Temperature-dependent postextrasystolic potentiation and Ca$^{2+}$ recirculation fraction in canine hearts

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Departments of 1Cardiovascular Physiology, 2Anesthesiology and Resuscitology, and 3Cardiovascular Medicine, Okayama University Graduate School of Medicine and Dentistry, Okayama, 700-8558; and 4National Cardiovascular Center Research Institute, Suita, Osaka, 565-8565, Japan

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Mizuno, Ju, Junichi Araki, Shunsuke Suzuki, Satoshi Mohri, Takeshi Mikane, Juichiro Shimizu, Hiromi Matsubara, Masahisa Hirakawa, Tohru Ohe, and Hiroyuki Suga. Temperature-dependent postextrasystolic potentiation and Ca$^{2+}$ recirculation fraction in canine hearts. Am J Physiol Heart Circ Physiol 282: H403–H413, 2002; 10.1152/ajpheart.00427.2000.—We have found that cardiac temperature proportionally changes O$_2$ cost of contractility, defined as O$_2$ consumption for myocardial total Ca$^{2+}$ handling normalized to contractility in terms of the end-systolic pressure-volume ratio (maximal elastance, $E_{\text{max}}$), in the canine left ventricle (temperature sensitivity, Q$^{10}$ of RF and O$_2$ cost of contractility (Q$^{10}$) of RF and O$_2$ cost of contractility (Q$^{10}$) of E$_{\text{max}}$). We have separately found that a decrease in the recirculation fraction (RF) of Ca$^{2+}$ within myocardial cells underlies an increased O$_2$ cost of E$_{\text{max}}$ in stunned hearts. We therefore hypothesized that a similar change in RF would underlie the Q$^{10}$ of O$_2$ cost of E$_{\text{max}}$. We tested this hypothesis by analyzing RF calculated from an exponential decay component of the transient alternans decay of postextrasystolic potentiation in the canine left ventricle. RF decreased from 0.7 to 0.5 as cardiac temperature increased from 33 to 38°C with Q$^{10}$ of 0.5, reciprocal to that of O$_2$ cost of E$_{\text{max}}$. We conclude that Q$^{10}$ of O$_2$ cost of E$_{\text{max}}$. Therefore, in the present study, we hypothesized that cardiac temperature would also change either RF or E$_{\text{max}}$ reactivity or both, and thereby change the O$_2$ cost of E$_{\text{max}}$. We tested this hypothesis in the canine heart. We used our recently developed method to obtain RF by extracting an exponential decay component from the transient alternans decay of postextrasystolic potentiation (PESP) (3, 10, 13, 16, 19, 26–28). We obtained interesting results to support that the temperature-dependent ATP-consuming activities in Ca$^{2+}$-handling processes could largely account for the temperature-dependent changes in both RF and E$_{\text{max}}$ reactivity, and hence Q$^{10}$ of O$_2$ cost of E$_{\text{max}}$.

MATERIALS AND METHODS

Surgical preparation. We used a standard-type excised, cross-circulated canine heart preparation that we have been consistently using in cardiac mechanoenergetics studies (18, 31). All procedures were conducted in conformity with the “Guiding Principles for Research Involving Animals and Human Beings” endorsed by the American Physiological Society as well as the Physiological Society of Japan. The details of the surgical preparations were described elsewhere (18, 31).

Briefly, in each of seven experiments, a metabolic support dog (20.2 ± 5.6 kg) and a heart donor dog (13.1 ± 1.5 kg), both adult mongrels, were anesthetized with pentobarbital sodium (25 mg/kg iv) and fentanyl (0.1–0.2 mg/h iv) after premedication with ketamine hydrochloride (25 mg/kg im). They were intubated, air-ventilated, and heparinized (15,000 units per support dog and 10,000 units per donor dog iv).

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Bilateral common carotid arteries and unilateral external jugular vein of the support dog were cannulated and connected to the arterial and venous cross-circulation tubes, respectively. The chest of the donor dog was opened midternally. 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.

The left atrium of the donor heart was opened, and all the left ventricular (LV) chordae tendineae were cut. 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 parahisian pacing.

A thin flabby latex balloon with an unstretched volume of ~50 ml mounted on a rigid connector was fit 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 allowed us to measure LV volume (LVV) accurately and control it precisely. LV pressure (LVP) was measured with a miniature pressure gauge (model P-7, Konigsberg Instruments; Pasadena, CA) placed inside the apical end of the balloon. The pressure gauge was calibrated against the fluid-filled pressure transducer.

LV temperature of the excised donor heart was maintained at 33, 36, and 38°C by cooling and warming the arterial cross-circulation tube through a thermostatic bath. LV temperature was measured with a thermistor placed between the endocardium and the balloon. 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 the present heart preparation.

An LV epicardial electrocardiogram was recorded with a pair of screw-in electrodes to trigger data acquisition. All LVP, LVV, and electrocardiogram signals were digitized at 2-ms intervals with an analog-digital converter (Lab-NB, National Instruments), displayed on a computer, and stored on a hard disk.

The systemic arterial blood pressure of the support dog served as coronary perfusion pressure of the excised donor heart. We prevented the tendency to hypotension and maintained systemic arterial pressure of the support dog around 90 mmHg by electrically stimulating Neiguan (PC-6) acupoints in bilateral forearms (32). Briefly, we inserted two stainless steel needles vertically into the acupoints. They were 3 cm above the transverse crease of the wrist and between the tendons of the long palmar muscle and the radial flexor muscle of the wrist. The stimulation was 5 V, 40 Hz, and 5-ms biphasic pulses. 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 weights of the LV, including the septum (84.0 ± 17.6 g), and RV (33.7 ± 6.3 g) were measured after each experiment.

Pacing. We used a fixed pacing stimulus pattern. It consisted of a single extrasystole (ES) inserted at an extrasystolic interval (ESI) of 320 ms after 10 or more regular beats. The first postextrasystolic beat (PES1) interval (PESI) was 500 ms with no compensatory pause. The 10 or more subsequent PESIs were also 500 ms. We allowed no compensatory pause because we wanted to know how the ES1 alone would affect the PESP beats at different LV temperatures. We have already found that even the PESP decay after no compensatory pause in PESI1 contained an alternans decay component, although smaller than after a compensatory pause (26). These pacing stimuli were produced with a stimulator controlled by a computer installed with LabView 3.1 (National Instruments).

Normalized contractility. To evaluate the beat-to-beat changes in LV contractility during each PESP decay, we used Emax (maximum elastance, or end-systolic pressure-volume ratio) as an index of ventricular contractility (30). In isovolumic contractions as we used, end systole corresponds to peak systole. Emax values of PES1–6 were calculated as the ratio of peak isovolumic LVP to LVV, identified as the LVP at which peak LVP was zero (30). These Emax values were normalized (nEmax) relative to the Emax of the preceding regular beats. The changes in nEmax values during the PESP decay were therefore proportional to those in the peak LVP values at a fixed LVV.

Curve fitting. We examined whether the obtained nEmax values of PES1–6 during each PESP decay at any LV temperature could be fit by the same equation that we had proposed and used in the previous studies (3, 10, 13, 16, 19, 26–28)

\[ nE_{\text{max}} = a \cdot \exp[-(i - 1)/\tau_{e}] + b \cdot \exp[-(i - 1)/\tau_{s}] \cos[\pi(i - 1)] + 1 \tag{1} \]

where \( nE_{\text{max}} \) is the normalized contractility of PESi \( (i = 1–6) \) relative to the preceding regular beats (Fig. 1, right). Coefficient \( a \) is the amplitude constant of the monoeponential decay term (first term). Coefficient \( b \) is the amplitude constant of the other exponential term multiplying the sinusoidal decay term (second term). Denominators \( \tau_{e} \) and \( \tau_{s} \) 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 PESi data points (11).

Ca2+ handling model. The conventional, monotonic, or exponential decay of the PESP has been accounted for by the beat-by-beat decreasing Ca2+ reactivity in E-C coupling from its transiently augmented level in PES1 toward the steady-state level of regular beats (21, 34, 35). The most potentiated PES1 is due to both an increased transsarcolemmal Ca2+ influx and an increased availability of sarcoplasmic reticulum (SR) Ca2+ and associated with the preceding extrasystole and PESI1 (37). The gradual monotonic decay over the following PES2–6 is due to the transsarcolemmal Ca2+ extrusion exceeding the influx and hence the gradually decreasing Ca2+ reusability over these beats. Stable regular beats are restored due to the Ca2+ homeostasis (3–5, 21).

Figure 1 illustrates the myocardial Ca2+ handling model that we modified to include the transient alternans decay (10, 13, 16, 19, 26–28). Intramyocardial Ca2+ recirculation and gradual Ca2+ extrusion could account for the monoexponential decay component to the homeostatic Ca2+ level (11, 21). The Ca2+ release from the SR would alternate transiently.
and could account for the sinusoidal decay component (1, 11, 25). This model indicates that the monoexponential decay term of our proposed Eq. 1 is related to the RF and the exponentially decaying sinusoidal term to the Ca\(^{2+}\) uptake-release characteristics of SR (see APPENDIX). We have proposed that this Ca\(^{2+}\) handling model enables us to assess the mass dynamics of total Ca\(^{2+}\) handling from cardiac mechanoenergetics at the organ level (3, 27).

Recirculation fraction. We calculated RF from the beat constant \(\tau_b\) in the first term of Eq. 1 best fit to the alternans decay over PES1–6 (10, 13, 16, 19, 26–28). We considered this RF to be equivalent to the RF obtained from the beat constant \(\tau\) of the conventional monotonic PESP (21, 34) (see APPENDIX). Both quantify the fraction of the total amount of Ca\(^{2+}\) handled in E-C coupling that recirculates intracellularly via the SR without being extruded transsarcolemmally. Reciprocally, \(1 - RF\) quantifies the fraction of the total amount of Ca\(^{2+}\) handled in E-C coupling that is extruded and enters transsarcolemmally, i.e., Ca\(^{2+}\) extrusion fraction.

The exponentially decaying PESP indicates theoretically that PESP beats have the same constant RF as that of the preceding regular beats at the same RI and PES1 except for the ESI (11, 21, 34). An extrasystole perturbs myocardial Ca\(^{2+}\) homeostasis and initiates the alternans decay without affecting RF (11, 21, 34). We reasonably assumed the same RF concept to hold in the monoexponential decay component of the alternans decay as in our previous studies (10, 11, 13, 16, 19, 26–28) (see APPENDIX).

Data analyses. We performed curve fitting of Eq. 1 by the least-squares method using LabView 3.1 (National Instruments) on a computer. We obtained \(a, b, \tau_a, \tau_b,\) and RF from the best-fit Eq. 1. We compared them among 33, 36, and 38°C. Goodness of the curve fitting was evaluated by correlation coefficient (r).

We obtained \(Q_{10}\) of \(a, b, \tau_a, \tau_b,\) RF, and \(E_{max}\). By definition, \(Q_{10}\) is equal to the ratio of a variable value \(M_{10}\) at temperature \((t + 10)°C\) over its value \(M_t\) at \(t°C\), i.e., \(Q_{10} = M_{10}/M_t\). When \(M_t\) is obtained at temperature \((t + 5)°C\) instead of \(M_{10}\), \(Q_{10} = (M_t/M_{10})^{1/2}\). Therefore, we obtained \(Q_{10}\) as the square of its change per 5°C rise from 33 to 38°C.

Statistics. Best-fit \(a, b, \tau_a, \tau_b,\) and RF values were presented as their means ± SD. They were compared among 33, 36, and 38°C by one-way repeated-measures ANOVA. When ANOVA was significant (\(P < 0.05\)), we performed multiple comparisons between LV temperature by Bonferroni’s test. We considered a \(P\) value <0.05 to indicate statistical significance. Correlation coefficient (r) of the curve fitting was obtained in each PESP decay. We used StatView 4.5 (Abacus Concepts; Berkeley, CA) to perform these statistics.

**RESULTS**

**PESP decay patterns.** Figure 2 depicts a representative set of the PESP decays at 33°C (Fig. 2A), 36°C (B), and 38°C (C) in one heart. Isovolumic LVV during regular beats and each PESP decay was maintained at a relatively small constant volume regardless of LV temperature. The small volume had to be chosen to avoid LVP of PES1 above 200 mmHg at 33°C. This turn caused a relatively low LVP (50–60 mmHg) in regular beats at 38°C. LVP over 200 mmHg tended to cause spontaneous extrasystoles, and in the worst case, damage the aortic valve resulting in herniation of the intraventricular balloon.

The three PESP decay patterns in Fig. 1 appear significantly different from each other. However, they shared essentially the same feature. At any temperature, PES1 was the greatest of all PES1–6 and regular beats (R1–3); PES2 was weaker than PES1; PES3 was stronger than PES2 at 33 and 36°C and nearly equal to PES2 at 38°C; and PES4 was weaker than PES3. PES5 was stronger than PES4 at 33 and 36°C and nearly equal to PES4 at 38°C. PES6 was weaker than PES5. In this way, the PESP always decayed in alternans over PES1–5 or 6 and gradually returned to the regular beat level. The transient alternans was most conspicuous at 33°C.

The amplitudes of peak LVPs of regular beats and PES1–6 decreased from Fig. 2, A to C. \(E_{max}\) of regular beats decreased from 12.7 ± 4.3 (means ± SD) mmHg/ml at 33°C to 11.7 ± 4.4 mmHg/ml at 36°C and 9.8 ± 3.9 mmHg/ml at 38°C. Simultaneously, the incomplete relaxation between regular beats as well as PES1–6 in Fig. 2A disappeared in Fig. 2, B and C. The width of the LVP wave gradually decreased from Fig. 2,
Fig. 2. Representative set of left ventricular (LV) pressure (LVP) tracings of postextrasystolic alternans decays at 33°C (A), 36°C (B), and 38°C (C) in one heart. Regular beat intervals (RIs) were fixed at 500 ms. Extrasystolic beat interval (ESI) was fixed at 320 ms. PESIs were fixed at 500 ms. Isovolumic LV volume in all beats were constant (12.9 ml/100 g LV) regardless of LV temperature.

A to C. These cardiodynamic changes were essentially the same as our previous observations (18, 24, 31).

Curve fitting. Figure 3 depicts a representative set of the alternating curves (solid curves) best fit with Eq. 1 to normalized $E_{\text{max}}$ (normalized $E_{\text{max}}$) of PES1–6 (closed circles) at 33°C (Fig. 3A), 36°C (B), and 38°C (C) in one heart. Figure 3 also shows their monoexponential decay terms (first term of Eq. 1, dashed curves) and exponentially decaying sinusoidal terms (second term of Eq. 1, dotted curves). The sinusoidal curves are meaningful only at integer $i$ values corresponding to PES1–6 and meaningless at other noninteger $i$ values (11).

As the temperature increased (Fig. 3, A to C), the amplitudes of the alternans as well as its exponential and sinusoidal components decreased. Amplitude constants $a$ and $b$ and beat constants $\tau_e$ and $\tau_s$ also decreased with increasing temperature. All the other hearts showed essentially the same results. The $r$ values obtained by fitting Eq. 1 to the alternans decays at 33, 36, and 38°C in seven hearts were 0.9997 ± 0.0005, 1.0000 ± 0.0001, and 0.9999 ± 0.0001, respectively. All these $r$ values were virtually 1. This result indicated that Eq. 1 excellently fit all alternans decays at 33, 36, and 38°C.

We have confirmed that the extremely high correlation was not due to the closeness of the numbers of unknown variables ($= 4$) and data ($= 6$) (10). Moreover, a polynomial equation even with four unknown variables ($y = a_1 + b_1^2 + c_1 + d$) did not fit the same PESI data with an almost unity $r$ comparable to those with Eq. 1. Furthermore, our recent theoretical study supported the reliability of Eq. 1 (11). We therefore considered that Eq. 1 could reasonably well characterize the alternans PESP for the purpose of RF calculation in a beating heart (3, 26).

Amplitude constants $a$ and $b$. Figure 4, A and B, plots means ± SD values (dimensionless) of amplitude constants $a$ and $b$ of Eq. 1 best fit to the alternans PESPs at 33, 36, and 38°C in the seven hearts. Both $a$ and $b$ decreased significantly with increasing temperature. Figure 4, C and D, plots means ± SD values (dimensionless) of amplitude constant ratios $a/(a + b)$ and $b/(a + b)$. Because $a + b$ is equal to the normalized magnitude of PES1, these ratios mean the fractional magnitudes of $a$ and $b$ in PES1. $a/(a + b)$ increased but $b/(a + b)$ decreased significantly as the temperature increased. These changes were consistent with the visual impression that the alternans as well as its oscillatory component gradually disappeared, and the monoexponential component became dominant as the temperature increased in Figs. 2 and 3.

Beat constants $\tau_e$ and $\tau_s$. Figure 5, A and B, plots means ± SD values (beats) of beat constants $\tau_e$ and $\tau_s$ of the Eq. 1 best fit to the alternans PESPs at 33, 36, and 38°C in the seven hearts. Both $\tau_e$ and $\tau_s$ decreased significantly with increasing temperature. Multiplication of $\tau_e$ and $\tau_s$ by RI in milliseconds converted these beat constants to time constants in time unit (in ms). Figure 5, C and D, plots means ± SD values (beats) of time constants $\tau_e$ times regular beat interval (RI) and $\tau_s$ times RI. Both time constants decreased significantly with increasing temperature. Because RI was fixed constant at 500 ms in the present study, these changes in time constants happened to be proportional to those in beat constants $\tau_e$ and $\tau_s$ per se.

Recirculation fraction. Figure 6A plots RF values (dimensionless) given by $\exp(-1/\tau_e)$ using $\tau_e$ of Eq. 1 best fit to the alternans decays at 33, 36, and 38°C in the seven individual hearts. This $RF = \exp(-1/\tau_e)$ is after the method by Morad and Goldman (21). We have confirmed the reliability of this RF calculation by a new discrete fitting method (11). Figure 6B plots their means ± SD values. RF decreased significantly with increasing temperature in every heart as well as on the
average after pooling all the hearts. RF decreased by 18% on average from 33°C to 36°C, by 13% on average from 36°C to 38°C, and by 28% on average from 33°C to 38°C.

Temperature sensitivity. Table 1 lists $Q_{10}$ of $a$, $b$, $\tau_e$, $\tau_m$, RF, and $E_{\text{max}}$. All the obtained $Q_{10}$ values were smaller than unity. Therefore, their $1/Q_{10}$ values were greater than unity. For example, RF had $Q_{10} = 0.5$ and $1/Q_{10} = 2$ on average. Similarly, $E_{\text{max}}$ of regular beats had $Q_{10} = 0.6$ and $1/Q_{10} = 1.7$.

**DISCUSSION**

Postextrasystolic alternans decay. The present result on $E_{\text{max}}$ confirmed the temperature dependence of $E_{\text{max}}$ of regular beats preceding PESP that we have reported previously (18, 24, 31). $Q_{10}$ of $E_{\text{max}}$ in the present study (Table 1) was similar to $0.44 \pm 0.13$ for 30–40°C in our previous study (18).

The present results (Fig. 2) show that the transient alternans decay of PESP is the most representative decay pattern at heart rates of 100–150 beats/min under normothermia in the excised, cross-circulated heart. This confirmed our previous studies (3, 10, 13, 16, 19, 26–28). Moreover, it supports for the first time the generality of the alternans PESP decay even under hypothermia (33°C) and hyperthermia (38°C) in the same canine heart at the pacing rate of 120 beats/min. Moreover, the alternans PESP at 33–38°C always fit precisely the same equation (Eq. 1) that we used in the previous studies (3, 10, 13, 16, 19, 26–28), namely, the sum of an exponentially decaying monotonic component and an exponentially decaying oscillatory component (Fig. 3).

The spontaneous heart rate range we naturally encounter in this type of canine heart preparation is 100–120 beats/min before atrioventricular block under normothermia (3, 10, 13, 16, 19, 26–28). Our recent unpublished observations show that the PESP decay appeared less alternating and more monotonic when heart rate decreased below 100 beats/min after atrioventricular block even under normothermia. The heart rate range above 100 beats/min seems to be the reason that the alternans PESP has been the representative type in our canine heart preparation under normothermia (3, 10, 13, 16, 19, 26–28). Nevertheless, PESP always contained an exponentially decaying monotonic component from which we were able to calculate RF over 33–38°C in this study.

Our present study has revealed that amplitude constants $a$ and $b$ and beat constants $\tau_e$ and $\tau_m$ of the two decay components of the alternans PESP decreased sensitively with increasing temperature (Figs. 4 and 5). Because $Q_{10} < 1$ of $a$, $b$, $\tau_e$, and $\tau_m$ are comparable to each other (Table 1), we suspect them to be inversely related to the temperature-dependent rates of chemical reactions in the Ca$^{2+}$ handling. In other words, $1/a$, $1/b$, $1/\tau_e$, and $1/\tau_m$ ($1/Q_{10} > 1$) may be more directly related to these chemical reactions.

$\tau_e$, $\tau_m$, and RF. In our Ca$^{2+}$ handling model (Fig. 1) (3, 10, 13, 16, 19, 26–28), $\tau_e$ of the monotonic decay component of the transient alternans PESP reflects essentially the same RF as $\tau_m$ of the monotonic PESP decay does (11, 21, 34, 35) (see APPENDIX). The present study revealed that $\tau_e$ and RF decreased with increasing temperature. Note, however, that we kept RIs and PESISs constant regardless of the temperature changes. Therefore, $\tau_e$ times RI, which has time units, also decreased in proportion to $\tau_e$ with increasing temperature. Therefore, $\tau_m$ times RI and $\tau_e$ alone have the same $Q_{10}$, although their dimensions are different. Their $Q_{10}$ ($<1$) and $1/Q_{10} (>1)$ suggest temperature accelerated chemical reactions behind the smaller RF.

The other beat constant $\tau_m$ also decreased with increasing temperature. In our previous studies, positive and negative inotropic interventions (catecholamines, Ca$^{2+}$, ryanodine, and postischemic stunning) did not significantly affect $\tau_e$ (3, 10, 13, 27). Exceptionally, 2,3-butanedione monoxime decreased both $\tau_m$ and $\tau_e$ (16). These results seem to support our contention that
τs reflects different Ca\(^{2+}\) kinetics from τe. We would therefore consider τe and τs to be related to different temperature-dependent chemical reactions in the Ca\(^{2+}\) handling. In our model (Fig. 1), τe seems to be related to the SR Ca\(^{2+}\) pump and sarcolemmal Na\(^+/\)Ca\(^{2+}\) exchange, whereas τs related to SR Ca\(^{2+}\) handling between the Ca\(^{2+}\) pump and ryanodine-sensitive release channel (Fig. 1). Although Q\(_{10}\) = 0.3 of τe and τs suggests temperature-dependent reactions, their association to any specific pumps, channels, and exchanges is beyond the present scope.

Cooling inotropism. The present Q\(_{10}\) = 0.6 for \(E_{\text{max}}\) is consistent with our previous results (18, 24, 31). However, O\(_2\) consumption for total Ca\(^{2+}\) handling is temperature independent (18, 24, 31). This leads to the temperature-dependent O\(_2\) cost of \(E_{\text{max}}\) with Q\(_{10}\) = 2 (18, 24, 31).

Multiple mechanisms (4) seem to underlie the temperature-dependent \(E_{\text{max}}\) and O\(_2\) cost of \(E_{\text{max}}\) with

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**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) at 33, 36, and 38°C. Plots show their means ± SD in 7 hearts. *P < 0.05 for significant difference of 38°C vs. 33°C by Bonferroni t-test after one-way repeated-measures analysis of variance.

**Fig. 5.** Temperature dependence of beat constants τe (A) and τs (B) and their multiplication with RI, i.e., τe × RI (C) and τs × RI (D) at 33, 36, and 38°C. Plots show their means ± SD of τe, τs, τe × RI and τs × RI in 7 hearts. *P < 0.05 for significant difference of 36 and 38°C vs. 33°C; **P < 0.005 vs. 33°C by Bonferroni t-test after one-way repeated-measures analysis of variance.
Q₁₀ < 1 and > 1, respectively, reciprocal to each other. Myocardial cooling inhibits SR Ca²⁺ uptake via the SR Ca²⁺ pump and the Ca²⁺ efflux via the Na⁺/Ca²⁺ exchange coupled with the Na⁺-K⁺ pump as well as via the sarcolemmal Ca²⁺ pump (4–6, 14, 22). Not only these ATPase-dependent processes but also ATPase-independent processes such as L-type Ca²⁺ channel and ryanodine-sensitive Ca²⁺ release channel have Q₁₀ of 2–4 (2). The cooling-suppressed Na⁺/Ca²⁺ exchange also suppresses the reverse mode of the Na⁺/Ca²⁺ exchange and increases Ca²⁺ transient (8). The Na⁺ pump is also inhibited by cooling (Q₁₀ around 3) (9) and intracellular Na⁺ rises (7), leading to increases in sarcoplasmic Ca²⁺ concentration, SR Ca²⁺ content, and Ca²⁺ availability for E-C coupling (4). The slowed Ca²⁺ handling not only decelerates but also prolongs contraction (Q₁₀ around 3) (4, 22). Cooling enhances the Ca²⁺ responsiveness so that contractile force increases for a given sarcoplasmic Ca²⁺ concentration (12). Cooling suppresses myosin ATPase and hence cross-bridge cycling, leading to a slower contraction and relaxation (4–6, 14). Cooling thus accounts for not only the positive inotropism and negative lusitropism seen in Fig. 2 but also for the decreased O₂ cost of Eₘₐₓ (18).

Our previous computer simulation of myocardial Ca²⁺ handling and cross-bridge cycling has simulated these temperature-dependent changes in myocardial mechanoenergetics to reasonable extent (17). In this simulation, we changed all ATP-consuming processes in the E-C coupling and force development. Therefore, we would speculate that the temperature-dependent changes in the parameters of the transient alternans PESP could largely be related to the ATP-consuming reactions in myocardial excitation, E-C coupling, and contraction.

**Ca²⁺ and ATP consumption.** A change in RF affects ATP consumption for myocardial total Ca²⁺ handling according to the different energy cost between the major internal and external Ca²⁺ handling routes (25, 31). The internal Ca²⁺ handling route via the SR is nominally twice more economical than the transsarcolemmal route (4, 27, 33).

In mass Ca²⁺ dynamics, the RF is exclusively related to the SR Ca²⁺ pump, and the 1 – RF is related predominantly to the sarcolemmal Na⁺/Ca²⁺ exchange coupled with the Na⁺-K⁺ pump (4, 27, 33). We had observed the economical O₂ cost of Eₘₐₓ in the hypothermic canine hearts (31). As the cause of this economical state, we previously speculated that the Ca²⁺ responsiveness of contractility increased primarily by cooling (18). However, the present study has revealed increases in τₑ and RF at hypothermia. Therefore, the decreased O₂ cost of Eₘₐₓ at hypothermia (18, 31) seems to have resulted from increases in Ca²⁺ responsiveness of contractility and RF. Similarly, the increased O₂ cost of Eₘₐₓ at hyperthermia (18, 24) seems to have resulted from decreases in Ca²⁺ responsiveness of contractility and RF.

**RF versus O₂ cost of Eₘₐₓ** Although the present Q₁₀ = 0.5 of RF is inversely proportional to the previous Q₁₀ = 2 of O₂ cost of Eₘₐₓ (18), this does not immediately mean that the latter is fully accountable by the former. Table 2 lists the equations relating total Ca²⁺ handling, its O₂ consumption (its V̇O₂, or total Ca²⁺ handling V̇O₂₂), RF, and Eₘₐₓ (3, 27). Equation A in Table 2 is the stoichiometric equation relating total Ca²⁺ handling, its V̇O₂₂, and RF. Equation B defines the reactivity (R) of Eₘₐₓ to total Ca²⁺ handling. The substitution of Eq. B into Eq. A yields Eq. C after conversion of the dimensions of V̇O₂₂ (from μmol/kg into ml O₂/100 g). Equation D defines the O₂ cost of Eₘₐₓ. The

Table 1. Q₁₀ of a, b, τₑ, τₑ, RF, and Eₘₐₓ

<table>
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<th>Variables</th>
<th>Q₁₀</th>
<th>1/Q₁₀</th>
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<td>a</td>
<td>0.34 ± 0.19</td>
<td>3.9 ± 2.3</td>
</tr>
<tr>
<td>b</td>
<td>0.15 ± 0.09</td>
<td>10.8 ± 11.5</td>
</tr>
<tr>
<td>τₑ</td>
<td>0.32 ± 0.16</td>
<td>3.8 ± 2.2</td>
</tr>
<tr>
<td>τₑ</td>
<td>0.24 ± 0.13</td>
<td>3.2 ± 1.0</td>
</tr>
<tr>
<td>RF</td>
<td>0.52 ± 0.15</td>
<td>2.0 ± 0.5</td>
</tr>
<tr>
<td>Eₘₐₓ</td>
<td>0.61 ± 0.15</td>
<td>1.74 ± 0.44</td>
</tr>
</tbody>
</table>

Values are means ± SD. a and b are dimensionless amplitude constants; τₑ and τₑ are beat constants in number of beats in Eq. 1; RF, recirculation fraction of Ca²⁺ within myocardial cells = exp(−1/τₑ); Eₘₐₓ is of regular beats immediately preceding postextrasystolic potentiation. Q₁₀, temperature sensitivity.
substitution Eq. D into Eq. C yields Eq. E. It relates O2 cost of Emax with RF and R. This relation (Eq. E in Table 2) indicates that O2 cost of Emax is proportional to 2 − RF and 1/R, but not 1/RF. This means theoretically that Q10 of O2 cost of Emax is neither proportional to nor accountable by Q10 of 1/RF, although they appear reciprocal to each other. Therefore, Q10 = 2 of O2 cost of Emax in our previous study (18) should not be directly accounted for by the present Q10 = 0.5 of RF.

Figure 7 shows a set of theoretical relations given by Eq. E in Table 2 with R as a parameter. Theoretically, the decrease in RF from 0.7 at 33°C to 0.5 at 38°C with Q10 of 0.5 in the present study (Fig. 6 and Table 1) causes an inversely proportional increase in O2 cost of Emax with Q10 of ~1.3 at any constant R, as shown by four slant lines. This Q10 is only ~65% of the Q10 = 2 of O2 cost of Emax (18). A theoretically possible mechanism to account for this discrepancy would be a simultaneous change in reactivity R in the same direction as O2 cost of Emax, as indicated by the heavy steep line connecting the two working points (open circles) at 33 and 38°C in Fig. 7. For a change in R to account for the Q10 = 2 of O2 cost of Emax, R has to decrease by ~40% from 0.022 to 0.018 with 5°C change from 33 to 38°C. This suggests the Q10 of R to be ~0.5 comparable to that of RF. This Q10 of R is comparable to Q10 = 0.5–0.6 of Emax in our previous studies (18, 24, 31).

In our previous study, we attributed the Q10 = 2 of O2 cost of Emax only to an increased R (18). Therefore, we neither knew nor studied the temperature-dependent RF. The present study indicates the comparable importance of RF and R in the temperature-dependent O2 cost of Emax. The results would provide insights into pathophysiolo gy of failing hearts as well as development of favorable cardiotoxic agents that operate without adversely increasing O2 consumption for myocardial Ca2+ handling and contractility (15).

Limitations. The present model of total Ca2+ handling has a limitation. Our Ca2+ handling model combines the oscillatory component of the SR Ca2+ handling with the Morad and Goldman model (21). Their model assumes that SR has little delay in the availability of sequestered Ca2+ for the next release (21). This holds as long as the mechanical restitution is fully recovered, namely, after a relatively long beat interval (37). However, beat intervals even without pacing in the present heart preparation are too short for the full recovery of mechanical restitution (20). This seems to generate the oscillatory component on top of the exponential decay (20) (see APPENDIX).

We calculated RF from the exponential component of the alternans PESP after removing its oscillatory component using Eq. 1. Therefore, any possible interactions between the exponential and oscillatory components are excluded in the RF calculation. We have separately shown that RF is independent of the magnitude of the oscillatory component in the alternans PESP (26). We have also observed no significant electrical alternans during the alternans PESP (26). This supported little if any alternation of transsarcolemmal Ca2+ influx (26). However, more direct evidence remains to be obtained to support our RF calculation.

Although RF seems to help our understanding of cardiac mechanoenergetics (3), one may suspect that the alternans PESP as we have reported in series (3, 10, 13, 16, 19, 26–28) may not occur in the intact in situ heart. At least we have confirmed its existence in intact in situ left ventricles of anesthetized open-chest dogs using a conductance volumetric catheter with a pressure gauge (unpublished observation). Generality of the alternans PESP remains to be confirmed in normal human hearts. However, when the alternans PESP does not occur, RF will simply be obtained by the conventional method (21, 34, 35).

One must be careful to interpret Q10 < 1 of RF obtained in this study. We fixed the heart rate against
the temperature-dependent chronotropism in the present study. However, when we changed the heart rate at a constant temperature of 37°C, RF increased with heart rate (26). Therefore, RF would remain unchanged \((Q_{10} = 0)\) or even increase \((Q_{10} > 0)\) under the temperature-dependent chronotropism. This situation seems to account for the previously reported decreased RF at a lower temperature accompanied by a lower pacing rate in guinea pig papillary muscles (29).

\(Q_{10}\) has its own limitation. In general, \(Q_{10} \geq 1.5\) of a variable suggests it related directly or indirectly to chemical processes, whereas \(1.4 > Q_{10} \geq 1\) related to physical processes. \(1/Q_{10} > 1.5\) and \(1.4 > 1/Q_{10} \geq 1\) suggests them to be inversely related to chemical and physical reactions, respectively. However, when a variable of interest is of a complex biological (physicochemical) system, its \(Q_{10}\) or \(1/Q_{10}\) could fall between 1.4 and 1/1.4 even when its subsystem(s) is or are related to chemical processes (23). Nevertheless, the strength of \(Q_{10}\) in this study is the elucidation that all \(1/Q_{10}\) values of RF and the other related variables characterizing the PESP are \(>1.4\). This suggests that they are closely related to chemical reactions most likely including ATPase activities and hence ATP consumption in myocardial \(Ca^{2+}\) handling, but not merely to physical processes such as diffusion. Attribution of RF and the other related variables in Eq. 1 to the individual processes of \(Ca^{2+}\) handling is beyond the present scope.

We therefore conclude that the present findings validated our tested hypothesis. The temperature dependence of the \(Ca^{2+}\)-handling chemical processes could largely account for the temperature-dependent change in \(O_2\) cost of \(E_{\text{max}}\). The involved chemical processes are related to the recirculation fraction of \(Ca^{2+}\) within myocardial cells (RF) and contractile reactivity (R) to released \(Ca^{2+}\). This finding would provide insights into better pathophysiological understanding and effective treatment of failing hearts.

**APPENDIX**

In relation to recirculation fraction (RF of \(Ca^{2+}\) within myocardial cells) briefly explained in MATERIALS AND METHODS, the entire sarcoplasmic \(Ca^{2+}\) that are recruited (i.e., released and then removed) for each E-C coupling could be lumped into two fractions. The major fraction is the \(Ca^{2+}\) that is released into the sarcoplasma via the ryanodine-sensitive \(Ca^{2+}\) channel from SR and sequestered by its \(Ca^{2+}\) pump back into the SR in each contraction (4, 5, 21, 33–35). The other fraction is the \(Ca^{2+}\) that enters transsarcolemmally into the sarcoplasma predominantly via L-type \(Ca^{2+}\) channel and auxiliarily via the reverse mode of \(Na^{+}/Ca^{2+}\) exchange (4, 5, 21). This fraction is extruded predominantly via the forward mode of \(Na^{+}/Ca^{2+}\) exchange and auxiliarily via the sarcolemmal \(Ca^{2+}\) pump (4, 5, 21). Note that the total \(Ca^{2+}\) flux or transport of our interest is of the order of 10–100 \(\mu\)mol/kg, which is 50–100 times greater than the much more popular, free \(Ca^{2+}\) or \(Ca^{2+}\) transient of the order of 0.1–2 \(\mu\)mol/l (3, 4).

In steady-state, regular beats, these internal and external fractions are balanced under \(Ca^{2+}\) homeostasis (4, 5, 21). The former fraction is RF of our present interest. However, no method has been developed to determine the RF directly in steady-state contractions of a beating whole heart.

The standard method to estimate the RF is to perturb the \(Ca^{2+}\) homeostasis by inserting an extrastole or a test stimulation and analyze the decay rate of the following potentiated beats on the basis of the Morad and Goldman model (21, 34, 35). Figure 8A graphically shows RF in regular beats and potentiated beats (PESi, \(i = 1–6\)) decaying exponentially, as reported to be the most representative (21, 34, 35). Here, we defined the constant height of the lower dashed zone relative to unity (RFo) as the RF of regular beats and the exponentially decaying height of the upper dashed zone relative to the potentiated component of PESi (RFp) as the RF of PESi above unity.

The model assumes that RFo maintains the same released \(Ca^{2+}\) as the regular beat level for the unity contractility component below the horizontal line at 1 even during PESi, whereas RFp suddenly appearing in PESi as the RF of potentiated component above the horizontal line at 1.

We could reasonably assume ventricular contractility or \(E_{\text{max}}\) is to be proportional to the total released \(Ca^{2+}\) on the basis of the constancy of \(O_2\) cost of \(E_{\text{max}}\) despite the 70% increase in \(E_{\text{max}}\) in our previous studies (30). Then PESi has the potentiated contractility or released \(Ca^{2+}\) of \(1 + a\times RF_p^{(i-1)}\), of which 1 is the regular beat level. The RF of PESi is then given by the ratio of the total RF to the total contractility or \(Ca^{2+}\), i.e., \((RF_o + a\times RF_p^{(i-1)})/\text{[1 + a RF_p^{(i-1)}]}\). If the \(Ca^{2+}\) pumps, exchangers, and channels, and hence their integrated systems, nonlinearly handle different amounts of \(Ca^{2+}\), RFp could differ from RFo.
and vary among PESi. However, the exponential decay of potentiated contractility indicates a constant rate of decay and hence requires such a nonlinear Ca2+ handling. For the above ratio to be always equal to RF, at all i values, RF must be equal to RF_i among PESi. Namely, RF = RF_0 = RF_i = RF_o + RF_p. Only under this condition, the above (RF_0 + a RF_p)/1 + a RF_p/(1 - i) is equal to (RF + a RF_i)/(1 + a RF_i/(1 - i)) = RF [1 + a RF_i/(1 - i)] = RF regardless of PESi and regular beats around PESP. This condition is nothing but what Morad and Goldman assumed (21).

There are two methods to obtain this RF. One is the slope method. Contractilities of PESi (i = 1–6) are plotted on the abscissa against those of next potentiated beats (PESi + 1) on the ordinate (21, 34, 35). They line up linearly toward regular beat contractility point, although not shown here (21, 34, 35). This relationship means that contractility and hence released Ca2+ decay at a constant rate over PESi toward the regular beat level. This constant decay rate obtained as the slope of the linear regression line indicates the RF (21, 35). Therefore, although RF is obtained from PESi, the same RF is assumed to hold during the regular beats before and after PESi (21, 34, 35). The other method is to fit a monoexponential function, a exp(-i - 1/δ), to PESi above the regular beat level (21, 34, 35). Mathematically, RF = exp(-1/δ) (21, 35).

However, neither of these conventional methods is applicable to the alternans PESi that we observed consistently in canine hearts in the present and previous studies (Fig. 8B) (3, 11, 27). We have developed Eq. 1 to extract the same monoexponentially decaying component as in Fig. 8A by peeling off the oscillatory decay component from the alternans PESi (3, 11, 26, 27). We have already confirmed that beat constant τ_u and hence RF obtained by this method, remain unchanged despite wide changes in the extrasystolic coupling interval and the first postextrasystolic interval (26). This constancy of τ_u and RF also held, although a and b of Eq. 1 simultaneously changed considerably (26). We have also found that τ_u and RF change sensitivity with pathological conditions of canine hearts (3, 10, 13, 16, 27). We have therefore concluded Eq. 1 to facilitate a better understanding of physiology and pathophysiology of myocardial Ca2+ handling in a beating heart, although yet limited to canine excised, cross-circulated (blood-perfused) hearts (3).

Moreover, we have successfully confirmed that the mechanical restitution mechanism (37) could account for the oscillatory component of the alternans PESP at a relatively high heart rate (Iribi G, Kajiyi F, Araki J, and Suga H, “A new myocardial calcium dynamics model in E-C coupling.” Presented as a poster A-462 at Experimental Biology 2001, March 31-April 4, 2001, Orlando, FL; unpublished observations). We (i.e., Iribi et al.) have concluded in this unpublished study that our RF calculation based on the model (Fig. 1) and Eq. 1 is theoretically reasonable.

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