured increment in VO_2 by electrical activation under the mechanically unloaded condition corresponds to the VO_2 for the nonmechanical activities of myocardium.

LV myocardial slice preparation. The heart and its slices are prepared as described before [109]. Briefly, the whole heart is excised after the Tyrode perfusion for 5–10 min. Tyrode solution at 18°C oxygenated with 100% O_2 is the perfusate. Its composition in mM is NaCl, 136.0; KCl, 5.4; $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, 1.0; $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$, 0.33; $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 0.9; glucose, 10.0; HEPES, 5.0; pH, corrected to 7.4 with NaOH at 30°C. The LV is longitudinally cut into three pieces. Each piece is cut into 300 μm thick slices in parallel with the epicardium with a microslicer. This cut is chosen so that most fibers are parallel-sectioned to fiber orientation according to the known fiber architecture of the heart wall. Consequently, 16–20 slices (average single-side surface area is approximately 30 mm^2) are obtained from each heart. The slices are stored 1–2 hours in 18°C Tyrode and oxygenated with 100% O_2 .

Experimental procedure. Five to 10 (8 ± 1) slices are placed between a pair of platinum field-stimulating electrodes in the airtight chamber filled with the prepared 2.24 ± 0.12 ml Tyrode solution oxygenated with 100% O_2 . After the slices are incubated for 10 min, oxygenation is stopped and the chamber is closed tightly to prevent any leaks of water and O_2 . MVO_2 (myocardial O_2 consumption per minute) of the quiescent slices without stimulation is obtained during the

first minute, and mVO_2 of the activated and free-shortening slices with electrical stimulation is obtained during the next 2 min at 30°C.

Image analysis of myocardial slices. For image analysis, myocardial slices of comparable thickness and surface area to those for oximetry are used in each experiment. A slice showing as much unidirectional motility as possible is selected for image analysis. The slice motility is analyzed as the relative changes (motility index, %) in the total number of pixels of the discriminated video signal of slice image in contraction and relaxation cycle, as previously proposed by Takaki *et al.* [115]. The motility index of intermittent seven contraction-relaxation cycles is averaged at 20-sec intervals throughout the latter 2 min of the electrical stimulation period (3 min).

The slices with thickness (300 μm) seem to be sufficiently oxygenated by diffusion, according to the published data and calculation [2, 109, 128, 129, 130]. These myocardial slices would be virtually free from mechanical constraints (preload, afterload, tethering, and shear stress) that they had *in situ*, though some internal viscoelastic loading may still be present. The O_2 consumption of unstimulated myocardium corresponds to the basal metabolism. The O_2 consumption of electrically stimulated (1 Hz) myocardium includes basal metabolism and O_2 consumption primarily for Ca^{2+} handling in the E-C coupling, but it does not include O_2 consumption for cross-bridge cycling. Thus O_2 consumption for the E-C coupling is obtained by subtracting basal metabolism from O_2 consumption of the stimulated myocardium. Basal metabolic mVO_2 is about $2 \times 10^{-2} \text{ mlO}_2 \cdot \text{min}^{-1} \cdot \text{g LV}^{-1}$. E-C coupling mVO_2 is about $0.9 \times 10^{-2} \text{ mlO}_2 \cdot \text{min}^{-1} \cdot \text{g LV}^{-1}$ ($0.15 \text{ mlO}_2 \cdot \text{beat}^{-1} \cdot \text{g LV}^{-1}$), corresponding to about 45% of basal metabolic mVO_2 . No arrhythmia occurred in slices shortened with 1:1 synchrony with stimulation throughout the experiment.

Basal metabolism. Myocardial mVO_2 measured in the absence of stimulation in normal Tyrode solution corresponds to basal metabolism, which is considered to include VO_2 for Na^+K^+ -ATPase to maintain membrane polarization. However, the VO_2 for Na^+K^+ -ATPase for electrical activation is negligibly small relative to the basal metabolic VO_2 [109]. This result is consistent with findings obtained by other methods [3, 15, 116, 131].

Basal metabolic mVO_2 is comparable to previous rat heart data in literature [119, 130] and before our study [109], but it is greater than those in dogs and rabbits (1.74, 1.25 or $1.02 \text{ mlO}_2 \cdot \text{min}^{-1} \cdot 100 \text{ g LV}^{-1}$) [80, 85, 108]. This species difference is consistent with the report that the basal metabolism of the rat myocar-

dium is greater than those of other species [114].

E-C coupling The increases in mVO_2 by stimulation represents E-C coupling VO_2 mainly for Ca^{2+} handling, since the residual cross-bridge cycling for free shortening is trivial in the mechanically unloaded slice contractions. The E-C coupling VO_2 in normal Tyrode solution corresponds to about 45% of the basal metabolism. This percent value seems to be smaller than the E-C coupling VO_2 value in canine heart preparations corresponding to 100–240% of the basal metabolism [47, 132]. However, the E-C coupling VO_2 was increased to 130% of basal metabolism by 2-Hz stimulation (our unpublished observation). Thus the present smaller percent value of the E-C coupling VO_2 is due to a lower stimulation frequency than in dogs.

(2) SR Ca^{2+} -ATPase blockade decreases O_2 use for E-C coupling of mechanically unloaded contracting rat heart slices

The composition of E-C coupling VO_2 is analyzed by using SR Ca^{2+} -ATPase inhibitors, thapsigargin (TG) and cyclopiazonic acid (CPA), and to evaluate the role of the SR Ca^{2+} handling in the myocardial E-C coupling. TG and CPA are well known to inhibit the SR Ca^{2+} ATPase in cardiac muscles and myocytes [112, 133–137]. The Ca^{2+} released from the SR by Ca^{2+} entry across the sarcolemma increases the intracellular Ca^{2+} in the E-C coupling, activating myocardial cells. Relaxation results as the Ca^{2+} is removed mainly back into the SR (by the Ca^{2+} -ATPase) and partly out of the cell (primarily by Na^+Ca^{2+} exchange) [138]. The Na^+Ca^{2+} exchange requires no ATP and thus no VO_2 , but it is dependent on the activity of the Na^+K^+ -ATPase, which maintains the Na^+ and K^+ ionic gradients and thus the membrane potential in a steady state. Therefore it is reasonable to hypothesize that E-C coupling VO_2 primarily includes VO_2 for the SR Ca^{2+} pump.

A blockade of the SR Ca^{2+} -ATPase by TG 0.1–1 μM or by CPA 10 μM significantly reduces O_2 consumption for the E-C coupling by 40% or 70% of the respective controls. Neither TG nor CPA reduces basal metabolic O_2 consumption. The magnitude of free shortening of the unloaded myocardial slices quantified by slice surface area reduction is small (about 1.5%) because of no external preload. TG 1 μM and CPA 10 μM markedly attenuated the already reduced free shortening. All these results reveal that the O_2 consumption of myocardial slices for the Ca^{2+} handling in the E-C coupling is mainly used for the SR Ca^{2+} -ATPase. This validation is possible by taking the advantage of mechanically unloaded myocardial slice preparation.

The inhibitory effect of TG was not dose-dependent from 0.1 to 1 μM . This may be explained by an incomplete blockade by TG of the SR Ca^{2+} -ATPase because of its restricted permeability to intracellular action sites of the myocardial slice [139, 140]. The validity of this speculation is supported by the results showing that the slice's free shortening is much less attenuated by TG 1 μM than by CPA 10 μM . The inhibitory effect of CPA is dose-dependent from 1 to 10 μM ; at 10 μM , CPA reduced the E-C coupling VO_2 by 70%. Therefore CPA 10 μM seems to exert an almost complete blockade on the SR Ca^{2+} -ATPase. When the TG and CPA data are taken together, the SR Ca^{2+} -ATPase consumes at least 70% of E-C coupling VO_2 .

In normal rat hearts, 80–87% Ca^{2+} recirculates intracellularly [122, 123], i.e., the SR Ca^{2+} -ATPase has a main role in Ca^{2+} handling. However, the Na^+ - Ca^{2+} exchange will become more important after an inhibition of the SR Ca^{2+} -ATPase [141]. Other energy consuming processes such as sarcolemmal Na^+ - K^+ -ATPase, therefore, may contribute to the cost of E-C coupling. However, this possibility may be excluded because TG or CPA has greatly reduced the slice motility, and thus sarcolemmal Na^+ - K^+ -ATPase may not have been stimulated.

Myocardial E-C coupling VO_2 in mechanically unloaded contractions mainly includes the VO_2 needed for the SR Ca^{2+} -ATPase, but basal metabolism does not include VO_2 for the SR Ca^{2+} pump in the rat LV slices. In conclusion, the SR Ca^{2+} -ATPase has a major role in Ca^{2+} handling in E-C coupling in the rat myocardium.

(3) mVO_2 and motility of mechanically unloaded LV slices in mouse hearts

The genetic manipulation of cardiac specific genes by transgenic mouse models holds promise for studies focused on exploring the specific role of contractile elements and Ca^{2+} -handling constituents in E-C coupling [142–144]. To interpret findings in such a genetically manipulated mouse, the evaluation of cardiac function in normal mouse should be accomplished in terms of the coupling of cardiac mechanical work and energetics because there are many differences between rat and mouse hearts [145]. Nevertheless, there are only a small number of reports on mouse cardiac mechanical work [146–149] and energetics [150].

An analysis of cardiac mechanical work and energetics in mice was recently performed [150, 151]. In the analysis, the feasibility of an isolated, balloon-

in-ventricle, isovolumically contracting, crystalloid-perfused mouse heart preparation for the studies of cardiac mechanoenergetics using the framework of ESPVR-PVA- VO_2 employed in larger species was tested [6, 48, 151]. The VO_2 -PVA relation is well fitted by a straight line, and the VO_2 -intercept value corresponding to mechanically minimally unloaded VO_2 in mouse LV has been reported [150, 151]. The VO_2 -intercept value includes VO_2 for total Ca^{2+} handling in E-C coupling and for basal metabolism, as in dogs and rats [5, 48, 108, 113, 152]. The VO_2 -intercept value, however, is threefold that in rat LV [7–11, 117]; thus residual mechanical-loading-derived VO_2 may be included.

To test the feasibility for studies of cardiac energetics by use of a mouse LV myocardial slice 300 μm thick, we performed the measurements of basal mVO_2 and mVO_2 for total Ca^{2+} handling in E-C coupling by using the same oximetry as that employed for rat LV 300- μm -thick slices. LV mouse myocardial slices of 300 μm thickness freely shorten by 1 Hz electrical field stimulation without external load.

Mouse LV myocardial slice preparations. The whole heart was excised under the perfusion with Tyrode solution oxygenated with 100% O_2 at 12 for 5 min. The composition of Tyrode solution (in mM) was 136.0 NaCl, 5.4 KCl, 1.0 $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, 0.33 $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$, 1.8 $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 10.0 glucose, and 5.0 HEPES, with pH adjusted to 7.4 with NaOH at 30°C. Each whole LV was cut into slices 300 μm thick along the LV long axis in parallel with the septum. Twelve to 16 slices (single-side surface area $\sim 23.0 \pm 7.5 \text{ mm}^2$; length and width $\sim 4.8 \pm 1.8$ and $\sim 5.2 \pm 1.9 \text{ mm}$, respectively) were taken from two mice. The slices were stored in oxygenated Tyrode solution at 18 for 30 min, subsequently at 25 for at least 30 min. The mVO_2 of slices were measured 2.5 h after the heart's excision.

Mechanically unloaded mVO_2 . In normal Tyrode solution, mean mVO_2 without electrical stimulation is $0.97 \pm 0.14 \text{ ml O}_2 \cdot \text{min}^{-1} \cdot 100 \text{ g LV}^{-1}$, and mean mVO_2 with electrical stimulation increases to $1.80 \pm 0.17 \text{ ml O}_2 \cdot \text{min}^{-1} \cdot 100 \text{ g LV}^{-1}$. Mean delta mVO_2 (= the mVO_2 with stimulation – the mVO_2 without stimulation) is $0.83 \pm 0.12 \text{ ml O}_2 \cdot \text{min}^{-1} \cdot 100 \text{ g LV}^{-1}$. There are no differences between mean mVO_2 with and without stimulation under Ca^{2+} free. The increases in extracellular Ca^{2+} concentrations up to 14.4 mM do not increase the mVO_2 without stimulation, but significantly increases the mVO_2 with stimulation up to 140% of control. The delta mVO_2 significantly increases up to 190% of the control in a dose-dependent manner.

(4) SR Ca^{2+} -ATPase blockade decreases the delta mVO_2 of contracting mouse heart slices

CPA 30 μM significantly reduces the delta mVO_2 to $0.27 \pm 0.06 \text{ ml O}_2 \cdot \text{min}^{-1} \cdot 100 \text{ g LV}^{-1}$ (35% of control). The combination of 5 mM BDM and 30 μM CPA does not further decrease delta mVO_2 , although BDM (3–5 mM) decreases it by 28–30% of control not in a dose-dependent manner (Ref. 3–5 mM BDM dose-dependently decreases the slice-free shortening). The delta mVO_2 of mouse LV slices during free shortening consists of energy expenditure for total Ca^{2+} handling in the E-C coupling and basal metabolism and probably no residual cross-bridge cycling.

The main part of delta mVO_2 is energy expenditure utilized for total Ca^{2+} handling in E-C coupling in mouse LV slices as in rat LV slices [109, 115]. This finding is evidenced by (1) an increase of delta mVO_2 in a Ca^{2+} concentration-dependent manner, and by (2) a decrease of delta mVO_2 by CPA in a dose-dependent manner.

However, in mouse LV slices, unlike in rat LV slices [109, 115], BDM 3–5 mM significantly decreases delta mVO_2 by 28–30%. Furthermore, after CPA 30 μM treatment, 35% of mVO_2 remained. Therefore it is possible that delta mVO_2 includes energy expenditure partly utilized for residual cross-bridge cycling. If 5 mM BDM directly inhibits cross-bridge cycling, the combination of 30 μM CPA and 5 mM BDM would further decrease delta mVO_2 . However, the combination does not further decrease delta mVO_2 , suggesting the possibility that BDM decreases delta mVO_2 not mediated by directly inhibiting cross-bridge cycling. The inhibitory effect of CPA on delta mVO_2 for Ca^{2+} handling in E-C coupling is maximal at 30 μM , since CPA does not further decrease delta mVO_2 at 100 μM . For this reason, the inhibitory effect of 5 mM BDM on delta mVO_2 for Ca^{2+} handling must be masked.

Consequently, in mouse slices, as in rat myocardial slices, the possibilities that delta mVO_2 for the residual cross-bridge cycling is negligibly small and that the BDM-induced definite decrease in delta mVO_2 is due to the inhibition of Ca^{2+} handling in E-C coupling are highly supportable. In fact, many reports show that BDM decreases energy expenditure utilized for total Ca^{2+} handling in E-C coupling [153–156].

The motility index indicates only residual cross-bridge cycling at a mechanically unloaded state, and thus motility is originally small. Therefore even at a lower Ca^{2+} concentration (3.6 mM), the motility index reached maximum value.

Basal mVO_2 in mice LV slices are unexpectedly smaller than those in rat LV myocardial slices (0.97 vs. $2.09 \text{ ml O}_2 \cdot \text{min}^{-1} \cdot 100 \text{ g}^{-1}$), but delta mVO_2 in mice

LV slices ($0.83 \text{ ml O}_2 \cdot \text{min}^{-1} \cdot 100 \text{ g}^{-1}$) does not differ from that in rat LV slices ($0.91 \text{ ml O}_2 \cdot \text{min}^{-1} \cdot 100 \text{ g}^{-1}$) [see Ref. 115]. Basal mVO_2 is believed to correlate reversely with body weight [114]. The reasons why basal mVO_2 is smaller in mouse LV slices than in rat LV slices remain to be clarified.

The VO_2 -PVA relation is well fitted by a straight line, and the value of VO_2 at zero PVA (VO_2 -intercept value) [150] theoretically corresponds to mechanically minimally unloaded VO_2 in mouse LV of whole heart preparations; the VO_2 -intercept value corresponds to mVO_2 with stimulation in mouse LV myocardial slices. The VO_2 -intercept value ($= 0.92 \mu\text{l O}_2 \cdot \text{beat}^{-1} \cdot \text{g LV}^{-1}$), however, is practically threefold of mVO_2 with stimulation ($0.30 \mu\text{l O}_2 \cdot \text{beat}^{-1} \cdot \text{g LV}^{-1}$) in mice LV myocardial slices. The measurements made in whole heart preparations [150] may be an overestimate resulting from the loss of oxygen across the epicardial surface of the perfused heart. Another overestimate may be due to energy expenditure for residual cross-bridge cycling, as described above. This might be possible, because it is difficult to make completely mechanically unloading conditions in the mouse whole heart preparation.

The motility index for mice LV slices is approximately 2.2%, whereas free shortening of mouse cardiac cells is 5–10% [157]. The relatively small motility index seems to be partly due to the heterogeneity of muscle fiber orientations of mouse LV slices, as in rat LV slices [115]. Nevertheless, the relative changes of the motility index could be beneficial to evaluate the effects of agents acting on residual cross-bridge cycling.

In conclusion, the mVO_2 of slices 300 μm thick from mouse LV under a quiescent condition represents basal mVO_2 used for cardiac basal metabolism and the increase in mVO_2 of the slices with 1 Hz field stimulation mainly represents energy expenditure used for total Ca^{2+} handling in E-C coupling in the heart.

VI. Acute failing heart models

(1) High Ca^{2+} -induced Ca overload failing rat hearts

Acute failing canine heart models have been produced by different procedures, such as hypercapnic acidosis [132], postacidotic stunning [158], postischemic stunning [159], and transient Ca^{2+} overloading [53]. These interventions have been associated with different abnormalities in cardiac mechanoenergetics, suggesting that acidosis, ischemia, and free radicals contribute to the observed contractile failure. In clinical settings, perfusion with blood cardioplegia after car-

diac arrest often results in stunned myocardium, even though no evidence of ischemia or acidosis is found. Ca^{2+} overload may contribute to the stunning of these hearts during cardioplegia. The mechanisms leading to contractile dysfunction after Ca^{2+} overload are well understood from various points of view, but Ca^{2+} overload heart following exposure to high Ca^{2+} in the absence of ischemia and acidosis is still poorly understood in terms of the coupling of mechanical work and energetics.

High Ca^{2+} infusion into the coronary circulation makes Ca^{2+} overload-induced contractile dysfunction in rat hearts independent of ischemia and acidosis. The blood Ca^{2+} concentration reached 9–15 mM. After the clearance of blood with high Ca^{2+} concentration for 20 min, the levels of Ca^{2+} decrease to 1.1–2.5 mM, and venous P_{O_2} values are from 39 to 45 mmHg. The values of arterial-venous differences in lactate throughout the experiment are from 0.19 to 0.25 mM, indicating no lactate production. pH is maintained at about 7.45.

In cross-circulated rat heart preparations following exposure to high Ca^{2+} , there is a contractile failure associated with a parallel downward-shift of the ESPVR and the linear LV PVA- VO_2 relation. The basal metabolic VO_2 is unchanged. This result suggests a decrease in oxygen consumption for total Ca^{2+} handling in E-C coupling.

After postischemic reperfusion, the proteolysis of a cytoskeletal protein, α -fodrin, a linker protein, ankyrin, or a regulatory element of the myofilament, troponin I, in rat hearts have been reported previously [160–163]. The proteolysis of fodrin or ankyrin is caused by the activation of a neutral protease, calpain, resulting from Ca^{2+} overload [160–162]. The proteolysis of fodrin in the homogenate of the heart with interventions under various Ca^{2+} loading conditions, including high Ca^{2+} infusion, was examined because fodrin is localized to the sarcolemma, the intercalated disks, and Z bands [161].

Although no proteolysis of ankyrin_R, troponin I, or a gap junction protein, connexin 43, is detected in the membrane fractions of LV myocardium from the high- Ca^{2+} -infused contractile failing hearts, a large amount of the 150 kD fragment of α -fodrin is detected in the heart after the clearance of blood with high Ca^{2+} concentration. This result seems to indicate that Ca^{2+} -overload protocol induces less cellular breakdown than ischemia-reperfusion injury does [159–162].

Relation between fodrin proteolysis and VO_2 intercept. The rat hearts subjected to the Ca^{2+} overloading protocol exhibit markedly decreased VO_2 intercepts of the LV VO_2 -PVA relations, but the exceptional hearts

rarely exhibit unchanged VO_2 intercepts. α -fodrin proteolysis in membrane fractions of LV myocardium is investigated in the hearts with the decreased VO_2 intercepts and other hearts with unchanged VO_2 intercepts. The amount of the proteolytic products in hearts with the decreased VO_2 intercepts is significantly increased, but in hearts with the unchanged VO_2 intercepts, it is not significantly increased. It seems quite likely that there are significant correlations between the decrease of VO_2 intercepts and membrane α -fodrin degradation.

A synthetic calpain inhibitor, calpain inhibitor-1 (N-acetyl-Leucine-Leucine-Norleucinal; ALLNoL; CI-1) (final concentration, 2.5–25 μM at coronary flow of 4 ml/min) [161, 164], perfused at 6 ml/h for 5 min before, for 40–45 min during, and for 5 min after the high Ca^{2+} infusion suppressed the contractile failure by high Ca^{2+} , the decrease in oxygen consumption for the total Ca^{2+} handling and membrane α -fodrin degradation. In conclusion, we found that the exposure to high Ca^{2+} induces contractile dysfunction by suppressing total Ca^{2+} handling in E-C coupling and degradation of membrane α -fodrin via activation of the calpain.

O_2 costs of PVA and LV contractility. The unchanged oxygen cost of PVA indicates an unchanged chemomechanical energy transduction rate after the Ca^{2+} overloading protocol. This suggests that there are no changes in the product of the PVA/ATP coupling ratio in the contractile machinery and in the ATP/ VO_2 coupling ratio in the mitochondria between normal and high Ca^{2+} -induced Ca^{2+} overloaded acute failing rat hearts [7].

The unchanged oxygen cost of LV contractility indicates the unchanged Ca^{2+} handling VO_2 in E-C coupling per unit change in LV contractility after the Ca^{2+} overloading protocol. This suggests that there are no changes in the sensitivity of the contractile machinery for Ca^{2+} , the Ca^{2+} /ATP in the total Ca^{2+} handling, and the ATP/ VO_2 in the mitochondria [5, 7, 48]. This suggests that the amount of Ca^{2+} handled in E-C coupling remains proportional to LV contractility.

Possible mechanisms for contractile dysfunction. The transient Ca^{2+} overload seems to have caused proteolysis of α -fodrin at the sarcolemma and a decrease in Ca^{2+} handling VO_2 by the activation of a Ca^{2+} -dependent protease, calpain. It has been proposed that α -fodrin maintains the integrity of the plasma membranes as a constituent of the membrane skeleton [165, 166]. Therefore it seems quite likely that the degradation of α -fodrin in membrane fractions would alter the properties of ion channels [161]. The decreased Ca^{2+} handling VO_2 in E-C coupling with unchanged oxygen costs of PVA and LV contractility reflected the

decreased total amount of Ca^{2+} handled, which may have been due to a suppression of the transsarcolemmal Ca^{2+} influx.

The possibility that a disruption of cytoskeletal proteins inactivates L-type Ca^{2+} channels has been reported [167, 168]. We speculate that the linkage of the L-type Ca^{2+} channel to the membrane α -fodrin acts to tether the channel in place, which somehow modulates the basal activity of the channel, and a loss of the linkage may impair its regulation.

(2) Ischemic-reperfused acute failing hearts

After ischemic-reperfusion (I-R), similar contractile failure would occur. All hearts underwent 15-minute global ischemia and 60-minute reperfusion. Mean ESP_{mLVV} and PVA_{mLVV} were significantly decreased, and mean VO_2 intercept of VO_2 -PVA relation was significantly decreased without change in its slope. To explore the mechanism for this, the effects of KB-R7943, a reverse mode Na^+ - Ca^{2+} exchanger inhibitor, were investigated. After KB-R7943 (10 μM) infusion for 1 min before ischemia and 5 min after reperfusion, the downwardshift of ESPVR and thus the decreases in mean ESP_{mLVV} and PVA_{mLVV} were reversed to the control level, though not completely. Mean VO_2 intercept and the slope of VO_2 -PVA relation were unchanged. The results suggest the contribution of reverse-mode Na^+ - Ca^{2+} exchanger to the Ca^{2+} handling failure in E-C coupling after I-R [see Ref. 223].

Role of the Na^+ - H^+ and Na^+ - Ca^{2+} exchange systems. The role of the Na^+ - Ca^{2+} and Na^+ - H^+ exchangers in failing hearts are vitally important at both processes of their generation and recovery [53, 73, 132, 158, 159]. The Na^+ - Ca^{2+} exchanger is electrogenic and sensitive to membrane potential. The time-course with which Ca^{2+} is extruded, is influenced by the time course of membrane potential. All the Ca^{2+} entering the cell during the initial depolarization seems to be extruded by the Na^+ - Ca^{2+} exchange [63]. It appears that Ca^{2+} influx via the Na^+ - Ca^{2+} exchanger (reverse mode) can also occur during the initial depolarization of cardiac cells. This process may be further stimulated by subsarcolemmal rises in Na^+ concentration caused by the Na^+ influx via the cardiac Na^+ channel. However, whether this occurs during normal E-C coupling remains controversial [63].

As the intracellular Ca^{2+} concentration subsequently begins to rise as a result of Ca^{2+} release from the SR, the reversal potential for the Na^+ - Ca^{2+} exchanger becomes greater than the plateau of the membrane potential. The Na^+ - Ca^{2+} exchanger then operates in a Ca^{2+} efflux- Na^+ influx “forward” mode. However, the weight of this exchanger in extrusion of cytosolic Ca^{2+} ,

especially in failing hearts, is still controversial [63, 73].

Mechanoenergetic disorders after a rapid correction of acidosis [158] is considered to result from transient Ca^{2+} overload via an accelerated Na^+ - Ca^{2+} exchanger (reverse mode) because of the heavily operating Na^+ - H^+ exchanger carrying H^+ out of myocytes at the time of rapid pH recovery. The same mechanisms may occur in the postischemic stunned myocardium [159] and the transient Ca^{2+} overload myocardium evoked by Ca^{2+} -free and high Ca^{2+} Tyrode perfusion [53] in canine hearts. At least in part, the Na^+ - Ca^{2+} exchanger (reverse mode) and/or the Na^+ - H^+ exchanger contribute to the above I-R acute failing rat hearts.

Cardioprotective effects of calpain inhibitor I. We investigated the cardioprotective effects of calpain inhibitor-1 (CI-1) for I-R acute failing hearts by using the framework of LV ESPVR-PVA- VO_2 relation and the O_2 costs of LV PVA and eEmax with Ca^{2+} . All hearts underwent 15-minute global ischemia (37°C). After a 60-minute blood reperfusion with an infusion of CI-1, LV ESPVR and VO_2 -PVA relation, and the O_2 costs of LV PVA and eEmax were obtained. Mean ESP_{mLVV} and PVA_{mLVV} are significantly decreased, and the mean VO_2 intercept of VO_2 -PVA relation is significantly decreased without a change in its slope after I-R, whereas mean ESP_{mLVV} and PVA_{mLVV} after I-R with CI-1 are hardly changed. The slopes of VO_2 -PVA relationships are unchanged after I-R with CI-1. Mean VO_2 intercepts of VO_2 -PVA relationships significantly ($P < 0.01$) decrease after I-R, but they are unchanged after I-R with CI-1. The relation between PVA-independent VO_2 and eEmax during the Ca^{2+} infusion is linear, and its slope indicates the O_2 cost of eEmax. There is no significant difference in the O_2 cost of eEmax after I-R with CI-1. A Western blotting of α -fodrin indicates that the proteolysis of α -fodrin is protected with CI-1. The immunostainings of α -fodrin, and the 145- and 150-kD proteolytic products of α -fodrin also confirm that CI-1 protects proteolysis of α -fodrin at membrane. The results indicate that the total Ca^{2+} handling is impaired after I-R injury, whereas calpain inhibitor-1 prevents the heart from I-R injury mediated via inhibiting the Ca^{2+} handling failure (see Ref. [233]).

VII. Chronic failing heart models

(1) Spontaneously diabetic mellitus rats

Left ventricular diastolic dysfunction in type II diabetes mellitus model rats. Although the principal pathophysiological feature of diabetes mellitus (DM)

is called diabetic microangiopathy, the presence of myocardial dysfunction “diabetic cardiomyopathy” independent of coronary artery disease has been well documented in streptozotocin-induced DM [169, 170]. The Otsuka Long-Evans Tokushima Fatty (OLETF) rat model of DM [171] manifests stable clinical and pathological features that resemble human type II DM. The causal link between microangiopathy and the pathogenesis of diabetic cardiomyopathy in OLETF rats has been investigated [172]. Early diastolic transmitral inflow exhibited prolonged deceleration time and low peak-velocity in the prediabetic stage [172]. Abnormal relaxation is usually among the earliest manifestations of cardiac dysfunction, and diastolic impairment is seen before systolic impairment [173], but the pathogenesis of diabetic cardiomyopathy is not well understood. Specifically, the coupling of mechanical work and energetics of the heart in diabetic cardiomyopathy is not well understood.

To gain insight into the pathogenesis of diabetic cardiomyopathy in terms of the coupling of LV mechanical work and energetics, LV mechanoenergetic studies on OLETF rat hearts were performed. It is hypothesized that diastolic dysfunction may manifest itself in early diabetic cardiomyopathy as a result of the dysfunction of SERCA2. If so, it is predicted that VO_2 for total Ca^{2+} handling in the E-C coupling may decrease, and the O_2 cost of LV contractility may increase because of a resultant activation of the Na^+ - Ca^{2+} exchanger and Na^+ - K^+ -ATPase [95, 141]. To prove this hypothesis, LV systolic and diastolic functions linked with intracellular Ca^{2+} handling were investigated in spontaneously DM, OLETF rats.

There were no significant differences between L-I OLETF (40–46 wk of age; early DM) and L-I control (40–52 wk) rats in body weights, whereas body weights in L-II OLETF (62–66 wk of age; late DM) rats were significantly ($P < 0.001$ unpaired t-test) lighter than those in L-II control rats (60–72 wk). The body weights of L-II OLETF rats were significantly ($P < 0.05$ unpaired t-test) lower than those of L-I OLETF rats, indicating severe DM in L-II OLETF rats. The fasted blood glucose levels in L-I and L-II OLETF rats were 390 ± 89 mg/dl and 484 ± 97 mg/dl.

Neither LV systolic function and mean coronary flow nor coronary flow reserve differed even in late DM rats. The amount of oxygen required for mechanical work and contraction was unaltered, though myosin isozyme was finally transformed from V_1 to V_3 . The maximum pacing rate decreased from 300 to 240 beats/min. To evaluate the LV relaxation rate or lusitropism, by using previously proposed “logistic” time constant (T_L) derived from a logistic model [174] and the con-

ventional “exponential” time constant (T_E), we analyzed LV isovolumic relaxation pressure-time curves at mLVV. The time constants of the hearts paced at approximately 300 beats/min in early DM versus the control rats were compared, along with those of the hearts paced at 240 beats/min in late DM. The LV relaxation rate was significantly ($P < 0.05$) slower only in late DM rats.

The O_2 consumption per min for total Ca^{2+} handling in E-C coupling, mainly consumed by SR Ca^{2+} ATPase (SERCA2), was decreased, without significant changes in basal metabolism or in mitochondrial oxidative phosphorylation. The protein level of SERCA2 in membranes was significantly ($P < 0.001$) lower in severe DM rats. Both the ADP/O ratio and the State III O_2 in late DM rat hearts showed a tendency to decrease but not to differ significantly from those in the control rats, though RCI in late DM rat hearts was significantly ($P < 0.05$) smaller than in the control rat hearts. In late DM rats, diabetic cardiomyopathy with lusitropic dysfunction and with unchanged coronary flow and coronary flow reserve were found, but up to 66 wks no systolic dysfunction was observed at 240 bpm pacing. From these results it seems very likely that mitochondrial respiratory function was not impaired even in late DM rat hearts. In conclusion, we found that the only lusitropic dysfunction resulting from the decreased amount of SERCA2 expression was related to generating diabetic cardiomyopathy in the spontaneously diabetic rats.

On the other hand, the possibility that the LV diastolic dysfunction is associated with an increase in cardiac collagen content at the prediabetic stage has been suggested [172]. Late DM rats showed a significant slowing of the LV relaxation rate compared with that in the control rats without significant differences in collagen contents. Therefore it seems very likely that LV diastolic dysfunction in the L-II OLETF rats was not simply related to the increased collagen content, but also was related to the decreased protein level of SERCA2.

Curved ESPVR, linear VO_2 -PVA relationship, and oxygen cost of PVA. No significant differences were found between late DM and the control rats in either ESP_{mLVV} or PVA_{mLVV} . Each mean slope of the VO_2 -PVA relationship (O_2 cost of PVA) in late DM and the control rats was almost the same as that previously reported [7, 8, 28, 99, 117]. Therefore the O_2 cost of PVA and thus the efficiency of chemomechanical energy transduction rate was unchanged, despite the different myosin isozymes in late DM rats, as in hypothyroid rats.

PVA-independent VO_2 . The significantly decreased PVA-independent VO_2 per minute with unchanged basal

metabolism in late DM rat hearts suggested a decrease in VO_2 for total Ca^{2+} handling in the E-C coupling per minute. The VO_2 for Ca^{2+} handling in the E-C coupling was primarily consumed by SERCA2 [115, 117]. Therefore the decrease in VO_2 for Ca^{2+} handling per minute indicated a decrease in O_2 consumption by SERCA2. This was causally related to the decreased pacing rate to prevent an incomplete LV relaxation. This decrease in the pacing rate was due to a dysfunction of SERCA2 resulting from the decreased amount of SERCA2.

O_2 cost of LV contractility, eE_{max} . In non-insulin-dependent diabetic hearts, no changes in the myocardial β -receptor population could be found, but the reduced responsiveness to a β -adrenergic agonist was reported [175]. The O_2 cost of LV contractility for dobutamine in late DM rat hearts, however, was unchanged. Furthermore, the O_2 cost of LV contractility for Ca^{2+} was also unchanged. All these results indicate that changes in the total Ca^{2+} handling VO_2 in the E-C coupling against unit change in LV contractility induced by Ca^{2+} or dobutamine were not altered on a beat-to-beat basis in the late DM rat hearts. When we decreased the pacing rate to 240 beats/min, from 300 beats/min, it was possible to maintain the unchanged systolic function and the unchanged O_2 cost of LV contractility in the late and severe DM rats.

LV diastolic dysfunction. In the late DM rat heart, the LV myosin isozyme is transformed to V_3 from V_1 . This transformation might be causally related to a significant slowing of the LV relaxation rate. However, in hypothyroid rat hearts where myosin isozyme is V_3 , both LV systolic and diastolic dysfunctions were found under the decreased pacing rate and the decreased VO_2 intercept without any change in the O_2 cost of PVA [11]. The decreased VO_2 intercept was due to the decreased VO_2 for Ca^{2+} handling in E-C coupling and the decreased basal metabolism [11]. These findings indicate that the LV mechanical work and energetic characterization very much differ between the hypothyroid and the late DM rats, in both of which myosin isozymes were the same V_3 . Therefore it seems unlikely that the transformation of myosin isozyme into V_3 is causally related to the LV diastolic dysfunction in the late DM rats, though the possibility for its partial contribution could not be excluded.

Although the LV systolic function was not impaired and the O_2 cost of LV contractility did not increase, we proved that diastolic dysfunction manifested itself because of the depressed expression of SERCA2 and that the decrease in VO_2 for Ca^{2+} handling in E-C coupling per minute manifested itself because of a decreased pacing rate in the late DM rat. In conclu-

sion, the LV lusitropic function in the late DM rat hearts was impaired by a depressed expression of SERCA2, and the impaired Ca^{2+} uptake function by SERCA2 was found to have an important role in the pathogenesis of diabetic cardiomyopathy in a type II DM rat model.

(2) Hypothyroid rats

Cardiac dysfunction. In canine and human hearts, LV myocardial myosin isozyme is V_3 in contrast to V_1 in normal adult rat hearts [176, 177]. It is well known that ATPase activity of V_1 myosin isozyme is higher than that of V_3 myosin isozyme and that the shortening velocity of V_1 -dominant myocardium is faster than that of V_3 -dominant myocardium [178, 179]. In the hypothyroid rat (V_3 -dominant myocardium), the isometric tension of native trabecular preparations substantially decreased [180] and peak developed tension of LV papillary muscle preparations significantly decreased [178], although the isometric tension of glycerinated trabecular preparations hardly changed. A decrease in LV contractility in *in situ* ejecting hearts of hypothyroid rats has been reported previously [97]. Nevertheless, it is still unknown whether the depressed contractility in *in situ* ejecting hearts of hypothyroid rats derives from the V_3 -dominant myocardium, the Ca^{2+} handling failure, or the abnormal pre- and/or afterload conditions resulting from hypothyroidism.

It is hypothesized that cardiac dysfunction in hypothyroidism is mainly caused by the Ca^{2+} handling failure. To prove this hypothesis, cardiac function was investigated in terms of the coupling of rat LV mechanical work and energetics without interference of preload and afterload in the excised, cross-circulated whole heart preparations from the hypothyroid rats.

Animal preparations. Drinking the water including 8-propylthiouracil (PTU, 0.8 mg/ml) for 4–5 wks made the rat hypothyroidism [178]. Decreased T3 and T4 levels in plasma, which were decreased to $34.2 \pm 7.1\%$ and $2.6 \pm 0\%$ of normal, respectively, confirmed hypothyroidism. Myosin isozyme in LV myocardium was V_3 ($95.1 \pm 2.0\%$ of total myosin isozyme) in the hypothyroid rats, whereas it was V_1 ($89.5 \pm 9\%$ of the total) in the normal rats. The LV, including the septum and the right ventricle, were 1.07 ± 0.08 and 0.29 ± 0.06 g in normal rats and 0.85 ± 0.10 and 0.21 ± 0.03 g in hypothyroid rats. The left and right ventricular weights were significantly different between the two groups.

LV inotropism and lusitropism were significantly depressed, and these mechanical dysfunctions are causally related to a marked decrease of myocardial VO_2 for the Ca^{2+} handling and for basal metabolism. The

former total Ca^{2+} handling VO_2 in E-C coupling was primarily consumed by SERCA2 [115, 117]. It is well known that thyroid hormone affects SERCA2 activity through the expression of both SERCA2 and PLB [181–188]. In fact, the higher protein levels of PLB and the lower protein levels of SERCA2 were found, although the oxygen costs of LV contractility for Ca^{2+} and for dobutamine in the hypothyroid rats did not differ from those in age-matched normal rats.

The results reveal that LV energetic changes associated with the decreased mechanical work in hypothyroid rats were mainly caused by the impairment of Ca^{2+} uptake via SERCA2. In conclusion, we find that the impairment of Ca^{2+} uptake plays an important role in the pathogenesis of cardiac dysfunction in hypothyroidism.

Curved ESPVR, linear VO_2 -PVA relationship, and oxygen cost of PVA. Marked differences in ESP_{mLVV} or PVA_{mLVV} were found between normal and hypothyroid rats, whereas each mean slope of the VO_2 -PVA relation (O_2 cost of PVA) in the two groups was found to be almost the same as that previously reported [7, 8, 28, 99, 117]. In hyperthyroid rabbits, where myosin isozyme is V_1 , the increased O_2 cost of PVA was observed [106], though in hyperthyroid dogs, where myosin isozyme remains V_3 , the unchanged O_2 cost of PVA was observed [189]. From these findings, it has been predicted that the O_2 cost of PVA is related to types of myosin isozyme. The results, however, indicate that no correlation exists between the O_2 cost of PVA and the types of myosin isozyme in the normal and the hypothyroid rats. The unchanged O_2 cost of PVA in the hypothyroid rats indicates that the efficiency of chemomechanical energy transduction (the inverse of O_2 cost of PVA) remained unchanged despite the different types of myosin isozyme.

PVA-independent VO_2 . The mean VO_2 intercept of the VO_2 -PVA relation (PVA-independent VO_2) in the hypothyroid rats was significantly smaller than in the normal rats. The VO_2 for total Ca^{2+} handling in the E-C coupling and for basal metabolism were both significantly smaller in the hypothyroid rats. The decrease in VO_2 for total Ca^{2+} handling reflects a decrease in the uptake activity of SERCA2 on a beat-to-beat basis, as mentioned above.

Thyroid hormone changes the expression of mitochondrially encoded respiratory genes [190]. In the hypothyroid state, the mitochondrial respiratory function may be depressed by the depressed gene expression, and further mitochondrial cytochrome oxidase activity is depressed [191]. Therefore it seems quite likely that hypothyroidism leads to a depressed mitochondrial respiration. This speculation is supported

by the present result showing the noticeably smaller basal metabolism in the left ventricle of the hypothyroid rats.

O_2 cost of LV contractility, eE_{max} . In the hypothyroid rats, the pacing of the heart at 300 beats/min failed, but the pacing at 262 beats/min succeeded. Although this reflects the dysfunction of SERCA2, the decreased pacing rate may lead to unchanged O_2 costs of LV contractility for Ca^{2+} and for dobutamine on a beat-to-beat basis in the hypothyroid rats.

In developing rat hearts, hypothyroidism decreased the function of SERCA2 and increased the function of Na^+ - Ca^{2+} exchanger [192, 193, 187]. In normal adult rats, 81% of the Ca^{2+} released from the SR was recirculated by SERCA2; Ca^{2+} handling relied largely on SR [122]. If this hypothyroidism decreased the uptake function of LV SERCA2 and increased the extrude function of LV Na^+ - Ca^{2+} exchanger, the O_2 cost of LV contractility might have increased [95]. But the unchanged O_2 cost of LV contractility in this hypothyroid rat indicates no change in the relative functions of SERCA2 and Na^+ - Ca^{2+} exchanger. This speculation is supported by a recent study showing no increase in LV Na^+ - Ca^{2+} exchanger protein in hypothyroid rats compared to that in normal rats [194]. The decreased total Ca^{2+} handling VO_2 in the E-C coupling in the hypothyroid rats suggests a decrease in the total amount of Ca^{2+} handled in the E-C coupling with no change in the recirculation fraction of Ca^{2+} uptaken by SR.

LV systolic and diastolic dysfunctions. In the excised hearts of the hypothyroid rats, at a pacing rate lower than normal, a significant slowing of the LV contraction and relaxation rates was found. In *in situ* hearts, the preload and afterload and the hormonal and neuronal influences could interfere with LV function. In contrast, in the cross-circulated (blood-perfused) excised hearts, LV function can be characterized by the myocardium *per se*. Therefore LV systolic and diastolic dysfunctions in myocardium seem to be actually caused in the hypothyroid rats.

In the hypothyroid rats, LV myosin isozyme V_1 was transformed to V_3 [177]. This transformation might be causally related to a significant slowing of the LV contraction and relaxation rates [178, 180]. But in the same-type heart preparations of the type II DM rats, where myosin isozyme is V_3 just as in hypothyroid rats, only diastolic dysfunction was found under the decreased pacing rate without any change in the VO_2 intercept of the VO_2 -PVA relation and the O_2 cost of PVA [9]. These findings indicate that the hypothyroid and the type II DM rats differ in the LV mechanical work and energetic characterization, though their

myosin isozymes are the same V_3 . Therefore it seems very unlikely that the only transformation of myosin isozyme into V_3 is causally related to the LV systolic and diastolic dysfunctions in the hypothyroid rats.

Not only systolic and diastolic dysfunctions and a decrease in VO_2 for Ca^{2+} handling in E-C coupling in physiological functional studies, but also a depressed expression of SERCA2 and an enhanced expression of PLB were found in the hypothyroid rats, as previously reported [183–187]. Furthermore, it has been reported that in hypothyroid mice, decreases in SERCA2a gene expression are accompanied by prolonged contraction and relaxation of papillary muscles [182]. It seems quite likely that the dysfunction of SERCA2 leads to a primary diastolic dysfunction and that the decreased Ca^{2+} -release resulting from the decrease in Ca^{2+} stored in SR leads to a secondary systolic dysfunction. On the other hand, the possibility of the final impairment of ryanodine receptor function cannot be excluded, because a lower level of expression of ryanodine receptor mRNA has been reported in 16-wk hypothyroid rabbits [195].

It is reported that in the failing rat heart model made by aortic banding, the ratio of SERCA2a to PLB decreased associated with worsening metabolism, i.e., a decreased ratio of phosphocreatine to ATP [196]. Similar to this failing heart model, in the hypothyroid rat heart the ratio of SERCA2a to PLB decreased, but this is not associated with worsening metabolism, i.e., unchanged O_2 costs of PVA and eE_{max} . In terms of the coupling of LV mechanical work and energetics, LV systolic and diastolic functions in the hypothyroid rats were impaired by the depressed Ca^{2+} uptake function by SERCA2. In conclusion, it was found that the impaired Ca^{2+} uptake function by SERCA2 plays an important role in the pathogenesis of heart failure in the hypothyroidism.

An overexpression of SERCA2a in the failing rat heart made by aortic banding improved cardiac function associated with an improvement of phosphocreatine to ATP ratio or the energy potential [196]. Furthermore, in the hypothyroid mice, an overexpression of SERCA2a also improves both the contraction and the relaxation of the papillary muscle [182]. Therefore, an overexpression of SERCA2a would be a promising strategy to rescue various cardiac dysfunctions, including the hearts in hypothyroidism.

VIII. Gene targeting for these failing-heart models

Our understanding of cardiac E-C coupling has improved significantly over the past 10 years, not only

by cardiac mechanoenergetic studies, but also by other studies. Furthermore, defects in the various steps of E-C coupling that characterize cardiac dysfunction have been identified in human and experimental models of heart failure, as mentioned above. The various abnormalities in ionic channels, transporters, kinases, and various signaling pathways could contribute to the failing phenotype. An important tool in dissecting the importance of the various changes in heart failure has been the use of cardiac gene transfer. To achieve effective cardiac gene transfer a number of obstacles remain, including appropriate vectors for gene delivery, appropriate delivery systems, and a better understanding of “the biology of the disease.” Gene transfer provides not only a potential therapeutic modality, but also an approach to identifying and validating molecular targets [197].

Cardiac myocytes from failing hearts are characterized by several cellular abnormalities in the E-C coupling processes [7, 9, 11, 198–201]. These include changes at the levels of the sarcolemma, SR, myofilaments, and mitochondria, all of which contribute to depressed mechanical functions and reserves. Identifying how the underlying mechanisms for these changes contribute to the observed pathology is frequently confused by simultaneous alterations in multiple signaling pathways in the complex environment of the failing or myopathic heart [202, 203]. For this reason, gene transfer has the distinct advantage of editing single genes at a time and allowing investigators to probe various mechanisms [204]. However, before this smooth transition, several various obstacles must be overcome, including choice of vectors, delivery methods, and most important a better understanding of the biology and mechanisms of heart failure [204], as described above in this review.

The major disadvantages to recombinant adenoviral gene transfer have been its transience and the intense inflammation and robust immune response it evokes. In animal models, adenoviral gene transfer to adult myocardium in vivo has generally been found to mediate high-level expression for approximately 1 week [204–206].

E-C coupling and the role of SERCA2a and PLB. E-C coupling in cardiac myocytes occurs as a coordinated sequence between intracellular Ca^{2+} handling proteins. As described above in this review, the understanding of E-C coupling in cardiac myocytes continues to develop [207]. To confirm all of a series of coordinated steps in E-C coupling, we offer the following descriptions. Electrical excitation across the sarcolemma leads to the opening of voltage-gated Ca^{2+} channels, allowing the entry of a small amount of Ca^{2+} ,

which triggers the release of a larger amount of Ca^{2+} from the SR through the ryanodine receptors (RYR2) [207, 208]. This increase in Ca^{2+} activates the myofilaments leading to contraction. Relaxation occurs when Ca^{2+} is dissociated from the myofilaments and is taken up by the SR Ca^{2+} -ATPase pump into the SR. The intracellular Ca^{2+} sparks arises from a cluster of RYR2 in close proximity to a voltage-dependent Ca^{2+} channel [207]. When a great many Ca^{2+} channels are activated, multiple Ca^{2+} sparks combine to increase cytosolic Ca^{2+} , which leads to myofilament activation [207, 208].

The Ca^{2+} pumping activity of SERCA2a is influenced by PLB, which inhibits SERCA2a in the unphosphorylated state [209, 210]. Phosphorylation by cAMP and Ca^{2+} -calmodulin-dependent protein kinases, however, reverses this inhibition.

The cardiac SR is composed of two main components: the junctional SR and the longitudinal tubules, which are not organized in relation to T tubules. For this reason, E-C coupling occurs mainly through diffusible signals: Ca^{2+} -induced Ca^{2+} release accounts for the opening of the SR Ca^{2+} release channels (RYR2), located on the junctional SR [211]. The RYR2 has a tetrameric structure composed of four monomers with a weight of ~564 kDa.

The SR Ca^{2+} -ATPase is localized in the longitudinal SR. Although five distinct Ca^{2+} -ATPase isoforms are found in skeletal, smooth, and cardiac muscles [212, 213], an alternative splicing of the SERCA2 gene gives rise to two distinct isoforms: SERCA2a and SERCA2b. Cardiac muscle expresses mainly the SERCA2a isoform, which is modulated by the phosphoprotein PLB.

The amount of Ca^{2+} in the SR is a very important determinant of the open probability of the RYR2 and directly affects the fraction of SR Ca^{2+} released for a given "spark." The maintenance of the SR Ca^{2+} is essential for E-C coupling. There is a strong positive correlation between SR Ca^{2+} -ATPase and contractility, based on experiments using SERCA2a inhibitors such as CPA and TG [115, 117, 214] as mentioned above in this review. SERCA2a is under the control of PLB, a small molecule and essential SR membrane phosphoprotein from a view of physiological function. This small protein has a hydrophilic domain that constitutes the cytosolic sector and a hydrophobic transmembrane domain. The latter domain inhibits the apparent affinity of SERCA2a for Ca^{2+} . PLB can be phosphorylated at distinct sites by different protein kinases: serine 10 by protein kinase C, serine 16 by cAMP-dependent protein kinase, and threonine 17 by Ca^{2+} -calmodulin-dependent kinase. The phosphory-

lation of each of these sites is associated with enhanced SR Ca^{2+} -ATPase activity [210, 215, 216]. Therefore PLB is a key regulator of cardiac mechanical function mediated by its effect on SERCA2a function.

E-C coupling and the role of Na^{+} - Ca^{2+} exchanger. Ca^{2+} is removed outside the cytosol by the sarcolemmal Na^{+} - Ca^{2+} exchanger, which has a high capacity but low affinity, and is the major Ca^{2+} extrusion mechanism of the cardiac myocyte [217], though its contribution varies among individual species [95, 115, 117]. This system returns Ca^{2+} concentrations to diastolic levels and may therefore contribute significantly to cardiac relaxation. The consequence of an increased activity of the Na^{+} - Ca^{2+} exchanger in failing hearts may compensate for the reduction in SR Ca^{2+} -ATPase activity [207]. An increased activity of the Na^{+} - Ca^{2+} exchanger should assist in cardiac relaxation, although Ca^{2+} release from the SR during systole might be reduced. This would be especially evident at higher rates of stimulation and thus lead to a blunted frequency response, as seen in myopathic human myocardium [218].

The Na^{+} - Ca^{2+} exchanger can operate to bring Ca^{2+} into the cell or to extrude Ca^{2+} from the cell [207, 217, 219–223]. It has been suggested that the relaxation abnormalities produced by the loss of SERCA2a activity could be, at least partially, compensated for by an increase in the activity of the Na^{+} - Ca^{2+} exchanger, as mentioned above [8, 95] and by others [207, 217, 219–222].

IX. Gene transfer of Ca^{2+} handling proteins

Gene transfer provides a unique opportunity to manipulate the expression of essential proteins and alter the expression of specific downstream signaling pathways implicated in the pathogenesis of heart failure. Cardiac mechanical dysfunction results from abnormalities in many subcellular mechanisms. Specifically, a single area of Ca^{2+} handling will be targeted: the Ca^{2+} handling proteins involved in E-C coupling (Fig. 3).

Recombinant adenoviral gene transfer has been instrumental in elucidating the molecular basis of the abnormalities in chronic failing hearts such as DM and hypothyroid failing heart models, and acute failing hearts such as Ca^{2+} overload and ischemic-reperfused acute failing heart models, as mentioned above. Many of these mechanoenergetic changes seen in E-C coupling can be halted and even reversed in these failing hearts (Fig. 3). The transfer of SERCA2a, for example, has been shown to lead to an increase in SR Ca^{2+} -ATPase activity, an increase in the amount of

Fig. 3. The relation between Ca²⁺ cycling proteins and integrative cardiac function in terms of mechanical work and energetics. E-C: excitation-contraction. VO₂: myocardial oxygen consumption per beat. SERCA2a: sarcoplasmic reticulum Ca²⁺-ATPase. PLB: phospholamban. AsPLB: antisense phospholamban. I-R: ischemic-reperfused.

Ca²⁺ released, a faster relaxation phase, and a decrease in diastolic Ca²⁺ [204, 206, 224–229].

In contrast, it has been shown that increasing PLB relative to SERCA2a in isolated myocytes simulates the abnormalities of Ca²⁺ handling seen in failing ventricular myocardium [204, 206, 224–229]. These include prolongation of the relaxation phase of the Ca²⁺ transient, a decrease in Ca²⁺ release, and an increase in the resting cytosolic Ca²⁺ concentration.

Furthermore, overexpressing SERCA2a can largely rescue the phenotype created by increasing the ratio of PLB/SERCA2a [204, 206, 224–229]. More recently, the restoration of SERCA2a in failing human cardiomyocytes has been shown to restore the free shortening function of these failing human cells to normal level [204, 206, 224–229]. This study validated the premise that targeting SERCA2a by gene transfer using Ad.SERCA2a (E1-deleted recombinant adenovirus vector carrying cDNA for SERCA2a) may offer a new therapeutic modality in small animals with heart failure.

Furthermore, cardiac metabolism measured by the ratio of creatine phosphate to ATP was also restored to normal in these studies. The restoration of the energetics is surprising (!), because enhanced contractility is usually associated with increased energy demand. The fact that gene transfer of SERCA2a is not associated with a compromise in cardiac energetics means that a beneficial remodeling occurred within the cardiac cell

restoring the metabolic machinery [197].

Ablation of PLB. The gene transfer of a dominant form of PLB has been shown to enhance contractile function both *in vitro* and in an animal model of heart failure [230–232]. Furthermore, antisense strategies showing the ablation of PLB (AsPLB) restored the free shortening function in human failing cardiac cells back to normal [206].

In conclusion, in terms of cardiac mechanical work and energetics in small animals, when the O₂ costs of PVA and LV contractility (eEmax) are evaluated by use of the framework of VO₂-PVA-eEmax, it could be determined whether a beneficial remodeling occurs within the heart, restoring the metabolic machinery. The result suggests that low O₂ costs holding hearts are energy-saving hearts, and that high O₂ costs holding hearts are energy-wasting hearts.

X. Perspectives

As shown in Fig. 3, AsPLB and SERCA2a gene transfer *in vivo* would rescue various types of failing hearts. Preliminary studies have been published recently [233]. The *in vivo* transfer of AsPLB prevented the formation of Ca²⁺ overload acute failing hearts induced by coronary infusion with high Ca²⁺. The downward shift of ESPVR and the upward shift of EDPVR and thus the decrease in PVA_{mLVV} were restored back to normal by AsPLB transfer. Simultaneously, the decreased

VO₂ intercept composed of O₂ consumption for Ca²⁺ handling in E-C coupling and basal metabolism were restored back to normal without changes in the O₂ costs of PVA and eEmax.

Gene transfer provides a unique opportunity to manipulate the expression of essential proteins and to alter the expression of specific downstream signaling pathways implicated in the pathogenesis of heart failure.

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