Effects of Repetitive Brief Ischemia on Contractile Efficiency and Oxygen Cost of Contractility in Dog Heart

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Abstract: It is unclear whether preceding repetitive brief ischemia causes any improvement in the energy efficiency of intracellular calcium cycling or crossbridge cycling that may lead to cardioprotection after subsequent sustained ischemia/reperfusion, a phenomenon called ischemic preconditioning. To address this issue, left ventricular (LV) contractility ($E_{\max}$) and the relation between myocardial oxygen consumption ($\dot{V}O_2$) and pressure-volume area (PVA, a measure of LV total mechanical energy) were assessed before (Control) and 20 min (Rep-20) and 60 min (Rep-60) after repetitive brief ischemia in 11 isolated, blood-perfused dog hearts. At Rep-20, $E_{\max}$ and PVA-independent $\dot{V}O_2$ (nonmechanical energy expenditure) decreased by 23.0 ± 19.5 and $13.9 \pm 18.0\%$, respectively (both $p < 0.05$). However, at Rep-60, both $E_{\max}$ and PVA-independent $\dot{V}O_2$ recovered to their respective control levels. The oxygen cost of contractility (the slope of the PVA-independent $\dot{V}O_2$ - $E_{\max}$ rela-

tion during CaCl_2 loading) remained constant (Control 0.0019 ± 0.0009 vs. Rep-60 0.0018 ± 0.0013 ml $\text{O}_2 \cdot \text{ml} \cdot \text{mmHg}^{-1} \cdot \text{beat}^{-1} \cdot 100 \text{ g}^{-2}$, ns), suggesting unchanged efficiency in Ca^{2+} cycling. Also, the contractile efficiency (the reciprocal of the slope of the $\dot{V}O_2$ -PVA relation, reflecting the efficiency of crossbridge cycling) was the same between the Control and Rep-60 (53.7 ± 16.7 vs. $55.4 \pm 14.4\%$, ns). Basal metabolism $\dot{V}O_2$ during KCl arrest was also similar to that in the normal heart. Nonmechanical energy expenditure was reduced in proportion to the decrease in LV contractility after repetitive brief ischemia, while both the contractile efficiency and oxygen cost of contractility remained constant. These results indicate that the heart, after repetitive brief ischemia but before sustained ischemia, has normal efficiencies of crossbridge cycling and Ca^{2+} cycling despite the transiently reduced contractility. [Japanese Journal of Physiology, 50, 515–524, 2000]

Key words: myocardial ischemia, energetics, pressure volume relation, myocardial oxygen consumption, ischemic preconditioning.

Hearts subjected to a brief period of ischemia attain tolerance to subsequent sustained ischemia, resulting in reduction of infarct size [1] or enhancement of recovery of contractile function during reperfusion [2]. This phenomenon is called ischemic preconditioning. As a mechanism underlying the salutary effect of ischemic preconditioning, a better preservation of ATP in the ischemic myocardium during the subsequent sustained ischemia has been reported [3, 4]. However, the preconditioning procedure (i.e., preceding brief is-

chemia) itself depresses the contractile function and reduces myocardial oxygen consumption, and hence, would result in better preservation of ATP in the preconditioned myocardium [1, 4, 5]. Therefore, it remains unknown whether the better preserved ATP is a result of a simple reduction of energy consumption or an increased metabolic efficiency of energy utilization in the preconditioned heart.

Left ventricular (LV) mechanoenergetics have been successfully assessed using the framework of a LV

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Fig. 1. A: Schematic illustration of left ventricular systolic pressure-volume area (PVA). PVA is the area surrounded by the end-systolic pressure-volume relation (ESPVR), end-diastolic pressure-volume relation (EDPVR), and systolic pressure-volume trajectory. The slope of ESPVR is defined as E_{max} (ventricular contractility index). PVA consists of both external work (EW) and potential energy (PE) in an ejecting contraction, or PE alone in an isovolumic contraction. **B: Schematic illustration of left ventricular oxygen consumption (VO_2) versus PVA relation and two components of VO_2 at a stable E_{max} .** Horizontal dashed line divides VO_2 into PVA-dependent and PVA-independent VO_2 . PVA-independent VO_2 reflects the VO_2 component for E-C coupling and basal metabolism. PVA-dependent VO_2 means mechanical work. The reciprocal of the slope of the VO_2 -PVA relation indicates contractile efficiency. **C: Parallel upward shift of the VO_2 -PVA relation caused by increases in PVA-independent VO_2 with enhancement of E_{max} .** The slope remains unchanged. **D: Relation between E_{max} and PVA-independent VO_2 .** The slope of this relation indicates oxygen cost of contractility.

contractility index (E_{max}), pressure-volume area (PVA), and myocardial oxygen consumption (VO_2) (Fig. 1) [6–11]. PVA is a specific area in the pressure-volume diagram circumscribed by the end-systolic pressure-volume line (E_{max} line), the end-diastolic pressure-volume curve, and the systolic pressure-volume trajectory (Fig. 1A). PVA correlates linearly with VO_2 in a load-independent manner in a stable contractile state, and the reciprocal of the slope of the linear VO_2 -PVA relation represents the chemomechanical energy transduction efficiency (contractile efficiency) of the contraction mechanism (Fig. 1B). In addition, during the enhancement of E_{max} with calcium or epinephrine, the PVA- VO_2 relation shifts upward with a proportional increase in the VO_2 -axis intercept (PVA-independent VO_2) (Fig. 1C). Because PVA-independent VO_2 consists of nonmechanical energy expenditure for excitation-contraction (E-C) coupling and basal metabolism, the slope of the linear relation between PVA-independent VO_2 and E_{max} represents the energy cost of E-C coupling (oxygen cost of contractility) (Fig. 1D).

Based on this E_{max} - VO_2 -PVA framework, we have found that contractile efficiency is decreased by more than 30% in the hyperthyroid rabbit heart [6] and that the oxygen cost of contractility is increased more than twofold in canine stunned myocardium after a single 15-min global ischemia [9]. In addition, the increased oxygen cost of contractility in the post-acidotic stunned myocardium has been found to be completely abolished by the administration of dimethylamiloride, an inhibitor of the Na^+ - H^+ exchange system, at the time of correction of acidosis [8]. However, neither contractile efficiency nor the oxygen cost of contractility has been studied in the preconditioned heart. If preceding brief ischemia could influence proteins involved in E-C coupling, it is not unreasonable to hypothesize that the benefit of preconditioning is related to a favorable mechanoenergetic alteration. We therefore hypothesized that preceding repetitive brief ischemia may cause an improvement in the energy efficiency of intracellular calcium cycling or crossbridge cycling that may lead to cardioprotection during and after subsequent sustained ischemia/reperfusion.

Accordingly, the purpose of the present study was to determine the energy utilization efficiency of the heart after repetitive brief ischemia. To this end, we assessed the relationship between LV contractility and myocardial oxygen consumption before and after repetitive brief ischemia in the isolated, blood-perfused dog heart utilizing the framework of $E_{\max}$ - $\dot{V}O_2$ -PVA.

METHODS

Surgical preparation. The investigation conforms with the Guiding Principles for the Care and Use of Animals approved by the Council of the Physiological Society of Japan. Experiments were performed in excised, cross-circulated (blood perfused) canine heart preparation (Fig. 2). The details of the surgical procedure have been described previously [7, 9]. Briefly, in each experiment two mongrel dogs (9–13 kg for heart donor and 12–25 kg for support) were anesthetized with sodium pentobarbital (25 mg/kg, I.V.) after premedication with ketamine hydrochloride (7 mg/kg, I.M.). Blood was heparinized (1,000 U/kg body weight) in both dogs. The left subclavian artery and the right ventricle (RV) of the donor dog heart were cannulated and connected to common carotid arteries and the external jugular vein of the support dog, respectively.

The heart of the donor dog was excised from the chest after cross circulation was started so that there was no interruption of coronary circulation. Systemic hypotension under cross circulation was prevented with indomethacin (0.5 to 1.0 mg/kg, I.V.). A thin latex balloon (unstressed volume of 50 ml) with a catheter-tip micromanometer (Millar PC-350, Millar Instrument, Houston, TX) was placed in the left ventricle (LV). The balloon was connected to a volume servo pump. The LV epicardial electrocardiogram (ECG) was recorded and heart rate was held constant by left atrial pacing.

Coronary blood flow was measured with an electromagnetic flowmeter (model MVF-2100, Nihon Kohden, Tokyo) by placing an in-line probe (model FF-05T, Nihon Kohden) in the coronary venous drainage tube from the right heart. We neglected LV Thebesian venous blood flow because of its small fraction (less than 3%) in the total coronary flow [10]. Coronary arteriovenous O_2 content difference (AVO_2D) was measured continuously with an AVOX analyzer (AVOX Systems, San Antonio, TX). This analyzer was calibrated against an IL-282-CO oxymeter (Instruments Laboratory, Lexington, MA) in each experiment.

Temperature of the heart was maintained at 35 to

Fig. 2. Schematic illustration of the isolated, cross-circulated heart preparation. ECG indicates electrocardiogram; LV, left ventricle.

37°C. The systemic arterial blood pressure of the support dog served as the coronary perfusion pressure of the excised heart. The mean level of systemic arterial pressure of the support dog was kept above 80 mmHg throughout each experiment by infusing either fresh blood obtained from the donor dog or 10% dextran-40 solution as needed. Arterial pH, pO_2 , and pCO_2 of the support dog were maintained within their physiological ranges.

Experimental protocol. The experimental protocol consisted of control volume run, control calcium run, three bouts of 5-min global ischemia, reperfusion 20 min (Rep-20) volume run, reperfusion 60 min (Rep-60) volume run, Rep-60 calcium run, and KCl arrest. All experiments were carried out under isovolumic contractions using a servo pump. Volume runs were performed in all 11 hearts, and both calcium runs and KCl arrest were done in 10 of the 11 hearts.

Volume run. LV volume was widely varied with the servo pump to cover a wide range of PVA in a stable, baseline contractile state. ECG, LV pressure, LV volume, coronary blood flow, and AVO_2D were measured in steady-state isovolumic contractions under each LV loading condition. Measurements were made at 5 to 7 different LV volumes. This run was called the control volume run.

Similar measurements were made at 20 and 60 min after the last ischemia/reperfusion and called Rep-20 and Rep-60 volume runs, respectively.

Calcium run. After the control volume run, we fixed LV volume at an arbitrarily determined moderate value in each experiment (14.1 ± 2.9 ml) where peak isovolumic pressure was 50–70 mm Hg. Calcium (2%

CaCl₂) was continuously infused into the coronary arterial tube at a starting rate of 0.02 mEq/min. The infusion rate was increased in steps every 5 min until $E_{\max}$ was nearly doubled. During the course of the enhancement of contractility, data were obtained at 5 to 6 different levels of contractility at the same preset volume in each experiment. The maximum infusion rate of calcium averaged 0.12 ± 0.05 mEq/min. After the measurements, the calcium infusion was discontinued, and 10 to 15 min was allowed to elapse until LV contractility returned to the pre-calcium control level. In our previous study, we have confirmed that this calcium run per se does not produce calcium-overloaded stunned myocardium in our preparation [9].

After the Rep-60 volume run, calcium infusion was repeated as the Rep-60 calcium run in the same manner as the pre-ischemic control calcium run.

Ischemia. The coronary arterial perfusion tube between the support dog and excised heart was clamped and held for 5 min. The LV volume was kept at the same preset volume (14.1 ± 2.9 ml) during ischemia where the calcium run had been performed. To avoid accidental air ejection from the LV into the decompressed ascending aorta, which would cause coronary air embolism during ventricular arrhythmia, the heart was immersed in a box filled with warmed saline (36°C) during the ischemic period.

When the tube clamp was released, the saline in the box was drained. This ischemic operation was repeated 3 times at intervals of 10 min. If ventricular fibrillation was provoked at reperfusion, direct current cardioversion was performed with a low energy of 5 or 10 J. In our previous study, we confirmed that this direct current countershock at 5–10 J does not affect $E_{\max}$ or the VO_2 -PVA relation [12].

KCl arrest. Ten hearts were finally arrested at V_0 by injecting KCl (3 to 4 ml bolus followed by continuous injection of 0.75 Eq/l at 3 ml/min) into the coronary arterial tube. VO_2 was determined as basal metabolic VO_2 .

Data analysis. All data were sampled at 2-ms intervals, and the digital data were analyzed with a signal processor (model 7T18, NEC San-Ei, Japan).

Oxygen consumption. VO_2 was obtained as the product of coronary blood flow and AVO₂D. It was divided by heart rate to obtain VO_2 per beat at a steady state. To minimize the contribution of RV VO_2 to the measured total VO_2 , the RV was kept collapsed by continuous hydrostatic drainage of the coronary venous blood. LV VO_2 was calculated in the same way as before [9], normalized for LV weight, and presented as ml O₂ · beat⁻¹ · 100 g⁻¹. LV (including the septum)

and RV weight measured at the end of each experiment averaged 71.8 ± 18.9 g and 35.1 ± 12.6 g, respectively.

Contractility. LV contractility was assessed by $E_{\max}$ in the same way as before [7, 9]. $E_{\max}$ was computed as the maximum value of the instantaneous ratio: $P(t)/[V(t) - V_0]$, where $P(t)$ and $V(t)$ are LV instantaneous pressure and volume, respectively, and V_0 is the volume at which peak isovolumic pressure is zero. $E_{\max}$ was normalized for LV weight and presented as mmHg · ml⁻¹ · 100 g.

Pressure-volume area (PVA). PVA was calculated in the same way as the previous study [9]. PVA was normalized for 100 g LV as mmHg · ml · beat⁻¹ · 100 g⁻¹.

VO_2 -PVA relation. The VO_2 and PVA data in each volume run were subjected to linear regression analysis to obtain a regression equation: $VO_2 = aPVA + b$, where slope a indicates the oxygen cost of PVA, $aPVA$ represents the PVA-dependent VO_2 , and intercept b represents PVA-independent VO_2 (Fig. 1B). The reciprocal of the slope ($1/a$) means contractile efficiency from PVA-dependent VO_2 to PVA. Contractile efficiency has been considered to represent the chemomechanical energy transduction efficiency of the contraction mechanism, and to reflect the efficiency of crossbridge cycling [6].

PVA-independent VO_2 . We calculated PVA-independent VO_2 for each contractile state in a calcium run in the same way as before [7, 9]. In brief, VO_2 of a contraction at an enhanced $E_{\max}$ consists of three components (Fig. 1C): an increase in PVA-independent VO_2 , PVA-dependent VO_2 , and PVA-independent VO_2 as the control value (b). The sum of the first and last VO_2 terms is the PVA-independent VO_2 of this contraction at the enhanced $E_{\max}$. The second VO_2 term, PVA-dependent VO_2 , was calculated as the product of the same slope value (a) as in the control period and the PVA of this contraction. PVA-independent VO_2 at each level of enhanced $E_{\max}$ was calculated as LV VO_2 minus $aPVA$.

Oxygen cost of contractility. The oxygen cost of contractility was defined as the slope of the relation between PVA-independent VO_2 and $E_{\max}$ in the control calcium run and Rep-60 calcium run in each heart (Fig. 1D). Because VO_2 for the basal metabolism is unchanged with calcium [11], the oxygen cost of contractility indicates an increase in VO_2 for E-C coupling for a given increase in contractility, and reflects the efficiency of intracellular Ca²⁺ cycling [7–9].

Statistics. All data are presented as mean $\pm$ SD. Data were analyzed for statistical significance using ANOVA followed by Student's t -test with Bonferroni's correction for multiple comparisons. Comparisons of

Fig. 3. A: Graph showing end-systolic pressure-volume data points in the control (○), reperfusion 20-min (Rep-20, ●), and reperfusion 60-min (Rep-60, ▲) volume runs. The slope of this relation is left ventricular contractility index ($E_{\max}$). **B:** Graph showing the myocardial oxygen consumption (VO_2)–pressure-volume area (PVA) data points obtained in the control, Rep-20, and Rep-60 volume runs in the same heart as in A. Note that $E_{\max}$ depression was accompanied by a decrease in the VO_2 intercept (PVA-independent VO_2) of the VO_2 -PVA relation, but the slope of the VO_2 -PVA relation remained unchanged.

the regression lines were performed by the analysis of covariance (ANCOVA) [13]. Values of $p < 0.05$ were considered statistically significant.

RESULTS

Volume run

Figure 3 shows a representative set of end-systolic pressure-volume (A) and the VO_2 -PVA (B) data points three volume runs in one heart. The VO_2 -PVA relation was highly linear over a wide range in each run ($r = 0.987$, $r = 0.978$, and $r = 0.959$, respectively). When $E_{\max}$ decreased from $6.06 \text{ mmHg} \cdot \text{ml}^{-1} \cdot 100 \text{ g}$ in the control volume run to $3.95 \text{ mmHg} \cdot \text{ml}^{-1} \cdot 100 \text{ g}$ at Rep-20, the VO_2 -PVA relation shifted downward with a decrease in the VO_2 intercept from 0.0406 to $0.0366 \text{ ml O}_2 \cdot \text{beat}^{-1} \cdot 100 \text{ g}^{-1}$ ($p < 0.01$ by ANCOVA from control). However, there was no significant change in the slope value ($p > 0.05$ by ANCOVA). At Rep-60, as $E_{\max}$ slightly recovered to $5.21 \text{ mmHg} \cdot \text{ml}^{-1} \cdot 100 \text{ g}$, the VO_2 -PVA relation shifted upward toward the control relation with an increase in the VO_2 intercept to $0.0400 \text{ ml O}_2 \cdot \text{beat}^{-1} \cdot 100 \text{ g}^{-1}$ without a significant change in the slope value. All other hearts showed similar changes.

Table 1 summarizes the averaged data of $E_{\max}$ and the VO_2 -PVA relation in the control, Rep-20, and Rep-60 volume runs. Figure 4 shows relative magnitudes of $E_{\max}$ (A), the slope of the VO_2 -PVA relation (B), and PVA-independent VO_2 values (C) compared with respective control values. Although both $E_{\max}$ and PVA-independent VO_2 decreased significantly (-23.0 ± 19.5 and $-13.9 \pm 18.0\%$, respectively, both $p < 0.05$) at Rep-20, they almost recovered at Rep-60. In contrast, the slope of the VO_2 -PVA relation did not

Table 1. Changes in cardiac mechanics and energetics.

	Control	Rep-20	Rep-60
$E_{\max}$ (mmHg · ml ⁻¹ · 100 g)	10.01 ±5.26	6.95 ±2.80 [†]	8.45 ±3.11
VO_2 -PVA relation			
PVA-independent VO_2 (ml O ₂ · beat ⁻¹ · 100 g ⁻¹)	0.0338 ±0.0045	0.0292 ±0.0078 [†]	0.0315 ±0.0084
Slope (10 ⁻⁵ ml O ₂ · mmHg ⁻¹ · ml ⁻¹)	1.33 ±0.30	1.36 ±0.30	1.29 ±0.37

Values are mean ± SD, $n = 11$. Control, Rep-20, and Rep-60 indicate values measured during control volume run, reperfusion 20-min volume run, and reperfusion 60-min volume run, respectively; $E_{\max}$, left ventricular contractility index; VO_2 , myocardial oxygen consumption; PVA, pressure-volume area; PVA-independent VO_2 , the VO_2 axis intercept of the VO_2 -PVA relation; Slope, slope of the VO_2 -PVA relation in the volume run. [†] Significantly different from control volume run ($p < 0.05$).

change significantly before and after ischemia/reperfusion.

Calcium run

Figure 5A shows the PVA-independent VO_2 - $E_{\max}$ relations in the control calcium run and the Rep-60 calcium run in a representative heart at the same LV volume. Both in the control and Rep-60, PVA-independent VO_2 increased with linear increases in $E_{\max}$ and correlation coefficient close to unity ($r = 0.989$ and $r = 0.991$, respectively). In addition, the two regression lines were almost superimposable with no significant slope difference by ANCOVA. As a result, the oxygen cost of contractility was also almost the same (0.00295 vs. $0.00384 \text{ ml O}_2 \cdot \text{mmHg}^{-1} \cdot \text{ml} \cdot \text{beat}^{-1} \cdot 100 \text{ g}^{-2}$) between the two calcium runs. Eight of the 10

Fig. 4. Bar graphs showing relative magnitudes of $E_{\max}$ (A), the slope of the PVA- $\dot{V}O_2$ relation (slope, B), and PVA-independent $\dot{V}O_2$ (C) in reperfusion 20-min (Rep-20), and reperfusion 60-min (Rep-60) volume runs compared with the control volume run.

Fig. 5. **A:** Plots and the linear regression lines of PVA-independent $\dot{V}O_2$ at $E_{\max}$ in the control calcium run (○) and reperfusion 60-min (Rep-60) calcium run (●) in the same heart. The slope of each line indicates the oxygen cost of contractility. Note that the two regression lines were almost superimposable. LV indicates left ventricle. **B:** Bar graph showing changes in the oxygen cost of contractility between the control and reperfusion 60-min (Rep-60) calcium runs in 10 hearts. Mean $\pm$ SD values are also indicated by bars

hearts showed similar results, whereas heart showed a significantly greater slope and the remaining heart showed a significantly smaller slope value in the Rep-60 calcium run than in the control calcium run by ANCOVA.

Figure 5B compares the oxygen cost of contractility between the control and Rep-60 calcium runs in 10 hearts. The average oxygen cost of contractility was similar between the control calcium run ($0.00187 \pm 0.00092 \text{ ml O}_2 \cdot \text{ml} \cdot \text{mmHg}^{-1} \cdot \text{beat}^{-1} \cdot 100 \text{g}^{-2}$) and the Rep-60 calcium run ($0.00184 \pm 0.00128 \text{ ml O}_2 \cdot \text{ml} \cdot \text{mmHg}^{-1} \cdot \text{beat}^{-1} \cdot 100 \text{g}^{-2}$).

KCl arrest

Basal metabolic $\dot{V}O_2$ during KCl arrest at the end of the experiment was $1.49 \pm 0.45 \text{ ml O}_2 \cdot \text{min}^{-1} \cdot 100 \text{g}^{-1}$ ($n=10$), which is very close to the previously reported values [9, 10] for normal canine hearts.

Circulating catecholamines

Circulating norepinephrine levels (normal range 0.05–0.4 ng/ml) measured in the arterial blood and coronary venous effluent, respectively, were 0.31 ± 0.18 and $0.25 \pm 0.21 \text{ ng/ml}$ for the control, 0.39 ± 0.17 and $0.36 \pm 0.21 \text{ ng/ml}$ at Rep-20, 0.48 ± 0.17 and $0.40 \pm 0.20 \text{ ng/ml}$ at Rep-60 (all not significant among time sequence).

DISCUSSION

The major findings of the present study are as follows: (1) contractile efficiency, which reflects the efficiency of crossbridge cycling, was unchanged before and after repetitive brief ischemia; (2) both $E_{\max}$ and PVA-independent $\dot{V}O_2$ transiently decreased at Rep-20, but recovered in parallel to a near control level at Rep-60;

(3) as a result, the oxygen cost of contractility, which reflects the efficiency of Ca^{2+} cycling, was unchanged before and after repetitive brief ischemia; and (4) basal metabolic VO_2 after repetitive brief ischemia was similar to the previously reported value in the normal heart. These findings suggest that neither the efficiency of crossbridge cycling nor the efficiency of Ca^{2+} cycling changed in the heart after repetitive brief ischemia.

LV contractility and VO_2 after repetitive brief ischemia. In the present study, E_{max} was depressed at Rep-20 and recovered to near the control level at Rep-60. Transient depression and early recovery of LV systolic performance after brief ischemia have been shown in previous studies [2, 14, 15]. This early recovery was in contrast to the delayed recovery observed in the stunned heart after single, 15-min ischemia/reperfusion, where E_{max} remained depressed significantly until 120 min after reperfusion [9]. Thus, there is no cumulative effect of 3 bouts of 5-min ischemia/reperfusion on LV contractility, despite the same total ischemic time as single, 15-min ischemia/reperfusion.

PVA-independent VO_2 decreased significantly at Rep-20 and recovered to near the control level at Rep-60 in proportion to the changes in E_{max} . These parallel changes in E_{max} and PVA-independent VO_2 are also concordant with our previous observations in post-ischemic stunned myocardium [9] 20 min after reperfusion and in the heart with decreased coronary perfusion pressure [16].

Contractile efficiency. The contractile efficiency, which is the reciprocal of the slope of the linear VO_2 -PVA relation, was unchanged throughout the experiment, while E_{max} was significantly depressed at Rep-20.

The contractile efficiency has been considered to represent the chemomechanical energy transduction efficiency of the contractile machinery, which is a product of the efficiencies of mitochondrial oxidative phosphorylation (efficiency from VO_2 to ATP) and crossbridge cycling (efficiency from ATP to PVA) [11]. Therefore, the unchanged contractile efficiency suggests that both the VO_2 to ATP and ATP to PVA efficiencies were unchanged after repetitive brief ischemia, or less likely, that an increase in either efficiency might have offset a decrease in the other efficiency. In any case, the unchanged contractile efficiency does not afford an energy-saving effect nor explain the cardioprotective mechanism of ischemic preconditioning.

Oxygen cost of contractility. The unchanged oxygen cost of contractility after repetitive brief is-

chemia is in sharp contrast with the markedly increased oxygen cost of contractility in stunned myocardium (2.2 times higher than the control) [9]. The increased oxygen cost of contractility in stunned myocardium has been attributed to reduced Ca^{2+} sensitivity of the myofilament [17, 18], microstructural damage of the cytoskeleton [19], and/or a decreased molar coupling ratio of the net sequestered Ca^{2+} to ATP consumed by Ca^{2+} pump ATPase due to increased Ca^{2+} permeability of the sarcoplasmic reticulum membrane [20, 21]. Thus, the unchanged oxygen cost of contractility after repetitive brief ischemia suggests that none of these mechanisms is impaired in the heart after repetitive brief ischemia.

The unchanged oxygen cost of contractility in the heart after repetitive brief ischemia may therefore indicate that such a heart consumes less oxygen for a given contractility than in stunned myocardium. However, this finding per se does not explain the reason why the preconditioned heart shows a cardioprotective effect as compared to a normal heart never subjected to preceding ischemia.

Possible mechanism of preconditioning effect. Then, how can we reconcile the present results with the mechanism underlying the cardioprotective effect of ischemic preconditioning from the viewpoint of cardiac mechanoenergetics? The present study has demonstrated that neither the contractile efficiency, oxygen cost of contractility, or basal metabolic VO_2 was changed significantly after repetitive brief ischemia. This indicates that preceding repetitive brief ischemia per se does not afford a novel intrinsic energy-saving mechanism to the heart during the subsequent 60-min period (except for the anticipated reduction of PVA-independent VO_2 resulting from the decrease in E_{max} 20 min after final brief ischemia). At first sight, this finding seems to contradict the "phosphocreatine (PCr) overshoot phenomenon" observed in the preconditioned heart, which would suggest that the energy-generating system is operating better than the energy-utilizing system [3, 22]. However, the specificity of the PCr overshoot phenomenon to the preconditioned heart has been controversial [23]. Furthermore, it is ATP and not PCr that is used directly in muscle contraction. In fact, the ATP level in the preconditioned myocardium before sustained ischemia has been shown to be similar to that in the control heart [3, 22]. Thus, the better preserved ATP after sustained ischemia in the preconditioned heart [3, 4] cannot be accounted for by improved energy utilization efficiency in the preconditioned heart.

There are several other possibilities for the cardioprotective mechanism of ischemic preconditioning.

First, the better preserved ATP in the preconditioned heart might be attributable to decreased energy consumption in the postischemic dysfunctioning myocardium. In fact, in the present study, PVA-independent $\dot{V}O_2$ decreased significantly along with the decrease in $E_{\max}$ soon after the final ischemia/reperfusion. However, this mechanism does not seem to have a critical role because the decrease in PVA-independent $\dot{V}O_2$ was only transient in the present study. Furthermore, the restoration of contractile function with dobutamine does not attenuate the cardioprotective effect of ischemic preconditioning [24].

Secondly, if enhanced anaerobic metabolism during ischemia contributes to the better preservation of ATP in the preconditioned heart, it may not be detected by the assessment of aerobic metabolic efficiency such as the $E_{\max}$ - $\dot{V}O_2$ -PVA analysis. However, the anaerobic glycolytic metabolism can produce only 2 mol of ATP from 1 mol of glucose compared with 38 mol of ATP produced in the aerobic metabolism [25]. This implies that the anaerobic metabolism can supply only 5.3% (2/38) of the ATP generated by the aerobic metabolism, which is negligible under normal contractile conditions. In addition, the anaerobic metabolism is thought to cease rapidly due to acidosis during no-flow ischemia [25]. However, whether or not the contribution of the anaerobic metabolism would be enhanced (or prolonged) in the preconditioned heart remains to be determined [3].

Thirdly, there remains a possibility that a switch to increased energy utilization efficiency is turned on only after the start of sustained ischemia in the preconditioned heart. In this case, the improved efficiency would not be detected before the onset of sustained ischemia, as in the present protocol. However, because the salutary effect of preconditioning with better preserved ATP has been reported not to last more than 20 min after the start of sustained ischemia [1, 4], the impact of this mechanism, if any, would not be large.

Finally, there is a possibility that the cardioprotective effect of ischemic preconditioning is afforded by a mechanism that attenuates myocardial injury during reperfusion after sustained ischemia. For example, Matsumura *et al.* [19] reported that Ca^{2+} overload after reperfusion activates intracellular protease, resulting in contractile dysfunction in postischemic myocardium. If ischemic preconditioning would attenuate reperfusion injury through a mechanism that is not associated with a change in the energy utilization efficiency, it would not be detected by metabolic assessment before reperfusion. In relation to this, Steenbergen *et al.* [26] suggested that ischemic precondition-

ing may attenuate the increases in the intracellular levels of Ca^{2+} , Na^+ , and H^+ during ischemia because of reduced stimulation of Na^+-H^+ and Na^+-Ca^{2+} exchange. Because our $E_{\max}$ - $\dot{V}O_2$ -PVA analysis has successfully detected increased oxygen cost in the post-acidotic stunned myocardium resulting from excessive operation of the Na^+-H^+ exchanger [8], this final possibility merits further investigation.

Study limitation. First, in the present protocol, we assessed ventricular mechanoenergetics only after repetitive brief ischemia and not during nor after sustained ischemia. However, if the heart is actually exposed to sustained ischemia, measurements for $\dot{V}O_2$ would not be possible during ischemia because of the absence of coronary venous effluent, and an accurate mechanoenergetic assessment would be greatly disturbed after reperfusion because of severe ischemia/reperfusion injury. In addition, the main purpose of the present study was to examine the hypothesis that any mechanoenergetic alteration might evolve before sustained ischemia.

Secondly, we did not confirm that 3 bouts of 5-min ischemia actually have the cardioprotective effect of preconditioning against subsequent sustained ischemia in our preparation. In the rat and rabbit, the preconditioning effect has been demonstrated regardless of the experimental preparation; in the *in situ* heart [5, 27], isolated buffer-perfused heart [28, 29], and blood-perfused heart [27]. In addition, a single, 5-min ischemia/reperfusion is known to be sufficient to induce ischemic preconditioning in the *in situ* dog heart [14, 30]. Therefore, it would not be unreasonable to expect that preconditioning is induced by 3 bouts of 5-min global ischemia in the isolated (cross-circulated) dog heart preparation. However, again, the main purpose of the present study was to examine the possibility of an evolving mechanoenergetic alteration before sustained ischemia rather than to confirm a preconditioning effect in the isolated dog heart preparation.

Conclusion. In conclusion, our mechanoenergetic analysis using the $E_{\max}$ - $\dot{V}O_2$ -PVA framework has demonstrated that, in isolated, blood-perfused dog heart, contractile efficiency and the oxygen cost of contractility remain constant after repetitive brief ischemia, whereas nonmechanical energy expenditure (PVA-independent $\dot{V}O_2$) is transiently reduced in proportion to the decrease in LV contractility ($E_{\max}$). These results indicate that the heart, after repetitive brief ischemia but before sustained ischemia, has normal efficiencies of crossbridge cycling and Ca^{2+} cycling despite the transiently reduced contractility.

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