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ALTERATIONS IN INTRACELLULAR CALCIUM AND TENSION OF ACTIVATED FERRET PAPILLARY MUSCLE IN RESPONSE TO STEP LENGTH CHANGES

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SUMMARY

1. To study the effects of mechanical constraints on the calcium (Ca^{2+}) affinity of cardiac troponin C, we analysed the tension and aequorin light (AL, intracellular Ca^{2+}) transients in response to a step length change in aequorin-injected ferret right ventricular papillary muscles. The muscle preparations were continuously activated with ouabain (10^{-4} M) (ouabain contracture) or with high frequency stimuli in the presence of ryanodine (5 μM) (tetanic contraction).

2. The tension transient in response to either the release or stretch was oscillatory: tension decreased rapidly during the release and then increased, after which it lapsed into a new steady level in a series of damped oscillations. The opposite was true for the stretch. The oscillatory responses were conspicuous and less damped in ouabain-activated preparations (oscillation frequency of 2·2–2·3 Hz at 22 °C and 4·5–4·6 Hz at 30 °C) and much more damped in ryanodine-treated preparations.

3. The transient AL response was also oscillatory, the time course of which corresponded to that of the transient tension response. Regardless of the difference in the time course of the transients in two different preparations and at two different temperatures, the increase in AL corresponded to the decrease in tension, likewise the decrease in AL to the increase in tension.

4. The mean level of AL after release was lower than the control level present just prior to the release in ouabain-activated preparations, but the AL after release finally returned to the nearly control level in ryanodine-treated preparations.

5. When the ryanodine-treated muscle was further treated with 2,3-butanedione monoxime (BDM) (20 mM), the tetanic tension decreased remarkably without affecting the AL signal. The tension transient of this preparation was quite similar to that of the resting muscle, which changed in a nearly stepwise fashion; AL was hardly affected by step length changes, as in the resting muscle, in spite of the higher AL level.

6. These results suggest that the Ca^{2+} affinity of cardiac troponin C is increased with an increase in tension (i.e. the cross-bridge attachment) and decreased with a

decrease in tension i.e. the cross-bridge detachment), and that the mean $[Ca^{2+}]_i$ is lowered by release, at least in a Ca^{2+}-overloaded condition, mainly through the sarcoplasmic reticulum.

INTRODUCTION

It is well established that cardiac muscle contraction occurs through the binding of calcium ion (Ca^{2+}) to a component of the thin filament, troponin C (Ebashi & Endo, 1968). The Ca^{2+} binding allows the actin and myosin to interact and leads eventually to the production of tension and shortening (i.e. mechanical events). There is now considerable evidence that the resulting mechanical events affect the Ca^{2+} affinity of cardiac troponin C (for reviews, see Stephenson & Wendt, 1984; Allen & Kentish, 1985; Cooper, 1990). Allen & Kurihara (1982) reported that in rat and cat ventricular muscles, an increase in the muscle length increases the tension but shortens the duration of the aequorin light (AL, a function of intracellular-free Ca^{2+} concentration, $[Ca^{2+}]_i$) without changing its amplitude. They also observed a transient increase in AL (i.e. extra Ca^{2+}) after a quick release in length. Housmans, Lee & Blinks (1983) reported a higher $[Ca^{2+}]_i$ during active shortening of cat papillary muscles than during isometric twitches. These observations were attributed to a decrease in the affinity of troponin C for Ca^{2+} resulting from a decrease in muscle length and/or active tension development, since the extra Ca^{2+} can be explained by the release of Ca^{2+} from troponin C into the myoplasm. However, the interpretation of these experiments is complicated by the possibility that Ca^{2+} release from the sarcoplasmic reticulum (SR) and/or Ca^{2+} entry across the surface membrane may be affected by muscle length. In addition, since their results were obtained from twitching preparations which had periodically altered electrical and mechanical properties, the interpretation is further complicated due to the time-dependent natures. To overcome these complications, Allen & Kentish (1988), recently, performed a length perturbation experiment similar to the earlier study (Allen & Kurihara, 1982) on skinned ferret cardiac muscles in which all membranes had been removed by Triton X-100, leaving troponin C as the major site of Ca^{2+} binding. They found that Ca^{2+} affinity of troponin C correlates more closely with the developed tension than the muscle length. This finding is extremely useful to identify the source of extract Ca^{2+} in response to a step length change in intact preparation which has normal SR and other Ca^{2+} transport systems, since the differing responses between the intact and Triton X-100 skinned preparations can be attributed to the Ca^{2+} transport system rather than the Ca^{2+} binding site of troponin C.

Therefore, we studied the tension and AL transients in response to step length changes in intact aequorin-injected ferret right ventricular papillary muscles continuously activated with a toxic dose of ouabain (ouabain contracture) so that the complication due to the time-dependent nature of twitch contraction might be avoided, as in the skinned preparation (Allen & Kentish, 1988). The same length perturbation experiment was also applied to the ryanodine-treated tetanized preparation, in which the Ca^{2+} handling of the SR is impaired (Sutko, Ito & Kenyon, 1985; Fabiato, 1985; Marban & Wier, 1985; Bers, Bridge & MacLeod, 1987; Hansford & Lakatta, 1987), to exclusively discern the contribution of the SR to extra Ca^{2+}. 2,3-Butane-dione monoxime (BDM) is known to selectively inhibit cross-bridge

cycling by a direct action on the contractile apparatus while leaving Ca^{2+} cycling relatively unaffected (Blanchard, Smith, Allen & Alpert, 1990; Kurihara, Saeki, Hongo, Tanaka & Suda, 1990; Morner & Wohlfart, 1991). In some of the ryanodine-treated tetanized preparations, the effects of BDM on the tension and AL transients were analysed to determine the direct relationship between the developed tension (i.e. cross-bridge cycling) and the $[Ca^{2+}]_i$.

The preliminary results of this study have already been presented (Saeki, Hongo, Tanaka & Kurihara, 1990; Saeki, Kurihara, Hongo & Tanaka, 1991).

METHODS

Preparation and apparatus

Male ferrets (about 800 g body weight) were anaesthetized with sodium pentobarbitone (80 mg/kg, I.P.).Hearts were quickly removed and thin right ventricular papillary muscles were excised and placed in a Petri dish that contained oxygenated normal Tyrode's solution. Each end of the excised muscle was tied with a silk thread to a small tungsten wire (125 μm in diameter) hook in a dissecting dish. The preparation was transferred to an experimental chamber and mounted horizontally between a length driver (JOCX-101A, General Scanning Co. Inc., CA, USA), which is capable of imposing step length changes within 4 ms, and a tension transducer (Kulite semi-conductor: BG-25, 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 solution was maintained at either 22 or 30 °C with a thermoelectric device (Haake, D-3, Germany) with an accuracy of $\pm$0·5 °C.

Solutions

Normal Tyrode solution use for dissecting the preparation and for the injection of aequorin had the following composition (mM): Na^+, 135; K^+, 5; Ca^{2+}, 2; Mg^{2+}, 1; Cl^-, 102; HCO_3^-, 20; HPO_4^{2-}, 1; SO_4^{2-}, 1; acetate, 20; glucose, 10; insulin, 5 units/l; 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 (*N*-2-hydroxyethyl-piperazine-*N'*-2-ethansulphonic acid) (Hepes-Tyrode solution), which had the following composition (mM): Na^+, 128; K^+, 5; Ca^{2+}, 2; Mg^{2+}, 1; Cl^-, 117; SO_4^{2-}, 1; acetate, 20; glucose, 10; insulin, 5 units/l; 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 measured after filling with aequorin solution, was used for the injection of aequorin. Aequorin was injected into 50–100 superficial cells of each preparation with 5–10 kg/cm^2 pressure while monitoring the membrane potential. Aequorin light 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. Improved light detection was facilitated by placing a concave mirror under the preparation. Details of the method were previously described by Allen & Kurihara (1982). All data were stored on a tape (NFR-3515W, Sony Magnescale Inc., Tokyo, Japan) and a computer for later analysis. In order to improve the signal-to-noise ratio, the light signals were recorded through a low-pass active filter with an appropriate time constant (10 Hz). Signals were averaged (32 records in the ouabain-activated preparations and 3 records in the ryanodine-treated preparations) with a computer or signal processor (7T07A, NEC-Sanei Co., Ltd, Tokyo, Japan). In the figures of the present study, the AL signals are expressed as fractional luminescence (L_f, logarithm of an AL signal normalized to maximal AL) and/or are expressed as photomultiplier current.

Experimental protocol

The muscle was initially stimulated to contract at 0·2 Hz with a square pulse of 5 ms duration and a voltage of 50% above the threshold level via a pair of platinum-black electrodes (3 mm

apart) that were mounted alongside the preparation in normal Tyrode solution. After a stabilizing period of 30–60 min, L_{max} was determined as the length of maximum isometric twitch tension. At this point, the length and diameter of the preparation were measured with an ocular micrometer. The muscle length ranged from 3·5 to 6·0 mm and averaged 4·8±0·7 mm (mean±S.D., $n = 13$), and

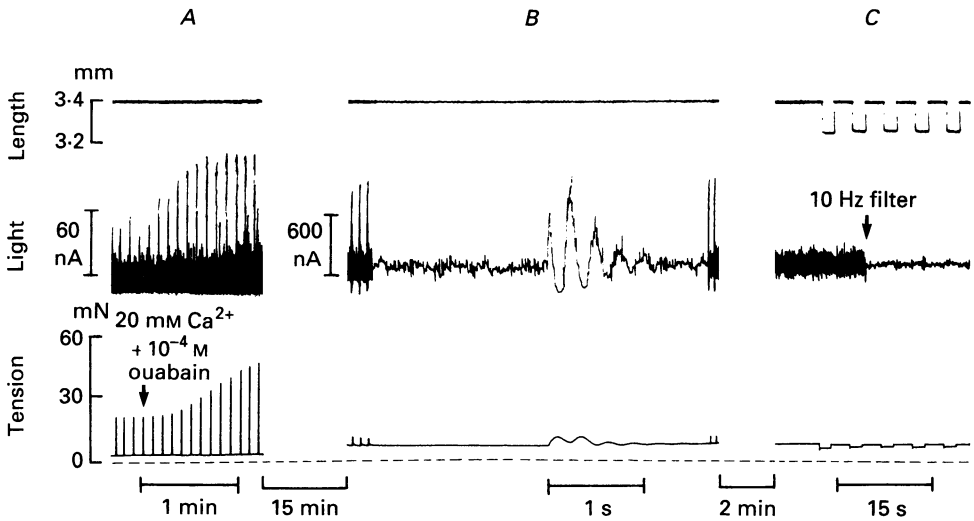

Fig. 1. The experimental protocol in ouabain-activated preparations. Upper trace is length; middle trace, aequorin light (AL); lower trace, tension. In *A*, high Ca^{2+} (20 mM) solution containing ouabain (10^{-4} M) was applied at the arrow. The muscle showed a transient increase (*A*) and then a continuous decrease in the systolic tension during which time the diastolic AL level and tension increased progressively (*B*). The twitch tension and AL signals became oscillatory after exposure to the ouabain solution for 16 min (*B*). After the muscle lapsed into a state of stable contracture with a negligible amount of twitch tension, electrical stimuli were stopped, and then the muscle was released and stretched stepwise (*C*). In order to improve the signal-to-noise ratio, the AL signals were recorded after a low-pass active filter of 10 Hz was applied at the arrow in *C*.

the diameter ranged from 500 to 900 μm and averaged 685±118 μm (mean±S.D., $n = 13$). These values were used as a reference for normalizing absolute muscle length and tension. Then, the electrical stimuli were stopped and aequorin was microinjected into the superficial cells. After completion of the injection, the muscle was stimulated again in the same manner as before, and the normal Tyrode solution was replaced with Hepes-Tyrode solution, and the muscle was stabilized for about 60 min until the twitch tension and AL signals became stable. Immediately after the stabilizing period, the solution was changed to Hepes–Tyrode solution containing a high concentration of Ca^{2+} (8–20 mM) and either 10^{-4} M ouabain (Sigma Chemical Co., St Louis, MO, USA) of 5 μM ryanodine (Agri Systems Inc., PA, USA), to obtain a continuously activated contractile state.

As seen in Fig. 1, ouabain produced a transient increase and then a continuous decrease in the systolic tension during which time the diastolic AL level and tension increased progressively (Fig. 1*A* and *B*). The twitch tension and AL signal became oscillatory (after exposure to 10^{-4} M ouabain for 16 min. Fig. 1*B*), as has been reported previously (Orchard, Eisner & Allen, 1983). Oscillatory increase in AL always preceded the oscillatory contraction, indicating that the AL increment was the cause of the contraction, as in normal twitch contraction. Finally, the muscle lapsed into a state of stable contracture with a negligible amount of twitch tension, which could be eliminated by the stopping electrical stimuli.

When the preparation was treated with ryanodine and stimulated at 0·2 Hz, the amplitudes of AL and tension started to decrease within 10 min. After 40–50 min of exposure to 5 μM ryanodine, a new steady state was attained with the developed tension averaging about 15% of initial twitch tension, which agreed with a previous report (Urthaler, Walker, Reeves & Hefner, 1989). Therefore,

the preparation was treated with ryanodine for at least 50 min before the tetanic experiment. To obtain tetanus tension, the muscle was stimulated with square pulses with a 40 ms duration at 10 Hz and a voltage of about threefold the threshold (Yue, Marban & Wier, 1986; Okazaki, Suda, Hongo, Konishi & Kurihara, 1990).

After the preparation was continuously activated with either ouabain or ryanodine, the muscle length was released and stretched stepwise by amounts of 2–12% of muscle length (Fig. 1*C*), as in an earlier study (Allen & Kentish, 1988).

It is important, however, to appreciate that 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 (Donald, Reeves, Reeves, Walker & Hefner, 1980). Additionally, 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 length changes. Therefore, we could not analyse the quantitative relationships among the AL, tension and length changes systematically in the present preparations, which would have different amounts of damaged ends.

RESULTS

AL and tension responses in resting and ouabain-activated preparation

Figure 1*C* shows the AL (middle trace) and tension (lower trace) transients in response to step change in length (upper trace, 3·7% of the initial muscle length) in the preparation activated with the solution containing 10^{-4} M ouabain and 20 mM $[Ca^{2+}]_o$ at 30 °C. The averaged responses of AL (middle trace) and tension (lower trace) are shown in the right panel of Fig. 2. As seen in this panel, the transient tension response to either release or stretch was oscillatory: tension decreased rapidly during the release and then increased, after which it lapsed into a new steady level in a series of damped oscillations. The opposite was true for the stretch back to the original length position, though not exactly symmetrical. The transient AL response was also oscillatory, the time course of which corresponded to that of the tension transient, though no such correspondence was observed during and immediately following the length change. The increase in AL corresponded exactly to the decrease in tension, and likewise the decrease in AL to the increase in tension, as estimated by vertical dashed lines. There was an increase in AL immediately following the release (for more clear identification, see Figs 3 and 4). The AL increment was preceded by a decrease in tension during the release, indicating that the AL increment was the consequence of the decrease in tension and/or the release in length. No detectable changes in AL were observed immediately following the stretch (see Figs 3 and 4). These features were commonly observed in all seven muscles studied. In contrast, the tension transient of the resting muscle in normal Tyrode solution changed in nearly stepwise fashion, being much simpler and smaller than that of the activated muscle (the left panel of Fig. 2). No detectable changes in AL were observed in association with the resting tension transients.

To test the effects of contracture level, we changed the concentration of $[Ca^{2+}]_o$ from 8 to 20 mM in the presence of ouabain, but the contracture level did not change appreciably, and averaged about 25% of the initial twitch tension.

Effects of initial muscle length on AL and tension transients in ouabain-activated preparations

Figure 3 shows the averaged AL (middle trace) and tension (lower trace) transient in response to the same amount (but different percentage of the initial muscle length) of step change in length at two different initial muscle lengths, L_{max} (left panel) and

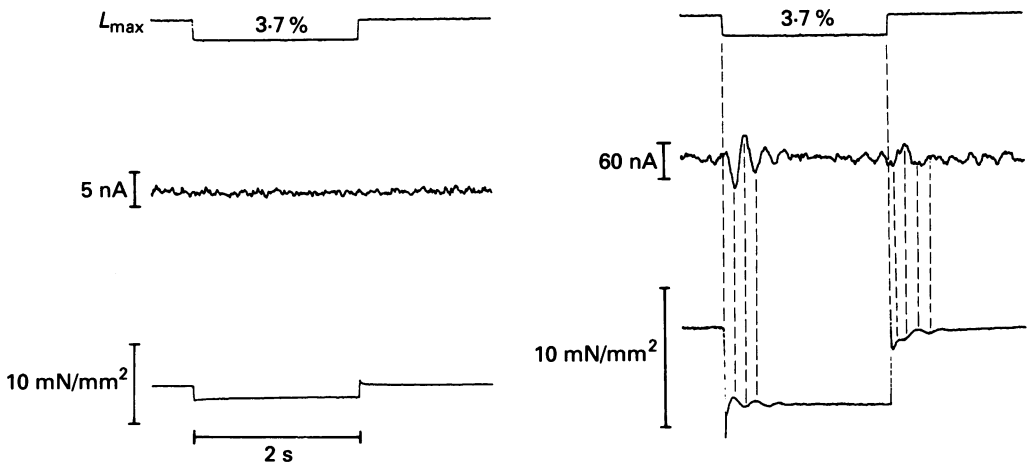

Fig. 2. Aequorin light (middle traces) and tension (lower traces) changes in response to step change in length (upper traces, 3·7% L_{max}) in resting (left panel) and during contracture induced by 20 mM Ca^{2+} and 10^{-4} M ouabain (right panel) at the initial muscle length of L_{max} and at 30 °C. Averaged records ($n = 32$).

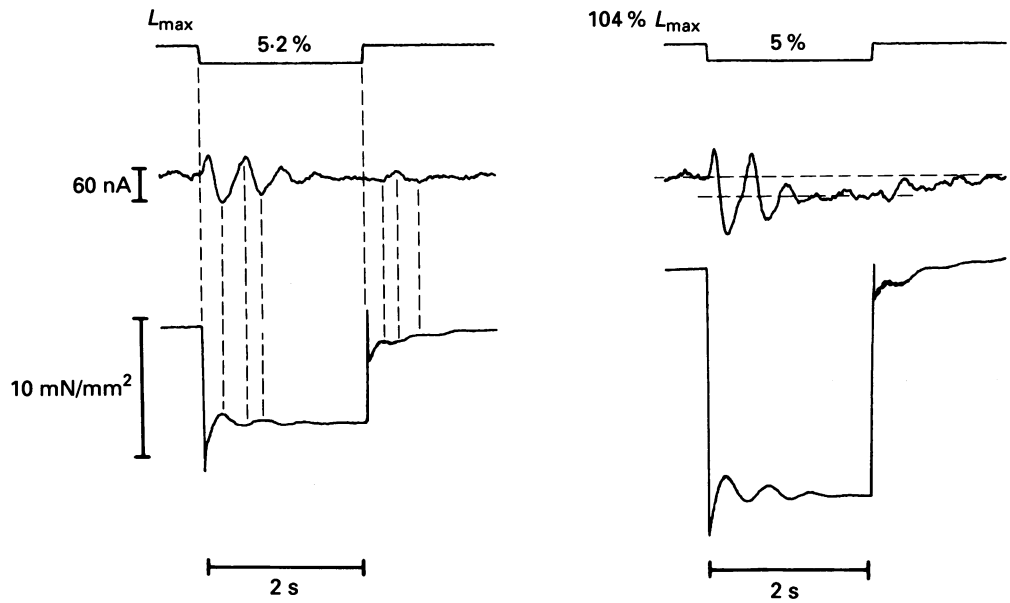

Fig. 3. Aequorin light (middle traces) and tension (lower traces) changes in response to the same amount (but different percentage of the initial muscle length) of step change in length (upper traces) at two different initial muscle lengths, L_{max} (left panel) and 104% L_{max} (right panel) at 22 °C during contracture induced by 8 mM Ca^{2+} and 10^{-4} M ouabain. Averaged records ($n = 32$).

104% L_{max} (right panel) in the preparation activated with the solution containing 10^{-4} M ouabain and 8 mM $[Ca^{2+}]_o$ at 22 °C. As seen in these traces, the oscillatory frequency was quite independent of the initial muscle length (described later),

though the amplitudes of AL and tension responses were greater at longer initial muscle lengths.

Lowering temperature (compare with the responses at 30 °C in Figs 2 and 4) markedly lengthened the oscillatory time courses of AL and tension transients, but

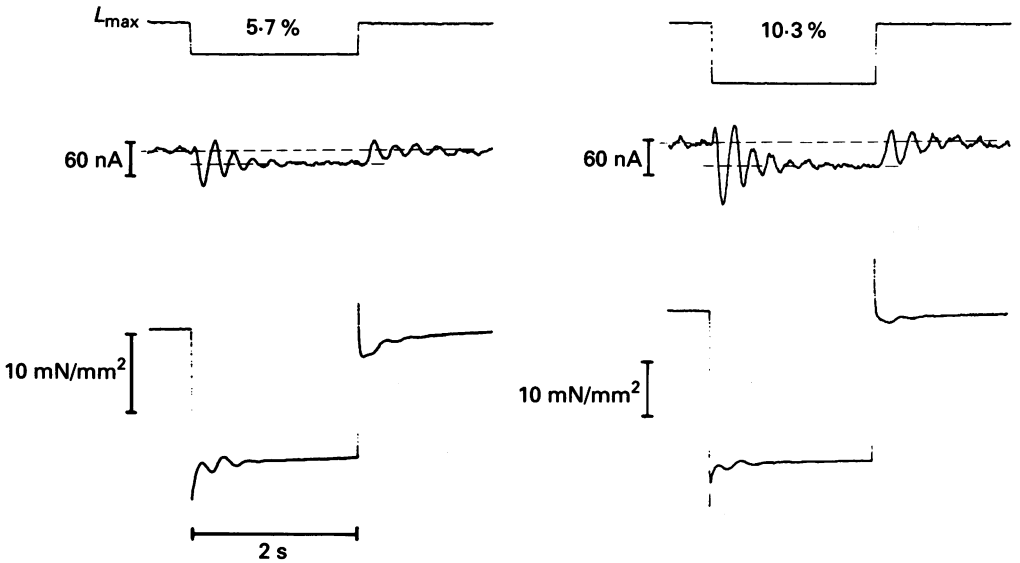

Fig. 4. Effects of the amplitude of step length change on aequorin light (middle traces) and tension (lower traces) responses at the initial muscle length of 104 % L_{max} and 30 °C in the muscle in contracture induced by 20 mM Ca^{2+} and 10^{-4} M ouabain. Averaged records ($n = 32$).

did not alter the characteristic relationships between AL and tension transients at 30 °C (described already for the right panel in Fig. 2); it was 2·2 ± 0·1 Hz (mean ± S.D.) at 22 °C and 4·5 ± 0·2 Hz at 30 °C in each of the five preparations studied at the initial muscle length of L_{max}, and 2·3 ± 0·2 Hz ($n = 6$) at 22 °C and 4·6 ± 0·2 Hz ($n = 7$) at 30 °C in the preparations studied at the initial muscle length of 102–105 % L_{max}. The AL increment immediately following the release, which was preceded by a decrease in tension during the release, was greater at longer initial muscle lengths.

The mean level of AL after release was lower than the control level present just prior to the release. This feature was much more clearly seen when the initial length was longer, as shown by horizontal dashed lines in the right panel in Fig. 3. This finding strongly suggests that $[Ca^{2+}]_i$ would be lowered by the Ca^{2+} uptake of the SR and/or the Ca^{2+} extrusion across the surface membrane in association with the length release.

Effects of the amplitude of length change on AL and tension transient in ouabain-activated-preparations

Figure 4 shows the averaged AL (middle traces) and tension (lower traces) transients in response to two different amplitudes of step length change (upper traces), 5·7 % (left panel) and 10·3 % (right panel) at the initial muscle length of

104 % L_{max} in the preparation activated with the solution containing 10^{-4} M ouabain and 20 mM $[Ca^{2+}]_o$ at 30 °C. As seen in these traces, the amplitudes of AL and tension responses were increased with an increase in the amplitude of step length change, while the time courses of both responses were quite independent of the amplitude of step length change.

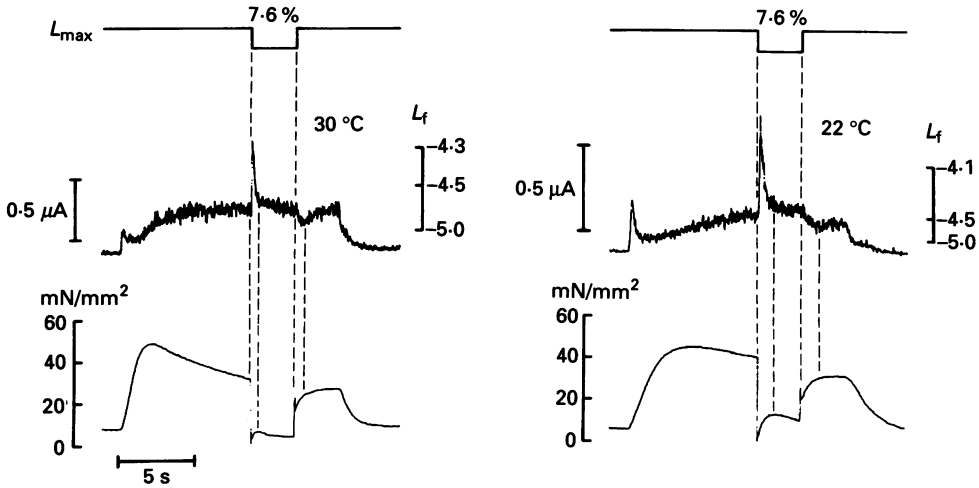

Fig. 5. Aequorin light (middle traces) and tension (lower traces) changes in response to step change in length (upper traces) at 30 °C (left panel) and 22 °C (right panel) during ryanodine-induced tetanic contraction. Averaged records ($n = 3$).

The amplitude of AL increment and tension reduction during and immediately following the release increased in parallel as the amplitude of the step release was increased. The reduction of the mean level of AL after release was greater at larger of step length changes, as indicated by horizontal dashed lines. These characteristics were confirmed in four additional preparations.

AL and tension transients at two different temperatures in ryanodine-treated preparations

Figure 5 shows the averaged AL (middle traces) and tension (lower traces) transients in response to step length change (upper traces, 7·6 % of the initial muscle length) during tetanic contraction in the preparation activated with the solution containing 5 μM ryanodine and 20 mM Ca^{2+} at 30 °C (left panel) and 22 °C (right panel). In most cases, the transient tension response was comprised of three different phases. The first phase was a prompt and large tension change during the length change; the second phase, a quick recovery of tension towards the value before the length change and the third, a delayed tension change in the same direction as the first phase. These responses are quite similar to those of glycerinated (Herzig & Rüegg, 1977; Steiger, 1977; Saeki, Kato, Horikoshi & Yanagisawa, 1984) and intact heart muscle in Ba^{2+} contracture (Steiger, 1977; Steiger, Brady & Tan, 1978; Saeki, Sagawa & Suga, 1980). This strongly implies that excitatory processes are not involved in the present

tension transients, though the membrane potential in ryanodine-treated tetanized preparations has been reported to vary from −25 to −60 mV (Marban, Kusuoka, Yue, Weisfeldt & Wier, 1986). In some preparations, the third phase was followed by a damped oscillation in AL and tension, as observed in the ouabain-activated preparations. The transient AL response to a step release in length comprised two phases: the first phase consisted of an initial rapid increase which was preceded by a decrease in tension during the release; the second phase consisted of a rapid decrease towards the initial control level, the time course of which was in phase with that of the second phase of rapid tension rise, as estimated by vertical dashed lines. In contrast, no detectable initial AL changes were observed for the step stretch, as observed in ouabain-activated preparations. There was a clear reduction of AL concurrent with the delayed tension rise in the third phase following the stretch, as indicated by vertical dashed lines. These features were commonly observed in all five preparations studied. The rate of the transient changes in both AL and tension was markedly decreased with the decrease in temperature from 30 to 22 °C, as observed in ouabain-activated preparations. This was in agreement with earlier observations on the tension transients (Steiger, 1977; Steiger *et al.* 1978; Saeki *et al.* 1980). We confirmed similar effects of temperature on AL and tension transients in four additional muscles. Both the reduction in AL and the tension rise in the second phase (for step release 9 s after the onset of tetanic contraction) were completed in about 200–400 ms at 30 °C and about 1·1 to 1·3 s at 22 °C. Both the reduction in AL and the tension rise in the third phase (for step stretch 12 s after the onset of tetanic contraction) were completed in about 400–800 ms at 30 °C and about 2·0–2·5 s at 22 °C.

When the $[Ca^{2+}]_o$ was decreased from 20 to 8 mM, the tetanus level and the amplitudes of both AL and tension transients were decreased, but the characteristic relationships between the AL and tension transients described above did not change.

Effect of initial muscle length on AL and tension transients in ryanodine-treated preparations

Figure 6 shows the averaged responses of AL (middle traces) and tension (lower traces) to the same amount (but different percentage of the initial muscle length) of step change in length (upper traces) at two different initial muscle lengths, L_{max} (left panel) and 103% L_{max} (right panel) during tetanic contraction in the preparation activated with the solution containing 5 μM ryanodine and 20 mM $[Ca^{2+}]_o$ at 30 °C. As seen in this figure, the characteristic relationships between AL and tension transients shown in Fig. 5 were not altered by changing the initial muscle length, and the time courses of both AL and tension transients were quite independent of the initial muscle length, in agreement with earlier observations on the tension transients (Steiger, 1977; Steiger *et al.* 1978; Saeki *et al.* 1980). However, the amplitudes of AL and tension responses were greater at longer initial muscle lengths. These effects of initial muscle length on AL and tension transients were confirmed in three additional preparations.

The amplitudes of AL and tension changes increased in parallel with an increase in the amplitude of step length change in all five preparations studied, as observed in the ouabain-activated preparations.

Effect of BDM on AL and tension transients in ryanodine-treated preparations

Figure 7 shows the averaged responses of AL (middle traces) and tension (lower traces) to the same amount of step change in length (upper traces, 7·6% of the initial muscle length) before (left panel) and after (right panel) the administration of 20 mM

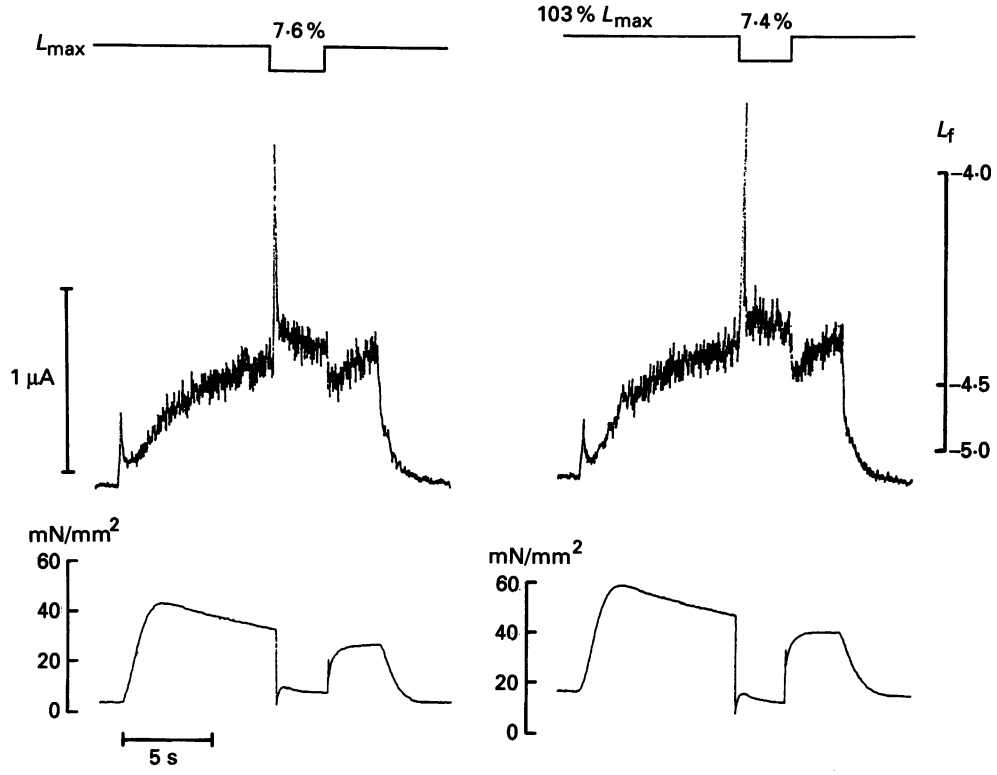

Fig. 6. Aequorin light (middle traces) and tension (lower traces) changes in response to the same amount (but different percentage of the initial muscle length) of step length change (upper traces) at two different initial muscle lengths, L_{max} (left panel) and 103% L_{max} (right panel) during tetanic contraction induced by 20 mM Ca^{2+} and 5 μM ryanodine. Averaged records ($n = 3$).

BDM during tetanic contraction in the preparation activated with the solution containing 5 μM ryanodine and 20 mM $[Ca^{2+}]_0$ at 30 °C. BDM selectively and almost completely inhibited the tetanic contraction without affecting the AL signal. The transient tension response was quite similar to that of the resting muscle (compare with the left panel in Fig. 2), indicating passive viscoelastic properties, and likewise no AL change was observed, as in the resting muscle. These BDM effects were completely reversible. When the BDM was removed from the solution and the same length perturbation experiment was performed, the muscle was as responsive to the transients as powerfully as before the administration of BDM. Similar results were obtained in two additional muscles.

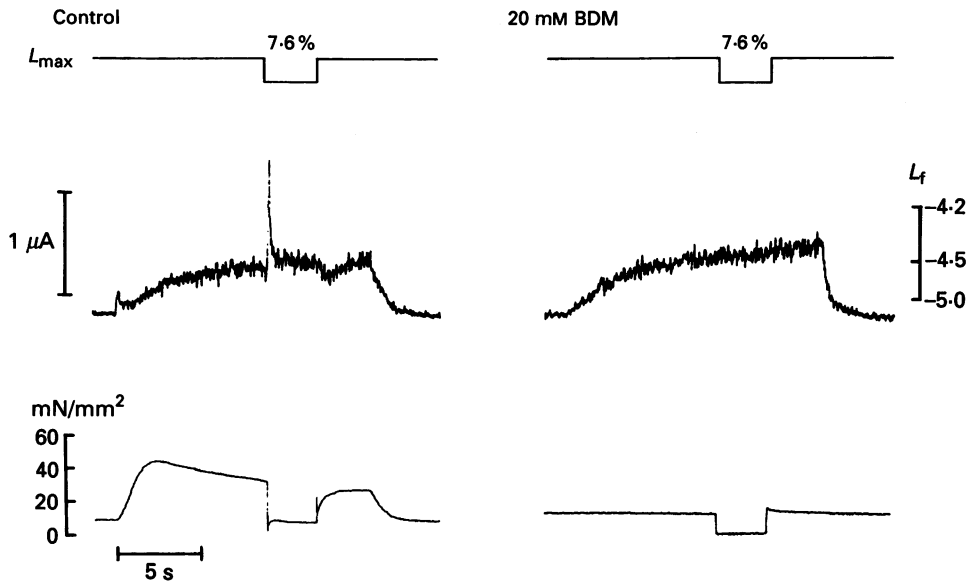

Fig. 7. Aequorin light (middle traces) and tension (lower traces) changes in response to step change in length (upper traces) at the initial muscle length of L_{max} and 30 °C before (left panel) and after (right panel) the administration of 20 mM 2,3-butanedione monoxime (BDM) during tetanic contraction induced by 20 mM Ca^{2+} and 5 μM ryanodine. Averaged records ($n = 3$).

DISCUSSION

The time course and frequency of oscillatory AL and tension transients in ouabain-activated preparations

The most important finding in the present study is that after the step change in length, the transient AL responses changed oscillatory in phase with the tension responses, except for the initial responses during and immediately following the length change in ouabain-activated preparations, even when the time course of the transients varied greatly at two different temperatures. This phenomenon seems to indicate that the AL changes are the consequence of the tension changes. If the AL changes are the cause of the tension changes, the increase in AL would always precede the tension development, as observed previously (Orchard *et al.* 1983) and seen in Fig. 1*B*. Furthermore, the fact that the increase in AL corresponded to the decrease in tension and the decrease in AL to the increase in tension strongly suggests that the Ca^{2+} affinity of troponin C is increased with an increase in tension (i.e. cross-bridge attachment) and decreased with a decrease in tension (i.e cross-bridge detachment), as suggested in earlier studies on cardiac muscles (Housmans *et al.* 1983; Hofmann & Fuchs, 1987*a*, *b*; Allen & Kentish, 1988), psoas muscles (Sweitzer & Moss, 1990) and barnacle muscles (Gordon & Ridgway, 1987, 1990).

Oscillatory tension transients similar to those of ouabain-activated preparations have been reported previously in glycerol-extracted insect flight muscles (Schadler,

Steiger & Rüegg, 1971), glycerol-extracted rabbit papillary muscles and intact rabbit papillary muscles activated by a high-K^+ and low-Na^+ solution (Steiger, 1977), and were actually observed in some of the present ryanodine-treated preparations. Therefore, the present oscillatory tension transients may be due to a more or less synchronized cross-bridge action rather than the oscillatory release of Ca^{2+} from the SR and other excitatory processes, as suggested previously (Schadler *et al.* 1971; Steiger, 1977).

Orchard *et al.* (1983) found 3–5 Hz spontaneous oscillations of AL and tension by a Fourier analysis in unstimulated ferret papillary muscles steadily activated in Na^+-free solution at 30 °C. They attributed those oscillations of $[Ca^{2+}]_i$ to the oscillatory release of Ca^{2+} from the SR, though could not identify the phase relationship (i.e. the causal relationship) between the AL and tension oscillations in their Fourier analysis, and concluded that the fluctuation of $[Ca^{2+}]_i$ presumably underlies the fluctuation of tension (i.e. the AL changes are the cause of the tension changes, in contrast to our interpretations for the present results). However, an interesting thing is that even at different experimental conditions, the oscillatory frequencies they observed are very close to those (4·5–4·6 Hz) observed at 30 °C in the present ouabain-activated preparations. Whether or not this is only a lucky coincidence remains to be studied.

AL and tension transients during and immediately following step length change

The amplitude of AL and tension changes during and immediately following the release increased in parallel with the increase in the step release from 2 to 12% of the initial muscle length (i.e. well above the length change required for cross-bridge detachment) in both ouabain-activated and ryanodine-treated tetanized preparations. If the forcible cross-bridge detachment associated with the step length changes was the main cause of Ca^{2+} release, we would expect to observe an AL signal which is independent of step size above a certain muscle length (i.e. the length change required for cross-bridge detachment, which is not necessarily 1–2% of the muscle length because of the presence of the damaged ends) regardless of the direction of the length changes. That is not the case in the present study. Therefore, it seems that the amount of Ca^{2+} released is determined chiefly by the magnitude of the change in tension (or length), and it is hardly affected by the sudden forcible detachment of cross-bridges, as suggested by Allen & Kentish (1988). Since no change in AL was observed when the muscle was in the resting state in normal Tyrode solution (Fig. 2) or when the active tension was suppressed by the administration of BDM (Fig. 7), the increase in AL may be due to the decrease in active tension associated with the release rather than the decrease in muscle length *per se*, which is thought to decrease the Ca^{2+} affinity of troponin C (Allen & Kurihara, 1982; Housmans *et al.* 1983; Stephenson & Wendt, 1984; Allen & Kentish, 1985; Cooper, 1990). On the hypothesis outlined above, it can be explained that the initial large decrease in tension produced a quite small increase in AL, whereas the subsequent small oscillatory changes in tension produced a large change in AL, since most of the decrease in initial tension is thought to be due to the reduction in passive tension associated with the length release (Allen & Kentish, 1988).

The tension increment following the rapid decrease in tension during the release might reduce the amplitude of the AL increment immediately following the release,

since it can be considered to increase the Ca^{2+} affinity of troponin C, as discussed already.

However, it must be appreciated that in addition to the limitations for the measurements of AL and step size of the length change described in the Methods the amplitude of rapid AL changes immediately following the release would be limited by the filter used (10 Hz), thus we were unable to analyse quantitatively the AL and tension transients during and immediately following the step length change.

The present finding both in ouabain-activated and ryanodine-treated tetanized preparations that no detectable change in AL was observed immediately following the stretch is consistent with the result in the Triton X-100 skinned cardiac preparations (Allen & Kentish, 1988). One possible explanation for this phenomenon is that during the stretch the cross-bridges are forcibly detached, whereas during the release some of them are pushed to a position where they will favourably detach in an ordinary way (i.e. not in a forcible way), thus the change in Ca^{2+} affinity of troponin C may only be brought about during the release. In the Triton X-100 skinned slow-twitch rat soleus loaded with the Ca^{2+}-sensitive photoprotein, obelin, the stretch in length has been reported to produce a clear decrease in light signal (Stephenson & Wendt, 1984). This might be due to the slower stretch in length, thus during the stretch there might be the attachment of cross-bridges (i.e. the increase in Ca^{2+} affinity of troponin C) which give rise to a decrease in the light signal. Voltage-clamped barnacle muscle fibres have been reported to produce an increase in AL concurrent with both a stretch and release in length (Gordon & Ridgway, 1990). This increment in AL has been ascribed to the cross-bridge detachment because of the similar amplitudes of the positive phase (i.e. AL increase) in response to stretch and to release for a length change of $> 2\%$. However, the AL increment for the stretch in barnacle seems to correspond well to the tension reduction following the initial rapid tension increment, thus may not be the cross-bridge detachment by the stretch *per se*, but by the decrease in tension.

The reduction of AL following the initial rapid AL increment in response to release corresponded quite well to the tension rise following the rapid decrease in tension during the release, and the reduction of AL in response to stretch to the delayed tension rise (Figs 5, 6 and 7). These results again suggest that the Ca^{2+} affinity of troponin C is increased with an increase in tension. Allen & Kentish (1988) did not observe the AL decrease associated with the tension rise in the second phase of the release response in Triton X-100-skinned preparations. The difference might be partly due to the greater tension rise in the second phase in the present ryanodine-treated preparations compared with that in the Triton X-100-skinned preparations, probably resulting from the difference in the speed of step length change (4 ms in the present study *vs.* 10 ms in Allen & Kentish's study). One may consider the possibility that the sarcolemmal Na^+–Ca^{2+} exchange (Reuter, 1974) and/or the Ca^{2+} pump decrease the $[Ca^{2+}]_i$ with a decrease in length. However, this is unlikely to explain the reduction of AL following the initial rapid AL increment in response to release, since in the ryanodine-treated tetanized preparations the AL decrease to the nearly control level concurrent with the tension rise in the second phase.

In ryanodine-treated tetanized preparations, endogenous catecholamine would be released by the strong repetitive stimulation (Okazaki *et al.* 1990). This may change

the time course of the tension transients (Berman, Peterson, Yue & Hunter, 1988), but may not seriously influence our interpretations for the relationships between the AL and tension transients, since the similar correlations between the two transients were always observed regardless of the difference in the time course of the transients at two different temperatures.

The mean level of AL after step length change

The other interesting finding is that in ouabain-activated preparations the mean level of AL after release was lower than the level present just prior to the step release, in contrast to the finding in the ryanodine-treated preparations. This may be related to their different Ca^{2+} transport capability. Since in ouabain-activated preparations the SR function is assumed to be normal (Orchard *et al.* 1983), in contrast to that of the ryanodine-treated preparations (Sutko *et al.* 1985; Fabiato, 1985; Marban & Wier, 1985; Bers *et al.* 1987; Hansford & Lakatta, 1987), it is strongly suggested that in ouabain-activated preparations the $[Ca^{2+}]_i$ might be lowered by the release (in other words, increased by the stretch) in length through the SR. There is another possibility that the Na^+–Ca^{2+} exchange (Reuter, 1974) and/or the Ca^{2+} pump across the surface membrane decrease the $[Ca^{2+}]_i$ with a decrease in length. However, these transport mechanisms may not be major determinants of the mean level of AL after step length changes, since we did not observe similar kinds of responses between the ouabain-activated and the ryanodine-treated tetanized preparations, both of which have the intact surface membrane (Orchard *et al.* 1983; Sutko *et al.* 1985; Marban & Wier, 1985; Hansford & Lakatta, 1987).

Le Guennec, White, Gannier, Argibay & Garner (1991) recently reported that in indo-1 loaded resting guinea-pig ventricular myocytes, the $[Ca^{2+}]_i$ increased with the stretch and returned to pre-stretch levels on return to resting cell length, although we did not observe any appreciable changes in AL in the resting muscle (left panel in Fig. 2). The discrepancy might be due to the use of different Ca^{2+} indicators. Aequorin is not as sensitive to a small change in $[Ca^{2+}]_i$ and on the other hand, fluorescent measurements are influenced by movement. Yet, the reason for the different results is not clear at present.

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