Dynamic relations among length, tension, and intracellular Ca\(^{2+}\) in activated ferret papillary muscles

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Saeki, Yasutake, Satoshi Kurihara, Kimiaki Komukai, Tetsuya Ishikawa, and Kiyohiro Takigiku. Dynamic relations among length, tension, and intracellular Ca\(^{2+}\) in activated ferret papillary muscles. Am. J. Physiol. 275 (Heart Circ. Physiol. 44): H1957–H1962, 1998.—To study the effects of mechanical constraints on the Ca\(^{2+}\) affinity of cardiac troponin C, we analyzed the tension and aequorin light (AL) responses to sinusoidal length changes (5–10% of the initial muscle length) in aequorin-injected, tetanized cardiac muscles. The amplitude of the quasi-sinusoidal tension and AL responses decreased with increasing length-perturbation frequency from 0.5 to 1 Hz at 24°C and from 1 to 3 Hz at 30°C. The increase in AL corresponded well to the increase in tension; likewise, the decrease in AL to the increase in tension and the tension response lagged behind the length change. A further increase in frequency (>1 Hz at 24°C and >3 Hz at 30°C) markedly increased the amplitude of the tension responses but decreased the amplitude of the AL responses. The increase in AL lagged behind the decrease in tension; likewise, the decrease in AL lagged behind the increase in tension, and the tension response led the length change. From previous mechanistic interpretations of the frequency dependence of the amplitude of tension response, we argue that the Ca\(^{2+}\) affinity of cardiac troponin C changes in parallel with the active tension (i.e., the number of active cross bridges) but not with the passive tension produced by the length perturbation-induced cross-bridge strain.

Cardiac troponin C; affinity; cross-bridge dynamics; aequorin dynamic relations among length, tension, and intracellular Ca\(^{2+}\). There is now considerable evidence that in cardiac muscle, physical parameters such as tension and length affect the Ca\(^{2+}\) affinity of troponin C (for reviews, see 1, 7, 17, 24). Saeki et al. (21) have previously shown that in aequorin-injected, tetanized ferret right ventricular papillary muscles there is a close correlation between the tension and aequorin light (AL, an indicator of intracellular Ca\(^{2+}\)) transients in response to a step change in length. Briefly, the transient AL response to a step release in length comprises two phases: the first phase consists of an initial rapid increase, which is preceded by a decrease in tension during the release [i.e., the first phase described by Huxley and Simmons (14)]; the second phase consists of a rapid decrease toward the initial control level, the time course of which is in phase with that of the second phase of rapid tension rise [i.e., the second phase described by Huxley and Simmons (14)]. In contrast, no detectable initial rapid AL change is observable for the step stretch, although there is a clear reduction of AL concurrent with the delayed tension rise after the stretch. These results are quite consistent with the results in Triton X-100 skinned cardiac preparations (2) and have been interpreted to indicate that the Ca\(^{2+}\) affinity of cardiac troponin C changes in phase with tension, since in these preparations the changes in AL could be considered to reflect the changes in myoplasmic Ca\(^{2+}\) due to the detachment from and/or the attachment to troponin C.

However, these interpretations could not be applicable to the relationship between the tension and AL transients during and immediately after the step-length change (particularly to no AL changes concurrent with the rapid tension rise following the stretch) because of the limitation for the time resolution in both tension and AL signals. In addition, the tension-dependent changes in the Ca\(^{2+}\) affinity of cardiac troponin C have not been fully understood in relation to the cross-bridge dynamics. Therefore, using the same preparation as that in the previous study (21) (aequorin-injected, tetanized ferret right ventricular papillary muscle), we studied the tension and AL responses to sinusoidal length changes of up to 5 Hz to analyze their dynamic interrelationships.

Sinusoidal analysis is particularly useful for studying cross-bridge kinetics because of its high resolving power (16, 20). In this method, any reaction significantly faster than the frequency of observation can be regarded as being at a local equilibrium (or steady state), whereas any reaction significantly slower than the frequency of observation does not occur in the time frame of the experiment. In between, we can analyze the dynamic length-tension-AL relations in the frequency domain.

MATERIALS AND METHODS

Preparation and apparatus. Male ferrets (800 g body wt) were anesthetized with pentobarbital sodium (80 mg/kg ip). Hearts were quickly removed, and thin right ventricular papillary muscles were excised and placed in a petri dish that contained oxygenated normal Tyrode solution. Each end of the excised muscle was tied with a silk thread to a small tungsten wire hook (125 µm in diameter) in a dissectioning dish. The preparation was transferred to an experimental chamber and mounted horizontally between a length driver (OCX-101A, General Scanning, CA) and a tension transducer (type BG-10, Kulite Semiconductor, NJ; compliance 2.5 µm/g, unloaded resonant frequency 1 kHz). The experimental chamber was equipped with a pair of platinum-black wire electrodes for stimulation. The temperature of the bathing solution was maintained at either 24° or 30°C with a thermostatic temperature-control device (type D-3, Haake, Germany) with an accuracy of ±0.5°C.
Solutions. Normal Tyrode solution used for dissecting the preparation and for the injection of aequorin was composed of the following (mM): 135 Na$^+$, 5 K$^+$, 2 Ca$^{2+}$, 1 Mg$^{2+}$, 102 Cl$^-$, 20 HCO$_3^-$, 1 HPO$_4^{2-}$, 1 SO$_4^{2-}$, 20 acetic acid, 10 glucose, and 5 U/l insulin; pH 7.34 at 30°C when equilibrated with 5% CO$_2$-95% O$_2$. After the injection of aequorin, normal Tyrode solution was replaced with Tyrode solution buffered with 5 mM HEPES (HEPES-Tyrode solution), which had the following composition (mM): 128 Na$^+$, 5 K$^+$, 2 Ca$^{2+}$, 1 Mg$^{2+}$, 117 Cl$^-$, 1 SO$_4^{2-}$, 20 acetic acid, 10 glucose, and 5 U/l insulin; pH was adjusted to 7.35 with NaOH at 30°C. The solution was oxygenated with 100% O$_2$.

Aequorin injection and measurement of light signals. Aequorin, purchased from Dr. J. R. Blinks, was dissolved in 150 mM KCl and 5 mM HEPES solution at pH 7.3, with a final aequorin concentration of 50–100 µM. A glass micropipette with a resistance of 30–80 MΩ was used to inject aequorin solution, was used for the injection of aequorin. Aequorin was injected into 100–200 superficial cells of each preparation with 5–10 kg/cm$^2$ pressure while the membrane potential was monitored. AL signals were detected with a photomultiplier (EMI 9789A, Ruislip, UK), which was mounted in a small housing. A 10-mm diameter quartz light guide, which was attached to the photomultiplier, was placed with its lower end just above the preparation (just below the surface of the Tyrode solution) to collect a large fraction of the total AL emitted by the preparation as possible and to make the efficiency of light collection independent of movements of the preparation associated with the length changes. Improved light detection was facilitated by placing a concave mirror under the preparation. This procedure was found to completely eliminate artifactual changes in AL collection associated with the length change (0.55 mm at most) performed in the present study. Details of the method were previously described by Allen and Kurihara (3). All data were stored on a tape (NFR-3515W, Sony Magnescan, Tokyo, Japan) and a computer for later analyses. To improve the signal-to-noise ratio, the AL signals were recorded after being filtered with a low-pass active filter of 10 Hz, which passed through a signal-to-noise ratio, the AL signals were recorded after being filtered with a low-pass active filter of 10 Hz, which passed through a low-pass active filter of 10 Hz, which passed through a low-pass active filter of 10 Hz, which passed through a low-pass active filter of 10 Hz.

RESULTS

Figure 1A shows a typical example of the averaged tension (middle trace) and AL (bottom trace) signals in response to 1-Hz sinusoidal length changes (top trace) from $L_{max}$ to 0.92 $L_{max}$ during tetanic contraction at 30°C. Although the tension and AL responses slightly deviated from sinusoidal waveforms because of the phase delay of AL responses produced at these higher perturbation frequencies, because of the large amplitude of length perturbation, we determined the frequency dependence of the amplitude and phase relations among length, tension, and AL changes. In contrast, the tension of the resting muscle changed in nearly triangular fashion with sinusoidal length perturbations being much smaller than that of the tetanized muscle, as shown in Fig. 1B.

Figure 2 shows the averaged tension (ΔT, middle traces) and AL (ΔAL, bottom traces) signals in response to various sinusoidal (1, 2, 3, and 5 Hz) length changes (ΔL, top traces) from $L_{max}$ to 0.92 $L_{max}$ during steady tetanic contraction at 30°C. The amplitude of tension responses first decreased with increasing frequency from 1 to 3 Hz and then increased with a further increase in frequency $>$3 Hz. In other words, there was a particular frequency (defined as the dip frequency) at which the amplitude of tension response became minimum (2-Hz at 30°C), as observed in earlier studies (12, 16, 20, 22, 23). The phase relation (i.e., the phase shift of tension response wave relative to sinusoidal length wave) showed a slight lag (i.e., the muscle tension at any length was higher during the shortening phase of the cycle than during lengthening) at 1 and 2 Hz (i.e., at the frequencies below the dip frequency), indicating...
that the viscous modulus of muscle is negative at these frequencies and that one obtains a positive net energy from the muscle during shortening and lengthening phases, but a pronounced lead was seen at the frequencies above the dip frequency (2 and 3 Hz). The AL response changed in phase with the tension response at the frequencies below the dip frequency, whereas it lagged behind the tension response at the frequencies above the dip frequency, as observed at 30°C. Although the data were not shown here, decreasing temperature produced a slight reduction of steady AL signal level without changing the steady tetanus level greatly, as observed in our previous study (16, 20, 22, 23). These features were more prominent in the shortening phase and less prominent in the lengthening phase, as shown by the vertical dashed lines. The phase relation in the lengthening phase might be affected by the resting elastic properties, which became significant with lengthening the preparation (see Fig. 1B). These features were more prominent in the shortening phase and less prominent in the lengthening phase, as shown by the vertical dashed lines. The phase relation in the lengthening phase might be affected by the resting elastic properties, which became significant with lengthening the preparation (see Fig. 1B). The amplitude of AL responses decreased with increasing frequency from 1 to 5 Hz. At 1 and 2 Hz (i.e., at the frequencies below the dip frequency), the increase in AL corresponded well to the decrease in tension. Likewise, the decrease in AL corresponded to the increase in tension. At 5 Hz (i.e., at the frequency above the dip frequency), however, the increase in AL lagged behind the decrease in tension. Likewise, the decrease in AL lagged behind the increase in tension. At 3 Hz (i.e., at the dip frequency), the phase relationships among tension, AL, and length changes could not be analyzed, because the waveforms of tension signal deviated significantly from sinusoidal waveforms.

When the temperature was decreased from 30° to 24°C, the dip frequency was shifted to 1 Hz, as seen in Fig. 3. The phase shift of the tension response wave relative to the sinusoidal length wave showed a small lag at the frequencies below the dip frequency (0.5 Hz), but a pronounced lead was seen at the frequencies above the dip frequency (2 and 3 Hz). The AL response changed in phase with the tension response at the frequencies below the dip frequency, whereas it lagged behind the tension response at the frequencies above the dip frequency, as observed at 30°C. Although the data were not shown here, decreasing temperature produced a slight reduction of steady AL signal level without changing the steady tetanus level greatly, as observed in our previous study (21). In the present study, we did not pay attention to these characteristics, which are thought to have no effects on the frequency-dependent relations among length, tension, and AL changes (23). In the resting muscles, the tension changed minutely in phase with the length independently of the frequencies, indicating there is little resting elastic properties with no obvious viscous resistance to the length changes. No detectable changes in AL were always observed regardless of the length perturbation frequency.

Figure 4 shows the average (means ± SD, n = 7) frequency dependence of the amplitude of tension (closed
circles) and AL (open circles) responses at 24 (left) and 30°C (right). The data were measured from the relations in shortening phase (i.e., the tension decrement-AL increment phase). The phase relationship between the tension and length changes at the dip frequencies (1 Hz at 24°C and 3 Hz at 30°C) could not be measured, because the waveforms of tension deviated significantly from sinusoidal waveforms (see Figs. 2 and 3).

**DISCUSSION**

The most interesting finding in the present study is that the relationship between the tension and AL changes observed at the sinusoidal length perturbations below the dip frequency is quite different from that above the dip frequency. With sinusoidal length perturbations below the dip frequency, the time course of AL responses changed in phase with the time course of tension responses, even when the dip frequency was quite different at two different temperatures. In addition, the amounts of phase delay of the tension and AL responses relative to the length changes below the dip frequency were significantly different at two different temperatures (Fig. 5) even though the length perturbations were identical in the two cases. These phenomena seem to indicate that the AL changes are the consequence of the tension changes rather than the length changes. Furthermore, the fact that 1) the amplitude of AL responses changed in parallel with the amplitude of tension responses, 2) the increase in AL corresponded to the decrease in tension, and 3) the decrease in AL corresponded to the increase in tension, suggests that the Ca2+ affinity of troponin C, a major Ca2+-binding, protein-regulating contraction, changes in phase with tension [as suggested in earlier studies on cardiac muscles (2, 11, 13, 17, 18, 21), psoas muscles (24), and barnacle muscles (9)], because the changes in AL can be considered to reflect the changes in myoplasmic Ca2+ due to the changes in Ca2+ binding to troponin C in the present ryanodine-treated tetanized preparations in which the Ca2+ handling of the sarcoplasmic reticulum is impaired (6, 10, 19).

In contrast to the above suggestions, with sinusoidal length perturbations above the dip frequency, the in-
crease in AL lagged behind the decrease in tension; likewise, the decrease in AL lagged behind the increase in tension. In addition, the amplitude of AL was decreased with an increase in frequency, despite an increase in the amplitude of tension responses. These results strongly suggest that tension changes associated with the sinusoidal length perturbations above the dip frequency are produced differently from those below the dip frequency, thus the relation to AL changes differs too.

Judging from the absolute values and their temperature dependence (Fig. 4) similar to those of the frequency where stiffness becomes minimum in the earlier small length perturbation experiments (12, 16, 20), we observed that the dip frequency in the present large length perturbation experiments is still considered to reflect the cycling rate of cross bridges. If this was the case, we can further interpret the present frequency dependence of tension changes as the same way as in the earlier study (5). The tension is expressed as follows as long as cross bridges act as independent tension generators in parallel.

\[ T = e N_p X \]

where \( T \) is tension, \( e \) is the cross-bridge elastance, \( N \) is the total number of cross bridges that are available to react per half sarcomere, \( p \) is the probability that an available cross bridge will be attached, and \( X \) is the average extension of an attached cross bridge (i.e., cross-bridge strain) from its unperturbed position. At very low perturbation frequencies, average cross-bridge extension would not be perturbed by applied length changes at all, because they would occur so slowly relative to the timescale of a cross-bridge cycle that any possible length change during a cross-bridge cycle would be near zero; \( X \) would be essentially the same value as the steady-state (unperturbed) value at very low perturbation frequency. As the perturbation frequency increases, length changes will begin to become apparent during a cross-bridge cycle, so that \( X \) will begin to be perturbed by length changes; thus \( X \) will begin to rise as the perturbation frequency increases. At a very high frequency, \( X \) will be exactly proportional to sinusoidal length changes, because the length changes occur fast enough to prevent any attachment or detachment on this timescale; thus \( X \) becomes the certain maximum value at very high frequencies.

If we reason in a similar manner for \( N_p \) for very low-frequency length perturbations, \( N_p \) would change essentially with length, following the length-tension relation. At a very high frequency, \( N_p \) would not change at all with length perturbations, because no attachment or detachment could occur on this timescale; thus \( N_p \) would be essentially the same value as the steady-state (unperturbed) value at a very high perturbation frequency. In between, \( N_p \) would decline around the frequency at which sinusoidal length perturbations began to challenge the cross-bridge cycling rate. Thus \( N_p \) might be expected to be a declining function of frequency with the steepest part of the descent occurring at frequencies that approach the cross-bridge cycling rate.

From the reasoning above, the amplitude of tension at low frequency is thought to be determined primarily by \( N_p \), with \( eX \) being essentially the steady-state (unperturbed) value. The amplitude of tension at high frequency is thought to be determined primarily by \( eX \), with \( N_p \) being essentially the steady-state (unperturbed) value. The dip in the amplitude of tension, observed at intermediate frequencies, can be considered to result from the interaction of a declining \( N_p \) countered by an increasing \( eX \) and should occur at a frequency of length oscillation at which the velocity of length change challenges the cross-bridge cycling rate. From these considerations, the tension-to-AL relationship below the dip frequency (i.e., the amplitude of AL response changed in parallel with the amplitude of tension response, and the increase in AL corresponded to the decrease in tension and the decrease in AL corresponded to the increase in tension) suggests that the \( \text{Ca}^{2+} \) affinity of cardiac troponin C changes in parallel with the active tension determined by the number of active cross bridges, and the tension-AL relation above the dip frequency (i.e., the amplitude of AL was decreased with an increase in frequency despite an increase in the amplitude of tension responses, and the increase in AL lagged behind the decrease in tension and the decrease in AL lagged behind the
increase in tension) suggests that the Ca\(^{2+}\) affinity of cardiac troponin C does not change with the passive tension produced by the length perturbation-induced cross-bridge strain. These suggestions also explain the reason why no detectable change in AL was observed immediately following the step-stretch (the length change far above the dip frequency) despite the large increase in tension, in earlier studies (2, 21).

One may question whether the present frequency dependence of tension changes is related only to the cycling rate of cross bridges, as in the earlier small length perturbation experiments (12, 16, 20, 22, 23). At large length changes in the present study, of course, a forcible detachment of cross bridges (“ uncoupling effect”) might contribute to the tension responses (4). This feature seems to be manifested particularly at higher frequencies because the reduction of mean tautenous tension during oscillations is associated with the increment of mean AL responses (see Figs. 2 and 3) but might not alter the present frequency dependence of the amplitude of tension changes (i.e., an index of the cycling rate of cross bridges) qualitatively.

It is certainly the case that in the present study, the changes in the muscle length are not identical with changes in the healthy central segment length because of the presence of the damaged ends of the preparation (8, 23), and thus, the damaged ends probably influence not only the size of the length changes but also produce the AL emission different from that of the healthy central regions when subjected to large length changes. However, this may not seriously influence our interpretations for the relationships between the AL and tension changes, because the frequency spectra of tension response have been reported to show qualitatively similar patterns among the central segment, the whole muscle, and the end region (23), and also because the time course of the calcium transient has recently reported to be unaffected by the damaged-end completion as far as the length change during contraction is within a limited range (15).

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