Simultaneous Measurements of Ionic Currents, Tension and Optical Properties of Voltage Clamped Skeletal Muscle Fibres

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Abstract. The paper presents the voltage clamp method for isolated skeletal muscle cells, which allows simultaneous measurements of ionic currents, tension and changes in their optical properties. Experimental results illustrate the range of possible applications. Measurements with two types of preparations, frog and crayfish, exhibiting differencies in EC coupling are compared. Basic characteristics of the voltage dependence of tension and birefringence during activation of contraction are described.

Key words: Skeletal muscle fibre — EC coupling — Voltage clamp method — Tension — Birefringence signal

Introduction

The excitation contraction coupling is a sequence of processes between electrical events occurring on the surface membrane and the force generated by contractile proteins. Depolarization of the sarcolemma spreads towards the cell longitudinal axes by a system of tubules radially invaginated from the surface membrane. The tubules are in aposition with terminal cisternae of the sarcoplasmic reticulum which forms specialized structures (diads or triads) transmitting the signal for the calcium release (Zachar 1971). The tubular membrane potential controls the calcium release into the myofilament space. To identify these processes and to analyse mutual interactions, functional relations between the input and output variables must be known.

We modified the voltage clamp method of Kovacs and Schneider (1978), to allow measurements of tension and optical properties on intact fibres. The use of different preparations is advantageous from the comparative point of view, however under the same experimental conditions only. The presented voltage clamp method for measurement of ionic currents, tension and optical properties

enables simultaneous observation of different steps of EC coupling. It is also quite universal and allows comparison of preparations with different properties of EC coupling signals. E.g. muscle cells of frog and crayfish represent two phylogenetically distinct species. Frog fibres operate on the sodium electrogenesis principle with action potentials of the 'all or nothing' type, and EC coupling being not affected by withdrawal of calcium ions from extracellular space (Armstrong et al. 1972). Crayfish fibres operate on the calcium electrogenesis principle, a gradual active response of the surface membrane with the contraction being dependent on the concentration of calcium ions in extracellular space, namely in the tubular system (Zacharová and Zachar 1967).

From optical methods we chose birefringence signal which reflects optical anisotropy i.e. the dependence of light speed on its direction relative to muscle cell geometry. This effect is a consequence of the interaction of light with electron orbitals of macromolecules, and thus provides information about molecular mechanisms of physiological processes in cells. A large change in birefringence following action potential and preceding contraction in skeletal muscle cell was first described by Baylor and Oetliker (1975).

The present paper describes voltage clamp of a terminal part of skeletal muscle cells and analyse the power of the method in investigating processes of excitation contraction coupling.

Materials and Methods

Experimental setup

We modified the voltage clamp method developed for dialyzed terminal segment of skeletal muscle cells (Kovacs and Schneider 1978), to be able to use intact fibres. One end of the tested fibre is isolated by two vaseline seals, which divide the chamber into three compartments (Fig. 1*A*). The compartment between the seals is grounded to increase the virtual seal resistance. The tested end of the fibre, of a lenght of about 0.3 mm from the tendon to the seal, is voltage clamped. Current is applied into the compartment containing the rest of the fibre to control voltage in the test compartment.

The tension of the terminal segment of the fibre in the test compartment was measured isometrically. We used a silicon strain gauge tension transducer (Marko et al. 1986) with a sensitivity of up to 1 μN. The segment of the fibre outside the test compartment is hyperpolarized at depolarizing pulses and does not contribute to mechanical response.

The test compartment is temperature clamped via a Peltier's battery and a thermistor in feedback. The thermistor is placed in the vicinity of the fibre in the test compartment. This system keeps temperature within 0—30°C with a deviation less then 0.1°C of the set value. A jump in temperature is equilibrated within 2 min.

Electrical circuits

The electrical scheme of the voltage clamp apparatus is on Fig. 1*B*. Changes in membrane potential are measured with a symmetrical differential amplifier between the test and the current compartment (amplifier A_S). A series resistance corresponding to membrane resistance of the fibre in the current

compartment (C) and to that of the sarcoplasm between the test and the current compartment, is compensated for by amplifier A_V. The potential in the test compartment is held at ground with operational amplifier A_E. The current passing the membrane in the test compartment is measured with amplifier A_R. Amplifier A_c is used for the application of current to the current compartment to clamp voltage in the test compartment. The system is adjusted and the series resistance is compensated for at the begining of each experiment similarly as described by Kovacs and Schneider (1978) (using microelectrode measurement). During the experiment the microelectrode is not in contact with the fibre. Fig. 1*C* illustrates the compensating procedure, and Fig. 1*D* shows an example of simultaneous measurement of current and tension in a frog skeletal muscle fibre.

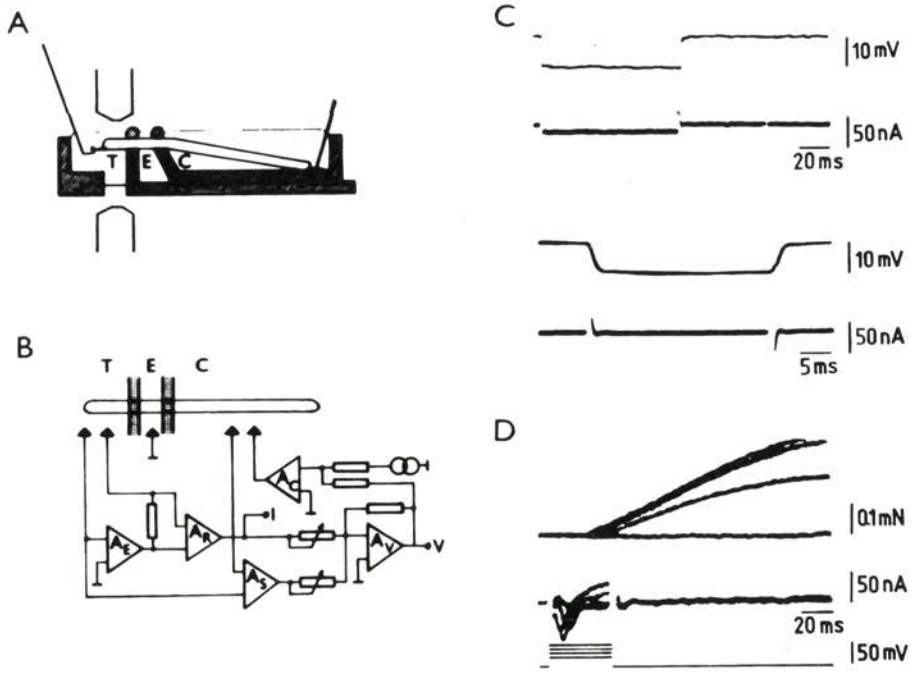

Fig. 1. Method for simultaneous measurements of tension, optical anisotropy and ionic currents of a voltage clamped intact skeletal muscle cell. (*A*) Design of the experimental chamber which has three compartments: T — test, E — ground and C — current compartment. In the test compartment a tendon of the fibre is fixed to a strain gauge transducer. The glass window at the bottom of the T-compartment allows measurement of optical properties in the activated part of the fibre. In compartment C the other end is fixed to the chamber. (*B*) Electronic circuits, which control voltage of the fibre membrane in the test compartment. The current is driven into compartment C. Amplifier A_E holds compartment T at virtual ground. A_R is a current measuring amplifier. Voltage between T and C compartments is measured by A_S. Input resistors of A_V are included to compensate for series resistance and to adjust voltage measurement circuit, respectively. (*C*) Examples of a membrane potential change measured with a microelectrode at hyperpolarizing pulses and corresponding membrane currents. (*D*) Simultaneous measurements of tension and ionic currents of a frog fibre in normal saline.

Optical measurements

The experimental layout allows measurements of optical properties of the activated part of the cell in the test compartment. We measured changes in optical anisotropy, the birefringence signal. Light linearly polarized in a plane oriented at 45° to the fibre's longitudinal axes was used. Behind the fibre the light was collected by an objective and passed to an analyser. Light intensity was measured with a photodiode (Fig. 2). Linearly polarized light passing the muscle fibre, which is optically anisotropic, changes its polarization, therefore light intensity behind crossed polarizers is not zero. Optical anisotropy of a muscle fibre is a result of a highly oriented structures such as contractile proteins and membranes of the sarcoplasmic reticulum. The volume densities of these structures in frog muscle fibre (Mobley and Eisenberg 1975) and in crayfish muscle fibre (Uhrík et al. 1980) may explain optical anisotropy and considerable changes of the latter during activation. These changes between the action potential and the onset of contraction normalized to the rest intensity I_0, are in the order of about 10^{-3}, and reflect the increase of calcium concentration in the sarcoplasm (Baylor and Oetliker 1975, 1977; Poledna and Morad 1983).

Preparations

Isolated muscle fibres were dissected from m. semitendinosus of the frog *Rana temporaria* and from m. extensor carpopoditi of the crayfish *Astacus fluviatilis*. The segment of the fibre to which vaseline seals were applied and where optical measurements were done had to be carefully cleaned from conective tissue.

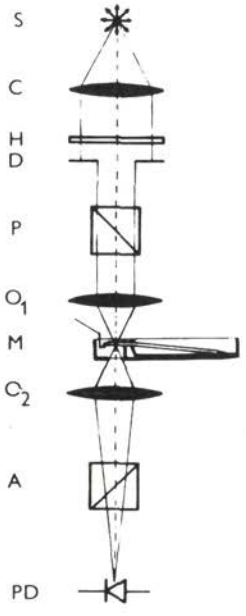

Fig. 2. The arrangement for optical measurements. Light from a halogen source (S) passes through a condensor (C), a heat filter (H), a diaphragm (D), a polarizer (P), an objective (O_1) an illuminated the fibre (M). Under the fibre light is collected by an objective (O_2), and its intensity behind analyser (A) is measured with a photodiode (PD).

Solutions

The frog saline had the following composition (in mmol/l): Na^+ 120, K^+ 2.5, Ca^{2+} 1.8, Cl^- 121, $TRIS^+$ 4, pH 7.1. A frog muscle fibre was mounted into the chamber in solution containing 10^{-7} mol/l TTX. This solution remained in the current compartment throughout the experiment to suppress the propagation of activation. In the grounded compartment, Na^+ ions were partially

isotonically replaced by 30 mmol/l TEA^+ and Cl^- ions were partially replaced by SO_4^{2-} ions to decrease membrane permeability. In the test compartment, the solution was changed according to the experimental protocol.

The crayfish saline (van Harreveld) contained (in mmol/l): Na^+ 205.3, K^+ 5.4, Ca^{2+} 13.5, Mg^{2+} 5.6, Cl^- 248.8, $TRIS^+$ 5. In the current compartment, Ca^{2+} was omitted and in the grounded compartment the crayfish saline was modified by partially replacing Na^+ ions by 100 mmol/l TEA^+.

Results

Under the voltage clamp conditions, the sarcolemma has a preset membrane potential, but the tubular membranes are electrically connected with the extracellular space through a series resistance of the tubular mouth and lumen. These resistances have been experimentally identified and measured (Falk and Fatt 1964; Poledna et al. 1976, 1978). Ionic currents flowing through a series resistance do not allow keeping the same membrane potentials on the surface and on the tubular membrane. In normal saline above the threshold depolarization, the active response of the tubular membrane is propagated inside the fiber.

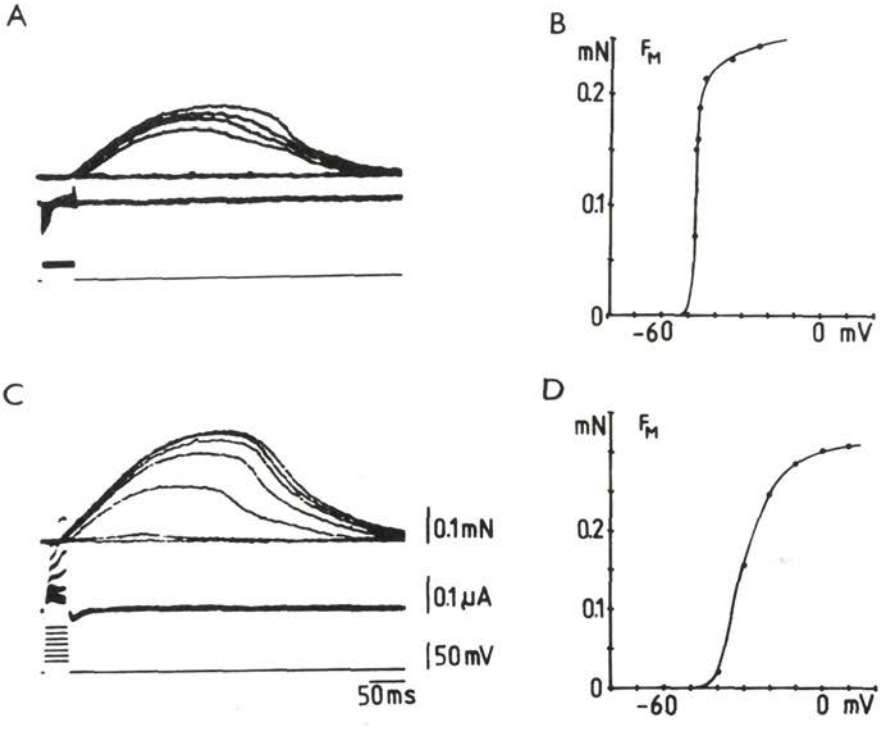

Fig. 3. Comparison of voltage dependence of frog muscle fibre contraction and their maxima in normal (*A*, *B*) and sodium free (*C*, *D*) saline.

The twitch reaches its maximal value within about 5 mV and the voltage — tension relationship is very steep (Fig. 3). A substitution of sodium ions by $TRIS^+$ in extracellular space changes twitch to a graded response over a range of about 40 mV. This implies that the process of calcium release into the myofilament space not only is triggered but also controled by tubular membrane voltage. It was therefore important to know voltage and time dependences of changes in optical anisotropy and contraction (Fig. 4). These experiments were done at 10°C and with 10^{-7} mmol/l of TTX added to normal saline. The maximum of contraction increased with depolarization up to saturation, which depended on the pulse length. A prolongation of depolarization increased time to peak up to about 200 ms. For long pulses the maximal value was reached

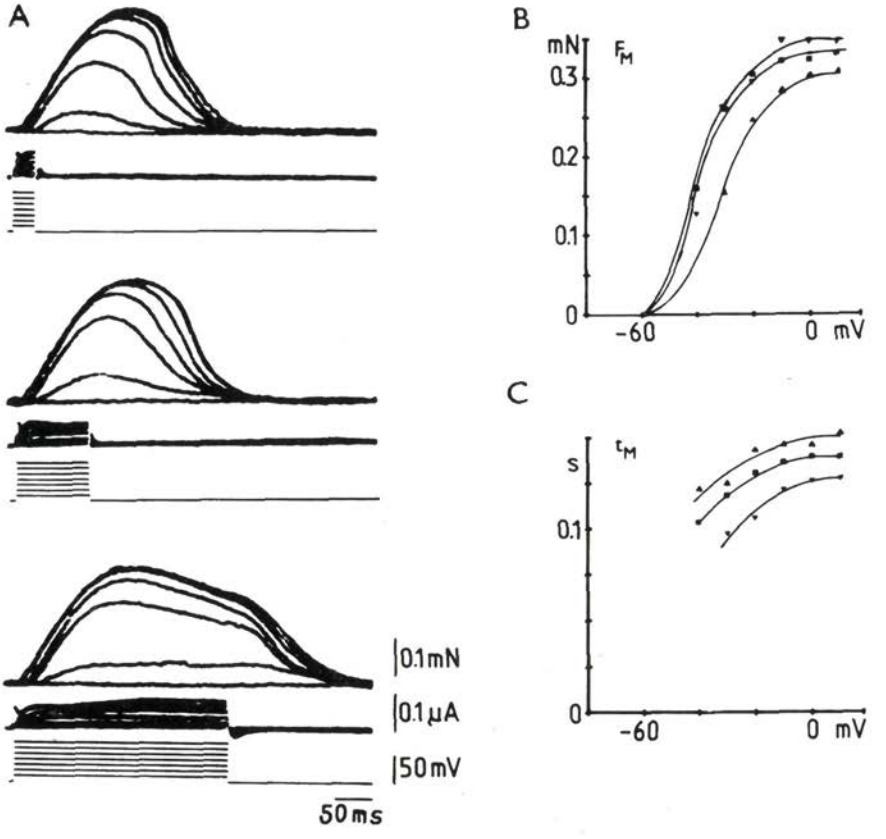

Fig. 4. Dependence of frog muscle fibre contraction (*A*), maximum of contraction (*B*) and time to peak (*C*) on the amplitude and length of depolarizing voltage clamp pulses in frog saline solution containing TTX. Symbols for different length of pulses: (▲) 30, (■) 100 and (▼) 300 ms.

before the end of the pulse and then the tension approximately exponentially approached a steady level. The maximum of contraction had an S-shaped voltage dependence and saturated at about zero membrane potential. The contraction threshold was between -60 and -50 mV.

Changes in optical anisotropy reflect also the activity of the sarcoplasmic reticulum. There are several hypotheses concerning the origin of these changes. Baylor and Oetliker (1977) supposed that the birefringence signal was a consequence of changes in the sarcoplasmic reticulum membrane potential related to calcium release. Kovacs et al. (1983) suspected changes in optical properties of the sarcoplasm due to changes in calcium concentration, and Poledna and Morad (1983) proposed, calcium ATP-ase in SR membranes as the source of anisotropy changes. Generally, a relationship of optical anisotropy and the rise in calcium concentration in the sarcoplasm has been accepted. From this point of view, only the latency of the optical signal and its rate of change are relevant (Fig. 5). This is because the signal may be later contamined by conformational changes of the troponin-tropomyosin complex and by motion artefacts. Latencies of the optical signal and contraction (Fig. 5) decrease for higher depolarizations and saturate. Their difference is almost voltage-independent. The maximal

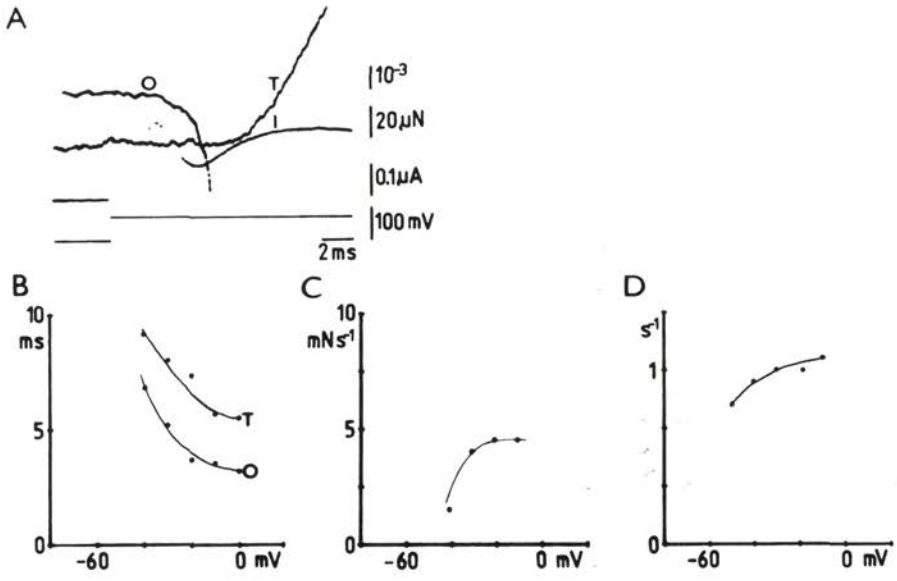

Fig. 5. (*A*) Simultaneous measurements of tension (*T*), changes in optical anisotropy (*O*) and membrane current (*I*) of frog muscle fibre in TTX saline. Voltage dependences of the latency of the birefringence signal (*B*), the maximal derivative of tension (*C*) and the maximal derivative of optical signal (*D*).

rate of change in both tension and the initial part of optical signal increase with depolarization and reach a steady value at zero membrane potential. Their time courses are qualitatively similar but the relation is not linear. A more detailed correlation of the measurements are necessary for quantitative description, since relationships between calcium concentration, contraction and optical anisotropy are not known.

The voltage dependence of contraction of crayfish muscle fibres differed from that of frog fibres. In crayfish, contraction saturated at zero membrane potential for short depolarizing pulses (up to 200 ms). For depolarizing pulses longer then 300 ms, in some fibres contraction did not reach saturation even at +20 mV, and maximum was reached after the end of the pulse (Fig. 6). Despite differences in EC coupling between frog and crayfish muscle fibres, the basic relationship of tension and optical anisotropy (Fig. 7) are similar.

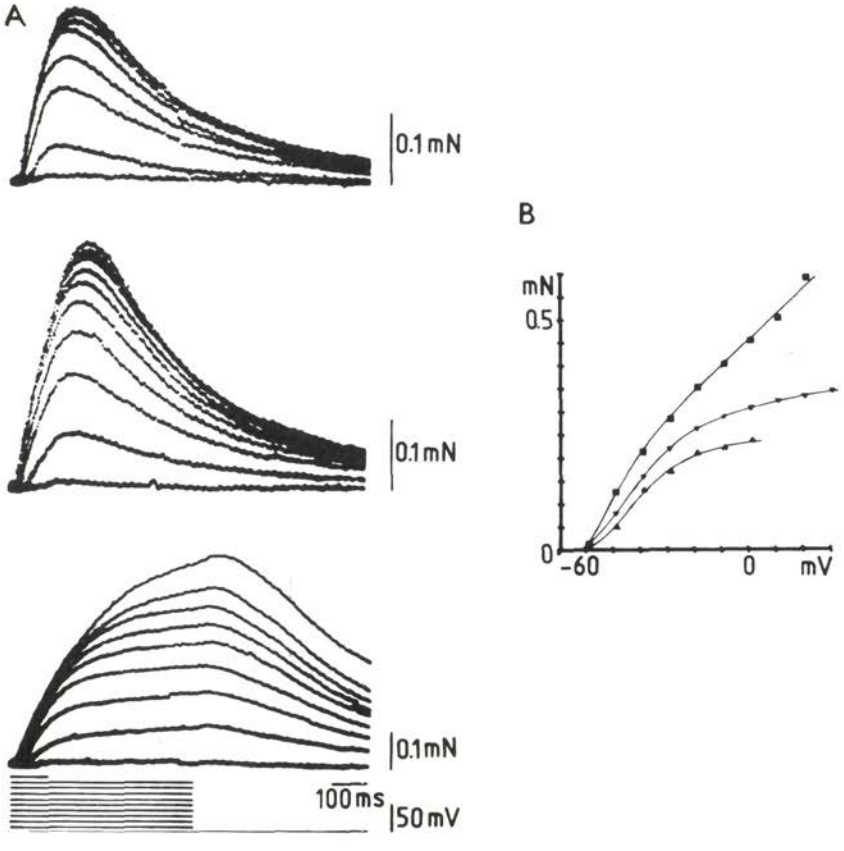

Fig. 6. (*A*) Voltage and pulse length dependence of crayfish muscle fibre contraction. (*B*) Voltage dependence of contraction maxima for pulse lengths (▲) 50, (▼) 100 and (■) 500 ms.

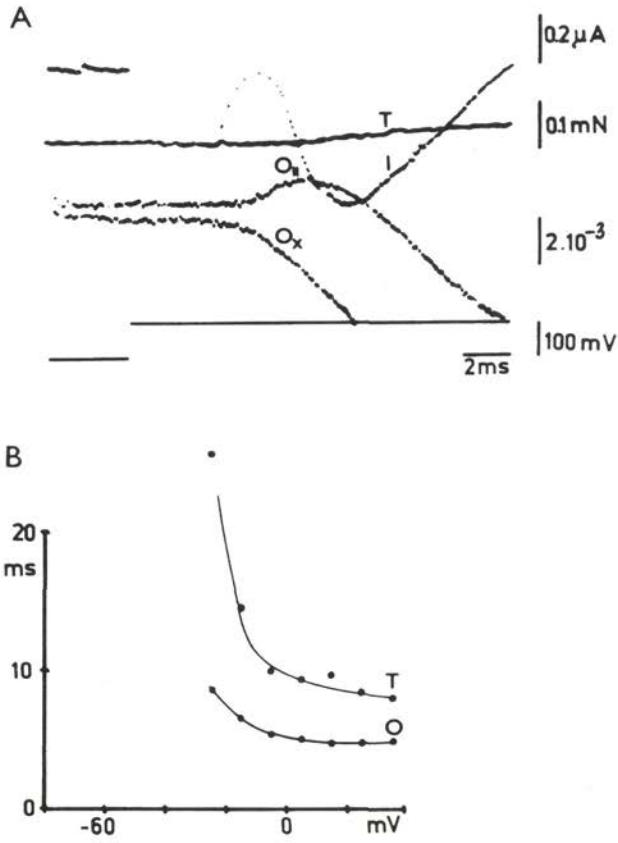

Fig. 7. (*A*) Simultaneous measurements of contraction (T), optical signal with crossed (O_x) and paralel ($O_{||}$) polarizers and membrane current (*I*) on crayfish muscle fibre. (*B*) Voltage dependence of latencies of the birefringence signal (O) and contraction (T).

Discussion

Experimental investigation of excitation contraction coupling is difficult owing to the necessity of preserving the integrity of morphologically and functionally complex intracellular structures such as the tubular system, the sarcoplasmic reticulum and their junctions in diads or triads.

The control of calcium release into the myofilament space by tubular membrane voltage has been obvious from the classical works by Hodgkin and Horowicz (1960) who studied frog skeletal muscle fibres, and by Zachar and Zacharová (1966) who worked with crayfish muscle fibres. In these works the membrane potential of the sarcolemma and the tubular system was controled by the concentration of potassium ions in the extracellular space. This method has a poor time resolution due to the limited speed of solution exchange and to the slow diffusion rate in the tubular system. This problem could by solved by electronic voltage clamp only. There had been developed several voltage clamp

methods and used to study EC coupling (Adrian et al. 1969; Costantin and Taylor 1973; Costantin 1974; Dudel et al. 1968; Caputo and Bolanos 1979; Caputo et al. 1984; Heistracher and Hunt 1969). These methods have had some restrictions concerned a potential range or a choice of preparations.

The method presented herein is a modification of the method developed by Kovacs and Schneider (1978). With an intact fiber, the input resistance in the current compartment increases. This was eliminated by the ground compartment, which raised the isolation resistance between the current and the test compartment. As the fiber is mechanicaly fixed in the current compartment, stretched and tied to a strain gauge transducer in the test compartment, contraction of the activated part of the fibre can be recorded. When the tested segment is depolarized, the fibre in the current compartment is partially hyperpolarized and does not contribute to the contractile response. To test this assumption, we activated the fibre by a hyperpolarizing pulse, however, no contraction was recorded by the transducer in the test compartment. The measured tension response from the tested part of the fibre is thus not affected by the rest of the fibre.

The voltage-tension relationships of frog fibres were compared with the results reported by Caputo and Bolanos (1979), Caputo et al. (1984) and Hodgkin and Horowicz (1960) and a good agreement was observed. A decrease of tension was found after the maximum was reached at long depolarizing pulses, this has not been described as yet. However this phenomenon is also observable on tension records shown in the above papers. It is probably a result of intracellular regulatory mechanisms of calcium cycling.

Our results of the voltage dependence of contraction in crayfish muscle fibres are comparable with those reported by Dudel et al. (1968). These authors observed saturation of contraction at about -20 mV, while we had some fibres showing a raise in the maximal tension even at $+20$ mV. All other results are in accordance.

Our voltage clamp method allows measurements of optical changes in the activated part of the fibre. We investigated optical anisotropy, which preceded the onset of contraction. This signal was first described by Baylor and Oetliker (1975). They proposed a hypothesis that the change in optical anisotropy was a consequence of potential changes in SR membranes, which accompanied calcium release, similarly as reported for the nerve membrane (Cohen et al. 1968). Changes in optical anisotropy correlate with calcium release from SR, measured by other methods (Oetliker et al. 1975).

Changes in optical anisotropy were compared with calcium release from SR by Kovacs et al. (1983), and they found a similarity in latencies of these processes, which for large depolarizations were about 6 ms at 2—4°C. Also, the rates of both processes correlated well. These authors supposed that the changes

in anisotropy of the sarcoplasm were due to changes in calcium concentration.

In single skeletal muscle cells, there are several processes, which change the optical anisotropy during activation of contraction. These predominantly include processes which result in measurable changes due to a large volume density of corresponding structures in a cell. SR membranes and contractile proteins should be considered. SR membranes are highly ordered, and may therefore contribute to optical anisotropy. SR membranes have a large protein content, 90% of which makes up the calcium pump (Blasie et al. 1981). At rest they are highly oriented and form the source of anisotropy. Due to calcium release and its increased concentration in the sarcoplasm, calcium is pumped into SR and states of calcium pump molecules are redistributed, resulting in anisotropy decrease; this agrees with the obtained experimental results. This hypothesis, proposed by Poledna and Morad (1983), has been based on effects of potentiators of contraction on the latency and rate of the birefringence signal. Calcium ions diffuse towards myofilaments and bind to troponin C. Conformational changes of the troponin — tropomyosin complex are a component of initiation of contraction. This process, which is delayed with respect to calcium release, must contribute to anisotropy changes, even if contraction is suppresed (e.g. by stretching the fibre). Owing to the above, the interpretation of the birefringence signal considering its whole time course and supposing only one process to occur may be incorrect. Due to this, we focused our attention on the latency of the birefringence signal and its derivation; with respect to the time sequence of EC coupling processes this corresponds to the beginning of calcium release into the sarcoplasm. The time course of contraction is another information about changes in calcium concentration, with the delay due to diffusion and conformation of the troponin-tropomyosin complex. Hence, the combination of these methods can yield similar information as do measurements with calcium sensitive dye (Poledna 1987) yet without interfering with the processes studied.

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