Voltage Dependence of Depolarization-Contraction Coupling Processes in Skeletal Muscle Cells

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Abstract. Changes of contraction and optical anisotropy of isolated skeletal muscle cells from *Rana temporaria* and *Astacus fluviatilis* were compared under voltage clamp conditions. The time course of the optical signal from frog muscle was shown to consist of two components which could be attributed to calcium binding both on the sarcoplasmic reticulum Ca-ATPase and troponin C. The optical signal from the crayfish muscle has no distinguishable components, and its onset probably reflects the start of Ca-ATPase activity. This hypothesis is supported by the analysis of effects of some pharmacological agents and conditioning depolarization on tension and optical signal.

Key words: Skeletal muscle fibre — EC coupling — Birefringence signal

Introduction

The activation of skeletal muscle cell contraction is controlled by calcium ions released into the sarcoplasm (for review see Zachar 1971). The time course of calcium concentration changes may help to better understand the relevant control processes. Direct measurements of the Ca dynamics are impossible. There are two ways how to solve this problem: by using calcium sensitive dyes, or by measuring some macroscopic processes which reflect the changes of calcium concentration. It has been shown (Baylor and Oetliker 1975; 1977 a, b, c; Baylor et al. 1984; Oetliker and Schümperli 1980; 1984; Suarez-Kurtz and Parker 1977; Taylor et al. 1982; Poledna and Morad 1983) that changes in anisotropy relate to changes of calcium concentration in the sarcoplasm. This provides the possibility of noninvasive monitoring of myoplasmic calcium ions by measuring anisotropy changes.

Proper interpretation of the results requires a sound understanding of the mechanisms underlying changes of the fibere anisotropy. Neither the distribution of free Ca^{2+} ions released from the SR nor of that entered from the extracellular space cannot be the sources of optical anisotropy. The change of optical anisotropy must be related to some Ca^{2+}-distribution dependent

process. This can be either electrostriction of sarcoplasmic reticulum membranes (Kerr's effect) resulting from Ca-release induced transmembrane potential changes (Baylor and Oetliker 1977 c; Oetliker et al. 1975; Oetliker and Schümperli 1980) or conformational changes after calcium binding on Ca-ATPase (Poledna and Morad 1983), troponin C (Suarez-Kurtz and Parker 1977) and/or some other regulatory protein (Baylor et al. 1984).

The aim of the present work was to analyse the changes of optical anisotropy and to identify their origin.

The type of electrogenesis determines also the control of the internal calcium concentration. Fibres with sodium electrogenesis release calcium from internal stores (terminal cisternae of the sarcoplasmic reticulum) (for review see Rüegg 1986). The activation of muscle cells with calcium electrogenesis is initiated by calcium inward current, which directly raises the sarcoplasmic calcium ion concentration and may act to trigger calcium release (Zacharová and Zachar 1967; Valko et al. 1967). These differences should be reflected in different time courses of optical anisotropy. A comparison of results obtained with both types of muscle fibres could provide insight into the mechanisms underlying the anisotropy changes. Therefore, experiments were performed with frog and crayfish skeletal muscle fibres.

Materials and Methods

Single muscle fibres were dissected from m. iliofibularis, and/or m. semitendinosus of the frog *Rana temporaria*, and from m. extensor carpopoditi of the crayfish *Astacus fluviatilis*.

Tension and optical anisotropy were recorded simultaneously in the voltage clamped terminal segment of the intact fibre. This part was electrically isolated by vaseline seals with the grounded pool. The tension was measured isometrically using a silicone tensometer. The birefringence changes were converted into changes in light intensity measured with a photodiode. The experimental set-up was described in details elsewhere (Marko et al. 1986; Poledna and Lacinová 1988). Experiments were performed at 10 °C, kept with Peltier elements and a thermistor in feedback configuration.

Frog fibres were dissected in Ringer's solution (in mmol/l: Na^+ 120; K^+ 2.5; Ca^{2+} 1.8; Cl^- 121; $TRIS^+$ 4; pH 7.1). TTX was added to all solutions in the experimental chamber. Na^+ ions were isotonically partially replaced by TEA^+ (30 mmol/l), and Cl^- ions by SO_4^{2-}; to decrease the surface membrane permeability.

The crayfish fibres were dissected in van Harreweld solution (in mmol/l: Na^+ 208.4; K^+ 5.4; Ca^{2+} 13.5; Cl^- 241; Mg^{2+} 5.6; $Tris^+$ 2; pH 7.4). During the experiment, 100 mmol/l Na^+ was isotonicaly replaced by TEA^+.

The experiments were controlled by a computer, providing also data acquisition through a 12-bit AD converter. The data were off line numerically proccessed (Lacinová 1988).

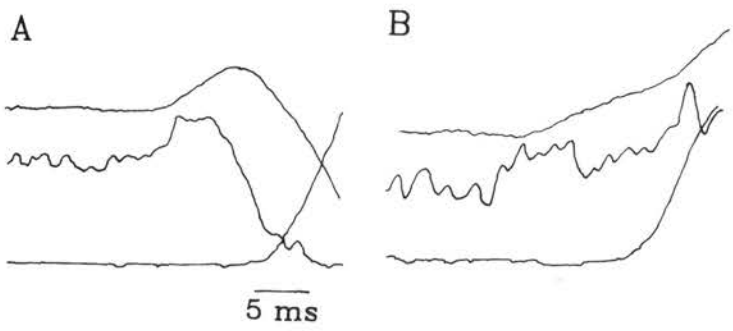

Fig. 1. Simultaneous measurements of tension (*lower trace*) and birefringence change (*upper trace*). The middle trace is the first derivative of the optical signal. The initial part of the optical signal has almost linear time course. This linear part ends before the onset of tension when a nonlinear one starts. Both components of the optical signal can have the same (*A*) or opposite (*B*) directions.

Results

The changes of optical anisotropy that precede contraction were analysed, and their relations to calcium release and contraction were studied.

Frog muscle

In the frog twitch skeletal muscle cell, the portion of optical signal preceding contraction has two distinguishable components differing in their time course and latency (Fig. 1). They can have the same (Fig. 1*A*) or opposite (Fig. 1*B*) direction. This effect could not be brought into direct correlation to the fibres width.

The signals measured with crossed and parallel polarizers were symmetrical, as could be expected for the anisotropy change (Baylor and Oetliker 1977b).

The optical signal started with an almost linear time course. The linearity is very well seen on its derivative, where the corresponding portion is constant (Fig. 1*A*). After subtraction of the linear part there remains a change of optical anisotropy, which still precedes the onset of tension. It is nonlinear and has a time course which parallels that of the contraction (Fig. 2).

The latency of the nonlinear component of the optical signal was determined after subtraction of linear part. The latencies of tension and of both componets of the optical signal decrease with increasing depolarizition (Fig. 3). Their differencies are almost voltage independent.

The maximal rate of changes of tension and the linear part of the optical signal reached saturation at about − 5 mV. This suggests that both phenomena

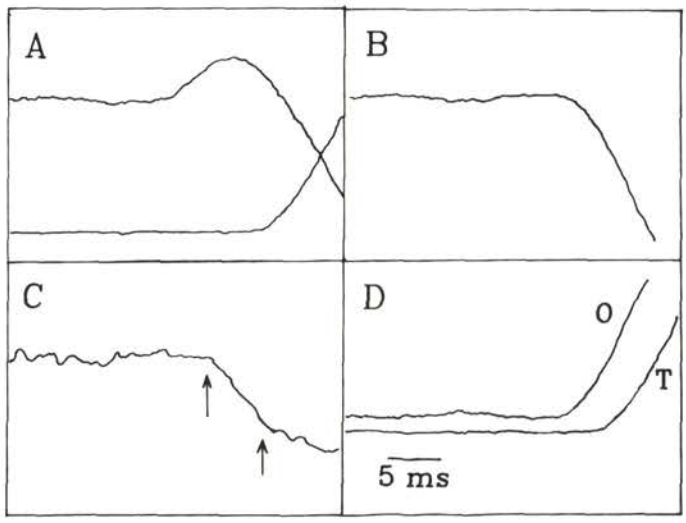

Fig. 2. After subtracting the linear part of the birefringence change from (*A*), the remaining signal is still nonlinear (*B*). This is obvious from its first derivative (*C*). Arrows show the onsets of the optical signal nonlinear part and of tension. *D*: A comparison of time courses of the nonlinear part of the optical signal (O) and tension (T).

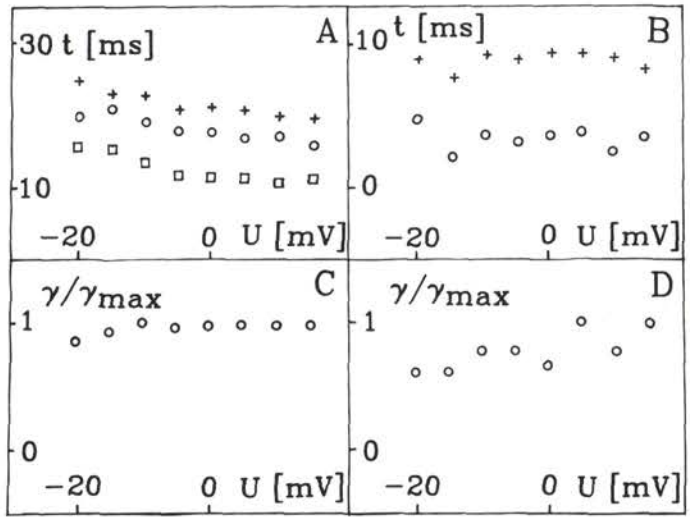

Fig. 3. *A*: Voltage dependences of latencies of tension (+) and of the linear (□) and nonlinear (o) part of the optical signal. *B*: The respective dependences of the differences. *C*: The voltage dependence of the maximal rate of tension. *D*: First derivative of the linear part of the optical signal. Voltage refers to membrane potential.

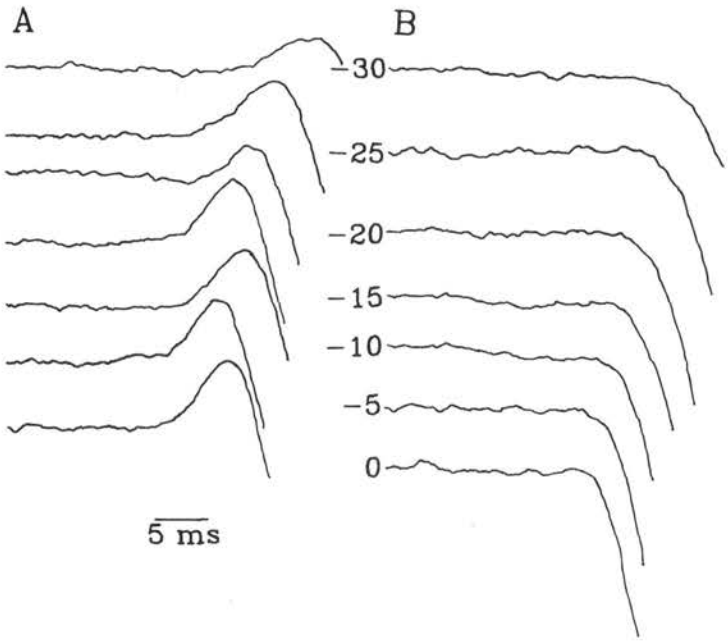

Fig. 4. The effect of caffeine on the time course of the optical signal *A*: normal saline; *B*: in the present of 2 mmol/l caffeine. Amplitudes of depolarizing pulses (refering to membrane potential) are shown at the traces.

are related to the same process, i.e. the release of calcium from TC, and the increase of Ca^{2+} concentration in the sarcoplasm.

The time course, voltage dependences and the relations of both components of the optical signal suggest that it may reflect the steps of E—C coupling following Ca-release. The linear component could correspond to calcium binding to Ca-ATPase of SR and to its conformational changes. The nonlinear component, which is delayed, could represent calcium binding to Ca-ATPase of SR and to its conformational changes. The nonlinear component, which is delayed, could represent calcium binding on troponin C. This interpretation is supported also by the similarity of time courses of the initial part of contraction and of the nonlinear component of the optical signal (Fig. 3), which implies relation to processes with similar kinetics.

This hypothesis was tested by modifying the E—C coupling: the sarcoplasmic Ca concentration was increased by caffeine and/or by conditioning depolarization.

Caffeine increases free calcium concentration in the sarcoplasm and higher

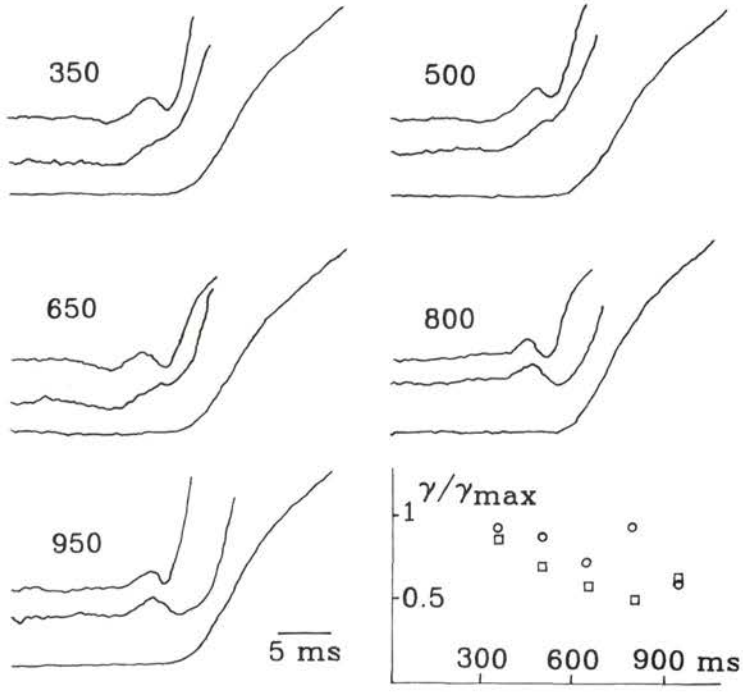

Fig. 5. Time course of the birefringence change after conditioning (*upper traces*) and testing (*middle traces*) pulse. The lower traces show the time course of tension development. The plot in the lower right part shows the dependence of the first derivative of the optical signal linear part on interpulse interval. Squares represent average values of the first derivative of the linear part conditioning and circles those after testing pulses (relative units).

concentrations evoke contractures (Sandow 1964; Sandow and Brust 1966). Two mmol/l caffeine was used as this concentration only potentiates contractions. Caffeine increased the rate of contraction, its amplitude, area under contraction curve, and decelerated relaxation. The linear component of the optical signal was completely missing (Fig. 4). The nonlinear component was not affected. Hence, an increase in the resting calcium concentration in the sarcoplasm suppressed the initial part of the optical signal.

Conditioning depolarization pulse modifies the conditions for activation of contraction with the second pulse. We used a pre-pulse and a testing pulse with the same amplitude and duration, chosen to evoke maximal contraction. The testing pulse could start only after the end of the relaxation period to allow measuring of optical anisotropy unbiased with any movement artefact. The minimal interval between the pulses was 350 ms for frog twitch muscle fibre, and

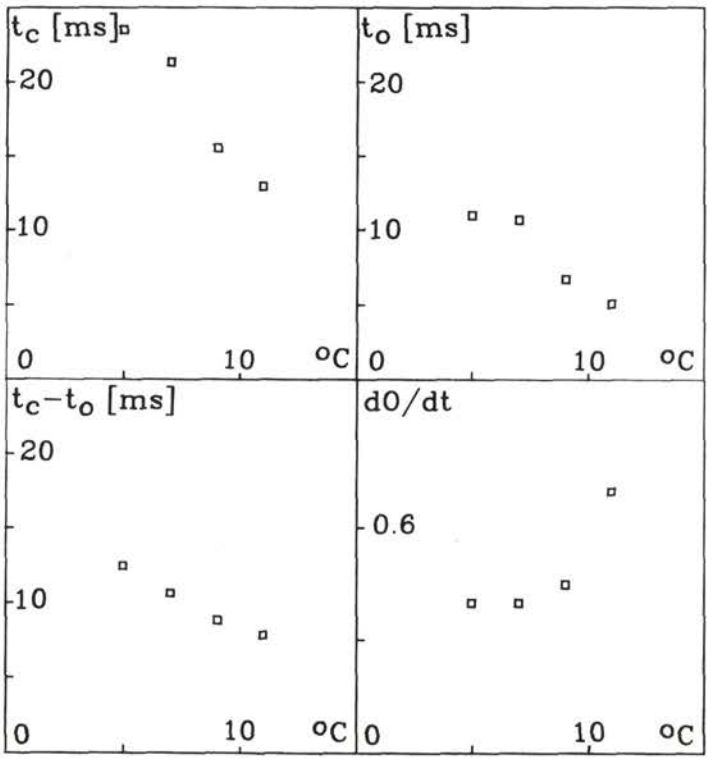

Fig. 6. The temperature dependences of the onset of contraction (t_c), optical signal (t_o), their difference (t_c—t_o), and of the first derivative of the linear part of the optical signal.

it was increased with a step of 100 ms. Under these conditions the tension was not influenced while the optical signal was modified. The linear part was suppressed and the nonlinear part had opposite time course (Fig. 5). The linear part was fully recovered after a 950 ms pause, the nonlinear one earlier, after approx. 800 ms. These time intervals may be seen as durations of processes, which are reflected by both parts of optical signal. The longer time period may be the interval needed for reuptake of Ca^{2+} ions by the calcium pump (Miledi et al. 1983), the shorter one may reflect resting state recovery of troponin C (Kress et al. 1986).

Also, the temperature effects on the birefringence change and tension were determined. The results are shown on Fig. 6. The onsets of both tension and

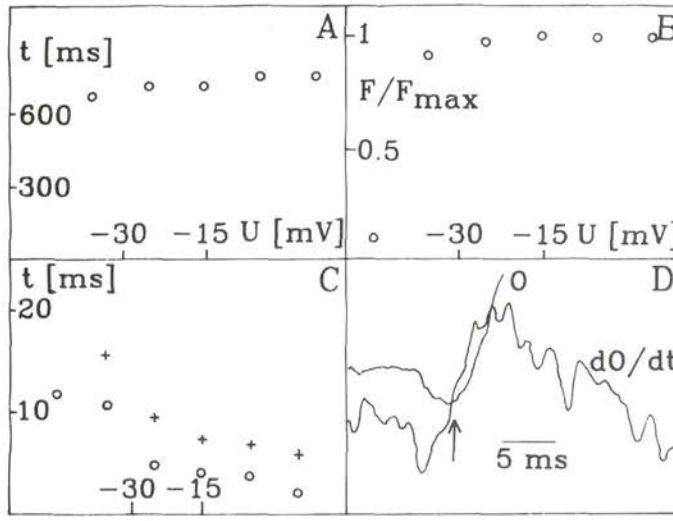

Fig. 7. Voltage dependences of time to peak tension (*A*), relative value of maximal tension (*B*), the onsets of tension (+) and optical signal (o) (*C*), and time course of the optical signal (*D*) and of its first derivative (dO/dt) (*D*). Voltage refers to membrane potential. The onset of tension in (*D*) is indicated by arrow. Isolated crayfish muscle fibre.

optical anisotropy change were speeded up, as were their first derivatives. Q_{10} for the first derivative of the optical signal was 2.1.

Crayfish muscle

The interval between the onset of the optical signal and tension is shorter in the crayfish than in the frog skeletal muscle fibre. The change of optical anisotroy is nonlinear and has no distinguishable components. The time course of the first derivative of the signal (Fig. 7) shows no discontinuity which could reflect the onset of another process. The symmetry of signals from parallel and crossed polarizers is strongly perturbed about 1 ms before the onset of tension (Fig. 8), where great changes of absorption occur.

The birefringence change appeared also at subthreshold depolarizations, where the inward calcium current is measurable. The optical signal correlates with the ascendent phase of the calcium current with some time delay (Fig. 8).

We modified the processes of activation of contraction by changing the external solution to obtain additional information about the nature of the change of optical anisotropy.

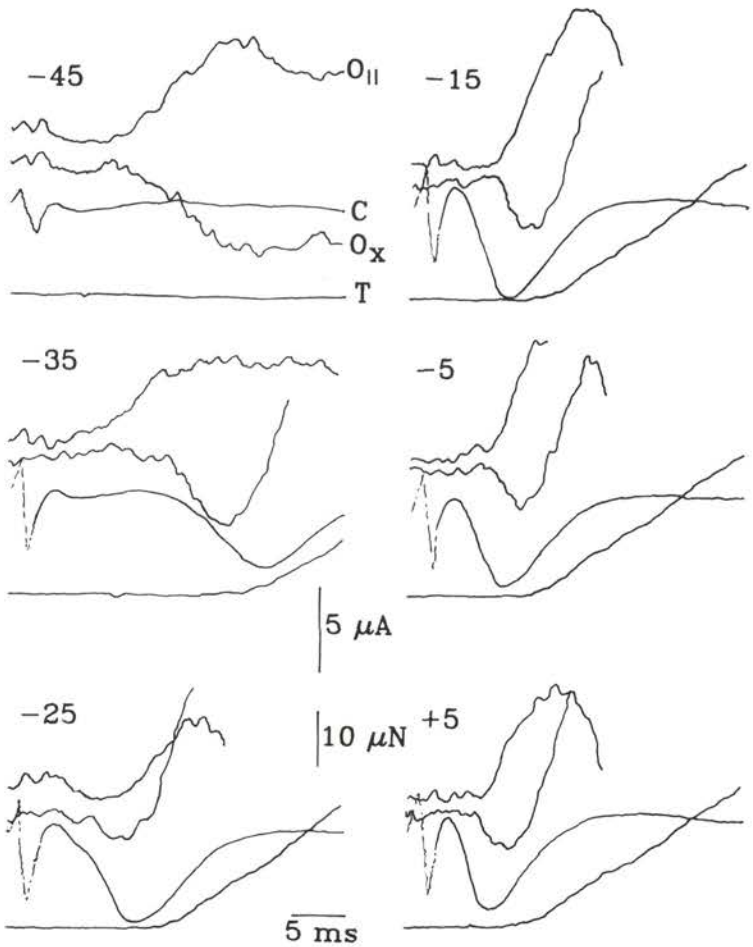

Fig. 8. The time courses of tension (T), ionic current (C) and optical signal measured with crossed (O_x) or parallel (O_{II}) polarizers. The amplitudes of depolarizing pulses refer to membrane potential. Isolated crayfish skeletal muscle fibre.

Replacing Ca^{2+} ions with 2-fold concentration of Sr^{2+} ions converts graded active response to all-or-nothing type (Fatt and Ginsborg 1958; Zacharová et al. 1962; Zacharová and Zachar 1967). Strontium is able to substitute calcium in the active response of surface and tubular membranes, it can bind also to Ca-ATPase, but it cannot activate contractile proteins (Stephenson and Williams 1980; Tomková and Kontšeková 1980). In our experiments the tension

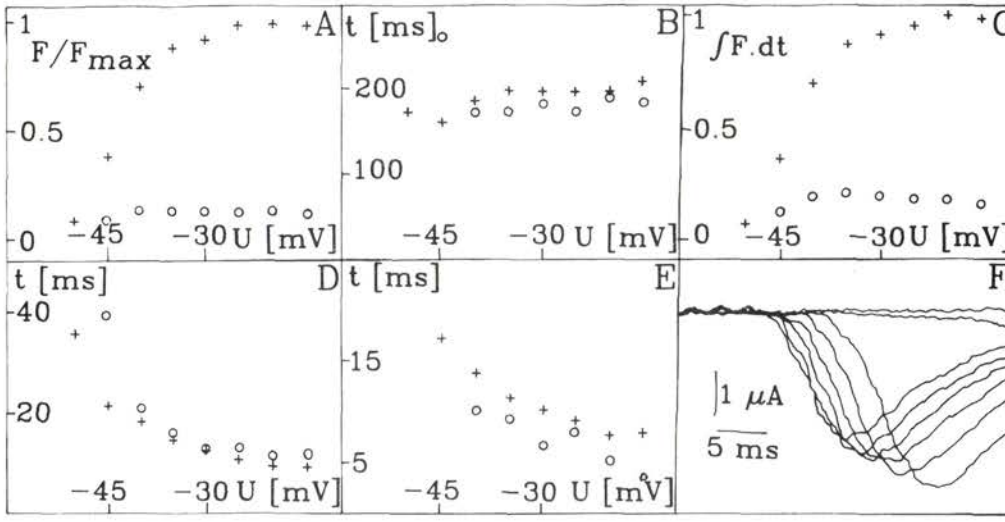

Fig. 9. Voltage dependences of maximal tension (*A*), time to peak tension (*B*), the area under tension time course (*C*), the onsets of tension (*D*), and of optical signal (*E*) in normal saline (+) and with Sr^{2+} ions substituted for Ca^{2+} ions (o). *F*: The strontium inward currents. The calcium inward currents were the same as shown in Fig. 10 (*F*).

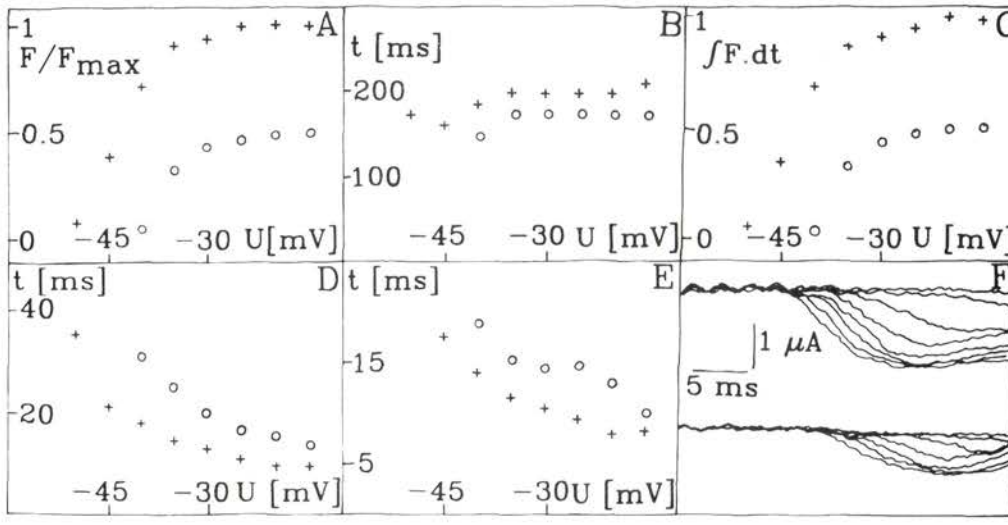

Fig. 10. Voltage dependences of maximal tension (*A*), time to peak tension (*B*), the area under tension time course (*C*), the onsets of tension (*D*) and optical signal (*E*) in normal saline (+) and in the presence 2 mmol/l Co^{2+} (o). F: The inward calcium currents in normal saline (*top*) and in the presence of 2 mmol/l Co^{2+} (*bottom*).

amplitude was decreased to about 20 % of its normal value, and the optical signal was increased and started earlier (Fig. 9). The strontium inward current was greater than the calcium inward current. The slope of the birefringence change started after the current set on.

Cobalt is known as a calcium channel blocker (for a review see Hagiwara and Byerly 1981). In our experiments 2 mmol/l cobalt was used. This concentration decreased both tension and calcium inward current to approx. 25 %. The contraction threshold was shifted toward higher depolarization by about 15—20 mV. The optical signal was influenced in the same way: the threshold was shifted and the amplitude was reduced (Fig. 10).

Discussion

The change of optical anisotropy is related to an elevated internal free calcium concentration. This fact was demonstrated by calcium sensitive dyes (Baylor and Chandler 1978; Baylor et al. 1981; 1982; 1984; Taylor et al. 1982; Kovács et al. 1983; 1987; Suarez-Kurtz and Parker 1977). However, the mechanisms of the birefringence signal development remain unclear.

Two components of the birefringence change were found in frog skeletal muscle cell. They differ in their kinetics. The optical signal may have at least two different sources. The possible sources of the birefringence change are conformational changes of the regulation proteins (Ca-ATPase, troponin C, parvalbumin) after calcium binding. The binding of Ca^{2+} ions on parvalbumin is too slow (Robertson et al. 1981) to be the source of the optical signal. The two components of the birefringence signal from frog muscle fibre could reflect the conformational changes of Ca-ATPase and troponin C respectively. The rate constants of these processes are similar (the rate constants of calcium binding on low-affinity troponin C sites or calcium pump are 0.12 $\mu mol^{-1}.1.s^{-1}$ (Robertson et al. 1981) and 0.35 $\mu mol^{-1}.1.s^{-1}$ (Inesi 1981) respectively), but the spatial distribution of the protein molecules is different. As compared with the longitudinal SR, the Ca-ATPase molecules are located in the Z-line region in concentrations at least about 1.5 times higher due to the larger area of the terminal cisternae membranes per unit length (Mobley and Eisenberg 1975). Troponin C molecules are distributed uniformly along the actin filament. So, the calcium binding on Ca-ATPase immediately after calcium release can be expected to be dominant. The linear part of the optical signal may reflect the calcium pump activation, while the nonlinear part would then reflect conformational changes of troponin C.

All the effects observed with calcium release modification are consistent with the above interpretation.

In the presence of caffeine the first part of the signal was missing. Caffeine is known to increase the resting Ca^{2+} concentration. A number of interpretations have been proposed for this effect, including potentiation of Ca-release (Endo 1977; Kovács and Szücs 1983) or Ca-ATPase inhibition (Chiarandini et al. 1970; Poledna and Morad 1983). Both the above mechanisms lead to optical signal inhibition: the first one raises the concentration of active Ca-ATPase. An additional activation of Ca pump following fibre depolarization was not measurable. The other mechanism proposed for caffeine effect to direct inhibition of birefringence signal from the Ca pump.

The specific Ca^{2+}-binding sites on troponin C have low affinity, but very high binding rate constant (Johnson et al. 1981). Therefore, increased resting calcium concentration only facilitates the binding of calcium released during activation.

The time required for total recovery of any part ot the birefringeence change is the interval needed by the process underlying the change return to the resting state. Interval of the full recovery of the anisotropy change correlates with the time for calcium uptake into SR vesicles (Miledi et al. 1983), or with the time for recovery of calcium inhibited calcium release (Schneider and Simmon 1988). It suggests a common origin of these processes, which could be the calcium pump.

The return of troponin C to its resting conformational state is a faster process as compared to the above processes (Kress et al., 1986). This may be reflected by a faster recovery of the nonlinear part of optical signal.

It seems that caffeine and conditioning depolarizations could affect the optical signal in a similar way. However, caffeine raises the sarcoplasmic calcium concentration permanently, and calcium binding proteins like troponin C and Ca-ATPase reach equilibrium levels and hence new initial-states. The equilibrium constants for calcium binding on troponin C or on the calcium pump are > 1 mmol/l (Hasselbach 1979) and about 0.2 mmol/l (Baylor et al. 1983) respectively. According to this difference, caffeine induced subthreshold calcium release affects predominantly the calcium pump signal. Conditioning pre-pulse releases calcium ions with a transient concentration change.

The value of Q_{10} for the optical signal linear part is similar to those measured by Weber et al. (1966) and Ogawa (1970) who studied temperature dependence of Ca-ATPase activity in SR vesicles.

E—C coupling processes in crayfish skeletal muscle cell are different (for a review see Rüegg 1986). This fact is reflected in the different time course of the birefringence change, measured on this preparation.

There are two main differences in the anisotropy change between frog and crayfish skeletal muscle. The time interval between the onsets of the optical signal and tension is shorter in crayfish than in frog muscle fibre and its time course is nonlinear. No data are available concerning the comparison of signals

calcium-sensitive dyes with birefringence change on this preparation; nevertheless, the optical signals and the calcium inward currents can be compared instead.

We have shown that the optical signal starts after the onset of the calcium inward current. It can be supposed that the optical signal from crayfish muscle fibre reflects calcium binding to intracellular regulatory protein molecules similar as it is the case in frog muscle cell.

Ca-ATPase and troponin C are occupied primarily by Ca^{2+} due to their fast rate constants. This binding and the resulting conformational changes can be supposed be the source of the birefringence change similar as it is the case in frog fibres. In crayfish muscle calcium ions enter the cell through surface and tubular membranes, and the calcium release sites are at the actin — myosin overlap. This is reflected in the earlier onset of tension as compared with frog muscle.

Prior to the onset of mechanical activity, crossbridges are formed. This process precedes measurable tension (Kress et al. 1986) and may be the source of absorption change.

The nonlinearity of the anisotropy change may reflect spatial spreading of activation. In the crayfish, time and spatial distribution of Ca^{2+} entering from the extracellular space trough the fibre volume must be considered. This process is rather complex, and it may be the reason for the optical signal nonlinearity in this preparation. The initial part of the signal may reflect both the activation of Ca-ATPase and the onset of the troponin signal.

The effects of Co^{2+} and Sr^{2+} ions support the hypothesis concerning the existence of a relation between intracellular Ca^{2+} concentration and the optical signal.

Co^{2+} blocks influx of Ca^{2+} ions. Its inhibiting effect on the birefringence change is simillar to the effect on the calcium inward current.

Sr^{2+} enters the fibre in higher amounts than does Ca^{2+}, but is not as effective in activating the contractile proteins. In skinned fibres strontium cannot induce any tension (Stephenson and Willians 1980). In intact fibres it may induce calcium release from the SR, because the contraction is inhibited less than in skinned fibres. The optical signal is, however, potentiated; it starts earlier and occurs with a higher rate. This confirms the relation between the anisotropy change and Ca-ATPase activation: Sr^{2+} cannot bind to troponin C, but can activate the calcium pump (Tomková and Kontšeková 1980). Sr^{2+} ions have lower affinity to Ca-ATPase than do Ca^{2+} ions, but strontium ions reach several times higher concentrations and therefore can initiate conformational change of troponin C.

The birefringence change in frog twitch skeletal muscle fibre can be used for monitoring both Ca-ATPase and troponin activities. In crayfish skeletal muscle fibre this signal provides information about internal Ca^{2+} distribution, but interpretation of the results presents difficulties.

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