Mechanoenergetics Characterizing Oxygen Wasting Effect of Caffeine in Canine Left Ventricle

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Abstract: Caffeine causes a considerable O₂ waste for positive inotropism in myocardium by complex pharmacological mechanisms. However, no quantitative study has yet characterized the mechanoenergetics of caffeine, particularly its O₂ cost of contractility in the $E_{\max}$ -PVA-VO₂ framework. Here, $E_{\max}$ is an index of ventricular contractility, PVA is a measure of total mechanical energy generated by ventricular contraction, and VO₂ is O₂ consumption of ventricular contraction. The $E_{\max}$ -PVA-VO₂ framework proved to be powerful in cardiac mechanoenergetics. We therefore studied the effects of intracoronary caffeine at concentrations lower than 1 mmol/l on left ventricular (LV) $E_{\max}$ and VO₂ for excitation-contraction (E-C) coupling in the excised cross-circulated canine heart. We enhanced LV $E_{\max}$ by

intracoronary infusion of caffeine after β -blockade with propranolol and compared this effect with that of calcium. We obtained the relation between LV VO₂ and PVA with $E_{\max}$ as a parameter. We then calculated the VO₂ for the E-C coupling by subtracting VO₂ under KCl arrest from the PVA-independent (or zero-PVA) VO₂ and the O₂ cost of $E_{\max}$ as the slope of the E-C coupling VO₂- $E_{\max}$ relation. We found that this cost was 40% greater on average for caffeine than for calcium. This result, for the first time, characterized integratively cardiac mechanoenergetics of the O₂ wasting effect of the complex inotropic mechanisms of intracoronary caffeine at concentrations lower than 1 mmol/l in a beating whole heart. [Japanese Journal of Physiology, 50, 257–265, 2000]

Key words: Cardiac energetics, $E_{\max}$, myocardial O₂ consumption, pressure-volume area, PVA.

The positive inotropism of caffeine (0.1–5 mmol/l) has a considerable O₂ wasting effect [1, 2] and various approaches have elucidated its mechanisms [2–4]. However, this O₂ wasting effect remains to be characterized by the $E_{\max}$ -PVA-VO₂ relation, which at present seems one of the most powerful integrative frameworks of cardiac mechanoenergetics [5, 6]. Here, $E_{\max}$ is the end-systolic maximum pressure-volume ratio (or elastance) and serves as an index of contractility reasonably independent of cardiac loading conditions [5–7]. PVA is the systolic pressure-volume (P - V) area that quantitates the total mechanical energy of ventricular contraction [5, 6, 8]. VO₂ is O₂ consumption by

ventricular contraction. The $E_{\max}$ -PVA-VO₂ framework is compatible with the Starling law of the heart [9]. In this framework, the O₂ cost of $E_{\max}$ serves as a physiologically sound quantitative measure of the O₂ demand for unit increment in $E_{\max}$ [5, 10–16].

We have already shown that calcium, catecholamines, digitalis, and phosphodiesterase inhibitors (DPI 201-106, OPC-8212, EMD-53998, milrinone, sulmazole, etc.) have comparable O₂ cost of $E_{\max}$ in the normal canine heart [5, 10, 11, 15]. We have also shown that the O₂ cost of $E_{\max}$ increases by 50–100% of the control in failing hearts produced by myocardial acidosis [12], postischemic and post-acidotic

Received on November 12, 1999; accepted on February 25, 2000

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stunning [13, 14], and ryanodine treatment [16]. Positive inotropism of cardiac cooling decreased the O_2 cost of $E_{\max}$, whereas negative inotropism of cardiac warming increased it at a Q_{10} of 2–3 between 30–40°C [17, 18]. However, no literature has yet documented a positive inotropism that has an abnormally high O_2 cost of $E_{\max}$. We suspected caffeine to be such an agent on the basis of its known pharmacological mechanisms, as listed in the following [2–4, 19–25].

Caffeine has complex pharmacological mechanisms for positive inotropism even after β -blockade [2–4, 19–25]. It increases cAMP by phosphodiesterase inhibition and augments the phosphorylation of various contractility-related proteins [2, 19, 20, 25]. It also increases the open probability of the ryanodine-sensitive calcium (Ca^{2+}) release channels in the sarcoplasmic reticulum (SR) [21, 23, 25]. This causes futile Ca^{2+} cycling analogous to ryanodine treatment at nanomolar concentrations, increasing the O_2 cost of $E_{\max}$ [16, 26]. On the other hand, caffeine increases the Ca^{2+} responsiveness of contractility [3, 4, 19, 22] so that a smaller amount of released Ca^{2+} achieves a comparable contractility. This compensates for the increased O_2 cost of $E_{\max}$ due to the futile Ca^{2+} cycling. Therefore, the O_2 wasting for excitation-contraction (E-C) coupling with caffeine should be determined as the balance of at least these opposing effects. The O_2 cost of $E_{\max}$ seems an appropriate measure to evaluate the net of these complex effects. However, the O_2 wasting effect of caffeine remains to be elucidated by the O_2 cost of $E_{\max}$ in a whole beating heart.

We tested our hypothesis that the O_2 cost of $E_{\max}$ could characterize integratively the O_2 wasting effect of caffeine despite its complex inotropic mechanisms. We used the left ventricle (LV) of a standard excised, cross-circulated canine heart preparation [10–16]. We found that the O_2 cost of $E_{\max}$ was 40% greater on average for caffeine than for calcium. We were thus able to obtain a quantitative result indicating evidently that caffeine, even at concentrations lower than 1 mmol/l, is a mechanoenergetically unique, O_2 -wasting, positive inotropic agent. This result supported our present hypothesis.

MATERIALS AND METHODS

$E_{\max}$ -PVA- VO_2 framework. The $E_{\max}$ -PVA- VO_2 framework enables an integrative assessment at the organ level of myocardial mechanoenergetics in different contractilities (Fig. 1) [5, 6]. $E_{\max}$ represents the recruitability of force-generating crossbridges per unit increase in ventricular volume according to the Starling law [6, 9]. PVA quantitates the total mechanical

Fig. 1. $E_{\max}$ -PVA- VO_2 framework of the left ventricle (LV). **A:** LV pressure-volume (P - V) diagram, explaining the definitions of $E_{\max}$ as an index of ventricular contractility and PVA as a measure of total mechanical energy of contraction. **B:** VO_2 -PVA relation in a constant $E_{\max}$. O_2 cost of PVA (a): slope of the VO_2 -PVA relation. PVA-independent VO_2 (b): VO_2 intercept of the VO_2 -PVA relation. **C:** VO_2 -PVA relations with comparable slopes (a) but different VO_2 intercepts (b) at three different $E_{\max}$ levels. The steeper VO_2 -PVA relation (heavy arrow) is a composite VO_2 -PVA relation traversing the three standard VO_2 -PVA relations at different $E_{\max}$ levels. **D:** PVA-independent VO_2 (b)- $E_{\max}$ relation. O_2 cost of $E_{\max}$ (c): slope of the PVA-independent VO_2 (b)- $E_{\max}$ relation. Basal metabolism (d): PVA-independent VO_2 intercept. For more details, see text.

energy produced by crossbridge cycling in each ventricular contraction [5]. PVA closely correlates with ventricular VO_2 per beat in a steady state (Fig. 1B). The slope (a) of the VO_2 -PVA relation represents the O_2 cost of PVA, and the VO_2 intercept (b) represents PVA-independent VO_2 . The VO_2 above basal metabolism is primarily for Ca^{2+} handling in the E-C coupling [26–30]. The PVA-dependent VO_2 above b seems exclusively related to crossbridge cycling [5].

The VO_2 -PVA relation usually ascends or descends in parallel with an increase or decrease in $E_{\max}$ with conventional inotropism (Fig. 1C). When $E_{\max}$ increases at a fixed ventricular volume, a VO_2 -PVA working point ascends along a steeper line traversing the parallel “standard” VO_2 -PVA lines. The steeper line is called a “composite” VO_2 -PVA line.

The elevation of the standard VO_2 -PVA relation closely correlates with $E_{\max}$ (Fig. 1D). The slope (c) of this PVA-independent VO_2 - $E_{\max}$ relation represents the O_2 cost of $E_{\max}$. Even a single composite VO_2 -PVA line reflects the O_2 cost of $E_{\max}$ [14]. This cost quantitates the energy wasting of the E-C coupling [5, 6, 26–30].

The O_2 cost of $E_{\max}$ is comparable among conven-

tional inotropic agents despite their different pharmacological mechanisms [5, 10, 11, 15]. However, postischemic stunning [14, 31], acidosis [12], post-acidotic stunning [13] and ryanodine treatment [16, 26, 29] increase this cost by 50–100%. Thus, our $E_{\max}$ -PVA- $\dot{V}O_2$ framework proved to quantitate the O₂ cost of $E_{\max}$ explicitly at the beating whole heart level [12–14, 16]. For this reason, we used the $E_{\max}$ -PVA- $\dot{V}O_2$ framework to characterize the O₂ wasting effect of caffeine.

Surgical preparation. We performed experiments on excised cross-circulated canine heart preparations as previously described in detail [5, 10–16]. Briefly, two mongrel dogs (13–22 kg) were anesthetized with sodium pentobarbital (30 mg/kg I.V.) after premedication with ketamine hydrochloride (7 mg/kg I.M.). Both dogs were heparinized (1,000 U/kg body weight). The heart donor dog (13.6 ± 1.3 (SD) kg) was thoracotomized under artificial ventilation. The left subclavian artery and the right ventricle were cannulated and connected to the bilateral common carotid arteries and external jugular vein, respectively, of the support dog (17.0 ± 2.7 kg) with arterial and venous cross-circulation tubing. Both systemic and pulmonary vessels to the heart were ligated. The heart was excised from the chest after starting cross-circulation without interruption of the coronary perfusion.

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

The cardiac temperature was kept at 35–37°C with heaters, and left atrial pacing kept the heart rate constant at 147 ± 6 beats/min. We repeatedly measured coronary arterial pH, PO_2 and PCO_2 and corrected them to normal as needed.

O₂ consumption. Coronary blood flow was measured with an electromagnetic flowmeter in the coronary venous cross-circulation. Coronary arteriovenous O₂ content difference (AVO₂D) was measured continuously with our custom-made O₂ content difference analyzer (PWA-200S, Shoen-Technica, Chiba, Japan) [5, 10–16]. LV O₂ consumption per minute was obtained as the product of the total coronary flow and AVO₂D and then divided by heart rate to obtain O₂ consumption per beat ($\dot{V}O_2$) on an online real-time basis with a computer [5, 10–16].

Right ventricular $\dot{V}O_2$ was minimized by keeping

the right ventricle collapsed with coronary venous hydrostatic drainage. The collapsed ventricle had zero PVA and hence no PVA-dependent $\dot{V}O_2$. LV PVA-independent $\dot{V}O_2$ was obtained by multiplying the biventricular PVA-independent $\dot{V}O_2$ with the LV to total ventricular weight ratio (0.78 ± 0.04) in each $E_{\max}$ [6, 13]. The total ventricular weight was 101.0 ± 18.0 g, and the LV weight including the interventricular septum was 78.7 ± 14.3 g.

Contractility index ($E_{\max}$). We sampled LV pressure (P) and volume (V) data at 2-ms intervals and processed them with the computer. As shown in Fig. 2, the LV $E_{\max}$ was determined as the end-systolic P - V ratio after subtracting V_0 (volume at which peak isovolumic P and PVA are zero) from the total volume [8]. We also determined $+\max dP/dt$, time to $E_{\max}$, and time to $-\max dP/dt$ from the rising phase of R of the epicardial ECG. The conventional exponential time constant of LVP decay during isovolumic relaxation with an assumption of zero asymptote was also determined.

Pressure-volume area (PVA). We calculated the PVA of each beat from the digitized P - V data on our computer [5, 10–16]. PVA is the area bounded by the systolic P - V segment, end-systolic P - V relation line and end-diastolic P - V relation curves (Figs. 1A and 2). PVA was normalized for 100 g LV in mmHg ml beat⁻¹ 100 g⁻¹.

Experimental protocol. We performed the experiments to obtain the O₂ cost of both PVA and $E_{\max}$ under caffeine and Ca²⁺ treatments as follows.

Baseline volume run. First, we performed the volume run to obtain the standard $\dot{V}O_2$ -PVA relation in all eight LVs. Steady-state isovolumic contractions at 6–40 ml were produced in baseline $E_{\max}$. $\dot{V}O_2$ -PVA data were obtained at several LV volumes.

Control volume run. Next, we gave propranolol at a constant rate of 1–2 mg/h into the coronary circulation for β -blockade in all LVs [23]. Steady-state isovolumic contractions were produced again in this controlled $E_{\max}$ run to obtain $\dot{V}O_2$ -PVA data at several LV volumes.

Caffeine and Ca²⁺ inotropism runs. Then, we performed inotropism runs to compare the effects of caffeine and Ca²⁺ in all LVs. Here, we fixed LVV at 22.8 ± 2.5 ml. We increased caffeine and Ca²⁺ infusion rates in steps every 5 min until $E_{\max}$ nearly doubled. The maximum dose of caffeine was 0.075 ± 0.016 mmol/min, corresponding to a coronary blood concentration of 0.90 ± 0.45 mmol/l (coronary flow: 110 ± 42 ml/min). The maximum dose of CaCl₂ was 0.080 ± 0.030 mmol/min, corresponding to 1.55 ± 0.65 mmol/l (coronary flow: 64 ± 25 ml/min). The Ca²⁺ run

Fig. 2. A representative set of tracings of coronary perfusion blood pressure (BP), isovolumic left ventricular (LV) pressure (LVP), constant LV volume (LVV), LV ECG (saturated QRS waves), coronary blood flow (CF), and coronary arteriovenous O_2 content difference (AVO_2D). The LVP-LVV diagrams show E_{max} and PVA. A shows control data. B and C show data under intracoronary caffeine infusion at 0.045 and 0.083 mmol/min, respectively. V_0 : end-systolic unstressed volume.

preceded the caffeine run in five LVs, and vice versa in three LVs.

Inotropism volume runs. We performed caffeine and Ca^{2+} volume runs at the end of the respective inotropism runs in all LVs. We waited for 15–30 min until the positive inotropic effects disappeared between the inotropism runs.

KCl run. Finally, we arrested all hearts at V_0 to obtain the basal metabolic VO_2 by slowly infusing KCl solution (0.3 mol/l) intracoronarily at a rate of 1–2 ml/min. This dose corresponded to an estimated intracoronary concentration of KCl of 10–20 mmol/l.

Data analyses. We analyzed the E_{max} -PVA- VO_2 data by our routine methodology [5, 10–18]. That which followed is briefly explained here because we explained them elsewhere [5, 10–18].

O_2 cost of PVA. We obtained a standard VO_2 -PVA regression line from the VO_2 -PVA data in each volume run by linear regression analysis. Its slope yielded the O_2 cost of PVA (a , Fig. 1B) in ml O_2 mmHg $^{-1}$ ml $^{-1}$. We obtained a composite VO_2 -PVA regression line from VO_2 -PVA data in each inotropism run by linear regression analysis.

PVA-independent VO_2 . We calculated PVA-independent VO_2 (intercept b) of each VO_2 -PVA value during each inotropism run as measured VO_2 minus PVA-dependent VO_2 (a , PVA, Fig. 1B).

O_2 cost of E_{max} . We obtained the relation between PVA-independent VO_2 (b) and corresponding E_{max} values at a fixed volume by linear regression analysis of

each inotropism run. The slope (c , Fig. 1D) of the regression line yielded the O_2 cost of E_{max} in ml O_2 ml mmHg $^{-1}$ beat $^{-1}$ 100 g $^{-2}$ [4, 9, 11–15]. Intercept d corresponding to basal metabolism, was 0.012 ± 0.003 ml O_2 beat $^{-1}$ 100 g $^{-1}$.

Statistics. We compared regression lines in the experimental runs separately for the standard VO_2 -PVA, composite VO_2 -PVA, and PVA-independent VO_2 - E_{max} relations in each heart by analysis of covariance (ANCOVA). Multiple comparisons of paired values among the runs in all LVs were made by analysis of variance (ANOVA) followed by the least significance difference (LSD) method. p values smaller than 0.05 were considered statistically significant. Data are presented as mean $\pm$ SD.

RESULTS

Figure 2 shows a representative set of simultaneous tracings of coronary BP (blood perfusion pressure), LVP (pressure), fixed LVV (volume), LV bipolar ECG, CF (coronary flow) and AVO_2D during a caffeine inotropism run. We increased the caffeine infusion rate from 0 (A) to 0.045 mmol/min (B) and maximally 0.083 mmol/min (C). This corresponded to an intracoronary caffeine concentration of 0.92 mmol/l. Caffeine maximally enhanced E_{max} from 2.4 to 5.5 mmHg ml $^{-1}$ 100 g, nearly doubled coronary flow, and considerably decreased AVO_2D . The Ca^{2+} inotropism run after the caffeine inotropism run increased E_{max}

Table 1. Mechanoenergetics compared between caffeine and Ca²⁺.

Inotropism runs	Caffeine			Ca ²⁺		
	Pre-caffeine		Maximum caffeine	Pre-Ca ²⁺		Maximum Ca ²⁺
EDV (ml)	22.8±2.4		22.8±2.4	22.8±2.4		22.8±2.4
EDP (mmHg)	4.1±2.2	NS	4.3±3.4	4.3±3.8	NS	3.5±2.6
ESP (mmHg)	64±15	<	112±13	69±21	<	111±15
CF (ml/min)	50±30	<	110±41	59±36	NS	63±25
AVO ₂ D (vol%)	9.9±3.8	>	6.0±2.0	8.2±2.8	<	9.8±2.7
E _{max} (mmHg/ml)	3.2±0.8	<	5.6±0.7	3.4±1.0	<	5.5±0.6
PVA (mmHg ml)	635±181	<	1,123±217	682±239	<	1,150±240
VO ₂ intercept (ml O ₂ /beat)	0.0194±0.0031	<	0.0266±0.0045	0.0189±0.0028	<	0.0242±0.0045
T _{max} (ms)	183±14	NS	185±13	185±11	>	175±10
+max dP/dt (mmHg/s)	694±152	<	1,193±138	738±198	<	1,238±180
−max dP/dt (mmHg/s)	530±142	<	995±100	590±205	<	1,020±158
Time to −max dP/dt (ms)	289±18	NS	286±24	295±23	>	271±18
τ (ms)	51.2±12.1	>	40.1±5.4	47.6±10.1	>	37.7±6.4

All values are normalized for 100 g LV. EDP, end-diastolic pressure at the preset isovolumic volume; ESP, end-systolic pressure; CF, coronary blood flow; AVO₂D, coronary arteriovenous O₂ content difference; E_{max}, end-systolic maximum elastance or pressure/volume ratio; PVA, systolic *P-V* area as a measure of total mechanical energy of ventricular contraction; VO₂ intercept, VO₂ (or *y*-axis) intercept of the VO₂-PVA regression line; T_{max}, time to E_{max} from the onset of contraction; +max dP/dt, maximum positive value of time derivative of LV pressure; −max dP/dt, maximum negative value of time derivative of LV pressure; time to −max dP/dt, time to −max dP/dt from the onset of contraction; τ, time constant of isovolumic relaxation pressure segment after −max dP/dt; < or >, significantly smaller or larger at *p*<0.05 by paired *t*-test; NS, insignificant. Maximum caffeine: 0.075±0.016 mmol/min. Maximum Ca²⁺: 0.080±0.030 mmol/min.

from 2.2 to 5.5 mmHg ml^{−1} 100 g in this LV. PVA increased in proportion to E_{max} as shown by the *P-V* diagrams.

Table 1 compares cardiac mechanoenergetics before and during the caffeine and Ca²⁺ inotropism runs at a fixed LV volume in all LVs. The pre-caffeine and pre-Ca²⁺ control data were obtained after β-blockade. Both maximum-caffeine and maximum-Ca²⁺ data were obtained at a maximally enhanced E_{max}. The E_{max} data evidently indicate comparable positive inotropism by caffeine and Ca²⁺. However, caffeine inotropism contrasted with Ca²⁺ inotropism by a nearly doubled coronary flow, a nearly 40% decreased AVO₂D, and unchanged T_{max} and time to −max dP/dt. Unlike Ca²⁺, caffeine enhanced lusitropism in terms of −max dP/dt and τ.

Figure 3 shows a representative set of the standard VO₂-PVA relations in baseline, control, Ca²⁺, and caffeine volume runs in an LV. Their slopes were not significantly different from each other (ANCOVA). The relation first descended from baseline to control and then ascended in both caffeine and Ca²⁺ volume runs. The caffeine and Ca²⁺ regression lines were significantly higher than the control (ANCOVA). The other LVs showed similar VO₂-PVA relations. The slope of each line yielded the O₂ cost of PVA. Its VO₂ intercept was the PVA-independent VO₂.

Figure 4A shows that the O₂ cost of PVA had no

Fig. 3. A representative set of standard VO₂-PVA regression lines in the baseline volume run (dotted line, solid circles), control (post-β-blockade) volume run (thick solid line, crosses), caffeine volume run (thin solid line, squares) and Ca²⁺ volume run (dashed line, open circles) in one LV. Equations indicate linear regression. *r*: correlation coefficient.

significant difference among the three volume runs in all LVs (ANOVA). Figure 4B shows that VO₂ intercept significantly increased from the control in both caffeine and Ca²⁺ volume runs, but had no significant difference between the caffeine and Ca²⁺ volume runs in all the LVs (ANOVA).

Figure 5A compares the composite VO₂-PVA re-

Fig. 4. O₂ cost of PVA (A) and VO₂ intercept (B) in control (post-β-blockade), caffeine, and Ca²⁺ volume runs (from top down) in all eight LVs. NS: insignificant. * $p < 0.05$ (ANOVA).

Fig. 5. A: A representative set of the composite VO₂-PVA regression lines in the caffeine (solid line, squares) and Ca²⁺ (dashed line, circles) inotropism runs in the same LV as in Fig. 3. The standard VO₂-PVA relation in control volume run (solid line) was transcribed from Fig. 3. Equations indicate linear regression. r : correlation coefficient. B: A representative set of PVA-independent VO₂-E_{max} regression lines in the caffeine (solid line, squares) and Ca²⁺ (dashed line, circles) inotropism runs in the same LV shown in A. Equations indicate linear regression. r : correlation coefficient.

Fig. 6. Composite VO₂-PVA slopes (A) in control (post-β-blockade), caffeine, and Ca²⁺ volume runs and O₂ cost of E_{max} (B) with caffeine and Ca²⁺ in all eight LVs. * $p < 0.05$ (ANOVA).

gression lines of the caffeine and Ca²⁺ inotropism runs with the control VO₂-PVA regression line in an LV. These composite VO₂-PVA regression lines were significantly steeper than the control (ANCOVA). The caffeine inotropism line was significantly steeper than the Ca²⁺ inotropism line (ANCOVA). Figure 5B compares the slopes of the PVA-independent VO₂-E_{max} regression lines in the caffeine and Ca²⁺ inotropism runs (namely the O₂ cost of E_{max}) in the same LV as in Fig. 5A. This cost was significantly greater with caffeine than with Ca²⁺ (ANCOVA). The other LVs had similar results.

Figure 6A shows that the caffeine inotropism run had a significantly steeper ($117 \pm 17\%$ of control, $p < 0.05$) slope of the composite VO₂-PVA regression line than the Ca²⁺ inotropism run (ANOVA). These slopes had significantly steeper slopes than the control in all LVs (ANOVA). Figure 6B shows the pooled results that caffeine had a greater ($141 \pm 52\%$ of control, $p < 0.05$) O₂ cost of E_{max} than Ca²⁺ in all LVs (ANOVA).

DISCUSSION

The present results indicate that intracoronary caffeine at concentrations lower than 1 mmol/l always has an obvious dose-dependent positive inotropism similar to Ca²⁺ in the canine LV. The O₂ cost of mechanical energy (PVA) is comparable between caffeine and Ca²⁺. However, the O₂ cost of E_{max} as a measure of energy wasting of E-C coupling is significantly (40% on average) greater for caffeine. Therefore, the results sup-

port our present hypothesis that the O₂ cost of $E_{\max}$ integratively characterizes the O₂ wasting effect of caffeine despite its complex inotropic mechanisms.

The coexistence of the positive inotropism and the increased O₂ cost of $E_{\max}$ in caffeine is unique among the positive inotropic agents we have studied [5, 10–16]. Catecholamines, ouabain, and the phosphodiesterase inhibitors that we had tested have virtually the same O₂ cost of $E_{\max}$ as Ca²⁺ in the normal canine LV [5, 10, 11, 15]. Pathological heart models such as postischemic stunned, acidotic, and post-acidotic stunned LVs as well as ryanodine-treated LV are characterized by a 1.5–2 times increase in O₂ cost of $E_{\max}$ [12–14, 16]. However, these failing heart models showed a depressed $E_{\max}$. Therefore, the caffeine-treated LV is the first model characterized by the unique mechanoenergetics consisting of a positive inotropism and its increased O₂ cost.

We could speculate about the Ca²⁺ handling in the caffeine-treated LV, taking advantage of the $E_{\max}$ -PVA- $\dot{V}O_2$ framework [5–9, 15]. Either an increased Ca²⁺ handling, an augmented Ca²⁺ responsiveness of contractility or both will enhance $E_{\max}$ generally [5, 6, 26–30]. Either an increased futile Ca²⁺ cycling, a suppressed Ca²⁺ responsiveness or both may increase the O₂ cost of $E_{\max}$ [26–30]. However, caffeine increases Ca²⁺ responsiveness [3, 4, 19]. Therefore, the caffeine-induced futile Ca²⁺ cycling via the SR [22, 23, 25], similar to ryanodine treatment [16, 29], could reasonably account for the O₂ wasting for contractility. However, caffeine at concentrations lower than 1 mmol/l enhanced both inotropism ($E_{\max}$) and lusitropism (τ) (Table 1) in the present blood-perfused canine heart, whereas ryanodine, also at nanomolar concentrations, depressed both $E_{\max}$ and τ in the same type of heart preparation [16]. This difference suggests that Ca²⁺ responsiveness and cAMP-dependent phosphorylation in addition to futile Ca²⁺ cycling [3, 4, 19, 20, 24], all augmented simultaneously by caffeine at concentrations lower than 1 mmol/l, played integratively important roles in the unique mechanoenergetics of caffeine.

Although caffeine increased coronary flow (Table 1), the Gregg phenomenon does not accompany the increased O₂ cost of $E_{\max}$ [31]. We could not suspect any other cardiodynamic parameters that we studied to be obviously responsible for the increased O₂ cost of $E_{\max}$ (Table 1). If caffeine at concentrations lower than 1 mmol/l augments random crossbridge cycling as in the rat myocardium [32], the O₂ cost of PVA would increase because random crossbridge cycling consumes more energy without effectively producing PVA. However, we doubt this possibility because the

O₂ cost of PVA remained unchanged in the present study (Figs. 3 and 4).

The present results all together seem to suggest the following integratively. The O₂ saving effect of caffeine, presumably due to the increased Ca²⁺ responsiveness with caffeine [3, 4, 19, 22], may be outweighed by the O₂ wasting effect of caffeine, presumably due to both futile Ca²⁺ cycling and augmented cAMP-dependent phosphorylation with caffeine [2, 19–21, 23, 25]. However, the present integrative method has an obvious limitation that these individual pharmacological mechanisms for the O₂ saving and wasting effects of caffeine cannot be analyzed reductionistically in the beating whole heart preparation simultaneously. Nevertheless, the present study is worthwhile since conventional reductionistic methods are still unable to elucidate the present integrative results.

Theophylline and sulmazole appear to have the same positive inotropic mechanisms as caffeine, particularly the inhibition of cyclic AMP phosphodiesterase, elevation of cyclic AMP level, and increased myofibrillar Ca²⁺ sensitivity in canine superfused myocardium preparation [33–36]. Although we have not studied the mechanoenergetics of theophylline, we have found that sulmazole has the same mechanoenergetic effects in the $E_{\max}$ -PVA- $\dot{V}O_2$ framework as milrinone, epinephrine, and even ouabain in the canine blood-perfused whole heart preparation [11]. Therefore, caffeine seems to have the oxygen wasting effect that sulmazole does not have in the canine blood-perfused heart. This suggests that the positive inotropic mechanisms of caffeine and sulmazole are not identical, at least in the canine blood-perfused heart preparation we have been using consistently. We would speculate that this difference may be accountable by the futile Ca²⁺ cycling with caffeine, as discussed above [21, 23, 25].

The present finding has the following clinical implication. The maximum concentration of caffeine, i.e., 1 mmol/l, used in this study is 10 times greater than the blood concentration, 0.1 mmol/l, usually reached in man after drinking a cup of regular coffee containing caffeine of 100 mg/cup [24, 37]. There are reports that more than 5–10 cups of coffee per day increase the risk of coronary heart disease by 1.4–2.5 times [38, 39]. If one takes these cups of coffee within a few hours, the blood concentration of caffeine may increase toward 1 mmol/l, the maximum concentration of caffeine used in this study. The present study suggests that a greater O₂ cost of $E_{\max}$ exists at any increased caffeine concentration up to 1 mmol/l, according to the linear composite $\dot{V}O_2$ -PVA and PVA-inde-

pendent VO_2 - $E_{\max}$ relations (Fig. 5A and B). Therefore, the caffeine-induced positive inotropic effect, accompanied by a significant O_2 wasting effect, may partly underlie the dose-dependent increase in the risk of coronary heart diseases.

In conclusion, the $E_{\max}$ -PVA- VO_2 framework has integratively characterized the unique mechanoenergetics of caffeine at intracoronary concentrations lower than 1 mmol/l at the beating whole heart level. Caffeine causes 1.4 times more energy wasting for excitation-contraction coupling than ordinary positive inotropism in the canine heart under β -blockade.

The first author (T.T.) greatly appreciated the continuous encouragement throughout the period of this study by Prof. Goro Kajiyama of the First Department of Internal Medicine, Hiroshima University School of Medicine, from which T.T. was on leave as a postdoctoral research fellow. This study was partly supported by Grants-in-Aid for Scientific Research (07508003, 08670052, 09307029, 09470009, 09670053, 10044330, 10558136, 10770307, 10877006, 11898028) from the Ministry of Education, Science, Sports and Culture, Research Grants for Cardiovascular Diseases (7C-2, 10C-5, 11C-1, 11C-7) from the Ministry of Health and Welfare, 1997–1998 Frontier Research Grants for Cardiovascular System Dynamics and the 1999 Grant for Cardiovascular Physiome Project from the Science and Technology Agency, and a research grant from Suzuken Memorial Foundation, all of Japan.

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