J. exp. Biol. 127, 173–189 (1987)
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THE ELECTRICAL CONSTANTS OF THE FIBRES FROM TWO LEG MUSCLES OF THE LOCUST *SCHISTOCERCA GREGARIA**

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Accepted 1 September 1986

SUMMARY

1. The passive electrical properties of the fibres from the M. extensor tibiae and the M. retractor unguis in the hindleg of the locust *Schistocerca gregaria* were investigated using short cable theory. The dependence on various physicochemical parameters was determined.

2. The sarcoplasmic resistivity (R_i) was the same in the extensor and in the retractor muscle. R_i was $\approx$175 Ωcm at 20°C.

3. The specific membrane resistance (R_m) was considerably lower in the retractor muscle ($\approx$5100 Ωcm^2) than in the extensor muscle ($\approx$13 000 Ωcm^2; $[K^+]_o$ = 10 mmol l^{-1}; temperature = 20°C). R_m increased by more than 100 % if the external potassium concentration was lowered from 10 to 5 mmol l^{-1} and it decreased by approximately 75 % if the calcium concentration was lowered from 2 to 0·2 mmol l^{-1}.

4. The specific membrane capacity (C_m) increased with fibre diameter. The different mean values for C_m in the extensor (8·5 μF cm^{-2}) and retractor muscle (6·3 μF cm^{-2}) can be accounted for by the different mean fibre diameters.

5. The temperature coefficients (Q_{10}) of the electrical constants were 0·74 for R_i, 0·48 for R_m, 1·01 for C_m and 1·21 for the resting membrane potential (temperature, 16–27°C).

6. There was close agreement between the membrane time constant (τ_m) derived from the decay of the excitatory junction potential (EJP) and that derived from injection of current pulses. Thus R_m and the length constant (λ) can be derived from the EJP and the fibre diameter if the sarcoplasmic resistivity and the specific membrane capacity are known.

7. The temporospatial dependence of miniature EJPs in a fibre can be predicted satisfactorily from the electrical constants as is demonstrated by an example given in the Appendix.

INTRODUCTION

Locust skeletal muscles are frequently used for physiological and pharmacological studies. Their electrical constants have been investigated by various authors (Usherwood, 1962; Henček, Mandelstam, Uhrik & Zachar, 1968; Washio, 1972;

* Dedicated to the late Graham Hoyle.

Key words: locust, cable constants, muscle, synaptic potential.

Duce & Scott, 1983), yet not in a comprehensive manner. The present study gives a detailed comparative account of the electrical constants of two different leg muscles. The dependence of the constants upon more frequently varying parameters like temperature or pH is also dealt with. In particular the effect of lowering the external potassium concentration $[K^+]_o$ was investigated, since salines with different $[K^+]_o$ have been used in different investigations (e.g. Hoyle, 1953; Walther, Hodgkiss & Hensler, 1982) and since $[K^+]_o$ in the haemolymph may vary with food supply (Hoyle, 1954).

The membrane constants determine the temporospatial characteristics of synaptic and miniature synaptic potentials within a fibre (e.g. Jack, Noble & Tsien, 1975). We demonstrate that the decay of an excitatory junction potential (EJP) offers a convenient way of estimating the membrane resistance and the length constant.

Knowing the electrical constants can be of considerable help for analysis of the amplitudes of miniature EJPs (e.g. Walther *et al.* 1982), a task which is more difficult to achieve in these polyterminally innervated fibres (e.g. Hoyle, 1955) than in fibres with a single 'endplate' region like those of mammals. In the Appendix it is demonstrated that the amplitude and the time to peak of a miniature synaptic potential can be predicted with reasonable accuracy from the measured membrane constants.

MATERIALS AND METHODS

Preparations

The jumping muscle (M. extensor tibiae; Hoyle, 1978) and the retractor of the claws (M. retractor unguis; Usherwood & Machili, 1968; Walther, 1980) from the hindleg of female locusts, *Schistocerca gregaria*, were used. For a better view of the fibres of the extensor muscle, the cuticle on the dorsal side was removed and the muscle spread out laterally. Muscles were mounted – stretched to their maximal *in situ* length – in a Perspex chamber which was perfused at a rate of approx. $1{\cdot}5\ \mathrm{ml\ min^{-1}}$. The experiments with three intracellular microelectrodes were carried out on muscle fibres innervated by a single excitatory, fast-type motoneurone. The fibres used were in the ventralmost fibre layer of the sixth to the twelfth bundles (counted from the distal end) of the jumping muscle and in the distally inserting ('white') bundle of the claw retractor, respectively. Additional experiments with two electrodes were performed in the extensor muscle mainly in the more distal fibres receiving innervation from both the fast (FETi) and the slow excitatory motoneurone (SETi).

The apparent diameter, d^*, of the impaled fibre was measured by means of an ocular micrometer. In histological cross-sections the muscle fibres are polygonal, often wedge-shaped (e.g. Hoyle, 1978; Walther, 1980, fig. 5; Walther, 1981, fig. 1). Therefore, in some of the experiments the preparations were fixed with 2·5% glutaraldehyde after the electrophysiological measurements had been finished. The muscle fibre bundles used were embedded in Spurr's resin and cut transversely in three different regions. From the sections, the circumference and the cross-sectional

area of an investigated fibre (which could be identified from its location within the bundle) were measured. Changes introduced by the histological procedure were determined from the width of a retractor unguis muscle, fixed in the same experiment, for making appropriate corrections. Diameters, d_c and d_a, were calculated for cylinders having either the same circumference or the same area, respectively, as measured for the fibre. The apparent diameter d^* was found to differ only by 1–9% from d_a. Therefore d^* was taken as an estimate of d_a in those preparations which were not processed histologically.

d_c was 6–16% (average 12%) larger than d^*. Thus $d^* \times 1{\cdot}12$ was used as an estimate of d_c. There was no significant effect on the muscle fibre diameter when the potassium concentration in the saline was changed from 5 to 10 mmol l^{-1} or from 10 to 5 mmol l^{-1}. This was specifically checked in three retractor unguis muscles over periods of about 1 h for each condition.

Solutions

Unless stated otherwise the saline consisted of (in mmol l^{-1}): NaCl, 150; KCl, 5 or 10; $CaCl_2$, 2; $MgCl_2$, 2; sucrose, 90; buffered with 2 mmol l^{-1} Tris maleate at pH 6·8. The preparations were allowed to equilibrate in the saline for at least 1 h before electrophysiological measurements were started. Usually, after changing a solution, measurements were resumed only after an equilibration time of at least 30 min. With changes of $[K^+]_o$ the equilibration times were extended up to 90 min.

When changing $[K^+]_o$ the $[Cl^-]_o$ was changed by the same amount, i.e. $[K^+]_o \times [Cl^-]_o$ was not held constant. Preparations which were kept for longer periods in 5 mmol l^{-1} K^+ saline often 'misbehaved' by twitching occasionally. Therefore it was advisable to perform the dissection in 10 mmol l^{-1} K^+ saline and to introduce the 5 mmol l^{-1} K^+ saline as late as possible.

Whereas in cockroach muscle the presence of bicarbonate in the saline has been reported to be necessary for stable resting potentials (Wareham, Duncan & Bowler, 1973), in both the retractor and the extensor preparation the resting potentials and input resistances were quite stable in HCO_3^--free saline for at least 12 h.

Electrophysiological techniques

Microelectrodes were filled with 3 mol l^{-1} KCl and had resistances around 20 MΩ (15–30 MΩ). In the shorter fibres (≤4·5 mm; M. extensor tibiae) the current electrode was inserted near the middle, whereas in longer fibres (approx. 10 mm; M. retractor unguis) it was placed about 1–2 mm from the middle. In the extensor fibres the voltage was recorded both from the middle of the fibre, i.e. approx. 50 μm away from the current electrode, and near its end. In the retractor fibres the two voltage electrodes were separated by approx. 200 μm and 1–2 mm from the current electrode, respectively. After impalement of a fibre the electrodes were allowed to seal in for at least 30 min before measurements were started.

Current was monitored *via* a virtual ground circuit. The amplitudes of the rectangular current pulses were adjusted to give steady-state hyperpolarizations

of 2·5–5 mV in 10 mmol l^{-1} K^+ saline and 4–10 mV in 5 mmol l^{-1} K^+ saline, respectively (measured in the mid-regions of the fibre). Electrotonic potentials were digitized and averaged ($N = 20$–60) by means of a Krenz TRC 4010 transient recorder linked to a Hewlett Packard 9920 S desktop computer.

Symbols and definitions

The abbreviations of Hodgkin & Nakajima (1972*a*) and Jack *et al.* (1975) are used.

- x distance along the fibre from the current electrode (cm);
- l_1, l_2 distance between current electrode and each end of the fibre (cm);
- d fibre diameter (cm);
- I applied current (nA);
- V_0 electrotonic potential produced at $x = 0$ (mV);
- R_{in} input resistance of fibre ($= V_0/I$) ($\Omega \times 10^6$);
- V_1 potential recorded near the current electrode (mV);
- V_2 potential recorded far from the current electrode (mV);
- R_i specific resistance of interior of fibre (Ωcm);
- r_i internal resistance per unit length of fibre; ($= 4R_i/\pi d^2$) (MΩ cm^{-1});
- R_m specific membrane resistance (kΩcm^2);
- λ length constant of fibre ($= \sqrt{R_m d/4R_i}$) (cm);
- τ_m membrane time constant (ms);
- C_m capacitance, i.e. specific low-frequency membrane capacity referred to the surface ($\mu F\, cm^{-2}$);
- RP resting membrane potential (mV).

Mean values are given ± standard error of mean.

Determination of membrane constants

The short cable approach of Weidmann (1952) and Hodgkin & Nakajima (1972*a*) was followed. The length constant λ was calculated from the relationship:

$$\frac{V_1}{V_2} = \frac{\cosh(l_2 - x_1)/\lambda}{\cosh(l_2 - x_2)/\lambda}, \tag{1}$$

(derived from equation 8 of Hodgkin & Nakajima, 1972*a*). l_2 is the distance between the current electrode and that end of the fibre which was facing the recording electrodes. The distances of the recording electrodes from the current electrode are given by x_1 and x_2, respectively (see fig. 1 of Hodgkin & Nakajima, 1972*a*). A program loop was run which computes the right side of equation 1 for increasing values of λ until it equals the left side. Using this value of λ, V_0 was calculated. r_i was determined from the input resistance R_{in}:

$$R_{in} = \frac{V_0}{I} = r_i \times \frac{\lambda}{\tanh(l_1/\lambda) + \tanh(l_2/\lambda)}, \tag{2}$$

(according to equation 8 of Hodgkin & Nakajima, 1972*a*). From λ and r_i, R_i and R_m were obtained. In a similar manner one can derive λ and R_m from measurements with two electrodes, making use of the R_i value obtained from the experiments with three electrodes. The membrane time constant τ_m was derived from the time course of the voltage change when current was applied. Using fig. 3 of Stefani & Steinbach (1969), for any given λ the percentage change (DVt) of the voltage which is reached after time $t = \tau_m$ was determined. For extensor fibres an average DVt of approx. 67 % and for retractor fibres an average DVt of approx. 83 % were found. Since the latter corresponds, in practice, to the case of an 'infinite' cable for retractor fibres, τ_m was also derived from a plot of the time to 50 % voltage change against x, using both the slope and the intercept (see Hodgkin & Rushton, 1946). The average of the three determinations was then taken.

Insertion of an electrode causes a drop in the resting membrane potential, which is followed by partial recovery. With three electrodes in one fibre a depolarization of a few millivolts (compared to the resting potential measured within the first seconds after impalement of the first electrode) persisted, e.g. $4{\cdot}2 \pm 0{\cdot}4$ mV ($N = 12$) in the extensor muscle at 10 mmol l^{-1} $[K^+]_o$. This indicates leakage through which injected current is partly shunted. Hence R_{in} and the constants derived from R_{in} will be underestimated unless some correction is made. Since the V/I relationship is linear (see Fig. 1A), an estimate of the leakage conductance (g) can be obtained from the equation:

$$g = \Delta RP/[(RP_0 - \Delta RP)R_{in}], \tag{3}$$

where ΔRP is the difference between the initial potential RP_0 and the final one, i.e. after the electrodes have sealed in. g was approx. 0·09 μmho (from 0·04–0·15 μmho), i.e. of the same order of magnitude as previously reported for frog (Hodgkin & Nakajima, 1972*a*) and stick insect muscle (Ashcroft, 1980). The effective current I_e, i.e. that current which leaves the fibre through the membrane, was obtained from:

$$I_e = I \times (1 - \Delta RP/RP_0). \tag{4}$$

I_e was used for the calculation of the constants in Tables 1–3. The effect of the correction for leakage can be appreciated by comparing R_m with the uncorrected value, R_m^*, which is also given.

Occasionally the electrotonic potential change showed an 'overshoot' both on the make and on the break of the current pulse. This was mainly found in fibres with low resting potentials. Records from such fibres were discarded.

RESULTS

Current/voltage relationships

In agreement with previous investigations (e.g. Lea & Usherwood, 1973*a*,*b*; Clements & May, 1977) linear current/voltage relationships were obtained with

hyperpolarizing currents in the tested range of 20 mV. This was true for 10 and 5 mmol l^{-1} $[K^+]_o$, both in the extensor (Fig. 1A) and in the retractor preparation. Fibres with low resting potentials (−50 to −55 mV) at 10 mmol l^{-1} $[K^+]_o$ showed delayed rectification within a range of 2–5 mV of hyperpolarization in accord with previously reported examples (e.g. Lea & Usherwood, 1973*b*).

Electrical constants

Tables 1–3 summarize the membrane constants for the retractor unguis muscle, at 10 mmol l^{-1} $[K^+]_o$, and for the extensor tibiae muscle at 5 and 10 mmol l^{-1} $[K^+]_o$, obtained from the analysis of electrotonic potential changes in a negative direction (Fig. 1B).

Decreasing $[K^+]_o$ from 10 to 5 mmol l^{-1} led to an approximately two-fold increase in R_m. The resting membrane potential (figures from Tables 1 and 2 corrected for a temperature of 20°C) rose to 76·4 mV, i.e. close to the value of 76·7 mV which is predicted from 59·2 mV at 10 mmol l^{-1} $[K^+]_o$ by the Nernst equation (see Leech, 1986, for comparable results in the retractor unguis preparation). The effects took more than 90 min to develop fully after changing from 10 to 5 mmol l^{-1} $[K^+]_o$ (cf. Hodgkin & Horowicz, 1959; Usherwood, 1968). While RP was almost the same in both kinds of muscle, R_m was about 2·5 times higher in the extensor tibiae than in the retractor unguis muscle. The different values for C_m in Tables 1 and 3 can be explained by the difference in average fibre diameter of the two muscles (Hodgkin & Nakajima, 1972*a*; see also below and Fig. 2).

The apparent specific internal resistance, R_i', was derived from the cable equations using d_c, i.e. the diameter of a cylinder having the same circumference as the fibre (see Materials and Methods). The cable approach treats the fibre as if it had been artificially blown up with a non-conducting medium (thus diluting the sarcoplasm) until it became a perfect cylinder of cross-sectional area $d_c^2 \times \pi/4$. R_i was derived from R_i', the cross-sectional area A and $d_c^2 \times \pi/4$ using the equation $R_i = R_i' \times 4A/(d_c^2 \times \pi)$ or, if the fibre's cross-sectional area had not been determined histologically, using the equation $R_i = R_i' \times d_a^2/d_c^2$ (see Materials and Methods; for an equivalent approach in crayfish, see Lnenicka & Mellon, 1983). The R_i figures for the extensor and the retractor muscles at 10 mmol l^{-1} $[K^+]_o$ (Tables 1, 3) do not

Fig. 1. Current/voltage relationship (A) and electrotonic potential change (B) obtained with injection of current into extensor tibiae muscle fibres. (A) After a series of measurements in 10 mmol l^{-1} $[K^+]_o$ (●: RP = −59·2 mV) $[K^+]_o$ was reduced to 5 mmol l^{-1}. Following an equilibration period of 90 min a second series was measured (★: RP = −69·4 mV) and $[K^+]_o$ was subsequently raised again to 10 mmol l^{-1}. 80 min later the third series (○: RP = −55·8 mV) was measured. The values were obtained from averaged signals (N = 5–10); temperature = 22·4°C for filled circles and asterisks and 22·7°C for open circles. Fibre diameter, 115 μm. (B) Response to hyperpolarizing current injected into the middle of a fibre. Upper record: 50 μm from the current electrode; middle record: 100 μm from the fibre end. Fibre length, 3850 μm; diameter, 125 μm; current, 5 nA; resting potential, −55 mV; temperature, 21·9°C; $[K^+]_o$, 10 mmol l^{-1}. Digitized, averaged signals (N = 50).

differ significantly from each other ($P > 0{\cdot}1$). The mean from the pooled data of both muscles at 10 mmol l^{-1} $[K^+]_o$ and 21·5 °C is 170·0+8·4 Ωcm ($N = 15$).

Quite similar R_m values (mean: 11 430 ± 1050 Ωcm^2; $N = 9$) were obtained from measurements with two electrodes at $[K^+]_o = 10$ mmol l^{-1} and 20·7 ± 0·5 °C in the extensor muscle (see Materials and Methods; for the C_m values see Fig. 2).

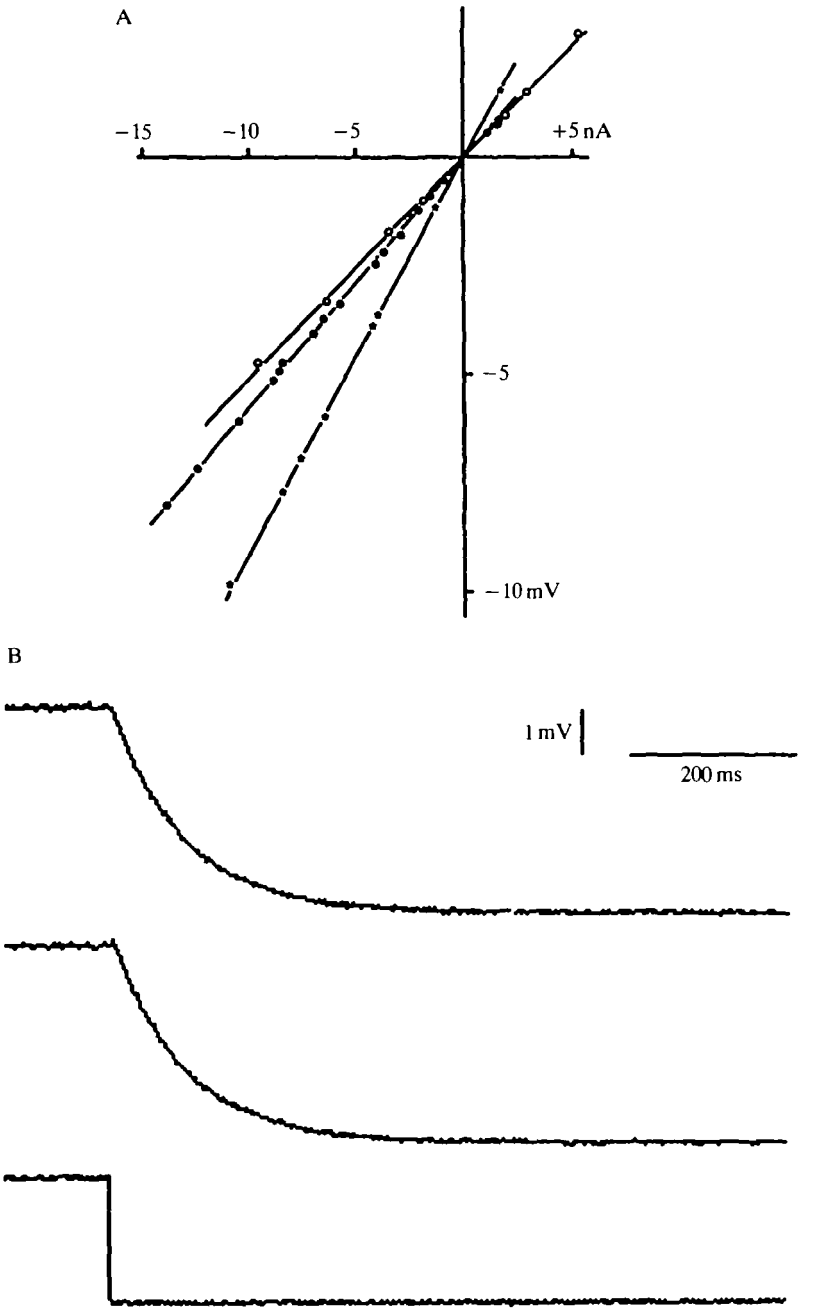

Fig. 1

Table 1. *Electrical constants for the extensor tibiae muscle in 10 mmol l^{-1} potassium saline*

RP (mV)	RP* (mV)	L (mm)	λ (mm)	d (μm)	R_{in} (MΩ)	R_i (Ωcm)	R_i' (Ωcm)	R_m (Ωcm^2)	R_m* (Ωcm^2)	τ_m (ms)	C_m ($\mu F\ cm^{-2}$)	r_i (M$\Omega\ cm^{-1}$)	Temperature (°C)
61·1†	56·9	4·03	4·37	122	0·76	168·7‡	211·5‡	12 170	11 230	94·2	8·46	1·49	21·5
±0·8	±0·7	±0·14	±0·20	±3·1	±0·07	±8·3	±10·4	±1040	±980	±8·8	±0·33	±0·08	±0·4

$N = 12$.
* Values not corrected for leakage.
† $N = 30$ (i.e. values from further fibres are included).
‡ $N = 11$ (i.e. the diameter of one fibre had not been measured); for R_i' see text.
For abbreviations see Materials and Methods.

Table 2. *Electrical constants for the extensor tibiae muscle in 5 mmol l^{-1} potassium saline*

RP (mV)	RP* (mV)	L (mm)	λ (mm)	d (μm)	R_{in} (MΩ)	R_i (Ωcm)	R_i' (Ωcm)	R_m (Ωcm^2)	R_m* (Ωcm^2)	τ_m (ms)	C_m ($\mu F\ cm^{-2}$)	r_i (M$\Omega\ cm^{-1}$)	Temperature (°C)
79·4†	69·9	3·94	5·86	120	1·41	173·3‡	217·4‡	22 800	21 160	181·2	8·70	1·69	21·8
±0·5	±2·0	±0·13	±0·44	±4·5	±0·16	±15·1	±18·7	±3100	±2760	±26·7	±0·77	±0·26	±0·4

$N = 6$.
* Values not corrected for leakage.
† $N = 35$ (i.e. values from further fibres are included).
‡ $N = 5$ (i.e. the diameter of one fibre had not been measured); for R_i' see text.
For abbreviations see Materials and Methods.

Table 3. *Electrical constants for the retractor unguis muscle in 10 mmol l^{-1} potassium saline*

RP (mV)	RP* (mV)	L (mm)	λ (mm)	d (μm)	R_{in} (MΩ)	R_i (Ωcm)	R_i' (Ωcm)	R_m (Ωcm^2)	R_m* (Ωcm^2)	τ_m (ms)	C_m ($\mu F\ cm^{-2}$)	r_i (M$\Omega\ cm^{-1}$)	Temperature (°C)
60·9†	57·9	9·52	2·01	65	0·51	178·9‡	221·8‡	4800	4680	31·9	6·3	5·60	20·65
±1·0	±0·5	±0·33	±0·11	±8	±0·06	±21·5	±22·1	±412	±390	±4·3	±0·6	±0·96	±0·36

$N = 5$.
* Values not corrected for leakage.
† $N = 7$ (i.e. values from further fibres are included).
‡ $N = 4$ (i.e. the diameter of one fibre had not been measured); for R_i' see text.
For abbreviations see Materials and Methods.

Effects of temperature, pH, HCO_3^- and Ca^{2+}

The influence of these parameters was studied in a restricted number of preparations and gave results similar to those reported previously for other insect muscles.

Temperature

A decrease (increase) in temperature results in an increase (decrease) of λ, τ_m, R_{in}, R_i and R_m while RP decreases (increases) and C_m is hardly affected (see also Ashcroft, 1980; Anwyl, 1977).

Four experiments (15·5–27 °C; two in the extensor, two in the retractor muscle) yielded the following Q_{10} values: $0{\cdot}74 \pm 0{\cdot}04\,\Omega$cm for R_i; $0{\cdot}48 \pm 0{\cdot}05\,\Omega cm^2$ for R_m; $1{\cdot}01 \pm 0{\cdot}03\,\mu F\,cm^{-2}$ for C_m; and $1{\cdot}21 \pm 0{\cdot}02$ mV for RP. Correction of the R_m values given in Tables 1–3 for 20 °C gives 13 050 and 27 250 Ωcm^2 for the extensor muscle in 10 and 5 mmol l^{-1} $[K^+]_o$, respectively, and 5140 Ωcm^2 for the retractor unguis muscle in 10 mmol l^{-1} $[K^+]_o$.

pH

In four experiments (extensor tibiae muscle; $[K^+]_o = 5$ mmol l^{-1}) increasing (decreasing) the pH caused an increase (decrease) in RP and a decrease (increase) in R_{in}, indicating changes in membrane conductance. Taking RP and R_{in} at pH 6·8 as 100 %, RP and R_{in} at pH 6·2 amounted to approximately 90 % and 136 %, respectively, and at pH 7·5 to about 106 % and 76 %, respectively. The pH effects were quickly and fully reversible. Washio (1971) gives similar results for the adductor coxae muscle.

HCO_3^-

Wareham *et al.* (1973) have reported a HCO_3^--sensitive, hyperpolarizing component of the resting membrane potential for cockroach muscle which was tentatively attributed to an electrogenic pump. To find out if HCO_3^- has a similar effect in the locust, six experiments in three extensor muscles were carried out, for which a pH of 7·5 was chosen since adding bicarbonate (10 mmol l^{-1}) at pH 6·8 led to a gradual alkalinization. Adding or withdrawing 10 mmol l^{-1} bicarbonate did not lead to a significant change of RP (see also Leech, 1986) or input resistance.

Ca^{2+}

For the investigation of neurally evoked excitatory junction potentials (EJPs) it is often necessary to reduce the extracellular $[Ca^{2+}]$ to prevent the muscle from twitching. The reduction of $[Ca^{2+}]_o$ may affect not only transmitter release but also the membrane constants. Therefore in three preparations (extensor tibiae) the membrane constants were determined both in normal and low $[Ca^{2+}]_o$ (Table 4). While R_m was drastically reduced in low $[Ca^{2+}]_o$ there was only a slight if any decrease in RP. This was confirmed in an additional experiment (extensor muscle) where the resting membrane potential in 0·2 mmol l^{-1} $[Ca^{2+}]_o$ and 3·8 mmol l^{-1} $[Mg^{2+}]_o$, measured immediately after impalement of the fibres, was $64{\cdot}5 \pm 0{\cdot}4$ mV

Table 4. *Effect of reducing $[Ca^{2+}]_o$ on electrical constants*

$[Ca^{2+}]_o$ (mmol l^{-1})	$[Mg^{2+}]_o$ (mmol l^{-1})	Temperature (°C)	RP (mV)	λ (mm)	R_i (Ωcm)	R_m (Ωcm^2)	τ_m (ms)	C_m ($\mu F\,cm^{-2}$)
2·0	2·0	22·4 ±0·5	54·7 ±0·7	5·00 ±0·17	180·4 ±17·6	13 180 ±2260	109·0 ±19·7	8·9 ±0·5
0·2	3·8	22·0 ±0·4	54·3 ±0·7	3·04 ±0·19	181·1 ±28·5	4620 ±670	38·0 ±5·6	8·9 ±0·8

Extensor tibiae muscle; $N = 3$; 10 mmol l^{-1} potassium saline.
For abbreviations see Materials and Methods.

($N = 23$) compared to 65·6 ± 0·6 mV ($N = 19$) in normal saline (at 23 °C). The effect of lowering $[Ca^{2+}]_o$ is similar to that observed in the adductor coxae muscle, albeit with Ca^{2+} concentrations one order of magnitude higher than used here (Washio, 1972).

Dependence of specific capacity on fibre diameter

Previously a linear dependence between the specific low-frequency capacity (capacitance) and the fibre diameter has been reported (e.g. Hodgkin & Nakajima, 1972*a*; Lnenicka & Mellon, 1983). This is commonly attributed to the contribution of the membranes extending into the interior of the muscle – in particular the transverse tubular system – which increases with the square of the fibre diameter.

The relationship between the C_m values from the extensor and the retractor muscles, when plotted against fibre diameter, covering a range from 50 to 140 μm (Fig. 2), shows a slope similar to that found in frog and in crayfish. The estimate of the specific capacity (3·4 $\mu F\,cm^{-2}$) for the surface membrane, obtained from the y-intercept of the fitted straight line, cannot be very accurate since it is based on a small number of values. It is certainly an overestimate, since it has been determined without considering the deviation of the fibre shape from a cylinder which causes an excess of surface membrane of roughly 12 % (see Materials and Methods). The contribution of sarcolemmal infoldings has also to be added (Huddart & Oates, 1970; Henček *et al.* 1968; Cochrane, Elder & Usherwood, 1972) and this increases the perimeter by at least 5 % in the fibres used here (unpublished observations). The zero diameter capacitance falls within the range reported for crustacean muscles (1·5–3·9 $\mu F\,cm^{-2}$; e.g. Falk & Fatt, 1964; Lnenicka & Mellon, 1983) and may not be very different from figures around 1 $\mu F\,cm^{-2}$ obtained in vertebrate skeletal muscles (e.g. Hodgkin & Nakajima, 1972*a*; Dulhunty & Franzini-Armstrong, 1977).

Estimation of cable constants from synaptic potentials

A simple way of estimating the cable constants would be of interest in various physiological and pharmacological investigations. If the time constant and the approximate fibre diameter can be determined then approximate values of R_m, λ and R_{in} can be calculated, provided the known values for C_m and R_i apply to the particular experimental conditions. τ_m can be derived from the falling phase of the

neurally evoked EJP: the current generating an EJP enters the polyterminally innervated muscle fibres of arthropods *via* many points. Thus the membrane is nearly uniformly depolarized and the voltage will decrease exponentially with the time constant of the membrane once the synaptic current has terminated. A precondition is that the EJP is not so large that it leads to an active, non-synaptic response.

In Fig. 3 the exponential decays are illustrated for EJPs evoked by stimulation of the slow excitor axon. The length constant of 4·2 mm and the membrane resistivity of 14 400 Ωcm^2 (for 21·5 °C; 10 mmol l^{-1} $[K^+]_o$; $R_i' = 215\ \Omega cm$) derived from these EJPs agree well with the figures of Table 1. In three experiments $[Ca^{2+}]_o$ and $[Mg^{2+}]_o$ were adjusted to reduce the amplitude of EJPs (evoked by stimulation of

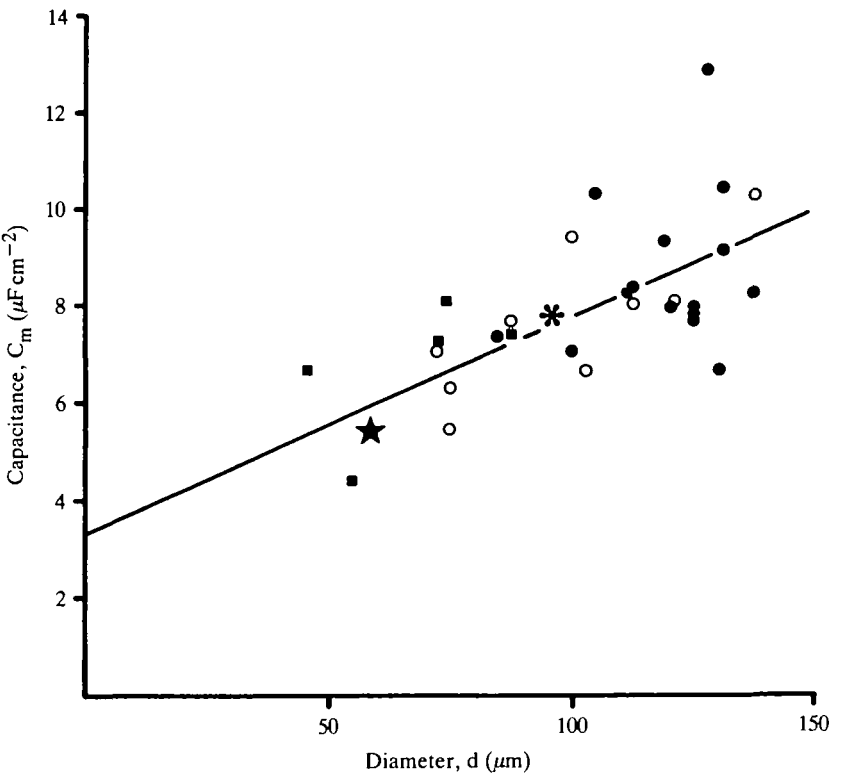

Fig. 2. Plot of capacitance (C_m) *versus* fibre diameter (d). ■, M. retractor unguis; ●, ○, M. extensor tibiae. Squares and filled circles are data obtained with three electrodes (see Tables 1–3); open circles, data from measurements with two electrodes (see text) using R_i obtained from the three-electrode measurements, i.e. 170 Ωcm at 21·5 °C. For the adductor coxae (★: Washio, 1972) and flexor tibiae muscles (✱: Henček, Mandelstam, Uhrik & Zachar, 1968) the values given by the authors have been corrected for R_i according to the figure given in the present study. Using all values from the extensor muscle, the relationship between d and C_m is best fitted by the linear equation $C_m = 0{\cdot}044d \pm 3{\cdot}4$. The diameters were determined optically during the experiments or from measurements of cross-sectional area (see Materials and Methods). Temperature, 20–23 °C.

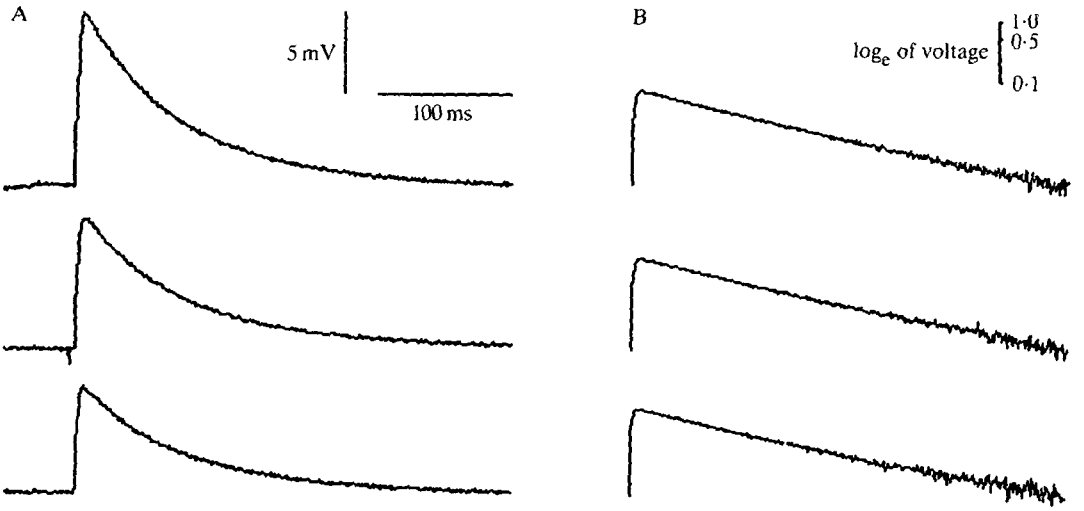

Fig. 3. Excitatory junction potentials in linear (A) and semilogarithmic presentation (B) for the extensor tibiae muscle. The slow excitor axon was stimulated. Nerve branches to fibres which otherwise would have twitched had been chopped. All records from one fibre. Average time constant $\tau_m = 79 \pm 0{\cdot}5$ ms; RP = 55 mV. Normal saline; $[K^+]_o$ = 10 mmol l^{-1}, 24·3°C. Fibre diameter, 94 μm. $R_m = 14\,400\ \Omega cm^2$ was derived from τ_m using $C_m = 7{\cdot}5\ \mu F\ cm^{-2}$ (see Fig. 2) and correcting for temperature (21·5°C) and shunt conductance around the electrode (0·04 μmho).

the fast excitatory motor axon) to 5–12 mV. The membrane time constant was determined both from the response to injected direct current (τ_{dc}) and from the decay of the EJP (τ_{EJP}). On average, τ_{EJP} was 98 ± 6 % of τ_{dc} ($N = 3$).

Since the various muscles do not obviously differ with regard to capacitance (see Fig. 2) it seems reasonable when estimating C_m to use the relationship $C_m = 0{\cdot}046d + 3{\cdot}2$, which is obtained if all the points in Fig. 2 are used. Frequently both in the initial and in the late phase of the logarithmically plotted decay of the EJP a slight upward deviation from linearity is seen. The reason for the initial deviation (up to 50 ms after the peak of the EJP) has not been investigated. As for the late deviations, a likely explanation is that they are due to small, ill-recognized miniature EJPs, the influence of which – in a logarithmic plot – increases strongly as the EJP approaches zero.

DISCUSSION

At 20°C the mean sarcoplasmic resistivity in 10 mmol l^{-1} $[K^+]_o$ is 179 Ωcm, which agrees well with the recently reported figures of 171 Ωcm in stick insect (in 20 mmol l^{-1} $[K^+]_o$; Ashcroft, 1980) and of 167 Ωcm in crayfish (in 5·4 mmol l^{-1} $[K^+]_o$; Lnenicka & Mellon, 1983). The individual values in the present study ranged from 126 to 227 Ωcm. Presumably this rather large variability can largely be accounted for by inaccurate determinations of fibre diameters. In frog muscle, according to Schneider (1970), a 5 mmol l^{-1} increase in $[K^+]_o$ results in a reduction of R_i by about 10 % and this relationship might also hold for insect muscles

(Ashcroft, 1980). The values determined in this study point in this direction, yet the differences were not statistically significant. A mean R_i of 176 Ωcm is obtained from all 20 measurements if those obtained in 5 mmol l^{-1} $[K^+]_o$ are corrected as suggested, while without such a correction R_i would be 180 Ωcm (at 20°C).

In agreement with studies on frog, crayfish and other insect muscles (e.g. Hodgkin & Nakajima, 1972*a*; Ashcroft, 1980; Lnenicka & Mellon, 1983) the range for R_m values is rather large whereas C_m values vary considerably less in fibres of approximately equal diameters. Furthermore, the dependency on fibre diameter, which has to be expected on account of the transverse tubular system (Hodgkin & Nakajima, 1972*b*) could clearly be shown for C_m (Fig. 2) but not for R_m. The large variation of the R_m values may have masked such a dependency (shown, for example, in frog; Hodgkin & Nakajima, 1972*a*).

For comparative reasons C_m and R_m values previously given for two other locust leg muscles (Washio, 1972; Henček *et al.* 1968) were recalculated for the R_i values from this study (see the legend to Fig. 2). While R_m in the extensor tibiae muscle is much higher than in the retractor unguis (4800 Ωcm^2), adductor coxae (3480 Ωcm^2) and flexor tibiae (2970 Ωcm^2) muscles, the C_m values of all four muscles agree reasonably within the C_m–diameter relationship (Fig. 2). Thus the large difference in R_m found here can hardly be accounted for by different amounts of membrane infolding (see Ashcroft, 1980), or the values for C_m should differ more. The high R_m value in the extensor tibiae muscle, therefore, most probably results from a particularly high resistance of the unit area of membrane. From these differences in R_m it should be noted that even within the same species the findings from one muscle cannot be generalized.

The strong effect of $[K^+]_o$ on R_m may be explained by a dependence of P_K on potential and on external potassium concentration, a dependence of G_K (for a given P_K) on potential (Hagiwara, 1983; Ruppersberg & Rüdel, 1985) and on a change of the internal Cl^- concentration as expected from changing $[K^+]_o \times [Cl^-]_o$ (e.g. Hodgkin & Horowicz, 1959).

R_m also markedly depends on $[Ca^{2+}]_o$. It could be argued that the decrease of R_m on reduction of $[Ca^{2+}]_o$ may result from an increased leak around the electrode tip. This is, however, unlikely since the measured membrane potential which should be affected by this leak was about the same in normal and reduced $[Ca^{2+}]_o$. The effect on R_m may not be specifically related to $[Ca^{2+}]_o$ since, for example, a reduction of $[Mg^{2+}]_o$ from 3·8 to 2·0 mmol l^{-1} in the presence of 0·2 mmol l^{-1} $[Ca^{2+}]_o$ led to a further reduction of R_m (from 6850 to 4850 Ωcm^2).

The C_m values reported in this study, corrected for the diameter, are similar to those reported previously for frog (e.g. Hodgkin & Nakajima, 1972*a*,*b*) and crayfish muscle (Lnenicka & Mellon, 1983). However, the C_m values ($\mu F\ cm^{-2}$) of 12·2 for the stick insect (Ashcroft, 1980), 7·1 for *Drosophila* larva (Jan & Jan, 1976) and 21·7 for a moth larva (Deitmer, 1977) are higher than those reported here if the dependence on diameter is assumed to be similar to that found for the locust (Fig. 2). Omission of the corrections for R_i and R_i' would lead to an increase of the C_m values calculated in this study by only 20 % and thus cannot account for the discrepancies.

One possible reason for the differences in membrane capacitance may be that the muscles investigated in moth, stick insect and fruitfly were all from the body wall and may differ in some respects from leg muscles.

APPENDIX

PROPAGATION OF A SYNAPTIC POTENTIAL MODELLED BY APPLICATION OF LINEAR CABLE THEORY

BY C. WALTHER

The passive propagation of voltage transients like (miniature) excitatory junction potentials [(m)EJPs] in a fibre depends upon the fibre's space constant, λ, and time constant, τ_m. Knowing these one can try to reconstruct the membrane response to a synaptic current (Jack & Redman, 1971; Gage & McBurney, 1973; Jack *et al.* 1975) by applying linear cable theory (Hodgkin & Rushton, 1946). From table 2 of Eisenberg & Johnson (1970) it can be deduced that, with fibre diameters like those of the insect preparations used here, the deviations from one-dimensional cable theory should not be important at distances greater than approx. 5 μm from an active synapse. The linear cable approach implies, however, that the membrane can be described electrically by the simple leaky capacitor model. This is not entirely adequate for the muscle membrane because of the transverse tubular system (see Jack *et al.* 1975). Nevertheless, the propagation of the endplate potential in frog muscle fibres has been described with reasonable success by this theory (Fatt & Katz, 1951; see also Jack *et al.* 1975). No comparable investigation seems to exist for an invertebrate muscle. One experiment was therefore devoted to this point.

The principle of this investigation was to inject current pulses mimicking the time course of a synaptic miniature current (e.g. see Usherwood & Machili, 1968). This was done in a retractor unguis fibre the cable constants of which had been determined by injection of rectangular current pulses (Fig. 4, upper right inset). Using these cable parameters, a computer program was run which simulated the synaptic miniature current by superposition of several rectangular pulses (see lower right inset of Fig. 4). The time to peak and the amplitude of the artificial miniature EPP were reconstructed by this method for various recording positions. The dashed lines in Fig. 4 and its left inset show the result.

The previous approaches assumed either that the synaptic charge transfer was instantaneous (Fatt & Katz, 1951) or that it took place as a rectangular current pulse of 2·5 ms (Jack *et al.* 1975). The present approach is thought to be more realistic. To avoid the complications of the short cable situation the program used is based on the equation for an 'infinite' cable. The figures for the cable constants derived by means of the 'infinite cable' approach were found to deviate, in the comparatively long retractor muscle, from the true values by 1–5%. In the present preparation λ (1·74 mm) should have been overestimated by less than 2%. Furthermore, a short cable behaves for a short period like an infinite cable (Jack *et al.* 1975).

The calculated amplitudes were slightly large, probably indicating that, due to capacitive loss, a somewhat smaller charge had been injected into the muscle fibre than had been measured by the virtual ground circuit. Fig. 4 shows quite clearly that

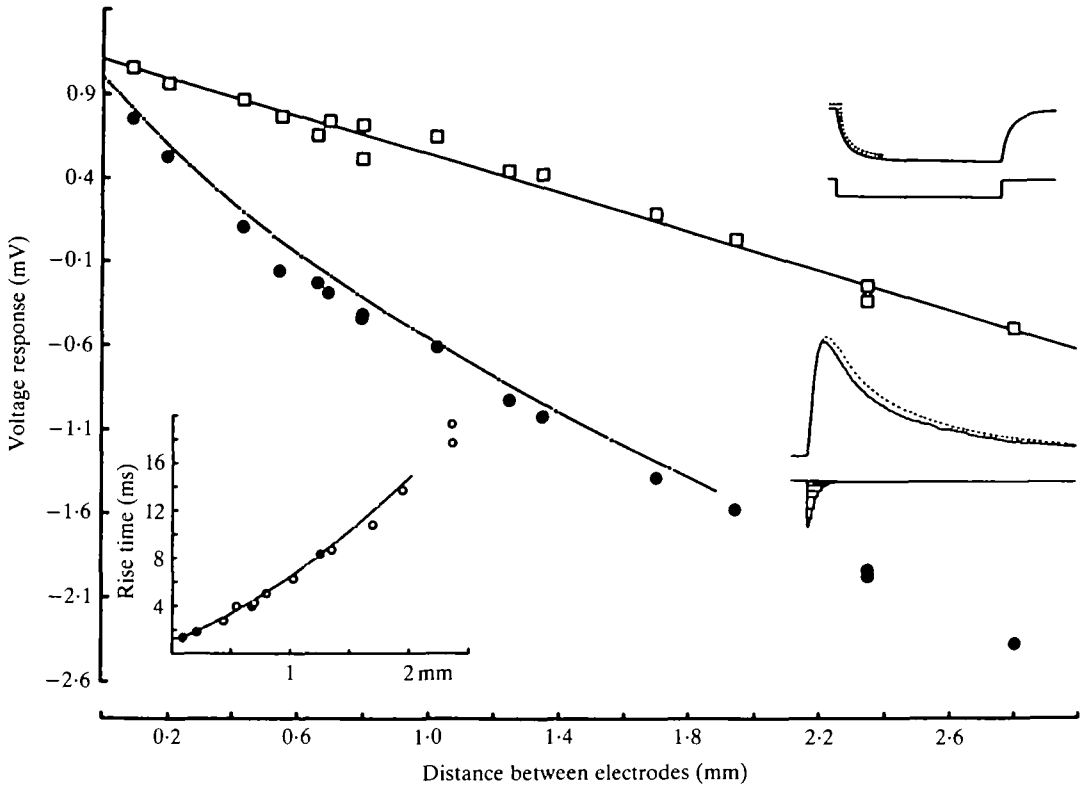

Fig. 4. Dependence of an artificially generated miniature 'synaptic' potential on the distance between the site of current injection and the site of recording. Retractor unguis muscle. x = 0 refers to the site of recording, i.e. a point 5·6 mm away from the distal end of the fibre; with increasing x the site of current injection was moved towards the distal end of the fibre. □, steady-state hyperpolarization with rectangular current pulse. From the straight line (fitted by eye) and an assumed $R_i' = 220\,\Omega$cm (see Table 3), $\lambda = 1{\cdot}74$ mm, $d_c = 64\,\mu$m, $R_m = 4310\,\Omega\text{cm}^2$, $\tau_m = 35$ ms and $C_m = 8{\cdot}1\,\mu\text{F cm}^{-2}$ were derived using the 'infinite' cable approach. ●, amplitude of artificially generated miniature 'synaptic' potential, an example of which is shown by the lower right inset (continuous line); small dots connected by dashes correspond to the amplitudes of mathematically reconstructed responses. Upper right inset: averaged response ($N = 32$) to 5 nA hyperpolarizing current pulse of 360 ms duration, injected at x = 435 μm; dotted line shows the first part of the mathematically reconstructed response based on the measured cable constants; this line has been displaced upwards for clarity. Lower right inset: response to injection of short 'synaptic' current pulse at x = 435 μm; sweep duration 82 ms; the smooth line of the bottom trace shows the current pulse of 32 nA amplitude; its simulation by superimposed rectangular pulses is also shown. Upper traces: voltage signal; the dotted line shows the response to the summed rectangular pulses, calculated from the cable constants. Continuous line, average of 32 recorded responses. The signals were negative going but for convenience have been plotted going upwards. Left inset: rise times of the responses to short 'synaptic' current pulses; the line corresponds to the rise times of mathematically reconstructed responses.

the spatial decay at short distances is steeper than exponential while the response to rectangular current pulses in this fibre is exponential (continuous line).

There is reasonable agreement between calculated and measured signals. This further supports the view that the propagation of synaptic potentials is not seriously complicated by the contribution of the transverse tubular system to the membrane properties. By means of rather more elaborate calculations (see Jack & Redman, 1971) it should also be possible for the condition of a short cable to determine the temporospatial propagation of mEJPs which has previously been estimated from injection of artificial miniature synaptic currents (Walther *et al.* 1982).

This investigation was supported from the Deutsche Forschungsgemeinschaft (SFB 138; Wa 223/3-). We thank Mrs R. Rach and S. Schäfer for technical assistance and Mrs I. Al-Aynein for typing the manuscript.

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