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EXCITATION–CONTRACTION COUPLING AND CHARGE MOVEMENT IN DENERVATED RAT EXTENSOR DIGITORUM LONGUS AND SOLEUS MUSCLES

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SUMMARY

1. K contractures and asymmetrical charge movement were recorded in extensor digitorum longus (e.d.l.) and soleus muscles that had been denervated for 2–68 days.

2. The relationship between maximum tension during a K contracture and membrane potential shifted to more negative potentials in denervated e.d.l. muscles (by −25 mV on average) and to a lesser extent in soleus (by −8 mV on average), and became steeper, more so in e.d.l. than soleus.

3. Apart from an early negative shift of −11 mV in the voltage dependence of tension in e.d.l. muscles during the first week, the other changes in K contractures following denervation occurred progressively during the first 3 weeks and then stabilized.

4. There was a clear difference in charge movement in denervated e.d.l. fibres but little change in denervated soleus fibres, so that the characteristics of charge movement in e.d.l. and soleus became very similar. The maximum amount of charge movement fell from an average normal value of 23 nC/μF to 6 nC/μF in e.d.l. within the first 2 weeks. The voltage sensitivity shifted to more negative potentials (by about −12 mV on average) within the first week. There was no significant change in the slope of the relationship between charge and membrane potential.

5. The effects of denervation on charge movement could only partly explain the changes in K contractures. The only obvious parallels were the early negative shift in the voltage dependence of charge movement and tension in denervated e.d.l. fibres. The other changes in K contractures in denervated fibres could be due to a change in the relationship between charge movement and Ca concentration in the myoplasm or an increase in the Ca affinity of the myofilaments.

6. Although charge movement fell to about a quarter of normal in denervated e.d.l. fibres, membrane capacity increased approximately 3-fold. A similar increase in capacity in soleus fibres was not associated with a change in charge movement.

7. Fewer indentations were seen in denervated than in normal e.d.l. fibres. The decrease paralleled the fall in charge movement.

INTRODUCTION

When the nerve to a mammalian skeletal muscle is sectioned, there are changes in many characteristics of the fibres including the amplitude and time course of the isometric twitch (Eccles, Eccles & Kozac, 1962). The contractile proteins are not altered during the first few weeks after denervation (Syrovy, Gutmann & Melichna, 1971; Jaweed, Herbison & Ditunno, 1975; Finol, Lewis & Owen, 1981) so that the changes in the twitch must be caused by changes in the electrical properties of the surface membrane or by changes in excitation–contraction (e.–c.) coupling. The time course of action potentials is different from normal after denervation (Lewis, 1972; Sellin & Thesleff, 1980) and it has been suggested that changes in electrical properties of the surface membrane are responsible for changes in the twitch (Finol *et al.* 1981).

It has recently been found (Dulhunty & Gage, 1983) that changes in twitches and K contractures in soleus muscle following spinal cord transection are associated with changes in asymmetrical charge movement assumed to be generated during excitation–contraction coupling (Schneider & Chandler, 1973). It was thought that the modified pattern of activity following removal of the influence of the upper motor neurones might have caused the changes. These were most marked in soleus fibres which partially adopted the charge movement characteristics of extensor digitorum longus (e.d.l.) fibres. Following denervation, muscles would be entirely deprived of neuronal input and hence their activity would be drastically altered.

We have therefore denervated e.d.l. and soleus muscles in order to determine what changes do occur in contractile activation and also to explore whether any differences that were discovered might be associated with changes in charge movement. K contractures were used as a measure of contractile activation.

METHODS

Adult male Wistar rats (body weight 300–450 g) were used for all experiments. Some animals were anaesthetized with halothane and denervated by removing a 0·5–1 mm segment of the sciatic nerve just distal to the sciatic notch. The animals were allowed to recover from the anaesthetic and were maintained for periods from 2 to 68 days. For experiments, normal and 'denervated' rats were killed with an overdose of halothane or chloroform and e.d.l. and soleus muscles removed and stored in a beaker containing normal Krebs solution (Solution A, Table 1) at room temperature. The muscles were then pinned out in a Petri dish lined with Sylgard (Dow Corning) in a low Cl control solution (Solution B, Table 1). Considerable care was taken to ensure that denervated preparations had not been reinnervated. Normal muscles and reinnervated muscles exhibited strong twitches when the main neurovascular bundle was cut or when nerve twigs in the connective tissue were cut during fine dissection. Any muscles demonstrating such twitch activity were rejected.

K contractures

Small bundles containing five to ten fibres were dissected free and mounted in a small volume bath designed for rapid solution change-over. The methods used for recording isometric twitch and tetanic tension, for eliciting and recording K contractures, and for measuring membrane potential in solutions containing different K ion concentrations (Solutions C, D, E and F, Table 1) have been described in detail previously (Dulhunty & Gage, 1983). Contractures were displayed on an oscilloscope and tension continuously recorded with a Hewlett Packard 7402A chart recorder. Oscilloscope traces of individual twitches were photographed with a polaroid camera. The temperature was maintained at 21 ± 1 °C during the experiments.

The solutions used are listed in Table 1. The experiments were done in low Cl solutions in order

to avoid the effects of the normally high Cl conductance on K contractures. K contractures elicited in fibres equilibrated in low external Cl ion concentrations (Solution B, Table 1) were invariably larger than contractures elicited in fibres in normal Krebs solution (Solution A, Table 1), even when the [K] × [Cl] products of the normal and high K solutions were the same. We attribute this to failure of high K to reach all parts of the T-system simultaneously. If the tubular length constant is small, as it is at normal Cl concentration (Dulhunty, Carter & Hinrichsen, 1984), different parts of the T-system would depolarize at different times and synchronous contractile activation of the entire fibre could not occur.

TABLE 1. Solutions. Ion concentrations are given in mM

Solution	Na	K	Ca	Cl	SO_4	Sucrose
A	150	3·5	2·5	160·5	—	—
B	80·5	3·5	8·0	16	45	170
C	160	40	8·0	16	100·6	—
D	120	80	8·0	16	100·6	—
E	40	160	8·0	16	100·6	—
F	—	200	8·0	16	100·6	—

In addition all solutions contained: 2·0 mM-TES (*N*-tris-(hydroxymethyl)-methyl-2-amino-ethanesulphonic acid) buffer, pH = 7·4 (adjusted with NaOH); 1 mM-Mg and 11·0 mM-glucose.

When bundles of fibres were exposed to the low Cl control solution (Solution B, Table 1), the twitch and tetanic tensions were initially depressed and then recovered over a period of about 1 h. In order to reduce the length of the experiment, the fine dissection, which normally took about 1 h, was done in the low Cl solution. For this reason all measurements of twitch and tetanic tension were made in the low Cl solution.

Asymmetrical charge movement

Asymmetrical charge movement was measured using the three-micro-electrode end-of-fibre voltage clamp (Adrian, Chandler & Hodgkin, 1970) as described previously (Dulhunty & Gage, 1983). Fibres were bathed in a solution which prevented contraction and minimized ionic conductance containing (mM): TEABr (tetraethylammonium bromide), 145; RbCl, 10; $CaCl_2$, 2; tetracaine HCl, 2; TTX (tetrodotoxin), 10^{-3}; TES (*N*-tris-(hydroxymethyl)-methyl-2-amino-ethanesulphonic acid) buffer, 2. The pH of the buffer was adjusted to 7·4 with NaOH.

RESULTS

K contractures in denervated fibres

K contractures recorded during exposure to a range of K concentrations in a normal e.d.l. (*A*) and an e.d.l. muscle denervated for 29 days (*B*) are shown in Fig. 1. When lower K concentrations (40 and 80 mM) were used, the contractures were clearly larger (relative to maximum contracture tension in 200 mM-K) in denervated than in normal fibres. On average, the relative K-contracture amplitude (i.e. K-contracture tension relative to tetanic tension) was greater in denervated than in normal e.d.l. fibres at all K concentrations (see Fig. 2). With higher K concentrations (160 and 200 mM, Fig. 1), the contractures decayed more rapidly in denervated fibres. The effects of denervation on the time course of K contractures will be described in more detail elsewhere.

Tension records normally show an early rapid peak in tension; this was presumably due to action potentials because the early peak was abolished by 10^{-6} M-TTX. It is interesting to note that the early peak was consistently more pronounced in

denervated e.d.l. fibres (Fig. 1*B*) particularly when lower (40 and 80 mM) K concentrations were used.

Similar results were obtained in normal and denervated soleus preparations (Fig. 1*C* and *D*). The early contraction peak was hidden by the slower phase of the

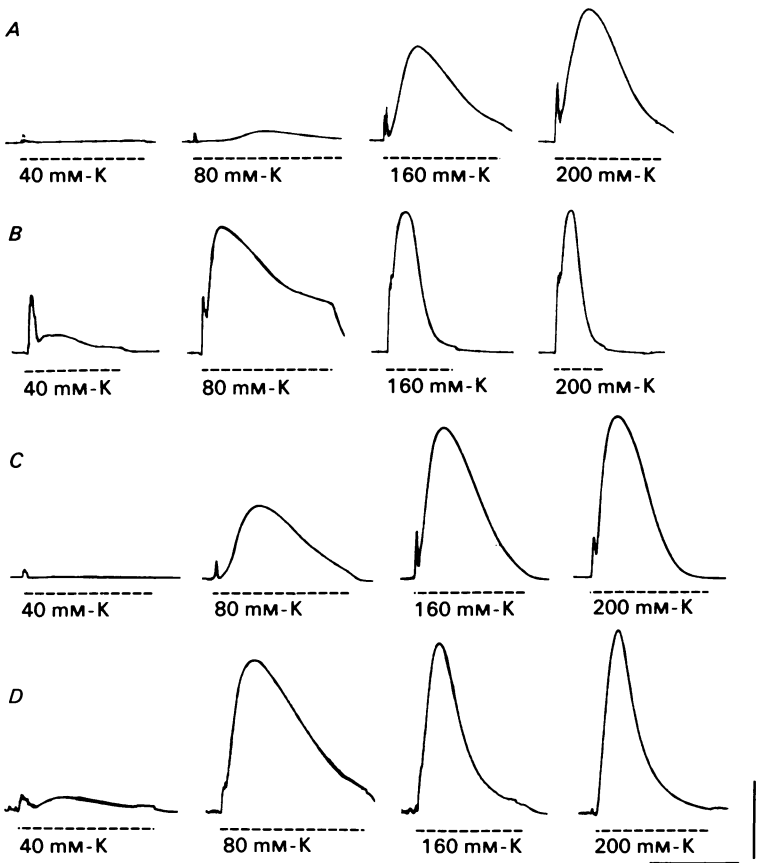

Fig. 1. K contractures recorded in a bundle of fibres from a normal (*A*) and a denervated (29 days) e.d.l. muscle (*B*). Similar results are shown for normal (*C*) and denervated (29 days) soleus (*D*). Bundles were exposed to solutions containing K concentrations as indicated for the times shown by the dashed lines. Temperature: 20–22 °C. Calibrations: vertical, 600 mg in *A*; 120 mg in *B*; 400 mg in *C*; and 180 mg in *D*. Horizontal, 20 s.

contracture in denervated fibres exposed to the 80, 160 and 200 mM-K solutions because of their faster rate of mechanical activation (unpublished observations).

Results such as those illustrated in Fig. 1 were obtained in fourteen to eighteen normal and denervated e.d.l. and soleus preparations and the averaged data are shown in Fig. 2 in which the peak tension during a K contracture relative to the peak tetanic tension (100 Hz stimulation) is plotted against membrane potential. Values for membrane potential were obtained in separate experiments in which potentials

were measured in the K solutions used to evoke the K contractures. The data used to calculate the average values shown in Fig. 2 were taken from muscles that had been denervated for longer than 15 days. A Boltzmann equation was fitted to the data (Dulhunty & Gage, 1983) to provide a basis for comparison of normal and denervated fibres. The equation is useful for this purpose but is not intended as a

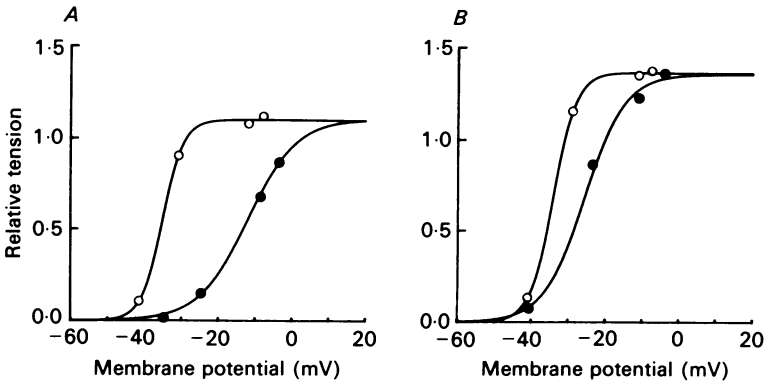

Fig. 2. The relationship between tension at the peak of a K contracture and membrane potential in bundles of fibres from fourteen normal (filled circles) and eighteen denervated (open circles, 15–67 days) e.d.l. muscles (*A*) and from fifteen normal (filled circles) and fifteen denervated (open circles, 16–50 days) soleus muscles (*B*). Tension is expressed relative to the maximum tetanic tension recorded immediately before the contracture and the membrane potentials for the different K concentrations were obtained in separate experiments. The standard error bars were always smaller than the dimensions of the symbols. The continuous lines show the best (least-squares) fit of eqn. (1) (see text) to the data. In *A*, $T_m = 1{\cdot}1$, $\overline{V} = -12$ mV and $k = 6{\cdot}6$ mV for the normal e.d.l. fibres and $T_m = 1{\cdot}1$, $\overline{V} = -35{\cdot}1$ mV and $k = 2{\cdot}8$ mV for the denervated e.d.l. fibres. In *B*, $T_m = 1{\cdot}4$, $\overline{V} = -25{\cdot}7$ mV and $k = 5{\cdot}4$ mV for normal soleus fibres and $T_m = 1{\cdot}4$, $\overline{V} = -34{\cdot}1$ mV and $k = 3{\cdot}03$ mV for denervated soleus fibres.

description of the complex relation between tension and membrane potential (see below). The continuous lines in Fig. 2 show the least-squares fit of the data to

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

where T is relative tension, T_m is the maximum relative tension, V is membrane potential, $\overline{V}$ is the potential at which T equals one-half T_m and $k = RT/AzF$ (R, T, z and F have their usual significance and A is a constant). In both e.d.l. and soleus preparations, denervation caused a marked shift to the left and a change in the slope of the tension–membrane potential curve. The effect was greater in the e.d.l. fibres (Fig. 2*A*).

The differences between normal and denervated muscles were not due to differences in action potentials or in membrane potential. In one experiment, K contractures were obtained from denervated e.d.l. and soleus muscles before and after application of 10^{-6} M-TTX, a concentration sufficient to block twitches and tetanic contractions. Neither the amplitude nor the time course of contractures were altered by the TTX.

TABLE 2. Relationship between tension during K contractures and membrane potential in bundles of muscle fibres dissected from normal and denervated e.d.l. and soleus muscles

	T_m	$\bar{V}$	k
E.d.l.			
Control (n = 14)	1·14±0·02	−11·4±1·01	6·20±0·32
Denervated (n = 18) 15–67 days	1·10±0·06	−35·4±0·47	2·60±0·17
Soleus			
Control (n = 15)	1·35±0·08	−26·6±0·96	4·74±0·21
Denervated (n = 15) 15–50 days	1·37±0·09	−34·2±0·54	3·08±0·19

The variables T_m, $\bar{V}$ and k were obtained from least-squares fits of eqn. (1) to results obtained from individual bundles and are expressed as mean±S.E. of the mean.

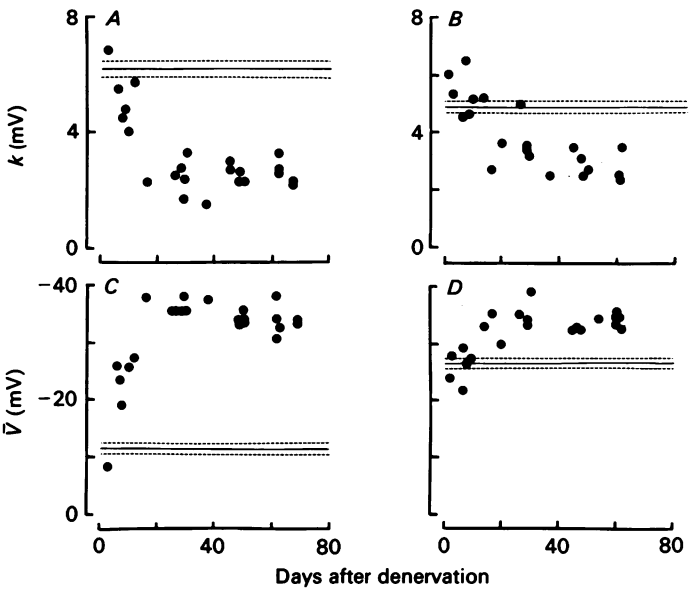

Fig. 3. The time course of the change in the voltage dependence of tension after denervation. The symbols show values for k and $\bar{V}$ obtained from the best (least-squares) fit of eqn. (1) to data from individual preparations denervated for the indicated times (horizontal axis, days after denervation). *A* shows k (mV) for e.d.l.; *B* shows k (mV) for soleus; *C* shows $\bar{V}$ (mV) for e.d.l.; *D* shows $\bar{V}$ (mV) for soleus. The continuous lines show the average normal data and the dashed lines indicate ±1 S.E. of the mean, i.e. k = 6·21±0·32 mV in *A* (fourteen preparations) and 4·74±0·21 mV in *B* (fifteen preparations) and $\bar{V}$ = −11·4±1·0 mV in *C* (fourteen preparations) and −26·6±1·0 in *D* (fifteen preparations).

In separate experiments (A. F. Dulhunty, unpublished observations), membrane potentials were measured in control and high K solutions. There was no difference between the average results for normal and denervated fibres when the membrane potentials were measured in the low external Cl solutions.

The average constants obtained from fitting eqn. (1) to *individual* tension–

membrane potential curves obtained in experiments with normal and denervated (more than 15 days) preparations are given in Table 2. The maximum relative tension was not significantly different from normal ($P > 0.025$, Student's t test). In each case, the values of $\bar{V}$ and k in denervated fibres were significantly different from the control values ($P < 0.0005$, Student's t test).

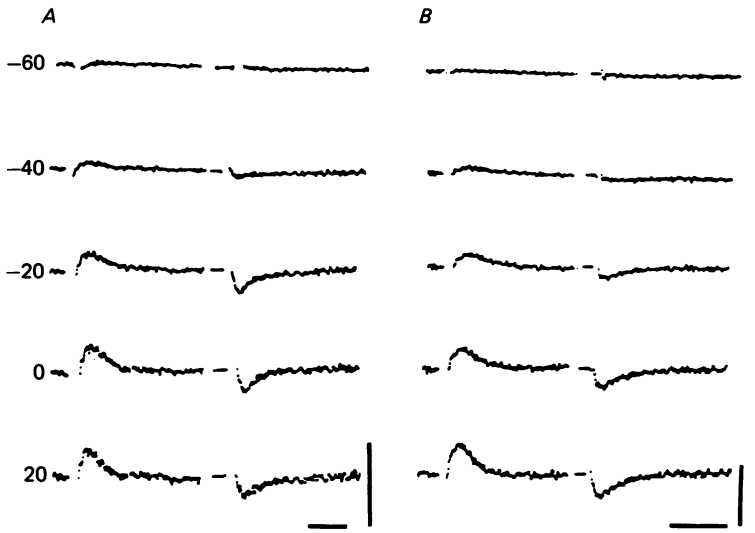

Fig. 4. Asymmetrical charge movement in *A*, a denervated e.d.l. fibre (14 days) and *B*, a denervated soleus fibre (8 days) in response to step changes in membrane potential from a holding potential of −90 mV to the potentials shown (the 2 ms loss of trace preceding many of the records was due to a difference in capacity currents because of the differences in the rate of achieving the new clamp potential with different steps). The fibre in *A* had a membrane capacity of 6·8 μF/cm^2 and a diameter of 54 μm. The fibre in *B* had a capacity of 7·0 μF/cm^2 and a diameter of 74 μm. Temperature, 12·3 °C in *A* and 14·1 °C in *B*. Calibrations: vertical, 3·39 μA/μF in *A* and 4·8 μA/μF in *B*; horizontal, 10 ms.

The development of the changes in the slope and voltage sensitivity of K-contracture tension after denervation are shown in Fig. 3. The values of k (Fig. 3*A* and *B*) and $\bar{V}$ (Fig. 3*C* and *D*) obtained from a least-squares fit of eqn. (1) to data from individual preparations have been plotted against time after denervation. The average values obtained from normal fibres are shown as continuous lines with the dashed lines indicating ± 1 S.E. of the mean (see Table 1). Although there is considerable scatter in the data, it is clear that the major reduction in k occurred within 20 days in both muscles. Similarly, most of the change in $\bar{V}$ occurred within the first 20 days in both muscles (Fig. 3*C* and *D*). There was one important difference between the effect of denervation on $\bar{V}$ in e.d.l. and soleus fibres: there was no discernible change in $\bar{V}$ in soleus fibres during the first week after denervation in contrast to an initial shift of about −10 mV in e.d.l. fibres so that the voltage dependence of tension was approximately the same in e.d.l. and soleus after 7 days. There was then a subsequent, similar shift in $\bar{V}$ of about −15 mV in both muscles.

Asymmetrical charge movement in denervated fibres

The change in voltage sensitivity of the tension–membrane potential curves may have been associated with a change in the potential dependence of asymmetrical charge movement, as occurs following spinal cord transection (Dulhunty & Gage,

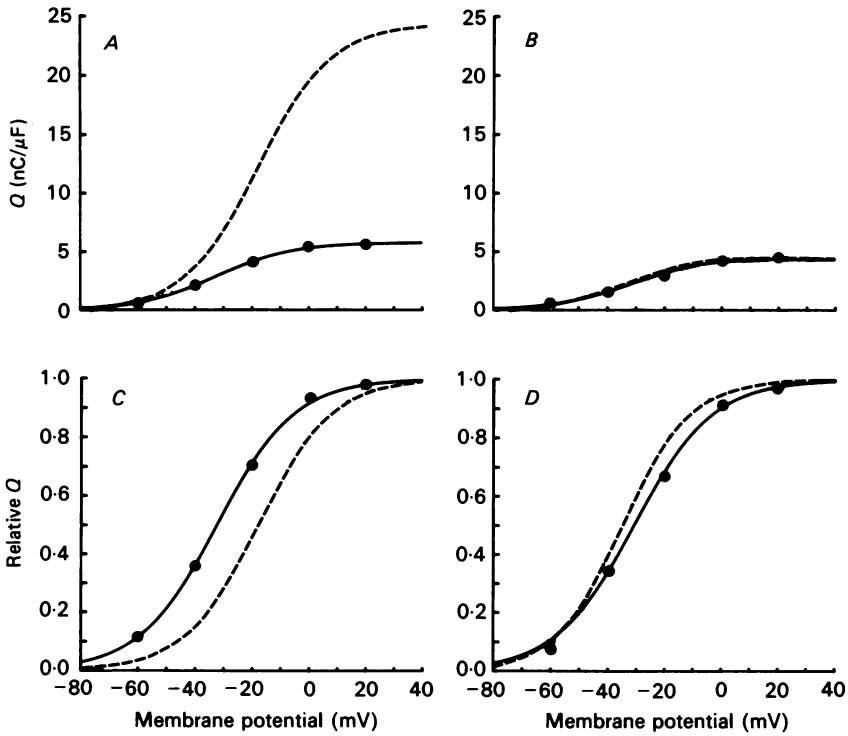

Fig. 5. The effect of denervation on the amount and voltage sensitivity of charge movement. Average values (filled symbols) of charge movement (Q, nC/μF) in thirty denervated (14–40 days) e.d.l. fibres are plotted against membrane potential (V) in A. A similar plot of data from sixteen denervated (18–40 days) soleus fibres is shown in B. The standard error bars are contained within the dimensions of the symbols. The continuous lines through the points show the best (least-squares) fit of eqn. (2) to the data. In A, $Q_m = 5{\cdot}8$ nC/μF, $\overline{V} = -18$ mV and $k = 12{\cdot}8$ mV. In B, $Q_m = 4{\cdot}6$ nC/μF, $\overline{V} = -30{\cdot}8$ mV and $k = 13{\cdot}7$ mV. The dashed lines show the least-squares fit of eqn. (2) to average data from normal fibres (Dulhunty & Gage, 1983). ($Q_m = 24{\cdot}3$ nC/μF, $\overline{V} = -18$ mV and $k = 12{\cdot}8$ mV for e.d.l.; $Q_m = 4{\cdot}35$ nC/μF, $\overline{V} = -34{\cdot}0$ and $k = 11{\cdot}6$ mV in soleus.) To illustrate the changes in $\overline{V}$ in denervated fibres, the data in A and B have been normalized to the same Q_m and replotted in C and D respectively.

1983). Asymmetry currents recorded from an e.d.l. fibre denervated for 14 days (A) and a soleus fibre denervated for 8 days (B) are shown in Fig. 4. These currents are very different from those seen in normal fibres where they are three to four times greater in e.d.l. than in soleus (Hollingworth & Marshall, 1981; Gage & Dulhunty, 1981; Dulhunty & Gage, 1983).

Asymmetry currents were measured in this way in e.d.l. and soleus fibres

denervated for more than 14 days. Averaged results are shown in Fig. 5 in which the amount of charge (Q), obtained by integrating the asymmetry currents, is plotted against membrane potential (V). The continuous lines show the least-squares fit of the data to a Boltzmann equation:

$$Q = Q_m/(1-\exp[-(V-\overline{V})/k]), \quad (2)$$

where Q_m is the maximum charge movement, $\overline{V}$ is the potential at which Q equals one-half Q_m and the other symbols have the same meaning as in eqn. (1). The dashed

TABLE 3. Relationship between charge movement and membrane potential in single fibres from normal and denervated e.d.l. and soleus muscles

	Q_m (nC/μF)	$\overline{V}$ (mV)	k (mV)
E.d.l.			
Control ($n = 8$)	23·4±2·6	−19·0±2·4	13·3±0·9
Denervated ($n = 22$) 14–40 days	5·9±0·3	−31·7±1·1	12·6±0·3
Soleus			
Control ($n = 24$)	4·3±0·5	−36·7±1·6	11·0±0·1
Denervated ($n = 16$) 18–40 days	4·4±0·3	−33·4±0·2	12·7±0·3

The variables Q_m, $\overline{V}$ and k were obtained from least-squares fits of eqn. (2) to results obtained from individual fibres and are expressed as mean±1 S.E. of the mean.

lines show the Boltzmann fits to average data obtained previously in normal preparations (Dulhunty & Gage, 1983). It can be seen that denervation caused a large reduction in Q_m in e.d.l. but not in soleus fibres. In order to compare $\overline{V}$ values, Q is normalized with respect to Q_m in Fig. 5*C* and *D*. The dashed lines again show average curves from normal fibres. There was a negative shift (about 10 mV) in $\overline{V}$ in e.d.l. but not in soleus, following denervation.

Average values of Q_m, $\overline{V}$ and k (obtained from least-squares fits of eqn. (2) to data from *individual fibres* denervated for more than 10 days) are listed in Table 3 and show the large significant fall in Q_m and negative shift in $\overline{V}$ in denervated e.d.l. fibres, but no significant change in denervated soleus fibres. The control data are taken from Dulhunty & Gage (1983). Denervation caused a small but significant decrease in the average slope (k) of the relationship between charge movement and membrane potential in soleus fibres ($P < 0{\cdot}005$, Student's t test) but there was no significant change in k in denervated e.d.l. fibres.

The time courses of the changes in Q_m and $\overline{V}$ after denervation are illustrated in Fig. 6 in which average values obtained from individual muscles denervated for different periods have been plotted against time after denervation. The continuous lines show the average normal values for each parameter and the dashed lines show ±1 S.E. of the mean (see Table 3). Despite the scatter in the data there does appear to be a marked difference between the time course of the changes in Q_m and $\overline{V}$ in the e.d.l. fibres. The decrease in Q_m was largely complete after 14 days (Fig. 6*A*).

The shift in $\bar{V}$ appeared to occur more rapidly and to be complete within a week: there was a negative shift in $\bar{V}$ (of $-12{\cdot}6$ mV) in fibres denervated for 6 days and there was no further significant change during the following 5 weeks (Fig. 6*C*). The data from soleus fibres shown for comparison in Fig. 6*B* and *D* demonstrate that denervation had little effect on charge movement in soleus.

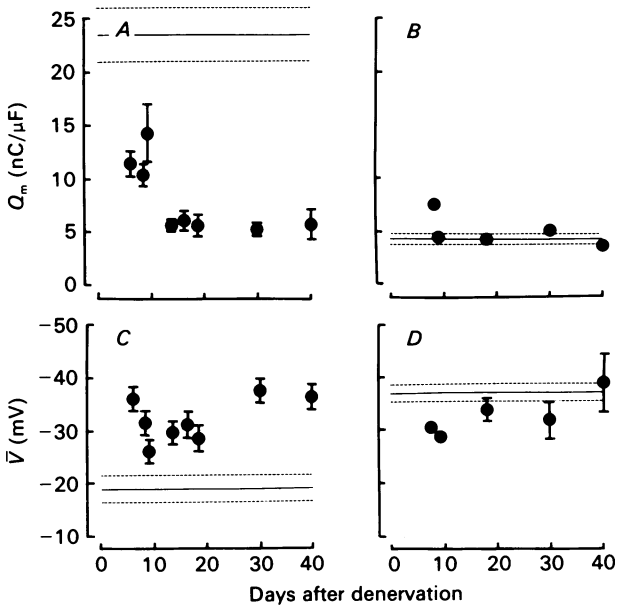

Fig. 6. The time course of the change in charge movement after denervation. The symbols show average values for Q_m (*A* and *B*) and $\bar{V}$ (*C* and *D*) obtained from the least-squares fit of eqn. (2) to data from individual fibres from muscles denervated for the indicated times (horizontal axis, days after denervation). The vertical bars indicate ± 1 S.E. of the mean where this is greater than the dimensions of the symbol. The horizontal lines show the average normal data and the dashed lines indicate ± 1 S.E. of the mean ($Q_m = 24{\cdot}3 \pm 0{\cdot}8$ nC/μF in *A* (eight fibres) and $4{\cdot}35 \pm 0{\cdot}59$ nC/μF in *B* (nine fibres): $\bar{V} = -18{\cdot}6 \pm 1{\cdot}1$ mV in *C* (eight fibres) and $-35{\cdot}8 \pm 1{\cdot}6$ mV in *D* (nine fibres)).

The difference in time course of the changes in Q_m and $\bar{V}$ was further emphasized by the observation that some e.d.l. fibres, denervated for 6–9 days, had charge movement of normal magnitude but the voltage sensitivity had shifted to more negative potentials. This is illustrated in Fig. 7. The Boltzmann curve fitted to the data from this fibre (Fig. 7*B*) gave a Q_m of 26 nC/μF, close to the average normal value of 23·4 nC/μF and much greater than values of Q_m in fibres denervated for longer times (Table 3). On the other hand, the $\bar{V}$ of $-28{\cdot}5$ mV is more negative than the average normal value of -19 mV and close to the average value of -31 mV in fibres denervated for longer times (Table 3).

As the shift in $\bar{V}$ of about -10 mV in denervated e.d.l. fibres occurred very soon after denervation and appears very similar to the early -10 mV shift in the tension–membrane potential curves in denervated e.d.l. fibres, the two may be closely

related. There appeared to be no slowly developing change in $\overline{V}$ for charge movement that could be correlated with the slowly developing −15 mV shift in the tension–membrane potential curve.

Passive electrical properties of denervated fibres

The passive electrical properties of fibres were recorded during charge movement experiments and some interesting effects of denervation were observed. The measure-

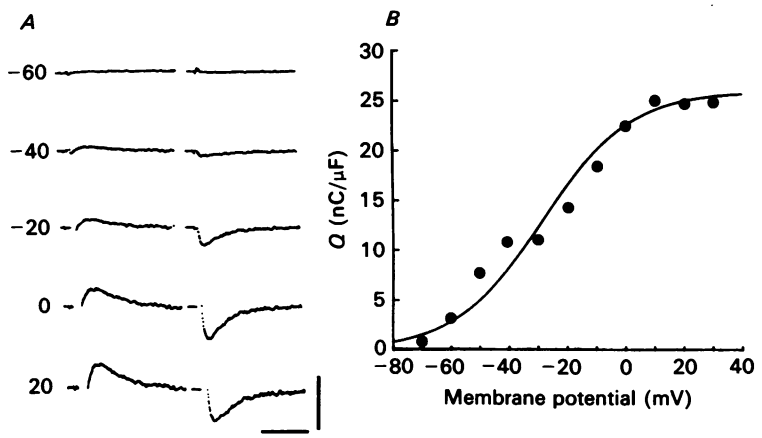

Fig. 7. Charge movement in an e.d.l. fibre denervated for 9 days, Q_m was within the range for normal fibres but $\overline{V}$ was shifted to a more negative membrane potential. Charge movement in *A* was recorded in response to step changes in membrane potential from a holding potential of −90 mV to the potentials shown beside each record. The fibre had a membrane capacity of 5·6 μF/cm^2 and a diameter of 52 μm. Temperature, 13·9 °C. Calibration: vertical, 6·1 μA/μF; horizontal, 10 ms. Charge movement, measured in this fibre (filled circles, nC/μF) is plotted against membrane potential in *B*. The curve shows the least-squares fit of eqn. (2) (see text) to the data and gave $Q_m = 26$ nC/μF, $\overline{V} = -28{\cdot}5$ mV and $k = 15$ mV.

ments were made in the solution used to record charge movement (see Methods) so that the Na, K and Cl conductances were reduced and the values obtained for input resistance, space constant and membrane resistance in normal fibres were predictably higher than values reported for fibres bathed in normal external solutions (Albuquerque & McIsaac, 1970; Dulhunty *et al.* 1984*a*).

The average values for electrical properties of the fibres are listed in Table 4. Average normal values taken from Dulhunty & Gage (1983) are included for comparison with the data from denervated fibres. The most significant effects of denervation were on the calculated diameter (column 4, Table 4) and on the 'specific' membrane capacity of the fibres (C_m column 6, Table 4). The other parameters (space constant (λ), resistance (r_m) and capacity per centimetre of fibre (c_m), and the 'specific' membrane resistance (R_m)) were not altered in a systematic way by denervation. The diameters of denervated fibres were significantly lower than the diameters of normal fibres ($P < 0{\cdot}0005$ for e.d.l. and $P < 0{\cdot}05$ for soleus, Student's *t* test).

Normally the membrane capacity, C_m, of fibres with a smaller diameter is less than that of larger diameter fibres (provided the volume fraction of the T-system, the geometry of the T-tubules and geometry of the fibre surface are constant) because of the relatively smaller contribution of the T-tubule membrane in smaller fibres (Hodgkin & Nakajima, 1972). It is surprising therefore that there was an increase

TABLE 4. Passive electrical properties of fibres from normal and denervated e.d.l. and soleus muscles

	λ (mm)	r_m (kΩ cm)	c_m (nF/cm)	d (μm)	R_m (Ω cm²)	C_m (μF/cm²)
E.d.l.						
Control (n = 6)	2·10±0·10	160±32	160±30	82·4±3·6	4137±663	6·20±0·90
Denervated (n = 22) 6–9 days	1·88±0·13	247±23	164±14	46·1±2·8	3637±430	11·80±0·95
Denervated (n = 31) 14–40 days	1·35±0·07	273±17	112±11	37·6±1·3	3259±225	9·76±0·96
Soleus						
Control (n = 8)	1·30±0·10	297±60	67·3±11·9	53·6±4·2	4993±610	4·00±0·40
Denervated (n = 3) 8 days	1·95±0·19	237±16	194±37	54·2±12·5	4147±1105	11·9±1·6
Denervated (n = 18) 18–40 days	1·47±0·18	282±48	158±19	43·6±3·1	3944±667	11·30±1·30

The variables (space constant, λ; membrane resistance per centimetre length, r_m; membrane capacity per centimetre length, c_m; calculated fibre diameter, d; specific membrane resistance, R_m and specific membrane capacity, C_m) were calculated as described previously (Dulhunty & Gage, 1983). The diameter, d, was not the observed 'diameter' but was calculated by assuming a value of 169 Ω cm for R_i (internal resistivity) at 20 °C and a Q_{10} of 1·37.

in the average membrane capacity of the denervated fibres even though the fibre diameter is reduced. Except for the e.d.l. fibres denervated for longer times, the increase in membrane capacity was statistically significant ($P < 0·001$, Student's t test). This increase in membrane capacity is consistent with the large increase in the volume fraction of the sarcotubular system (i.e. T-system plus sarcoplasmic reticulum) reported in denervated e.d.l. and soleus fibres (Engel & Stonnington, 1974).

The membrane capacities listed in Table 4 are similar to those described for low Cl solutions in other studies (Hollingworth & Marshall, 1981; Dulhunty *et al.* 1984*a*) but are significantly higher than capacities for normal or denervated fibres bathed in Krebs solutions with normal Cl concentrations (see, e.g. Albuquerque & McIsaac, 1970). This difference in values arises from the fact that the T-tubule space constant in mammalian fibres is short in solutions containing a high Cl ion concentration (Dulhunty *et al.* 1984*a*).

DISCUSSION

The results show that denervation of fast (e.d.l.) and slow (soleus) rat muscles shifts the voltage dependence of K contractures to more negative potentials, more so in e.d.l. than soleus. Denervation also causes a change in the magnitude and a similar

shift in the voltage sensitivity of charge movement in e.d.l. but not in soleus fibres. The net effect after denervation for 3 weeks or longer is that the voltage dependence of K contractures is much the same in the two types of fibre. Charge movement also has similar (soleus type) characteristics in the two types of muscle following denervation.

Close examination of the time courses of these changes suggests that the change in voltage dependence of K contractures is slower to develop fully than the change in voltage dependence of charge movement in e.d.l. fibres. In e.d.l. fibres, the early negative shift in the voltage for half-maximal charge generation could be responsible for the initial -10 mV shift in the tension–membrane potential curve (see Results). The later negative shift and increase in slope of the tension–membrane potential curve, seen in both e.d.l. and soleus fibres, must be due to a change in some other aspect of excitation–contraction coupling. This conclusion is supported by evidence from Gutman & Sandow (1965) who found that denervated mammalian fibres are more than normally sensitive to caffeine. Since the contractile proteins do not appear to be altered during the first few weeks after denervation (Syrovy *et al.* 1971; Jaweed *et al.* 1975; Finol *et al.* 1981), it is more likely that denervation affects the relationship between charge movement and Ca concentration in the myoplasm.

There are morphological changes in denervated fibres which could be associated with an increase in the amount of Ca released from the sarcoplasmic reticulum. Engel & Stonnington (1974) found that there was an increase in the volume fraction of the sarcotubular system in e.d.l. and soleus fibres which developed during the first 20 days after denervation. The greater volume of sarcoplasmic reticulum may release more Ca and hence increase the Ca concentration in the myoplasm by a greater amount. If the maximum Ca concentration during a K contracture were greater than the concentration required to saturate the contractile proteins, the increase in the internal Ca concentration could cause a shift in the tension–membrane potential curve to more negative membrane potentials and would lead to an increase in the slope of the relationship between tension and membrane potential. Alternatively, the affinity of the contractile proteins for Ca may increase in denervated muscle.

The early -10 mV shift in the voltage sensitivity of tension and charge movement occurs during the period of rapid turnover in the constituents of the surface membrane (Jeffrey, Leung & Rostas, 1979; Sellin & Thesleff, 1980). It is interesting that, following denervation, some membrane components remain unchanged in soleus but change in e.d.l. to resemble those in soleus (Cotrufo & Savettieri, 1977; Jeffrey *et al.* 1979). Modifications in membrane composition could well influence the voltage sensitivity of membrane processes. For example, the voltage sensitivity of a reaction occurring within the membrane could be affected by alterations in surface charge or in the dipole moment or position of molecules involved in the voltage-sensitive reaction. If a change in surface charge were responsible for the shift in the voltage sensitivity of tension and charge movement, then other voltage-dependent processes should be similarly affected. In accord with this possibility, Thesleff & Ward (1975) have reported evidence for a shift of Na inactivation to more negative membrane potentials in denervated e.d.l. fibres and the records in Figs. 1 and 2 indicate greater action potential activity with depolarization of denervated e.d.l. fibres, which could be due to a shift in the voltage dependence of Na activation.

The amount of charge movement must be related to the nature or number of the charge-generating molecules, presumably proteins, within the membrane (Dulhunty & Gage, 1983). A change in the characteristics of charge movement probably requires a turnover of protein molecules within the T-tubule or the sarcoplasmic reticulum membranes. It is interesting to note that the time course of the change in the amount of charge movement in e.d.l. fibres following nerve section is similar to the time course of other structural changes in the sarcotubular membranes following denervation (Engel & Stonnington, 1974; Dulhunty, Gage & Valois, 1984*b*).

A correlation between the amount of charge movement and the number of indentations in the terminal cisternae of rat e.d.l. and soleus muscles has been suggested previously: the two increase in parallel following spinal cord transection (Gage & Dulhunty, 1981; Dulhunty & Gage, 1983; Dulhunty, Gage & Valois, 1981, 1983). Further evidence to support a role for the indentations in excitation–contraction coupling is provided by the fact that there is a parallel change in the numbers of indentations and the amount of charge movement after denervation. The number of indentations in denervated e.d.l. fibres were significantly lower than normal (Dulhunty *et al.* 1984*b*). Typical freeze-fracture replicas from a normal and a denervated e.d.l. fibre are shown in Pl. 1. The rows of indentations in the terminal cisternae of the normal fibre can be clearly seen (Pl. 1 *A* and *B*) but they are absent in the denervated fibre (Pl. 1 *C* and *D*).

There is little, if any, change in charge movement in denervated soleus fibres, in spite of removal of the normal pattern of activation. It can be concluded that the normal pattern of activity in soleus does not control the characteristics of charge movement. On the other hand, the higher rates of activity of soleus muscles that occur following upper motor neurone lesions *do* modify charge movement (Dulhunty & Gage, 1983). It appears that e.d.l. fibres, when deprived of their sporadic, high-frequency pattern of activation, adopt the charge movement characteristics of normal or denervated soleus fibres.

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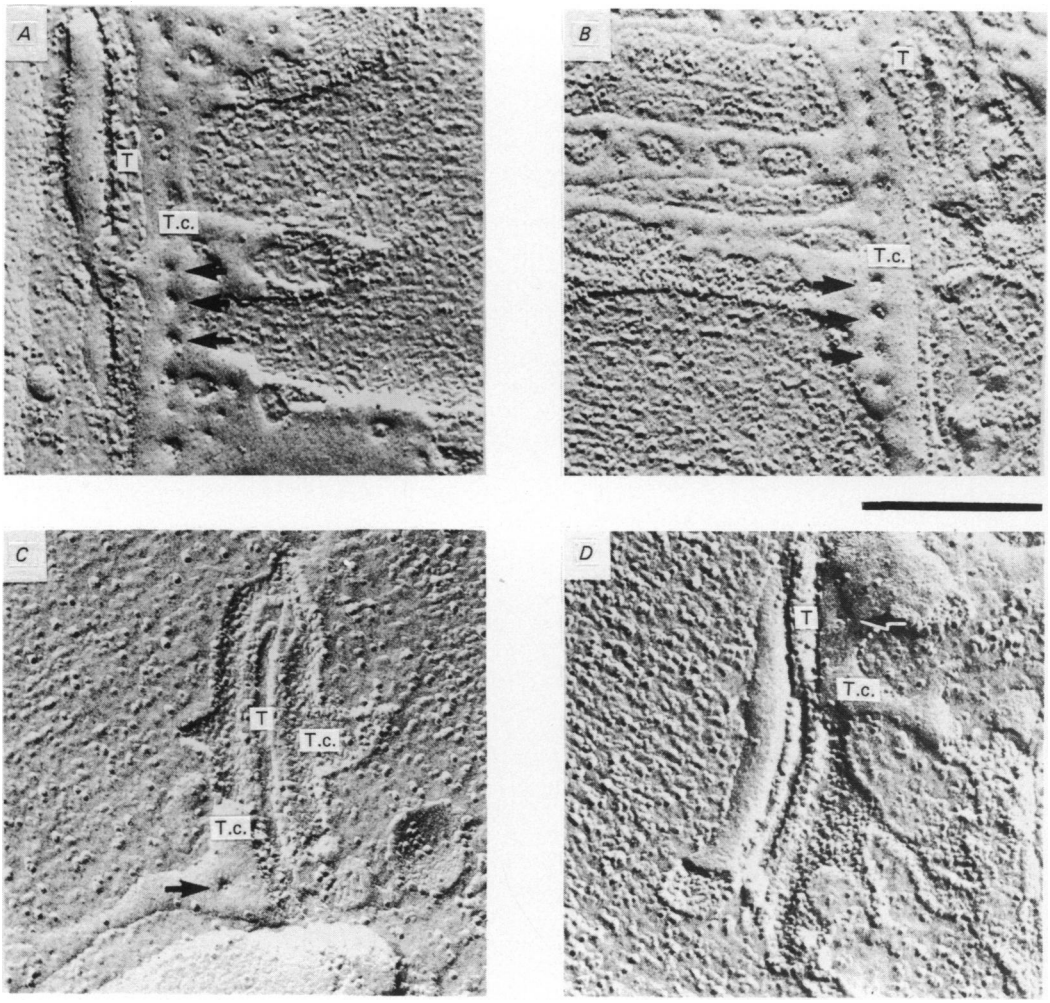
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EXPLANATION OF PLATE

Typical freeze-fracture replicas of two normal e.d.l. fibres (*A* and *B*) and two e.d.l. fibres denervated for 48 days (*C* and *D*). The T-tubules (T) and terminal cisternae (T.c.) have been labelled. Rows of indentations (arrows) are clearly seen in the terminal cisternae of normal e.d.l. fibres but are scarce in the terminal cisternae of the denervated fibres. The calibration bar is 270 nm.