Intracellular Calcium Transients and Developed Tension in Rat Heart Muscle

A Mechanism for the Negative Interval-Strength Relationship

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ABSTRACT The purposes of the present study were to determine (*a*) whether changes of intracellular [Ca^{2+}] (Ca_i) can account for the decrease of developed tension observed in rat heart muscle when stimulation rate is increased, and (*b*) whether the effect of stimulation rate on Ca_i is altered in conditions in which the rate of repriming of the sarcoplasmic reticulum (SR) is altered, as when perfusate [Ca^{2+}] (Ca_o) is increased, and in heart muscle from senescent animals. The photoprotein aequorin was used to monitor Ca_i in rat papillary muscles. In muscles from 6-mo-old rats, increasing the stimulation rate in the range 0.2–0.66 Hz led to parallel decreases of both the aequorin light transient and developed tension when Ca_o was 2 mM. When Ca_o was increased to 4 mM, changes in the stimulation rate had less effect on both the light transient and tension. At 8 mM Ca_o, changing the stimulation rate had no effect on either the light transient or developed tension. Papillary muscles from 24-mo-old rats, in which SR function is likely to be depressed, exhibited a prolonged Ca^{2+} transient and twitch. At a Ca_o of 4 or 8 mM, increasing the stimulation rate from 0.33 to 0.66 Hz still led to decreases in the size of the aequorin light transient and developed tension in these muscles. Developed tension and aequorin light responded to increases of Ca_o in the same way in both groups of muscles. We conclude that under the conditions of our experiments, developed tension is determined by Ca_i. The negative interval-strength relationship observed when Ca_o is in the physiological range can be accounted for by a time-dependent recycling of Ca^{2+} by the SR. The effects of increasing Ca_o and the age-related differences observed at high Ca_o can also be accounted for using this model.

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INTRODUCTION

The tension developed by rat heart muscle varies greatly with changes of stimulation rate. Unlike most other species, however, the rat exhibits a negative interval-strength relationship: a decrease in the stimulation rate leads to an increase in developed tension (Hoffman and Kelly, 1959).

Two main proposals have been made to account for this "rest potentiation." First, it has been suggested (Henry, 1975) that some metabolic substrate, essential for the development of tension, is replenished only slowly between contractions. Second, it has been suggested (Lee et al., 1970) that there is a time-dependent intracellular recycling of Ca^{2+} between contractions in the rat heart. There is abundant evidence for intracellular recycling of Ca^{2+}, in many types of muscle, from autoradiographic (Winegrad, 1970), anatomical (Simpson and Rayns, 1968), and pharmacological (Chapman and Leoty, 1976) studies, and many models of excitation-contraction coupling (e.g., Kaufman et al., 1974) have been based on such recycling. Thus, as the interstimulus interval is increased, there is more time for Ca^{2+} to be recycled and ready for the next release, so that more Ca^{2+} is released in response to the next stimulus and more tension is developed. This is supported by the preliminary observation that the size of the Ca^{2+} transient parallels developed tension when the stimulation rate is changed in rat heart muscle (Allen and Kurihara, 1980). Rest potentiation and the negaive interval-strength relationship are abolished by caffeine and ryanodine (Sutko and Willerson, 1980; Kort and Lakatta, 1983), drugs that inhibit normal sarcoplasmic reticulum (SR) function. This suggests that SR Ca^{2+} cycling may have a major role in the negative interval-strength relationship.

The present study was designed to investigate whether the decrease in tension with increasing stimulation rate can be attributed to a decline in the size of the Ca^{2+} transient, and whether the results could be explained by a model of time-dependent repriming of the SR. Since the rate of Ca^{2+} uptake and recycling by the SR is increased by raising $[Ca^{2+}]$ (Fabiato and Fabiato, 1975; Froehlich et al., 1978; Chiesi et al., 1981; Capogrossi and Lakatta, 1985), and decreased in heart muscle from senescent animals (Froehlich et al., 1978; Narayanan, 1981), we have examined the effect of these changes on the interval-strength relationship, using the photoprotein aequorin to monitor intracellular $[Ca^{2+}]$ (Ca_i) in rat papillary muscles.

METHODS

Animals

Wistar rats from the Gerontology Research Center's Aging Colony were used in the present study. The young adult animals studied were between 5 and 7 mo old and the senescent animals were 24 mo old. At ~24 mo, the mortality rate in this population of rats is 50% (Lakatta et al., 1975).

Solutions

The standard superfusate used in these studies contained (mmol/liter): 135 Na^+, 5 K^+, 2 Ca^{2+}, 1 Mg^{2+}, 104 Cl^-, 20 HCO_3^-, 1 HPO_4^{2-}, 20 acetate, 10 glucose, 4×10^{-5} insulin. This solution was equilibrated with nominal 5% CO_2/95% O_2 to give a pH of ~7.35.

Preparation

The muscle preparation was essentially the same as that described by Allen and Kurihara (1982) and Allen and Orchard (1983*a*). Briefly, rats were anesthetized with chloroform. A thin (<0.9 mm; range, 0.4–0.9 mm), uniform papillary muscle was dissected from the right ventricle and mounted horizontally in a muscle bath between a fixed hook and a tension transducer (UC2, Statham Inc., Oxnard, CA). The effective volume of the muscle bath was 0.25 ml. The muscle was superfused with Tyrode solution at a rate of 12 ml min^{-1}, and stimulated via punctate electrodes with 2-ms pulses at 1.2 times threshold using a Grass stimulator (SD 9, Grass Instrument Co., Quincy, MA). The temperature of the muscle bath was maintained at 30°C. The length of the muscle was adjusted to that at which it developed maximum tension in response to stimulation.

Aequorin

The photoprotein aequorin (purchased from Dr. J. R. Blinks, Mayo Medical School, Rochester, MN) was then injected into 30–100 superficial cells of the preparation using methods that have been described previously (Allen and Kurihara, 1982). Aequorin light was monitored using a photomultiplier tube (9893B/350, Thorn-EMI Gencom Inc., Plainview, NY). The output of the photomultiplier tube, as well as tension, and a stimulus marker were displayed on a four-channel pen recorder (2400S, Gould Inc., Cleveland, OH), and recorded on an FM tape recorder (3968A, Hewlett-Packard Co., San Diego, CA) for later offline analysis. Since the aequorin light signal is intrinsically noisy, it is necessary to average the responses to several stimuli to obtain an adequate signal-to-noise ratio. This was done using a microcomputer (MNC-11, Digital Equipment Corp., Maynard, MA), sampling at a rate of 1 kHz. All references to the aequorin light transient in the present paper refer to this averaged record. Such averaging may obscure transient changes in the size and time course of the light transient. However, in the present study, aequorin light and tension were obtained in steady state conditions, as judged by developed tension. This potential drawback of averaging does not, therefore, limit the interpretation of the present results. This computer was also used to obtain data levels and times on the digitized, averaged signal.

Although the use of aequorin as an indicator of Ca_i in heart muscle is now well established, and has been discussed recently (see Blinks et al., 1982), some relevant points need to be discussed. First, aequorin is a relatively poor indicator of Ca_i in the range that is thought to exist in resting heart muscle (Blinks et al., 1982). In the present study, we estimate that we could detect a $[Ca^{2+}]$ of >200 nM. Second, the amount of light emitted by aequorin is proportional to $[Ca^{2+}]^{2.5}$. This means that spatial inhomogeneities of $[Ca^{2+}]$ will result in an overestimate of Ca_i (Blinks et al., 1982). Third, the calibration procedure used to convert aequorin light to $[Ca^{2+}]$ depends on an in vitro calibration curve (see Allen and Blinks, 1979), which is obtained in conditions that are thought to mimic the intracellular environment. The calibration curve used to convert aequorin light to $[Ca^{2+}]$ in the present study was obtained in 2 mM Mg^{2+}, 154 mM K^+, pH 7.2, at 30°C, and is given by

$$[Ca^{2+}] = \{[1 - (FL)^{0.33}] - (FL)^{0.33} \cdot K_{TR}\}/K_R \cdot [(FL)^{0.33} - 1],$$

where $K_{TR} = 152$, $K_R = 4.13 \times 10^6$, and FL is the fractional luminesence, which was obtained as described by Allen and Blinks (1979).

Although aging may introduce additional problems in the interpretation of aequorin light transients—if, for instance, there is different aging of superficial and central cells, or if the intracellular environment changes with age—we know of no evidence for such

changes. The same aequorin calibration curve was therefore used to convert light signals to $[Ca^{2+}]$ in the two age groups.

Experimental Program

Control conditions were taken as a perfusate $[Ca^{2+}]$ (Ca_o) of 2 mM and a stimulation rate of 0.33 Hz. The muscle was stimulated at 0.33 Hz, at each Ca_o, before and after changing the stimulation rate to either 0.66 or 0.2 Hz. The order in which changes of the stimulation rate and Ca_o were tested was randomized between muscles. Results have been normalized either (*a*) to 0.33 Hz at the Ca_o being examined, when the stimulation rate was changed, or (*b*) to 2 mM Ca_o, 0.33 Hz when Ca_o was increased. This approach was taken to obviate the effect of aequorin consumption, which was small but significant over the time course of the experiments.

Data Analysis

The effect of age on the aequorin light transient and twitch tension was tested using unpaired *t* tests. In muscles in which Ca_o and/or the stimulation rate were varied, comparisons were made using a two-way analysis of variance followed by paired *t* tests if necessary, with each muscle serving as its own control. Significance was taken as $P < 0.05$. All values are expressed as means ± SEM.

RESULTS

Effect of Changing the Stimulation Rate at Different Ca_o in 6-Mo-Old Muscles

Fig. 1*A* shows continuous chart recordings of aequorin light and tension from a representative muscle from a 6-mo-old rat when the stimulation rate was increased from 0.33 to 0.66 Hz and decreased from 0.33 to 0.2 Hz, in a Ca_o of 2 mM. Fig. 1*B* shows aequorin light transients and twitches averaged during the periods indicated in Fig. 1*A*. Increasing the stimulation rate from 0.33 to 0.66 Hz resulted in a decrease in the height of both the aequorin light transient and twitch tension. Decreasing the stimulation rate increased both the light transient and twitch tension. Table I shows the mean changes in the light transient and twitch tension when the stimulation frequency was changed when Ca_o was 2, 4, and 8 mM.

There were no significant changes in the time course of either aequorin light or the twitch when the stimulation rate was changed. Resting tension and light did not show any detectable changes.

In 4.0 mM Ca_o, the changes of light and tension observed with either increases or decreases in stimulation rate were smaller than those observed in a Ca_o of 2.0 mM. Fig. 2 shows a typical response of a 6-mo-old muscle to changes of stimulation rate when Ca_o was 8 mM. Changes of the stimulation rate had no effect on either the size of the light transient or twitch tension. These results are compatible with the previous suggestion that repriming of SR Ca^{2+} release is time dependent (see Introduction) and that increasing Ca_o can alter this dependence.

Effect of Age on the Aequorin Light Transient and Developed Tension

We examined the effect of changes in stimulation rate in muscles from 24-mo-old animals, in which SR function might be expected to be depressed (Froehlich et al., 1978).

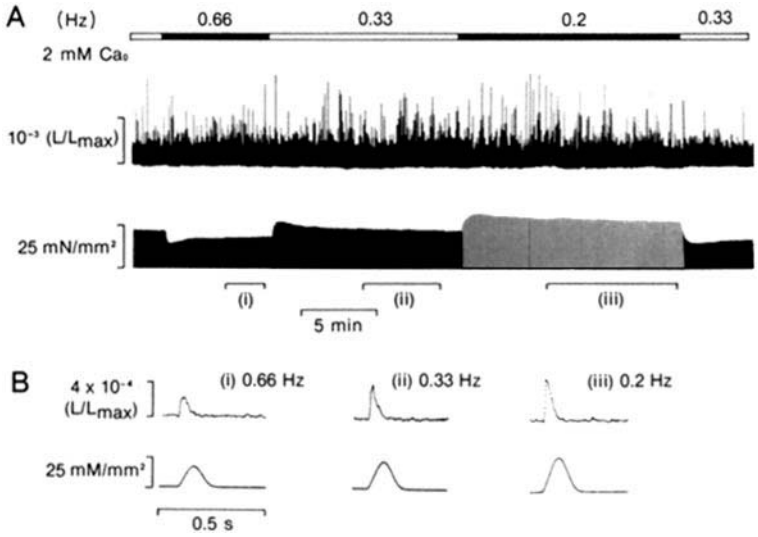

FIGURE 1. The effect of changing stimulation frequency, at a Ca_o of 2 mM, on aequorin light and developed tension in a 6-mo-old papillary muscle. (*A*) Chart records of aequorin light (top) and tension (below). The frequency of stimulation is shown above the record. (*B*) Aequorin light transients (top) and twitch tension (bottom) averaged (n = 100) during the periods shown in the top panel. The aequorin light signal is expressed as fractional luminesence (L/L_{max}). See Allen and Blinks (1979) for details.

Fig. 3 shows averaged light and tension records obtained from papillary muscles from 6- and 24-mo-old rats. The twitch is longer and the rate of decline of the aequorin light transient is lower in the older muscles.

Table II shows mean values of the magnitude and time course of the aequorin light transient and developed tension from muscles in the two age groups in control conditions.

TABLE I

Effect of Stimulation Rate on the Size of the Aequorin Light Transient and Twitch Tension in 2, 4, and 8 mM Ca_o

	Peak light (percent of 0.33 Hz)			Peak tension (percent of 0.33 Hz)		
Frequency (Hz)	0.66	0.33	0.2	0.66	0.33	0.2
(*A*) 6-mo muscles						
2 mM Ca_o	68.8±5.6*	100	126.5±8.5*	68.4±4.3*	100	114.0±5.6*
4 mM Ca_o	88.1±12.0	100	114.6±18.3	85.1±9.5	100	103.9±6.9
8 mM Ca_o	99.5±9.0	100	101.8±4.6	94.8±6.5	100	105.0±0.8*
(*B*) 24-mo muscles						
2 mM Ca_o	76.5±9.0*	100	114.2±9.8	72.8±4.8*	100	120.7±10.0
4 mM Ca_o	62.3±7.7*	100	130.0±15.8	71.4±3.7*	100	108.6±6.7
8 mM Ca_o	83.2±10.8	100	89.0±6.0	84.0±3.7*	100	104.0±3.3

Values are means ± SEM and are normalized to the value at 0.33 Hz. The asterisk denotes a significant effect of stimulation changes from the value at 0.33 Hz.

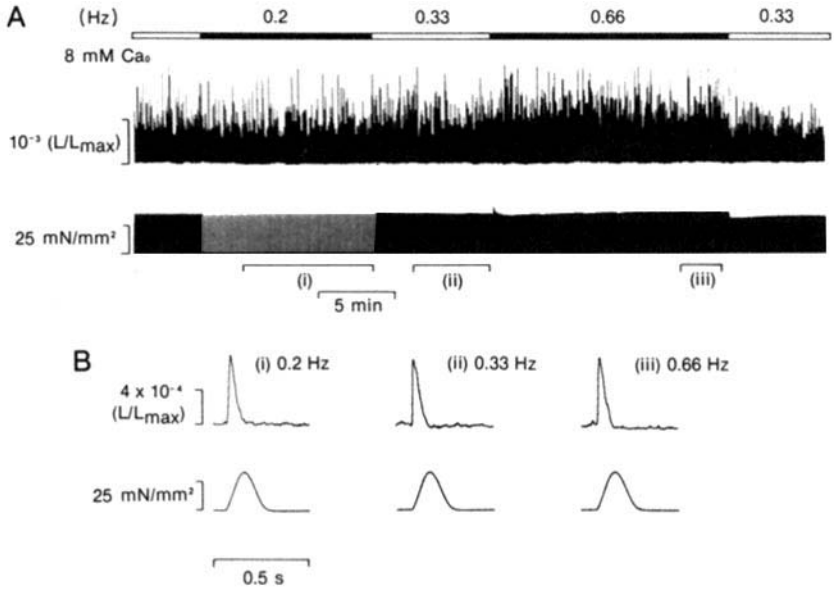

FIGURE 2. The effect of changing stimulation frequency, at a Ca_o of 8 mM, on aequorin light and developed tension in the same experiment as that shown in Fig. 1. (*A*) Chart records of aequorin light (top) and tension (below). The frequency of stimulation is shown above the record. (*B*) Aequorin light transients (top) and twitch tension (bottom) averaged (n = 100) during the periods shown in the top panel.

There were no significant differences in the size of the muscles in the two age groups, nor was there any difference in resting tension or in the tension developed by each age group. The estimated peak [Ca^{2+}] values in the two age groups were not significantly different, and were all within the range reported previously by Allen and Kurihara (1980). The observation that there were no significant differences in peak [Ca^{2+}] or peak tension in the two age groups

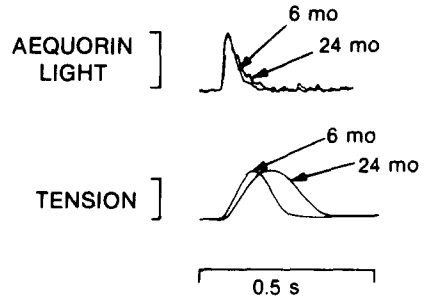

FIGURE 3. Averaged (n = 75) Ca^{2+} transients (top) and twitch tension (bottom) from a 6-mo-old and a 24-mo-old muscle. The light and tension records have been scaled to the same height and superimposed. The light calibration bar represents a fractional luminesence of 4×10^{-4} for the 6-mo muscle and 3.5×10^{-4} for the 24-mo muscle. The tension calibration bar represents a tension of 27 mN/mm^2 for the 6-mo muscle and 29 mN/mm^2 for the 24-mo muscle.

supports the observation in skinned fibers that there is no difference in the Ca^{2+} sensitivity of the contractile proteins between the two groups (Bhatnagar et al., 1984). However, the time to peak tension and the half-time of relaxation of developed tension ($T_{1/2}$ tension) were significantly longer in the older muscles than in the younger muscles. The half-time of the decline of the aequorin light transient ($T_{1/2}$ light) was ~50% longer in the 24-mo muscles.

The results in Table II and Fig. 3 are compatible with the observation that Ca^{2+} sequestration by the SR is slower in muscles from senescent animals than in muscles from young animals (Froehlich et al., 1978; Narayanan, 1981) and that this is a cause of the prolonged contraction duration in senescent myocardium (Lakatta et al., 1975).

Effect of Changing the Stimulation Rate at Different Ca_o in 24-Mo-Old Muscles

Changing the stimulation frequency when Ca_o was 2 mM had similar effects on the light transient and developed tension as those observed in the young muscles

TABLE II

Summary of Measured Variables for Muscles in the Two Age Groups Studied, Under Control Conditions

	6 mo		24 mo	
Muscle diameter (mm)	0.68±0.04	(11)	0.70±0.05	(7)
Time to peak tension (ms)	88.8±3.1*	(11)	110.4±6.8*	(7)
$T_{1/2}$ tension (ms)	54.9±2.3*	(11)	73.4±4.4*	(7)
Resting tension (mN/mm^2)	5.6±0.9	(11)	8.4±1.5	(7)
Developed tension (mN/mm^2)	25.6±5.0	(11)	29.4±10.7	(7)
$T_{1/2}$ light (ms)	21.3±1.5*	(11)	32.9±4.3*	(7)
L/L_{max}	2.49×10^{-4} $\pm 6.04 \times 10^{-5}$	(4)	1.24×10^{-4} $\pm 3.25 \times 10^{-5}$	(5)
Estimated peak Ca_i (M)	2.18×10^{-6} $\pm 2.30 \times 10^{-7}$	(4)	1.62×10^{-6} $\pm 2.03 \times 10^{-7}$	(5)

Values are means ± SEM. Numbers in brackets are number of animals. The asterisk denotes a significant difference between the two groups.

(Table I, *B*). However, when Ca_o was 4 or 8 mM, the response of 24-mo-old muscles to changes of the stimulation rate was different from that in the young muscles. While a reduction in the stimulation frequency from 0.33 to 0.2 Hz had no significant effect on the light transient or developed tension, neither tension nor light was maintained as the frequency of stimulation was increased from 0.33 to 0.66 Hz. In a Ca_o of 4.0 mM, the peak of the aequorin light transient decreased significantly to 62.3 ± 7.7% of control, and developed tension decreased significantly to 71.4 ± 3.7% of control. In a Ca_o of 8.0 mM, increasing the stimulation rate significantly decreased developed tension to 84.0 ± 3.7% of control and the peak of the aequorin light transient to 83.2 ± 10.9% of control. An example of this behavior is illustrated in Fig. 4.

This result indicates that heart muscle from old animals differed from that of young animals when the stimulation frequency was increased and Ca_o was elevated. In young muscles, the decrease in the aequorin light transient and the associated twitch caused by a reduction in the interstimulus interval could be

overcome by increasing Ca_o. In muscles from older animals, however, elevation of Ca_o was not able to compensate for increases in the stimulation rate, and both peak light and tension declined at 4 and 8 mM Ca_o, as the stimulation rate was increased to 0.66 Hz.

Effect of Changing Ca_o

It was possible that the different response of old muscle to changes of the stimulation rate at 4 and 8 mM Ca_o was because old heart muscle responded differently to an increase of Ca_o. To investigate this possibility, we examined the effect of increasing Ca_o in steps from 2 to 8 mM in both 6-mo and 24-mo muscles stimulated at a control frequency of 0.33 Hz.

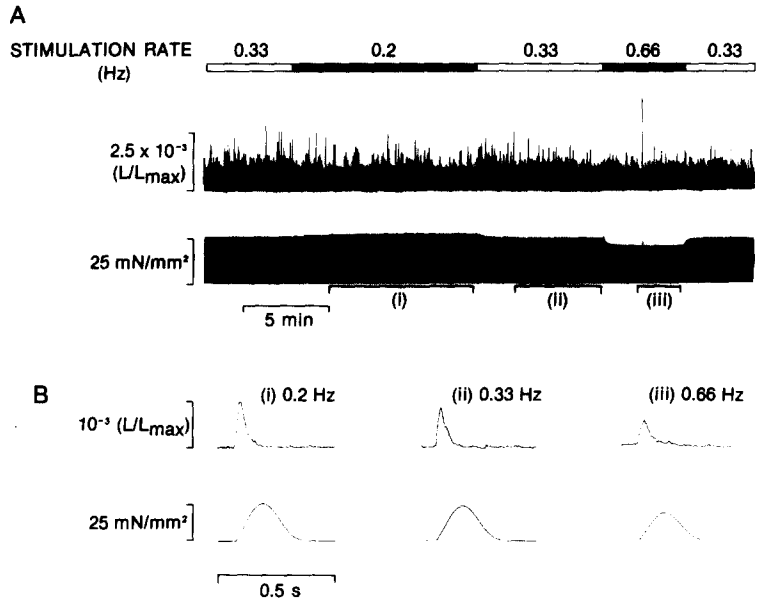

FIGURE 4. The effect of changing stimulation frequency, at a Ca_o of 8 mM, on aequorin light and twitch tension in a 24-mo-old papillary muscle. (*A*) Chart records of aequorin light (top) and tension (below). The stimulation frequency is shown above the record. (*B*) Aequorin light transients (top) and tension (below) averaged (n = 100) during the periods indicated in the top panel.

Fig. 5 illustrates the effect of increments of Ca_o on tension and aequorin light transients in a 24-mo-old muscle. The top panel shows that increasing Ca_o from 2 to 4 mM resulted in a large increase in developed tension. Further increases of Ca_o resulted in small decreases of developed tension in this muscle.

The lower panel in Fig. 5*A* shows averaged aequorin light and tension records from the periods marked in the top panel. This shows that the changes in developed tension were paralleled by changes in the peak of the aequorin light transient. Table III lists the mean effect of increasing Ca_o on the aequorin light transient and tension in 6- and 24-mo muscles. Increasing Ca_o from 2 to 4 mM caused large increases of the aequorin light transient and developed tension in

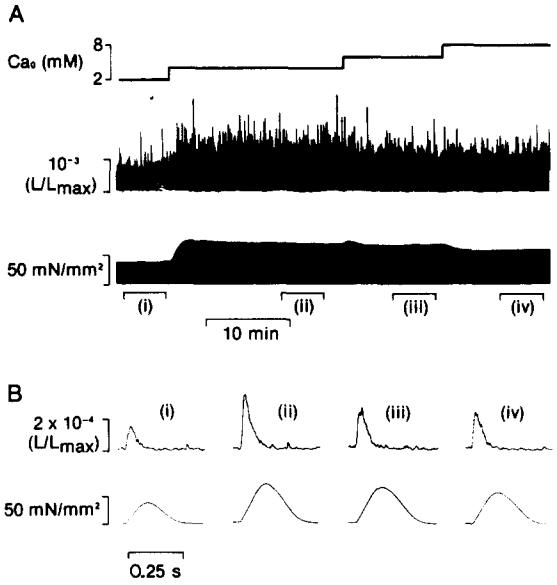

FIGURE 5. The effect of increasing Ca_o on aequorin light transients and tension in a 24-mo-old papillary muscle. (*A*) Chart recordings of aequorin light (top) and tension (bottom). The solution protocol is shown above the record. (*B*) Aequorin light transients (top) and twitch tension (bottom), averaged (n = 100) during the periods shown in the top panel.

both age groups. Further increases in Ca_o had little effect on peak aequorin light or tension. Resting tension and resting light, measured in the 50 ms before the stimulus, did not change significantly.

The changes of aequorin light and tension observed in 6-mo-old muscles stimulated at 0.33 Hz on increasing Ca_o from 2 to 8 mM were not significantly different from those observed in 24-mo-old muscles. These results indicate that the amount of Ca^{2+} initiating a contraction reaches a maximum when Ca_o is ~4 mM; it cannot be increased, and may even decrease, when Ca_o is increased further. More importantly for the present study, it indicates that the different response of old muscle to changing the stimulation rate at a high Ca_o is not because Ca_i responds differently to changes of Ca_o in old muscle.

TABLE III

Effect of Increasing Ca_o on the Size of the Aequorin Light Transient and Twitch Tension in the Two Age Groups Studied

	Light				Tension			
Ca_o	2	4	6	8	2	4	6	8
6 mo (n = 7)	100	189±40	192±28	186±33	100	147±16	133±13	133±17
24 mo (n = 4)	100	188±21	193±42	177±65	100	165±29	181±56	180±58

The stimulation frequency was 0.33 Hz throughout; values are normalized to those in 2 mM Ca_o. There were no significant differences between the two groups.

Relationship Between Peak Aequorin Light and Tension

The relationship between the changes in the size of the light transient and the change in developed tension observed in all the protocols in the present study is shown in Fig. 6. In all the conditions studied, the size of the aequorin light transient always changed in the same direction, and usually by approximately the same amount, as developed tension. In each group of muscles, the changes of peak light were never significantly different from the change of peak tension. This close relationship between peak force and light was maintained across the entire range of Ca_o and stimulation frequencies in Table I and this relation did

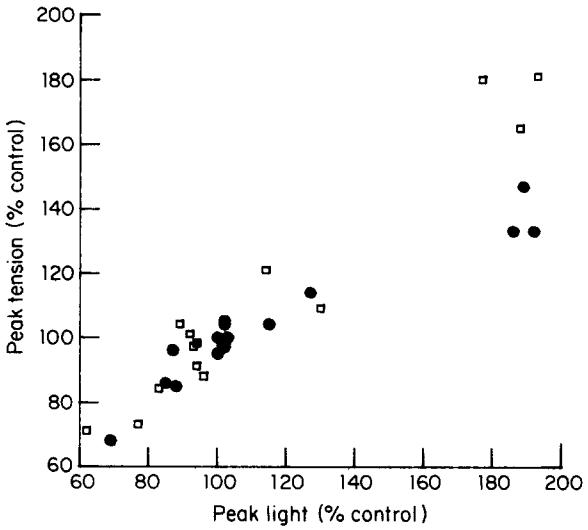

FIGURE 6. The relationship between changes in the size of the aequorin light transient and twitch tension. Data from Tables II and III were normalized in the same way. The data from the 6-mo muscles alone are best fitted to the line $y = 5.3x^{0.63}$ ($r = 0.93$), and the 24-mo muscles to the line $y = 2.05x^{0.85}$ ($r = 0.94$). The data from both age groups can be fitted to the linear function $y = 29.6 \pm 0.68x$ ($r = 0.84$), or to the power function $y = 3.22x^{0.74}$ ($r = 0.89$).

not differ in the two age groups. The only exception to this was when Ca_o was increased at 0.33 Hz in the young muscles. Although the response of the muscles did not differ significantly from that of the old muscles, tension appeared to increase less than aequorin light.

DISCUSSION

Relationship Between Stimulation Rate, Ca_o, and Ca_i

Changes of the stimulation frequency caused parallel changes in developed tension and the size of the aequorin light transient. Therefore, at 2 mM Ca_o, the decrease in developed tension observed as the stimulation frequency was increased could be attributed to a decrease in the amount of Ca^{2+} released. We

will consider, therefore, (*a*) possible sources of Ca^{2+} and (*b*) mechanisms that could alter the amount of Ca^{2+} released in response to each stimulus.

POSSIBLE SOURCES OF ACTIVATING CA^{2+} Ca^{2+} enters the cytoplasm from the SR and via the "slow" inward current (I_{si}). It seems unlikely that I_{si} was a major source of Ca^{2+} in the present study since Willerson et al. (1978) have shown that when Ca_o is within the range used in the present study, blockers of I_{si} have little effect on developed tension. Much evidence, e.g., time-dependent repriming of caffeine contractures (Chapman and Leoty, 1976), supports the idea that Ca^{2+} replenishment of the SR in cardiac muscle is time dependent, and rat heart muscle, in particular, appears to depend on the SR for most of its activating Ca^{2+} (Bers, 1985). When SR Ca^{2+} cycling is inhibited by caffeine or ryanodine (Sutko and Willerson, 1980; Kort and Lakatta, 1983), rat heart muscle exhibits rest decay, rather than rest potentiation, which suggests that the SR Ca^{2+} cycling plays a major role in the negative interval-strength relationship observed under normal conditions.

MECHANISMS THAT COULD ALTER THE AMOUNT OF CA^{2+} RELEASED BY THE SR It has been suggested (Henry, 1975) that the decrease of developed tension observed when the stimulation frequency is increased at 2 mM Ca_o is due to a lack of metabolic substrate. The present study has shown that for this to be so, the lack of substrate must decrease the size of the Ca^{2+} transient. Very severe metabolic inhibition can decrease the size of both the Ca^{2+} transient and twitch tension (Allen and Orchard, 1983*b*). It seems unlikely, however, that metabolic deprivation can account for the decrease in the size of the Ca^{2+} transient as the stimulation rate was increased in the present study. Inspection of the first column of Table I, *A*, shows that in higher Ca_o, increasing the stimulation rate has less effect on Ca_i and tension. If the decrease in the size of the Ca^{2+} transient observed in 2 mM Ca_o as the stimulation rate was increased was due to metabolic insufficiency, then it is difficult to see how increasing Ca_o could have this effect.

It seems more likely, therefore, that another mechanism must determine the amount of Ca^{2+} released by the SR as the stimulation rate is changed. Two possibilities remain: there may be a time-dependent replenishment of a trigger for Ca^{2+} release, or the SR itself may require time to reload its Ca^{2+} release site.

Restitution of the action potential is probably not the determinant of Ca^{2+} release from the SR in rat cardiac muscle, since if two stimuli are placed sufficiently close in time (i.e., 100 ms, which is much shorter than the shortest interstimulus interval used in this study), an electrical response can be elicited with little or no contractile response (Lee et al., 1970). The mechanism coupling the action potential to Ca^{2+} release may require restitution and may be a determinant of the restitution time for Ca^{2+} release. However, blocking Ca^{2+} entry via I_{si}, which can act as a trigger for Ca^{2+} release from the SR (Fabiato, 1983), does not affect developed tension when Ca_o is within the range used in the present study (Willerson et al., 1978). These results suggest that the time-dependent restitution of Ca^{2+} release depends on another mechanism. It seems most likely that SR Ca^{2+} loading and cycling is the major determinant of the restitution time for subsequent Ca^{2+} release.

Thus, increasing the stimulation rate at 2 mM Ca_o gives the SR less time to

recycle Ca^{2+}, and this results in a decrease in Ca^{2+} release, manifested as a decrease in the size of the Ca^{2+} transient and developed tension. However, an increase in Ca_o, which increases Ca_i (Sheu and Fozzard, 1982) and hence the rate at which the SR pumps Ca^{2+} (Froehlich et al., 1978), decreases the time required before a given release of Ca^{2+} can occur (Lee et al., 1970). Hence, the Ca^{2+} release was not decreased as the stimulation rate was increased at higher Ca_o.

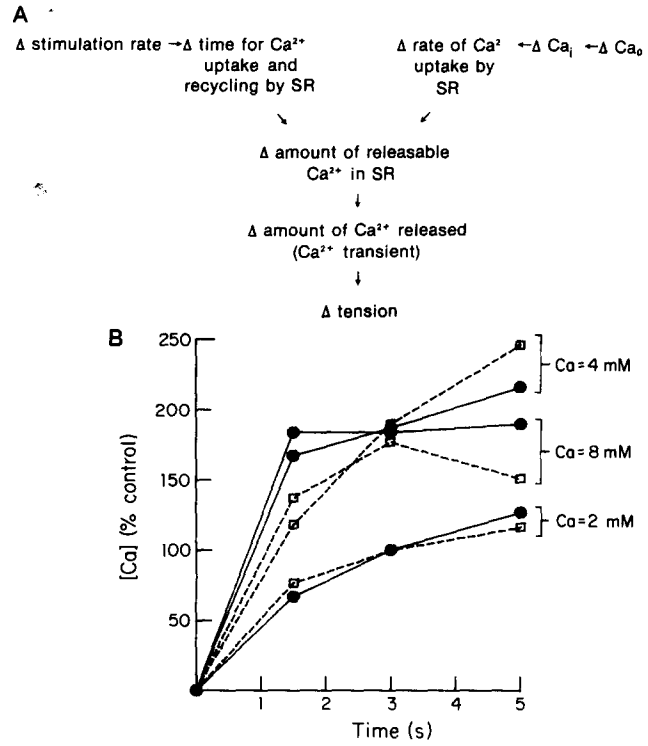

FIGURE 7. A model relating the amount of Ca^{2+} released by the SR to stimulation rate and Ca_o (see text for details). (*A*) A schematic diagram showing how changes in stimulation rate and Ca_o could alter the amount of Ca^{2+} available for release by the SR. (*B*) The amount of Ca^{2+} released (the size of the Ca^{2+} transient) as a function of Ca_o and interstimulus interval in the two age groups studied. The abscissa gives the time between stimuli (the time available for the SR to load with Ca^{2+}). The data are taken from the present study (Tables I and III). The changes at each Ca_o are normalized to the value at 0.33 Hz at that Ca_o (as in Table I). The vertical separation was taken from the values in Table III. This approach was taken in order to obviate the effects of time on the preparation, which makes direct comparisons of the response a long time apart difficult.

Fig. 7*A* shows a schematic diagram of this hypothesis, which is the simplest that can explain all the present results. Fig. 7*B* shows aequorin light data taken from Tables I and III, and is assumed to represent the amount of Ca^{2+} released by the SR (see above) as a function of both Ca_o and time after the previous stimulus. At 2 mM Ca_o, slowing the stimulation rate allows more time for SR

Ca^{2+} loading and recycling, and hence leads to a larger subsequent Ca^{2+} release and twitch. At 4 mM Ca_o, SR Ca^{2+} loading and recycling is faster, and the curve relating the size of the light transient to time is flat after 3 s. Therefore, in young muscles, the recycling process is complete in 3 s and slowing the stimulation rate does not lead to further increases in Ca^{2+} release. However, increasing the stimulation rate will decrease the time available for Ca^{2+} loading and recycling, so the Ca^{2+} release site will not be fully replenished. In young muscles, the decrease will not be as great as that observed in 2 mM Ca^{2+}, since the SR is on a flatter part of the curve relating SR Ca^{2+} uptake to time.

In 8 mM Ca_o, the SR Ca^{2+} cycling is complete in <1.5 s in young muscles, so that changes in the interstimulus interval within the range studied had no effect on releasable Ca^{2+}, and hence no effect on Ca_i and tension.

In old muscles, however, increasing the stimulation rate still led to a decrease in Ca_i and tension when Ca_o was 4 and 8 mM. Previous studies have shown that if the interstimulus interval is reduced sufficiently, old heart muscle will not respond to a second stimulus, whereas at the same interstimulus interval, young muscles responded with a contraction (Lakatta et al., 1975). This indicates that Ca^{2+} recycling is slower in old muscles. There are four possible explanations for this. First, Ca^{2+} may dissociate from troponin more slowly in the old muscles. However, the observation that both developed tension and the Ca^{2+} transient are prolonged argues against this explanation (Allen and Kurihara, 1982). Second, the action potential is shorter in rat ventricular muscle than in other species, and is repolarizing at about the time that the Ca^{2+} transient is declining. The action potential is prolonged in heart muscle from senescent animals (Wei et al., 1984) and this may modify Ca^{2+} uptake by the SR of old animals. Third, age-related differences may occur in the mechanisms that govern Ca^{2+} release. There is some evidence for this from work on mechanically skinned cell fragments, in which fragments from older animals require a greater Ca^{2+} trigger for release to occur (Fabiato, 1982). Fourth, the SR of the older animals has a lower rate of Ca^{2+} sequestration than that of younger animals. There is certainly evidence for this from work on isolated SR vesicles (Froehlich et al., 1978; Narayanan, 1981). The age difference in the rate of Ca^{2+} uptake by the SR becomes more marked as $[Ca^{2+}]$ is increased (Froehlich et al., 1978), which could explain why age-related differences were seen at a Ca_o of 8 mM, but not at 2 mM.

It is of interest that in some muscles, raising Ca_o higher than 4 mM led to a decrease in the light transient and developed tension. There have been recent reports of a type of Ca^{2+} overload in which the Ca^{2+} transient becomes larger, while developed tension declines (Allen et al., 1984). It was suggested that this was due to spontaneous Ca^{2+} release from the SR of some cells of the preparation between stimuli. This may be the explanation for the small decrease of tension observed when Ca_o was increased in the 6-mo-old muscles (see Table III). The present results also show another type of "overload," in which both the size of the Ca^{2+} transient and tension decrease as Ca_o is increased (e.g., Fig. 5). The cause of this decrease is unclear, but may also be due to spontaneous release of Ca^{2+} from the SR during the diastolic period when Ca_o is high.

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