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ASYMMETRICAL CHARGE MOVEMENT IN SLOW- AND FAST-TWITCH MAMMALIAN MUSCLE FIBRES IN NORMAL AND PARAPLEGIC RATS

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SUMMARY

1. Asymmetrical charge movements (Q) were recorded from the voltage-clamped ends of muscle fibres in extensor digitorum longus (e.d.l.) and soleus muscles from rats. Tetracaine (2 mM) was added to solutions to prevent contraction.

2. In both muscles the relationship between Q and membrane potential (V) was S-shaped and could be described by the Boltzmann-type equation $Q = Q_m/(1+\exp[-(V-\overline{V})/k])$ where Q_m was the maximum charge, $\overline{V}$ the membrane potential at which $Q = Q_m/2$, and k a 'slope factor'. On average, Q_m was 5–6 times greater in e.d.l. than in soleus fibres and charge movement occurred at more negative potentials in soleus than in e.d.l. fibres, $\overline{V}$ being $-36{\cdot}7$ mV in the former and $-19{\cdot}0$ mV in the latter, a difference of about 18 mV.

3. The threshold for contraction, determined using a two-electrode voltage clamp, was more negative in soleus than in e.d.l. fibres. For 500 ms depolarizations, the difference was 12 mV.

4. The relationship between tension and membrane potential during potassium contractures was S-shaped and, when fitted by the Boltzmann-type equation, gave $\overline{V}$ values of -25 mV for soleus and -14 mV for e.d.l. fibres.

5. In paraplegic rats, the threshold for contraction in soleus fibres shifted about 12 mV to more positive potentials, but there was no change in e.d.l. fibres so that there was no significant difference between the two muscles.

6. In paraplegic rats the relationship between tension and membrane potential during potassium contractures also shifted to more positive potentials in soleus fibres, whereas there was no change in e.d.l. fibres.

7. These changes in the voltage sensitivity of contractile activation in soleus fibres from paraplegic rats were associated with a parallel shift in the voltage sensitivity of charge movement so that the average $\overline{V}$ shifted from $-36{\cdot}7$ mV in normal rats to a value of $-14{\cdot}2$ mV in paraplegic rats. There was also a four-fold increase in Q_m in soleus fibres from paraplegic rats.

8. The difference between the voltage sensitivity of contractile activation and charge movement in e.d.l. and soleus fibres in normal rats supports the hypothesis that the two are closely related: even stronger support comes from the observation of the parallel shift in the voltage sensitivity of contractile activation and charge movement in soleus fibres in paraplegic rats.

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INTRODUCTION

Mechanical activation of skeletal muscle fibres depends upon the release of calcium ions from the sarcoplasmic reticulum following depolarization of the transverse tubules (T-tubules). The nature of the signal which passes between the T-tubule membrane and the sarcoplasmic reticulum is unknown. However, it is thought that a slow asymmetric charge movement which can be recorded from muscle fibres may be related to this step in excitation–contraction coupling (Schneider & Chandler, 1973). The evidence for an association between this charge movement and contractile activation in amphibian muscle is that the two phenomena: (a) occur within the same range of membrane potentials (Schneider & Chandler, 1973; Horowicz & Schneider, 1981*a*, *b*); (b) show similar inactivation properties with prolonged depolarization (Chandler, Rakowski & Schneider, 1976); and (c) are similarly affected by glycerol treatment (Chandler *et al.* 1976; Dulhunty, Gage & Barry, 1981*a*). Other evidence comes from studies of mammalian fibres where both contractile activation and charge movement in slow-twitch fibres occur in a more negative potential range than in fast-twitch fibres (Dulhunty, 1980; Hollingworth & Marshall, 1980, 1981; Gage & Dulhunty, 1981*a*, *b*).

The experiments reported here were done in order to examine the correlation between the voltage dependence of contractile activation and charge movement in fast-twitch and slow-twitch rat muscle fibres and to determine whether the voltage dependence of contractile activation and the voltage dependence of charge movement were altered in a parallel manner following upper motor neurone lesions. Mid-thoracic spinal cord transection was used to disconnect upper motor neurones from motor neurones innervating extensor digitorium longus (e.d.l.) and soleus muscles. The procedure has been shown to alter the contractile properties of slow-twitch muscle fibres (e.g. soleus) so that they resemble normal fast-twitch (e.g. e.d.l.) fibres (Buller, Eccles & Eccles, 1960). It was found that the voltage dependence of both contraction and charge movement in soleus fibres was shifted to more positive membrane potentials and that charge movement increased after spinal cord transection, so that the soleus fibres became more like normal e.d.l. fibres. The results indicate that the characteristics of charge movement must be influenced by upper motor neurones and provide further support for the idea that charge movement is involved in excitation–contraction coupling.

Some of these results have been reported briefly elsewhere (Gage & Dulhunty, 1981*a*, *b*).

METHODS

Experiments were done on e.d.l. and soleus fibres from normal and paraplegic, adult, male, Wistar rats (150–300 g). Paraplegia was induced in weanling rats (21–24 days old) under ether anaesthesia by transecting the spinal cord in the mid-thoracic region. The animals were kept in separate cages in the laboratory under constant supervision and survived well (two out of thirty died post-operatively). They suffered some discomfort from urinary retention during the first 2 weeks but then adjusted and, apart from the paraplegia, appeared healthy and normal. The animals were kept for 6–12 weeks post-operatively before being killed for an experiment. At that time they were smaller than their normal litter mates, the hind-limbs particularly being less well developed. The e.d.l. and soleus muscles were smaller than muscles from normal animals of the same age and the fibre diameter was less than normal (see Table 3 below).

Muscles were removed from the hind limbs of anaesthetized rats and dissected so that the ends of individual muscle fibres were clearly visible. Preparations were then mounted on silicone rubber (Sylgard, Dow Corning) in a Perspex bath through which solutions flowed. Solutions used in different experiments are listed in Table 1. Temperature, controlled by a Peltier element and monitored by a thermistor placed near the muscle, was 20 ± 1 °C for tension experiments and 12 ± 1 °C for charge movement experiments.

TABLE 1. Solutions

(A) Contraction

Solution	Na	K	Ca	Cl	SO_4	Sucrose
A	145	3·5	1·8	151	—	—
B	80	3·5	8·0	16	43	170
C	4	80·0	8·0	16	43	170
D	—	200·0	8·0	16	100	—

In addition all solutions contained 1 mM-magnesium, 11 mM-glucose and 2 mM-TES (*N*-tris-(hydroxymethyl)-methyl-2-amino-ethanesulphonic acid) buffer, adjusted to pH = 7·4 with NaOH.

(B) Charge movement

Solution	TEA	Rb	Ca	Cl	Br	Co
E	145	10	2	14	145	—
F	145	10	2	34	145	10

Both solutions also contained 2 mM-tetracaine, 5×10^{-7} M-TTX, and 2 mM-TES buffer, adjusted to pH = 7·4 with NaOH. Figures refer to concentration (mM).

Isometric contraction. Bundles of fibres (cross-sectional area less than 0·25 mm^2) were mounted in a small volume bath (0·5 ml) at rest length. The maximum rate of solution flow through the bath was 2 ml/s. The tendon at one end was held in spring-loaded clamping forceps and the other tendon was attached to an Akers semiconductor force transducer. The final length of the preparation was set so that twitch tension was maximal (i.e. about 1·2 times rest length).

Twitch and tetanus. The preparation was stimulated at 0·05 Hz through large platinum electrodes which extended along either side of the bath. The pulse duration was set at 0·2 or 0·5 ms and the pulse amplitude was increased until the stimulus was supramaximal for the twitch. Tetanic contractions were elicited at intervals of not less than 5 min using a pulse frequency that gave maximum tetanic tension (normally between 80 and 100 Hz).

Potassium contracture tension. A potassium contracture was elicited by suddenly exposing the preparation to a solution containing a high potassium ion concentration (20–200 mM). The experiments were done under conditions of low external chloride ion concentration. The preparation was initially equilibrated in the control solution (Solution B, Table 1) before the control twitch and tetanic tension were recorded. The high-potassium solutions had a higher than normal ionic strength when the potassium concentration was greater than 84 mM. In order to determine whether there was an effect of ionic strength on potassium-contracture tension, some experiments were done using solutions that had the same ionic strength as the 200 mM-potassium solution (Solution D, Table 1), having potassium concentrations from 20 to 200 mM. Ionic strength was kept constant by varying the Na_2SO_4 concentration so that the sulphate concentration was always 100 mM. In all solutions, Na_2SO_4 was replaced by K_2SO_4 to increase the potassium-ion concentration.

The preparations were maintained in the high potassium solution until contracture inactivation was complete, and then returned to the control solution (Solution B, Table 1). A second contracture was not elicited until the twitch and tetanic tension had recovered to a steady-state value. The recovered steady-state tension was within 5% of the tension recorded prior to the contracture Results from preparations showing a greater reduction in tension were rejected. The maximum potassium-contracture tension was normalized to the maximum tension during a tetanus recorded just before the contracture, and plotted as a function of the steady-state membrane potential measured in the high-potassium solution. Potassium contractures recorded from rat muscle did not demonstrate a 'slow' component (Dulhunty, 1980) and maximum contracture tension could be unambiguously recorded.

Membrane potential in high external potassium solutions. Thin sheets of muscle fibres were dissected

from muscles and set up for micro-electrode impalement. After equilibration in Solution B (Table 1), control membrane potentials were recorded before exposure to a high-potassium solution. Membrane potentials were recorded from at least ten fibres after 5 min in a high-potassium solution. The preparation was allowed to recover for 15–20 min in Solution B (Table 1) before a new set of control potentials was recorded. If the average membrane potentials recorded before and after exposure to the high external potassium ion concentration were within 1 mV of each other, the experiment was continued and the preparation exposed to another high-potassium solution. The average membrane potential recorded in each solution is listed in Table 2. It is interesting to note that the depolarization for a particular potassium concentration was reduced in solutions with raised ionic strength.

TABLE 2. Membrane potentials (V) recorded in the high-potassium solutions used to elicit potassium contractures (see text)

Solution	K (mM)	V (mV)	n
Control*	3·5	−82 (0·4)	36
I	40	−37 (0·8)	11
	80	−18 (0·8)	17
II	40	−44 (1·7)	10
	80	−23 (0·4)	20
	120	−16 (0·8)	11
	160	−10 (0·9)	10
	200	−4 (0·7)	10

* Solution B, Table 1.

N.B. Solution I was similar to Solutions B and C, Table 1. Solution II was similar to Solution D, Table 1 having a higher than normal ionic strength.

The results are expressed as the average membrane potential (1 S.E. of the mean in parentheses) with the number of observations given in the last column.

Strength–duration curves. Strength–duration curves for activation threshold were determined in individual fibres using a two micro-electrode, point-voltage-clamp technique (Adrian, Chandler & Hodgkin, 1969), with visual determination of contraction threshold, as described previously (Dulhunty, 1980). The measurements were done in normal Krebs solution (Solution A, Table 1), containing 2×10^{-7} g/ml tetrodotoxin, at room temperature (20 ± 1 °C).

Asymmetrical charge movement. A voltage-recording electrode filled with 3 M-KCl (2–5 MΩ) was inserted at S1, a distance (l) of 300–500 μm from the end of a muscle fibre. A current-passing electrode, filled with a mixture of 1·8 M-potassium citrate and 0·8 M-KCl (2–5 MΩ), was inserted at a distance of approximately $2l + 50$ μm from the end of the fibre. Repetitive small hyperpolarizing current pulses were passed through the current electrode as it was inserted so that a change in membrane potential was detected by the electrode at S1 when the same cell was penetrated by the current electrode. A second voltage-recording electrode was then inserted at S2, a distance $2l$ from the end of the fibre and about 50 μm (l') from the current electrode. The membrane potential was recorded differentially between the voltage electrode at S1 and a similar electrode positioned extracellularly close by.

Command voltages were delivered to the clamp amplifier from a twelve bit digital-to-analog converter controlled by a microcomputer: the ±5 V output of the D to A converter was reduced to ±204·8 mV at the summing junction of the clamp amplifier. Pulses were blunted exponentially with a time constant of 0·4 ms. The steady-state difference in membrane potential recorded at S1 and S2, the membrane potential recorded differentially at S1 and the current passed through the current electrode were filtered with a fourth-order Bessel filter (3 dB down at 1 or 2 kHz) and stored on magnetic disk.

Cable properties of fibres and membrane current were calculated using the equations presented by Adrian, Chandler & Hodgkin (1970). Briefly, the space constant (λ), internal resistance (r_i) and

membrane resistance (r_m) per centimetre length of fibre were calculated from

$$\lambda \cong \sqrt{\left(\frac{3l^2 V_1(\infty)}{2\Delta V(\infty)}\right)},$$

$$r_i \cong \frac{V_1(\infty) \exp\left[(2l+l')/\lambda\right]}{I(\infty)\,\lambda \cosh\left(l/\lambda\right)},$$

$$r_m = r_i \cdot \lambda^2,$$

where V_2 and V_1 were the voltages recorded at S2 and S1, $V_1(\infty)$ and $\Delta V(\infty)$ were the steady-state values of V_1 and V_2-V_1, and $I(\infty)$ was the steady-state value of the clamp current (recorded with a virtual ground circuit). Fibre diameter was calculated from r_i by assuming an internal resistivity of 170 Ω cm at 20 °C and a Q_{10} of 1·37. Membrane capacity was calculated from the integral of ΔV recorded in response to a 10 mV depolarizing voltage step from a holding potential of −90 mV.

For measuring asymmetric capacity currents preparations were first equilibrated in Solution E or Solution F (Table 1). Linear capacity and leakage currents were subtracted from records by appropriately scaling the current recorded during a voltage step from −90 to −80 mV. In order to improve the signal-to-noise ratio, currents generated by sixteen to forty 'control' steps and four 'test' steps were averaged before subtraction. Any remaining flat 'pedestal' of current following a capacity current was assumed to be ionic and subtracted from the capacity current. For records in which there was a sloping base line, also presumed due to residual ionic current, a straight line fitted to the sloping line was used to calculate base-line values during the capacity current, a technique described in detail by Horowicz & Schneider (1981*a*). Charge (Q, nC/μF) was calculated from the ratio of the integral of the ΔV transient (after subtraction of linear currents) to the integral of the V_1 transient generated by a 10 mV depolarizing voltage step from a holding potential of −90 mV.

RESULTS

Normal rats

Cable properties. In order to record charge movement in voltage-clamped muscle fibres, a solution was used that reduced sodium, potassium and chloride conductance and inhibited contraction (Solution E, Table 1). Passive electrical properties of e.d.l. and soleus muscle fibres were measured in this solution.

The cable constants of six e.d.l. and eight soleus muscle fibres from normal rats are shown in Table 3. Both types of muscle fibre had a higher than normal value for membrane resistance (i.e. 4000–5000 Ω cm² compared with 500–1000 Ω cm² in normal e.d.l. and soleus fibres, Luff & Atwood, 1972; Dulhunty, unpublished observations). This had the effect of increasing the length constant (λ). The smaller effective capacity of the soleus fibres (compared with the e.d.l. fibres) in normal rats can probably be attributed to the smaller average diameter of the soleus fibres, and perhaps to a less extensive T-tubule system in soleus fibres (Eisenberg, Kuda & Peter, 1974). There were no significant differences in the cable properties of the e.d.l. and soleus fibres.

Asymmetrical charge movement. Depolarization of e.d.l. and soleus fibres produced asymmetrical charge movement as illustrated in Fig. 1. The traces were obtained by averaging several sweeps and subtracting the scaled capacitive and leakage currents (see Methods). In e.d.l. fibres, this charge movement became apparent at membrane potentials between −40 and −50 mV, whereas in soleus fibres, charge movement could be detected at more negative potentials. The difference in voltage dependence of charge movement in an e.d.l. and a soleus fibre is apparent in Fig. 1. It can also be seen in Fig. 1 that the charge movement in the e.d.l. fibre was greater than in the

TABLE 3. Cable constants measured in e.d.l. and soleus muscle fibres from normal and paraplegic rats in Solution E containing TEABr and tetracaine

Muscle	n	Diameter (μm)	τ (ms)	λ (mm)	Effective capacity (μF cm^{-2})	R_m (Ω cm^2)
Normal						
E.d.l.	6	82·4	25·9	2·1	6·2	4137
		(3·6)	(4·3)	(0·1)	(0·9)	(663)
Soleus	8	53·6	19·8	1·7	4·0	4993
		(4·2)	(3·5)	(0·2)	(0·4)	(610)
Paraplegic						
E.d.l.	8	43·0	18·6	1·3	5·0	3829
		(2·5)	(1·9)	(0·1)	(0·4)	(464)
Soleus	30	47·6	17·1	1·3	5·2	3429
		(1·7)	(1·5)	(0·1)	(0·3)	(341)

Mean values from n fibres are shown with ± 1 S.E. of the mean in parentheses.

soleus fibre. In fact, in many soleus fibres it was difficult to detect any charge movement above the noise.

When the amount of asymmetrical charge at the onset of a depolarizing pulse (Q_{on}) was compared with the amount of charge at the turn-off of the pulse (Q_{off}) it was apparent that, with greater depolarizations, Q_{off} was greater than Q_{on}. This is illustrated in Fig. 2*A* in which Q_{on} (measured from the records shown in Fig. 1*A*) is plotted against Q_{off}. It is clear that in both the soleus (circles) and e.d.l. (squares) fibres, Q_{off} tended to be larger than Q_{on}, especially at more positive potentials. It has been suggested that such an effect may be due to contamination of the 'off-current' by calcium current which can be suppressed by adding cobalt to the extracellular solution (Horowicz & Schneider, 1981*a*). It was found in several experiments that addition of cobalt (10 mM) reduced Q_{off}. Records of charge movement recorded from an e.d.l. fibre and a soleus fibre in such a solution are shown in Fig. 1*B* and a plot of Q_{on} against Q_{off} for these two fibres are shown in Fig. 2*B*. It can be seen that there was good equality of Q_{on} and Q_{off} in the presence of cobalt. Although the addition of cobalt might seem desirable to suppress calcium currents, a shift in Q–V curves to the right was observed in the presence of cobalt, presumably because of an effect on surface charge. For this reason, cobalt was not used and charge movement was measured from Q_{on}, rather than Q_{off}.

The relationship between charge (Q, nC/μF) and membrane potential was S-shaped, appearing to saturate at more positive potentials in both e.d.l. and soleus fibres. Graphs of Q against membrane potential for an e.d.l. (squares) and soleus (circles) fibre are shown in Fig. 3. Similar curves were obtained in a number of each type of fibre (see Table 4). It was shown by Schneider & Chandler (1973) that the relationship between such charge movement (Q) and potential (V) conforms to predictions from a simple, two-state Boltzmann model for distribution of charge in an electrical field:

$$Q = Q_m/(1+\exp[-(V-\overline{V})/k]), \quad (1)$$

where Q_m is the maximum charge movement, $\overline{V}$ is the potential at which Q equals half Q_m and $k = RT/AzF$, R, T, z and F having their usual significance and A being

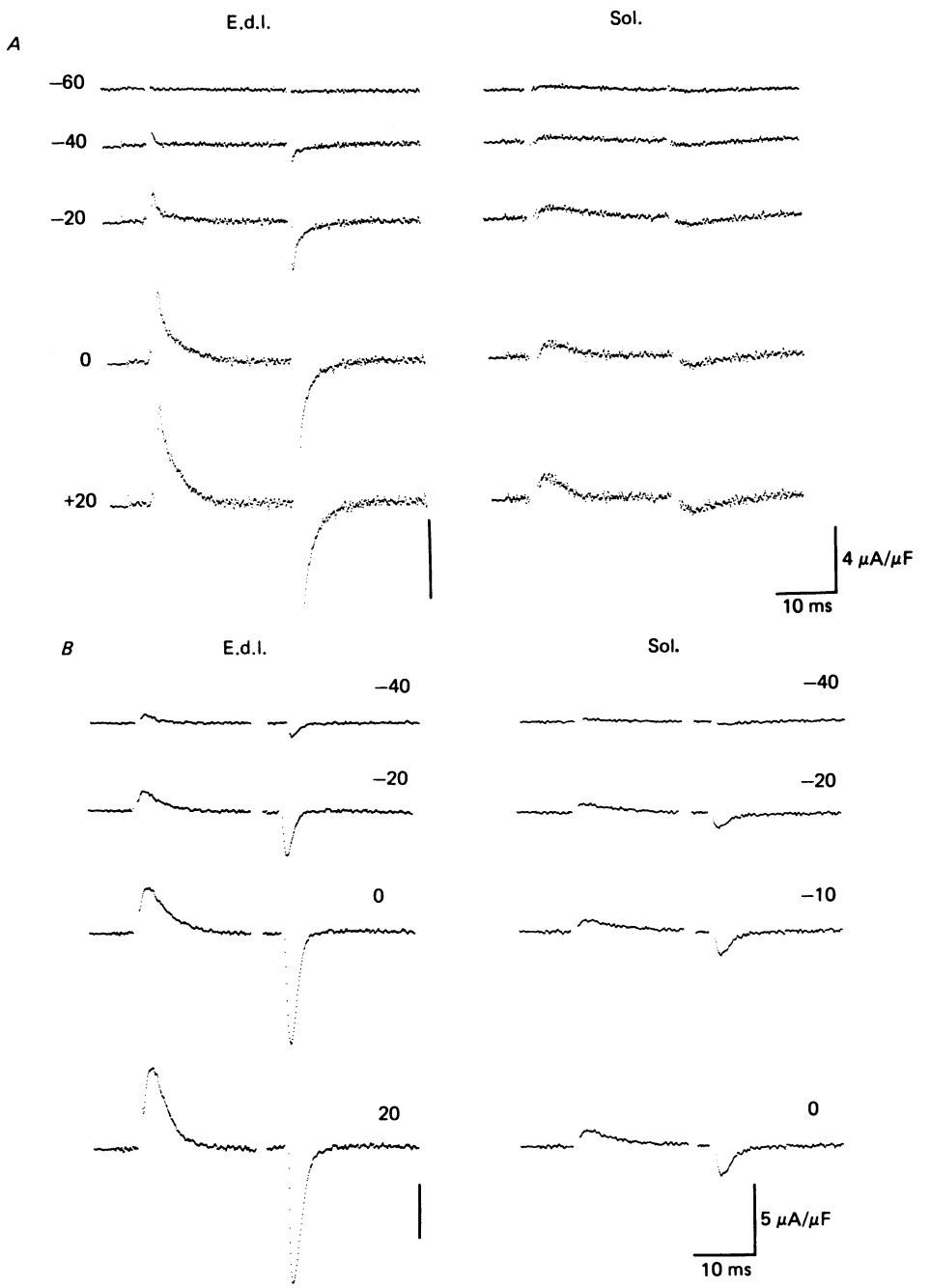

Fig. 1. Asymmetrical charge movement recorded in extensor digitorum longus (e.d.l.) and soleus (sol.) muscle fibres from normal rats as described in Methods. The records in *A* were obtained in Solution E whereas the records in *B* were obtained in Solution F containing 10 mM-cobalt. Holding potential, −90 mV. The control pulse used for subtraction of linear currents was repeated sixteen times in *A* and forty times in *B*. The numbers beside each trace denote the membrane potential during the pulse. Temperature: 11·8 °C in *A*, 11 °C in *B*.

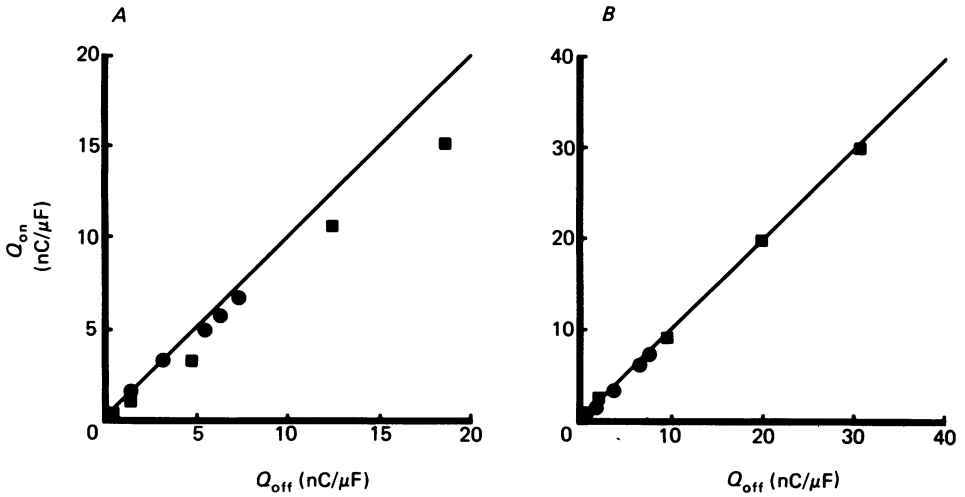

Fig. 2. Graphs of the area of an asymmetrical current generated by a step depolarization (Q_{on}) against the area of the asymmetrical current generated by turning off the step depolarization (Q_{off}) in Solution E (*A*) and Solution F (*B*) containing 10 mM-cobalt, in e.d.l. (squares) and soleus (circles) fibres. Measurements were taken from the same fibres as in Fig. 1. Straight lines represent equality of Q_{on} and Q_{off}.

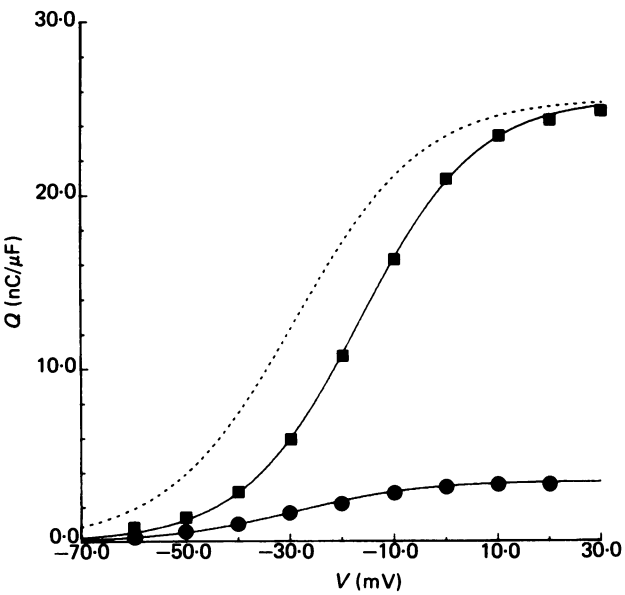

Fig. 3. Charge movement (Q, nC/μF), recorded in an e.d.l. (squares) and a soleus (circles) fibre from a normal rat, plotted against clamp potential. The lines through the points show least-squares fits of eqn. 1 to the data (see text). For the e.d.l. fibre, this curve gave Q_m = 25·7 nC/μF, $\overline{V}$ = −16·5 mV and k = 11·3 mV whereas for the soleus fibre, the values were Q_m = 3·5 nC/μF, $\overline{V}$ = −29 mV and k = 12·3 mV. The dashed line shows the curve for the soleus fibre (circles) scaled up to the same maximum value as for the e.d.l. fibre and illustrates the difference in the voltage-sensitivity of charge movement in the two types of fibre.

a constant. The continuous lines through the points in Fig. 3 show a least-squares fit of this equation to the data. In the e.d.l. fibre, the best fit was obtained with $Q_m = 25{\cdot}7$ nC/μF, $\overline{V} = -16{\cdot}5$ mV and $k = 11{\cdot}3$ mV, whereas in the soleus fibre the best fit was given by $Q_m = 3{\cdot}5$ nC/μF, $\overline{V} = -29$ mV and $k = 12{\cdot}3$ mV. The main differences between the two types of fibre are that the maximum charge movement in the e.d.l. fibre is much greater than in the soleus fibre and that the potential for equal distribution of charge ($\overline{V}$) is more negative by 12·5 mV in the soleus than in the e.d.l. fibre. Similar differences between e.d.l. and soleus fibres have been reported by Hollingworth & Marshall (1981). Results obtained in eleven e.d.l. and eleven soleus fibres are shown in Table 4. On average, maximum charge movement was 5–6 times larger in e.d.l. than in soleus fibres. The true difference is probably greater as charge movement could not be measured in many soleus fibres. The difference in average $\overline{V}$ values was 17·7 mV.

TABLE 4. Charge movement in normal rats

Fibre type	n	Q_m (nC/μF)	$\overline{V}$ (mV)	k (mV)
E.d.l.	11	23·4 ± 2·6	−19·0 ± 2·4	13·3 ± 0·9
Soleus	11	4·3 ± 0·5	−36·7 ± 1·6	11·0 ± 0·1

Mean values (± 1 S.E. of the mean) of Q_m, $\overline{V}$ and k obtained from least-squares fits of eqn. 1 to experimental data. n = the number of fibres from which data were obtained.

Muscle contraction. It has been reported previously that, in the mouse, the voltage dependence of contractile activation is different in e.d.l. and soleus muscles, equivalent activation occurring at more negative potentials in soleus (Dulhunty, 1980). It was thought worthwhile to confirm that there is a similar difference in the voltage dependence of contraction in rat e.d.l. and soleus muscles. Two methods were used. The first was to voltage clamp a small area of the surface membrane of a muscle fibre to determine the 'strength–duration' curve for just-visible contraction (Adrian, Chandler & Hodgkin, 1969). The second was to depolarize a muscle fibre by raising the extracellular potassium concentration and to measure the tension developed as a function of membrane potential.

Strength–duration curves. The relationship between the level of membrane potential and the time for which it had to be maintained for just-visible contraction is shown for e.d.l. (squares) and soleus (circles) fibres in Fig. 4. It is clear that less depolarization was needed to activate soleus than e.d.l. fibres at all pulse durations and this is consistent with the difference in the voltage dependence of charge movement. With the longest pulses used (500 ms), the difference in the threshold for contraction was 12 mV.

Potassium contractures. The relationship between tension and membrane potential in the two types of fibre was examined using potassium contractures. Membrane potentials corresponding to different potassium concentrations were measured in an initial series of experiments (Table 2). In other experiments, relative tension (T, tension during the plateau of a potassium contracture relative to tetanic tension recorded at 80–100 Hz) was measured for series of potassium concentrations and average values are shown plotted against membrane potential in Fig. 5. Although

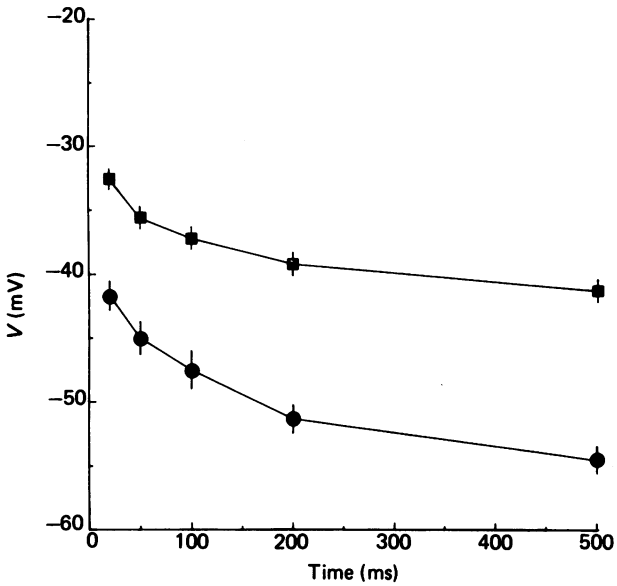

Fig. 4. Strength–duration curves recorded using a two micro-electrode voltage-clamp technique. The most negative clamp potential for just visible local contraction is plotted against the duration of the clamp pulse. Vertical lines show ±1 S.E. of the mean. It can be seen that more positive potentials were required for contraction in e.d.l. fibres (squares, fourteen fibres) than in soleus fibres (circles, fifteen fibres). Temperature, 20–22 °C.

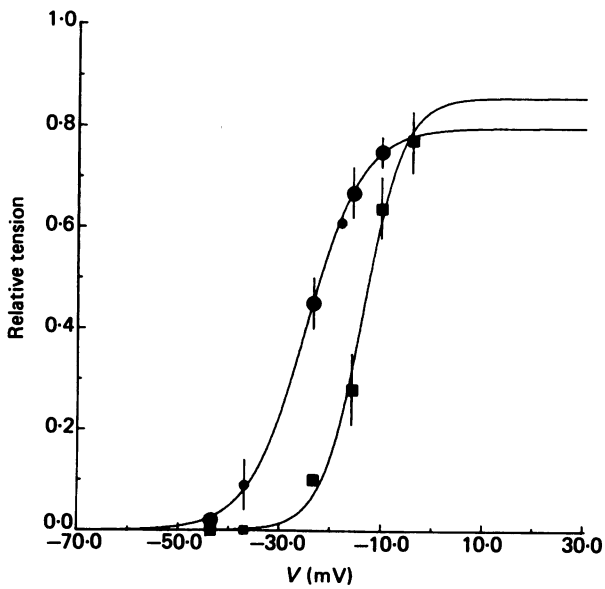

Fig. 5. Relative tension (tension during a potassium contracture relative to maximal tension developed during tetanus) plotted against membrane potential for e.d.l. (squares) and soleus (circles) fibres in normal rats. The lines through the points are best fits of eqn. 2 to the data. Smaller symbols and larger symbols denote results obtained in solutions with normal and raised ionic strength, respectively. Vertical lines show ±1 S.E. of the mean. The lines are described by T_m (maximum relative tension) = 0·86, $\bar{V}$ = 13·8 mV and k = 3·9 mV for the e.d.l. fibres (n = 5) and by T_m = 0·8, $\bar{V}$ = −25 mV and k = 5·5 mV for the soleus fibres (n = 5).

the relationship between tension and membrane potential involves a complex sequence of reactions, not a single, first order reaction, it was convenient for purposes of comparison to fit a Boltzmann-type equation

$$T = T_m/(1+\exp[-(V-\overline{V})/k]) \quad (2)$$

to experimental points as before (continuous lines, Fig. 5). (T_m is maximal relative tension and the other symbols have the same meaning as in equation 1.) Such fits gave half-maximum tension values at -25 mV in soleus muscles and $-13{\cdot}8$ mV in e.d.l. fibres, a difference of 11·2 mV.

It is clear that the relationship between tension and membrane potential was steeper in e.d.l. fibres ($k = 3{\cdot}9$ mV) than in soleus fibres ($k = 5{\cdot}5$ mV) and much steeper in both types of muscle than the relationship between Q and membrane potential where the k values were 2 to 3 times larger.

Paraplegic rats

Because of the differences in charge movement in e.d.l. and soleus fibres, it was decided to examine charge movement in these muscles following spinal cord transection, which is known to cause a change in the properties of slow-twitch muscle fibres towards those of fast-twitch muscle fibres (Buller *et al.* 1960; Davey, Dunlop, Hoh & Wong, 1981). We confirmed this change in contractile properties ina preliminary series of experiments.

Muscle contraction. Records of twitches and potassium contractures in e.d.l. and soleus muscles from normal and paraplegic rats are shown for comparison in Fig. 6. The left-hand column shows isometric twitches, the right-hand column, potassium contractures, and the middle column shows isometric twitches before and after a tetanus (80–100 Hz for 1·5–2 s). In e.d.l. fibres, there was little difference in the time course of the twitch or potassium contracture, or in post-tetanic potentiation, in normal (e.d.l.-n.) and paraplegic (e.d.l.-t.) rats. However, there were marked differences in the contractile characteristics of soleus fibres in normal (sol.-n.) and paraplegic (sol.-t.) rats. Average values of twitch contraction and relaxation times, twitch/tetanus ratio and post-tetanic potentiation are given in Table 5. It is clear that these properties were not significantly changed in e.d.l. fibres from paraplegic rats. In contrast, the properties of soleus fibres did change towards those of e.d.l. fibres. The twitch contraction and relaxation times of soleus fibres from paraplegic rats were half their normal values, the twitch/tetanus ratio was twice normal and post-tetanic potentiation, normally not seen in soleus fibres, was now apparent (Fig. 6).

In the experiments reported here, the properties of soleus muscle fibres were never converted completely to those of e.d.l. muscle fibres. It is clear from the records shown in Fig. 6 and from the data in Table 5 that soleus fibres isolated from animals after spinal cord transection had slower contraction and relaxation times, a smaller twitch/tetanus ratio and less post-tetanic potentiation than e.d.l. fibres in the same animals.

Strength–duration curves. Following spinal cord transection, when the characteristics of soleus muscle fibres had changed as described above, the 'rheobase' of strength–duration curves had also shifted as illustrated in Fig. 7. More depolarization was now required to produce contraction. There was little change in the e.d.l. fibres and the

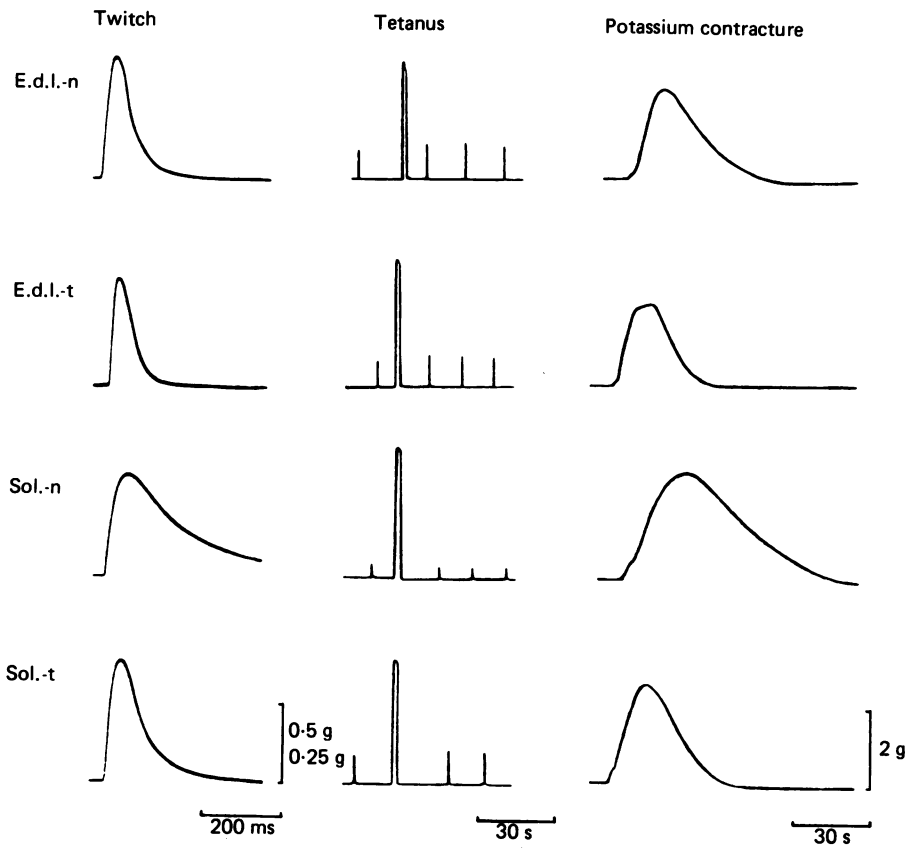

Fig. 6. Isometric twitches (column 1) and potassium contractures (column 3, solution D) recorded in small bundles of muscle fibres. Isometric twitches preceding and following a tetanus are shown in column 2. Records were obtained from normal e.d.l. (e.d.l.-n.) and soleus (sol.-n) fibres and from e.d.l. and soleus fibres following spinal cord transection (e.d.l.-t and sol.-t, respectively). Vertical calibration denotes 0·5 g for all records in column 1 except for sol.-n. where it denotes 0·25 g. The vertical calibration shows 2 g for columns 2 and 3. Horizontal calibration denotes 200 ms for column 1 and 30s for columns 2 and 3.

strength–duration curves for e.d.l. and soleus fibres were very similar in paraplegic rats (Fig. 7) in contrast to the differences seen in normal rats (Fig. 4).

Potassium contractures. The relationship between relative tension and membrane potential during potassium contractures in e.d.l. (*A*) and soleus (*B*) fibres from paraplegic rats is shown in Fig. 8. As before, the continuous lines show least-squares fits of eqn. 2 to the points. The experimental data recorded from soleus fibres from paraplegic rats were now best fitted with a value for $\overline{V}$ of -16 mV in contrast to the value of -25 mV in normal rats. Again the voltage dependence of contractile activation had shifted to more positive values (closer to e.d.l.) in soleus muscle fibres from paraplegic rats compared with normal.

Cable properties. Cable properties measured in e.d.l. and soleus fibres immersed in solution E (Table 1) are shown in Table 3. The diameter of fibres from paraplegic rats was less than in the control animals and this appeared to be a real change. However,

TABLE 5. Isometric contractile properties of e.d.l. and soleus muscle fibres from normal animals and from paraplegic animals 6–7 weeks after spinal cord transection

	Normal rats		Paraplegic rats	
	Sol.	E.d.l.	Sol.	E.d.l.
Twitch				
C.t. (ms)	89	35	46	31
	(8)	(2)	(2)	(1)
50% d.t. (ms)	162	53	81	45
	(13)	(4)	(4)	(4)
Twitch/tetanus ratio	0·12	0·28	0·22	0·27
	(0·01)	(0·03)	(0·01)	(0·03)
P.t.p.	0·98	1·26	1·14	1·29
	(0·02)	(0·12)	(0·03)	(0·03)
Potassium contracture				
C.t. (s)	18·4	9·5	10·4	8·2
	(4·0)	(1·3)	(1·2)	(1·0)
50% d.t. (s)	26·0	10·8	7·7	7·2
	(6·0)	(1·0)	(0·2)	(0·7)

Twitch contraction time (c.t.) and 50% decay times (50% d.t.) are given. Post-tetanic potentiation (p.t.p.) is the ratio of the amplitude of a twitch elicited immediately before a tetanus to that recorded immediately after a tetanus. Contraction times and 50% decay times for potassium contractures are also given. The results are expressed as means ± S.E. of the mean in parentheses. Five muscles of each type were used.

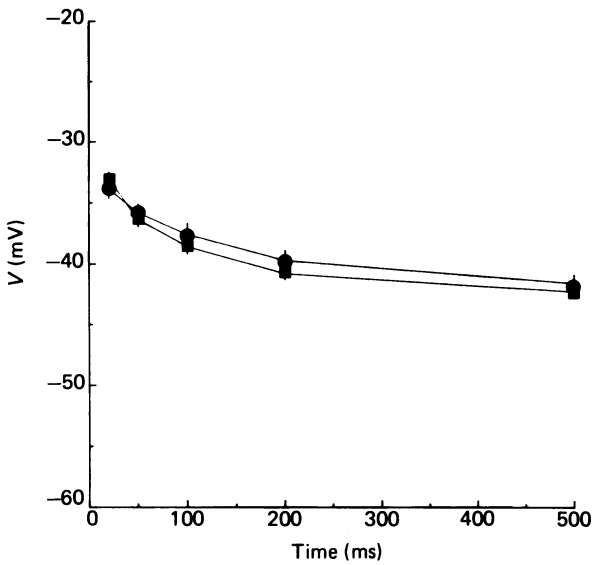

Fig. 7. Strength–duration curves, recorded as in Fig. 3, in e.d.l. (squares, $n = 23$) and soleus (circles, $n = 24$) fibres from paraplegic rats. Vertical lines show ±1 S.E. of the mean. Temperature, 20–22 °C.

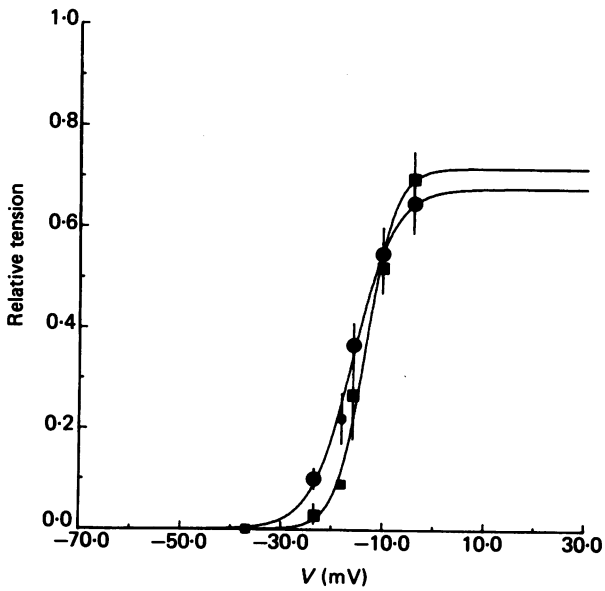

Fig. 8. Relative tension during potassium contractures plotted against membrane potential in e.d.l. (squares, $n = 5$) and soleus (circles, $n = 5$) fibres from paraplegic rats. Smaller symbols and larger symbols denote results obtained in solutions with normal and raised ionic strength, respectively. The best fit of eqn. 2 to the data (continuous lines) gave values of $T_m = 0{\cdot}72$, $\overline{V} = -13{\cdot}5$ and $k = 2{\cdot}8$ mV for the e.d.l. fibres and $T_m = 0{\cdot}68$, $\overline{V} = -16{\cdot}0$ mV and $k = 4{\cdot}0$ mV for the soleus fibres. Temperature, 20–22 °C.

TABLE 6. Charge movement in paraplegic rats

Fibre type	n	Q_m (nC/μF)	$\overline{V}$ (mV)	k (mV)
E.d.l.	8	15·6±1·5	−19·3±3·0	12·4±2·4
Soleus	24	15·7±0·7	−14·2±1·3	12·8±0·5

Mean values (±1 S.E. of the mean) of Q_m, $\overline{V}$ and k obtained from least-squares fits of eqn. 1 to experimental data from e.d.l. and soleus fibres. n = the number of fibres from which data were obtained.

the cable properties were not significantly different in the two types of fibre and were comparable with cable properties measured in the control fibres (Table 3).

Asymmetrical charge movement. Charge movement was not significantly changed (at the 1 % level) in e.d.l. fibres from paraplegic rats, as can be seen in Table 6. However, both the amount of charge movement and its voltage dependence were clearly changed in soleus muscle fibres ($P \ll 0{\cdot}005$ for both). Average values for Q recorded from twenty-four soleus fibres from paraplegic rats are plotted against membrane potential in Fig. 9 and the line through the points shows the least-squares fit of eqn. 1. The least-squares fit of eqn. 1 to average data from soleus fibres in normal rats (Table 2), shown as a continuous line below, illustrates the increase in charge movement in soleus fibres of paraplegic rats. When this curve is scaled up to the same Q_m as for the paraplegic rats (dashed line), the shift in the voltage dependence of charge movement to more positive potentials in paraplegic rats is evident. In the paraplegic rats, the average Q_m had increased from a normal value of 4·3 nC/μF to 15·7 nC/μF and the average $\overline{V}$ had shifted from −36·7 to −14·2 mV.

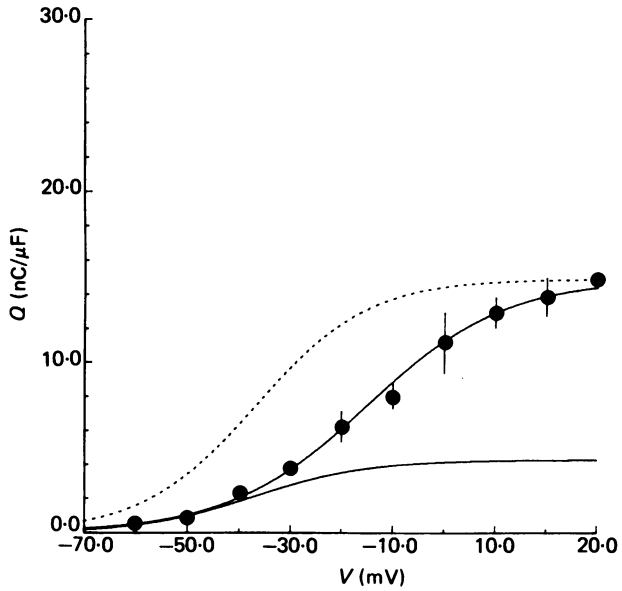

Fig. 9. Charge movement (Q, nC/μF) plotted against clamp potential in soleus muscle fibres from paraplegic rats. The circles represent average values obtained from twenty-four fibres (see Table 6) and the vertical lines show ± 1 S.E. of the mean. The best fit of eqn. 1 to the points (continuous line through the points) gave $Q_m = 15{\cdot}7$ nC/μF, $\overline{V} = -14{\cdot}2$ mV and $k = 12{\cdot}8$ mV. The best fit of eqn. 1 to average values of Q *vs.* V in eleven soleus muscle fibres from normal rats is shown (lower continuous line) for comparison and the change in $\overline{V}$ is illustrated by the dashed line obtained by scaling up the lower line to a Q_m of 15·7 nC/μF.

DISCUSSION

The origin of the asymmetric charge movement that can be recorded from skeletal muscle fibres is uncertain, but evidence has been accumulating that it is associated with excitation–contraction coupling. The close similarity between the voltage dependence of tension (Figs. 4 and 5) and charge movement (Fig. 3 and Table 4) in e.d.l. and soleus muscle fibres strongly supports the hypothesis that charge movement is the voltage-sensing process in excitation–contraction coupling. Hollingworth & Marshall (1981), who have recorded charge movements in rat e.d.l. and soleus muscles also, found a similar difference in voltage sensitivity, but their Q_m values were larger and their $\overline{V}$ values were slightly more negative (e.g. 5 mV in e.d.l.) than values reported here. The differences may be related to their use of hypertonic sucrose rather than tetracaine to prevent contraction. In frog muscle, tetracaine causes a slight positive shift in $\overline{V}$ but appears not to depress charge movement (Almers & Best, 1976) except in hypertonic solutions (Huang, 1982). Even if tetracaine did depress some of the charge movement, the remaining charge had properties that changed in accord with the contractile properties of the muscle fibres (following spinal cord transection) suggesting a close relationship between the two.

The reason for the difference in the voltage sensitivity of charge movement in the two types of muscle in normal rats is not clear. It may reflect different properties of the protein molecules which move to generate charge movement or differences

in the electric field 'seen' by these molecules. Differences in electric field could be due to non-homogeneities in membrane structure or surface charge, or to differences in the position of the proteins within the membrane. It may be relevant that biochemical analysis of the composition of e.d.l. and soleus plasmalemma has revealed significant differences in the lipid, protein and carbohydrate fractions from the two muscles (Smith & Appel, 1979; Jeffery, Leung & Rostas, 1981). Furthermore, freeze-fracture replicas of plasmalemma from the two muscles have a very different appearance (Ellisman, Rash, Staehelin & Porter, 1976). Soleus membrane has many more particles and this may represent a difference in the protein content or it may reflect the depth to which the protein molecules penetrate the lipid phase of the membrane. The different voltage sensitivity of charge movement in e.d.l. and soleus fibres could be explained if the voltage-sensitive protein in soleus fibres were more deeply embedded in the membrane and saw a different fraction of the membrane field.

The reason for the smaller amount of asymmetrical charge movement in the slow-twitch fibres is also not clear. Since the areas of junctional contact per unit area of T-tubule membrane are the same in e.d.l. and soleus fibres (A. F. Dulhunty, unpublished observations) the different amounts of charge moved in the two preparations cannot reflect a difference in contact area. It is possible that there is a difference in the density of protein molecules in the junctional area or a difference in the number of charged groups on each protein molecule. It is interesting that the relative amount of charge movement in e.d.l. and soleus fibres is similar to the relative number of indentations in their terminal cisternae; there are fewer indentations in normal soleus fibres than in normal e.d.l. and the number of indentations in soleus fibres increases following spinal cord transection (Dulhunty, Gage & Valois, 1981*b*). These indentations may be closely related to charge movement. It is also interesting that the calcium transient, measured with aequorin (Eusebi, Miledi & Takahashi, 1980), is smaller in soleus fibres than in e.d.l. fibres. This could be partly due to a smaller area of membrane in the terminal cisternae available for release of calcium ions (Eisenberg *et al.* 1974; Eisenberg & Kuda, 1975, 1976) or to a lower calcium capacity per unit volume of sarcoplasmic reticulum (Briggs, Poland & Solaro, 1977) in soleus fibres. Alternatively, the smaller calcium transient in soleus fibres may be related to the smaller charge movement. The fact that the contractile proteins in soleus fibres need fewer calcium ions for activation (Stephenson & Williams, 1981) is in line with what appears to be a general adaptation of the soleus sarcoplasmic reticulum to store less calcium and to release fewer calcium ions when activated.

There was considerable variability in the amount of charge recorded from soleus and e.d.l. fibres. This was particularly apparent in soleus fibres where the charge was often too small to measure above the noise. Presumably much of the variability can be attributed to the mixed populations of motor units in the muscles. It would have been interesting, but not feasible, to have compared the charge movement of fibres within one motor unit to see how closely the relationship between contraction time and charge movement was maintained.

The relationship between tension and voltage is clearly steeper than the relationship between charge movement and voltage in fibres from both muscles (Figs. 3 and 5). If charge movement is the only voltage-sensitive step in excitation–contraction coupling, then one of the subsequent steps must be non-linear, perhaps involving a

co-operative process. For example, the relationship between charge movement and calcium release from the sarcoplasmic reticulum, or between calcium ion concentration and tension may be non-linear. It is known that the relationship between membrane potential and calcium release (measured in amphibian muscle using calcium indicators; Baylor, Chandler & Marshall, 1979) is steeper than the relationship between membrane potential and charge movement. Furthermore, the relationship between calcium concentration and tension in skinned muscle fibres (using calcium-buffered solutions) is highly non-linear (Stephenson & Williams, 1981). It is not surprising, therefore, that the relationship between membrane potential and tension, involving as it does these non-linear steps, should be steeper than the relationship between membrane potential and charge movement.

Although the slope of the relationship between membrane potential and charge movement was similar in e.d.l. and soleus fibres, the slope of the relationship between membrane potential and tension (see k values) was greater in e.d.l. than in soleus fibres by a factor of 1·4. This must reflect a difference in some later step or steps in excitation–contraction coupling in fibres from the two muscles. Indeed, it has been shown that the slope of the relationship between buffered calcium concentration and tension in skinned muscle fibres is steeper in e.d.l. than in soleus fibres (Stephenson & Williams, 1981).

Most observations of the relationship between charge movement and mechanical activation have been made in amphibian preparations and are difficult to compare with the results presented here. Like mammalian muscles, amphibian muscles do contain fast- and slow-twitch fibres (Lannergren, 1979). There are no descriptions of charge movement or mechanical activation in the different twitch fibre types. However, it has been found that the voltage dependence of tension is similar in slow tonic fibres and twitch fibres (Gilly & Hui, 1980*a*; Caputo & de Bolanos, 1979; Hodgkin & Horowicz, 1960; Nasledov, 1969). The voltage dependence of charge movement is also similar in slow tonic and twitch fibres, but the maximum charge movement in the tonic fibres is one quarter to one third of that in the twitch fibres (Gilly & Hui, 1980*b*).

The effects of spinal cord transection on the magnitude and voltage dependence of charge movement in soleus fibres are very striking. The change could be due to the removal of a 'trophic' factor originating in the upper motor neurone or it could be due to an altered pattern of lower motor neurone activity. The latter seems more likely. It is well known that the pattern of impulse activity in a motor nerve is of great importance in determining many of the characteristics of the muscles it innervates (for review, see Salmons & Henriksson, 1981). Furthermore, muscle properties can be modified by imposing patterns of direct stimulation on the muscle after denervation (Lømo, Westgaard & Dahl, 1974). It is becoming increasingly clear that most, if not all, of the differences between fast- and slow-twitch fibres are subject to modification when the pattern of activity is altered. There have been several studies of changes in muscle fibres from animals with transected spinal cords. In all cases the major changes have been in soleus fibres which tend to adopt the characteristics of fast-twitch fibres. The twitch in soleus becomes faster than normal, post-tetanic potentiation becomes apparent, the temperature dependence of the twitch amplitude increases (Buller *et al.* 1960; Davey *et al.* 1981), myosin isoenzymes

are converted to a fast (e.d.l.) type (Hoh, Kwan, Dunlop & Kim, 1980), the surface-to-volume ratio of the sarcoplasmic reticulum increases (Davey *et al.* 1981) and the freeze-fracture appearance of the terminal cisternae membrane becomes similar to that of normal e.d.l. fibres (Dulhunty *et al.* 1981). We have now found that the characteristics of charge movement in soleus fibres change towards those of e.d.l. fibres. It is well known that impulse activity increases in the lower motor neurones after damage to upper motor neurones and that this can lead to spasticity in the muscle groups involved. Most of the changes that have been recorded in soleus muscles after spinal cord transection are consistent with a simple alteration in the activity pattern towards that normally seen by e.d.l. fibres. The changes are not as complete as those seen during controlled stimulation experiments and the transformation of a number of morphometric parameters is marginal (Davey *et al.* 1981). However, this is hardly surprising since it would be unlikely that a perfect transformation of activity would result simply from cord transection.

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