Superior Logistic Model for Decay of Ca^{2+} Transient and Isometric Relaxation Force Curve in Rabbit and Mouse Papillary Muscles

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SUMMARY

A decrease in myocardial intracellular calcium concentration ($[\text{Ca}^{2+}]_i$) precedes relaxation, and a monoexponential function is typically used for fitting the decay of the Ca^{2+} transient. However, a logistic function has been shown to be a better fit for the relaxation force curve, compared to the conventional monoexponential function. In the present study, we compared the logistic and monoexponential functions for fitting the $[\text{Ca}^{2+}]_i$ declines, which were measured using the aequorin method, and isometric relaxation force curves at 4 different onsets: the minimum time-derivative of $[\text{Ca}^{2+}]_i$ ($d[\text{Ca}^{2+}]_i/dt_{\min}$) and force ($dF/dt_{\min}$), and the 10%, 20% and 30% lower $[\text{Ca}^{2+}]_i$ levels and forces over the data-sampling period in 7 isolated rabbit right ventricular and 15 isolated mouse left ventricular papillary muscles. Logistic functions were significantly superior for fitting the $[\text{Ca}^{2+}]_i$ declines and relaxation force curves, compared to monoexponential functions. Changes in the normalized logistic $[\text{Ca}^{2+}]_i$ decline and relaxation force time constants at the delayed onsets relative to their 100% values at $d[\text{Ca}^{2+}]_i/dt_{\min}$ and $dF/dt_{\min}$ were significantly smaller than the changes in the normalized monoexponential time constants. The ratio of the logistic relaxation force time constant relative to the logistic $[\text{Ca}^{2+}]_i$ decline time constant was significantly smaller in mouse than in rabbit. We conclude that the logistic function more reliably characterizes the $[\text{Ca}^{2+}]_i$ decline and relaxation force curve at any onset, irrespective of animal species. Simultaneous analyses using the logistic model for decay of the Ca^{2+} transient and myocardial lusitropism might be a useful strategy for analysis of species-specific myocardial calcium handling. (Int Heart J 2007; 48: 215-232)

Key words: Lusitropism, Logistic function, Monoexponential function, Curve fitting, Relaxation time constant, Goodness of fit, Calcium handling, Species differences, Onset

CARDIAC contraction and relaxation are related to myocardial intracellular calcium concentrations ($[\text{Ca}^{2+}]_i$).^{1,2)} In mammalian cardiac muscles, a Ca^{2+} transient

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precedes the cross-bridge (CB) attachment that leads to force development. However, the attachment of the CB influences the affinity of troponin (Tn) for $[Ca^{2+}]_i$, which secondarily alters the relative timing and amplitude of the $[Ca^{2+}]_i$ decline and the relaxation force.^{3,4)} This feedback mechanism leads to a steeper or more cooperative force- $[Ca^{2+}]_i$ relationship, so that a given rise in systolic $[Ca^{2+}]_i$ produces a greater force than is evident in the absence of feedback.

A monoexponential function has been used for fitting the decay of the Ca^{2+} transient in the isovolumic left ventricle (LV) in excised rat hearts,⁵⁻⁷⁾ and in mouse,⁸⁻¹⁰⁾ rabbit,⁹⁻¹¹⁾ rat,¹¹⁻¹³⁾ canine⁹⁾ and human myocytes.⁹⁾ Therefore, the monoexponential $[Ca^{2+}]_i$ decline time constant ($Ca\tau_E$) has been used as an index of $[Ca^{2+}]_i$ decline. The monoexponential function has also been proposed as a reasonably good fit for the isovolumic relaxation LV pressure curve in the excised canine heart,¹⁴⁾ leading to use of the monoexponential relaxation pressure time constant ($P\tau_E$) as an index of relaxation rate or lusitropism.¹⁵⁾ The relationships between $P\tau_E$,⁵⁻⁷⁾ LV weight,⁶⁾ cell relengthening⁸⁾ or half-life of relaxation and $Ca\tau_E$ ¹¹⁾ have also been examined.

Despite the widespread use of monoexponential functions, the relaxation pressure curves may not be precisely characterized by this function as shown in semilogarithmic plots of pressure versus time¹⁶⁾ and the first time-derivative of pressure (dP/dt) versus time.¹⁷⁾ Furthermore, a logistic function has been proven to fit the isovolumic relaxation LV pressure curve in the excised canine heart more accurately, compared to the conventional monoexponential function,¹⁸⁾ and a logistic relaxation pressure time constant ($P\tau_L$) has been proposed and found to be a better ventricular lusitropic index than the $P\tau_E$.^{18,19)} Moreover, the logistic function provides a better fit to the isometric relaxation force curve in the ferret right ventricular (RV) papillary muscle, compared to a monoexponential function,²⁰⁾ and the logistic relaxation force time constant ($F\tau_L$) has been proposed as a better myocardial lusitropic index than the monoexponential relaxation force time constant ($F\tau_E$).²⁰⁾ Furthermore, logistic fitting has been used in both animal experiments, such as for the rabbit RV papillary muscle²¹⁾ and trabeculae,²¹⁾ and calf,²²⁾ rat²³⁾ and ferret hearts,²⁴⁾ and clinical settings.²⁵⁾

The time of the maximum rate of LV pressure fall (ie, the minimum time-derivative of pressure (dP/dt_{min})) has been employed as an index of activity of the relaxation system.²⁶⁾ dP/dt_{min} was first defined as the starting point of the relaxation pressure curve for monoexponential fitting,¹⁴⁾ and subsequently monoexponential fitting from the start of dP/dt_{min} has been used widely.^{16,17,27)} However, it is evident that there is a considerable gap between the best-fit monoexponential curve and the actual relaxation curve near dP/dt_{min} .¹⁸⁾ Similarly, the maximum rate of decline (ie, the minimum time-derivative of force (dF/dt_{min})) has been used as the onset of relaxation,²⁸⁾ however, other starting points for relaxation have

also been used.²⁹⁾ Furthermore, the maximum rate of the $[\text{Ca}^{2+}]_i$ decline (ie, minimum time-derivative of $[\text{Ca}^{2+}]_i$ ($d[\text{Ca}^{2+}]_i/dt_{\min}$)) has been used as the onset of the $[\text{Ca}^{2+}]_i$ decline;⁵⁾ but other starting points for the $[\text{Ca}^{2+}]_i$ decline have been used.⁶⁾ Therefore, the best starting point for the $[\text{Ca}^{2+}]_i$ decline and relaxation force curve remains unclear, and whether and how the onset of relaxation affects both logistic and monoexponential fitting remain to be elucidated.

In the present study, we compared logistic and monoexponential fitting of the decay of Ca^{2+} transients and relaxation force curves with different onsets for data sampling periods in different animal muscles.

METHODS

This study protocol was approved by the Animal Investigation Committee of The Jikei University School of Medicine. All procedures were conducted in conformity with the Guiding Principles for Research Involving Animals and Human Beings endorsed by both the American Physiological Society and the Physiological Society of Japan.

Surgical preparation: Seven male albino rabbits (Japan White, 2.0-2.5 kg) and 15 mice (C57BL6J, 25-30 g) were anesthetized with pentobarbital sodium (50 mg/kg i.v.). After a surgical plane of anesthesia was achieved, the hearts were quickly removed. The coronary blood was washed out with Tyrode solution via the aorta. The RV wall of rabbit or the LV wall of mouse was opened and a thin papillary muscle was isolated. The muscle was mounted horizontally in an experimental chamber, immersed in a bath, and continuously perfused with Tyrode solution at 30°C. One end of the muscle was connected to the lever of an electromagnetic motor (JCCX-101A, General Scanning, Watertown, MA, USA) that was used to alter muscle length, and the other end was attached to the arm of a tension transducer (BG-10, Kulite Semiconductor Products, Leonia, NJ, USA; compliance, 2.5 $\mu\text{m/g}$, unloaded resonant frequency, 0.6 kHz). A pair of black platinum electrodes was placed in parallel with the muscle, which was stimulated by a single square pulse of 5 ms duration at 0.2 Hz; the strength of the stimulation was 1.5-fold the threshold. The muscle was slowly stretched and adjusted to a length where the tension reached a maximum ($L_{\max}$).

Tyrode solution: N-2-hydroxy-ethylpiperazine-N'-2-ethanesulfonic acid (HEPES)-buffered Tyrode solution was used during muscle dissection and as a perfusate. The composition of the solution was as follows (in mmol/L): 136.9 NaCl, 5.4 KCl, 0.5 MgCl_2 , 0.33 NaHPO_4 , 5 HEPES, and 5 glucose. The pH was adjusted to 7.40 ± 0.05 with NaOH at 24°C and equilibrated with 100% O_2 . The temperature of the solution was continuously monitored with a thermocouple and maintained at $30 \pm 0.5^\circ\text{C}$.

Measurement of $[Ca^{2+}]_i$ with aequorin: Aequorin was purchased from Dr. J.R. Blinks (Friday Harbor, WA). It was dissolved in 150 mM KCl and 5 mM N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid at pH 7.0, at a final aequorin concentration of 50-100 μ mol/L. Using a glass micropipette, aequorin was pressure-injected into 150-200 superficial cells of the preparation while the membrane potential was monitored. Aequorin light signals were detected with a photomultiplier (EMI 9789A, Ruislip, UK) mounted in a small housing,³⁰⁾ and the aequorin light signal and tension were recorded simultaneously. Aequorin light signals were converted to $[Ca^{2+}]_i$ using an *in vitro* calibration curve.^{31,32)} The constants used in the experiment were as follows: n , 3.14; KR, 4,025,000; KTR, 114.6.³³⁾ Sixty-four aequorin light signals were averaged to improve the signal-to-noise ratio of $[Ca^{2+}]_i$. $[Ca^{2+}]_i$ levels were sampled at 1 ms intervals and digitized with an A/D converter. All $[Ca^{2+}]_i$ data were stored on tape (NFR-3515W, Sony Magnescale, Tokyo) and a computer (PC-9801, NEC, Tokyo) for later analysis.

$[Ca^{2+}]_i$ decline: First, $d[Ca^{2+}]_i/dt$ was obtained by differentiating the sampled data after digital smoothening using an 11-point, nonweighted moving average of digitized $[Ca^{2+}]_i$ signals. The relationship of fitting of the $[Ca^{2+}]_i$ decline with the starting point was studied using 4 starting points: $d[Ca^{2+}]_i/dt_{min}$ (0%Ca), and 10%, 20% and 30% lower $[Ca^{2+}]_i$ levels (-10%Ca, -20%Ca and -30%Ca, respectively). Here, 100% indicates the $[Ca^{2+}]_i$ level at $d[Ca^{2+}]_i/dt_{min}$, rather than the peak $[Ca^{2+}]_i$ level. The endpoint of the $[Ca^{2+}]_i$ decline was defined as the point when the fall in isometric force returned to the resting tension level. The endpoint of the $[Ca^{2+}]_i$ decline corresponded to the time when $[Ca^{2+}]_i$ returned to close to the resting $[Ca^{2+}]_i$ level; so-called zero μ mol/L. Therefore, 4 individual $[Ca^{2+}]_i$ decline terms from 0%Ca, -10%Ca, -20%Ca and -30%Ca to the endpoint were examined: 0%CaD, -10%CaD, -20%CaD and -30%CaD, respectively.

Functions for $[Ca^{2+}]_i$ decline: The following logistic function was used to fit the $[Ca^{2+}]_i$ decline, using the least squares method in DeltaGraph 4.0 (DeltaPoint, Monterey, CA, USA):

$$Ca(t) = Ca_A / [1 + \exp(t/Ca\tau_L)] + Ca_B \quad \text{Eq. 1}$$

where Ca_A is the logistic $[Ca^{2+}]_i$ decline amplitude constant, t is the time from $d[Ca^{2+}]_i/dt_{min}$, $Ca\tau_L$ is the $[Ca^{2+}]_i$ decline time constant of the exponential term in the denominator, and Ca_B is the logistic $[Ca^{2+}]_i$ decline non-zero asymptote. The logistic curve given by Eq.1 decreases monotonically and apparently exponentially, but not in an S-shaped manner; despite its exponential appearance, Eq.1 is not mathematically exponential.

The following monoexponential function was also used to fit the $[Ca^{2+}]_i$ decline:⁵⁻⁷⁾

$$Ca(t) = Ca_0 \exp(-t/Ca\tau_E) + Ca_\infty \quad \text{Eq. 2}$$

Where Ca_0 is the monoexponential $[Ca^{2+}]_i$ decline amplitude constant, t is the time from $d[Ca^{2+}]_i/dt_{min}$, $Ca\tau_E$ is the monoexponential $[Ca^{2+}]_i$ decline time constant, and Ca_∞ is the monoexponential $[Ca^{2+}]_i$ decline non-zero asymptote.

Tension: Tension was measured using a storage oscilloscope (7TO7A, NEC San-ei, Tokyo). The force signals were sampled at 1 ms intervals and digitized with an A/D converter. All tension data were simultaneously stored on a computer (PC-9801, NEC, Tokyo) for later analysis.

Relaxation force curve: First, dF/dt was obtained by differentiating data that was digitally smoothened using an 11-point, nonweighted moving average of digitized force signals. The relationship of these data with the starting point of fitting for the relaxation force curve was determined. The starting points were the tension at dF/dt_{min} (0%RF), and the 10%, 20% and 30% lower tensions (-10%RF, -20%RF and -30%RF, respectively). Here, 100% indicates the tension at dF/dt_{min} , rather than the peak isometric tension. The endpoint of relaxation was identified as the point when the isometric force returned to the resting tension level. The time of this endpoint corresponded to the time when the developed tension reached zero mN/mm^2 . From the preliminary data, we defined the endpoints as 900 and 490 ms from the muscle twitch in rabbit and mouse, respectively. Therefore, 4 individual isometric relaxation force terms from 0%RF, -10%RF, -20%RF and -30%RF to the endpoint of relaxation were examined: 0%RFC, -10%RFC, -20%RFC and -30%RFC, respectively.

Functions for relaxation force curve: The following logistic function was used to fit the isometric relaxation force curve using the least squares method.²⁰⁾

$$F(t) = F_A / [1 + \exp(t/F\tau_L)] + F_B \quad \text{Eq. 3}$$

where F_A is the logistic relaxation force amplitude constant, t is the time from dF/dt_{min} , $F\tau_L$ is the logistic relaxation force time constant, and F_B is the logistic relaxation force non-zero asymptote.

The following monoexponential function was also used to fit the isometric relaxation force curve:

$$F(t) = F_0 \exp(-t/F\tau_E) + F_\infty \quad \text{Eq. 4}$$

where F_0 is the monoexponential relaxation force amplitude constant, t is the time from dF/dt_{min} , $F\tau_E$ is the monoexponential relaxation force time constant, and F_∞ is the monoexponential relaxation force non-zero asymptote.

Calculation: Ca_A , $Ca\tau_L$ and Ca_B were calculated by Eq. 1 and Ca_0 , $Ca\tau_E$ and Ca_∞ by Eq. 2 for 0%CaD, -10%CaD, -20%CaD and -30%CaD in each muscle. Similarly, F_A , $F\tau_L$ and F_B were calculated by Eq. 3, and F_0 , $F\tau_E$ and F_∞ by Eq. 4 for 0%RFC, -10%RFC, -20%RFC and -30%RFC in each muscle. The comparison was repeated for all 28 $[Ca^{2+}]_i$ declines and relaxation force curves in 7 rabbit muscles, and for all 60 $[Ca^{2+}]_i$ declines and relaxation force curves in 15 mouse muscles.

Starting points: For the different starting points from $d[Ca^{2+}]_i/dt_{min}$ for the $[Ca^{2+}]_i$ decline, we selected $[Ca^{2+}]_i$ levels relative to the peak $[Ca^{2+}]_i$ level of the same ratio as the tension at 0%RF (dF/dt_{min}), -10%RF, -20%RF and -30%RF relative to the peak isometric tension (0%CaF, -10%CaF, -20%CaF and -30%CaF). Ca_A , Ca_{τ_L} and Ca_B were calculated by Eq. 1 and Ca_0 , Ca_{τ_E} and Ca_{∞} by Eq. 2 for the different individual $[Ca^{2+}]_i$ declines from the four %CafS to the endpoint $[Ca^{2+}]_i$ level: 0%CaFD, -10%CaFD, -20%CaFD and -30%CaFD, respectively.

Statistical analysis: The goodness of fit was evaluated by comparing the correlation coefficient (r) of the logistic fit with r of the monoexponential fit. A paired Student t test was applied to r after Fisher's Z transformation: $Z = 1/2 [\ln(1+r) - \ln(1-r)]$.³⁴⁾ The residual (the best-fit logistic or monoexponential value at a sampling point of the best-fit curves minus the actual value) was analyzed relative to the corresponding $[Ca^{2+}]_i$ decline and relaxation force curve. The residual mean squares (RMS) also quantifies the goodness of fit, and this was calculated as the residual sum of squares divided by the residual degrees of freedom, which indicates the number of data points analyzed minus the number of parameters in the function.³⁵⁾ Logistic and monoexponential RMS values were compared using the Wilcoxon signed-rank test.

Ca_{τ_L} , Ca_{τ_E} , Ca_B and Ca_{∞} were compared among the four %CaDs or %CaFDs, and F_{τ_L} , F_{τ_E} , F_B and F_{∞} were compared among the four %RFCs by one-way repeated measures analysis of variance (RANOVA). When the RANOVA was significant ($P < 0.05$), multiple comparisons among the four %CaDs, %CaFDs or %RFCs were performed using the post-hoc Scheffé test.

nCa_{τ_L} and nCa_{τ_E} were analyzed for -10%CaD, -20%CaD and -30%CaD after normalization relative to their 100% values at 0%CaD, and $n\tau_L$ and $n\tau_E$ were analyzed for -10%RFC, -20%RFC and -30%RFC after normalization relative to their 100% values at 0%RFC. The differences in Ca_B (dCa_B) between 0%CaD and each %CaD, in Ca_{∞} (dCa_{∞}) between 0%CaD and the corresponding %CaD, in FB_B (dFB_B) between 0%RFC and each %RFC, and in F_{∞} (dF_{∞}) between 0%RFC and the corresponding %RFC were examined. nCa_{τ_L} with nCa_{τ_E} at each %CaD, $n\tau_L$ with $n\tau_E$ at each %FC, dCa_B with dCa_{∞} at each %CaD, and dFB_B with dF_{∞} at each %RFC were also compared using the paired Student t test.

The intersection of Ca_{τ_L} at 0%CaD and F_{τ_L} at the corresponding 0%RFC (I_{τ_L} at 0%SP), and the intersection of Ca_{τ_E} at 0%CaD and F_{τ_E} at the corresponding 0%RFC (I_{τ_E} at 0%SP) were plotted. Similarly, the intersections of Ca_{τ_L} at -10%CaD, -20%CaD and -30%CaD and F_{τ_L} at the corresponding -10%RFC, -20%RFC and -30%RFC (I_{τ_L} at -10%SP, -20%SP and -30%SP), as well as Ca_{τ_E} at the three %CaDs and F_{τ_E} at the corresponding %RFCs (I_{τ_E} at -10%SP, -20%SP and -30%SP) were plotted. The ratio of F_{τ_L} at the %RFC relative to Ca_{τ_L} at the corresponding %CaD (F/Ca_{τ_L}) was compared with the ratio of F_{τ_E} at the %RFC

relative to $\text{Ca } \tau_E$ at the corresponding %CaD ($F/\text{Ca } \tau_E$) by the paired Student t test. Furthermore, $F/\text{Ca } \tau_L$ and $F/\text{Ca } \tau_E$ at each %SP in rabbit were compared with these data in mouse, again using the unpaired Student t test.

Analyses were performed using Microsoft Excel 98 (Microsoft-Japan, Tokyo), Statcel (OMS, Saitama, Japan) and StatView 5.0 (SAS Institute Inc, Cary, NC, USA) software. The measured and calculated values are expressed as the mean $\pm$ SD. A P value < 0.05 was considered to indicate statistical significance.

RESULTS

Muscle preparation: Muscle characteristics, $[\text{Ca}^{2+}]_i$ levels at the four %CaDs and tension values at the four %RFCs in the 7 rabbit RV and 15 mouse LV papillary muscles are listed in Table I.

Table I. Muscle Characteristics, $[\text{Ca}^{2+}]_i$ Declines, and Relaxation Force Curves at 4 Starting Points

	Rabbit	Mouse
Muscle diameter (mm)	0.73 ± 0.13	0.63 ± 0.11
Muscle length (mm)	3.30 ± 0.77	2.01 ± 0.43
Peak $[\text{Ca}^{2+}]_i$ level ($\mu\text{mol/L}$)	0.73 ± 0.10	1.70 ± 0.39
$[\text{Ca}^{2+}]_i$ level at 0%Ca ($\mu\text{mol/L}$)	0.43 ± 0.05	1.26 ± 0.44
$[\text{Ca}^{2+}]_i$ level at -10%Ca ($\mu\text{mol/L}$)	0.38 ± 0.08	1.14 ± 0.39
$[\text{Ca}^{2+}]_i$ level at -20%Ca ($\mu\text{mol/L}$)	0.34 ± 0.06	1.00 ± 0.35
$[\text{Ca}^{2+}]_i$ level at -30%Ca ($\mu\text{mol/L}$)	0.30 ± 0.06	0.88 ± 0.30
$d[\text{Ca}^{2+}]_i/dt_{\min}$ at 0%Ca ($\mu\text{mol/L/ms}$)	-10.9 ± 2.8	-20.9 ± 4.6
Peak tension (mN/mm^2)	15.6 ± 2.6	8.3 ± 6.1
Tension at 0%RF (mN/mm^2)	9.0 ± 1.1	5.6 ± 4.0
Tension at -10%RF (mN/mm^2)	8.1 ± 1.1	5.0 ± 3.6
Tension at -20%RF (mN/mm^2)	7.3 ± 0.9	4.5 ± 3.2
Tension at -30%RF (mN/mm^2)	6.4 ± 0.8	3.9 ± 2.8
$dF/dt_{\min}$ at 0%RF ($\text{mN/mm}^2/\text{ms}$)	-70.5 ± 13.6	-79.8 ± 52.0
dF/dt at -10%RF ($\text{mN/mm}^2/\text{ms}$)	-67.8 ± 12.8	-78.7 ± 51.7
dF/dt at -20%RF ($\text{mN/mm}^2/\text{ms}$)	-67.2 ± 13.3	-77.0 ± 51.3
dF/dt at -30%RF ($\text{mN/mm}^2/\text{ms}$)	-64.7 ± 12.7	-73.5 ± 49.5
$[\text{Ca}^{2+}]_i$ level at 0%CaF ($\mu\text{mol/L}$)	0.42 ± 0.07	1.15 ± 0.25
$[\text{Ca}^{2+}]_i$ level at -10%CaF ($\mu\text{mol/L}$)	0.39 ± 0.07	1.03 ± 0.23
$[\text{Ca}^{2+}]_i$ level at -20%CaF ($\mu\text{mol/L}$)	0.35 ± 0.05	0.94 ± 0.19
$[\text{Ca}^{2+}]_i$ level at -30%CaF ($\mu\text{mol/L}$)	0.30 ± 0.05	0.81 ± 0.17

Values are the mean $\pm$ SD in 7 rabbit RV and 15 mouse LV papillary muscles.

Figure 1. Representative logistic and monoexponential fits for $[Ca^{2+}]_i$ declines and relaxation force curves. **A** and **B** show representative logistic and monoexponential fits (thick dashes), respectively, for one 0%CaD (thin dots); and **C** and **D** show representative logistic and monoexponential fits, respectively, for one 0%RFC in rabbit RV papillary muscle. **E** and **F** show representative logistic and monoexponential fits, respectively, for one 0%CaD; and **G** and **H** show representative logistic and monoexponential fits, respectively, for one 0%RFC in mouse LV papillary muscle. Insets list the best-fit logistic and monoexponential $[Ca^{2+}]_i$ decline amplitude constants (Ca_A and Ca_0), time constants (CaT_L and CaT_E), non-zero asymptotes (Ca_B and Ca_∞), correlation coefficients (r) and residual mean squares (RMS), and the best-fit logistic and monoexponential relaxation force amplitude constants (F_A and F_0), time constants (FT_L and FT_E), non-zero asymptotes (F_B and F_∞), r and RMS.

Table II. Logistic (Ca_A) and Monoexponential $[Ca^{2+}]_i$ Decline Amplitude Constants (Ca_0), and Logistic (F_A) and Monoexponential Relaxation Force Amplitude Constants (F_0) at 4 Starting Points

	0%CaD	-10%CaD	-20%CaD	-30%CaD
Rabbit				
Ca_A ($\mu\text{mol/L}$)	0.81 ± 0.11	0.71 ± 0.12	0.61 ± 0.11	0.54 ± 0.07
Ca_0 ($\mu\text{mol/L}$)	0.43 ± 0.06	0.38 ± 0.06	0.33 ± 0.06	0.29 ± 0.04
Mouse				
Ca_A ($\mu\text{mol/L}$)	2.50 ± 0.87	2.27 ± 0.77	1.98 ± 0.67	1.71 ± 0.58
Ca_0 ($\mu\text{mol/L}$)	1.35 ± 0.47	1.22 ± 0.41	1.06 ± 0.36	0.92 ± 0.31
	0%RFC	-10%RFC	-20%RFC	-30%RFC
Rabbit				
F_A (mN/mm^2)	18.7 ± 2.4	16.6 ± 2.2	14.9 ± 1.9	12.9 ± 1.7
F_0 (mN/mm^2)	10.1 ± 1.3	8.9 ± 1.2	8.0 ± 1.1	7.0 ± 0.9
Mouse				
F_A (mN/mm^2)	11.3 ± 8.1	10.1 ± 7.2	8.9 ± 6.3	7.7 ± 5.5
F_0 (mN/mm^2)	6.1 ± 4.4	5.4 ± 3.9	4.8 ± 3.4	4.1 ± 2.9

Values are the mean $\pm$ SD in 7 rabbit RV and 15 mouse LV papillary muscles.

Curve fitting: The best-fit logistic and monoexponential curves for one 0%CaD in a rabbit papillary muscle are shown in Figures 1A and 1B, respectively, and those in a mouse papillary muscle are shown in Figures 1E and 1F, respectively. The best-fit logistic and monoexponential curves for 0%RFC in the same rabbit and mouse papillary muscles were almost identical (Figures 1C, 1D, 1G and 1H).

Amplitude constant: Ca_A and Ca_0 values for the four %CaDs, and F_A and F_0 values for the four %RFCs in rabbit and mouse are listed in Table II.

Goodness of fit: The logistic r values for 0%CaD in rabbit and for 0%CaD and -10%CaD in mouse were significantly greater than the corresponding monoexponential r values (Table III), and the logistic RMS values for 0%CaD and -10%CaD in rabbit and for the four %CaDs in mouse were significantly smaller than the corresponding monoexponential RMS values. The logistic r values for the four %RFCs in both rabbit and mouse were significantly greater than the cor-

Table III. Correlation Coefficients (r) and Residual Mean Squares (RMS) of Logistic and Monoexponential Fits for $[\text{Ca}^{2+}]_i$ Declines and Relaxation Force Curves at 4 Starting Points

	0%CaD	-10%CaD	-20%CaD	-30%CaD
Rabbit				
Logistic r (Z)	0.9570 ($1.91 \pm 0.18^*$)	0.9425 (1.76 ± 0.19)	0.9268 (1.64 ± 0.21)	0.9065 (1.51 ± 0.23)
Monoexponential r (Z)	0.9548 (1.88 ± 0.18)	0.9416 (1.75 ± 0.19)	0.9253 (1.62 ± 0.21)	0.9058 (1.50 ± 0.22)
Logistic RMS ($\mu\text{mol/L}$) ²	$0.00077 \pm 0.00030^\#$	0.00076 ± 0.00030	0.00069 ± 0.00028	0.00077 ± 0.00033
Monoexponential RMS ($\mu\text{mol/L}$) ²	0.00081 ± 0.00031	0.00078 ± 0.00032	0.00071 ± 0.00028	0.00077 ± 0.00031
Mouse				
Logistic r (Z)	0.9969 ($3.23 \pm 0.41^*$)	0.9963 ($3.15 \pm 0.41^*$)	0.9953 (3.03 ± 0.42)	0.9940 (2.90 ± 0.44)
Monoexponential r (Z)	0.9949 (2.98 ± 0.40)	0.9946 (2.95 ± 0.42)	0.9938 (2.88 ± 0.44)	0.9924 (2.78 ± 0.44)
Logistic RMS ($\mu\text{mol/L}$) ²	$0.00068 \pm 0.00055^\#$	$0.00066 \pm 0.00055^\#$	$0.00063 \pm 0.00055^\#$	$0.00061 \pm 0.00056^\#$
Monoexponential RMS ($\mu\text{mol/L}$) ²	0.00107 ± 0.00072	0.00094 ± 0.00065	0.00082 ± 0.00061	0.00075 ± 0.00061
	0%RFC	-10%RFC	-20%RFC	-30%RFC
Rabbit				
Logistic r (Z)	0.9999 ($4.84 \pm 0.61^{**}$)	0.9999 ($4.91 \pm 0.33^{**}$)	0.9999 ($4.97 \pm 0.42^{**}$)	0.9999 ($4.96 \pm 0.60^*$)
Monoexponential r (Z)	0.9978 (3.41 ± 0.16)	0.9982 (3.52 ± 0.19)	0.9986 (3.63 ± 0.24)	0.9990 (3.78 ± 0.30)
Logistic RMS (mN/mm^2) ²	$0.0027 \pm 0.0021^\#$	$0.0013 \pm 0.0009^\#$	$0.0009 \pm 0.0005^\#$	$0.0008 \pm 0.0005^\#$
Monoexponential RMS (mN/mm^2) ²	0.0411 ± 0.0109	0.0187 ± 0.0089	0.0126 ± 0.0065	0.0076 ± 0.0012
Mouse				
Logistic r (Z)	0.9996 ($4.39 \pm 0.40^{**}$)	0.9998 ($4.55 \pm 0.33^{**}$)	0.9998 ($4.66 \pm 0.25^{**}$)	0.9998 ($4.69 \pm 0.39^{**}$)
Monoexponential r (Z)	0.9971 (3.27 ± 0.17)	0.9976 (3.37 ± 0.18)	0.9981 (3.48 ± 0.20)	0.9985 (3.61 ± 0.23)
Logistic RMS (mN/mm^2) ²	$0.0026 \pm 0.0033^{##}$	$0.0012 \pm 0.0014^{##}$	$0.0006 \pm 0.0008^{##}$	$0.0005 \pm 0.0011^{##}$
Monoexponential RMS (mN/mm^2) ²	0.0197 ± 0.0248	0.0123 ± 0.0151	0.0071 ± 0.0087	0.0039 ± 0.0046

r values are the means after Z transformation (Z), and Z and RMS values are the mean $\pm$ SD in 7 rabbit RV and 15 mouse LV papillary muscles; *: $P < 0.05$, **: $P < 0.001$ compared with the monoexponential r ; #: $P < 0.05$, ##: $P < 0.001$ compared with the monoexponential RMS.

Figure 2. Logistic ($\text{Ca}\tau_l$) and monoexponential $[\text{Ca}^{2+}]_i$ decline time constants ($\text{Ca}\tau_e$) and logistic ($F\tau_l$) and monoexponential relaxation force time constants ($F\tau_e$) at 4 starting points. **A-H** show the means $\pm$ SD in 7 rabbit RV and 15 mouse LV papillary muscles, respectively. **A** and **E** show $\text{Ca}\tau_l$ (closed squares) and $\text{Ca}\tau_e$ (open circles), respectively, for 0%CaD, -10%CaD, -20%CaD and -30%CaD; *, $P < 0.05$, **, $P < 0.001$ compared with 0%CaD; †, $P < 0.001$ compared with -10%CaD; ‡, $P < 0.05$ compared with -20%CaD. **B** and **F** show the relative $n\text{Ca}\tau_l$ (closed squares) and $n\text{Ca}\tau_e$ (open circles), respectively, for -10%CaD and -30%CaD normalized with respect to their 100% values at 0%CaD; §, $P < 0.05$, §§, $P < 0.001$ between $n\text{Ca}\tau_l$ and $n\text{Ca}\tau_e$; NS indicates no significant difference. **C** and **G** show $F\tau_l$ (closed squares) and $F\tau_e$ (open circles), respectively, for 0%RFC, -10%RFC, -20%RFC and -30%RFC; **, $P < 0.001$ compared with 0%RFC; †, $P < 0.05$, ††, $P < 0.001$ compared with -10%RFC; ‡, $P < 0.001$ compared with -20%RFC. **D** and **H** show the relative $nF\tau_l$ (closed squares) and $nF\tau_e$ (open circles), respectively, for -10%RFC, -20%RFC and -30%RFC normalized with respect to their 100% values at 0%RFC; §§, $P < 0.001$ between $nF\tau_l$ and $nF\tau_e$.

responding monoexponential r values, and the logistic RMS values for the four %RFCs in both rabbit and mouse were significantly smaller than the corresponding monoexponential RMS values.

Time constant: $\text{Ca}\tau_l$ and $\text{Ca}\tau_e$ and $F\tau_l$ and $F\tau_e$ decreased significantly for the delayed %CaDs and %RFCs in both rabbit and mouse (Figures 2A, 2C, 2E and 2G). However, there were significant differences between $n\text{Ca}\tau_l$ and $n\text{Ca}\tau_e$ in mouse (Figure 2F), and between $nF\tau_l$ and $nF\tau_e$ in rabbit and mouse (Figures 2D and 2H). These data show that the change in $F\tau_l$ was smaller than that in $F\tau_e$ in rabbit, and that changes in $\text{Ca}\tau_l$ and $F\tau_l$ were smaller than in $\text{Ca}\tau_e$ and $F\tau_e$ in mouse. There were significant differences between $F/\text{Ca}\tau_l$ and $F/\text{Ca}\tau_e$ at 0%SP in rabbit (Figure 3A) and at the four %SPs in mouse (Figures 3E-H). Both $F/\text{Ca}\tau_l$ and $F/\text{Ca}\tau_e$ at each %SP were significantly smaller in mouse (Figures 3E-H) than in rabbit (Figures 3A-D).

Figure 3. Relationships between logistic $[\text{Ca}^{2+}]_i$ decline time constant ($\text{Ca}\tau_L$) and logistic relaxation force time constant ($F\tau_L$), and between monoexponential $[\text{Ca}^{2+}]_i$ decline time constant ($\text{Ca}\tau_E$) and monoexponential relaxation force time constant ($F\tau_E$) at 4 starting points. **A-D** show $I\tau_L$ (closed squares) and $I\tau_E$ (open circles) at 0%SP, -10%SP, -20%SP and -30%SP in 7 rabbit RV papillary muscles, respectively. **E-H** show $I\tau_L$ (closed squares) and $I\tau_E$ (open circles) at the four %SPs in 15 mouse LV papillary muscles, respectively. The dotted lines show identical lines. There are significant differences between $F/\text{Ca}\tau_L$ and $F/\text{Ca}\tau_E$ at 0%SP in rabbit ($P < 0.05$) and 0%SP, -10%SP, -20%SP and -30%SP in mouse ($P < 0.0001$). $F/\text{Ca}\tau_L$ and $F/\text{Ca}\tau_E$ at the four %SPs are smaller in mouse than in rabbit ($P < 0.005$).

Non-zero asymptote: Ca_B and Ca_∞ and F_B and F_∞ increased significantly for the delayed %CaDs and %RFCs in both rabbit and mouse (Figures 4A, 4C, 4E and 4G). However, there were significant differences between $d\text{Ca}_B$ and $d\text{Ca}_\infty$ and between dF_B and dF_∞ (Figures 4B, 4D, 4F and 4H). These data show that differences in Ca_B and F_B were smaller than in Ca_∞ and F_∞ .

Starting point: The logistic r values for -10%CaFD and -20%CaFD in rabbit and for the four %CaFDs in mouse were significantly greater than the corresponding monoexponential r values (Table IV). The logistic RMS values for -10%CaFD and -20%CaFD in rabbit and for the four %CaFDs in mouse were significantly smaller than the corresponding monoexponential RMS values. The changes in $\text{Ca}\tau_L$, $\text{Ca}\tau_E$, Ca_B and Ca_∞ for delayed %CaFDs were almost the same as the changes in the respective data for delayed %CaDs.

Figure 4. Logistic (Ca_B) and monoexponential $[Ca^{2+}]_i$ decline non-zero asymptotes (Ca_∞), and logistic (F_B) and monoexponential relaxation force non-zero asymptotes (F_∞) at 4 starting points. **A-H** show the means $\pm$ SD in 7 rabbit RV and 15 mouse LV papillary muscles, respectively. **A** and **E** show Ca_B (closed squares) and Ca_∞ (open circles), respectively, for 0%CaD, -10%CaD, -20%CaD and -30%CaD; *: $P < 0.05$, **: $P < 0.001$ compared with 0%CaD; †: $P < 0.05$, ††: $P < 0.001$ compared with -10%CaD. **B** and **F** show dCa_B (closed squares) between 0%CaD and the other %CaDs, and dCa_∞ (open circles) between the corresponding %CaDs, respectively; §: $P < 0.05$, §§: $P < 0.001$ between dCa_B and dCa_∞ . **C** and **G** show F_B (closed squares) and F_∞ (open circles), respectively, for 0%RFC, -10%RFC, -20%RFC and -30%RFC; *: $P < 0.05$, **: $P < 0.001$ compared with 0%RFC; †: $P < 0.05$, ††: $P < 0.001$ compared with -10%RFC. **D** and **H** show dF_B (closed squares) between 0%RFC and the other %RFCs, and dF_∞ (open circles) between the corresponding %RFCs, respectively; §: $P < 0.05$, §§: $P < 0.001$ between dF_B and dF_∞ .

Table IV. Correlation Coefficients (r) and Residual Mean Squares (RMS) of Logistic and Monoexponential Fits for $[Ca^{2+}]_i$ Declines at 4 Starting Points From the $[Ca^{2+}]_i$ Level Relative to Peak $[Ca^{2+}]_i$ of the Same Ratio as the Tension for 0%RF (dF/dt_{min}), -10%RF, -20%RF and -30%RF Relative to the Peak Isometric Tension

	0%CaFD	-10%CaFD	-20%CaFD	-30%CaFD
Rabbit				
Logistic r (Z)	0.9594 (1.94 ± 0.31)	0.9449 ($1.78 \pm 0.36^*$)	0.9288 ($1.65 \pm 0.36^*$)	0.9126 (1.54 ± 0.32)
Monoexponential r (Z)	0.9415 (1.85 ± 0.27)	0.9416 (1.75 ± 0.35)	0.9250 (1.62 ± 0.34)	0.9103 (1.53 ± 0.32)
Logistic RMS ($\mu\text{mol/L}$) ²	0.00093 $\pm$ 0.00037	0.00092 $\pm$ 0.00038#	0.00091 $\pm$ 0.00039#	0.00091 $\pm$ 0.00040
Monoexponential RMS ($\mu\text{mol/L}$) ²	0.00100 $\pm$ 0.00035	0.00097 $\pm$ 0.00038	0.00095 $\pm$ 0.00038	0.00093 $\pm$ 0.00041
Mouse				
Logistic r (Z)	0.9965 ($3.18 \pm 0.32^*$)	0.9959 ($3.09 \pm 0.34^*$)	0.9951 ($3.01 \pm 0.35^*$)	0.9940 ($2.90 \pm 0.38^*$)
Monoexponential r (Z)	0.9950 (3.00 ± 0.37)	0.9942 (2.92 ± 0.37)	0.9934 (2.85 ± 0.36)	0.9920 (2.76 ± 0.37)
Logistic RMS ($\mu\text{mol/L}$) ²	0.00067 $\pm$ 0.00056#	0.00063 $\pm$ 0.00055#	0.00061 $\pm$ 0.00056#	0.00057 $\pm$ 0.00056#
Monoexponential RMS ($\mu\text{mol/L}$) ²	0.00092 $\pm$ 0.00065	0.00083 $\pm$ 0.00060	0.00078 $\pm$ 0.00061	0.00073 $\pm$ 0.00061

r values are the means after Z transformation (Z), and Z and RMS values are the mean $\pm$ SD in 7 rabbit RV and 15 mouse LV papillary muscles; *: $P < 0.05$, *: $P < 0.001$ compared with the monoexponential r ; #: $P < 0.05$ compared with the monoexponential RMS.

DISCUSSION

Goodness of logistic fit: The results demonstrate that the goodness of logistic fitting for decays of the Ca^{2+} transient and relaxation force curves is superior to that for the monoexponential function, regardless of onset and irrespective of animal species (for rabbit and mouse muscles). Changes in the logistic fit for $[\text{Ca}^{2+}]_i$ declines and relaxation force curves with delayed starting points are smaller than those for the corresponding monoexponential fits. Such superiority of logistic fitting for the $[\text{Ca}^{2+}]_i$ decline over the conventional monoexponential function has not been reported previously, whereas the superiority of logistic fitting for the relaxation force curve has been found in previous studies.¹⁸⁻²⁵⁾ Therefore, logistic fitting enables $\text{Ca}\tau_L$ and $F\tau_L$ to be obtained as more reliable indices for decay of the Ca^{2+} transient and myocardial lusitropism.

Calcium handling and relaxation: $[\text{Ca}^{2+}]_i$ is a central factor in excitation-contraction (E-C) coupling. As systolic $[\text{Ca}^{2+}]_i$ falls, Ca^{2+} dissociates from the Tn complex and decreases the interaction between actin and myosin, thereby lessening the pressure or force generation and initiating relaxation. The lusitropic performance of the myocardium depends on how promptly $[\text{Ca}^{2+}]_i$ is sequestered into the sarcoplasmic reticulum (SR).³⁶⁾ The Ca^{2+} affinity of the Tn complex also determines relaxation, and the rate of relaxation depends on which of these processes is rate-limiting.^{3,4)} A linear relationship between $F\tau_E$ and $\text{Ca}\tau_E$ has been shown, even in low flow ischemia caused by graded reductions of coronary flow⁵⁾ and with inhibition of the SR Ca^{2+} -ATPase with cyclopiazonic acid.⁷⁾ In contrast, there is no relationship between $F\tau_E$ and $\text{Ca}\tau_E$ in mild to moderate hypertrophic hearts.⁶⁾ Taken together, these data support the contention that cardiac lusitropism is caused by the rate of the $[\text{Ca}^{2+}]_i$ decline. Our results show that $F/\text{Ca}\tau_L$ differs from $F/\text{Ca}\tau_E$, suggesting that $F\tau_L$ and $\text{Ca}\tau_L$ are determined by recycling of Ca^{2+} stores and the Tn- Ca^{2+} affinity, which are more accurate and reasonable indices than $F\tau_E$ and $\text{Ca}\tau_E$. Therefore, simultaneous analyses of the decay of the Ca^{2+} transient and myocardial lusitropism using a logistic model might be useful for studying myocardial calcium handling.

Species difference: The rate of $[\text{Ca}^{2+}]_i$ decline and cardiac lusitropism are dependent on the species, and each species has its own time constants. The integrated dynamic balance of $[\text{Ca}^{2+}]_i$ is determined by Ca^{2+} influx through the sarcolemma via voltage-dependent L-type Ca^{2+} channels, by the SR Ca^{2+} load, and by the system of Ca^{2+} removal via the Na^+ - Ca^{2+} exchanger from the myocardium to the extracellular medium. Species differences are mainly dependent on the duration of Ca^{2+} release from the SR induced by Ca^{2+} influx through L-type Ca^{2+} channels and the Na^+ - Ca^{2+} exchanger.³⁷⁾ Specifically, the rate of $[\text{Ca}^{2+}]_i$ decline reflects the activities of the SR Ca^{2+} -ATPase and the Na^+ - Ca^{2+} exchanger. For instance, $\text{Ca}\tau_E$

is smaller in mouse myocytes than in rabbit myocytes,⁹⁾ the SR Ca^{2+} content is greater in mouse than in rabbit, and the peak increase in L-type Ca^{2+} current is smaller in mouse. The SR Ca^{2+} ATPase is about 2- to 3-fold faster in rat myocytes than in rabbit myocytes, whereas the Na^{+} - Ca^{2+} exchanger is about 2- to 3-fold faster in rabbit than in rat.¹¹⁾ The SR Ca^{2+} ATPase is more powerful than the Na^{+} - Ca^{2+} exchanger, and the dominance is much more marked in rat (approximately 13-fold) than in rabbit (2- to 5-fold). Our results show that the $[\text{Ca}^{2+}]_i$ level in rabbit is smaller than that in mouse, although tension in rabbit is larger than in mouse (Table I). $\text{Ca}\tau_L$ and $\text{Ca}\tau_E$ and $F\tau_L$ and $F\tau_E$ in rabbit are slower than those in mouse (Figure 2). $\text{Ca}\tau_L$ in rabbit and mouse is 53 and 40 ms, respectively, and $F\tau_L$ in rabbit, ferret²⁰⁾ and mouse is 65, 54 and 33 ms, respectively. $F/\text{Ca}\tau_L$ in mouse is smaller than in rabbit (Figure 3) suggesting that the SR in mouse might show higher function than in rabbit. These findings confirm a substantial species difference in SR Ca^{2+} -ATPase and Na^{+} - Ca^{2+} exchanger activities, and suggest that a smaller animal and a more rapid heart rate are associated with greater SR Ca^{2+} -ATPase activity.

The logistic fit for decay of the Ca^{2+} transient is slightly superior to the monoexponential fit at some onsets in rabbit, but not significantly so for any onsets, whereas in mouse the logistic fit is superior at all onsets. However, we note that this result may also be due to the lower signal to noise ratio for cardiac muscle characterization in rabbit (Figures 1A and 1B) and the fewer number of samples (just 7 rabbit muscles were examined).

Onset: A poor fit near the onset is a well-known problem in monoexponential fitting of the myocardial relaxation curve. In the early phase, close to $dF/dt_{\min}$, the actual tension is smaller than the monoexponential fitted values; moreover, the tension residuals of the monoexponential fit near the onset (even with delayed starting points) are greater than those of the corresponding logistic fit. $F\tau_E$ values are calculated from the slope of plots of dF/dt versus force at the terminal of the isometric twitch force curve.²⁸⁾ However, the phase-plane curve is not linear, even after $dF/dt_{\min}$; this curve in the logistic function has a downward convexity, whereas it is linear in the monoexponential function.¹⁸⁾ $P\tau_E$ is determined from the inverse slope of the linear plots of the semilogarithm of $P(t)$ in the monoexponential fitting versus time. However, the early plots appear not to be linear; rather, they are concave to the origin.¹⁶⁾ The semilogarithmic curve of the logistic function is slightly concave to the origin, whereas the same curve for the monoexponential function is theoretically linear.¹⁸⁾ Moreover, $P\tau_E$ is determined from the slope of plots of dP/dt versus time in the monoexponential fit; however, the initial rate shortly after $dP/dt_{\min}$, to 10 or 20 ms, is nearly constant, before decreasing continuously toward the asymptote rather than monoexponentially.¹⁷⁾ The rate of increase in $dP(t)/dt$ of the logistic function gradually accelerates with time during

the early phase of relaxation and decelerates with time during the late phase of relaxation. In contrast, the rate of increase in $dP(t)/dt$ of the monoexponential function continuously decelerates throughout relaxation. As a result, the $dP(t)/dt$ curve of the logistic function is blunt near the onset, whereas that of the monoexponential function is sharp. Taken together, these findings lead to substantial differences between the logistic and monoexponential functions. The problems with the monoexponential fit near the starting point are resolved by the logistic fit, and these findings support the contention that the logistic fit for myocardial relaxation is superior to monoexponential fitting for any onset.

Logistic model: The study shows that logistic fitting for the $[\text{Ca}^{2+}]_i$ decline and relaxation force curve is excellent, but this is still an empirical model. However, we suspect that all the mechanisms underlying myocardial relaxation,¹⁾ such as membrane repolarization, E-C coupling, CB kinetics, and the inhomogeneity of these processes during myocardial relaxation, manifest as a whole or in an integrative manner as apparent logistic lusitropism. We hypothesize that a whole force curve can be expressed as the difference between S-shaped logistic curves for the rising and falling components.³⁸⁾ Computer simulation of the Ca^{2+} transient, Tn- Ca^{2+} binding, and CB kinetics has also shown that the cumulative CB attachment and detachment curves are fitted reasonably by a logistic function.³⁹⁾ Therefore, the mechanisms underlying $[\text{Ca}^{2+}]_i$ decline and cardiac relaxation may have a logistic nature.

There is no agreement on the optimal method for determining the relaxation time constant. Many mathematically equivalent models have been suggested, but not all of these have been evaluated with different onsets.²¹⁾ Langer⁴⁰⁾ has reported a considerable improvement in the goodness of fit, compared with the logistic Eq. 3; however, theoretically a function with a greater number of parameters should fit the same data better than a logistic or monoexponential function. Therefore, we suggest that the logistic function gives the best fit for myocardial relaxation using 3 parameters.

Limitations: Regarding the endpoint of the $[\text{Ca}^{2+}]_i$ decline and relaxation force curve, the final 10% has been deleted in previous studies of the monoexponential fitting,⁶⁾ since the terminal phase is primarily determined by a Ca^{2+} -independent process.¹⁾ However, since $F\tau_L$ and $P\tau_L$ are independent of the endpoint in muscle²⁰⁾ and LV,^{18,19)} we selected the endpoints of the $[\text{Ca}^{2+}]_i$ decline and the relaxation force curve as zero $\mu\text{mol/L}$ and zero mN/mm^2 , respectively.

The temperature in the experimental model may represent another limitation of the study. We investigated Tyrode-superfused papillary muscles at 30°C , whereas almost all data concerning force in rat¹²⁾ and mouse myocytes,⁸⁾ and pressure in isolated canine^{18,19)} and rat hearts^{5,6)} have been obtained at 37°C . The other previous experiments have been performed at 30°C ²⁰⁾ or below,^{11,28)} and it is well-

known that such lowering of temperature causes considerable prolongation of the relaxation force²⁹⁾ or pressure.⁴¹⁾ Therefore, $F\tau_L$ and $F\tau_E$ are likely to increase with decreasing temperature, and the shape of the logistic or exponential function may change. Therefore, repeating the experiments at physiological temperature in muscles of various species would be of value.

The $[Ca^{2+}]_i$ level was measured using aequorin, unlike previous studies that have used indo-1,^{5,6,11,12)} fura-2⁸⁾ and fluo-3.^{9,10)} Aequorin binds $[Ca^{2+}]_i$ and decreases in concentration with time. Since Ca^{2+} affinity is dependent on the fluorescence Ca^{2+} indicator,^{42,43)} the general applicability of the logistic function for $[Ca^{2+}]_i$ decline remains to be tested.

Finally, the isometric relaxation might, by itself, influence the physiological conditions.²⁸⁾ In order to provide physiological relevance, the results need to be related to the functional situation in myocardial fibers. The relevant isovolumic pressure fall in the ventricle does not imply isometricity of the fibers, due to changes in the ventricular shape. Therefore, the appropriateness of different models to describe $[Ca^{2+}]_i$ decline and myocardial relaxation should be examined for different fiber lengths from L_{max} .^{4,20,28)}

Conclusion: The logistic function fits the decay of the Ca^{2+} transient and the isometric twitch relaxation force curve more precisely than the monoexponential function in isolated rabbit RV and mouse LV papillary muscles. Therefore, the logistic model may characterize the $[Ca^{2+}]_i$ decline and myocardial lusitropism more reliably than the monoexponential model, and $Ca\tau_L$ and $F\tau_L$ may serve as more reliable indices of $[Ca^{2+}]_i$ decline and myocardial lusitropism. $F/Ca\tau_L$ in mouse is smaller than in rabbit, and analyses using the logistic model for the decay of the Ca^{2+} transient and relaxation force curve might be useful for understanding species-specific myocardial calcium handling.

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