Load Independence of Temperature-Dependent Ca^{2+} Recirculation Fraction in Canine Heart

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Abstract: Intramyocardial Ca^{2+} recirculation fraction (RF) critically determines the economy of excitation-contraction coupling. RF is obtainable from the exponential decay of the postextrasystolic potentiation of left ventricular (LV) contractility. We have shown that RF remains unchanged despite increasing LV volume (LVV) at normothermia, but decreases with increasing temperature at a constant LVV. However, it remains unknown whether the temperature-dependent RF was not due to the simultaneously changed peak LV pressure (LVP) at a constant LVV. We hypothesized that this temperature-dependent RF would be independent of the simultaneous change in LVP. We used nine excised, cross-circulated canine hearts and allowed their LVs to contract isovolumically. During stable regular beats at 500 msec intervals, we inserted an extrasystolic beat at 360

msec interval followed by the postextrasystolic beats (PESs) at 500 msec intervals. We equalized the temperature-dependent peak LVPs of the regular beats at 36°C and 38°C to the peak LVP level of the stable regular beat at 33°C by adjusting LVV. We fitted the same equation: $nE_{\max} = a \cdot \exp[-(i-1)/\tau_e] + b \cdot \exp[-(i-1)/\tau_s] \cos[\pi(i-1)] + 1$, used before to the normalized $E_{\max}$ (maximum elastance) values of PESi ($i = 1-6$) relative to the regular beat $E_{\max}$. RF given by $\exp(-1/\tau_e)$ decreased by 19% to 38°C from 33°C. The temperature coefficient (Q_{10}) of $1/\text{RF}$ was significantly greater than 1.3. The present results indicated a similar temperature dependence of RF and its Q_{10} to those we observed previously without equalizing peak LVP. Thus, the temperature-dependent RF is independent of ventricular loading conditions. [The Japanese Journal of Physiology 54: 319–329, 2004]

Key words: alternans, excitation-contraction coupling, calcium handling, sarcoplasmic reticulum, Q_{10} .

The recirculation fraction (RF) of Ca^{2+} within myocardium is defined as the fraction of Ca^{2+} that recirculates via sarcoplasmic reticulum (SR) in the total Ca^{2+} handled (i.e., released and removed) in excitation-contraction (E-C) coupling [1, 2]. The other fraction of Ca^{2+} is transsarcolemmally extruded primarily by the sarcolemmal (SL) $\text{Na}^+/\text{Ca}^{2+}$ exchange [3]. The internal Ca^{2+} handling via SR is twice as economical as the transsarcolemmal Ca^{2+} extrusion since the Ca^{2+} : ATP stoichiometry is nominally 2:1 for the SR Ca^{2+} pump versus 1:1 for the SL $\text{Na}^+/\text{Ca}^{2+}$

exchange coupled with the Na^+/K^+ pump. Therefore, RF is an essential parameter in cardiac mechanoeconomics since it critically determines the energetic economy of E-C coupling [4–6]. On this basis, we have successfully calculated the total amount of Ca^{2+} handling by combining RF which is obtainable from the exponential decay of the postextrasystolic potentiation of left ventricular (LV) contractility with O_2 consumption of canine hearts under various physiological and pathological conditions [7–12].

We have recently shown that RF remains

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unchanged despite increasing LV volume (LVV) at normothermia [13], but it decreases with increasing temperature from 33°C to 38°C at a constant LVV [14]. However, increasing temperature simultaneously decreased ventricular contractility and thus peak isovolumic LV pressure (LVP) at a constant LVV because of the negative inotropism of warming [15–22]. Therefore, it remains unknown whether the temperature-dependently decreased LVP at a constant LVV affected the temperature-dependent RF. Based on our previous results [13, 14], we hypothesized that the temperature-dependent RF would primarily be due to the temperature effect per se rather than the simultaneously changed peak LVP.

In the present study, we tested this hypothesis experimentally, using the LV of the excised, cross-circulated canine heart preparation of our expertise. We obtained RF at 33°C, 36°C, and 38°C while eliminating the temperature-dependent changes in peak isovolumic LVP of the regular beats by adjusting LVV. The present results yielded essentially the same temperature dependence of RF as the previous study [14], i.e., whether or not we changed LVV to compensate for the temperature-dependent LVP. The present results validated our present hypothesis. Thus, the temperature-dependent RF is independent of the simultaneous change in LVP because of the negative inotropism of warming.

MATERIALS AND METHODS

Surgical preparation. We used the excised, cross-circulated canine heart preparation of the same standard type that we have been consistently using in cardiac mechanoenergetic studies [6, 20, 23]. This investigation conforms to the guiding principles for the care and use of animals in the field of physiological sciences of the Physiological Society of Japan. The details of the surgical preparations were described elsewhere [4–14, 17, 24–26].

Briefly, in each of nine experiments, we anesthetized a metabolic support dog (22.3 ± 5.8 kg, mean $\pm$ SD) and a heart donor dog (12.8 ± 2.9 kg), both adult mongrels, with pentobarbital sodium (25 mg/kg, i.v.) and fentanyl (0.1–0.2 mg/h, i.v.) after premedication with ketamine hydrochloride (25 mg/kg, i.m.). They were intubated, air-ventilated, and heparinized (15000 units per support dog and 10000 units per donor dog, i.v.).

We cannulated the bilateral common carotid arteries and the unilateral external jugular vein of the support dog and connected them to the arterial and venous cross-circulation tubes, respectively. The chest of the

donor dog was opened midsternally. The arterial and venous cross-circulation tubes from the support dog were cannulated into the left subclavian artery and the right ventricle (RV) via the right atrial appendage of the donor dog, respectively. All systemic and pulmonary vascular connections to the heart were ligated. The metabolically supported beating heart was excised from the chest of the donor dog under continuous cross circulation from the support dog. The coronary perfusion of the excised donor heart was never interrupted during the surgical preparation.

We opened the left atrium of the donor heart and cut all the LV chordae tendineae. Complete atrioventricular block was made by chemical (0.2–0.5 ml injection of 36% formaldehyde solution) or electrical (direct current of 20–30 J) ablation of the His bundle. A bipolar pacing electrode was placed on the upper portion of the ventricular septal endocardium via the left atrium for para-Hisian pacing.

We fitted a thin flabby latex balloon with an unstretched volume of ~50 ml mounted on a rigid connector into the LV. The connector was secured at the mitral annulus. The balloon was connected to our custom-made volume servo pump (AR-Brown, Tokyo, Japan). Both the balloon and the water housing of the servo pump were primed with water. The servo pump enabled us to measure LVV accurately and to control it precisely. LVP was measured with a miniature pressure gauge (model P-7, Konigsberg Instruments, Pasadena, CA, USA) placed inside the apical end of the balloon.

We measured LV temperature of the excised donor heart with a thermistor placed between the endocardium and the LV balloon. It was maintained at 33°C, 36°C, and 38°C by cooling and warming the arterial cross-circulation tube with a thermostatic bath. We used this temperature range because sustained alternans tended to occur after a premature beat below 33°C, whereas spontaneous premature beats tended to occur frequently above 38°C in this type of heart preparation [14].

We recorded an LV epicardial electrocardiogram (ECG) with a pair of screw-in electrodes to trigger data acquisition. All LVP, LVV, and ECG signals were digitized at 2-msec intervals and processed on a LabVIEW (National Instruments, Austin, TX, USA)-installed computer.

The systemic arterial blood pressure of the support dog served as the coronary perfusion pressure of the excised donor heart. We successfully prevented the tendency to hypotension and maintained mean systemic arterial blood pressure of the support dog around 90 mmHg by electrically stimulating Neiguan (PC-

6) acupoints in the bilateral forearms, as usual in our laboratory [27]. Briefly, we inserted one stainless steel needle vertically into the acupoint 3 cm above the transverse crease of each wrist between the tendons of the long palmar muscle and the radial flexor muscle. The stimulation was 5 V-40 Hz biphasic pulses with 5-msec width. Systemic arterial blood pressure was maintained stable in each experiment by transfusing whole blood reserved from the donor dog or 6% hydroxyethylated starch solution, or by infusing methoxamine (5–30 mg/h), as needed. Arterial pH, PO₂, and PCO₂ of the support dog were repeatedly measured with a blood gas analyzer and maintained within their physiological ranges with supplemental O₂ and intravenous NaHCO₃, or by adjusting the ventilator setting.

The LV, including the septum, weighed 78.2 ± 22.5 g and the RV 32.3 ± 11.6 g after each experiment.

Pacing protocol. We used a fixed protocol of LV pacing. It consisted of a single extrasystolic beat (ES) inserted with an extrasystolic interval (ESI) of 360 msec after 10 or more regular beat intervals (RIs) of 500 msec (120 beats/min). The first postextrasystolic beat interval (PESI1) was 500 msec with no compensatory pause. The 10 or more subsequent PESIs were also 500 msec. We have already found that even the postextrasystolic potentiation (PESP) decay after no compensatory pause in PESI1 contained an alternans decay component, though smaller than after a compensatory pause [25].

Equalized peak isovolumic LVP. We equalized peak isovolumic LVPs of the regular beats at 36°C and 38°C to that at 33°C by appropriately adjusting LVV with the servo pump to compensate for the negative inotropism of warming [15–22]. This is the major difference of the experimental conditions in the present study from those in the previous one [14].

Normalized contractility. We used E_{max} (maximum elastance, or end-systolic pressure-volume ratio) as a reasonably reliable, load-independent index of ventricular contractility [6, 20, 23] to evaluate the beat-to-beat changes in LV contractility during each PESP decay. In the isovolumic contraction, end systole corresponds to the peak LVP. We calculated E_{max} values (mmHg/ml) of the 1st–6th postextrasystolic beats (PES1–6) as the ratio of peak isovolumic LVP to LVV minus V₀ that we identified as the LVV at which peak LVP was zero. We normalized these E_{max} values of PES1–6 relative to the E_{max} of the preceding three regular beats (R3–1) and designated them as nE_{max} (dimensionless).

Curve fitting. We examined whether the

obtained nE_{max} values of PES1–6 during each PESP decay at any LV temperatures and volumes could be fitted by the following equation.

$$\text{nE}_{\text{max}} = a \cdot \exp[-(i-1)/\tau_e] + b \cdot \exp[-(i-1)/\tau_s] \cos[\pi(i-1)] + 1 \quad (1)$$

where nE_{max} is the normalized contractility of PES_i ($i = 1-6$) relative to the regular beat E_{max} (Fig. 1, right). We had proposed this equation [4] and have been using it in our previous studies [5–14]. Coefficient a is the amplitude constant (dimensionless) of the monoexponential decay term (first term). Coefficient b is the amplitude constant (dimensionless) of the other exponential term multiplying the sinusoidal decay term (second term). Denominators τ_e and τ_s are the beat constants of the first and second exponential terms, respectively. Their unit is the number of beats but not time such as milliseconds. Subscripts e and s refer to exponential and sinusoidal. Although we used the cosine function in Eq. 1, the term could be any oscillatory or alternating function as long as it fits the discrete PES_i data points [28].

We performed the curve fitting of Eq. 1 to the observed PESP decay at 33°C, 36°C, and 38°C by the least-squares method. We obtained a , b , τ_e , and τ_s from the best-fitted Eq. 1. The goodness of the curve fitting was evaluated by correlation coefficient (r).

Ca²⁺ handling model. The conventional monotonic or exponential PESP decay has been accounted for by the beat-by-beat decreasing Ca²⁺ release in E-C coupling from its once transiently augmented level in PES1 toward the steady-state level of the regular beats [1–3, 29–34]. The most potentiated PES1 is considered to be due to both the increased transsarcolemmal Ca²⁺ influx and the increased Ca²⁺ released from SR because of the disturbed Ca²⁺ homeostasis [1–3]. The gradual monotonic decay of the potentiated contractility over PES2–6 is considered to be due to the transsarcolemmal Ca²⁺ extrusion exceeding the preceding influx and then the gradually decreasing Ca²⁺ sequestered by and released from SR over these beats. Stable regular beats are considered to be restored as a result of these Ca²⁺ homeostatic mechanisms.

Figure 1 illustrates the myocardial Ca²⁺ handling model that we had proposed by modifying the conventional model [1] to account for the PESP transient alternans decay. Intramyocardial Ca²⁺ recirculation with gradual Ca²⁺ extrusion over the PESP decay could account for the monoexponential component (monotonic component) decaying to the homeostatic Ca²⁺ level [1, 28]. The transient alternation of Ca²⁺ release from SR during the PESP decay could account

Fig. 1. Schematic diagram of our myocardial Ca^{2+} handling model. E_{max} : contractility index, maximum elastance, or end-systolic pressure-volume ratio. SM: sarcomere. SR: sarcoplasmic reticulum. SL: sarcolemma. RF: recirculation fraction of Ca^{2+} within myocardial cells, namely, the fraction being sequestered via the SR Ca^{2+} pump (ATPase) and released via the SR Ca^{2+} channel (upward arrow through SR) in the total Ca^{2+} handled in excitation-contraction coupling (thick downward arrow between SR and SM). $\text{XF} = 1 - \text{RF}$: extrusion fraction of Ca^{2+} removed predominantly via the SL $\text{Na}^{+}/\text{Ca}^{2+}$ exchange and slightly via the SL Ca^{2+} pump (ATPase) and entering via the SL Ca^{2+} channel (transsarcolemmal outward and inward arrows, respectively). The top right graph depicts a best-fitted Eq. 1 curve (see MATERIALS AND METHODS) characterizing the postextrasystolic potentiation alternans decay. The middle right graph depicts the exponentially decaying oscillatory component (second term) of the best-fitted Eq. 1. The bottom right graph depicts the exponentially decaying monotonic component (first term) of Eq. 1. In these graphs, a : amplitude constant of the first term of Eq. 1, τ_e : beat constant of the first term of Eq. 1, b : amplitude constant of the second term of Eq. 1, and τ_s : beat constant of the second term of Eq. 1. $n\text{E}_{\text{max}}$: normalized E_{max} values of the 1st–6th postextrasystolic beats (PES1–6) relative to three preceding regular beats. PESi: i -th postextrasystolic beat ($i = 1$ –6).

for the exponentially decaying sinusoidal decay component (oscillatory component) [28–30]. This model indicates that the monoexponential decay term of Eq. 1 is related to the RF and the exponentially decaying sinusoidal term is related to the Ca^{2+} handling within the SR. We have proposed this Ca^{2+} handling model that enables us to assess the mass dynamics of total Ca^{2+} handling from cardiac mechanoenergetics at the organ level [4–14, 24–26].

Recirculation fraction (RF). We calculated RF (dimensionless) given by $\exp(-1/\tau_e)$ from beat constant τ_e in the first term of Eq. 1 best fitted to the alternans decay over PES1–6 as usual. We considered this RF equivalent to the RF obtained by $\exp(-1/\tau)$ from beat constant τ of the conventional monotonic decay [1, 2, 30]. Either RF quantifies the fraction of Ca^{2+} that recirculates intracellularly via SR without being extruded transsarcolemmally in the total amount of Ca^{2+} handled in E-C coupling (Fig. 1). We normalized the RF values at 36°C and 38°C with respect to that at 33°C in each heart.

The exponential PESP decay theoretically indi-

cates that the PESi ($i = 1$ –6 or so) has the same constant RF as the regular beats at the same RI and PESi except for the ESI [1, 2, 30], as we have explained in detail [14]. An ESI seems to perturb myocardial Ca^{2+} homeostasis and then initiate the alternans decay by an inherent feedback system without affecting RF [29, 35]. We reasonably assumed the same RF concept to hold in the monotonic component of the PESP alternans decay in our previous studies [4–14, 24–26].

Temperature coefficient (Q_{10}). The temperature coefficient Q_{10} (dimensionless) indicates temperature sensitivity. In general, $Q_{10} \geq 2$ of a variable evidently suggests that it is related directly or indirectly to a chemical process (such as pump, channel, and exchange) whereas $1.5 \geq Q_{10} \geq 1$ may be related to a physical process (such as diffusion).

By definition, Q_{10} is equal to the ratio of a variable value M_{10} at temperature $(t + 10)^\circ\text{C}$ over its value M_0 at $t^\circ\text{C}$, i.e., $Q_{10} = M_{10}/M_0$. When M_5 is obtained at temperature $(t + 5)^\circ\text{C}$ instead of M_{10} , the above equation becomes $Q_{10} = (M_5/M_0)^2$ where exponent 2 derives from 10/5. Therefore, we obtained Q_{10} as the

square of its change per 5°C rise from 33°C to 38°C.

Statistics. In each PESP decay, we obtained mean $\pm$ standard deviation (SD) of peak LVP and LVV; the measured regular beat Emax; the best-fitted a , b , τ_e , and τ_s ; the calculated RF; the normalized RF; and the correlation coefficient (r). We used the one-way repeated measures analysis of variance (ANOVA) to compare the paired data of each of peak LVP and LVV; the measured regular beat Emax; the best-fitted a , b , τ_e , and τ_s ; the calculated RF; the normalized RF in the individual hearts among 33°C, 36°C, and 38°C. When the ANOVA was significant ($P < 0.05$), we performed Bonferroni's multiple t -test to compare each of the paired variables between the three temperatures. Here, we used a P value of 0.0167 ($= 0.05/3$) after Bonferroni's correction to indicate the statistical significance of every paired comparison.

Since all the individual and mean Q_{10} values of the best-fitted a , b , τ_e and τ_s , RF, and regular beat Emax were smaller than unity (see RESULTS), we obtained Q_{10} values of their reciprocals (i.e., $1/a$, $1/b$, $1/\tau_e$, $1/\tau_s$, $1/\text{RF}$, and $1/\text{Emax}$). $1/\tau_e$ and $1/\tau_s$ are rate constants. Then, we statistically obtained Q_{10} for each of these 6 parameters that started to have a statistically significant difference from the mean Q_{10} values of their reciprocals of the respective parameters in the nine hearts by means of a Student's t -test with $P = 0.05$.

We used StatView 4.5 (SAS Institute Inc., Cary, NC, USA) to perform these statistics as in the previous study [14].

RESULTS

Postextrasystolic potentiation (PESP) decay pattern.

Figure 2 depicts a representative set of the PESP decays at 33°C (Fig. 2A), 36°C (Fig. 2B), and 38°C (Fig. 2C) in one excised, cross-circulated canine heart. Peak amplitudes of LVP of all regular beats were maintained at almost 70 mmHg despite the increasing LV temperature. Peak LVPs of the regular beats had to be set relatively low to prevent peak LVPs of PES1 and other spontaneous and sporadic arrhythmias from exceeding 200 mmHg at 33°C. Peak LVP exceeding 200 mmHg tended to tear the mitral annulus by the suture and cause herniation of the balloon from the LV chamber.

In Fig. 2, peak LVP of PES1 was the greatest of all PES1-6 and PES2 was weaker than PES1 at all temperatures. However, PES3 was stronger than PES2 at 33°C but nearly equal to PES2 at 36°C and weaker than PES2 at 38°C. PES4 was weaker than PES3 at all temperatures. PES5 was nearly equal to PES4 at 33°C but weaker than PES4 at 36°C and 38°C. PES6 was slightly weaker than PES5 at all temperatures. In this way, PES always decayed more or less in transient alternans over PES1-6 and gradually returned to the regular beat level. The transient alternans was the most obvious at 33°C.

The incomplete relaxation during the regular beats and PES1-6 in Fig. 2A disappeared as temperature increased, as shown by the lack of a constant diastolic pressure period before the onset of next beat in Fig. 2B and C. Simultaneously, the LVP curve grad-

Fig. 2. Representative set of left ventricular pressure (LVP) tracings of the three steady-state regular beats (R3, R2, and R1) before the extrasystole (ES), ES per se, and the 1st–6th postextrasystolic beats (PES1–6) decaying in transient alternans at 33°C (A), 36°C (B), and 38°C (C) in an excised, cross-circulated canine heart. Regular beat (R) intervals (RIs) were fixed at 500 msec. Extrasystolic beat interval (ESI) was fixed at 360 msec. Postextrasystolic beat intervals (PESIs) were restored to 500 msec. Peak LVPs of all regular beats were kept constant by increasing LV volume with increasing temperature despite the negative inotropism of warming.

ually narrowed from Fig. 2A to C.

We observed in all the other hearts that PES1-6 at 33°C, 36°C, and 38°C decayed almost like those in Fig. 2A–C. On average, peak LVPs of R3-1 were 67.0 ± 11.8 , 66.5 ± 15.6 , and 56.2 ± 14.6 mmHg at 33°C, 36°C, and 38°C in the nine hearts, respectively. These LVPs had no statistically significant difference among the three temperatures. LVVs for these LVPs were 12.3 ± 2.6 , 13.9 ± 3.3 and 16.3 ± 3.4 ml, respectively. LVV at 38°C was significantly ($P < 0.0167$) greater than that at 33°C. Regular beat Emax values were 12.9 ± 7.5 , 9.8 ± 4.6 , and 6.2 ± 2.8 mmHg/ml, respectively. Emax at 38°C was significantly ($P < 0.0167$) smaller than that at 33°C.

Curve fitting. Figure 3 depicts a representative set of the alternating solid curves best fitted by Eq. 1 to nEmax of PES1-6 (closed circles) at 33°C (Fig. 3A), 36°C (Fig. 3B), and 38°C (Fig. 3C) in the same PESP decays already shown in Fig. 2A–C, respectively. Figure 3 also shows their monoexponential decay components (first term of Eq. 1, dashed curves) and their exponentially decaying sinusoidal components (second term of Eq. 1, dotted curves). Note that the sinusoidal curves are meaningful only at integer i values corresponding to PES i ($i = 1-6$) and meaningless at other non-integer i values [28].

Thus, as LV temperature increased from Fig. 3A

to C, the amplitudes of the PESP decay as well as the monotonic and oscillatory components decreased. Both their amplitude constants a and b and beat constants τ_e and τ_s decreased simultaneously as shown in the insets of Fig. 3A–C. These curves in Fig. 3A–C evidently indicated that PES1-6 decayed in transient alternans. Although the PESP decay at 38°C may look monotonic, it was not purely monoexponential, as the positive b value evidently indicates. Thus, the PESP decay always had both a monotonic and an oscillatory component at the three temperatures.

All the other hearts showed essentially the same results. We did not at all encounter a purely exponential PESP decay under the para-Hisian LV pacing protocol as used in this and previous studies [13, 14, 25].

The correlation coefficients (r) obtained by fitting Eq. 1 to the PESP decay were 0.9998 ± 0.0001 , 0.9999 ± 0.0001 , and 0.9999 ± 0.0001 at 33°C, 36°C, and 38°C in the nine hearts, respectively. All these r values were almost 1, as in all our previous studies [7–14, 25].

Amplitude constants a and b . Figure 4A and B plot amplitude constants a and b of Eq. 1 best fitted to the PESP decays at 33°C, 36°C, and 38°C in the nine hearts. The absolute magnitudes of both a and b decreased significantly with increasing LV temperature. Figure 4C and D plot amplitude constant

Fig. 3. Three representative solid curves of Eq. 1 (see MATERIALS AND METHODS) best fitted to the normalized contractility (nEmax) data points (closed circles) of the 1st–6th postextrasystolic beats (PES1-6) at 33°C (A), 36°C (B), and 38°C (C) in the same excised, cross-circulated canine heart shown in Fig. 2. Each best-fitted Eq. 1 curve consists of an exponentially decaying monotonic component (first term of Eq. 1, dashed curve) and an exponentially decaying oscillatory component (second term of Eq. 1, dotted curve). Eq. 1 is $nEmax = a \cdot \exp[-(i-1)/\tau_e] + b \cdot \exp[-(i-1)/\tau_s] \cos[\pi(i-1)] + 1$, the first term of Eq. 1 is $nEmax = a \cdot \exp[-(i-1)/\tau_e] + 1$, and the second term of Eq. 1 is $nEmax = b \cdot \exp[-(i-1)/\tau_s] \cos[\pi(i-1)] + 1$. In insets, a : amplitude constant of the first term of Eq. 1, τ_e : beat constant of the first term of Eq. 1, b : amplitude constant of the second term of Eq. 1, and τ_s : beat constant of the second term of Eq. 1. r : correlation coefficient.

ratios $a/(a+b)$ and $b/(a+b)$. Because $a+b$ is equal to the normalized magnitude of PES1 [25], these ratios mean the fractional magnitudes of a and b in PES1. The relative magnitude of $a/(a+b)$ increased but that of $b/(a+b)$ decreased significantly with increasing LV temperature.

Beat constants τ_e and τ_s . Figure 5A and B plot beat constants τ_e and τ_s of Eq. 1 best fitted to the PES1 decays at 33°C, 36°C, and 38°C in the nine hearts. Both τ_e and τ_s decreased significantly with increasing LV temperature. Multiplying τ_e and τ_s by RI converted these beat constants to time constants in msec. Figure 5C and D plot time constants $\tau_e \cdot \text{RI}$ and $\tau_s \cdot \text{RI}$ at 33°C, 36°C, and 38°C. Both $\tau_e \cdot \text{RI}$ and $\tau_s \cdot \text{RI}$ decreased as LV temperature increased. Because RI was fixed constant at 500 msec, these changes in $\tau_e \cdot \text{RI}$ and $\tau_s \cdot \text{RI}$ were proportional to those in τ_e and τ_s *per se*.

Recirculation fraction (RF). Figure 6A plots RF values given by $\exp(-1/\tau_e)$ using τ_e of Eq. 1 best fitted to the PES1 decays at 33°C, 36°C, and 38°C in the nine hearts. RF decreased significantly with increasing LV temperature. Figure 6B plots the normalized RF values at 36°C and 38°C with respect to the RF value at 33°C in each of the nine hearts. On average, RF decreased by $6.6 \pm 10.9\%$ from 33°C to 36°C and by $19.2 \pm 13.3\%$ from 33°C to 38°C. The

normalized RF decreased significantly with increasing LV temperature.

Temperature sensitivity. Table 1 first lists the Q_{10} values of a , b , τ_e , τ_s , RF, and regular beat Emax. All these Q_{10} values were significantly ($P < 0.05$) smaller than unity. The present study has elucidated that all Q_{10} values of $1/a$, $1/b$, $1/\tau_s$, and regular beat $1/\text{Emax}$ characterizing the PES1 decay were significantly greater than 2. Table 1 next lists the Q_{10} values of the reciprocal values of a , b , τ_e , τ_s , RF, and regular beat Emax. All these Q_{10} values were significantly ($P < 0.05$) greater than unity. Table 1 shows in the right most parentheses the minimal Q_{10} values that are significantly different from the mean Q_{10} values of the respective parameters in the nine hearts (exceptionally eight hearts for $1/b$). The Q_{10} values of $1/a$, $1/b$, $1/\tau_s$, and $1/\text{Emax}$ were significantly greater than 2 but $1/\tau_e$ and $1/\text{RF}$ were smaller than 2.

DISCUSSION

Main findings. The principal novel finding of the present study is that the temperature dependence of RF holds even when the temperature-dependent change in LVP because of the negative inotropism of warming was eliminated by adjusting LVV. RF significantly decreased by 19% with increasing LV tem-

Fig. 4. Temperature dependence of amplitude constants a (A) and b (B) and their relative magnitudes to $a+b$, i.e., $a/(a+b)$ (C) and $b/(a+b)$ (D). Plots show their mean $\pm$ standard deviation (SD) at 33°C, 36°C, and 38°C in the nine excised, cross-circulated canine hearts. *: $P < 0.0167$ ($= 0.05/3$) and **: $P < 0.00167$ ($= 0.005/3$) for a statistically significant difference by Bonferroni's multiple t-test after a one-way repeated measures analysis of variance.

Fig. 5. Temperature dependence of beat constants τ_e (A) and τ_s (B) and their multiplications with regular beat intervals (RI), i.e., time constants $\tau_e \cdot RI$ (C) and $\tau_s \cdot RI$ (D). Plots show their mean $\pm$ standard deviation (SD) at 33°C, 36°C, and 38°C in the nine excised, cross-circulated canine hearts. *: $P < 0.0167$ ($= 0.05/3$) and **: $P < 0.00167$ ($= 0.005/3$) for a statistically significant difference by Bonferroni's multiple t -test after a one-way repeated measures analysis of variance.

Fig. 6. Temperature dependence of recirculation fraction (RF). Plots show mean $\pm$ standard deviation (SD) of RF (A) at 33°C, 36°C and 38°C and those of the normalized RF values at 36°C and 38°C with respect to 33°C (B) in the nine excised, cross-circulated canine hearts. *: $P < 0.0167$ ($= 0.05/3$) and **: $P < 0.00167$ ($= 0.005/3$) for a statistically significant difference by Bonferroni's multiple t -test after a one-way repeated measures analysis of variance.

perature from 33°C to 38°C. The Q_{10} value of $1/RF$ was significantly greater than 1.3.

Thus, the present results combined with the previous results [14] evidently confirmed for the first time the temperature dependence of RF independently of LVP and LVV.

Curve fitting. Our present study has revealed that amplitude constants a and b , and beat constants τ_e and τ_s of the monotonic and oscillatory components of the PESP decay decreased significantly with

increasing LV temperature (Figs 4 and 5). Although the PESP decay at 38°C appeared monotonic, it decayed evidently in transient alternans consisting of a monotonic component and an oscillatory component. This also corroborated the generality of the PESP decay in the canine heart preparation even under reasonably physiological conditions.

A comparison of the temperature dependence of a , b , τ_e , and τ_s between the present study (Figs 4 and 5) and the previous one (Figs. 4 and 5 of Ref. [14])

Table 1. Temperature coefficients (Q_{10}) of a , b , τ_e , τ_s , RF, and regular beat Emax and their reciprocals.

Parameters	Q_{10}	Parameters	Q_{10}
a	$0.27 \pm 0.13 (<1)$	$1/a$	$4.6 \pm 2.3 (>3.1)$
b	$0.15 \pm 0.10 (<1)$	$1/b$	$8.1 \pm 4.7 (n=8)$ (>4.9)
τ_e	$0.55 \pm 0.31 (<1)$	$1/\tau_e$	$2.3 \pm 1.1 (>1.6)$
τ_s	$0.37 \pm 0.17 (<1)$	$1/\tau_s$	$3.2 \pm 1.4 (>2.3)$
RF	$0.67 \pm 0.23 (<1)$	$1/\text{RF}$	$1.6 \pm 0.5 (>1.3)$
Emax	$0.28 \pm 0.13 (<1)$	$1/\text{Emax}$	$4.9 \pm 3.2 (>2.8)$

Q_{10} values are means $\pm$ standard deviation (SD). a and b are amplitude constants (dimensionless) in the best-fitted Eq. 1. τ_e and τ_s are beat constants in the number of beats in the best-fitted Eq. 1. $\text{RF} = \exp(-1/\tau_e)$ is the recirculation fraction of Ca²⁺ via sarcoplasmic reticulum in excitation-contraction coupling within myocardial cells. Emax is the ventricular contractility index in terms of maximum elastance or end-systolic pressure-volume ratio of steady-state regular beats. Q_{10} values of all parameters $a \sim \text{Emax}$ were smaller than 1 ($P < 0.05$). In the Q_{10} column on the right, the Q_{10} values of the reciprocals of parameter $a \sim \text{Emax}$ were significantly greater than the Q_{10} values in the parentheses ($P < 0.05$). $1/\tau_e$ and $1/\tau_s$ are rate constants. Number of hearts (n) = 9, except for Q_{10} of $1/b$ ($n = 8$). Q_{10} of $1/b$ ($n = 9$) was 16.5 ± 25.6 because of an extremely large Q_{10} of $1/b$ (= 83.6) as a result of a very small Q_{10} value of b (= 0.01) in one heart. However, $P = 0.063$ for $n = 9$ was obtained for its $Q_{10} > 2.0$. After removing this exceptional case of $1/b$, $P < 0.05$ for $n = 8$ resulted in its $Q_{10} > 4.9$.

impresses their similarity despite the different peak LVP and LVV conditions. A more careful comparison of them indicates that a , b , and τ_s decreased rapidly from 33°C to 36°C and slightly from 36°C and 38°C in the present study, whereas they decreased more linearly from 33°C to 38°C in the previous study [14]. This difference may be due to the controlled LVV with increasing temperature in the present study in contrast to the constant LVV in the previous study [14]. Linearly increasing LVV from 10 ml to 18 ml at a constant temperature has shown to decrease a and b first rapidly and then gradually (Fig. 3 of Ref. [13]). This volume range is comparable to that in the present study. Therefore, it is most likely that the equalized peak LVP by controlling LVV has rapidly decreased a and b with increasing temperature from 33°C to 38°C in the present study as compared to the previous results [14].

Increasing LVV from 10 ml to 14 ml kept τ_s constant but that from 14 ml to 18 ml increased τ_s at a constant temperature in our previous study [13]. Thus, it is most likely that the increased LVV increased τ_s and stopped decreasing τ_s during warming from 36°C to 38°C in the present study (Fig. 5B).

However, the temperature-dependent decreases in

τ_e are comparable whether LVV was changed in the present study or fixed in the previous study [14]. This seems to be due to the reasonably constant τ_e independent of the changes in LVV [13].

Beat constants τ_e and τ_s . As in the previous study [14], RI was fixed at 500 msec regardless of LV temperature. Therefore, time constants in msec calculated as beat constants τ_e and τ_s times RI, namely, $\tau_e \cdot \text{RI}$ and $\tau_s \cdot \text{RI}$, were proportional to τ_e and τ_s , respectively (Fig. 5). We showed previously that both $\tau_e \cdot \text{RI}$ and $\tau_s \cdot \text{RI}$ were unchanged even when τ_e and τ_s changed with changing RI [25]. This suggests that time constant, but not beat constant, reflects the heart-rate-independent recovery speed of transiently perturbed sarcoplasmic Ca²⁺ toward Ca²⁺ homeostasis [2, 3]. Therefore, we fixed RI while LV temperature was changed to study the direct effects of temperature on RF. If LV was not paced and RI was shortened chronotropically as temperature increased, beat constant τ_e in beat number would increase with the temperature-dependently decreasing RI and would be greater at a higher temperature than those we observed (Fig. 5).

The other beat constant τ_s also decreased with increasing temperature. In our previous studies [7, 9–12], various positive and negative inotropic interventions did not significantly affect τ_s though they affected τ_e and RF variously. Exceptionally, 2,3-butanedione monoxime decreased both τ_e and τ_s [8]. These results seem to support our Ca²⁺ handling model (Fig. 1) where τ_s reflects different Ca²⁺ kinetics from τ_e . We have therefore been considering τ_e and τ_s to be different temperature-dependent chemical processes in the Ca²⁺ handling. In our model (Fig. 1), τ_e is attributed to the combination of the SR Ca²⁺ pump and the SL Na⁺/Ca²⁺ exchange coupled with the Na⁺/K⁺ pump [1, 3], whereas τ_s is attributed to the Ca²⁺ handling between the SR Ca²⁺ pump and the ryanodine-sensitive Ca²⁺ release channel. Therefore, $Q_{10} = 0.55$ of τ_e and 0.37 of τ_s seem to characterize the temperature-dependent processes in these Ca²⁺ handling processes [15, 16, 18, 21, 22, 36, 37]. However, their association with any specific pumps, channels, and exchanges is beyond the present scope.

Recirculation fraction (RF). As τ_e decreased with increasing temperature, $\text{RF} = \exp(-1/\tau_e)$ also decreased (Figs. 5C and 6) in a similar manner to the previous one [14]. This similarity indicates practically no effect of LVP and LVV loading conditions on RF. This finding seems also consistent with virtually no effect of the changes in peak LVP on RF in our previous study [13]. This is a considerable advantage when we obtain RF because we do not need to spec-

ify or fix peak LVP and LVV in the isovolumic contraction mode. Whether this holds in the ejection contraction mode remains unknown.

We would speculate the smaller RF at the higher temperature to reflect the increased Ca^{2+} fraction of the transsarcolemmal removal and the decreased Ca^{2+} fraction within SR [36, 37] during each cardiac cycle. Since RF increases with increasing heart rate and thus decreasing beat interval [25], we fixed the pacing rate of both RI and PESI regardless of LV temperature in the present study. Therefore, there was no effect of heart rate *per se* on the temperature dependence of RF in the present study as in the previous study [14].

Temperature sensitivity. Since Q_{10} values of a , b , τ_e , τ_s , RF and regular beat E_{max} were significantly smaller than 1 in the present study, we calculate their reciprocals as in our previous study [14]. The present study has elucidated that all Q_{10} values of $1/a$, $1/b$, $1/\tau_s$, and regular beat $1/E_{\text{max}}$ characterizing the PESP decay were significantly greater than 2. This result suggests that a , b , τ_s , and regular beat E_{max} were closely related to one or more chemical processes in the Ca^{2+} handling and resultant mechanical contraction as in our previous study [14].

The slightly smaller temperature dependency of Q_{10} of $1/\tau_e$ (significantly > 1.6 , $P < 0.05$) and $1/\text{RF}$ (significantly > 1.3 , $P < 0.05$) did not statistically indicate their relations to chemical processes. However, when a variable of interest is of a complex biological (physicochemical) system, its Q_{10} could fall below 2 even when its subsystem(s) is (are) related to one or more chemical processes [14]. In fact, both τ_e and RF are well known to characterize the Ca^{2+} handling chemical processes consisting of at least the SR Ca^{2+} pump (internal process) and the SL $\text{Na}^+/\text{Ca}^{2+}$ exchange coupled with the Na^+/K^+ pump (external process) [1, 3]. Since RF is determined by the relative magnitude of these two processes, τ_e and RF may appear less temperature-dependent than a , b , τ_s , and E_{max} , though both are related to chemical processes.

The contemporary reductionistic knowledge is that the temperature sensitivities of all the Ca^{2+} transporters are comparable among themselves [3]. According to this knowledge, one may expect that both internal and external Ca^{2+} handling processes would have comparable Q_{10} values, canceling each other and thus resulting in a temperature-independent RF with the net Q_{10} of almost 1. Against this expectation, the present study as well as the previous one [14] show the significantly decreased RF with increasing temperature (Fig. 6), resulting in $Q_{10} > 1.3$ for $1/\text{RF}$ (Table 1). From this unexpected result, we would speculate that the transsarcolemmal Ca^{2+} extru-

sion processes, consisting primarily of the SL $\text{Na}^+/\text{Ca}^{2+}$ exchange and the Na^+/K^+ pump, may have a greater temperature coefficient than the internal Ca^{2+} recirculation processes via SR, primarily the SR Ca^{2+} pump. A further elucidation of the relative contributions of all the Ca^{2+} transporters involved in E-C coupling and attribution of RF and the other variables of Eq. 1 to the individual processes of Ca^{2+} handling are beyond the present scope and warrant further studies.

Conclusions. The present results show that LV RF of Ca^{2+} via SR in E-C coupling within myocardial cells has intrinsic temperature dependence in the excised, cross-circulated canine heart preparation. This dependence remained unchanged though the temperature-dependent changes in peak LVP were eliminated by adjusting LVV. Thus, the temperature-dependent change in RF would be the effect of cardiac temperature *per se* independent of LVP and LVV loading conditions.

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