Effects of Intracoronary Caffeine on Left Ventricular Mechanoenergetics in Ca^{2+} Overload Failing Rat Hearts

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Abstract: How different the effects of caffeine on cardiac mechanoenergetics in failing hearts are from those of normal hearts remains to be fully elucidated. First we successfully instituted a new experimental model of acute mild heart failure in the rat by 0.005 mM Ca^{2+} Tyrode perfusion. These failing hearts neither decreased left ventricular end-systolic pressure nor increased left ventricular end-diastolic pressure, indicating unchanged left ventricular mechanics. However, their myocardial mitochondrial respiratory function examined by respiratory control index (RCI) and oxygen consumption rate in state III (State III O_2) was significantly depressed compared with normal hearts. From these results, we judged that this Ca^{2+} protocol could make mild Ca^{2+} overload acute failing hearts and that this model would be appropriate for comparing the effects of

caffeine on cardiac mechanoenergetics between normal hearts and these failing hearts. We investigated the effects of caffeine on cardiac mechanoenergetics above a concentration of 0.05 mM that corresponds to the maximum blood concentration after a healthy human subject drinks a cup of coffee or tea. We obtained results indicating that caffeine depressed left ventricular systolic and diastolic functions and decreased a measure of total mechanical energy per beat in terms of systolic pressure-volume area (PVA) more severely in these failing hearts at concentrations (20-fold higher than the concentration in a cup of coffee) lower than those in normal hearts. This result implies that these acute failing hearts are Ca^{2+} overloaded. [Japanese Journal of Physiology, 48, 373–381, 1998]

Key words: caffeine, Ca^{2+} overload, rat heart.

Coffee and black tea containing caffeine have been drunk as favorite beverages by healthy subjects. However, patients with failing hearts are prohibited or limited to drinking them as protection from aggravating cardiac failure. However, how different the effects of caffeine on cardiac mechanoenergetics in failing hearts are from those of normal hearts remains to be fully elucidated.

Caffeine is the main component in coffee and tea that is pharmacologically effective on the cardiovascular system. Caffeine is well known to have cardiotonic and diuretic actions mediated via the central nervous system *in vivo* and has been widely used as a pharma-

cological tool for releasing calcium (Ca^{2+}) from the sarcoplasmic reticulum (SR) *in vitro* [1–3]. The effects of caffeine on cardiac systolic intracellular calcium concentration [Ca^{2+}]_i and contraction are complex as reported with increases mediated by inhibiting the cyclic AMP phosphodiesterase activity and decreases by inhibiting the release of Ca^{2+} from SR [4–7]. Caffeine has also been reported to increase the sensitivity of myofilaments to Ca^{2+} [4, 8, 9], to inhibit Ca^{2+} uptake by the SR [2, 5, 6, 10], to change the Ca^{2+} current [2], and to cause a cellular Ca^{2+} overload [7,11]. However, the effect of caffeine on a mild-type Ca^{2+} overload failing heart has not been investigated.

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We recently developed a new experimental model of mild-type acute failing canine heart by perfusion with a short-term Ca^{2+} -free and high- Ca^{2+} Tyrode solution, which is presumably Ca^{2+} overload failing heart [12, 13]. To get an insight into the above question, we first developed a different mild-type acute failing heart as an experimental model in the rat by using Langendorff perfusion with 0.005 mM Ca^{2+} -Tyrode solution for 5–5.5 min. Since neither end-systolic pressure (ESP) nor end-diastolic pressure (EDP) was changed in this heart failure model, we successfully compared the effects of intracoronary caffeine on left ventricular (LV) mechanoenergetics between the normal and the mild Ca^{2+} overload failing rat hearts. An evaluation of LV mechanoenergetics has been performed by using our newly proposed approach to the combination of a curved end-systolic pressure-volume relation and a linear O_2 use–pressure-volume area (PVA: a measure of total mechanical energy) relation [14–16]. The minimum concentration of caffeine tested in this study was determined by measuring caffeine blood concentration when healthy human subjects drank caffeine solution (100 mg) equivalent to the amount in a cup of coffee or black tea.

MATERIALS AND METHODS

Determination of caffeine blood concentration in human subjects. Healthy human subjects donating blood samples for the measurement of caffeine concentration were prohibited to have caffeine drinks in their meals on the test day. Before drinking the caffeine solution (100 mg/200 ml), and 1 and 3 h after drinking it, 5 ml of blood was sampled each time. Blood caffeine concentrations have been reported to reach a maximum 1 h after consumption [17]. One hundred milligrams of caffeine is a representative amount included in a cup of coffee. The blood was immediately centrifuged at 4,000 rpm and plasma was collected. After the clean-up treatment of completely removing proteins and lipids, plasma caffeine was measured by high-performance liquid chromatography.

Rat heart preparation. Eighteen retired breeder male Wistar rats, weighing 510–760 g, were anesthetized with pentobarbital sodium (50–75 mg/kg, I.P.) and intubated. All rats were heparinized (2,000 U/rat, I.P.). The chest was opened midsternally under artificial ventilation. The ascending aorta was then rapidly cannulated, and the heart was perfused aerobically in the Langendorff mode at $36 \pm 2^\circ\text{C}$ with perfusion fluid (containing, in mM: 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, 15.0; HEPES, 5.0; pH, corrected to 7.4 with NaOH at 37°C when gassed with 100% O_2) at a constant pressure (95 cmH_2O). The beating heart was then excised from the chest. Coronary perfusion of the excised heart was never interrupted during the preparation.

In all the hearts, the LV apex was punctured with a 21-gauge cannula to drain the thebesian flow and aortic regurgitant fluid, if any. The left atrium was opened and all LV chordae tendinae were cut. A thin latex balloon (balloon material volume, 0.13 ml) was fitted into the LV. The balloon, primed with water, was connected via a polyethylene tube to a pressure transducer (Life Kit DX-312, Nihon Kohden, Tokyo, Japan) and a 0.5 ml precision glass syringe with fine scales (minimum scale: 0.005 ml). LV pressure (LVP) was measured with this transducer. The balloon did not develop any pressure and thus was unstretched as long as its volume was below approximately 0.4 ml. Therefore LV volume (LVV) was changed and measured by adjusting intraballoon water volume with the syringe in 0.05 ml steps between 0.18 and 0.33 ml and fixed constant by a three-way stopcock. We used only isovolumic contractions throughout this study. Systolic unstressed volume (V_0) should be determined as the volume at which peak isovolumic pressure and thus PVA were zero. Although residual PVA was small, LV peak pressure sometimes did not become zero because of the volume of the balloon material per se. Thus V_0 was determined as the volume-axis intercept of the end-systolic P - V relation by curve fitting with Eq. 1 (see below).

An LV epicardial electrocardiogram (ECG) was recorded with a pair of needle electrodes. Heart rate was maintained constant at 300 beats/min by an electrical pacing of the right atrium throughout each experiment approximately 30% higher than the sinus rhythm (213 ± 37 beats/min: mean $\pm$ SD). The coronary flow rate (CF) was obtained by directly measuring the coronary outflow volume per minute. LV wet weight was 1.37 ± 0.19 g ($n=18$).

Experimental protocol. LV ESP and EDP were obtained at four varied LVVs (mean volume: 0.20 ml/g) in each heart (volume run). “Control volume run” was performed without any inotropic interventions. After the control volume run, a “caffeine inotropism run” at the constant LVV (0.18 ± 0.02 ml/g) was performed in the normal hearts ($n=9$) from 0.05 mM. In this inotropism run 5 min after the onset of 4 mM caffeine infusion, a “caffeine volume run” was performed at the same LVVs as used in the control volume run ($n=5$). After the caffeine inotropism run, a 20 min washout was performed by normal Tyrode so-

lution. In the other 9 hearts, after control volume run a Ca^{2+} overload heart was made by low Ca^{2+} Tyrode and normal Tyrode perfusions (see below). The caffeine inotropism run at the constant LVV (0.17 ± 0.02 ml/g) was started in these hearts 4–10 min after the Ca^{2+} overloading protocol. In this inotropism run 5 min after the onset of a 2 mM caffeine infusion, a “caffeine volume run” was performed at the same LVVs as in the control volume run ($n=5$). After a caffeine inotropism run, a 20 min washout was performed by normal Tyrode solution.

Caffeine protocols in the normal and Ca^{2+} overload hearts ($n=9$ each): 15 mM caffeine solution was infused into the coronary perfusion tubing at graduated rates of 0.045 ± 0.007 – 5.2 ± 1.7 ml/min (calculated concentration: 0.05–8 mM).

Ca^{2+} overloading protocol ($n=9$): The heart was perfused with 0.005 mM Ca^{2+} Tyrode solution for 5–5.5 min at a flow rate of 15 ± 1.8 ml/min (10.8–16.4 ml/min), then perfused with normal Tyrode solution for 1.5 h.

Data analysis. We determined PVA by the method described below because the ESPVR of the rat LV was an upward convex curve unlike the linear ESPVR of the adult canine LV [14–16, 18, 19]. We attempted to fit the end-systolic and end-diastolic pressure (P)-volume (V) points by the following equations:

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

$$P_{ed} = A' \{1 - \exp[-B'(V - V_u)]^{-1}\}, \quad (2)$$

where P_{es} is end-systolic peak isovolumic pressure, P_{ed} is end-diastolic isovolumic pressure, V is isovolumic volume, V_0 and V_u are the volume intercepts of the nonlinear curves best fitted to the end-systolic and end-diastolic P - V points, and A and A' and B and B' are fitting parameters. Equation 1 is the same as the equation we used in our previous study on puppy LV mechanics [20]. Although AB represents only the slope of the curve at V_0 , the dimensions of AB are mmHg/ml and the same as those of $E_{\max}$, which indicates the slope of the linear ESPVR of the adult canine LV [18–20]. Thus we also calculated AB values.

PVA is defined as the P - V area circumscribed by the curvilinear ESPVR, EDPVR, and the systolic portion of the ventricular P - V trajectory [21, 22]. The area under the ESPVR is obtained by integration of Eq. 1 best fitted to the data. The area under the EDPVR was reasonably assumed to be zero within the same LVV range [14–16]. However, in the Ca^{2+} overload hearts, especially after caffeine infusion, the

EDPVR shifted upward. Therefore the area under the EDPVR could not be negligible. The area under the EDPVR is obtained by an integration of Eq. 2 best fitted to the data and was subtracted from the area under the ESPVR to obtain a net PVA. PVA was then normalized by LV mass to 1 g. Representative PVAs of the rat LV are shown by the striped area in Figs. 5 and 6.

In the present study, we calculated AB and PVA at an LVV of 0.20 ml/g [$\text{PVA}_{0.20}$] to assess LV mechanoenergetics. In our laboratory, Tachibana *et al.* [15] had proposed that *in situ* rat hearts PVA at an appropriate LVV could be used to evaluate LV mechanoenergetics despite the curvilinear LV ESPVR. This was based on the data showing a linear VO_2 -PVA relation by Hata *et al.* [14] and Takaki *et al.* [16]. As the appropriate LVV, we chose 0.20 ml/g. This was a medium volume in the range of changed volume (0.18–0.33 ml) for data sampling.

Measurement of mitochondrial oxygen consumption.

A separate group of 12 retired breeder male Wistar rats, weighing 430–710 g, were anesthetized with pentobarbital sodium (50–75 mg/kg, I.P.) and intubated. These hearts were prepared by the same method as used for the preset mechanoenergetic study. Normal hearts ($n=6$) were perfused by the normal Tyrode solution for 1.5 h without intervention. Ca^{2+} overload hearts ($n=6$) were perfused for 1.5 h after the same Ca^{2+} overloading protocol, as described above. From each of these hearts, LV myocardial mitochondria were prepared after removing the atria and the right ventricular free wall. LV wet weight was 1.13 ± 0.16 mg ($n=12$).

A differential centrifugation was used according to the method of Hatefi *et al.* [23]. Immediately after the mitochondrial preparation, the oxygen consumption of mitochondria was measured polarographically with an oxygen electrode installed in a closed cell (UC-12; Central, Tokyo, Japan) at 25°C. The incubation medium contained 0.3 M mannitol, 10 mM KH_2PO_4 , 2.5 mM MgCl_2 , and 0.1 mM ethylenediaminetetraacetate (EDTA) with pH 7.4 in a total of 0.7 ml. First, 0.03 ml of mitochondrial suspension (6.27 ± 2.08 mg protein/ml) was added; then 0.2 M succinic acid (0.03 ml) was added as a substrate to the incubation medium. One minute later, 10 mM adenosine diphosphate (ADP) (0.01 ml) was added. The rate of oxygen consumption in State III (State III O_2) and the respiratory control index (RCI) were determined. State III O_2 was calculated from mitochondrial oxygen consumption in n atoms (1 mol $\text{O}_2 = 2$ atoms O) of oxygen consumed per min per mg mitochondrial protein during State III respiration. RCI (dimensionless) was taken as

the ratio between the rates of oxygen consumption before and after the addition of ADP.

We have previously confirmed more than 95% intactness of the mitochondria prepared by the present differential centrifugation method from the heart, using an electron-microscopic examination [24].

Statistics. In each experiment, end-systolic and end-diastolic P - V points were fitted by the nonlinear equations (Eqs. 1 and 2). A comparison of paired individual correlation coefficients (R) was performed by paired t -test. A comparison of nonpaired individual values was performed by pooled t -test. A value of $p < 0.05$ was considered statistically significant. All data are expressed as mean $\pm$ SD.

RESULTS

Caffeine blood concentration in human subjects

Before the caffeine solution was consumed (100 mg), caffeine was not detected in the human blood samples. At 1 and 3 h after consumption, 0.10 ± 0.05 and 0.06 ± 0.02 mM ($n=6$) of caffeine were detected. This result indicated that in healthy human subjects, blood caffeine concentration reached maximally 0.06–0.10 mM after a subject drank a cup of coffee or tea. Therefore we investigated the effect of caffeine above a concentration of 0.05 mM.

Effects of caffeine on LV mechanoenergetics in normal rat hearts

Figure 1 illustrates simultaneously recorded traces of LVP, LVV, ECG, and coronary flow (CF) obtained by changing LVV in steps from 0.18 to 0.33 ml in a normal rat heart. LV end-systolic pressure (ESP) and end-diastolic pressure (EDP) increased with increased LVV. LV ESP was 64.8 ± 8.6 mmHg ($n=18$) and EDP

was 6.4 ± 2.7 mmHg ($n=18$) at the medium LVV of 0.17 ± 0.02 ml/g. The coronary flow rate was 14.6 ± 1.93 ml/min at the same medium LVV.

Figure 2a summarizes the LV pressure–caffeine concentration relations in the normal rat hearts ($n=9$). Intracoronary infusion of caffeine slightly increased ESP up to 1 mM, but at 2 mM and higher it decreased ESP and then increased. At 4 mM, ESP maximally decreased to 70% of control ($p < 0.05$), and EDP increased to 4.5-fold of control ($p < 0.01$). At 6 mM, the decrease in ESP was reversed to an increase to 85% of the control and EDP reached 7-fold of control ($p < 0.01$), resulting in a decrease in LV-developed pressure to 10 mmHg (caffeine contracture). After the 20-min washout with Tyrode solution, ESP reached a level of 80% of the control and EDP recovered to the control level (8 mmHg).

Fig. 1. Simultaneously recorded tracing of left ventricular pressure (LVP), volume (LVV), electrocardiogram (ECG), and coronary flow (CF) during increasing LVV from 0.18 to 0.33 ml (from most leftward to rightward traces) in a normal heart.

Fig. 2. Left ventricular (LV) pressure–caffeine concentration relations in normal hearts (a) and Ca^{2+} overload hearts (b).

Caffeine in Ca²⁺ Overload Hearts

Fig. 3. Simultaneously recorded tracing of left ventricular pressure (LVP) and electrocardiogram (ECG) during Ca²⁺ overloading protocol.

Fig. 4. Respiratory control index (RCI) and State III O₂ of myocardial mitochondria in normal and Ca²⁺ overload hearts.

Ca²⁺ overloading protocol; perfusion with 0.005 mM Ca²⁺ Tyrode solution

After a control volume run, LVP and ECG were continuously recorded at LVV=0.20 ml/g, and 0.005 mM Ca²⁺ Tyrode solution was perfused into the coronary artery for 5.5±0.16 min. The perfusate was then changed to normal Tyrode solution, resulting in a mild Ca²⁺ paradox. An example is shown in Fig. 3. During perfusion with 0.005 mM Ca²⁺ Tyrode solution, the heart arrested. After perfusion with normal Tyrode solution, EDP slightly increased, but decreased gradually. ESP transiently increased, but recovered to the pre-0.005 mM Ca²⁺ Tyrode solution perfusion level.

Respiratory function of myocardial mitochondria from 0.005 mM Ca²⁺ Tyrode solution-perfused excised heart

Figure 4 summarizes the data of respiratory control index (RCI; dimensionless) and State III O₂ (ng atom · ml⁻¹ · mg protein⁻¹) of myocardial mitochondria from the normal (Normal) and 0.005 mM Ca²⁺ Tyrode solution-perfused hearts (Ca²⁺ overload). Both RCI and State III O₂ in the 0.005 mM Ca²⁺ Tyrode solution-perfused hearts were significantly smaller than those in the normal hearts. From these results, we judged that this protocol could make Ca²⁺ overload acute failing hearts as in canine blood-perfused excised hearts, despite the different LV mechanics [12,13].

Effects of caffeine on LV mechanoenergetics in Ca²⁺ overload hearts

In the Ca²⁺ overload hearts, intracoronary infusion of caffeine was performed as in normal hearts. Figure 2b summarizes the LV pressure–caffeine concentration relations in the Ca²⁺ overload hearts (*n*=9). Caffeine at concentrations lower than those in the normal hearts decreased ESP and increased EDP more severely than in the normal hearts. From 0.5 mM, ESP decreased while EDP increased in a concentration-dependent manner. At 2 mM, ESP decreased to 72% of the control, while EDP increased to 7.5-fold of control (*p*<0.01), then increased up to 8 mM. At 4 mM, ESP decreased maximally (*p*<0.01), then increased up to 90% of the control at 8 mM. Caffeine at higher than 4 mM induced a tonic contracture (caffeine contracture). In 3 of 9 hearts, 8 mM caffeine infusion arrested the beating. After a 20-min washout with Tyrode solution, ESP recovered to 70% of the control, whereas EDP remained increased at a level of 9-fold the control.

ESP and EDP in the normal hearts were 51.2±8.8 (*n*=9) and 6.38±4.87 mmHg (*n*=9) (Fig. 2a) at LVV=0.18±0.02 ml/g, and those in the Ca²⁺ overload hearts were 44.0±7.75 and 24.2±6.3 mmHg (*n*=9) at LVV=0.17±0.02 ml/g during 2 mM caffeine infusion. EDP in the Ca²⁺ overload hearts was significantly higher than in the normal hearts (Fig. 2b).

Figure 5a shows an LV pressure–caffeine concentration relation at a constant LV volume (=0.17 ml/g) in a normal heart. Figure 5b indicates the control ESPVR, EDPVR, and PVA. Figure 5c indicates the ESPVR, EDPVR, and PVA 5 min after the onset of an infusion of 4 mM caffeine. The ESPVR shifted downward, and the EDPVR shifted upward at larger LV volumes. PVA at 0.20 ml/g was reduced in this heart.

Table 1a summarizes all the mechanoenergetic indexes in the normal hearts (*n*=5). None of the indexes showed significant differences between control and 2–4 mM caffeine infusion.

Figure 6a shows an LV pressure–caffeine concentration relation at a constant LVV (=0.19 ml/g) in a Ca²⁺ overload heart. Figure 6b indicates the control ESPVR, EDPVR, and PVA in a Ca²⁺ overload heart.

Fig. 5. An example of left ventricular (LV) pressure-caffeine concentration relation in a normal heart (a) and end-systolic, end-diastolic pressure-volume relations and pressure-volume area (PVA) in control (b) at A in (a) and during infusion of caffeine (4 mM) (c) at B in (a).

Figure 6c indicates the ESPVR, EDPVR, and PVA 5 min after the onset of an infusion of 2 mM caffeine in a Ca^{2+} overload heart. The ESPVR shifted markedly downward, and the EDPVR shifted markedly upward at all the LVVs. ESP and PVA at 0.20 ml/g were markedly reduced, and EDP at 0.20 ml/g was markedly increased in this heart.

Table 1b summarizes all these mechanoenergetic indexes in the Ca^{2+} overload hearts ($n=5$). Respective correlation coefficients between the best-fit curves to the ESPVR and EDPVR were sufficiently high, that is, 0.989 ± 0.023 and 0.994 ± 0.009 in control, and 0.996 ± 0.007 and 0.993 ± 0.008 during 2–4 mM caffeine infusion in the normal hearts, and 0.994 ± 0.007 and 0.983 ± 0.017 in control and 0.994 ± 0.009 and 0.976 ± 0.032 during 1–2 mM caffeine infusion in the Ca^{2+} overload hearts. ESP, EDP, and PVA at 0.20 ml/g during 1–2 mM caffeine infusion were significantly different from those of control in the Ca^{2+} overload hearts. However, no significant difference in AB was noted between the control and during the infusion of 1–2 mM caffeine.

DISCUSSION

The LV mechanics in the rat heart treated with the present Ca^{2+} overloading protocol was different from the canine one treated with Ca^{2+} -free, high- Ca^{2+}

coronary perfusion [12]. Our newly made experimental model of failing hearts in the rat neither decreased ESP nor increased EDP; that is, at least LV mechanics appeared to be unchanged. However, mitochondrial respiratory function was significantly depressed as in canine Ca^{2+} overload hearts [13]. From these results, we judged this mild failing rat heart model to be appropriate for comparing the effect of caffeine on LV mechanoenergetics between normal and Ca^{2+} overload failing hearts.

In isolated rat ventricular myocytes, at high caffeine concentrations (1 mM or above) the threshold Ca^{2+} for regenerative Ca^{2+} -induced Ca^{2+} release will be reduced to below the resting $[\text{Ca}^{2+}]_i$, thus producing a diastolic increase in $[\text{Ca}^{2+}]_i$ [25]. At lower caffeine concentrations (0.05–0.5 mM), the threshold is higher than the resting $[\text{Ca}^{2+}]_i$, and caffeine serves only to enhance the regenerative Ca^{2+} release produced during systole [25]. Thus in normal hearts, LV ESP must have increased at lower caffeine concentrations. In fact, LV ESP slightly increased, though statistically not significantly, at lower caffeine concentrations (0.05–1 mM), as shown in Fig. 2a. However, at concentrations higher than 1 mM of caffeine, LV ESP decreased and EDP increased, and at 6 mM caffeine contracture occurred. This result suggests that at 1–6 mM caffeine produced an elevation of diastolic $[\text{Ca}^{2+}]_i$ levels resulting in cellular Ca^{2+} overload [7, 11, 25].

Table 1. Variables of cardiac mechanoenergetics before and during caffeine infusion.

a. Normal hearts

Exp. No.	Control						Caffeine 2–4 mM					
	AB	V_0 (ml/g)	V_u (ml/g)	ESP (0.20 ml/g)	EDP (0.20 ml/g)	PVA (0.20 ml/g)	AB	V_0 (ml/g)	V_u (ml/g)	ESP (0.20 ml/g)	EDP (0.20 ml/g)	PVA (0.20 ml/g)
1	1,225	0.088	0.133	72.5	5.8	2.67	1,442	0.105	0.093	47.0	34.0	0.90
2	691	0.103	0.159	62.8	13.4	2.93	1,191	0.134	0.154	48.0	18.4	1.56
3	2,062	0.076	0.108	91.8	18.0	7.80	1,030	0.062	0.112	76.2	26.9	5.29
4	1,074	0.066	0.133	78.9	13.3	5.77	569	0.054	0.124	57.2	17.6	3.94
5	492	0.125	0.117	48.2	3.2	6.60	238	0.016	0.155	38.0	8.7	3.25
Mean	1,109	0.092	0.130	70.8	10.7	5.15	894	0.074	0.128	53.3	21.1	2.99
SD	608	0.023	0.019	16.4	6.1	2.27	486	0.046	0.027	14.5	9.6	1.78

b. Ca^{2+} overload hearts

Exp. No.	Control						Caffeine 1–2 mM					
	AB	V_0 (ml/g)	V_u (ml/g)	ESP (0.20 ml/g)	EDP (0.20 ml/g)	PVA (0.20 ml/g)	AB	V_0 (ml/g)	V_u (ml/g)	ESP (0.20 ml/g)	EDP (0.20 ml/g)	PVA (0.20 ml/g)
1	732	0.075	0.155	63.0	6.1	4.31	615	0.112	0.141	43.6	19.3	1.49
2	905	0.031	0.135	70.2	6.6	7.41	1,461	0.117	0.120	54.5	16.4	2.30
3	1,840	0.095	0.110	77.9	8.2	5.75	1,218	0.094	0.109	60.1	24.0	2.89
4	2,832	0.074	0.089	87.9	14.4	7.69	1,300	0.059	0.096	64.6	29.7	4.80
5	510	0.038	−0.004	66.5	21.1	4.28	905	0.092	0.089	62.8	33.1	2.20
Mean	1,364	0.063	0.097	73.1	11.3	5.89	1,100	0.095	0.111	57.1*	24.5*	2.73*
SD	965	0.027	0.062	9.9	6.4	1.63	338	0.023	0.021	8.5	7.0	1.25

* Significantly different from control. AB is obtained from the best-fit curve to the end-systolic P - V points. AB represents only the slope of the curve at V_0 . V_0 is the volume intercept of the best-fit curve to the end-systolic P - V points. Systolic unstressed volume. V_u is the volume intercept of the best-fit curve to the end-diastolic P - V points. Diastolic unstressed volume. ESP is end-systolic pressure value of the best-fit curve at left ventricular volume of 0.20 ml/g. EDP is end-diastolic pressure value of the best-fit curve at left ventricular volume of 0.20 ml/g. PVA is obtained by subtracting the area under the best-fit curve to the end-diastolic P - V points from the area under the best-fit curve to the end-systolic P - V points at left ventricular volume of 0.20 ml/g.

Even at lower caffeine concentrations (above 0.5 mM) than those in the normal heart, LV ESP began to decrease and EDP started to increase in the present Ca^{2+} overload heart. The increase in EDP was much larger than that in the normal heart at 2 mM, and 4 mM caffeine contracture occurred. The increased EDP did not recover after a sufficient washout in contrast to almost complete recovery in the normal heart. These results indicate that caffeine can cause severer cellular Ca^{2+} overload in the Ca^{2+} overload heart than in the normal heart at concentrations lower than those in the normal heart. These results support the possibility that the present failing heart model is a mild Ca^{2+} overload heart despite normal ESP and EDP.

Thus we successfully made the present mild failing rat heart model, though the rat heart easily became a severe Ca^{2+} overload by means of a Ca^{2+} paradox induced by perfusions with Ca^{2+} -free and normal Tyrode solutions (our unpublished observation), which have been used in the canine heart [12]. These results altogether indicate that even lower concentrations (1–2 mM) of caffeine could cause severer cardiac dysfunction in Ca^{2+} overload hearts than in the normal heart. However, these concentrations were much higher than those included in a cup of coffee or black tea. Although the present results were obtained in the *ex vivo* animal experiments, they might suggest the possibility that human subjects with cardiac failure

Fig. 6. An example of left ventricular (LV) pressure-caffeine concentration relation in a Ca^{2+} overload heart (a) and end-systolic, end-diastolic pressure-volume relations and pressure-volume area (PVA) in control (b) at A in (a) and during infusion of caffeine (2 mM) (c) at B in (a).

can drink daily a few cups of coffee or black tea.

In conclusion, the present results have indicated that caffeine worsens left ventricular systolic and diastolic functions in Ca^{2+} overload acute failing hearts more severely than in normal hearts at concentrations lower than those in normal hearts, though these results were observed only in the rat. The rat heart can easily become a severe Ca^{2+} overload and thus may respond excessively to caffeine. Even in the rat Ca^{2+} overload acute failing hearts, a 20-fold concentration of caffeine included in a cup of coffee is needed to depress cardiac function.

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