Effects of Ca^{2+} and Epinephrine on Ca^{2+} Recirculation Fraction and Total Ca^{2+} Handling in Canine Left Ventricles

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Abstract: We investigated the effects of intracoronary Ca^{2+} and epinephrine on the intracellular Ca^{2+} recirculation fraction (RF) and total Ca^{2+} handling in the left ventricle (LV) of the excised cross-circulated canine heart preparation. We analyzed LV postextrasystolic potentiation (PESP) following a spontaneous extrasystole that occurred sporadically under constant atrial pacing. All PESP decayed in alternans and none decayed monotonically. We extracted an exponential decay component from the alternans PESP, determined its beat constant (τ_e), and calculated $\text{RF} = \exp(-1/\tau_e)$. Increased intracoronary Ca^{2+} slightly increased τ_e and RF, but epinephrine did not change them, although both agents en-

hanced LV contractility 2–3 times. Neither Ca^{2+} nor epinephrine affected the sinusoidal decay of the alternans PESP. These results indicate that RF via the sarcoplasmic reticulum was slightly augmented by Ca^{2+} , but not by epinephrine. We combined these RF data with LV Ca^{2+} handling O_2 consumption data and obtained 40–110 $\mu\text{mol/kg}$ as the total amount of Ca^{2+} handled in one cardiac cycle in the control and enhanced contractile states. These results indicate that this new LV-level approach seems to better the understanding of the Ca^{2+} mass dynamics responsible for the mechanoenergetics enhanced by inotropic interventions. [Japanese Journal of Physiology, 48, 123–132, 1998]

Key words: heart, excitation–contraction coupling, oxygen consumption, postextrasystolic potentiation, beat constant.

The intracellular Ca^{2+} recirculation fraction (RF) in a steady-state beat has conventionally been determined from the beat constant (τ) of the exponential function of the monotonically decaying postextrasystolic (PES) potentiation (PESP) interrupting the stable beats [1–3]. RF is considered to be an integrative measure of the mass dynamics of Ca^{2+} handled in the excitation–contraction (E–C) coupling of each cardiac cycle [4]. RF is known to change under various inotropic interventions in different animal species [1, 2, 5–8]. A greater RF means that a greater fraction of the total amount of Ca^{2+} involved in the E–C coupling of

each cardiac cycle recirculates intracellularly via the sarcoplasmic reticulum (SR) and that inversely a smaller fraction of the total Ca^{2+} is extruded transsarcolemmally primarily by the $\text{Na}^+/\text{Ca}^{2+}$ exchange and enters the cell via the Ca^{2+} channel [1–11]. Since these intracellular and transsarcolemmal Ca^{2+} handlings have different energetics for ion transport [9–11], we consider that RF is a key parameter in cardiac energetics.

We previously found that the comparable RF values were obtained from τ not only of the monotonically decaying PESP, but also of the exponentially decaying

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Abbreviations: ES, extrasystole; E_{max} , end-systolic maximum elastance or pressure-volume ratio as an index of ventricular contractility; LV, left ventricle; PES1–6, 1st–6th postextrasystolic beats; PESP, postextrasystolic potentiation; PVA, systolic pressure-volume area as a measure of total mechanical energy of cardiac contraction; RF, intracellular Ca^{2+} recirculation fraction; τ_e , beat constant; VO_2 , cardiac oxygen consumption.

component of the transient alternans PESP in the left ventricle (LV) of canine hearts [12, 13]. We then found that RF obtained from τ of the two types of PESP decreased 16%, to 0.47, from 0.56, on average in the canine LV whose contractility was halved by intracoronary infusion of ryanodine at nanomolar concentrations [14, 15]. Using the decreased RF, we could account partly for the O_2 waste of these ryanodine-treated failing hearts [14, 15]. However, we recognized that RF could not fully account for this O_2 waste because of probable futile Ca^{2+} cycling via the SR made leaky to Ca^{2+} by ryanodine [14, 15].

We have found that Ca^{2+} and epinephrine infused intracoronarily had normal O_2 cost of contractility, although these two agents have pharmacologically different positive inotropic mechanisms [16–18]. We therefore hypothesized that the comparable O_2 cost of contractility between Ca^{2+} and epinephrine would be associated with comparable RF values. To test this hypothesis in the present study, we obtained RF in control and enhanced contractile states with Ca^{2+} and epinephrine and compared the RF values among the three contractile states in canine hearts. We then combined these RF values with LV O_2 consumption $\dot{V}O_2$ for the excitation–contraction (E–C) coupling and made a new attempt to calculate the total amount of Ca^{2+} handled in the E–C coupling in the different contractile states. We obtained results supporting the hypothesis.

METHODS

Surgical preparation. We retrieved PESP data from the strip-chart recording of our previous canine heart experiments on the mechanoenergetic effects of intracoronary Ca^{2+} and epinephrine [18]. The materials and methods of the heart preparation were described in detail in the previous paper [18]. Briefly, two adult mongrel dogs were anesthetized with pentobarbital sodium (30 mg/kg, I.V.) after premedication with ketamine hydrochloride (7 mg/kg, I.M.) in each experiment. Both dogs were heparinized (10,000 units per dog). The bilateral common carotid arteries and external jugular vein of the support dog (body weight = 15.0 ± 1.3 (SD) kg) were cannulated with arterial and venous cross-circulation tubes. The heart donor dogs (14.1 ± 0.8 kg) were thoracotomized under artificial ventilation. The left subclavian artery was cannulated with the arterial cross-circulation tube and the right ventricle with the venous cross-circulation tube via the right atrium. All the systemic and pulmonary vascular connections to the heart were ligated and severed. The cross-circulated heart was excised from the chest without stopping coronary circulation

as usual.

The left atrium was opened, all the LV chordae tendineae were cut, and a flabby rubber balloon with an unstretched volume of 50 ml was fitted into the LV chamber. The balloon was connected to our custom-made volume servo pump (International Servo Data, Tokyo, Japan), and both the balloon and the water housing of the pump were primed with water.

The temperature of the heart was kept constant at 36°C with heaters in the water bath containing the arterial cross-circulating tube and under the acrylic box containing the heart. The left atrium was electrically paced at a constant rate in each heart (150 ± 6 beats/min). This pacing rate was chosen not to be exceeded by the sinus rate even under the positive chronotropism of epinephrine at its maximum infusion rate. Coronary arterial pH, PO_2 , and PCO_2 were occasionally measured and corrected as needed.

Experimental protocol. The experimental protocol consisted of three runs: control, Ca^{2+} , and epinephrine. These runs had been designed to compare the mechanoenergetic ($\dot{V}O_2$ –PVA– E_{max} relation [17]) effects of Ca^{2+} and epinephrine, as originally described in detail [18]. Here, E_{max} is an index of LV contractility identified as the end-systolic maximum elastance of the LV or the slope of the end-systolic pressure–volume relation line [19]. PVA (abbreviation of systolic pressure–volume area) is a measure of the total mechanical energy of LV contraction [17].

Control run. First, in a stable control contractility, steady-state isovolumic LV contractions were produced at 5–7 different volumes (<40 ml). Peak isovolumic pressure of the LV ranged from 0 mmHg at V_0 (systolic unstressed or dead volume, 6–7 ml) to 120–180 mmHg. At each volume load, E_{max} and $\dot{V}O_2$ –PVA data of the LV were obtained in steady-state beats and documented in detail in the previous paper [18].

Ca^{2+} and epinephrine runs. We then fixed the isovolumic volume at an intermediate level (14–21 ml) where peak isovolumic pressure was 70–90 mmHg in the control contractility. Then either Ca^{2+} or epinephrine was infused into the coronary circulation, and the dose was gradually increased until E_{max} increased about 2–3 times. Ca^{2+} preceded epinephrine in six experiments and vice versa in the other six. The maximum intracoronary infusion rate of Ca^{2+} was 0.08 mmol/min, corresponding to a calculated increment of intracoronary Ca^{2+} concentration by approximately 1.5 mmol/l on the average. The maximum intracoronary infusion rate of epinephrine was 1.3 μ g/min, corresponding to a calculated intracoronary concentration of approximately 25 μ g/l on the average. At each enhanced contractility, E_{max} and $\dot{V}O_2$ –PVA data

were obtained in steady-state beats. All these data were previously documented in detail [18].

Data analysis. In the original study [18], we used only the mechanoenergetic data of steady-state beats and excluded all the data contaminated by spontaneous PESP. In the present study, however, we retrieved the PESP parts from the strip-chart tracings to calculate RF of the immediately preceding steady-state beats and combined it with the previously documented $\text{VO}_2\text{-PVA-}E_{\text{max}}$ data [18].

We surveyed all the PESP following single sporadic extrasystoles in all 12 hearts subjected to the previous study [18]. Any PESP following multiple extrasystoles were excluded. Peak isovolumic LV pressures of the stable regular beats, the extrasystole itself, and the first through 6th (occasionally 5th) PES beats were measured. The PESP returned to regular beats by the 5th or 6th PES beat. Peak isovolumic pressures of these PES beats were all normalized to that of the preceding stable regular beat at the same isovolumic volume. Since LV volume was kept constant in each set of these beats, the normalized pressure changes indicated normalized contractility changes in terms of E_{max} .

We had recorded only stable contractions without any arrhythmias at a high speed (2.5 or 5 cm/s) on both a strip-chart recorder and a computer hard disk in the original study [18]. In the present study, however, we analyzed the contractility changes during PESP to obtain RF in the same way as before [12, 13, 15]. We found that almost all the PESP had been recorded at a low speed (1 or 2 mm/s) on a strip-chart recorder alone. To obtain reliable data for the present analysis, we therefore used a precision-scale magnifying glass to read peak isovolumic pressures on the strip charts with a resolution of 1 mmHg. This precise reading was possible whether PESP were recorded at a high or low speed. Thus, the speed of the tracing did not affect the PESP data subjected to the present analysis.

Curve fitting and beat constant (τ_e). To the normalized pressures in each PESP, we fitted

$$y = a \cdot \exp[-(x-1)/\tau_e] + b \cdot \exp[-(x-1)/\tau_s] \cdot \cos[\pi(x-1)] + 1 \quad (1)$$

in the same way as previously done [13, 15]. Here, y is the normalized contractility of each of the PES beats; x ($=1-6$) is the ordinal number of a PES beat; a and b are the normalized magnitudes of the two decay components in PES1; τ is time constant of the respective exponential functions (see below); π is the circular constant.

Equation 1 has been proven to be a physiologically reasonable formula to describe the transient alternans

type of PESP [13, 15]. It contains two exponential terms. The first represents the exponential decay component; the second multiplies the unit-amplitude sinusoidal term to make the alternans decay component. τ_e and τ_s are beat constants of the respective exponential functions, where subscripts e and s refer to exponential and sinusoidal. These τ_e and τ_s were expressed in the number of beats, and they are called beat constants instead of time constants [4, 13, 15]. Only τ_e was needed to calculate RF which is independent of τ_s [13, 15].

When the resultant a in Eq. 1 was negligibly small (<0.02), the resultant τ_e became either abnormally small (<0.5 beats) or large (>5 beats). We judged such a too-small or a large τ_e to be unreliable because of decreases in the signal-to-noise ratio of the measured pressures with decreases in the amplitude (a) of the first exponential term decreased, as previously discussed in detail [15]. The transient alternans PESP with $a < 0.02$ appeared purely sinusoidal. We then eliminated the exponential decay term (i.e., the first term) from Eq. 1 and left the exponentially decaying sine term (i.e., the second term), namely,

$$y = b \cdot \exp[-(x-1)/\tau_s] \cdot \cos[\pi(x-1)] + 1. \quad (2)$$

We refitted Eq. 2 to these alternans PESP cases with $a < 0.02$.

The alternans PESP better fitted by Eq. 1 were grouped as pattern A. Those better fitted by Eq. 2 were grouped as pattern B. Although Eq. 2 did not yield τ_e for RF, we utilized τ_s to compare the sinusoidal decay component between patterns A and B.

Curve fitting by the method of least squares was performed by DeltaGraph (Delta Point, Monterey, CA) on a Power Macintosh computer. The coefficient of determination (=squared correlation coefficient, r^2) was obtained as a measure of the goodness of fit as in our previous studies [13, 15]. In statistics, r^2 quantitates the fraction of the variance of data attributable to the best-fit function; $1-r^2$ quantifies the fraction of the data's variance left unaccountable by the best-fit curve. More quantitatively, $r^2=0.990$, for example, means that as much as 99% of the data's variance could be accounted for by the best-fit function, leaving only 1% of the data variance unattributable to this function. Thus even $r^2=0.990$ means that the curve fitting is excellent. As for the reliability of the present curve fitting to patterns A and B PESP, refer to the precise examination we performed in our previous study [15].

Recirculation fraction (RF). We calculated RF from τ_e by $\text{RF} = \exp(-1/\tau_e)$ [4, 13, 15]. RF is a well-accepted concept of mass Ca^{2+} dynamics of the

myocardium to account for the monotonic decay of the PESP over several beats [1–8]. We previously proposed for the first time that RF is obtainable from τ_e (in Eq. 1) of the exponential decay component of the alternans PESP [13]. RF quantifies the fraction of the total Ca^{2+} handled in the E–C coupling that intracellularly recirculates mostly via the Ca^{2+} pump and Ca^{2+} release channel of the SR without being extruded transsarcolemmally [4, 13, 15]. $1 - \text{RF}$ quantifies the fraction of the total Ca^{2+} handled in the E–C coupling that is transsarcolemmally extruded primarily via the $\text{Na}^+/\text{Ca}^{2+}$ exchange (see below) and enters the cell mostly via the sarcolemmal Ca^{2+} channel [4, 13, 15].

On the basis of the exponential decay of PESP to the stable contractility level of regular beats, RF has been assumed to be constant during a series of the regular steady-state beats and during the PESP except for the extrasystolic beat (see below) [1–8]. We made a similar assumption on the RF calculated from the exponential decay component of the alternans PESP in the present study, as in the previous study [15].

Ca^{2+} handling. We now used the RF for the first time to divide the $\dot{V}\text{O}_2$ for total Ca^{2+} handling in the E–C coupling into two fractions, one for RF and the other for $1 - \text{RF}$. This was not possible in the ryanodine-treated hearts because of possible futile Ca^{2+} cycling via the SR made leaky to Ca^{2+} by nanomolar ryanodine [14, 15]. However, we assumed that futile Ca^{2+} cycling via the SR would be minimal, if at all, in the normal canine hearts in control and enhanced contractile states with intracoronary Ca^{2+} and epinephrine [16–18]. We considered this assumption reasonable because the O_2 cost of $E_{\max}$ (or slope of the Ca^{2+} handling $\dot{V}\text{O}_2$ – $E_{\max}$ relation) was independent of ordinary positive and negative inotropic agents in normal canine LVs [16–18]. Therefore, for the sake of simplicity, we assumed no futile Ca^{2+} cycling via the SR in the present analysis.

First, we chose average values for $\dot{V}\text{O}_2$ per beat of mechanically unloaded contractions with zero PVA, or PVA-independent $\dot{V}\text{O}_2$ [16, 17], as their representative values in the control, Ca^{2+} , and epinephrine runs from the $\dot{V}\text{O}_2$ data in the original study [18]. Second, we calculated $\dot{V}\text{O}_2$ for total Ca^{2+} handling by subtracting basal metabolic $\dot{V}\text{O}_2$ determined under KCl arrest from those PVA-independent $\dot{V}\text{O}_2$ values. These values represented Ca handling $\dot{V}\text{O}_2$ in the respective contractilities [17, 18]. Third, these representative Ca handling $\dot{V}\text{O}_2$ values were combined with average RF values newly obtained in the present study. To this end, we developed the following equation (see APPENDIX).

$$\text{Total } \text{Ca}^{2+} = 6 \times 10^7 \times (\text{Ca}^{2+} \text{ handling } \dot{V}\text{O}_2 / 22,400) / (1 - \text{RF}/2), \quad (3)$$

where the unit of total Ca^{2+} is micromol/beat per kg LV and that of Ca^{2+} handling $\dot{V}\text{O}_2$ is ml O_2 /beat per 100 g LV. Constant 6 derives from the nominal atomic P : O ratio of 3, which is equivalent to the molecular P : O_2 ratio of 6 in the ATP production consuming O_2 in the myocardium under normal aerobic conditions [17]. 10^7 converts Ca^{2+} in mol/100 g LV to micromol/kg LV. Constant 22,400 (ml O_2) is the gas volume of 1 mol of O_2 (STPD, namely, 0°C , 1 atm, and dry).

To derive the Ca^{2+} : ATP : $\dot{V}\text{O}_2$ stoichiometry values in the intracellular and transsarcolemmal Ca^{2+} handling routes, we made the following assumptions, which are identical with those made in our previous study [15]. Briefly, both myocardial Ca^{2+} and Na^+ must be in a state of homeostasis in each steady-state contraction [10, 11]. We therefore reasonably assumed that the total extruded Ca^{2+} , primarily via the $\text{Na}^+/\text{Ca}^{2+}$ exchange and secondarily via the sarcolemmal Ca^{2+} pump, was balanced with the total Ca^{2+} influx primarily via the sarcolemmal Ca^{2+} channel and secondarily, if any, via the $\text{Na}^+/\text{Ca}^{2+}$ exchange in each steady-state contraction [4, 9–11, 15]. Simultaneously, we assumed the released Ca^{2+} via the SR Ca^{2+} channel to be equal to the sequestered Ca^{2+} via the SR Ca^{2+} pump in each steady-state contraction [4, 10, 11]. Likewise, we assumed the net Na^+ influx via the $\text{Na}^+/\text{Ca}^{2+}$ exchange to be balanced with the Na^+ efflux mostly via the Na^+/K^+ pump in each steady-state contraction [10, 11, 15]. We also assumed the Na^+ influx via the sarcolemmal Na^+ channel for membrane excitation to be balanced with its efflux by the Na^+/K^+ pump in each steady-state contraction. If these intrabeat ionic balances did not exist in steady-state contractions, contractility of these beats would fluctuate, contradicting the definition of the steady-state contractions [10, 11, 15].

These Ca^{2+} and Na^+ handlings are known to be closely coupled with myocardial energetics in steady-state contractions [15, 17, 20, 21]. The SR Ca^{2+} pump ATPase normally has a 2Ca^{2+} : 1ATP stoichiometry [20, 22]. Since the Na^+ handling via the Na^+/K^+ pump ATPase for membrane excitation requires only 1% of total myocardial $\dot{V}\text{O}_2$, this $\dot{V}\text{O}_2$ for membrane excitation can reasonably be neglected in myocardial energetics [15, 17, 20]. Therefore most of the total Na^+ handling energy was assumed to be consumed by the Na^+/K^+ pump to extrude the Na^+ that entered in exchange with Ca^{2+} via the $\text{Na}^+/\text{Ca}^{2+}$ exchange [15, 17, 20].

Since the $\text{Na}^+/\text{Ca}^{2+}$ exchange has a 3Na^+ : Ca^{2+}

Fig. 1. Representative examples of simultaneous tracings of left ventricular isovolumic pressure and epicardial electrocardiogram during a series of regular beats (R), a single spontaneous extrasystole (ES) of a ventricular origin, and postextrasystolic potentiation beats (PES1-6) under intracoronary Ca^{2+} infusion at a dose of 0.06 mmol/min (A) and epinephrine infusion at a dose of 1.0 $\mu\text{g}/\text{min}$ (B).

stoichiometry and the Na^+/K^+ pump has a $3\text{Na}^+ : 1\text{ATP}$ stoichiometry [23], the overall $\text{Ca}^{2+} : \text{ATP}$ stoichiometry via the transsarcolemmal route is considered to be $1\text{Ca}^{2+} : 1\text{ATP}$ in steady state [15, 20]. Moreover, the sarcolemmal Ca^{2+} pump, which normally contributes secondarily to Ca^{2+} extrusion, also has a $1\text{Ca}^{2+} : 1\text{ATP}$ stoichiometry [10]. Therefore we could assume in the steady-state contractions used for VO_2 measurement [18] that the $1\text{Ca}^{2+} : 1\text{ATP}$ stoichiometry of the transsarcolemmal Ca^{2+} handling related to $1 - \text{RF}$ was only half as economical (or twice as wasteful) of the $2\text{Ca}^{2+} : 1\text{ATP}$ stoichiometry of the intracellular Ca^{2+} handling via the SR related to RF [15, 20]. Because of this twofold difference in energetics of Ca^{2+} handling, the shift of the Ca^{2+} handling from the intracellular (or internal) route to the transsarcolemmal (or external) route as reflected in the decreased RF decreases the economy (or increases the energy waste) of Ca^{2+} handling in the E-C coupling, as previously described [15]. We also assumed mitochondrial Ca^{2+} handling to be a negligible fraction of total Ca^{2+} handling in normal hearts [10, 11], as in our previous study [15]. After Eq. 3 was solved, total Ca^{2+} was divided into two parts, one for RF and the other for $1 - \text{RF}$.

Statistics. One-way analysis of variance (ANOVA) was used to compare the pooled mean τ_e , τ_s , and RF values of individual parameters among the three different contractilities: control, Ca^{2+} , and epinephrine. The F test was used to examine the significance of the variation of these data among the three inotropic conditions. When the F test resulted in $p < 0.05$, the least significant difference (LSD) was used to test the significance of the multiple compar-

isons [24]. $p < 0.05$ indicates a statistically significant difference. We used StatView v.4.5 (Abacus Concepts, Inc., Berkeley, CA) to perform these statistics.

The representative values for the total Ca^{2+} were calculated by combining the mean RF values in the three respective contractility runs with the mean values for the Ca^{2+} handling VO_2 obtained at the ends of these runs. Because of the limited numbers of PESP cases on the three contractile states, we unfortunately could not obtain RF as a function of increasing contractility in each inotropism. Therefore we pooled RF values in each inotropism and could perform no statistical tests to compare the resultant total Ca^{2+} values among control, Ca^{2+} , and epinephrine.

RESULTS

Figure 1 depicts representative tracings of LV isovolumic pressure and ECG in a PESP in the Ca^{2+} run (A) and in the epinephrine run (B). Both PESP cases obviously demonstrate transient alternans. The extrasystole (ES) was weaker than the regular beat (R); the first PES interval had a compensatory pause. The first PES beat (PES1) was stronger than R, the second (PES2) was weaker than R, and the third (PES3) was stronger than R. Thus the PESP decayed in alternans over several beats. These alternans PESP resemble those that we documented previously [12, 13, 15]. All other alternans PESP in any of the control, Ca^{2+} , and epinephrine runs resembled the curves in Fig. 1. We found no monotonically decaying PESP, which has long been considered to be representative in normal hearts [25].

Figure 2 depicts representative examples of the

Fig. 2. Representative cases of curve fitting to an alternans PESP obtained under control (A), intracoronary Ca^{2+} infusion at a dose of 0.02 mmol/min (B), and epinephrine at a dose of 0.5 $\mu\text{g}/\text{min}$ (C). All these PESPs belong to pattern A. The squared correlation coefficient as the goodness of the fitting was 0.999 in the three cases. The

transient alternans curve (solid) indicates $a \cdot \exp[-(x-1)/\tau_e] + b \cdot \exp[-(x-1)/\tau_s] \cdot \cos[\pi(x-1)] + 1$ with the best-fit parameters. PES1-6 corresponds to $x=1-6$. The dashed exponential curve indicates $a \cdot \exp[-(x-1)/\tau_e] + 1$ with the best-fit parameters. The dotted exponential curve indicates $b \cdot \exp[-(x-1)/\tau_s] + 1$ with the best-fit parameters.

best-fit curves of pattern A in control (A), Ca^{2+} (B), and epinephrine runs (C). The solid curves were best fitted to the data (closed circles). The two other types of curves, i.e., the exponential decay component curve (dashed) and the exponential term (dotted) of the unit-amplitude sinusoidal decay curve, were drawn by using the best-fit parameters of Eq. 1. All the other pattern A PESP cases could be decomposed into similar curves regardless of the inotropic conditions. As described in METHODS, pattern A PESPs had $a > 0.02$. The other PESPs with $a < 0.02$ were grouped into pattern B, which obviously did not have the exponential decay component and appeared to be decaying purely sinusoidally.

The goodness of the curve fitting was always excellent with virtual unity coefficients of determination (r^2): 0.999 ± 0.001 in control, 0.999 ± 0.002 under Ca^{2+} , and 0.999 ± 0.001 under epinephrine. These r^2 values indicate that as much as 99.9% on average of the variance of the PESP contractility changes was always accounted for by the fitted curve with only 0.1% residual variance unattributable to it. This indicates that the curve fitting was excellent for our purpose, as in our previous study [15]. The numbers of pattern A PESP cases were 47 in the control run, 34 in the Ca^{2+} run, and 34 in the epinephrine run. Those of pattern B PESP cases were 40 in the control run, 45 in the Ca^{2+} run, and 16 in the epinephrine run. As a whole, 216 PESP cases were analyzed in the 12 hearts.

Beat constant (τ_e) of the exponential decay component, which was obtainable only in pattern A, was significantly greater by 24–28% in the Ca^{2+} run than in both the control and epinephrine runs ($p < 0.008$ by LSD), as shown in Table 1. However, no significant

difference existed between control and epinephrine ($p > 0.7$).

The other beat constant (τ_s) values, which were obtainable in both patterns A and B, were 0.900 ± 0.219 beats in control, 0.868 ± 0.127 beats under Ca^{2+} , and 0.858 ± 0.195 beats under epinephrine in pattern A. They were 0.854 ± 0.305 beats in control, 0.799 ± 0.179 beats under Ca^{2+} , and 0.848 ± 0.220 beats under epinephrine in pattern B. These τ_s values were all comparable. ANOVA showed no significant differences of τ_s among these three different inotropic runs or between patterns A and B.

Magnitude constants a and b and their ratio a/b varied widely and showed no significant differences among the three different inotropic runs. Both a and b are known to be functions of the beat intervals of ES and PES1 [26]. Since these intervals changed spontaneously, widely but reciprocally under a constant atrial pacing [18], we kept a , b , and a/b out of our present interest.

The significantly increased τ_e under Ca^{2+} resulted in a significant ($p < 0.019$) but slight increase in RF, by 13–15% on average, from both control and epinephrine runs, as shown in Table 1. Because of the nonlinear relation between τ_e and RF according to $\text{RF} = \exp(-1/\tau_e)$, the percent change of RF became smaller than that of τ_e in their working ranges. There was no significant difference in RF between control and epinephrine ($p > 0.67$).

We then combined the representative RF values in the three different contractile states with the representative Ca^{2+} handling VO_2 values in the corresponding contractility runs. As their representative values, we chose their mean values in Table 1 (see METHODS).

Table 1. The total amount of Ca^{2+} handled in the excitation–contraction coupling, the fractional amounts of internally recirculating and transsarcolemmally extruded Ca^{2+} in control, and the enhanced contractile states with Ca^{2+} and epinephrine.

Mechanoenergetic and Ca^{2+} variables	Control ($N=47$)	Ca^{2+} ($N=34$)	Epinephrine ($N=34$)
$E_{\max}$ (mmHg/(ml/100 g LV))	4.7 ± 2.1	$11.3 \pm 3.2^*$	$10.8 \pm 3.9^*$
Ca^{2+} handling VO_2 (ml O_2 /beat/100 g LV)	0.011 ± 0.004	$0.029 \pm 0.005^*$	$0.028 \pm 0.004^*$
τ_e (beats)	1.85 ± 0.72	$2.30 \pm 0.75^*$	$1.79 \pm 0.69^\#$
RF (dimensionless)	0.55 ± 0.14	$0.62 \pm 0.11^*$	$0.54 \pm 0.13^\#$
Total Ca^{2+} ($\mu\text{mol}/\text{beat}/\text{kg LV}$)	40	111	102
Internally recirculating Ca^{2+} ($\mu\text{mol}/\text{beat}/\text{kg LV}$)	22	69	55
Transsarcolemmally extruded Ca^{2+} ($\mu\text{mol}/\text{beat}/\text{kg LV}$)	18	42	47
$E_{\max}/\text{Total } \text{Ca}^{2+}$ (mmHg/(ml/100 g LV))/($\mu\text{mol}/\text{beat}/\text{kg}$)	0.12	0.10	0.11

Mean $\pm$ SD for the top 4 rows. Representative values for the bottom 4 rows. N , number of postextrasystolic transient alternans cases used to obtain τ_e and RF; $E_{\max}$, an index of cardiac chamber contractility identified as the end-systolic maximum elastance of the chamber (left ventricle in the present study) or the slope of the end-systolic pressure–volume relation line; LV, left ventricle. Ca^{2+} handling VO_2 , left ventricular O_2 consumption (VO_2) for total Ca^{2+} handling by subtracting basal metabolic VO_2 under KCl arrest from the VO_2 of mechanically unloaded contractions; τ_e , decay time constant in the number of beats obtained from the exponential decay component of the postextrasystolic transient alternans; RF, intracellular Ca^{2+} recirculation fraction calculated from τ_e ; Total Ca^{2+} , total amount of Ca^{2+} handled in the excitation-coupling in a steady-state beat. * $p < 0.05$ between control and Ca^{2+} and between control and epinephrine by least significant difference. # $p < 0.05$ between Ca^{2+} and epinephrine by least significant difference.

Using Eqs. 3–5, we obtained total Ca^{2+} values as shown in Table 1. We found that all the total Ca^{2+} , intracellularly recirculating Ca^{2+} , and transsarcolemmally extruded Ca^{2+} increased 2–3 times in proportion to both the Ca^{2+} handling VO_2 and $E_{\max}$ values, which also increased approximately 2.5 times with either Ca^{2+} or epinephrine administration. The ratio of total Ca^{2+} to $E_{\max}$ was comparable among control, Ca^{2+} , and epinephrine runs, as shown in the bottom row of Table 1.

DISCUSSION

We are the first to succeed in obtaining RF (intracellular Ca^{2+} recirculation fraction) by using the transient alternans PESP [12, 13, 15], although other investigators have obtained RF by using the monotonic PESP [1–8]. We previously showed that RF values obtained from these two types of PESP were comparable not only in canine normal LVs [13], but also in the canine failing LVs produced by intracoronary infusion of ryanodine at nanomolar (<40 nmol/l coronary blood, but not micromolar) concentrations [15]. Although we wanted to obtain RF from the two types of PESP in the present study as we did in the previous study [13, 15], we could not do so simply because PESP always decayed in alternans in the atrially paced hearts at a constant rate [18]. We had found that PESP could decay monotonically only when the first PES interval was noncompensatory [12, 13]. The first PES interval always had a compensatory pause under a

constant atrial pacing [15]. In the present study we therefore gave up on obtaining RF from the monotonic PESP by using the conventional method.

The τ_e , τ_s , and RF values in control contractile state in the present study are comparable to those ($\tau_e = 1.89$ beats, $\tau_s = 0.90$ beats, and $\text{RF} = 0.55$ on average) in the control contractile state before ryanodine treatment in our previous study [15]. This means high reproducibility of these variables in the control contractile state of the LV in the canine excised cross-circulated heart preparation that we have established as a standard heart preparation and that we have preferably long been using [12–19].

In contrast to the significant 20% decrease in RF in the ryanodine-treated failing hearts [15], RF increased by 13% with intracoronary Ca^{2+} , but not with epinephrine. According to the definition of RF, we interpreted that the RF increased by Ca^{2+} indicates that a slightly greater fraction of the total Ca^{2+} recruited in the E–C coupling was intracellularly handled via the SR in an enhanced contractility with Ca^{2+} , but not with epinephrine. This means energetically that a greater fraction of the total Ca^{2+} was handled more economically in an enhanced contractility with Ca^{2+} , according to the twofold difference of the $\text{Ca}^{2+} : \text{ATP}$ stoichiometry between the internal and external Ca^{2+} handling routes [15, 20] (see METHODS).

These increased τ_e and RF with Ca^{2+} in the canine heart are consistent with the rat heart data obtained by the conventional method [6], although no such result had been reported in canine hearts. No changes in τ_e

and RF with epinephrine have been reported in canine hearts [7] consistent with our present result, although epinephrine increased τ_e and RF in guinea pig hearts [7] and isoproterenol decreased them in rat heart muscles [6]. Thus changes in τ_e and RF with Ca^{2+} and beta agonists seem to be dependent not only on the inotropic agents, but also on the animal species. Therefore tacit assumption of a constant RF (e.g., 0.7) without its measurement may not always be appropriate [20].

Our speculation follows on the mechanisms of the different responses of τ_e and RF to Ca^{2+} and epinephrine that enhanced $E_{\max}$ 2–3 times. Beta agonists accelerate both Ca^{2+} transient and force curves in contrast to Ca^{2+} administration [27]. These accelerations seem attributable primarily to the phosphorylation of the proteins, such as the sarcolemmal and SR Ca^{2+} channels, troponin I, and phospholamban, that are directly related to the internal and external Ca^{2+} handlings [22, 27]. The increased total Ca^{2+} or free Ca^{2+} may have secondarily affected these Ca^{2+} handlings dose-dependently [23, 28]. Therefore no significant change in RF with epinephrine, despite the increased total Ca^{2+} , suggests that both internal and external Ca^{2+} handlings are equally accelerated. In contrast, Ca^{2+} administration is known to affect the Ca^{2+} handling processes dose-dependently without the phosphorylation [23, 28]. The slightly increased RF under Ca^{2+} suggests that the internal Ca^{2+} handling was slightly more facilitated than the external Ca^{2+} handling by the increased Ca^{2+} .

Apart from τ_e , we would consider τ_s to reflect primarily the delay of Ca^{2+} releasability from the SR after Ca^{2+} uptake by the SR [13, 15, 29]. This delay seems to mean a time delay of Ca^{2+} release from the SR after Ca^{2+} uptake [29] and seems to cause the common time constant of both extrasystolic restitution and PESP curves as a function of the extrasystolic coupling interval [29]. In fact, transient mechanical alternans has been simulated with a Ca^{2+} delay within the SR [30, 31].

We could interpret the unchanged τ_s with Ca^{2+} and epinephrine to indicate that the alternans PESP decay consists of at least two different Ca^{2+} handling mechanisms; one is dependent on inotropism, and the other is independent of it. Interestingly, even ryanodine did not affect τ_s in our previous study [15]. This suggests that τ_s is not related to the ryanodine-sensitive Ca^{2+} release channel of the SR. We could not observe electrical alternans in the monophasic action potential of the LV epicardium during an alternans PESP (Shimizu, unpublished observation). This suggests that it is unlikely that the alternans PESP decay was caused by

alternating Ca^{2+} influx.

No previous methods [20, 21] have enabled assessment of the total amount of Ca^{2+} handled in each E–C coupling in a beating whole LV. Although Alpert *et al.* have obtained total Ca^{2+} for an E–C coupling by combining tension-independent heat and an assumed RF in a contracting myocardium preparation [20], this method as such is not directly applicable to a beating whole heart. Although our present integrative method is indirect, the obtained total Ca^{2+} values fall within or near the range of the total Ca^{2+} handling data measured at cellular and tissue levels (20–100 $\mu\text{mol/kg}$ wet myocardium) [9–11, 20, 21, 32].

The limitations of the present study are discussed. First, we assumed no futile Ca^{2+} cycling via the SR in the present study. This assumption may be reasonable in the normal hearts under increased Ca^{2+} and epinephrine in the present study in contrast to the ryanodine-treated hearts in the previous study [15]. The comparable values for the total $\text{Ca}^{2+}/E_{\max}$ ratio (Table 1) among control, Ca^{2+} , and epinephrine suggest that neither Ca^{2+} nor epinephrine has augmented futile Ca^{2+} cycling from control. This is consistent with our previous observation that the Ca^{2+} handling VO_2 – $E_{\max}$ relations were superimposable on each other whether $E_{\max}$ was enhanced with Ca^{2+} or beta agonists or depressed with Ca^{2+} blockers or beta antagonists in normal hearts [16–18]. Unfortunately, no method has been developed to assess the magnitude of futile Ca^{2+} cycling at an LV level.

Second, we used the nominal P : O ratio of 3. However, the P : O ratio measured in the intact myocardium may be 5–30% smaller than 3 when there is futile ATP use [33]. Equation 3 indicates that total Ca^{2+} would then be 5–30% smaller than those listed in Table 1 in proportion to the decreased P : O ratio. Third, we assumed that unloaded VO_2 minus basal metabolism is entirely Ca^{2+} handling VO_2 [14–18]. Although there is an opinion that residual cross-bridge cycling consumes a considerable amount of VO_2 within the Ca^{2+} handling VO_2 [34], we favor our assumption on the basis of our findings directly obtained in the excised cross-circulated canine LV [35, 36].

To conclude, the present results indicate that intracellular Ca^{2+} recirculation fraction via the SR was slightly augmented by Ca^{2+} , but not by epinephrine, despite the 2–3 times enhanced $E_{\max}$ by either agent. By combining these recirculation fraction data with Ca^{2+} handling VO_2 data, for the first time we were able to obtain reasonable values for the total amount of Ca^{2+} handled in each cardiac cycle in control and in enhanced contractile states. The present heart-level integrative approach seems to better the understanding

of the Ca^{2+} mass dynamics responsible for the altered mechanoenergetics under different inotropic interventions.

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APPENDIX

We obtained Eq. 3 (see METHODS) as follows. Given total Ca^{2+} ($\mu\text{mol/kg}$) and RF, then

the amount of Ca^{2+} ($\mu\text{mol/kg}$) that recirculates internally in each steady-state beat
 $= \text{RF} \times \text{total } \text{Ca}^{2+}$,

and

the amount of Ca^{2+} ($\mu\text{mol/kg}$) that is extruded

transsarcolemmally in the same steady-state beat
 $= (1 - \text{RF}) \times \text{total } \text{Ca}^{2+}$.

The division of these two fractions of total Ca^{2+} by the respective $\text{Ca}^{2+} : \text{ATP}$ stoichiometries of the internal and external Ca^{2+} handling routes described below Eq. 3 yields

the amount of ATP ($\mu\text{mol/kg}$) hydrolyzed for handling $\text{RF} \times \text{total } \text{Ca}^{2+} = \text{RF} \times \text{total } \text{Ca}^{2+} / 2$,

and

the amount of ATP ($\mu\text{mol/kg}$) hydrolyzed for handling $(1 - \text{RF}) \times \text{total } \text{Ca}^{2+}$
 $= (1 - \text{RF}) \times \text{total } \text{Ca}^{2+}$.

The addition of these two amounts of ATP yields

the total amount of ATP ($\mu\text{mol/kg}$) for total Ca^{2+} handling
 $= \text{RF} \times \text{total } \text{Ca}^{2+} / 2 + (1 - \text{RF}) \times \text{total } \text{Ca}^{2+}$
 $= (1 - \text{RF}/2) \times \text{total } \text{Ca}^{2+}$.

The division of this total amount of ATP by the $\text{P} : \text{O}_2$ ratio of 6 (= $\text{P} : \text{O}$ ratio of 3) described below Eq. 3 yields

VO_2 for total Ca^{2+} handling or Ca^{2+} handling VO_2
 $(\mu\text{mol/kg}) = (1 - \text{RF}/2) \times \text{total } \text{Ca}^{2+} / 6$.

The multiplication of this VO_2 in $\mu\text{mol/kg}$ by 10^{-6} and 22,400 (ml O_2 /mol) yields

VO_2 for total Ca^{2+} handling or Ca^{2+} handling VO_2 (ml O_2 /kg)
 $= 10^{-6} \times 22,400 \times (1 - \text{RF}/2) \times \text{total } \text{Ca}^{2+} / 6$.

A further division of this VO_2 by 10 yields

Ca^{2+} handling VO_2 (ml O_2 /100 g)
 $= 10^{-7} \times 22,400 \times (1 - \text{RF}/2) \times \text{total } \text{Ca}^{2+} / 6$.

The solution of this equation for total Ca^{2+} handled in each beat yields

total Ca^{2+} ($\mu\text{mol/kg}$) $= 6 \times 10^7 \times$
 $(\text{Ca}^{2+} \text{ handling } \text{VO}_2 / 22,400) / (1 - \text{RF}/2)$,

where the unit of Ca^{2+} handling VO_2 is ml O_2 /100 g.

This is Eq. 3.