A new integrative method to quantify total Ca$^{2+}$ handling and futile Ca$^{2+}$ cycling in failing hearts

Shimizu, Juichiro, J unichi Araki, J u Mizuno, Shinyu Lee, Yi Syuu, Shingo Hosogi, Satoshi Mohri, Takeshi Mikane, Miyako Takaki, Tad W. Taylor, and Hiroyuki Suga. A new integrative method to quantify total Ca$^{2+}$ handling and futile Ca$^{2+}$ cycling in failing hearts. Am. J. Physiol. 275 (Heart Circ. Physiol. 44): H2325–H2333, 1998.—Ca$^{2+}$ handling in excitation-contraction coupling requires considerable O$_2$ consumption (VO$_2$) in cardiac contraction. We have developed an integrative method to quantify total Ca$^{2+}$ handling in normal hearts. However, its direct application to failing hearts, where futile Ca$^{2+}$ cycling via the Ca$^{2+}$-leaky sarcoplasmic reticulum (SR) required an increased Ca$^{2+}$ handling VO$_2$, was not legitimate. To quantify total Ca$^{2+}$ handling even in such failing hearts, we combined futile Ca$^{2+}$ cycling with Ca$^{2+}$ handling VO$_2$ and the internal Ca$^{2+}$ recirculation fraction via the SR. We applied this method to the canine heart mechanoenergetics before and after intracoronary ryanodine at nanomolar concentrations. We found that total Ca$^{2+}$ handling per beat was halved after the ryanodine treatment from ~60 µmol/kg left ventricle before ryanodine. We also found that futile Ca$^{2+}$ cycling via the SR increased to >1 cycle per beat after ryanodine from proportionally zero before ryanodine. These results support the applicability of the present method to the failing hearts with futile Ca$^{2+}$ cycling via the SR.

Excitation-contraction coupling; contractility; postextrasystolic potentiation; sarcoplasmic reticulum; ryanodine

Excitation-contraction (E-C) coupling requires Ca$^{2+}$ on the order of 20–100 µmol/kg myocardium to be bound with various intracellular Ca$^{2+}$ binding sites, including troponin C and calmodulin, in each cardiac contraction (1, 3, 6, 8, 10, 18, 22–24). These total Ca$^{2+}$ handling (flux or transport) values have been obtained either biochemically or physiologically using isolated contracting myocardial preparations. However, these analytic methods cannot be used readily to quantify total Ca$^{2+}$ handling in a beating whole heart (9, 29). Although the popular Ca$^{2+}$ transient methods detect sarcoplasmic free Ca$^{2+}$ concentrations on the order of 0.1–2 µmol/l in beating myocardium and whole heart preparations, they represent only a small fraction of the total Ca$^{2+}$ handling, left unbound with Ca$^{2+}$ binding sites (3, 5, 6, 11, 13, 16, 18, 22). Therefore, there has been no appropriate method to quantify total Ca$^{2+}$ handling in a beating whole heart.

We recently attempted to quantify total Ca$^{2+}$ handling in the left ventricle (LV) of the canine beating heart by combining LV myocardial O$_2$ consumption (VO$_2$) and the so-called intracellular Ca$^{2+}$ recirculation fraction (RF, the fraction of total released Ca$^{2+}$ that is sequestered by the sarcoplasmic reticulum (SR) Ca$^{2+}$-ATPase pump) (29). RF was obtained from the exponential decay constant of the postextrasystolic potentiation (PESP) (2, 9, 12, 14, 20, 21, 29, 33). Assuming no futile Ca$^{2+}$ cycling via the SR in normal hearts, we were able to estimate total Ca$^{2+}$ handling in control and enhanced contractile states produced with Ca$^{2+}$ and epinephrine (29). However, we could not apply this method to the failing hearts produced by infusing intracoronary ryanodine because this intervention led to energy-wasting, futile Ca$^{2+}$ cycling from the SR rendered leaky to Ca$^{2+}$ (9, 31). This is a serious limitation of our previous method (29). Accordingly, we have developed a new method that enables us to quantify total Ca$^{2+}$ handling in failing hearts that includes the energy-wasting Ca$^{2+}$ handling due to futile Ca$^{2+}$ cycling.

This new integrative method was applied to the mechanoenergetics and RF data of hearts treated with ryanodine to produce contractile failure and, presumably, futile Ca$^{2+}$ cycling (31) to estimate the feasibility of this method.

Methods

Background. Our cardiac mechanoenergetic framework separates VO$_2$ for E-C coupling from the total VO$_2$ of a beating heart (15, 25–27, 31). E-C coupling VO$_2$ is primarily due to the energy requirement of Ca$^{2+}$ handling because Na$^+$ handling energy for membrane excitation is negligibly small (1, 3, 25, 29). The present method integrates this framework with the RF concept and the different molar Ca$^{2+}$-ATPase stoichiometries of the energetically major Ca$^{2+}$ handling routes (1, 3, 8, 9, 17, 30). Figure 1 depicts these major intracellular (internal) and transsarcolemmal (external) Ca$^{2+}$ handling routes, including the sarcolemmal and SR Ca$^{2+}$ channels, the Ca$^{2+}$- and Na$^+$-K$^-$-ATPase pumps, and the Na$^+$/Ca$^{2+}$ exchangers (1, 3, 4, 8, 9, 11, 12, 17, 19, 22, 29, 30). Although we neglected the Na$^+$ handling energy for membrane excitation, we retained...
the Na\(^+\) handling energy by the Na\(^+\)-K\(^+\)-ATPase pump coupled with the Na\(^+\)/Ca\(^{2+}\) exchange (see below) (1, 3, 9, 17, 25, 29).

RF decreases and 1 − RF (the other fraction of total Ca\(^{2+}\) transported transsarcolemmally primarily by the Na\(^+\)/Ca\(^{2+}\) exchanger) reciprocally increases in various types of failing hearts (9, 14, 20). A constant RF and Ca\(^{2+}\) and Na\(^+\) homeostasis are maintained in steady-state beats. Otherwise, gradual changes in RF and internal Ca\(^{2+}\) and Na\(^+\) concentrations would alter myocardial contractility over beats, disrupting the steady state (3, 12, 29). To maintain the Ca\(^{2+}\) and Na\(^+\) homeostasis, the Na\(^+\)/K\(^+\) pump is coupled with the Na\(^+\)/Ca\(^{2+}\) exchange to remove the exchanged Na\(^+\) influx (3).

Ca\(^{2+}\) handling VO\(_2\) will increase with an internal-to-external shift of Ca\(^{2+}\) handling because the Na\(^+\)/Ca\(^{2+}\) exchange-Na\(^+\)-K\(^+\) pump system has the 1 Ca\(^{2+}\):1 ATP stoichiometry in contrast to the 2 Ca\(^{2+}\):1 ATP stoichiometry of the SR Ca\(^{2+}\) pump (see below) (1, 3, 9, 29). In fact, our studies have shown that ryanodine infused at nanomolar (not micromolar) concentrations into the coronary circulation of the excited cross-circulated canine heart preparation stably decreased both LV contractility and RF without significantly decreasing Ca\(^{2+}\) handling VO\(_2\) (9, 31). Therefore, we suspected that not only total Ca\(^{2+}\) handling but also RF are major determinants of the Ca\(^{2+}\) handling VO\(_2\) (9, 29).

We previously proposed an original formula to calculate total Ca\(^{2+}\) handled or transported in the E-C coupling process from experimentally obtained Ca\(^{2+}\) handling VO\(_2\) and RF values in normal hearts with presumably no futile Ca\(^{2+}\) cycling (29). This formula was successfully applied for the first time to canine normal LVs in control and contractile states enhanced by intracoronary Ca\(^{2+}\) and epinephrine (29). However, we could not apply this formula legitimately to pathological hearts that are presumably wasting Ca\(^{2+}\) handling VO\(_2\) due to futile Ca\(^{2+}\) cycling via the Ca\(^{2+}\)-leaky SR (9, 31). Therefore, we could not obtain an estimate of total Ca\(^{2+}\) handling in ryanodine-treated failing hearts, although we successfully estimated both Ca\(^{2+}\) handling VO\(_2\) and RF in these hearts (9). If futile Ca\(^{2+}\) cycling is occurring, total Ca\(^{2+}\) handling cannot simply be divided by RF into the internal and external fractions (9). Therefore, we developed the following new method.

New method. Figure 1A illustrates the Ca\(^{2+}\) handling model for our original method (29) and Fig. 1B illustrates that for our new method. Both have the internal and external Ca\(^{2+}\) handling routes in common. Both methods require Ca\(^{2+}\) handling VO\(_2\) and RF to divide the Ca\(^{2+}\) handling VO\(_2\) into the two major Ca\(^{2+}\) handling routes with the twofold different molar Ca\(^{2+}\):1 ATP stoichiometries (see below) (1, 3, 9, 17, 29, 30). However, the new method incorporates the futile Ca\(^{2+}\) cycling and the resultant extra VO\(_2\) into the original method (29), as shown by the third route (hatched loop) in Fig. 1B.

As shown in both Fig. 1A and Fig. 1B, Ca\(^{2+}\) enters the cell via the sarcolemmal Ca\(^{2+}\) channel and simultaneously Ca\(^{2+}\) is released into the sarcoplasm from the SR via its Ca\(^{2+}\) release channel (3, 11). Most of the Ca\(^{2+}\) is then bound to the Ca\(^{2+}\) binding sites, including not only troponin C to elicit cross-bridge cycling but also calmodulin, mitochondria, etc. (3, 6, 10, 11, 13, 16, 18, 22−25). The SR Ca\(^{2+}\) pump sequesters a considerable fraction, which is the RF, of the total Ca\(^{2+}\) by hydrolyzing ATP at a molar stoichiometry of 2 Ca\(^{2+}\):1 ATP (1, 3, 4, 7, 8, 30). The remaining fraction (1 − RF) of the total Ca\(^{2+}\) is predominantly extruded by the Na\(^+\)/Ca\(^{2+}\) exchanger and secondarily by the sarcolemmal Ca\(^{2+}\) pump (although not shown, but see below). The Na\(^+\)/Ca\(^{2+}\) exchanger coupled with the Na\(^+\)-K\(^+\) pump maintains Ca\(^{2+}\) and Na\(^+\) homeostasis at a molar stoichiometry of 1 Ca\(^{2+}\):1 ATP (see below) (1, 3, 4, 8, 17). Note that this stoichiometry is one-half that of the SR Ca\(^{2+}\) pump. In other words, the SR Ca\(^{2+}\) pump is economical and the Na\(^+\)/Ca\(^{2+}\) exchange coupled with the Na\(^+\)-K\(^+\) pump is one-half economical, or twice as wasteful, in myocardial Ca\(^{2+}\) handling.

We have assumed that Ca\(^{2+}\) cycling via the SR during each contraction is only once in normal hearts; i.e., the Ca\(^{2+}\)
transiently released and then sequestered by the SR Ca\(^{2+}\) pump during the same beat is no more released until the next beat (3, 29, 30). However, extra Ca\(^{2+}\) cycling is assumed to occur when the SR becomes leaky to Ca\(^{2+}\) in abnormal hearts such as those treated with ryanodine at nanomolar concentrations (3, 4, 9, 19, 31). Ryanodine at nanomolar concentrations bound to the SR Ca\(^{2+}\) release channel fixes the channel one-half open and makes it permeable to Ca\(^{2+}\) (in contrast to the complete channel closure achieved at micromolar concentrations of ryanodine) (4, 19, 31). In the Ca\(^{2+}\)-leaky SR, part of the Ca\(^{2+}\) sequestered by the Ca\(^{2+}\) pump may leak out and be sequestered again during the same beat. Hence, the internal Ca\(^{2+}\) handling includes extra Ca\(^{2+}\) cycling (N) above and beyond the normal Ca\(^{2+}\) cycle in each beat. N > 0 represents the existence of the futile Ca\(^{2+}\) cycling (4, 31) as modeled in Fig. 1B. The new method described in this paper takes the futile Ca\(^{2+}\) cycling into consideration. As for the reactivity (R), see below.

Formulation. We propose the following equation to calculate the amount of ATP consumed for total Ca\(^{2+}\) handling that includes the futile Ca\(^{2+}\) cycling

\[
\text{Ca}^{2+}\text{ handling ATP} = (\text{total Ca}^{2+}\text{ handling}) \cdot (\text{RF}/2 + (1 - \text{RF}) + N \times \text{RF/2})
\]

where the units of both Ca\(^{2+}\) handling ATP and total Ca\(^{2+}\) handling are micromoles per kilogram wet myocardium, N is dimensionless. As for the details of RF, see Recirculation fraction below.

In Eq. 1, (total Ca\(^{2+}\) handling)RF gives the amount (µmol/kg) of Ca\(^{2+}\) handling via the internal route (i.e., SR), and (total Ca\(^{2+}\) handling)(1 - RF) gives the amount (µmol/kg) of Ca\(^{2+}\) handling via the external route. These two terms were used in the original method (Fig. 1A) to determine total Ca\(^{2+}\) handling in normal hearts, which were assumed to have no futile Ca\(^{2+}\) cycling (29). The new term in this equation, (total Ca\(^{2+}\) handling)N × RF, is equal to N × (total Ca\(^{2+}\) handling)RF estimates the amount (µmol/kg) of Ca\(^{2+}\) handling by futile cycling (N > 0) via the SR (Fig. 1B).

The denominator 2 of two RF/2 terms in Eq. 1 is the coefficient 2 in the molar stoichiometry of 2 Ca\(^{2+}\)·1 ATP of the SR Ca\(^{2+}\) pump ATPase (1, 8, 30). The value of 2 for both (total Ca\(^{2+}\) handling)RF and (total Ca\(^{2+}\) handling)N × RF terms in Eq. 1 converts the respective quantities of Ca\(^{2+}\) handling via the SR into the respective amounts of ATP hydrolyzed for the normal and futile Ca\(^{2+}\) handling by the SR Ca\(^{2+}\) pump. The externally handled term, (total Ca\(^{2+}\) handling)(1 - RF), has 1, but not 2, in the denominator because its net stoichiometry is simply 1 Ca\(^{2+}\)·1 ATP as the result of the 3 Na\(^{+}\)/Ca\(^{2+}\) exchange and the 3 Na\(^{+}\)·2 K\(^{+}\)-ATP pump (1, 8, 17). The sarcolemmal Ca\(^{2+}\) pump, which secondarily removes Ca\(^{2+}\) from the cytoplasm, also has a 1 Ca\(^{2+}\)·1 ATP stoichiometry (3). As a result, RF/2 + (1 - RF) + N \times RF/2 is an overall stoichiometric factor to convert total Ca\(^{2+}\) handling into Ca\(^{2+}\) handling ATP.

Mitochondrial oxidative phosphorylation starting with such metabolic substrates as lactate and pyruvate has a nominal stoichiometry of atomic ratio of 3 P:1 O\(_2\) or molecular ratio of 6 P:1 O\(_2\), where P is the high-energy phosphate of ATP. The P-to-O ratio increases by ~5% when the metabolic substrate is glucose and decreases by a similar percentage when the substrates are free fatty acids. Therefore, the P-to-O ratio falls between 2.83 and 3.17 in practice (25). On the average, 3 is a reasonable P-to-O ratio in blood-perfused hearts under aerobic conditions where the metabolic substrates are physiological mixtures of lactate, glucose, and free fatty acids (25).

Table 1. Numerical listing of the assumptions and parameters of the present Ca\(^{2+}\) handling model

<table>
<thead>
<tr>
<th>Assumptions and Parameters</th>
<th>Normal Heart</th>
<th>Failing Heart</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of futile Ca(^{2+}) cycling, cycles/beat</td>
<td>0</td>
<td>≥0</td>
</tr>
<tr>
<td>Molar Ca(^{2+})-ATP stoichiometry of SR Ca(^{2+}) pump (dimensionless)</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Molar Ca(^{2+})-ATP stoichiometry of SL Ca(^{2+}) pump (dimensionless)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Molar Ca(^{2+})-ATP stoichiometry of SL Na(^{+})/Ca(^{2+}) exchange coupled with SL Na(^{+})-K(^{+}) pump (dimensionless)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Atomic P-to-O ratio of oxidative phosphorylation (dimensionless)</td>
<td>3</td>
<td>3</td>
</tr>
</tbody>
</table>

SR, sarcoplasmic reticulum; SL, sarcolemma. For details of assumptions and parameters, see text.

Table 1 is a numerical listing of the assumptions and parameters of the present Ca\(^{2+}\) handling model.

Equation 1 is modified to obtain Ca\(^{2+}\) handling VO\(_2\)

\[
\text{Ca}^{2+}\text{ handling VO}_2 = (\text{RF/2} + (1 - \text{RF}) + N \times \text{RF/2})
\]

where the unit of Ca\(^{2+}\) handling VO\(_2\) is micromoles per kilogram, and ½ converts ATP in micromoles per kilogram to VO\(_2\) in micromoles per kilogram.

Solving Eq. 2 for total Ca\(^{2+}\) handling yields

\[
\text{Total Ca}^{2+}\text{ handling} = 6\times(\text{Ca}^{2+}\text{ handling VO}_2) / \text{RF/2 + (1 - RF) + N \times RF/2}
\]

where both total Ca\(^{2+}\) handling and Ca\(^{2+}\) handling VO\(_2\) have the same dimensions of micromoles per kilogram.

To use a unit of milliliters per 100 grams for Ca\(^{2+}\) handling VO\(_2\), we modified Eq. 3 to

\[
\text{Total Ca}^{2+}\text{ handling} = 6\times10^7\times[(\text{Ca}^{2+}\text{ handling VO}_2)/22,400] / \text{RF/2 + (1 - RF) + N \times RF/2}
\]

where the unit of total Ca\(^{2+}\) handling is micromoles per kilogram and that of Ca\(^{2+}\) handling VO\(_2\) is milliliters per 100 grams myocardium (STPD, namely, 0°C, 1 atm, and dry).

Dividing both terms by Ca\(^{2+}\) handling VO\(_2\), Eq. 4 became

\[
\text{Total Ca}^{2+}\text{ handling} / \text{(Ca}^{2+}\text{ handling VO}_2) = 2.680 / \text{RF/2 + (1 - RF) + N \times RF/2}
\]

where 2.680 is a rounded-off value of 6 × 10^7/22,400. The left-hand term (total Ca\(^{2+}\) handling)/(Ca\(^{2+}\) handling VO\(_2\)) is the ratio of total Ca\(^{2+}\) handling to the VO\(_2\) needed for its handling. We designated this ratio as the "Ca\(^{2+}\) handling economy."

Figure 2A shows a family of curves derived from Eq. 5 with N as a parameter. This graph shows that total Ca\(^{2+}\) handling cannot be estimated even when both Ca\(^{2+}\) handling VO\(_2\) and RF are known unless we know N, which is not directly measurable.

However, when we assumed N = 0 in the normal hearts, we could estimate (total Ca\(^{2+}\) handling)/(Ca\(^{2+}\) handling VO\(_2\)) from RF (listed in Table 2), as indicated by the direction and order of arrows 1 and 2 in Fig. 2A. This is nothing but solving Eq. 5 for (total Ca\(^{2+}\) handling)/(Ca\(^{2+}\) handling VO\(_2\)) by substi-
We obtained (totalCa\textsuperscript{2+})\textit{E}_{\text{max}}\text{ has been considered to quantify the incremental number or amount of attached cross-bridges per unit increment in ventricular volume (26). \textit{E}_{\text{max}} has been widely used as a practically useful index of contractility to characterize ventricular performance and mechanical energy (15, 25, 26).

By definition, \textit{R} was given as

$$R = \frac{\text{\textit{E}_{\text{max}}}}{(\text{total Ca}\textsuperscript{2+} \text{ handling})} \tag{6}$$

where the unit of total Ca\textsuperscript{2+} handling is micromoles per kilogram and that of \textit{E}_{\text{max}} is millimeters mercury per milliliter per 100 grams. Therefore, the unit of \textit{R} is millimeters mercury per milliliter per 100 grams per micromole per kilogram.

Figure 2B shows a family of curves derived from Eq. 6 with \textit{R} as a parameter. This graph shows that the response of \textit{E}_{\text{max}} to total Ca\textsuperscript{2+} handling increases as an increasing function of \textit{R}. This indicates that total Ca\textsuperscript{2+} handling can be uniquely determined from \textit{E}_{\text{max}} if \textit{R} is given and vice versa. Inversely, the \textit{R} value could be determined if both \textit{E}_{\text{max}} and total Ca\textsuperscript{2+} handling are given.

In the normal hearts, we obtained \textit{R} values from their \textit{E}_{\text{max}} and total Ca\textsuperscript{2+} handling (listed in Table 2), the latter having been estimated in Fig. 2A, as the intersection of arrows 3 and 4 in Fig. 2B.

We then assumed that \textit{R} remained unchanged by ryanodine at nanomolar concentrations, taking advantage of its pharmacological specificity. It selectively fixes the ryanodine-sensitive Ca\textsuperscript{2+} channel of the SR one-half open without affecting the Ca\textsuperscript{2+} sensitivity of the troponin C and Ca\textsuperscript{2+} responsiveness of the contractile proteins (4, 19, 31). Therefore, we obtained total Ca\textsuperscript{2+} handling from the known \textit{E}_{\text{max}} (as listed in Table 2) using the same \textit{R} line as indicated by arrows 5 and 6 in Fig. 2B.

Then, this total Ca\textsuperscript{2+} handling was divided by its Ca\textsuperscript{2+} handling \textit{V}_{\text{O2}} to obtain Ca\textsuperscript{2+} handling economy on the y-axis of Fig. 2A. Arrows 7 and 8 start from the Ca\textsuperscript{2+} handling economy and RF to obtain \textit{N} as the intersection of these arrows. In reality, we solved Eqs. 5 and 6 for \textit{N} by first substituting the same \textit{R} as the normal \textit{R} into Eq. 6 and then substituting \textit{RF} and the obtained total Ca\textsuperscript{2+} handling into Eq. 5.

The method and steps we used to obtain the Ca\textsuperscript{2+} handling-related variables listed in Table 2 are described above. However, if we were not allowed to assume the unchanged \textit{R} in the failing hearts, Eqs. 5 and 6 could not have been solved for \textit{R} and \textit{N}. For such a case, refer to APPENDIX.

Recirculation fraction. We obtained the \textit{RF} in each contractile state by two different methods. The first method was the conventional one based on using the monotonically decaying PESP (12, 14, 20, 33), and the second one was our recently developed method to use the transient alternans PESP (2, 9, 21, 29). We had shown that RFs obtained by the two different methods were essentially the same in normal hearts (21). We had also shown that the same held either before or after ryanodine treatment (9). However, RFs obtained by the two different methods were significantly decreased equally by ryanodine treatment (9). We utilized these previously obtained RF data (9) in the present study.

The details of RF determination were described in our previous paper (9). Briefly, we measured peak isovolumic pressures of regular beats and the 1st to 6th postextrasystolic beats (PES1–6) of both monotonic and alternans decay types of PESP (9, 21). These pressures were normalized with respect to the regular beat pressure. The normalized pressure

![Fig. 2. Theoretical relations among total Ca\textsuperscript{2+} handling, Ca\textsuperscript{2+} handling \textit{V}_{\text{O2}}, RF, \textit{E}_{\text{max}}, \textit{R}, and \textit{N}. A: family of curves relating Ca\textsuperscript{2+} handling economy [(total Ca\textsuperscript{2+} handling)/(Ca\textsuperscript{2+} handling \textit{V}_{\text{O2}})] to RF with \textit{N} as a parameter, derived from Eq. 5. B: family of curves relating \textit{E}_{\text{max}} to total Ca\textsuperscript{2+} handling with \textit{R} as a parameter, derived from Eq. 6. Arrows 1–8 indicate steps to obtain estimates of unknown variables from known variable values.](http://ajpheart.physiology.org/)

**Fig. 2. Theoretical relations among total Ca\textsuperscript{2+} handling, Ca\textsuperscript{2+} handling \textit{V}_{\text{O2}}, RF, \textit{E}_{\text{max}}, \textit{R}, and \textit{N}.**

**A.** Number of futile Ca\textsuperscript{2+} cycling (N = 0 cycles).

**B.** Reactivity \textit{R} = 0.1 ([mmHg/ml 100g]/[\mu mol/kg]).

---

**Here, \textit{E}_{\text{max}} is a mechanical load-independent index of ventricular contractility that Suga et al. proposed in 1973 (28); see also Ref. 26. \textit{E}_{\text{max}} represents the maximum elastance of the LV chamber at the end of systole or the slope of the LV end-systolic pressure-volume relation line (26, 28).**

---

**Arrows 1–8 indicate steps to obtain estimates of unknown variables from known variable values.**
values of every set of PES1–6 of the monotonic type were fitted by an exponential equation

\[ y = a \cdot \exp \left[ -\frac{(x - 1)}{\tau_a} \right] + 1 \]  

where \( a \) is the amplitude of PES1, \( x \) is the ordinal number of PES1–6, and \( \tau_a \) is the beat constant of the exponential decay of the PES (9). Those of the alternans type were fitted by an exponential-sinusoidal equation

\[ y = a \cdot \exp \left[ -\frac{(x - 1)}{\tau_a} \right] + b \cdot \exp \left[ -\frac{(x - 1)}{\tau_s} \right] \cdot \cos \left[ \pi (x - 1) \right] + 1 \]  

where \( a \) and \( b \) are the amplitudes of the exponential and sinusoidal decay components of PES1 and \( \tau_s \) is the beat constant of the sinusoidal decay. We had shown that time constant \( \tau_s \) but not \( \tau_a \) was related to RF (9, 21, 29).

We then calculated RF using \( RF = \exp(-1/\tau_a) \) (9, 12, 21, 29), where \( 1 \) means 1 beat and hence \( 1/\tau_a \) is a dimensionless (beat/beat) fraction of 1-beat interval relative to \( \tau_a \). Therefore, \( \exp(-1/\tau_a) \) indicates the decremental fraction of PES (namely, RF, also dimensionless) within one beat, as first shown by Morad and Goldman (12). This formula has been used for years by other investigators (14, 33).

RF during steady-state beats cannot be directly assessed, but it can be assessed during PESP intervening in the steady-state beats. In the contemporary model of total Ca\( ^{2+} \) handling, a constant RF in steady-state beats is assumed to hold during the successive PESP (12, 14, 20, 33). The basic assumption to obtain RF from the PESP decay has been that the contractility changes are proportional to the beat-to-beat changes in the total Ca\( ^{2+} \) released into the sarcoplasm and bound to Ca\( ^{2+} \) binding sites including troponin C (12, 14). This assumption seems reasonable on the basis of the linear relationship between myocardial tension and total Ca\( ^{2+} \) bound intracellularly within the working range of contractile force development (3, 6).

The constancy of RF over each monotonically decaying PESP has been confirmed in a number of studies by the exponential curve fitting of the decay (9, 14, 20, 21, 33). A constant RF is mathematically equivalent to a constant ratio of PES pressure decay (12). This RF has usually been obtained as the slope of a linear regression line of contractility of the next beats (PES\( \times x + 1 \)) on contractility of the present beats (PES\( \times x \)) (12, 14, 20, 33), where \( x \) is the ordinal number of the PES beats. The same RF is mathematically obtainable from the beat constant \( \tau_a \) of the exponential decay of PESP by \( RF = \exp(-1/\tau_a) \) (12, 14, 20, 33).

Although these ratio and time constant methods are theoretically equivalent, we preferred to use the time constant method because we were able to directly fit a nonlinear curve to the PESP decay by the least-squares method using DeltaGraph (Delta Point, Monterey, CA) on a Power Macintosh computer (Apple Japan, Tokyo, Japan). We had obtained RF values before and after ryanodine treatment (9). Their mean \( \pm \) SD values obtained from both monotonic and alternans types documented in the original paper (31). Briefly, ryanodine was continuously infused at a constant rate of 1.4 \( \pm \) 0.5 nmol/min into the cross-circulated heart via the coronary arterial circulation for \( \sim \)1 h (31). Because coronary flow was 51 \( \pm \) 21
ml·min⁻¹·100 g LV⁻¹, we calculated the intracoronary concentration of ryanodine to be 29 ± 13 nmol/l coronary blood. Note that ryanodine at nanomolar (not micromolar) concentrations did not abolish the Ca²⁺ release function of the SR (19, 31). E max gradually fell to nearly one-half of control over 1 h. We obtained mechanoenergetics data, including LV pressure and volume, E max, total VO₂, and unloaded VO₂, in steady-state beats with constant atrial pacing. We found that spontaneous PESP occurred sporadically. Basal metabolic VO₂ was measured under KCl arrest at the end of each experiment. Ca²⁺ handling VO₂ was obtained by subtracting basal metabolic VO₂ from unloaded VO₂ at zero pressure-volume area.

RESULTS AND DISCUSSION

To test the feasibility of the present method in cases with N > 0, we applied it to our previous mechanoenergetics data obtained in the ryanodine-treated failing canine LVs (9, 31). Table 2 lists the experimentally obtained mechanoenergetics data (31). The E max and VO₂ values are the mean ± SD values during control conditions before the ryanodine treatment and in the failing condition after the ryanodine treatment (9, 31).

The RF values in Table 2 are the data that we had calculated from the beat constants (rₐ) of the exponential decay of the monotonically decaying PESP and the exponential decay component of the transient alternans PESP (9).

We assumed N = 0 in the control contractile state before ryanodine and N > 0 in the depressed contractile state after ryanodine treatment (9, 31). We also assumed that R remained virtually unchanged by the ryanodine treatment at nanomolar concentrations (4, 9, 31). The bases of these assumptions are described in METHODS.

Table 2 lists all the resultant Ca²⁺-related values calculated from the mechanoenergetics and RF data by the present method. Although the RF values obtained from the monotonics and alternans PESPs were not significantly different (9), we combined these RF values separately with the same representative set of mechanoenergetic data as shown by the two PESP-type columns (patterns A and C) in Table 2. The resultant total Ca²⁺ handling values for both RF values are comparable either before or after ryanodine. The resultant total Ca²⁺ handling values were considerably smaller after ryanodine than before. Moreover, these total Ca²⁺ handling values in both control and ryanodine-treated hearts fell within the physiological range (20–100 µmol/kg) documented in the literature (1, 3, 6, 8, 10, 18, 22–24).

We obtained total Ca²⁺ handling of 56 (from pattern A) and 60 (from pattern C) µmol/kg at a baseline E max of 4.2 mmHg·ml⁻¹·100 g. This E max value is approximately one-third to one-fourth of the maximum E max with high doses of intracoronary Ca²⁺ or catecholamines (15, 28). Considering the maximum Ca²⁺ binding capacity of 20–100 µmol/kg of intramyocardial sites including troponin C and calmodulin (3, 6, 10, 16, 22, 23), the ~60 µmol/kg of Ca²⁺ could be interpreted as a rather high value for the baseline E max.

However, our present Ca²⁺ handling values are dynamic values calculated on a per-beat basis, whereas the apparently maximum Ca²⁺ binding capacity values were biochemically determined under steady-state conditions. The rate constants of Ca²⁺ binding to troponin C and the other Ca²⁺ binding sites (18, 22) indicate that Ca²⁺ binding to them is not immediate after the Ca²⁺ release. This suggests that part of the released Ca²⁺ may be removed before effective binding to the Ca²⁺ binding sites. Our simulation study of Ca²⁺ kinetics, similar to that of Robertson et al. (18), has shown that a significant fraction (20–30%) of the total released Ca²⁺ has been sequestered by the SR by the time of one-half peak force development (13). Therefore, our total Ca²⁺ handling values could be considered reasonable if part of the total released Ca²⁺ is removed by the SR Ca²⁺ pump immediately after its release and is not used effectively for E-C coupling (3, 7).

The high-affinity Ca²⁺ binding sites (50–70 µmol/kg, 2 Ca²⁺ per 1 troponin C molecule) of troponin C (25–35 µmol/kg) seem to be almost fully saturated even at a low diastolic free Ca²⁺ level (3, 6, 10, 23). Therefore, the total Ca²⁺ handling we obtained appears to be related to the beat-to-beat changes in the amount of Ca²⁺, which are proportional to contractile force development in excess of the stably bound amount of Ca²⁺ independent of the twitch force development. The beat-to-beat change in Ca²⁺ includes that bound to the low-affinity Ca²⁺ specific sites (25–35 µmol/kg, 1 Ca²⁺ per 1 troponin C molecule) of troponin C as well as other Ca²⁺ bound to calmodulin and all other Ca²⁺ binding sites on a per-beat basis (3, 7).

Because we assumed the same R after ryanodine (see Table 2) as that before ryanodine, the resultant Ca²⁺ handling was proportional to E max before and after ryanodine (Fig. 2B). Despite the considerably decreased total Ca²⁺ handling from 56–60 to 31–33 µmol/kg after ryanodine, Ca²⁺ handling VO₂ was only little decreased after ryanodine, as seen in Table 2. This disproportionately high value for Ca²⁺ handling VO₂ in the ryanodine-treated heart is now accounted for by the combination of the significantly decreased RF and markedly increased N despite the nearly halved total Ca²⁺ handling associated directly with E-C coupling.

The present method has several limitations. First of all, the present model may appear too simple to calculate accurately total Ca²⁺ handling because of the numerous assumptions incorporated into the model. However, we have included the major ATP-consuming processes of Ca²⁺ handling and neglected minor ones on the basis of the contemporary knowledge (1, 3, 4, 8, 9, 11, 12, 17, 19, 22, 29, 30). Although the new knowledge of other minor Ca²⁺ handling processes is available (3, 11, 22), it cannot readily be incorporated into our model, because it was obtained by reductionistic methodology in isolated, but not in situ, myocytes of animals other than dogs. For example, we neglected the contribution of the reverse mode of the Na⁺/Ca²⁺ exchanger to the transsarcolemmal Ca²⁺ entry according to its relatively small contribution in normal rabbit myocytes (3). However, this contribution is yet unknown in canine hearts. Therefore, even if we adopt reductionistic knowledge obtained in rabbit myocytes into the
present model, this may not guarantee improvement of the model. Nevertheless, the present study warrants further efforts to improve the integrative approach toward better understanding of myocardial Ca\textsuperscript{2+} handling by integrative methods.

Despite this limitation, we would consider that our estimates of total Ca\textsuperscript{2+} handling in Table 2 appear reasonably realistic in the light of the literature data (1, 3, 6, 8, 10, 18, 22–24). Any of Eqs. 1–5 indicates mathematically that a relatively small error in RF, N, and Ca\textsuperscript{2+} handling VO\textsubscript{2} due to our neglect of minor Ca\textsuperscript{2+} handling processes would produce a comparably small error in total Ca\textsuperscript{2+} handling.

In relation to this limitation, we assumed N = 0 in the normal hearts before the ryanodine treatment and the same R value before and after ryanodine to estimate total Ca\textsuperscript{2+} handling. As described in Formulation and Appendix, if we had not used these assumptions, we could not have obtained estimates of N, R, and total Ca\textsuperscript{2+} handling values after ryanodine treatment. However, if one were satisfied with mechanoenergetic differentiation between normal and failing hearts, the assumptions for N and R are not required. Then, comparison of the R-N relation between them would be a very helpful new method (Fig. 3C).

Second, we used mechanoenergetics and PESP data documented only in our previous studies (9, 31) to test the feasibility of our new method. We had already applied the part with N = 0 to a baseline E\textsubscript{max} in a different group of normal hearts (29). There, the representative data were E\textsubscript{max} of 4.7 mmHg/ml, Ca\textsuperscript{2+} handling VO\textsubscript{2} of 0.011 ml O\textsubscript{2}/beat, both per 100 grams, and RF of 0.55. All these values are close to the present control data listed in Table 2. N was assumed to be 0 in the normal hearts in control and enhanced contractile states with Ca\textsuperscript{2+} and epinephrine (29) as in the normal hearts in control contractile state before ryanodine in the present study. Then, the calculated total Ca\textsuperscript{2+} handling was 40 µmol/kg, which is of the same order as the present control value for total Ca\textsuperscript{2+} handling. This similarity among different groups of normal hearts supports the feasibility of the present method for the hearts with N = 0. However, the feasibility of the formula with N > 0 was tested for the first time in this study. We must admit that the present method remains to be tested using various types of failing hearts whose Ca\textsuperscript{2+} handling processes are abnormal, although some limitation remains, as explained in Appendix.

A third limitation would be our assumption that residual cross-bridge cycling does not contribute to the Ca\textsuperscript{2+} handling VO\textsubscript{2}. Although residual cross-bridge cycling appeared to contribute considerably to unloaded VO\textsubscript{2} in rabbit hearts (34), we have obtained evidence supporting the view that residual cross-bridge cycling energy, if any, is negligibly small in the Ca\textsuperscript{2+} handling VO\textsubscript{2} of canine blood-perfused hearts (32).

Other limitations are that we only used average RF and mechanoenergetics data to calculate total Ca\textsuperscript{2+} handling and that the intracoronary ryanodine concentrations were at most, 40 nmol/l. Because we only used PESPs following spontaneous extrasystoles, which occurred sporadically, we could not deal with a larger number of matched sets of mechanoenergetics and RF data. Therefore, we had to use the average data as listed in Table 2 as representative values. As for the ryanodine concentration, E\textsubscript{max} gradually decreased to one-half of the control over 1 h during continuous intracoronary infusion of ryanodine at 1.4 nmol/min (31). E\textsubscript{max} was already low enough to judge the heart to be in a failing state (31). Moreover, end-diastolic pressure started to rise in isovolumic contractions at a fixed intermediate volume (31). We could not obtain any stable data thereafter. Because of these limitations, future controlled studies are warranted.
In conclusion, our present heart-level method may contribute to a better understanding of total Ca\(^{2+}\) handling in ryanodine-treated, failing hearts characterized by futile Ca\(^{2+}\) cycling. We consider this to be so because no better methods are yet available to achieve the same goal. When using this method, one must realize the limitations remaining to be overcome. The utility of this integrative analysis method may be increased by conquering these limitations.

**APPENDIX**

We eliminated total Ca\(^{2+}\) handling by combining Eqs. 5 and 6 and obtained

\[
E_{\text{max}}/(\text{Ca}^{2+}\text{ handling } V_{O_2}) = 2.680R/[RF/2 + (1 - RF) + N \times RF/2]
\]  

(9)

Here, \(E_{\text{max}}/(\text{Ca}^{2+}\text{ handling } V_{O_2})\) is equal to the reciprocal of the slope that we have designated as the “\(O_2\) cost of \(E_{\text{max}}\)” that is the ratio of Ca\(^{2+}\) handling \(V_{O_2}\) to \(E_{\text{max}}\) (13, 15, 25, 26, 31).

Therefore, we rewrote Eq. 9 as

\[
O_2\text{ cost of } E_{\text{max}} = \frac{[RF/2 + (1 - RF) + N \times RF/2]/2.680}{R}
\]  

(10)

We designated the reciprocal of the \(O_2\) cost of \(E_{\text{max}}\) as “\(E_{\text{max}}\) economy,” as listed in Table 2. In Eq. 10, both \(O_2\) cost of \(E_{\text{max}}\) and \(RF\) are measurable, but \(N\) and \(R\) are unknown and to be obtained. Rearranging Eq. 10 yields

\[
N = \frac{[5.360R(\text{Cost of } E_{\text{max}}) + RF - 2]/RF}{2}
\]  

(11)

Equation 11 indicates that \(N\) is a linear function of \(R\) with both \(O_2\) cost of \(E_{\text{max}}\) and \(RF\) as parameters. Figure 3A shows a family of representative R-N relations for a given \(O_2\) cost of \(E_{\text{max}}\) with \(RF\) as a parameter. Figure 3B shows another family of representative R-N relations for a given \(RF\) with \(O_2\) cost of \(E_{\text{max}}\) as a parameter. Thus, when both \(O_2\) cost of \(E_{\text{max}}\) and \(RF\) are given, one R-N relation line is specified.

Figure 3C shows four specific R-N relations for the representatives of \(O_2\) cost of \(E_{\text{max}}\) and \(RF\) (listed in Table 2) in the normal hearts and the ryanodine-treated, failing hearts. The upper two lines are of the failing hearts. The two lower lines are of the normal hearts. In both pairs, the upper line corresponds to the normal heart as studied by postextrasystolic transient alternans. We consider this to be so because no better methods are yet available to achieve the same goal. When using this method, one must realize the limitations remaining to be overcome. The utility of this integrative analysis method may be increased by conquering these limitations.