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ROLE OF SODIUM AND POTASSIUM PERMEABILITIES IN THE DEPOLARIZATION OF DENERVATED RAT MUSCLE FIBRES

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SUMMARY

1. Na^+ and K^+ flux measurements and membrane potential (V_m) determinations were performed on normal and denervated rat extensor digitorum longus (e.d.l.) muscles.

2. The mean V_m in normal muscle fibres was $-74{\cdot}6$ mV. During the first week after denervation V_m fell about 20 mV following an S-shaped time course.

3. In that period the Na^+ permeability (P_{Na}) increased and the K^+ permeability (P_K) decreased, so that by the sixth day post-denervation, the P_{Na}/P_K ratio was increased by a factor of 2·7.

4. The decrease in P_K preceded the increase in P_{Na}.

5. No major contribution to the fall of V_m by a reduced activity of an electrogenic Na^+ pump could be detected.

6. A good agreement was found between the experimental values of the depolarization and those calculated using the constant-field equation assuming Cl^- is at equilibrium and no significant change of the intracellular K^+ concentration ($[K^+]_i$) during the first week after denervation.

7. It is concluded that the depolarization promoted by denervation in e.d.l. rat muscle fibres can be fully explained in terms of changes in P_{Na} and P_K.

INTRODUCTION

It has been repeatedly shown that depolarization is one of the earliest changes that can be detected in rat skeletal muscle after denervation (Ware, Bennett & McIntyre, 1954; Locke & Solomon, 1967; Albuquerque & Thesleff, 1968; Albuquerque, Shuh & Kauffman, 1971; Bray, Hawken, Hubbard, Pocket & Wilson, 1976; Robbins, 1977; Wareham, 1978; Shabunova & Vyscocil, 1982; Leader, Bray, Macknight, Mason, McCaig & Mills, 1984).

Through the years such depolarization has been attributed to several mechanisms, by different authors. Thus, Locke & Solomon (1967), McArdle & Albuquerque (1975) and Bray *et al.* (1976) based on measurements of membrane potential (V_m) in the

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presence and absence of ouabain proposed that after denervation muscles depolarize due to an inhibition of an electrogenic Na^+ pump. Robbins (1977), on the other hand, found that the Na^+–K^+ pump of denervated and control e.d.l. muscles was similarly sensitive to ouabain and suggested that the depolarization is mainly due to an increase in P_{Na} rather than to a reduction of the activity of an electrogenic Na^+ pump.

Experiments by Wareham (1978) have also indicated that P_{Na} is increased in denervated e.d.l. and soleus rat muscles. A similar conclusion had been previously drawn by Creese, El-Shafie & Vrbová (1968) working with rat diaphragm. In this same preparation Klaus, Lullmann & Muscholl (1960) detected a fall of P_K of the order of 33–50% after denervation.

The purpose of these experiments was to determine P_{Na} and P_K from measurements of V_m and passive Na^+ and K^+ fluxes in normal and denervated paired e.d.l. rat muscles during the first week after denervation.

Our results indicate that the changes in P_{Na} and P_K can account by themselves for the depolarization observed during this period.

METHODS

All experiments were performed on denervated and contralateral normal e.d.l. muscles from Wistar rats.

Denervation was done under ether anaesthesia by transaction of the peroneal nerve 5 mm from the fibular head. At different times during the first week after denervation both denervated and control muscles were removed and placed in a Krebs solution of the following composition (mM): NaCl, 135; KCl, 5; $CaCl_2$, 2; $MgCl_2$, 1; Na_2HPO_4, 1; $NaHCO_3$, 15; glucose, 11; bubbled with 95% O_2 and 5% CO_2 in a constant-temperature bath at 25 °C (pH 7·20–7·30).

Na^+ influx

Following the technique previously described in detail (Venosa, 1974) paired muscles fastened to light stainless-steel holders by means of thin surgical threads were first exposed to Krebs solution containing 2×10^{-6} M-tetrodotoxin (TTX) for about 30 min and then transferred to an identical medium labelled with $^{24}Na^+$ for 15–17 min. TTX was used to preclude the possibility that spontaneous activity (fibrillation potentials) could increase the Na^+ uptake through voltage-gated channels in denervated muscles under resting conditions. The isotope loading period was followed by a wash-out period lasting 110 min during which muscles were bathed in a series of twelve tubes each containing 2·7 ml of non-radioactive Krebs solution. To minimize the loss of intracellular $^{24}Na^+$ the washing solution contained 5×10^{-4} M-ouabain. Control experiments showed that this concentration of ouabain maximally inhibited the cardioglycoside-sensitive fraction of the Na^+ influx in this preparation. At the end of the wash-out period the muscles were placed in tubes containing 2·7 ml of distilled water and all the tubes were counted in a gamma counter. From the counts per minute (c.p.m.) remaining in the muscles at the end of the experiment and the c.p.m. in the wash-out tubes corrected for radioactive decay, the $^{24}Na^+$ content of a given muscle at the beginning of each collecting period was calculated by back addition. The semilogarithmic plot of c.p.m. remaining in a muscle as a function of the wash-out time has a slow single-exponential component corresponding to the release of the isotope from the intracellular compartment. Its extrapolation to time zero (by regression analysis of the last five points) represents the intracellular c.p.m. at the end of the loading period. The influx was calculated using the following equation (Venosa, 1974):

$$J_i^{Na} = \frac{(\text{c.p.m.})_i}{A S_0 60} \frac{k_o}{(1 - e^{-k_o t})}, \quad (1)$$

where J_i^{Na} is the Na^+ influx (pmol cm^{-2} s^{-1}), $(\text{c.p.m.})_i$ is the intracellular c.p.m. at the end of the loading period, and A is the surface membrane area of muscle assuming a surface to mass ratio

similar to that estimated for soleus muscle, i.e. 1300 $cm^2\ g^{-1}$ (Clausen & Hansen, 1974). It has been shown (Albuquerque & McIsaac, 1970) that membrane capacitance of e.d.l. muscles tends to increase after denervation. This suggests that the reduction in the fibre volume under those conditions (see Fig. 3) by no means implies a decrease in membrane area. Therefore, the estimation of A in denervated muscles was done using the weight of the contralateral normal ones. S_0 represents the specific activity of the labelled solutions (c.p.m./pmol Na^+) and k_o is the $^{24}Na^+$ efflux rate coefficient determined 90 min after the beginning of an efflux run when k_o was reasonably well established. The mean k_o from thirty-three normal muscles in the presence of normal Krebs solution was $0{\cdot}0164 \pm 0{\cdot}0008\ min^{-1}$ and it was not significantly affected by denervation. Thus the mean denervated/control ratios for 2, 4, 6 and 8 days after denervation were: $1{\cdot}20 \pm 0{\cdot}41$ ($n = 8$); $1{\cdot}10 \pm 0{\cdot}31$ ($n = 7$); $1{\cdot}16 \pm 0{\cdot}17$ ($n = 3$) and $1{\cdot}01 \pm 0{\cdot}24$ ($n = 6$), respectively. Even if a 20% change in k_o were statistically significant, that would alter the term containing k_o in eqn (1) by only about 3% for the loading times used in these experiments. Therefore, the above value of k_o was used in all Na^+ influx calculations.

K^+ influx

The technique was identical to that used for Na^+ influx measurements except that the loading solution was labelled with $^{42}K^+$ and contained in addition to TTX, 5×10^{-4} M-ouabain (to block active K^+ uptake). In all the experiments before the loading period, muscles were exposed for 30 min to unlabelled Krebs solution containing the same concentration of ouabain and TTX as the radioactive one. To reduce the release of $^{42}K^+$ from the intracellular compartment the washing unlabelled Krebs solution contained no K^+ and 4 mM-procaine. J_i^K was calculated using eqn (1) for K^+. In K^+ efflux experiments k_o for normal muscles was $0{\cdot}00434 \pm 0{\cdot}00037\ min^{-1}$ ($n = 12$). For 6-days-denervated muscles k_o was not significantly different from the control ones (denervated/control: $0{\cdot}96 \pm 0{\cdot}05$, $n = 4$). For 2- and 4-day-denervated muscles, on the other hand, the ratios were $0{\cdot}73 \pm 0{\cdot}1$ ($n = 4$) and $0{\cdot}86 \pm 0{\cdot}01$ ($n = 4$), respectively, both being statistically different from 1 ($P < 0{\cdot}02$). Although, as mentioned before, these changes in k_o have very little effect on the term containing k_o in eqn (1), they were considered in the calculation of J_i^K because of their statistical significance.

Na^+ and K^+ efflux

Muscles were exposed to normal Krebs solution containing either $^{24}Na^+$ or $^{42}K^+$ for about 2·5 h and then washed in a series of tubes containing 2·7 ml of unlabelled Krebs solution as previously described (Venosa & Horowicz, 1973) and the efflux was expressed in terms of efflux rate coefficient (i.e. fractional loss).

The isotopes were obtained from Comisión Nacional de Energía Atómica.

Membrane potential (V_m) measurements

V_m was measured by means of conventional electrophysiological techniques, using glass microelectrodes filled with 3 M-KCl with resistances ranging from 5 to 20 MΩ.

Statistics

Student's t test was used to estimate the statistical significance of differences.

RESULTS

V_m measurements

In order to estimate P_{Na} and P_K during the first week after denervation, V_m measurements were performed at different times during that period.

Figure 1 shows the V_m of paired denervated and control muscles as a function of time after denervation. The mean V_m from twenty control muscles was $-74{\cdot}6 \pm 0{\cdot}4$ mV (± 1 S.E.; $n = 283$ fibres). It can be seen that the time course of depolarization in denervated muscles was S-shaped and after the fourth day V_m tended to a steady level around -53 mV. Table 1 shows all V_m measurements, which include the data

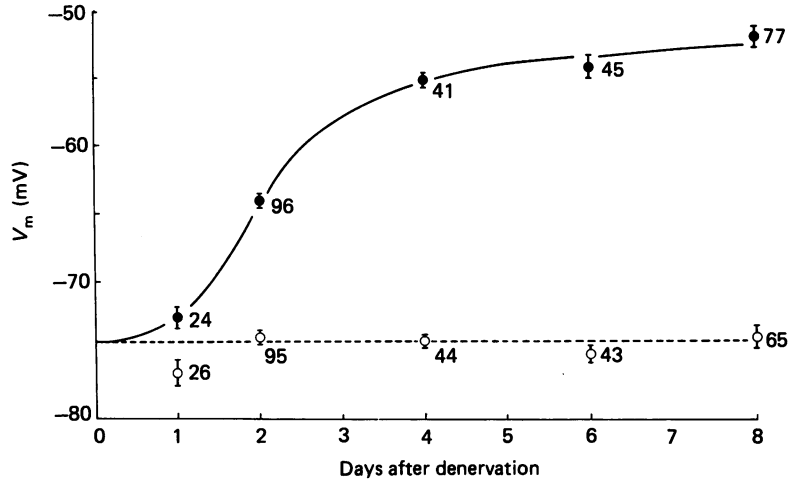

Fig. 1. Resting membrane potential (V_m) as a function of time after denervation. Each pair of experimental points represents the mean of between three and five pairs of control (○) and denervated (●) muscles. The dashed line represents the weighted mean of all control muscles ($n = 17$). The figures next to the experimental points correspond to the number of fibres sampled and the bars represent ±1 S.E. of the mean.

TABLE 1. V_m in control and denervated e.d.l. muscles

Days after denervation	V_m (mV)	Muscles/ fibres
0	−74·6±0·4	20/283
1	−72·6±0·8	3/26
2	−62·4±0·4	6/191
4	−54·6±0·3	5/84
6	−55·5±0·5	5/92
8	−52·8±0·7	3/73

Values are mean±1 S.E.

from Fig. 1 and the controls from Table 2 (second column). Table 2 illustrates the effect of 2×10^{-6} M-TTX on V_m in both control and 2- to 6-day-denervated muscles. It can be seen that while TTX has no effect on V_m of normal muscles it does produce a small but significant hyperpolarization in denervated preparations, as previously shown by Albuquerque & Warnick (1972).

Na^+ influx and P_{Na}

J_i^{Na} was measured in pairs of normal and denervated muscles (2, 4, 6 and 8 days after denervation). Figure 2 illustrates a typical experiment where a 6-day-denervated e.d.l. and the contralateral normal muscle were first exposed to normal Krebs solution labelled with $^{24}Na^+$ and containing 2×10^{-6} M-TTX for 16 min and then washed in unlabelled medium as described in Methods. Under these conditions only TTX-insensitive Na^+ influx was measured. The Figure shows a semilogarithmic plot of the radioactivity remaining in the muscles during the wash-out period and the extrapolation of the slow component to time zero which indicates the intracellular c.p.m. at the end of the uptake period.

TABLE 2. Effect of 2×10^{-6} M-TTX on V_m

Days after denervation	V_m (mV) Control	TTX	ΔV_m	Ratio V_m	P	d.f.	Muscles
0	$-75{\cdot}8 \pm 2{\cdot}1$	$-75{\cdot}9 \pm 2{\cdot}5$	0·1	1·00	n.s.	78	3
2	$-60{\cdot}1 \pm 2{\cdot}2$	$-62{\cdot}8 \pm 2{\cdot}5$	2·7	1·04	< 0·003	190	4
4	$-53{\cdot}9 \pm 2{\cdot}0$	$-56{\cdot}4 \pm 2{\cdot}4$	2·5	1·05	< 0·002	87	3
6	$-56{\cdot}7 \pm 2{\cdot}7$	$-58{\cdot}7 \pm 2{\cdot}7$	2·0	1·04	< 0·02	89	3

Values are mean ± 1 S.D. Degrees of freedom, d.f.

J_i^{Na} is given by eqn (1) and P_{Na} can be obtained from eqn (2) (Hodgkin & Katz, 1949):

$$P_{Na} = J_i^{Na} \frac{RT}{V_m F} \frac{1 - e^{V_m F/RT}}{[Na^+]_o}, \quad (2)$$

where $[Na^+]_o$ is the external Na^+ concentration, V_m is the corresponding value of the resting membrane potential from Table 1 corrected by the slight hyperpolarization effect of TTX on denervated muscles illustrated in Table 2 (fifth column), and F, R and T have their usual meaning.

It should be pointed out that in six control experiments the substitution of NaCl by Tris Cl in the Krebs solution did not affect $^{24}Na^+$ efflux, suggesting that Na^+ for Na^+ exchange does not occur to any appreciable extent across the sarcolemma of e.d.l. muscle fibres. Consequently J_i^{Na} was taken as passive. Table 3 and Fig. 5 illustrate the post-denervatory changes of P_{Na}. It is apparent that until the second day after denervation there was no significant change in P_{Na}, followed by a sharp increase between the second and the fourth day. By the sixth day it reached a relative (denervated/control) steady value of 1·66.

In experiments like the one shown in Fig. 2 the difference at time zero between the total c.p.m. and the c.p.m. corresponding to the extrapolation of the intracellular component represents the extracellular $^{24}Na^+$ space. From this value, S_0, and the weights in paired normal and denervated muscles, the relative fibre volume (denervated/control) can easily be calculated. It was found that this parameter decreases in denervated muscles. Figure 3 shows the time course of the reduction in fibre volume that takes place during the first week following denervation. It is worth noting that the relative fibre volume of 1-week-denervated e.d.l. muscles is attained by frog sartorii not before 40–50 days after denervation (Kotsias, Venosa & Horowicz, 1984).

K^+ fluxes and P_K

Figure 4 shows a typical passive $^{42}K^+$ influx experiment performed on a pair of denervated (6 days) and control muscles (see Methods). P_K was calculated using eqn (2) for K^+ on the reasonable assumption that with the active K^+ transport blocked by ouabain the measured J_i^K was all passive (in control experiments we found that Na^+ efflux was maximally reduced by that concentration of ouabain). V_m measurements showed that ouabain produced a slight depolarization in innervated as well as in denervated muscles which might be due to the inhibition of an electrogenic Na^+–K^+ pump (see Table 4). It is not unlikely that a small accumulation of K^+ in the

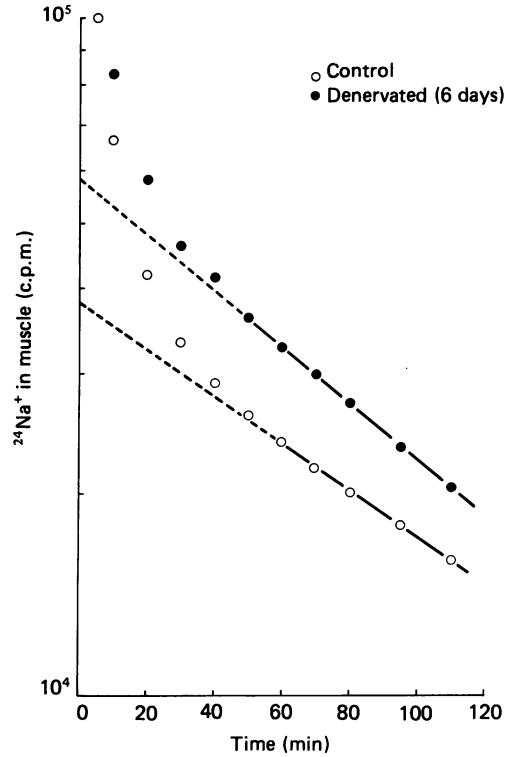

Fig. 2. Wash-out of $^{24}Na^+$ from a pair of control (○) and 6-day-denervated (●) e.d.l. muscles in the presence of Krebs solution containing 5×10^{-4} M-ouabain. Just before the wash-out both muscles had been exposed for 16 min to Krebs solution labelled with $^{24}Na^+$. The dashed lines represent the intracellular components of the efflux and their extrapolations to time zero the respective intracellular contents of $^{24}Na^+$ at the end of the loading period. Na^+ influx and P_{Na} were calculated as previously described (see Methods).

TABLE 3. P_{Na} in control and denervated e.d.l. muscles

Days after denervation	J_i^{Na} (pmol cm^{-2} s^{-1})	P_{Na} (pm s^{-1})	P^d_{Na}/P^c_{Na}	n
0	4·60±0·50	93·4±10·4	0·94±0·14	6 pairs
2	3·65±0·36	86·8±13·5		
0	3·34±0·39	67·9±8·0	1·55±0·25	6 pairs
4	4·02±0·39	104·1±6·4		
0	3·93±0·69	79·7±14·0	1·66±0·14	6 pairs
6	5·29±0·66	131·0±16·0		

Values are means±1 S.D.

extracellular space as a result of the pump inhibition might also contribute to the observed depolarization. This depolarization is similar, in absolute value, to the hyperpolarization promoted by TTX so that the two effects roughly cancelled out in these experiments where the loading solution contained both drugs. Therefore, the values of V_m used in the calculation of P_K from influx experiments were those shown in Table 1.

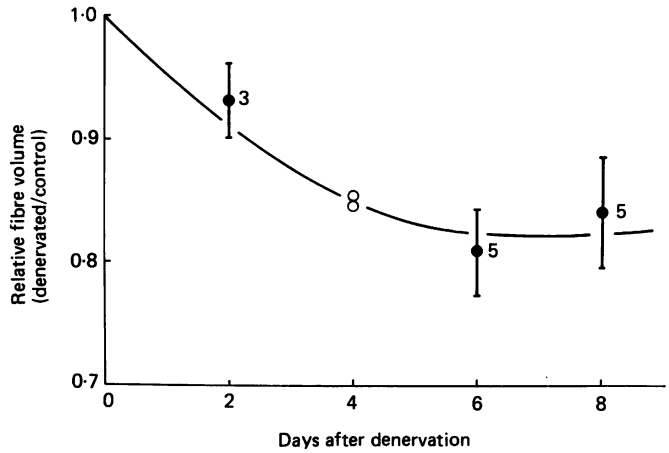

Fig. 3. Relative fibre volume (denervated/control) as a function of time after denervation. Filled circles and vertical bars represent means ± 1 S.E. and the figures next to them the number of muscle pairs used. The open circles are single experiments.

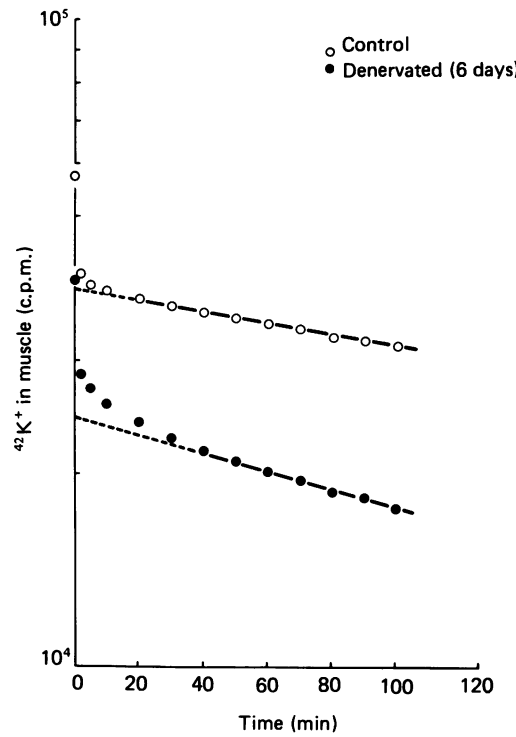

Fig. 4. Wash-out of $^{42}K^+$ from an influx experiment performed on a pair of control (○) and denervated (●, 6 days) muscles, similar to that shown in Fig. 2 for $^{24}Na^+$.

In Table 5 the data from J_i^K experiments are summarized. P_K of denervated (d) relative to control (c) muscles was also calculated from efflux experiments using the following expression:

$$\frac{P_K^d}{P_K^c} = \frac{k_o^d}{k_o^c} \frac{v^d V_m^c (1 - e^{-V_m^d F/RT})}{v^c V_m^d (1 - e^{-V_m^c F/RT})}, \quad (3)$$

where v is the fibre volume and k_o is the K^+ efflux rate coefficient (fractional loss).

TABLE 4. Effect of ouabain (5×10^{-4} M) on V_m

Days after denervation	V_m (mV) Control	V_m (mV) Ouabain (5×10^{-4} M)	ΔV_m (mV)	Ouabain / Control	P	d.f.
0	-74.9 ± 0.4	-70.3 ± 0.6	-4.6	0·94	0·0001	241
2	-59.7 ± 0.7	-60.9 ± 0.8	1·2	1·02	n.s.	88
4	-57.5 ± 0.5	-52.9 ± 0.4	-4.6	0·97	0·0001	78
6	-54.6 ± 0.6	-52.9 ± 0.5	-1.7	0·92	< 0·02	93

Values are mean ± 1 S.E. Abbreviations: d.f., degrees of freedom; n.s., not significant.

P_K from efflux experiments can be calculated using the constant-field flux equation (Hodgkin & Katz, 1949):

$$P_K = J_o^K \frac{RT}{V_m F} \frac{e^{V_m F/RT} - 1}{[K]_i e^{V_m F/RT}}. \quad (4)$$

J_o^K is related to k_o by (Keynes & Lewis, 1951):

$$J_o^K = k_o [K^+]_i \frac{v}{A}, \quad (5)$$

where A is the fibre surface membrane area. From eqns (4) and (5):

$$P_K = k_o \frac{v}{A} \frac{RT}{V_m F} (1 - e^{-V_m F/RT}). \quad (6)$$

On the assumption that A does not change significantly after denervation (see Methods) eqn (3) is obtained.

Table 5 (lower section, third column) shows the P_K^d/P_K^c ratio calculated using eqn (3). Although the estimates from both unidirectional fluxes clearly indicate that denervation reduces P_K, the relative decrease in this parameter calculated from J_o^K is larger than that computed from J_i^k determinations at all times after denervation. At present we have neither a simple explanation for such a difference nor a good reason to prefer one set of values over the other. Thus, it seemed reasonable to consider the mean P_K^d/P_K^c ratios (see Table 5, lower section) to estimate the post-denervation changes of the P_{Na}/P_K ratio (Table 6). As can be seen in Table 6 P_{Na}/P_K underwent a marked change following denervation: by the sixth day it had increased by a factor of 2·7.

Figure 5 shows a normalized plot of the time course of the changes in V_m, P_{Na}, P_K and P_{Na}/P_K.

It is interesting to calculate the magnitude of the depolarizations (ΔV_m) that the observed changes in the P_{Na}/P_K ratio would produce and to compare them with the

TABLE 5. P_K in control (c) and denervated (d) muscles

From K^+ influx

Days after denervation	J_i^K (pmol cm^2 s^{-1})	P_K (pm s^{-1})	P_K^d/P_K^c	n (pairs)
0	1·88±0·11	1257±73	0·64±0·00	4
2	1·05±0·06	755±63		
0	1·75±0·06	1175±38	0·80±0·05	4
4	1·10±0·05	940±42		
0	2·19±0·08	1470±48	0·78+0·03	4
6	1·37±0·05	1120±41		

From K^+ efflux

	k_o^d/k_o^c	P_K^d/P_K^c	n (pairs)	Mean P_K^d/P_K^c*
2	0·75±0·06	0·49±0·02	4	0·57
4	0·86±0·01	0·42±0·01	4	0·61
6	0·96±0·03	0·46±0·01	4	0·62

Values are means±1 S.E. All control–denervated differences are statistically significant ($P < 0·02$). k_o values were measured at 110 min from the beginning of the efflux experiments. The values of V_m and v^d/v^c used to calculate P_K^d/P_K^c with eqn (3) are those shown in Figs 1 and 3, respectively. * From J_i^K and J_o^K.

TABLE 6. P_{Na} and P_K in control (c) and denervated (d) e.d.l. muscles

Days after denervation	P_{Na}^d/P_{Na}^c	P_K^d/P_K^c	$\frac{P_{Na}^d/P_K^d}{P_{Na}^c/P_K^c}$
2	0·94	0·57	1·65
4	1·55	0·61	2·54
6	1·66	0·62	2·68

experimental ΔV_m values. Assuming Cl^- is at equilibrium in e.d.l. muscles (McCaig & Leader, 1984), V_m relates to the ionic distribution across the sarcolemma by eqn (7) (Hodgkin & Horowicz, 1959):

$$V_m = \frac{RT}{F} \ln \frac{[K^+]_o + \alpha[Na^+]_o}{[K^+]_i}, \tag{7}$$

where $\alpha = P_{Na}/P_K$.

If $[K^+]_i$ does not change significantly during the first week post-denervation (see Discussion), ΔV_m would be determined by the change in α and given by eqn (8):

$$\Delta V_m = V_m^d - V_m^c = \frac{RT}{F} \ln \frac{[K^+]_o + \alpha^d[Na^+]_o}{[K^+]_o + \alpha^c[Na^+]_o}. \tag{8}$$

In control muscles the mean P_{Na} and the mean P_K were 83·9±3·7 pm s^{-1} (±1 S.E., $n = 18$) and 1256±45 pm s^{-1} ($n = 12$), respectively, yielding $\alpha^c = 0·067$. Multiplying α^c by the factor listed in the last column of Table 6, the values of α^d for 2-, 4- and 6-day-denervated muscles are obtained so that the corresponding ΔV_m values can easily be calculated using eqn (8). Table 7 shows the calculated and the measured

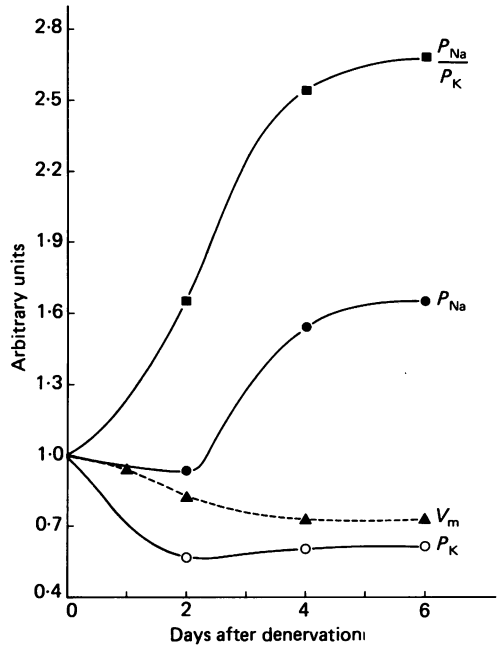

Fig. 5. Normalized plot of the time course changes of V_m (▲), P_{Na} (●), P_K (○) and P_{Na}/P_K (■) in e.d.l. muscle fibres after denervation. V_m values represent the denervated/control ratios from the paired muscles in Fig. 1.

TABLE 7. Post-denervatory depolarization (ΔV_m)

Days after denervation	ΔV_m (mV)		Calculated / Measured	Measured − calculated
	Calculated	Measured		
2	9·3	10·0	0·93	0·7
4	18·2	19·1	0·95	0·9
6	19·3	21·0	0·92	1·7

Calculated values calculated using eqn (8). Measured values from data plotted in Fig. 1.

ΔV_m values. As can be seen, the agreement is quite good. Thus, the conclusion that can be drawn from these data is that the depolarization promoted by denervation in rat e.d.l. muscles can be accounted for by an increase in the P_{Na}/P_K ratio due to both an increase in P_{Na} and a decrease in P_K.

DISCUSSION

The depolarization of uninjured muscle fibres bathed in their normal saline is most likely due to one or more of the following changes: (a) decrease in P_K; (b) increase in P_{Na}; (c) decrease in $[K^+]_i$; and (d) stoppage of an electrogenic Na^+ pump. Each of these alterations have been invoked by different authors as the cause of the post-denervatory depolarization seen in rat skeletal muscle.

Based on the observation that the fall in V_m produced by ouabain in normal

muscles was absent or considerably diminished in denervated muscles, it has been suggested that the post-denervatory depolarization would be due to the inhibition of an electrogenic Na^+ pump (Locke & Solomon, 1967; Bray *et al.* 1976). Our own results, however, do not support this notion. In our hands ouabain (5×10^{-4} M) produced a modest depolarization in both control and denervated muscles (see Table 4). The discrepancy between these results and those of Locke & Solomon (1967) and Bray *et al.* (1976) might be partly due to the fact that we use e.d.l. rather than gastrocnemius, soleus or diaphragm muscles. One can estimate the contribution of an electrogenic Na^+ pump in normal muscle fibres. For example, let us assume that the pump extrudes only Na^+ (Na^+/K^+ coupling ratio = ∞) and that all the Na^+ efflux were active. Then, for a membrane resistance (R_m) of the order of 545 Ω cm^2 (Albuquerque & McIsaac, 1970) and in steady state ($J_o^{Na} = J_i^{Na} = 3{\cdot}83$ pmol cm^{-2} s^{-1}; see Table 3), the contribution of the pump (V_p) to the membrane potential would be given according to Ohm's law, by $V_p = J_o^{Na} F R_m = 0{\cdot}20$ mV. This suggests that the inhibition of an electrogenic Na^+ pump would not play any relevant role in the development of the post-denervatory depolarization.

An alternative explanation for the depolarizing effect of ouabain would be that suggested by Robbins (1977), namely that the drug would increase P_{Na}. But it still does not clarify the qualitative and quantitative differences between our data and those of Locke & Solomon (1967) and Bray *et al.* (1976) with regard to the effect of the drug on V_m of control and denervated muscles. We checked this point by measuring J_i^{Na} in the presence and in the absence of 5×10^{-4} M-ouabain in paired muscles. P_{Na} of control muscles (P_{Na}^c) was calculated assuming $V_m = -74{\cdot}6$ mV (see Results) and that of the companion muscles (pre-equilibrated) for 30 min in unlabelled ouabain–Krebs solution, P_{Na}^{oua}, by taking $V_m = -74{\cdot}6 \times 0{\cdot}94 = 69{\cdot}9$ mV (see Table 4). The mean P_{Na}^{oua}/P_{Na}^c ratio was $1{\cdot}04 \pm 0{\cdot}24$ ($n = 4$) indicating that ouabain does not alter P_{Na} in e.d.l. muscle fibres ($P > 0{\cdot}45$).

Robbins (1977) estimated P_{Na} using Li^+ virtually as an isotope of Na^+ and on the assumption that, as in frog muscle (Keynes & Swan, 1959), Li^+ is not actively transported by rat muscle fibres, he concluded that during the first 2 days after denervation V_m is reduced because of an increase of P_{Na}. The present results indicate that in e.d.l. muscles the changes in both P_{Na} (increase) and P_K (decrease) occur and suffice to account for the depolarization observed after denervation. Moreover, it should be noted that although the P_{Na}/P_K ratio seems to determine by itself the degree of depolarization, the decrease in P_K precedes the increase in P_{Na} so that during the first 2 days following denervation, and at variance with Robbins' (1977) results, the early increase in P_{Na}/P_K ratio appears to be entirely due to the fall in P_K with no significant change in P_{Na} (Fig. 5).

The other change that could affect V_m is a drop in $[K^+]_i$. The published data, however, indicate that $[K^+]_i$ either does not change or at the most might decrease by only about 10% at the end of the first week following nerve transection (Drahota, 1960; Kovacs, Vissy & Went, 1968; Robbins, 1977; Arrizurieta de Muchnik, Sosa, Kotsias & Muchnik, 1981; Kotsias, Muchnik, Arrizurieta, Losavio & Sosa, 1985). Actually, the good agreement between the measured depolarizations and those calculated from changes in P_{Na}/P_K ratio (Table 7) makes it unnecessary to assume any significant reduction in $[K^+]_i$ to explain the fall of V_m.

It should be mentioned that the effect of denervation on ionic permeabilities apparently are time and muscle-type dependent. For instance, in e.d.l. muscles denervated for 15–18 days an increase in K^+ conductance (g_K) and a decrease in Cl^- conductance (g_{Cl}) has been observed (Camerino & Bryant, 1976). Also, it was reported that in rat gastrocnemius and soleus muscle while g_{Cl} begins to decrease during the first week post-denervation, g_K decreases during the first week and increases after that period (Lorkovič & Tomanek, 1977).

It has been shown that in e.d.l. muscle fibres like in twitch frog muscle fibres, the resting P_K exhibits inward-going rectification (Duval & Lèoty, 1978) so that it decreases as the difference between V_m and the K^+ equilibrium potential ($V_m - E_K$), i.e. the outward driving force on K^+, increases. It might be possible, therefore, that after denervation a small reduction in P_K by making V_m less negative with no alteration in E_K would increase the outward driving force on K^+ which in turn would decrease P_K further. In frog muscle fibres the reduction in P_K apparently represents a decrease in the K^+ conductance of an inward rectifying channel which is partially offset by an increase in the conductance of a linear high-resistance pathway (Venosa & Kotsias, 1985). At present we do not know if a similar process underlies the post-denervatory fall of P_K in rat e.d.l. muscle.

In summary, our results strongly suggest that the depolarization that develops during the first week after denervation in e.d.l. rat muscle fibres can be fully accounted for by a fall in P_K and an increase in P_{Na}.

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