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Extracellular Potential Field of Excited Isolated Frog Muscle Fibres Immersed in a Volume Conductor

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Abstract. The extracellular potential field of isolated frog muscle fibres immersed in a volume conductor was studied at radial distances up to 3 mm during excitation. The shape of the field distant from both the point of the origin of the excitation and the end of the fibre as well as changes in the field when depolarization wave approached the fibre end were described. Different amplitude decrease rates in individual phases of the extracellular potential and the peak-to-peak amplitude at different temperatures were found. Extracellular potentials at long radial distances were recorded using an averaging technique. The shape of the extracellular potentials at long radial distances over the fibre and beyond its end were very similar to the shape of extraterritorial potentials of a single motor unit.

Key words: Muscle fibre — Action potentials — Extracellular potentials — Potential field — Volume conductor

Introduction

The extracellular potential field of excited single frog muscle fibres was studied by Håkansson (1957). Further studies of this problem are needed because:

a) Extracellular potentials recorded by Håkansson over muscle fibres were biphasic and the potential field described consisted of a positive and a negative area.

Theoretically, extracellular potentials over an excitable fibre should be similar to the second space derivative of the action potential (Lorente de Nó 1947) and hence they should be three-phasic — positive-negative-positive. Three-phasic extracellular potentials of single muscle fibres were observed experimentally (Katz and Miledi 1965; Gydikov et al. 1981). Results obtained by Håkansson (1957) may be explained on the assumption that under certain conditions the amplitude of the second positive phase is very small and not clearly visible. A potential field composed of three areas will corespond to three-phasic extracellular potentials (Lorente de Nó 1947).

b) The muscle fibres are relatively short. It is not correct to consider them as infinitely long, as it is often accepted for nerve fibres. When the depolarized area reaches the end of the muscle fibre, the distribution of the potentials in the volume conductor changes in a complicated manner (Gydikov and Kosarov 1972a, 1972b). Håkansson did not study the effect of the muscle fibre terminal region.

c) Håkansson (1957) investigated the potential field at radial distances smaller than 1.5 mm. At larger radial distances, the amplitude of the extracellular potentials is low and they cannot be discriminated from the noise level. The averaging technique allows to increase the signal-to-noise ratio at longer radial distances.

Materials and Methods

Investigations were carried out on 22 frog muscle fibres isolated from the gastrocnemius muscle. The majority of the fibres were from *Rana ridibunda* and a few from *Rana temporaria*. The terminal parts of the isolated fibres were cleaned since parts of the tendon and the fascia were left attached to the ends and were used to fix the fibres. The fibres were stretched to 120% of their normal length and immersed in a double-walled chamber filled with Ringer's solution containing (in mmol/l): 115.0 NaCl; 2.0 KCl; 1.8 $CaCl_2$; 0.68 NaH_2PO_4; 1.3 Na_2HPO_4 (pH 7.0—7.2). Between the walls of the chamber water of different temperatures was circulated: 10 °C, 15 °C, 20 °C and 25 °C. The temperature of the bathing solution was checked by a thermometer. Upon changing the temperature the solution was stirred and enough time was allowed to reach a new steady-state.

The fibres were stimulated extracellularly by 0.5—1.0 ms electrical pulses of suprathreshold intensity. The stimulating electrode was mounted in a small plexiglass chamber filled with Ringer's solution; the fibre was placed in a slot of the chamber. The chamber served to decrease stimulation artifacts. Consecutive stimuli were applied at intervals longer than 2 min.

Extracellular potentials were recorded by glass-coated tungsten electrodes. The electrode resistance was about 150 kΩ. Records were taken from different points. The axial distance to the end of the fibres was changed in steps of 100 μm over the fibre and beyond its end. The radial distance was changed in steps of 50 μm up to 1 mm. Displacement of the electrode in the radial direction was controlled by a stepping motor allowing changes with an accuracy of 1 μm. From a series of extracellular potentials recorded at different axial and radial distances, the extracellular potential field was constructed for several post-stimulus intervals. Suitable time intervals were chosen at which the depolarization area was still far from the end of the fibre and at which the area passed through the terminal part of the fibre and disappeared. The terminal part of the muscle fibres studied narrowed conically (Schwarzacher 1960; Gydikov et al. 1981). The extracellular potential field at a given interval after the stimulus was constructed, as the amplitude of the potential at the end of this interval was measured for all the points of recording. Data obtained for points with the same axial distance but different radial distances were plotted and a curve was fitted by eye (Fig. 1). Radial distances corresponding to a potential amplitude of 0; 10; 20; 40; 100; 200; and 400 μV were read from the plot. Corresponding points determinated by axial and chosen radial coordinates were plotted to obtain the extracellular potential field. The points relative to every axial distance studied were then connected by equipotential curves.

In some fibres extracellular potentials were recorded at a radial distance of 3 mm and at different axial distances. Average extracellular potentials were obtained from 256 times stimulated fibres. A great amplification was used (5 μV/cm). The averaged responses were significantly deformed by the stimulus artifact, due to the high gain of the amplifier. To avoid this deformation, the fibres were injured

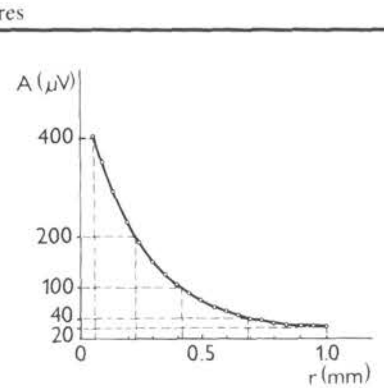

Fig. 1. Relationship between the amplitude of the extracellular potentials (A, μV) of a single frog muscle fibre recorded at a constant axial distance, and the radial distance (r, mm); the plot was used to estimate the radial coordinate of the equipotential lines.

mechanically after the averaging when the action potentials disappeared. Another series of 256 stimuli were applied and the pure artifact was averaged using another channel of the averager. The two records from the channels of the averager were electronically subtracted from each other and the extracellular potential at long radial distance was obtained almost free of any artifact. Due to the necessity of injuring the fibre after the recording we could not take records at more points and construct the extracellular potential field at long radial distances. However, the extracellular potentials obtained by this method were sufficient to draw several conclusions concerning the features of the field at long distances.

Results

Fig. 2*A* shows extracellular potentials recorded at a constant axial distance over a muscle fibre of *Rana ridibunda*. The axial distance from the point of origin of the excitation was 8 mm and from the end 9 mm at different radial distances. Upon increasing the radial distance the peak-to-peak amplitude of the potentials decreased. The amplitude of the negative phase near the membrane decreased (in terms of percent) more quickly than that of the initial positive phase. At longer radial distances, the amplitude of negative phase decreased more slowly than that of the initial positive phase. The amplitude of second positive phase decreased very slowly. With increasing radial distance the maximum of the first positive phase shifted slightly forwards and the negative and the second positive maxima shifted slightly backwards over time.

Fig. 2*B* shows extracellular potentials recorded from the same fibre at a constant axial distance over the terminal conical part of the fibre at different radial distances. The peak-to-peak amplitude of the potentials also decreased with increasing radial distance. The sharpest percentual decrease was observed with the amplitude of the first negative phase. A slower decrease was observed with the amplitude of the first positive phase. The amplitude of the terminal negative phase

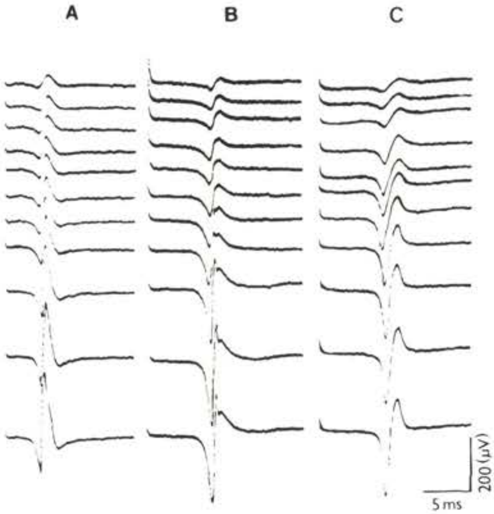

Fig. 2. Changes in the extracellular potentials of an isolated muscle fibre (*Rana ridibunda*) with the radial distance: *A* — at a constant axial distance from both the point of origin of the excitation and the end; *B* — at a constant axial distance over the conical terminal part of the fibre; *C* — at a constant axial distance beyond the end of the fibre. From top to bottom, records at 1000; 900; 800; 700; 600; 500; 400; 300; 200; 100; and 50 μm radial distance.

decreased more slowly. As a result the potential shape showed complex changes with increasing radial distance. Near the membrane potentials composed of two negative maxima were observed. At longer radial distances, the first negative maximum disappeared, the positive maximum shifted forwards as the first negative maximum shifted backwards over time.

Fig. 2*C* shows extracellular potentials recorded from the same fibre at a constant axial distance of 1 mm beyond the end of the fibre and at different radial distances. With increasing radial distance the peak-to-peak amplitude decreased. The amplitude of the positive phase decreased (as expressed in percentual values) more quickly than that of the negative phase.

The radial distance dependent amplitude decrease of the first positive and the negative phase of the potentials recorded over the fibre and its end (*Rana ridibunda*) is shown in Fig. 3*A*.

Fig. 3*B* shows changes in the two amplitude ratio upon increasing the radial distance.

Results obtained with a fibre from *Rana temporaria* are shown in Fig. 4. Almost identical changes in extracellular potentials were observed with increasing radial distance. Similar results were obtained with other muscle fibres of the two species studied.

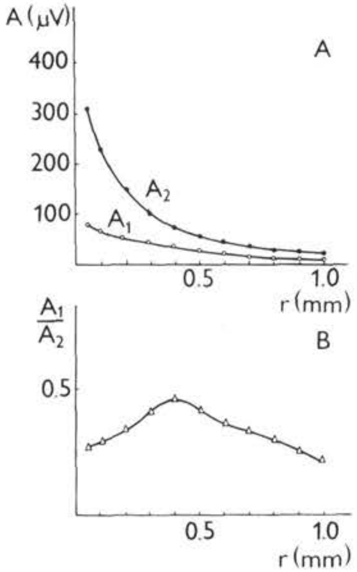

Fig. 3. Relationship between the amplitude (A, μV) of the different phases of the extracellular potentials of frog muscle fibres, and the radial distance (r, mm). A — at a constant axial distance over the fibre; ○ — initial positive phase (A_1), ● — negative phase (A_2); B — ratio $\frac{A_1}{A_2}$.

Fig 4. Changes in the extracellular potential of an isolated muscle fibre with the radial distance. A fibre from *Rana temporaria*. For other symbols see Fig. 2.

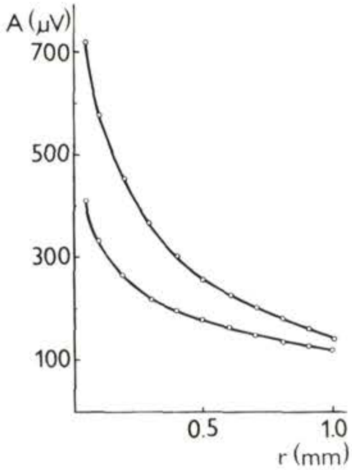

Fig. 5. Relationship between the peak-to-peak amplitude of the extracellular potentials (*A*, μV) (muscle fibre from *Rana ridibunda*) and the radial distance (*r*, mm) (constant axial distance over the fibre); upper curve: at 25 °C; lower curve: at 10 °C.

The amplitude of different phases with increasing radial distance was temperature dependent (Fig. 5). Upon increasing temperature the amplitudes of the different potential phases recorded near the membrane increased considerably. Changes at longer radial distances were smaller. The amplitude decrease of the negative phase with increasing radial distance was slower at a low temperature (10 °C) than that of the first positive phase even near the membrane.

Fig. 6 shows the extracellular potential field of a muscle fibre from *Rana ridibunda* at six different time intervals after the stimulus (increasing in steps of 0.4 ms) at 20 °C. The potential field shape did not change at the first two time intervals, and it shifted axially by approximately 0.8 mm, corresponding to a velocity of excitation spreading of about 2 m/s. As seen from Fig. 6*A* and *B*, the potential field was composed of a central negative area and two lateral positive areas. With increasing radial distances the zero equipotential line separating the negative area from the second positive area was significantly inclining and the negative area broadened.

At the end of the subsequent time intervals (Fig. 6 *C*, *D*, *E*, *F*) the spreading of the extracellular potential fileld was accompanied by changes in the configuration: a) The forefront positive area first became more intensive, shortened and then gradually disappeared; b) The negative area also shortened in space, its intensity first increased and then decreased. However, at the end of the subsequent time intervals the intensity of the negative area increased again (Fig. 6*F*) and decreased

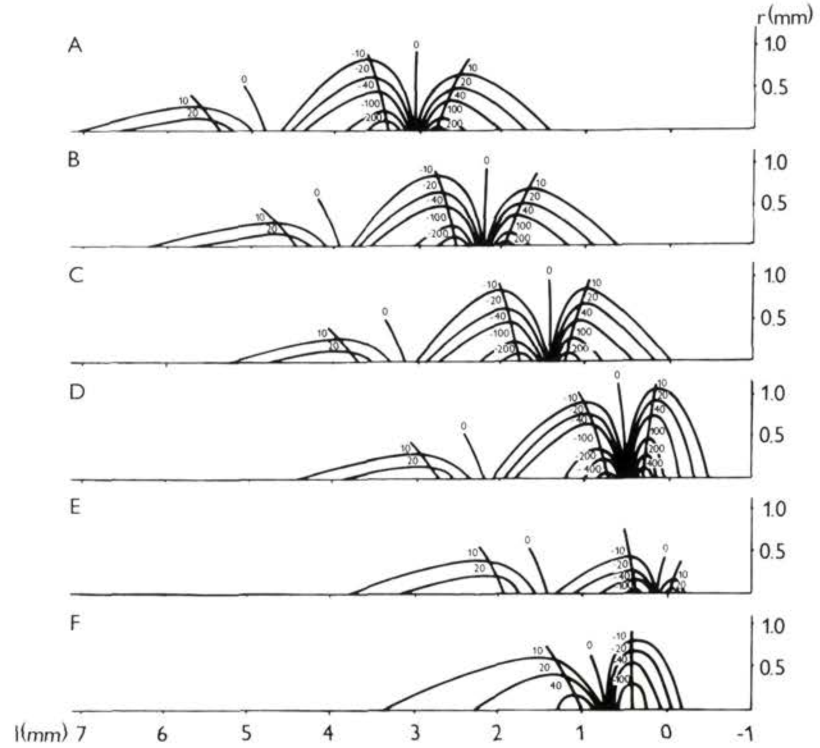

Fig. 6. Extracellular potential field of an active isolated muscle fibre from *Rana ridibunda* (20 °C). Intervals between records *A*, *B*, *C*, *D*, *E* and *F* — 0.4 ms, each. Abscissa — axial distance (l mm), measured to the end point of the fibre originate — radial distance (*r*, mm).

thereafter as the area disappeared (not shown). c) The second positive phase intensity first gradually increased as well and then decreased, became shorter in length and disappeared (not shown). Similar results were obtained at 25 °C. The intensity of the positive area of the field corresponding to the second positive phase of the extracellular potentials was very low at temperatures lower than 20 °C. Only a positive and a negative area were observed at temperatures 15° and 10 °C.

The shape of the extracellular potentials at long radial distances (Fig. 7) was changed. When recorded over the fibre they were composed of an initial positive, a negative, occasionally a second positive, and a terminal positive phase. When recorded beyond the end of the fibre they were composed of an initial positive and a terminal negative phase. The maximum of the positive terminal phase recorded over the fibres coincided in time with the maximum of the terminal negative phase recorded beyond the end of the fibres.

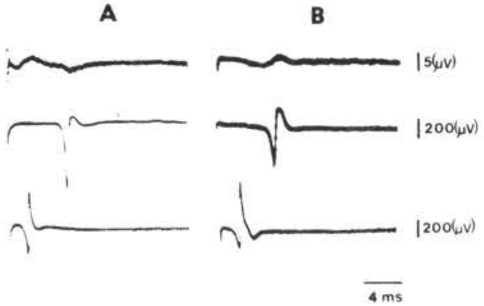

Fig. 7. Extracellular potentials of muscle fibres from *Rana ridibunda* at short and long radial distances. *A* — from top to bottom: radial distance 3 mm, axial distance 2 mm; radial distance 50 μm, axial distance 0 mm; radial distance 50 μm, axial distance 2 mm. *B* — from top to bottom: radial distance 3 mm, axial distance 0 mm; radial distance 50 μm, axial distance 0 mm; radial distance 50 μm, axial distance 2 mm. The axial distance was measured as a distance to the end point of the fibre.

Discussion

The results show that, in accordance with the theoretical predictions (Lorente de Nó 1947; Brooks and Eccles 1947), the extracellular potentials recorded at long distances from the end of the muscle fibres are usually three-phasic and the extracellular potential field consists of a negative area surrounded by two positive areas. This is evident at temperatures higher than 20 °C. At temperatures lower than 20 °C the amplitude of the second positive phase of the extracellular potentials, as well as the intensity of the second positive area of the extracellular potential field were very low. At low temperatures, biphasic potentials and potential field consisting of two areas (a positive and a negative one) were observed as described by Håkansson (1957).

The shape of the extracellular potential changes significantly upon shifting the recording electrode to the very end of the fibre (Gydikov et al. 1981). Over the fibre andat long distance from both the point of origin and the end they were similar to the second space derivative of the intracellular action potential. At points over the very end and beyond the end of the fibre they were similar to the first space derivative of the intracellular action potential. It was shown that over the fibre close to its end, the shape of the extracellular potentials may be considered as a sum of two potentials. One of them is similar to the second space derivative of the action potential, and the other one is a positive-negative potential similar to the first space derivative of the action potential at the very end of the fibre. At points over the terminal part of the fibre the extracellular potentials may show two negative maxima. Based on the investigations of Lorente de Nó (1947), Brooks and Eccles

(1947), Katz and Miledi (1964) the extracellular potentials were expected to have shapes similar to the second space derivative of the intracellular action potential at points over the fibre, as well as similar to the first space derivative at the end beyond the end. In an earlier paper (Gydikov et al. 1981) we described the transition area over the terminal part of the fibres. The present investigations of the extracellular potential field in this area and beyond the end of the muscle fibres supplied new data about the events due to the finite length of the muscle fibres. Now it has become evident that the two negative maxima of the extracellular potentials in the region over the fibres and close to the end (Fig. 2*B* and Fig. 4*B*) are not due to continued negative areas of the potential field. The two negative maxima are determined by time dependent changes in the intensity of the negative area: an increase, followed by a decrease, another increase, and a final decrease with gradual disappearance.

The shape changes of the extracellular potential field at the end of the muscle fibre are probably due: 1) to the transition of the extracellular potentials from a similarity with the second space derivative of the intracellular action potential to a similarity with the first space derivative, and 2) to the effect of the conically narrowed terminal part of the muscle fibre (Schwarzacher 1960; Gydikov et al. 1981). Further experimental and model investigations are required to specify the contributions of these factors.

New models should be developed considering the finite length of the muscle fibres, to explain some other observations reported in the present paper particularly in the transitional area near the end of the fibres:

a) The temperature dependence of the peak-to-peak amplitude decrease and the amplitude decrease of the individual phases of the extracellular potentials with increasing radial distance.

b) The shifts of the individual potential maxima with increasing radial distance. At longer radial distances (3 mm) above the muscle fibre and beyond the end of the fibre extracellular potentials have a shape very similar to that of the extraterritorial potentials of single motor units (Gydikov and Kosarov 1972a, b; Kosarov et al. 1974). The records indirectly suggest that at longer radial distances the extracellular potential field intensity decreases and its shape changes significantly. A terminal positive phase similar to that of the extraterritorial single motor unit potentials (Gydikov and Kosarov 1972a) is observed over the fibre. Obviously, this phase appears when the depolarized area reaches the fibre end.

The shape changes of the extracellular potentials with increasing radial distance were gradual. The significant shape differences between records obtained from near the membrane and at long radial distances (3 mm) were mainly due to different amplitude decay of individual phases with increasing radial distance and to broadening of the phases. Another reason for this difference was the much more pronounced effect of the finite length of the muscle fibre on the extracellular potentials recorded at long radial distances, as is evident from the appearance of a terminal positive phase.

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