Relationship Between Force and Intracellular $[Ca^{2+}]$ in Tetanized Mammalian Heart Muscle

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ABSTRACT   To determine features of the steady state $[Ca^{2+}]$-tension relationship in intact heart, we measured steady force and intracellular $[Ca^{2+}]$ ($[Ca^{2+}]_i$) in tetanized ferret papillary muscles. $[Ca^{2+}]_i$ was estimated from the luminescence emitted by muscles that had been microinjected with aequorin, a Ca^{2+}-sensitive, bioluminescent protein. We found that by raising extracellular $[Ca^{2+}]$ and/or by exposing muscles to the Ca^{2+} channel agonist Bay K 8644, tension development could be varied from rest to an apparently saturating level, at which increases in $[Ca^{2+}]_i$ produced no further rise in force. 95% of maximal Ca^{2+}-activated force was reached at a $[Ca^{2+}]_i$ of 0.85 ± 0.06 μM (mean $\pm$ SEM; $n = 7$), which suggests that the sensitivity of the myofilaments to $[Ca^{2+}]_i$ is far greater than anticipated from studies of skinned heart preparations (or from previous studies using Ca^{2+}-sensitive microelectrodes in intact heart). Our finding that maximal force was reached by ~ 1 μM also allowed us to calculate that the steady state $[Ca^{2+}]_i$-tension relationship, as it might be observed in intact muscle, should be steep (Hill coefficient of >4), which is consistent with the Hill coefficient estimated from the entire $[Ca^{2+}]_i$-tension relationship derived from families of variably activated tetani (6.08 ± 0.68; $n = 7$). Finally, with regard to whether steady state measurements can be applied directly toward understanding physiological contractions, we found that the relation between steady force and $[Ca^{2+}]_i$ obtained during tetani was steeper than that between peak force and peak $[Ca^{2+}]_i$ observed during physiological twitches.

INTRODUCTION

The strength of contraction in heart muscle is intimately related to intracellular free $[Ca^{2+}]$ ($[Ca^{2+}]_i$). The traditional approach to characterizing the relationship between force and $[Ca^{2+}]_i$ has been to measure the steady state $[Ca^{2+}]$-tension relationship in "skinned" cardiac muscle preparations in which the sarcolemma has been rendered hyperpermeable (Winegrad, 1971), disrupted chemically

(Endo and Kitazawa, 1978), or removed mechanically (Fabiato and Fabiato, 1975). $[Ca^{2+}]$ and other constituents thought to affect contraction can then be controlled in the myofilament lattice.

By following this approach, considerable insight has been gained into the relationship between $[Ca^{2+}]$ and force, but it has been difficult to determine whether the findings obtained from skinned preparations can be extrapolated directly to intact heart muscle. First, potential differences between the intracellular constituents of intact and skinned muscle (Fabiato, 1982; Spedding, 1982) could cause the steady state $[Ca^{2+}]$-tension relation in skinned heart muscle to deviate from that which exists in intact cells. The constituents in question include pH, $[MgATP^{2-}]$, and $[P_i]$, as well as enzymes and cofactors that regulate phosphorylation of the inhibitory subunit of troponin; all of these are believed to affect the relationship between force and $[Ca^{2+}]$ (Brandt et al., 1982; Winegrad, 1984). Another possible limitation of the results from skinned preparations is that the $[Ca^{2+}]_i$ transients of physiological contractions may be brief enough so that in this case force and $[Ca^{2+}]$ cannot be described by a steady state relationship.

We therefore investigated whether the steady state relationship between $[Ca^{2+}]_i$ and force could be estimated from measurements of $[Ca^{2+}]_i$ and force obtained during rapid repetitive stimulation (tetanization) of intact ferret papillary muscles. $[Ca^{2+}]_i$ was estimated from the luminescence emitted by muscles that had been microinjected with aequorin, a Ca^{2+}-sensitive, bioluminescent protein. In particular, we addressed the following questions: (a) Can force development in intact muscle be varied from a resting level to the maximum level that can be activated by Ca^{2+}? (b) How do data obtained during steady force development (tetanus) in intact papillary muscles compare with results from skinned muscle preparations? (c) Does the $[Ca^{2+}]_i$-tension relationship derived from tetani predict the relationship between peak force and peak $[Ca^{2+}]_i$ obtained from twitches, as might be expected from data in skinned heart cells (Fabiato, 1981)?

Preliminary reports of this work have appeared (Yue et al., 1985; Marban et al., 1985).

METHODS

Experimental Setup

Hearts were excised from 8–12-wk-old English ferrets that had been anesthetized with sodium pentobarbital (80 mg/kg, intraperitoneal). Papillary muscles 0.60 ± 0.14 mm (mean ± SD; $n = 12$) in external diameter were rapidly dissected from the right ventricle and placed in a continuously perfused bath chamber. The septal end of the muscle was held fixed by a hook, and the tendinous end was attached to a force transducer (model BG-10, Kulite Semiconductor Products, Ridgefield, NJ). Two types of superfusion solutions were used (Table I). In three experiments, extracellular $[Ca^{2+}]$ ($[Ca^{2+}]_o$) was increased to as high as 72 mM by isosmotic substitution of $CaCl_2$ for NaCl in solution B. The perfusate temperature was kept constant at 30°C by a Peltier device (Cambridge Thermionic Corp., Cambridge, MA) located beneath the bath. Muscles were stimulated at 0.66 Hz during a 1-h recovery period (in 1 mM $[Ca^{2+}]_o$), after which 40–200 superficial cells were pressure-injected with aequorin (purchased from J. R. Blinks, Rochester, MN) as

described previously (Wier, 1980; Allen and Kurihara, 1980). The preparation was allowed to equilibrate for at least 1 h after the microinjection of aequorin, after which time aequorin should have diffused throughout the cells (assuming a radial diffusion coefficient of 1×10^{-11} m^2 s^{-1}; Campbell et al., 1979). A parabolic mirror was then positioned above the muscle, the muscle length was adjusted to l_{max}, and an opaque box was lowered over the bath to shield the preparation from external light during the experiments.

Measurements

Luminescence from the preparation was conducted, via a Lucite light guide (6 mm diam), to a photomultiplier tube (9893 B/350, EMI, Fairfield, NJ) located beneath the preparation. Photons were counted using an amplifier/discriminator (model 1121A, Princeton Applied Research, Princeton, NJ), stored in digital form as counts per second, and signal-averaged (model 4203, Princeton Applied Research) when required to improve the signal-to-noise ratio. Force signals were recorded simultaneously on magnetic tape (model 3964, Hewlett-Packard, Co., Palo Alto, CA), and later converted to digital form.

Estimation of [Ca^{2+}]$_i$

The method of estimating [Ca^{2+}]$_i$ was analogous to that discussed previously (Allen and Blinks, 1978; Wier and Hess, 1984). Briefly, luminescence signals, L (in counts per second), were normalized by the maximal rate of light emission, L_{max} (determined as

TABLE I

Composition of Superfusion Solutions

	NaCl	KCl	MgCl$_2$	NaHCO$_3$	HEPES	Na$_2$HPO$_4$	NaC$_2$H$_3$O$_2$	Glucose	CaCl$_2$
Solution A	92	5	1	20	0	1	20	10	1–10
Solution B	108	5	1	0	5	0	20	10	1–15

Solution A was equilibrated with 95% O$_2$, 5% CO$_2$, resulting in pH 7.35. Solution B was equilibrated with 100% O$_2$, and pH was adjusted to 7.35. All values are millimolar.

described below). The normalized light signal, termed the fractional luminescence (L/L_{max}), could then be used to obtain an upper-limit estimate of spatial-average [Ca^{2+}]$_i$ by referring to an in vitro calibration curve (Yue and Wier, 1985). The curve we obtained in this study by the method of Blinks et al. (1978) can be described by an equation of the form:

$$\frac{L}{L_{max}} = \left(\frac{1 + K_R[\text{Ca}^{2+}]_i}{1 + K_{TR} + K_R[\text{Ca}^{2+}]_i} \right)^3 ,$$

(1)

with $K_R = 4.131 \times 10^6$ M^{-1} and $K_{TR} = 151.7$ determined in vitro (154 mM KCl, 2 mM MgCl$_2$, 5 mM PIPES, pH 7.2, 30°C).

L_{max} was determined at the end of the experiment from the integral of light emitted when the preparation was lysed with 4% Triton X-100 and exposed to saturating [Ca^{2+}]. Before normalizing a particular signal L, however, the L_{max} estimated at the end of the experiment was adjusted for aequorin consumption (10–15% for an entire experiment) by taking into account the integral of all the light emitted from the time at which a particular signal L was recorded to the end of the experiment (Wier et al., 1983).

Raw [Ca^{2+}]$_i$ signals, calculated point by point from unfiltered L/L_{max} signals with Eq. 1, were filtered by a low-pass window filter (symmetric FIR digital filter) whose impulse response was:

$$h(0) = 2 f_c/f_s;$$

$$h(\pm k) = \frac{\sin(2\pi k f_c/f_s)}{\pi k} [0.54 + 0.46 \cos(\pi k/M)],$$

where $2M + 1$ sampled points were used to derive a single filtered point, f_c was the cutoff frequency, and f_s was the sampling frequency (167–1,000 Hz) (Ziemer et al., 1983).

Tetanization of Cardiac Muscle

To enable tetanization of muscles, 5 μM ryanodine, a plant alkaloid believed to inhibit the release of Ca^{2+} from sarcoplasmic reticulum (Sutko and Kenyon, 1983), was added to the perfusate. After ~20 min of exposure, drug effects reached steady state, and fused tetani could be reliably elicited by rapid stimulation (10 Hz) with rectangular stimulus pulses of long duration (40 ms). An additional ~30 min was allowed for equilibration before starting experimental protocols. Bay K 8644 (300 nM), a Ca^{2+} channel agonist (Schramm et al., 1983), was sometimes added to the perfusate to help produce maximally activated tetani. Although caffeine also enables tetanization of heart muscle (Henderson et al., 1971; Forman et al., 1972), ryanodine is preferable in that it does not appear to alter the sensitivity of the myofilaments to $[Ca^{2+}]$ (caffeine effect, see Wendt and Stephenson, 1983; ryanodine result, see Fabiato, 1985a). Bay K 8644 is also believed to leave myofilament sensitivity to $[Ca^{2+}]$ unchanged (Gross et al., 1985).

We confirmed that rapidly delivered extracellular current pulses of long duration did not themselves produce luminescence in the experimental chamber.

RESULTS

Response of Muscle to Rapid Stimulation After Exposure to Ryanodine

We found that ferret papillary muscles could be tetanized after exposure to ryanodine, as first noted for cat myocardium by Strobeck et al. (1980). Stimulation for 2–4 s at 10 Hz rapidly produced a steady "plateau" of force (Fig. 1, top trace). Superimposable records were obtained when tetani were separated by "rest" periods lasting 30–60 s (during which time single twitches were stimulated at 0–0.66 Hz). This stability allowed us to average luminescence signals from several consecutive tetani to improve the signal-to-noise ratio. As shown in Fig. 1, aequorin luminescence and calculated $[Ca^{2+}]_i$ (middle and bottom traces, respectively, derived from 32 responses) also reached a plateau during the tetanus.

To determine the mean level of the signal during the plateau of the tetanus, raw $[Ca^{2+}]_i$ signals (Fig. 1, bottom trace, points) were low-pass-filtered at 10 Hz as described above (Fig. 1, bottom trace, solid curve). This filtering did not attenuate the peaks of the $[Ca^{2+}]_i$ twitch transients bracketing the tetanus as shown.

Effect of Varying $[Ca^{2+}]_o$ on the Cardiac Tetanus

In contrast to skeletal muscle fibers, we found that striking changes in the level of force and $[Ca^{2+}]_i$ reached during the plateau of tetani in ventricular muscle could be produced by varying $[Ca^{2+}]_o$. Resting force and $[Ca^{2+}]_i$ are shown in trace 1 of Fig. 2, A and B. As $[Ca^{2+}]_o$ was raised from 1 to 5 mM, a progressive increase occurred in the magnitude of the steady levels of $[Ca^{2+}]_i$ (Fig. 2B, traces

2-4) and force (Fig. 2A, traces 2-4). When $[Ca^{2+}]_o$ was raised even further (up to 10 mM in solution A and up to 15 mM in solution B, Table I), the level of $[Ca^{2+}]_i$ reached during the tetanus increased, whereas force was not enhanced appreciably beyond the level in trace 4 (records not shown). Further and dramatic increases in $[Ca^{2+}]_i$, but not in force, could be obtained by the addition of Bay K 8644, a Ca^{2+} channel agonist, to the solutions with high $[Ca^{2+}]_o$ (Fig. 2, A and B, trace 5). In this case, $[Ca^{2+}]_i$ continued to rise slowly during the tetanus, but force remained almost constant throughout the plateau at a level no greater than that in high $[Ca^{2+}]_o$ before the addition of the Ca^{2+} channel agonist. Similar findings were obtained in a total of seven muscles.
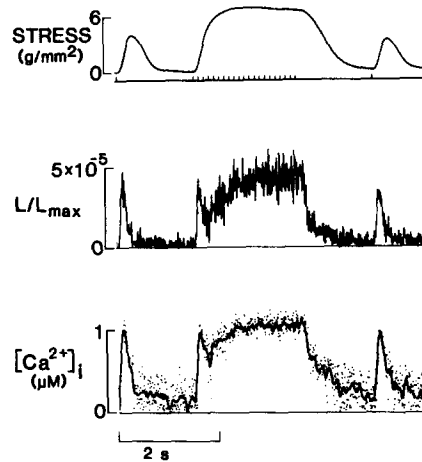

FIGURE 1. Signal-averaged (32 sweeps) records of developed stress (top trace), aequorin luminescence (middle trace), and $[Ca^{2+}]_i$ (bottom trace) obtained from a tetanized ferret papillary muscle in 6 mM $[Ca^{2+}]_o$. The unfiltered $[Ca^{2+}]_i$ record (calculated from unfiltered luminescence [middle trace]) appears as points in the bottom trace. The filtered (10-Hz low-pass) $[Ca^{2+}]_i$ record is the solid curve in the bottom trace. Stimuli were delivered at the times indicated by the marks displayed below the upper trace. Solution A was the perfusate. Preparation LD.

Maximum Ca^{2+}-activated Force Is Reached

The results in Fig. 2 imply that steady force development in intact papillary muscles can be varied from a resting level (trace 1) to the maximum level that can be activated by Ca^{2+} (trace 5). Four features of the results argue strongly that maximal Ca^{2+}-activated force was in fact reached.

(a) Within a single, highly activated tetanus, $[Ca^{2+}]_i$ rose slowly throughout most of the tetanus (Fig. 2B, trace 5), while force remained virtually constant for >1.5 s (Fig. 2A, trace 5). The simplest interpretation is that force cannot increase beyond this plateau level despite the continuing increase in $[Ca^{2+}]_i$ because maximal Ca^{2+}-activated force has been reached. This interpretation might be questioned if $[Ca^{2+}]_i$, derived from injected, superficial cells, is unrepresentative of the true $[Ca^{2+}]_i$ throughout the muscle. In this case, one could

argue that maximal Ca^{2+}-activated force has not been reached; the rising super-ficial $[Ca^{2+}]_i$ would therefore be accompanied by increasing force development in the outer layers of muscle. Since force measured from the entire fiber is constant, we would be forced to postulate that $[Ca^{2+}]_i$ and force are declining deep in the fiber, at a rate just sufficient to offset the increasing force develop-
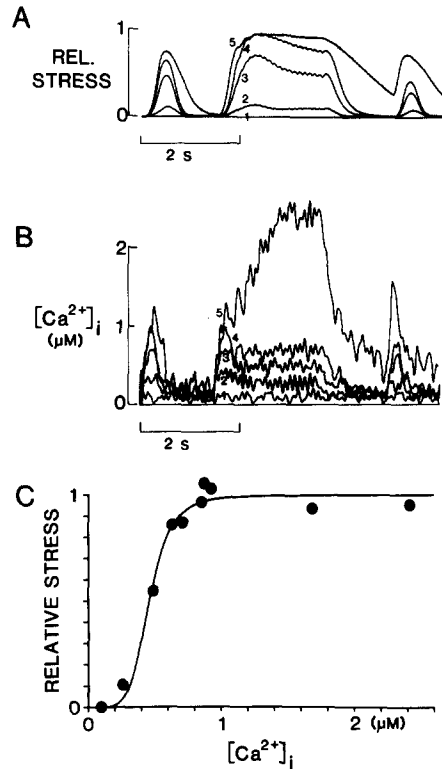

FIGURE 2. Different steady levels of force and $[Ca^{2+}]_i$ obtained at various $[Ca^{2+}]_o$. (A) Stress normalized by the maximum stress derived from a Hill curve that was fitted to the data (see panel C). (B) $[Ca^{2+}]_i$ records after low-pass-filtering at 10 Hz. The $[Ca^{2+}]_o$ (in solution B) corresponding to the various traces was: (1) 1 mM, unstimulated; (2) 1 mM; (3) 3 mM; (4) 5 mM; (5) 13 mM and 300 nM Bay K 8644. All records are the average of 2–24 responses. (C) Stress normalized relative to the maximal force achieved plotted as a function of $[Ca^{2+}]_i$. Points are plotted from records 1–5 and from five other tetani (not shown). $[Ca^{2+}]_i$ and tension were measured 1 s after the initial spike in $[Ca^{2+}]_i$ (see text for details). The solid curve is a Hill function (text Eq. 2) with $n = 5.59$ and $K_{1/2} = 0.46$ μM. Preparation 2TT.

ment in the outer layers. It seems implausible that this cancellation should be fortuitously exact over the entire duration of the tetanus.

(b) Several tetani obtained at relatively high $[Ca^{2+}]_o$ (with or without Bay K 8644) yielded a range of $[Ca^{2+}]_i$ greater than that in trace 4 (only the case in trace 5 is shown in Fig. 2B, but all points are plotted in Fig. 2C). However, the

force attained in all of these tetani was nearly the same. Again, the simplest interpretation is that maximal Ca^{2+}-activated force has been reached. Alternatively, one might argue that increases in force produced by the outer layers of muscle were offset almost precisely, from one tetanus to the next, by decreases in force development deep in the preparation. It is unlikely, however, that such cancellation would result in the same level of net force produced by the entire fiber for several tetani with different levels of superficial $[Ca^{2+}]_i$.

To verify that the phenomena described above represented a genuine saturation of force with respect to $[Ca^{2+}]_i$, we varied $[Ca^{2+}]_o$ over an even broader range in three experiments, by isosmotic substitution of $CaCl_2$ (up to 72 mM) for NaCl. The decrease in $[Na^+]_o$ would also be expected to augment $[Ca^{2+}]_i$ via
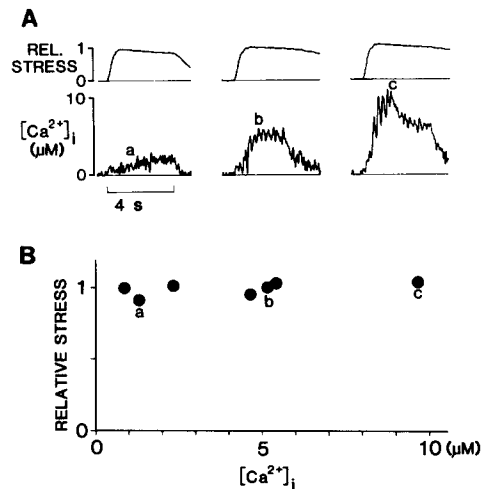

FIGURE 3.   Stress and $[Ca^{2+}]_i$ during highly activated tetani. (*A*) Records obtained in modified solution B containing 1 μM Bay K 8644, 93 mM NaCl, 10 mM $CaCl_2$ (*a*), 48 mM NaCl, 40 mM $CaCl_2$ (*b*), or 26 mM NaCl, 55 mM $CaCl_2$ (*c*). (*B*) Points represent the relation between force and $[Ca^{2+}]_i$ at 1.5 s after the onset of tetani, and include data from traces *a–c* in panel *A* (as labeled). Records are derived from the average of one to two sweeps. Preparation HOT.

Na/Ca exchange. Fig. 3 shows the results of a representative experiment. Panel *A* shows tetani obtained in $[Ca^{2+}]_o$ of 10, 40, and 55 mM. The plot of force vs. $[Ca^{2+}]_i$ reached in these (labeled *a–c*) and other tetani (panel *B*) indicates that despite variations in $[Ca^{2+}]_i$ from ~1 up to 10 μM, maximal tension did not change (measurements made 1.5 s after the onset of tetani). These results make it even less plausible that the saturation of force could be explained artifactually by the offsetting effects of rising force in superficial layers and declining force deep in the middle of the muscle.

(*c*) The small fluctuations of $[Ca^{2+}]_i$ about a steady mean level (synchronous with stimulation pulses), which were sometimes evident during the plateau phase of the cardiac tetanus (Fig. 2*B*), were accompanied by "ripples" in force at

intermediate levels of $[Ca^{2+}]_i$ (Fig. 2A, traces 3 and 4). The high-gain display of these signals (Fig. 4A, a, traces AC-coupled) indicates that force (solid line) and $[Ca^{2+}]_i$ (points) were closely correlated, both being approximately sinusoidal. The cross-correlogram between these signals (Fig. 4B, a) demonstrates quantitatively that the signals share a dominant frequency of 10 Hz, and that $[Ca^{2+}]_i$ leads force by ~20 ms. In contrast, there is no significant fluctuation of force to match $[Ca^{2+}]_i$ oscillations during the plateau of a highly activated tetanus (Fig. 2, A and B, trace 5), as would be expected if the mean level of $[Ca^{2+}]_i$ reached during a tetanus were significantly greater at all times than the minimum required for maximal Ca^{2+}-activated force to be reached. This is demonstrated rigorously by the high-gain AC-coupled display of these traces (Fig. 4A, b; force, solid line;
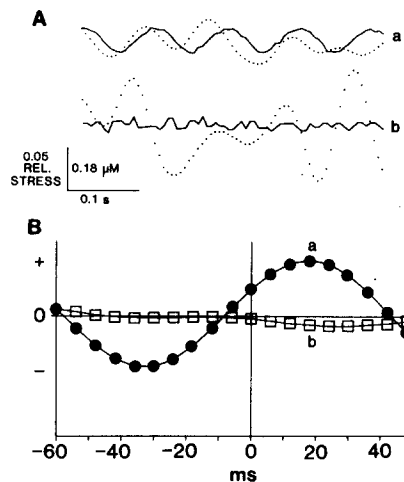

FIGURE 4. Temporal correlation of $[Ca^{2+}]_i$ and force fluctuations during the plateau of tetani. (A) $[Ca^{2+}]_i$ (points) and force (solid line) during the plateaus of tetani 3 (a) and 5 (b) from Fig. 2. The records were AC-coupled (i.e., high-pass-filtered $[Ca^{2+}]_i$ and tension with a cutoff frequency of 0.5 Hz). (B) Cross-correlograms of $[Ca^{2+}]_i$ and the force records depicted in A. The abscissa shows τ in milliseconds.

$[Ca^{2+}]_i$, points), and by the flat cross-correlogram for these signals (Fig. 4B, b). These oscillations are distinct from myofilament-generated oscillations in force, which occur at constant $[Ca^{2+}]$ (Fabiato and Fabiato, 1978a).

(d) The average (± SEM) maximal stress (6.41 ± 0.57 g/mm^2, $n = 12$) compares favorably with the maximal stress obtained from single, skinned cardiac cells (11.67 g/mm^2 in Fabiato, 1981) after taking into account the ratio of cellular to total volume (0.6 in Page, 1962; $11.67 \times 0.6 = 7$ g/mm^2).

Activity-related Changes in Myofilament Sensitivity to $[Ca^{2+}]_i$?

The results in Fig. 2 also suggest that contractile activity itself may alter the relationship between force and $[Ca^{2+}]_i$. At intermediate levels of $[Ca^{2+}]_i$, plateau force declined gradually despite constant levels of $[Ca^{2+}]_i$ (Fig. 2, A and B, traces

3 and 4). This can be explained by the proposition that sustained activation of force induces a gradual rightward shift of an S-shaped [Ca^{2+}]$_i$-tension relationship, i.e., a reduction of myofilament sensitivity to [Ca^{2+}]$_i$. Three aspects of the results favor this hypothesis.

(a) The relationship between peak force and peak calculated [Ca^{2+}]$_i$ for the post-tetanic twitch (Fig. 5, crosses) is slightly depressed compared with that for the pre-tetanic twitch (Fig. 5, open circles; same preparation as in Fig. 2). These data favor a genuine reduction in myofilament sensitivity to [Ca^{2+}]$_i$, and are inconsistent with mechanisms for declining force based solely upon sarcomere length shortening during the tetanus.

(b) Tetanic plateau force declines primarily at intermediate [Ca^{2+}]$_i$ (Fig. 2A, traces 3 and 4), but is nearly constant at both low and high [Ca^{2+}]$_i$ (Fig. 2A, traces 2 and 5). This can be explained by a rightward shift of the [Ca^{2+}]$_i$-tension relationship with only a small decline in maximum Ca^{2+}-activated force: intermediate force values are most sensitive to small shifts in the position of a sigmoid curve along the [Ca^{2+}]$_i$ axis. Among the factors capable of producing an activity-
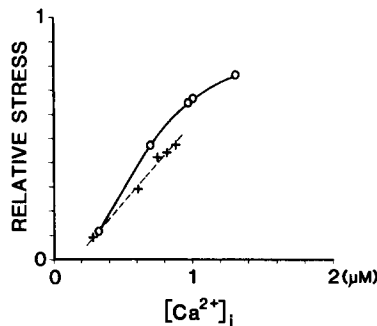

FIGURE 5. Relationship between peak stress and peak [Ca^{2+}]$_i$ obtained from twitches just before (O) and just after (+) tetani. Curves were fitted by eye. Preparation 2TT.

related shift in myofilament sensitivity are P$_i$ and/or H$^+$. The relatively small decline in force during tetani at high [Ca^{2+}]$_i$ (e.g., Fig. 3A) is not inconsistent with accumulation of P$_i$ or H$^+$ as a mechanism for the more prominent decline of force at intermediate [Ca^{2+}]$_i$; these factors cause a considerable rightward shift in the Ca^{2+}-tension relation, in addition to depressing maximal force (Kentish, 1984; Fabiato and Fabiato, 1978b).

(c) Since the rate of decline of relative stress (in 4–5 mM [Ca^{2+}]$_o$) did not correlate with fiber diameter (P > 0.40, n = 7), it is not likely that radial inhomogeneities in extracellular ion depletion (Hilgemann et al., 1983), pO$_2$, stimulus current density, or [Ca^{2+}]$_i$ contributed significantly to the decline in plateau force.

Steady State Relationship Between Force and [Ca^{2+}]$_i$

To minimize the effects of the gradual shifts in myofilament sensitivity on our estimate of the steady state [Ca^{2+}]$_i$-tension relationship, we related the mean

levels of $[Ca^{2+}]_i$ and force just 1 s after the initial spike of $[Ca^{2+}]_i$ seen often at the beginning of tetani (Fig. 2B, traces 2–5). Since force relaxed completely in <1 s when $[Ca^{2+}]_i$ fell quickly at the end of rapid stimulation (Fig. 2, A and B, traces 2–4), we reasoned that the memory of force for previous levels of $[Ca^{2+}]_i$ was <1 s. Hence, force measured at this time should reflect only the plateau level of $[Ca^{2+}]_i$ and whatever small reduction in myofilament sensitivity to $[Ca^{2+}]_i$ might have occurred. Figs. 6 and 2C show plots of steady force vs. $[Ca^{2+}]_i$ for measurements made in this manner.

$[Ca^{2+}]$-tension relations during tetani can be characterized not only by the level of maximal Ca^{2+}-activated force, but also by their relative steepness. The conventional index of steepness is the coefficient derived from a best-fitting Hill function (see Eq. 2), as illustrated in Fig. 6. The solid curve is a Hill function fitted to the data by the Taylor series method of nonlinear least-squared error estimation (Draper and Smith, 1981). The Hill coefficient in this case was 9.11, $[Ca^{2+}]_i$ at half-maximal stress $(K_{1/2})$ was 0.69 μM, and $[Ca^{2+}]_i$ at 95% maximal stress $(Ca_{0.95})$ was 0.95 μM. The Hill coefficient in Fig. 2C was 5.59, but this may be less reliable since there are fewer points in the critical middle portion of
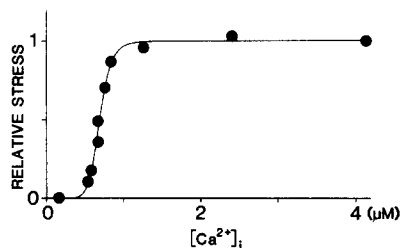

FIGURE 6. Estimate of the steady state relationship between $[Ca^{2+}]_i$ and force obtained in intact heart muscle. The solid curve is the least-squared error fit of the Hill equation to the data (see text for parameters). Preparation MUSA.

the curve. The results obtained in a total of seven experiments are summarized in Table II and Fig. 7. The mean values expressed in Table II were derived from curves fitted individually to the data of each experiment; a single curve was not fitted to data pooled from all experiments (which can artifactually decrease the Hill coefficient, as noted by Brandt et al., 1982). Such a $[Ca^{2+}]_i$-tension relationship provides an attractive explanation for the observation that, although small fluctuations of $[Ca^{2+}]_i$ could often be observed in tetani corresponding to all levels of mean $[Ca^{2+}]_i$ (Fig. 2B, traces 2–5), corresponding fluctuations of force were obvious only in tetani at intermediate levels of $[Ca^{2+}]_i$ (Fig. 2, A and B, traces 3 and 4 only; Fig. 4, a only). Fluctuations in force are expected to be small at low or high levels of $[Ca^{2+}]_i$ (Fig. 2A, traces 2 and 5; Fig. 4, b), where the slope of the estimated $[Ca^{2+}]_i$-tension relationship approaches zero.

TABLE II

Estimated Steady State Relationship Between Force and [Ca²⁺]ᵢ

Buffer solution	Maximum Ca²⁺-activated stress	Hill coefficient	$K_{\frac{1}{2}}$	$Ca_{0.95}$
	g/mm^2		μM	μM
A	4.73±0.93	6.48±0.79	0.44±0.03	0.70±0.02
	(n = 3)	(n = 3)	(n = 3)	(n = 3)
B	6.97±0.59	5.78±1.01	0.55±0.04	0.97±0.07
	(n = 9)	(n = 4)	(n = 4)	(n = 4)
A and B pooled	6.41±0.57	6.08±0.68	0.50±0.04	0.85±0.06
	(n = 12)	(n = 7)	(n = 7)	(n = 7)

Results are expressed as means ± SEM. $K_{\frac{1}{2}}$ is the [Ca²⁺]ᵢ at which force is one-half maximal, and $Ca_{0.95}$ is the [Ca²⁺]ᵢ at which force is 95% of the maximum.

Comparison of Relationships Between Force and [Ca²⁺]ᵢ Obtained from Tetani and from Twitches

Can the relationship between steady force and [Ca²⁺]ᵢ derived from tetani predict the relationship between peak force and peak [Ca²⁺]ᵢ obtained from twitches? In Fig. 8, three distinct relationships are present: the curve for tetani (solid circles) is shifted the most to the left, the curve for twitches after exposure to ryanodine is slightly displaced to the right (open circles), and the curve for twitches before exposure to ryanodine is shifted the most to the right (solid triangles). Similar results were obtained in a total of seven muscles.

Differences in the time course of [Ca²⁺]ᵢ signals corresponding to the three modes of contraction may underlie the disparities in the relationships between force and calculated [Ca²⁺]ᵢ shown in Fig. 8A. Representative force and calculated [Ca²⁺]ᵢ signals for the different types of contraction are displayed in Fig. 8B. Time to peak force and peak [Ca²⁺]ᵢ were typically 350 and 500 ms, respectively, for twitches after ryanodine exposure, and 40 and 250 ms, respectively, for twitches before ryanodine exposure. It appears that the slower the change in [Ca²⁺]ᵢ during twitch contraction, the closer is the correspondence between the curves relating force and [Ca²⁺]ᵢ during twitches and at steady state.
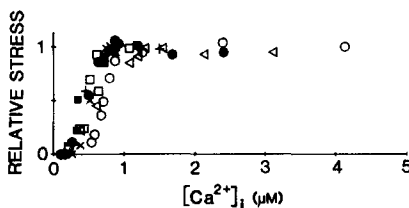

FIGURE 7. Estimates of the steady state [Ca²⁺]ᵢ-tension relationship from all muscles in which complete curves were obtained (n = 7). Different symbols correspond to different preparations. The maximal stress used for normalization was that obtained from the least-squared error fits of the Hill equation to the data in each preparation.
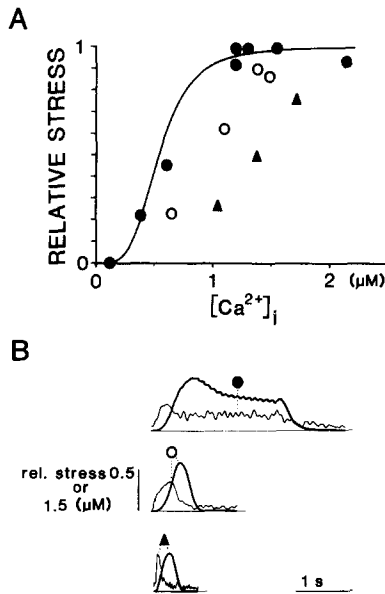
A

B

FIGURE 8. Relationship between $[Ca^{2+}]_i$ and force obtained during tetani and during twitch contractions. (A) The filled circles represent the relationship between steady force and $[Ca^{2+}]_i$ obtained from tetani as described in the text for Fig. 6. The open circles and solid triangles are the relationships between peak force and peak $[Ca^{2+}]_i$ for twitches after and before exposure to ryanodine, respectively. (B) Representative force (thick trace) and $[Ca^{2+}]_i$ (thin trace) signals from which the graph in A was constructed (average of 12–16 responses). Top trace, tetanus; middle trace, twitch after exposure to ryanodine; bottom trace, twitch before exposure to ryanodine. $[Ca^{2+}]_i$ records for the tetanus and ryanodine twitch were low-pass-filtered at 10 Hz; the $[Ca^{2+}]_i$ record for the physiological twitch was filtered at 30 Hz. Solution B was the perfusate. Preparation MCSS.

DISCUSSION

We have answered affirmatively the first question raised in the Introduction: force can be varied in intact heart muscle from rest to the maximal Ca^{2+}-activated level. This allows us to proceed to consider the remaining questions.

Evidence for Greater Myofilament Sensitivity to $[Ca^{2+}]$ in Intact Muscle Than in Skinned Preparations

The finding that maximum Ca^{2+}-activated force is attained by an apparent $[Ca^{2+}]_i$ (from aequorin measurements) of ~1 μM ($Ca_{0.95}$ = 0.85 ± 0.06 μM, Table II) provides strong evidence that the myofilament sensitivity in intact muscle is considerably greater than that observed in skinned fibers. We argue as follows.

For a muscle to develop maximal force, the $[Ca^{2+}]_i$ within it must everywhere exceed that required for maximal activation of the myofilaments. Thus, the $[Ca^{2+}]_i$ required for maximal activation must be less than or equal to the spatial average $[Ca^{2+}]_i$ present during maximal force production. Our estimate of the

$[Ca^{2+}]_i$ required for maximal activation (~ 1 μM) provides an upper-limit estimate of spatial average $[Ca^{2+}]_i$ at this point (Blinks et al., 1982; Yue and Wier, 1985). Hence, our results imply that the $[Ca^{2+}]_i$ required for maximal activation to be approached should be less than or equal to 1 μM.

A steady state $[Ca^{2+}]$-tension relationship that approaches maximal force by ~ 1 μM would be shifted far to the left compared with the majority of curves obtained in skinned fibers. Taking the data as originally reported, $Ca_{0.95}$ values from representative skinned muscle studies cluster around 10 μM (Marban et al., 1980), although one record from skinned dog Purkinje fiber yielded $Ca_{0.95} = 1.4$ μM (Stern et al., 1983, Fig. 9B). Nevertheless, all are shifted to the right relative to our results. Hence, the evidence suggests that the myofilament sensitivity to $[Ca^{2+}]_i$ may be considerably greater in intact muscle than in skinned preparations.

This suggestion is dependent on the accuracy of our estimate of $[Ca^{2+}]_i$, which is based upon the use of a particular calibration curve obtained in vitro under ionic conditions that are believed to mimic the intracellular environment. What would happen to the major conclusions raised so far if there were a substantial, unanticipated error in the calibration curve (e.g., if ryanodine affects aequorin luminescence; Fabiato, 1985a)?

The determination of maximal force would still be valid, since it depends only on the saturation of force with respect to $[Ca^{2+}]_i$-dependent aequorin luminescence.

The factor that would be affected is the value at which maximal Ca^{2+}-activated force is reached. Nevertheless, it is unlikely that the substantial apparent differences in $Ca_{0.95}$ obtained from intact vs. skinned preparations could arise from the use of an inappropriate aequorin calibration curve. Fig. 9 shows three aequorin calibration curves obtained in vitro under very different conditions: the solid line at the center is the curve that we have used in this study (Eq. 1), the dashed curve at the left was obtained at 20°C in 0 mM $MgCl_2$, and the dashed curve at the right was obtained at 20°C in 6 mM $MgCl_2$ (dashed calibration curves are from Moore, 1984, and include pre-equilibration with Mg^{2+}). In order for our L/L_{max} data to have given a $Ca_{0.95}$ equal to one of the closer values from skinned preparations (5.01 μM, Wendt and Stephenson, 1983), the assumed calibration curve would have had to pass through the circle shown in Fig. 9. Such a curve would differ greatly from any that has been determined for aequorin.

Implicit in Fig. 9 is the effect of another possible error, under- or overestimation of L_{max}. A 100% underestimation of L_{max} (i.e., a doubling of L/L_{max} from ~ 2.41 to 4.82×10^{-5}) would change the value for $Ca_{0.95}$ from 0.85 to 1.15 μM. This effect is relatively small and would not influence the basic conclusions.

Hence, after consideration of potential errors in our estimate of $[Ca^{2+}]_i$, we are still left with the conclusion that myofilament sensitivity to $[Ca^{2+}]_i$ appears to be considerably greater in intact muscle than in skinned fibers. The source of the discrepancy between our results and those in skinned preparations is not clear at this time. It will be important to confirm our results with other $[Ca^{2+}]_i$ indicators, as alternative methods become available. In addition, the considerable

differences among the results from various skinned fiber preparations themselves should be investigated further.

Evidence That the Steady State $[Ca^{2+}]_i$-Tension Relation Is Steep in Intact Myocardium

If maximal Ca^{2+}-activated force is approached by $\sim$1 μM and force is relaxed at resting $[Ca^{2+}]_i$, we can set a lower limit on the steepness of the steady state $[Ca^{2+}]_i$-tension relationship. In the analysis to follow, we show that the Hill coefficient (used as an index of steepness) of the $[Ca^{2+}]_i$-tension relationship should be >4 in our preparation.
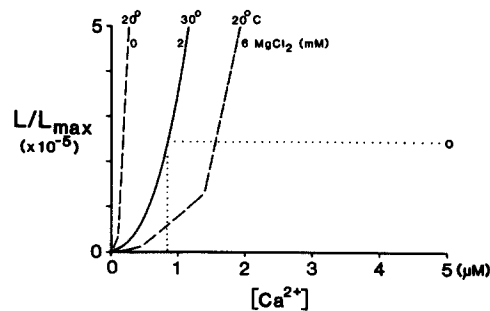

FIGURE 9. Aequorin calibration curves measured in vitro. The solid curve at the center (obtained at 30°C and pH 7.2 with 2 mM MgCl$_2$, 5 mM PIPES, 154 mM KCl) was obtained and used in the present study. The dashed curves were obtained at 20°C at pH 7.0 in 0 (left) and 6 (right) mM MgCl$_2$ (both in 5 mM PIPES, 150 mM KCl) (dashed curves are from Moore, 1984). The open circle represents a point on the hypothetical calibration curve that would have been required for the data in the present study to have given a $Ca_{0.95}$ value equal to one of the lowest of the values for skinned heart muscle in the literature (Wendt and Stephenson, 1983).

The Hill equation can be expressed:

$$S_n = \frac{[Ca^{2+}]_i^n}{K_{1/2}^n + [Ca^{2+}]_i^n},$$
(2)

where S_n is stress normalized by maximal stress, n is the Hill coefficient, and $K_{1/2}$ is the $[Ca^{2+}]_i$ required for $S_n = 0.5$. Our experimental results require that S_n be >0.95 for a $[Ca^{2+}]_i$ of >1 μM. From Eq. 2, we have the following constraint on the possible values of $K_{1/2}$ and n:

$$0.05263 > (K_{1/2})^n,$$
(3)

where $K_{1/2}$ is in micromolar units. This constraint is represented by the entire region above the solid curve in Fig. 10. A second requirement is that the muscle be relaxed at resting $[Ca^{2+}]_i$. Resting $[Ca^{2+}]_i$ in ferret papillary muscles has been determined to be 0.26 μM using Ca^{2+}-sensitive microelectrodes (Marban et al., 1980). Thus, we assume that S_n is <0.05 for a $[Ca^{2+}]_i$ of <0.26 μM. From Eq. 2, we have an additional constraint on the possible values of $K_{1/2}$ and n:

$$19 < (K_{1/2}/0.26)^n. \tag{4}$$

This constraint is represented by the entire region above the dashed curve in Fig. 10. The shaded region represents the values of $K_{1/2}$ and n, which satisfy both constraints (Eqs. 3 and 4). By inspection of the shaded region, we see that the Hill coefficient should be >4.

A more exact determination of the Hill coefficient depends on the accurate measurement of the entire $[Ca^{2+}]$-tension relationship, including the steeply ascending portion. The steady state $[Ca^{2+}]_i$-tension relationships that we have determined suggest that the Hill coefficient is ~6 (Table II), but three factors reduce the certainty of our direct determination of the Hill coefficient.

First, since the calculated $[Ca^{2+}]_i$ derives from superficial, injected cells, whereas tension reflects the entire muscle, a nonuniform $[Ca^{2+}]_i$ throughout the muscle could distort our determination of the steeply ascending portion of the $[Ca^{2+}]_i$-tension relationship. Second, changes in myofilament sensitivity consequent to activity would tend to displace the relationship to the right. Third, the
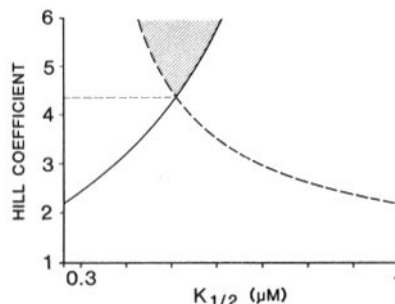

FIGURE 10.   Estimates of the Hill coefficient for the $[Ca^{2+}]_i$-tension relationship in vivo. The solid curve represents a lower limit for the Hill coefficient, given the constraint that 95% of maximal force is reached by a $[Ca^{2+}]_i$ of 1 μM. The dashed curve represents a lower limit for the Hill coefficient, given the constraint that <5% of maximal force is expressed at resting $[Ca^{2+}]_i$ (0.26 μM). The apex of the shaded region is the lower limit for the Hill coefficient, given both of the above constraints.

sarcomere length probably became shorter as tetani were increasingly activated, because of the presence of compliant damaged ends (Krueger and Pollack, 1975). This would tend to make our curves less steep than the true $[Ca^{2+}]_i$-tension curve. Hence, we conclude that the Hill coefficient of the steady state $[Ca^{2+}]_i$-tension relationship is >4, but we cannot be certain that it is necessarily ~6, as suggested by the best fit to our data. It should be emphasized that the determinations of maximal stress and of $Ca_{0.95}$ are not subject to these complications.

A Hill coefficient (n) of >4 is far larger than has been reported in most skinned fiber studies in heart or skeletal muscle (see Chapman, 1983, for a review of heart muscle and Brandt et al., 1982, for a review of skeletal muscle). When special measures are taken to maintain sarcomere length constant in skinned cardiac muscle, Hill coefficients as high as 5.4 have been found (Ter

Keurs, H. E. D. J., personal communication), although, as discussed above, the sarcomere length was not constant in our preparation. Fabiato and Fabiato (1978b, Fig. 1B, solid circles) and Stern et al. (1983, Fig. 9B) have reported steady state [Ca^{2+}]-tension relations that, by our calculations, demonstrate an n of >3 for heart muscle, while Moisescu (1976) and Brandt et al. (1982) have reported n values of ~4 and ~5.5, respectively, for skeletal muscle.

A Hill coefficient far greater than the number of Ca^{2+}-binding sites on troponin (three) is not necessarily unexpected, as recently clarified by Shiner and Solaro (1984). Since steady force generation during a tetanus approximates a system at steady state, but not at equilibrium, the Hill coefficient for Ca^{2+} activation of force may be far greater than the number of Ca^{2+}-binding sites on troponin that are responsible for activation (Shiner and Solaro, 1984). In light of this consideration, our estimate of the Hill coefficient can be reconciled with the proposal that only one of the three Ca^{2+}-binding sites of troponin regulates the activation of contraction (Robertson et al., 1982). Furthermore, cooperative interactions among neighboring troponin and/or actin molecules could make the [Ca^{2+}]$_i$-tension relation even steeper (Hill, 1983). Finally, the existence of multiple stationary states may also contribute to large Hill coefficients (Shiner and Solaro, 1982).

Interpretation of the Differences Among the Relationships Between Force and [Ca^{2+}]$_i$ Obtained During Tetani and During Twitches

Our results (Fig. 8) suggest that the slower the change in [Ca^{2+}]$_i$ accompanying twitch contraction, the more closely the relationship between peak force and calculated [Ca^{2+}]$_i$ approaches the [Ca^{2+}]$_i$-tension relationship derived from tetani. Fabiato (1985b, footnote 13) has found that the relationship between peak force and peak [Ca^{2+}]$_i$ for fast transients in skinned skeletal muscle cells is shifted to the right with respect to the steady [Ca^{2+}]-tension relation, which is consistent with our results. One explanation for our results is that as [Ca^{2+}]$_i$ transients become briefer, there is insufficient time for force and [Ca^{2+}]$_i$ to approach steady state. Another possibility is that as [Ca^{2+}]$_i$ transients rise and decline more rapidly, spatial gradients of [Ca^{2+}]$_i$ become more severe. [Ca^{2+}]$_i$ is certainly more likely to be spatially uniform during the plateau of the tetanus than during phasic twitch contractions (cf. Cannell and Allen, 1984). Then, if spatial gradients of [Ca^{2+}]$_i$ are present during twitch contraction, the calculated [Ca^{2+}]$_i$ would overestimate spatial average [Ca^{2+}]$_i$ (Yue and Wier, 1985), resulting in an apparent rightward shift of the relationship between peak force and peak [Ca^{2+}]$_i$.

We are unable to distinguish between these two possibilities. Nevertheless, because of the first possibility, it is clear that agreement of the steady state [Ca^{2+}]-tension relationship and the relationship between peak force and peak [Ca^{2+}]$_i$ for very slow twitches (e.g., those resulting from Ca^{2+}-induced release of Ca^{2+} in skinned cells) cannot be taken as evidence that the steady state curve would predict the relationship between peak force and peak [Ca^{2+}]$_i$ for physiological contractions as well (see Fabiato, 1981).

Comparison with Previous Studies in Intact Muscles

Numerous investigators using Ca^{2+}-sensitive microelectrodes (Marban et al., 1980) or aequorin (Allen and Blinks, 1978; Allen and Kurihara, 1980; Allen and

Orchard, 1983; Morgan et al., 1983; Marban and Wier, 1984; Hess and Wier, 1984; Wier and Hess, 1984) have attempted to make inferences about the $[Ca^{2+}]$-tension relation in intact heart muscle. However, $[Ca^{2+}]$-tension relations comparable to those in skinned muscle remained elusive because maximal force was not achieved, and most of the relations obtained in intact heart were not at steady state. The use of variably activated tetani may help fill this gap by enabling the determination of maximal Ca^{2+}-activated force at steady state in intact myocardium. In particular, it should be possible to determine whether maximal Ca^{2+}-activated force is depressed by P_i accumulation (Kentish, 1984) in hypoxia, or augmented by β-adrenergic stimulation (Winegrad, 1984).

Measurements of $[Ca^{2+}]_i$ during contractures (Marban et al., 1980; Marban and Wier, 1984; Kort et al., 1985) or during tonic tension (Wier and Hess, 1984) have yielded fragments of $[Ca^{2+}]$-tension relations that do not appear to saturate even at $[Ca^{2+}]_i$ up to 10 μM, in contrast to our results showing saturation at a $[Ca^{2+}]_i$ of ≥ 1 μM. There are two possible explanations for this discrepancy. First, the phenomenon of an activity-related decrease in myofilament sensitivity to $[Ca^{2+}]_i$ (discussed above) would require that a higher $[Ca^{2+}]_i$ be present for a given level of contractile activation during a contracture lasting up to 40 min than during a tetanus lasting <4 s. Second, the presence of spontaneous $[Ca^{2+}]_i$ oscillations during tonic tension would linearize the apparent $[Ca^{2+}]$-tension relation and shift it to the right (Kort et al., 1985). In the present study, the situation is much improved because tetani are brief, and because ryanodine minimizes spatiotemporal $[Ca^{2+}]_i$ inhomogeneity (Wier et al., 1983; Eisner et al., 1984; Kort et al., 1985).

REFERENCES

Allen, D. G., and J. R. Blinks. 1978. Calcium transients in aequorin-injected frog cardiac muscle. *Nature.* 273:509–513.

Allen, D. G., J. R. Blinks, and F. G. Prendergast. 1977. Aequorin luminescence: relation of light emission to calcium concentration—a calcium independent component. *Science.* 196:996–998.

Allen, D. G., and S. Kurihara. 1980. Calcium transients in mammalian ventricular muscle. *European Heart Journal.* 1(Suppl. A):5–15.

Allen, D. G., and C. H. Orchard. 1983. The effect of hypoxia and metabolic inhibition on intracellular calcium in mammalian heart muscle. *Journal of Physiology.* 339:102–122.

Blinks, J. R., P. H. Mattingly, B. R. Jewell, M. van Leeuwen, G. C. Harrer, and D. G. Allen. 1978. Practical aspects of the use of aequorin as a calcium indicator: assay, preparation, microinjection, and interpretation of signals. *Methods in Enzymology.* 57:292–328.

Blinks, J. R., W. G. Wier, P. Hess, and F. G. Prendergast. 1982. Measurement of Ca^{2+} concentrations in living cells. *Progress in Biophysics and Molecular Biology.* 40:1–114.

Brandt, P. W., R. N. Cox, M. Kawai, and T. Robinson. 1982. Regulation of tension in skinned muscle fibers. Effect of cross-bridge kinetics on apparent Ca^{2+} sensitivity. *Journal of General Physiology*. 79:997–1016.

Campbell, A. K., T. J. Lea, and C. C. Ashley. 1979. Coelenterate photoproteins. *In* Detection and Measurement of Free Ca^{2+} in Cells. C. C. Ashley and A. K. Campbell, editors. Elsevier/North-Holland, New York. 13–72.

Cannell, M. B., and D. G. Allen. 1984. Model of calcium movements during activation in the sarcomere of frog skeletal muscle. *Biophysical Journal*. 45:913–925.

Chapman, R. A. 1983. Control of cardiac contractility at the cellular level. *American Journal of Physiology*. 245:H535–H552.

Draper, N. R., and H. Smith. 1981. Applied Regression Analysis. 2nd edition. Wiley Interscience, New York. 462–468.

Eisner, D. A., C. H. Orchard, and D. G. Allen. 1984. Control of intracellular ionized calcium concentration by sarcolemmal and intracellular mechanisms. *Journal of Molecular and Cellular Cardiology*. 16:137–146.

Endo, M., and T. Kitazawa. 1978. E-C coupling studies on skinned cardiac fibers. *In* Biophysical Aspects of Cardiac Muscle. M. Morad and M. Tabatabai, editors. Academic Press, Inc., New York. 307–327.

Fabiato, A. 1981. Myoplasmic free calcium concentration reached during the twitch of an intact isolated cardiac cell and during calcium-induced release of calcium from the sarcoplasmic reticulum of a skinned cardiac cell from the adult rat or rabbit ventricle. *Journal of General Physiology*. 78:457–497.

Fabiato, A. 1982. Calcium release in skinned cardiac cells: variations with species, tissues, and development. *Federation Proceedings*. 41:2238–2244.

Fabiato, A. 1985a. Effects of ryanodine in skinned cardiac cells. *Federation Proceedings*. In press.

Fabiato, A. 1985b. Rapid ionic modifications during the aequorin-detected calcium transient in a skinned canine cardiac Purkinje cell. *Journal of General Physiology*. 85:189–246.

Fabiato, A., and F. Fabiato. 1975. Contractions induced by a calcium-triggered release of calcium from the sarcoplasmic reticulum of single skinned cardiac cells. *Journal of Physiology*. 249:469–495.

Fabiato, A., and F. Fabiato. 1978a. Myofilament-generated tension oscillations during partial calcium activation and activation dependence of the sarcomere length-tension relation of skinned cardiac cells. *Journal of General Physiology*. 72:667–699.

Fabiato, A., and F. Fabiato. 1978b. Effects of pH on the myofilaments and the sarcoplasmic reticulum of skinned cells from cardiac and skeletal muscles. *Journal of Physiology*. 276:233–255.

Forman, R., L. E. Ford, and E. H. Sonnenblick. 1972. Effect of muscle length on the force-velocity relationship of tetanized cardiac muscle. *Circulation Research*. 31:195–206.

Gross, R., M. Beckem, M. Kayser, M. Schramm, R. Taniel, and G. Thomas. 1985. Effect of the calcium agonistic dihydropyridine Bay K 8644 on the heart. *In* Bayer-Symposium IX: Cardiovascular Effects of Dihydropyridine-type Calcium Antagonists and Agonists. A. Fleckenstein, editor. Springer-Verlag, Berlin/Heidelberg. In press.

Henderson, A. H., R. Forman, D. L. Brutsaert, and E. H. Sonnenblick. 1971. Tetanic contraction in mammalian cardiac muscle. *Cardiovascular Research*. 5(Suppl. I):96–100.'

Hess, P., and W. G. Wier. 1984. Excitation-contraction coupling in cardiac Purkinje fibers. Effects of caffeine on the intracellular $[Ca^{2+}]$ transient, membrane currents, and contraction. *Journal of General Physiology*. 83:417–433.

Hilgemann, D. W., M. J. Delay, and G. A. Langer. 1983. Activation-dependent cumulative depletions of extracellular free calcium in guinea pig atrium measured with antipyrylazo II and tetramethylmurexide. *Circulation Research.* 53:779–793.

Hill, T. L. 1983. Two elementary models for the regulation of skeletal muscle contraction by calcium. *Biophysical Journal.* 44:383–396.

Kentish, J. C. 1984. Effects of inorganic phosphate on the force-[Ca²⁺] relationship of skinned muscles from rat ventricle. *Journal of Physiology.* 358:75P. (Abstr.)

Kort, A. A., E. G. Lakatta, E. Marban, M. D. Stern, and W. G. Wier. 1985. Fluctuations in intracellular calcium concentration and their effect on tonic tension in canine cardiac Purkinje fibres. *Journal of Physiology.* 367:291–308.

Krueger, J. W., and G. H. Pollack. 1975. Myocardial sarcomere dynamics during isometric contraction. *Journal of Physiology.* 251:627–643.

Marban, E., T. J. Rink, R. W. Tsien, and R. Y. Tsien. 1980. Free calcium in heart muscle at rest and during contraction measured with Ca²⁺-sensitive microelectrodes. *Nature.* 286:845–850.

Marban, E., and W. G. Wier. 1984. The influence of beta-adrenergic stimulation on the relation between intracellular [Ca²⁺] and tension in intact mammalian cardiac muscle. *Biophysical Journal.* 45:277a. (Abstr.)

Marban, E., and W. G. Wier. 1985. Ryanodine as a tool to determine the contributions of calcium entry and calcium release to the calcium transient and contraction of cardiac Purkinje fibers. *Circulation Research.* 56:133–138.

Marban, E., W. G. Wier, and D. T. Yue. 1985. Determination of maximal force in intact heart muscle. *Circulation.* 72(Suppl. III):328. (Abstr.)

Moisescu, D. G. 1976. Kinetics of reaction in calcium-activated skinned muscle fibres. *Nature.* 262:610–613.

Moore, E. D. W. 1984. Effects of pre-equilibration with Mg²⁺ on the kinetics of the reaction of aequorin with Ca²⁺. *Journal of General Physiology.* 84:11a. (Abstr.)

Morgan, J. R., W. G. Wier, P. O. Hess, and J. R. Blinks. 1983. Influence of Ca²⁺-channel blocking agents on calcium transients and tension development in isolated mammalian heart muscle. *Circulation Research.* 52(Suppl. 1):47–52.

Page, E. 1962. Cat heart muscle in vitro. III. The extracellular space. *Journal of General Physiology.* 46:201–213.

Robertson, S. P., J. D. Johnson, M. J. Holroyde, E. Kranias, J. D. Potter, and R. J. Solaro. 1982. The effect of TnI phosphorylation on static and kinetic calcium binding by cardiac TnC. *Journal of Biological Chemistry.* 257:260–263.

Schramm, M., G. Thomas, R. Towart, and G. Franckowiak. 1983. Novel dihydropyridines with positive inotropic action through activation of Ca²⁺ channels. *Nature.* 303:535–537.

Shiner, J. S., and R. J. Solaro. 1982. Activation of thin-filament-regulated muscle by calcium ion: considerations based on nearest-neighbor lattice statistics. *Proceedings of the National Academy of Sciences.* 79:4637–4641.

Shiner, J. S., and R. J. Solaro. 1984. The Hill coefficient for the Ca²⁺ activation of striated muscle contraction. *Biophysical Journal.* 46:541–543.

Spedding, M. 1982. Comparison of Ca⁺⁺-antagonists and trifluoperazine in skinned smooth muscle fibres. *British Journal of Pharmacology.* 75:25P. (Abstr.)

Stern, M. D., A. A. Kort, G. M. Bhatnagar, and E. G. Lakatta. 1983. Scattered-light intensity fluctuations in diastolic rat cardiac muscle caused by spontaneous Ca⁺⁺-dependent cellular mechanical oscillations. *Journal of General Physiology.* 82:119–153.

Strobeck, J. E., J. Krueger, and E. H. Sonnenblick. 1980. Load and time considerations in the

force-length relation of cardiac muscle. *Federation Proceedings.* 39:175–182.

Sutko, J. L., and J. L. Kenyon. 1983. Ryanodine modification of cardiac muscle responses to potassium-free solutions. Evidence for inhibition of sarcoplasmic reticulum release. *Journal of General Physiology.* 82:385–404.

Wĕndt, I. R., and D. G. Stephenson. 1983. Effects of caffeine on Ca-activated force production in skinned cardiac and skeletal muscle fibres of the rat. *Pflügers Archiv European Journal of Physiology.* 398:210–216.

Wier, W. G. 1980. Calcium transients during excitation-contraction coupling in mammalian heart: aequorin signals of canine Purkinje fibers. *Science.* 207:1085–1087.

Wier, W. G., and P. Hess. 1984. Excitation-contraction coupling in cardiac Purkinje fibers. Effects of cardiotonic steroids on the intracellular [Ca^{2+}] transient, membrane potential, and contraction. *Journal of General Physiology.* 83:395–415.

Wier, W. G., A. A. Kort, M. D. Stern, E. G. Lakatta, and E. Marban. 1983. Cellular calcium fluctuations in mammalian heart: direct evidence from noise analysis of aequorin signals in Purkinje fibers. *Proceedings of the National Academy of Sciences.* 80:7367–7371.

Winegrad, S. 1971. Studies of cardiac muscle with a high permeability to calcium produced by treatment with ethylene diaminetetraacetic acid. *Journal of General Physiology.* 58:71–93.

Winegrad, S. 1984. Regulation of cardiac contractile proteins: correlations between physiology and biochemistry. *Circulation Research.* 55:565–574.

Yue, D. T., E. Marban, and W. G. Wier. 1985. Relationship between force and [Ca^{2+}] in tetanized ventricular myocardium. *Biophysical Journal.* 47:353a. (Abstr.)

Yue, D. T., and W. G Wier. 1985. Estimation of intracellular [Ca^{2+}] by nonlinear indicators: a quantitative analysis. *Biophysical Journal.* 48:533–537.

Ziemer, R. E., W. H. Tranter, and D. R. Fannin. 1983. Signals and Systems: Continuous and Discrete. Macmillan Publishing Co., New York. 373–376.