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SPONTANEOUS ACTIVITY IN DENERVATED MOUSE DIAPHRAGM MUSCLE

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SUMMARY

1. Intracellular electrodes were used to study the discrete depolarizations which trigger fibrillation potentials in chronically denervated mouse diaphragm muscles. Provided that the muscles were perfused on both sides spontaneous activity was maintained *in vitro*.

2. Discrete spontaneous depolarizations, present only in the centre of the muscle, were recorded from the third day of denervation reaching a maximum in prevalence 9–12 days after sectioning of the nerve. These potentials had random occurrence and nearly constant amplitude and frequency within a fibre, dependence of amplitude and frequency on membrane potential, and low temperature dependence.

3. The spontaneous activity was enhanced and could be initiated in previously quiescent fibres by lowering the external Ca concentration. The activity was reduced by increasing external Ca and was abolished at 15 mM-$[Ca]_o$. Tetrodotoxin (10^{-7} M) blocked spontaneous activity.

4. The spontaneous activity was enhanced by the catecholamines isoprenaline and adrenaline (0·5–10 μg/ml.). This effect of isoprenaline was accompanied by an increase in the rate of rise and the amount of overshoot of the action potential.

5. Ouabain (10^{-6}–10^{-4} M) or K^+-free solutions reversibly blocked spontaneous activity. Ouabain (10^{-4} M) reduced the rate of rise and the amount of overshoot of the action potential.

6. Detubulation of muscle fibres with glycerol or the presence of hypertonic solutions abolished spontaneous activity which could not be restarted by reducing Ca or by the addition of isoprenaline.

7. The results support the suggestion that the spontaneous discrete depolarizations which give rise to fibrillation potentials in denervated muscle result from regenerative sodium conductance increases within the transverse tubular system of the muscle fibres. Catecholamines and ouabain could affect this activity either directly, through an action on membrane excitability, or indirectly via the Na^+–K^+ pump.

INTRODUCTION

Spontaneous action potentials (fibrillation) appear in chronically denervated mammalian skeletal muscle. Two types of fibrillation have been described. One type of fibrillation is generated by spontaneous rhythmic membrane potential oscillations by a mechanism similar to anode break excitation (Thesleff, 1963; Thesleff & Ward, 1975). In another type of fibrillation, characterized by irregular discharging fibres, discrete, spontaneous and randomly occurring depolarizations serve as triggers for action potentials (Purves & Sakmann, 1974).

Chronically denervated mammalian skeletal muscle has been shown to produce contractures to applied catecholamines both *in vivo* (Bowman & Zaimis, 1961; Bowman & Raper, 1965; Turkanis, 1969) and *in vitro* (Montagu, 1955; Bhoola & Schachter, 1961; Paterson 1963; Bhoola, Evans & Smith, 1972). The increase in tension produced by catecholamines in denervated muscle is accompanied by an increase in fibrillation (Luco & Sánchez, 1959; Bowman & Raper, 1965).

The purpose of the present work was to study the mechanism underlying the second type of fibrillation, i.e. the spontaneous discrete depolarizations which trigger action potentials and to determine if catecholamines enhance this activity. The study was carried out in the chronically denervated mouse hemidiaphragm muscle, since in that tissue fibrillation almost exclusively seemed to originate from discrete depolarizations and this activity was well maintained *in vitro* (see Methods).

METHODS

Experiments were made on adult male NMRI mice weighing about 20 g or in some instances on male Sprague–Dawley rats weighing 150–180 g. The left hemidiaphragm muscle was denervated by sectioning the phrenic nerve under ether anaesthesia. The muscle was removed at various times after denervation and placed in a constant temperature bath ($\pm 0{\cdot}5°$ C) perfused with an oxygenated fluid for the recording of spontaneous electrical activity. A few experiments were made on either the chronically denervated extensor digitorum longus or the soleus muscle of the mouse.

To maintain the fibrillatory activity of denervated muscles *in vitro* it proved necessary to mount the muscle in such a way as to allow the flow of perfusion fluid on both sides of the muscle. With this mounting and a perfusion rate of approximately 3 ml./min (bath volume 20 ml.) fibrillatory activity was maintained for more than 6 h in the mouse diaphragm muscle. If the rate of flow was reduced or if the fluid was not allowed to circulate on both sides of the muscle fibrillations stopped within 30 min.

Unless otherwise stated the composition of the bathing fluid was in mM: NaCl 135; $NaHCO_3$ 15·0; Na_2HPO_4 1·0; KCl 5·0; $CaCl_2$ 4·0; $MgCl_2$ 1·0; glucose 11·0 and the temperature 37° C. K^+-free solutions were obtained by substituting KCl with NaCl. The pH of the solutions were 7·2–7·4.

Conventional 3 M-KCl micro-electrodes were used for intracellular recording and electrodes filled with 2 M-K citrate for current passing. The input resistance of single fibres and the characteristics of the action potential were obtained by standard intracellular recording techniques, as previously described (Redfern & Thesleff, 1971).

To obtain a value for the amount of spontaneous activity present in the muscle under various experimental conditions the recording micro-electrode was randomly inserted into surface fibres in the region of the former end-plate and the presence or absence of activity noted during a recording time of 1–2 min. The results are expressed as number of fibres with activity/number of fibres examined. The number of muscles tested is given in parentheses after each value.

In many experiments mechanical activity accompanying fibrillation made intracellular recording difficult. In such instances dantrolene Na 3 μg/ml. was added to the bathing fluid for a period of 30 min after which the muscle was returned to normal fluid. Dantrolene sodium is known to attenuate contractions without affecting action potentials (Ellis & Bryant, 1972). Dantrolene had, in the concentrations used, no apparent effect on the spontaneous subthreshold potentials which were the subject of this study. For example, the number of active fibres in normal bathing fluid was 244/493 (22) compared to 110/240 (12) in the presence of dantrolene Na.

To affect the transverse tubular system of the fibres some muscles were detubulated according to the method of Eisenberg & Eisenberg (1968). In these experiments isolated muscles were soaked in oxygenated bathing fluid containing 600 mM glycerol for 30 min, after which they were returned to isotonic bathing fluid for 1 h before the experiment commenced. In other experiments muscles were soaked in solutions made hypertonic by addition of 350 mM sucrose or 135 mM-NaCl to the normal bathing fluid in order to osmotically shrink the tubular system.

To locally reduce the Ca concentration 10 mM ethylenebis (oxyethylenenitrilo) tetra-acetic acid (EGTA) was applied to the muscle fibres by microperfusion through a glass pipette 50–100 μm in diameter.

Tetrodotoxin 3 × crystalline was obtained from Sankyo Co. Ltd, G-strophanthin (ouabain) was supplied by Sandoz Ltd, (−) adrenaline bitartrate from Sigma Chemical Co. Ltd, and (−) isoprenaline bitartrate was a gift from Hässle Ltd. Solutions of isoprenaline and adrenaline in isotonic bathing fluid were prepared immediately prior to use and their doses refer to the salt.

RESULTS

Characteristics of spontaneous activity

In denervated mouse hemidiaphragm muscle fibres spontaneous, discrete and in many instances threshold depolarizations were recorded from the third day of denervation, reaching a maximum in prevalence 9–12 days after sectioning of the nerve. The graph in Fig. 1 shows the percentage of fibres in which this type of activity was present at various times after denervation. Similar potentials could be recorded in the chronically denervated extensor digitorum longus or soleus muscles of the mouse but in these muscles they were much less frequent and were never observed earlier than 10 days after denervation.

Fig. 2 illustrates the different types of potentials recorded. The potentials could have a sharp rising phase followed by exponential decay or a

plateau-like appearance, frequently with either irregular or regular inflexions indicating a complex composition.

The frequency of the spontaneous potentials was relatively constant within a fibre but varied between 0·1 and 25/s among the fibres in a muscle as shown in the diagram in Fig. 3. Their amplitude similarly varied from 0·5 to 9·0 mV among fibres but was relatively constant within a single

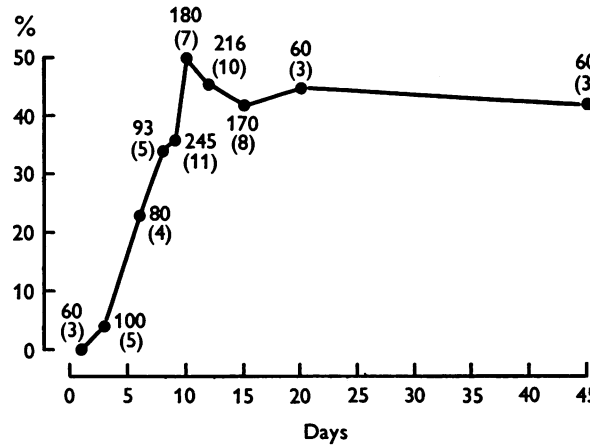

Fig. 1. Time course of the development of spontaneous activity in denervated mouse diaphragm muscle. The number of fibres with activity is expressed as a percentage of the fibres investigated. The figures indicate the number of fibres examined, the number of muscles tested is given in parentheses under each value.

fibre as exemplified by the histograms from three individual fibres shown in Fig. 4. Depending upon potential amplitude and the threshold level of the fibre the potentials were either subthreshold or threshold (see Fig. 2).

Spontaneous depolarizations of the type illustrated in Fig. 2*C* were only observed in the centre part of the muscle-strip, i.e. around the former end-plate region. In 340 fibres (seventeen muscles) examined in regions close to the tendon, similar potentials were never encountered. However, spikes triggered by the local depolarizations were propagated along the fibre and could be recorded at tendon regions. An example of such a fibrillation potential lacking a pre-potential is shown in Fig. 2*D*.

We confirmed the observation of Purves & Sakmann (1974) that the frequency and amplitude of the spontaneous depolarizations were dependent upon the level of the resting potential of the fibre. Hyperpolarization of the membrane by local current injection reduced the frequency and increased the amplitude of these potentials while depolarization of the membrane had the opposite effect. Above -100 mV and below -30 mV

the spontaneous activity was abolished. As described by Purves & Sakmann (1974) tetrodotoxin in a concentration of 10^{-7} M blocked all signs of spontaneous activity. The activity re-appeared when tetrodotoxin was removed from the bathing medium.

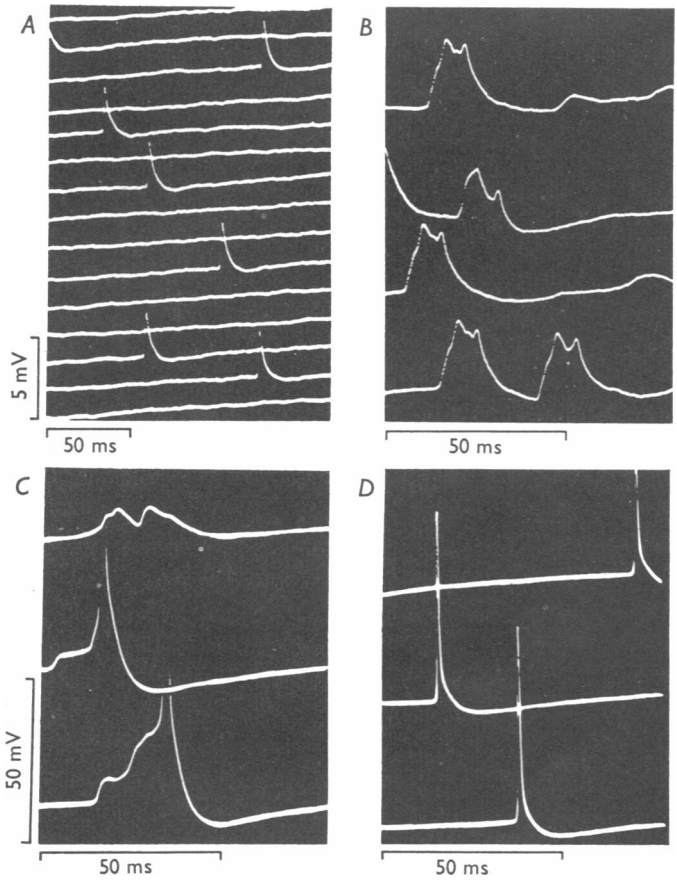

Fig. 2. Representative spontaneous potentials recorded in 9–10 days denervated mouse diaphragm muscles. *A*, discrete subthreshold depolarizations. *B*, depolarizations of complex appearance. *C*, subthreshold depolarizations summating to give rise to action potentials. *D*, fibrillation potentials lacking a prepotential, as recorded in the region of the tendon (records retouched for greater contrast).

Effects of calcium

The dependence upon membrane potential and the blocking effect of tetrodotoxin indicated, as suggested by Purves & Sakmann (1974), that the discrete depolarizations were caused by a regenerative increase in the

Na conductance of the membrane, similar to that associated with the normal action potential. Since the Ca ion concentration markedly affects the regenerative Na carrying system (Frankenhaeuser & Hodgkin, 1957)

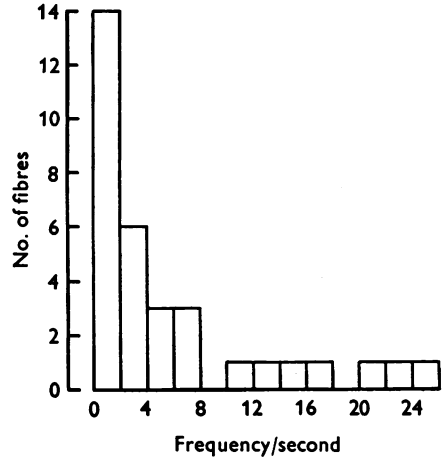

Fig. 3. Frequency distribution of spontaneous potentials in an 11 days denervated muscle. The mean frequency is 4·5/s (n = 33).

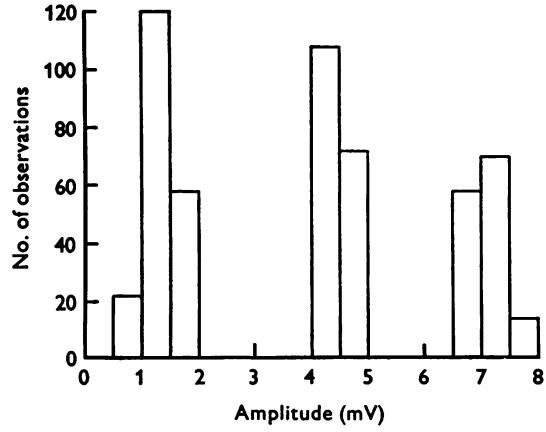

Fig. 4. Amplitude distribution of discrete spontaneous potentials in three fibres in a 9 days denervated mouse diaphragm showing the narrow distribution of amplitudes within each fibre.

it was of interest to examine the effects of high and low Ca concentrations on spontaneous activity. As shown by the diagram in Fig. 5 increasing the external Ca concentration decreased spontaneous activity and at 15 mM abolished it completely in all fibres. Lowering the Ca concentration to 0·5 mM enhanced spontaneous activity by approximately 20 %. The resting

membrane potential underwent only minor changes (± 5 mV) in the presence of these Ca concentrations (Fig. 5). A more drastic effect of the external Ca concentration is shown by an experiment in which the area of a fibre,

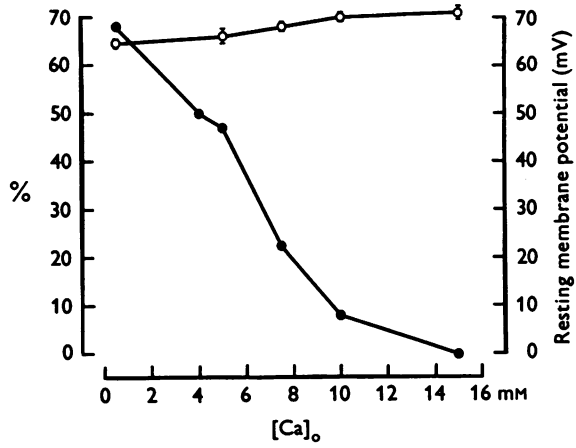

Fig. 5. The effect of changing the external calcium concentration $[Ca]_o$, on the amount of spontaneous activity in 10–12 days denervated mouse diaphragm muscles and on the resting membrane potential of the muscle fibres. ● number of fibres with activity expressed as a percentage of 120 fibres (six muscles) examined. ○ mean resting membrane potential ± S.E. from sixty fibres (four muscles).

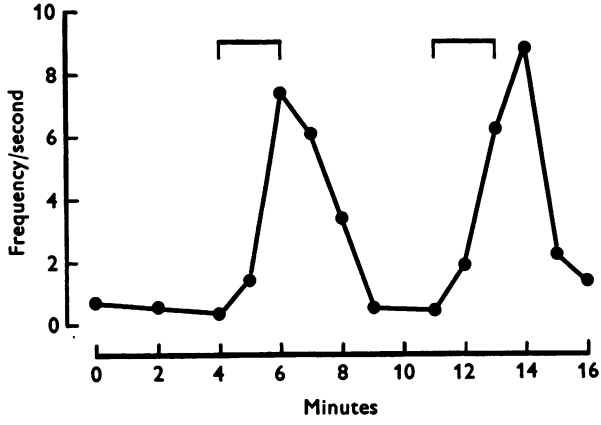

Fig. 6. Effect of EGTA on the frequency of spontaneous potentials as recorded in a single muscle fibre of a 10 days denervated mouse diaphragm. The horizontal bars indicate the period of local superfusion with EGTA (10 mM).

which was spontaneously active, is locally superfused by a solution containing EGTA to reduce the ambient Ca level. Fig. 6 illustrates such an experiment and shows a marked and reversible increase in the spontaneous

potential frequency during the period of superfusion with EGTA. This technique was also very effective in inducing activity in previously quiescent fibres provided that the superfusion occurred in the centre part of the muscle. It was, however, not possible to induce spontaneous activity by superfusing the tendon part of the muscle.

Effects of temperature

The possibility that the temperature of the bathing fluid affected the amount of spontaneous activity in the muscle tissue was examined by determining the number of fibres with discrete depolarizations at 37, 22 and 14° C. At 37 and 22° C activity was recorded in 85/160 (eight) and 55/100 (five) fibres, respectively, whilst at 14° C the number of active fibres was 9/60 (three). Thus reducing the temperature of the bathing fluid from 37 to 22° C had little effect on spontaneous activity, whereas lowering the temperature to 14° C decreased the number of spontaneously active fibres by 38 %. Reducing the temperature also altered the time course of the spontaneous depolarizations, increasing their amplitude and slowing both their rise time and decay. Clearly, therefore, temperature does affect these spontaneous potentials but the dependence is not marked.

Effects of isoprenaline and adrenaline

Isoprenaline in a concentration above 0·1 μg/ml. increased the percentage of fibres with spontaneous activity and produced a maximum effect at 0·5 μg/ml. At that concentration the number of active fibres had increased by about 29 % (from 40/90 (four) to 66/90 (four)), i.e. to about 73 % of the total number of fibres. Increasing the concentration of isoprenaline up to 10 μg/ml. had no further effect on the number of fibres active. Fig. 7 shows that isoprenaline, when added in a concentration of 1 μg/ml., also augments the frequency of spontaneous potentials as recorded in a single fibre. The full effect of isoprenaline on spontaneous activity required 1–3 min to develop and upon removal of the drug spontaneous activity only slowly, within approximately 30 min, reverted to control values. Adrenaline had similar effects but was in general less active (Fig. 7).

Effects on electrical membrane properties

Conceivably catecholamines could stimulate spontaneous activity by a number of different mechanisms. The possibility existed that they directly affected the membrane properties in such a way as to facilitate regenerative sodium conductance or that they influenced metabolic events in the cell which in turn enhanced membrane excitability. We therefore examined the effects of isoprenaline on a number of electric membrane properties,

the study being carried out in the innervated and in the chronically denervated rat hemidiaphragm preparation in which the fibre diameter is larger than in the mouse (Gauthier & Padykula, 1966). Insertion of two closely spaced micro-electrodes into the relatively thin fibres of the mouse diaphragm muscle caused damage to the membrane as indicated by a drop in resting membrane potential. The results obtained are shown in Table 1. It is noticeable that in the presence of isoprenaline the resting

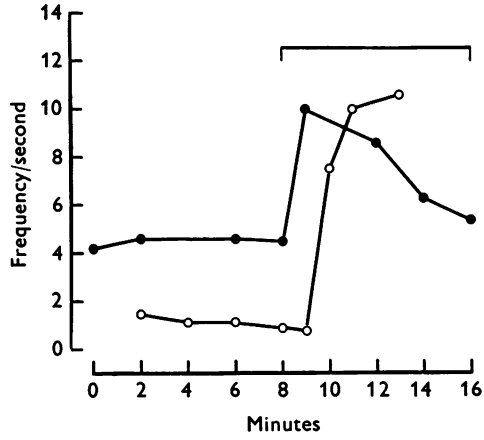

Fig. 7. Effect of isoprenaline and adrenaline on the frequency of spontaneous potentials as recorded in single fibres of a 9 days denervated mouse diaphragm muscle. The horizontal bar indicates the period of bath perfusion with drug. ○, isoprenaline, 1 μg/ml.; ● adrenaline, 1 μg/ml.

membrane potential increased by about 5 mV in chronically denervated as well as in the innervated muscles and that the drug increased the maximal rate of rise of the action potential by about 100 V/s in denervated muscles. The amount of overshoot was also increased and the duration of the spike shortened in the presence of isoprenaline, both effects occurring only in the chronically denervated preparation. These actions of isoprenaline on the action potential were not the result of membrane hyperpolarization since the recordings were made at fibres locally polarized to −90 to −100 mV (see Redfern & Thesleff, 1971). From these results it is evident that isoprenaline enhanced regenerative sodium conductance across the membrane of the denervated muscle, since it increased both rate of rise and amount of overshoot of the action potential. This could explain its facilitatory effect on spontaneous activity but does not exclude the possibility of an action by other mechanisms.

TABLE 1. Membrane properties of rat diaphragm muscle fibres recorded at the end-plate, before and 5–20 min after addition of isoprenaline (0·5 μg/ml.), or ouabain (10^{-4} M). The values are means ± S.E. and the figures in parentheses give the number of muscle fibres/the number of muscles examined.

RMP, resting membrane potential; MRR, maximum rate of rise of the action potential; E_{crit}, critical level for action potential generation; OS, amplitude of overshoot of action potential; Duration, duration of action potential at zero potential level

	RMP (mV)	Input resistance (MΩ)	MRR V/S	E_{crit} (mV)	OS (mV)	Duration (ms)
Innervated muscle:						
Control	74 ± 0·7 (36/3)	0·40 ± 0·060 (20/2)	736 ± 22·8 (36/3)	45 ± 0·6 (36/3)	31 ± 1·4 (36/3)	0·9 ± 0·03 (36/3)
+ Isoprenaline	78 ± 0·8** (27/3)	0·43 ± 0·059 (20/2)	706 ± 17·8 (27/3)	46 ± 0·6 (27/3)	31 ± 1·0 (27/3)	0·9 ± 0·04 (27/3)
+ Ouabain	65 ± 0·7** (22/3)	—	670 ± 15·2* (22/3)	47 ± 1·0 (22/3)	29 ± 1·2 (22/3)	0·9 ± 0·02 (22/3)
10 and 11 days denervated muscles:						
Control	64 ± 0·6 (67/6)	0·63 ± 0·039 (20/2)	507 ± 15·3 (67/6)	41 ± 0·9 (67/6)	25 ± 0·9 (67/6)	1·3 ± 0·06 (67/6)
+ Isoprenaline	69 ± 0·8** (37/4)	0·65 ± 0·035 (20/2)	612 ± 15·9** (37/4)	42 ± 0·8 (37/4)	32 ± 0·9** (37/4)	1·2 ± 0·04* (37/4)
+ Ouabain	61 ± 0·5** (33/4)	—	442 ± 12·8* (33/4)	43 ± 0·8 (33/4)	18 ± 0·6** (33/4)	1·4 ± 0·06 (33/4)

* Significant at $P < 0·05$; ** significant at $P < 0·001$.

Effects of ouabain

Hyperpolarization of the membrane without a concomitant change in input resistance in the presence of isoprenaline (Table 1) suggested that the drug stimulated an electogenic Na pump. It was therefore of interest to see if a blocker of the metabolically driven sodium extrusion mechanism, such as ouabain, would affect the spontaneous activity in denervated muscle and its enhancement by isoprenaline.

Ouabain in a concentration of 10^{-4} M abolished spontaneous activity in all fibres, reducing the number of active fibres from 100/160 (eight) to 0/130 (eight). In the presence of 10^{-6} M ouabain the number of active fibre was reduced from 38/80 (four) to 20/80 (four), i.e. by about 23%. The blocking effect of ouabain was irreversible and occurred within a few minutes after its addition to the bathing fluid as shown by Fig. 8. In that experiment discrete depolarizations with a frequency of about 7/S were recorded in a single fibre, when ouabain 10^{-4} M was introduced into the bath the frequency within 8 min gradually declined to zero. In the presence of 10^{-4} M ouabain it was impossible to initiate spontaneous activity either by

passing hyperpolarizing or depolarizing currents, or by reducing calcium concentration or by addition of isoprenaline.

As shown by the values in Table 1, ouabain (10^{-4} M) within the period of 20 min decreased membrane potential and reduced the rate of rise and

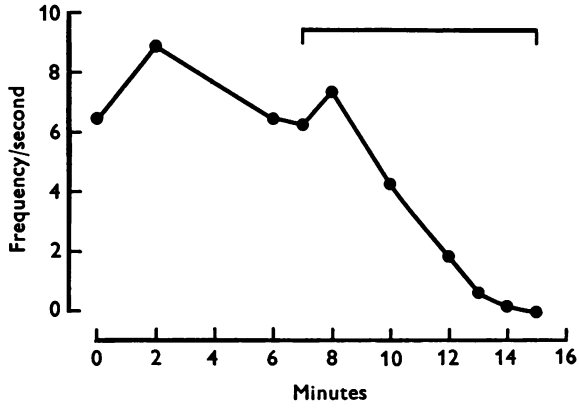

Fig. 8. Effect of ouabain on the frequency of spontaneous potentials as recorded in a single muscle fibre of a 10 days denervated mouse diaphragm. The horizontal bar indicates the period of bath perfusion with ouabain (10^{-4} M).

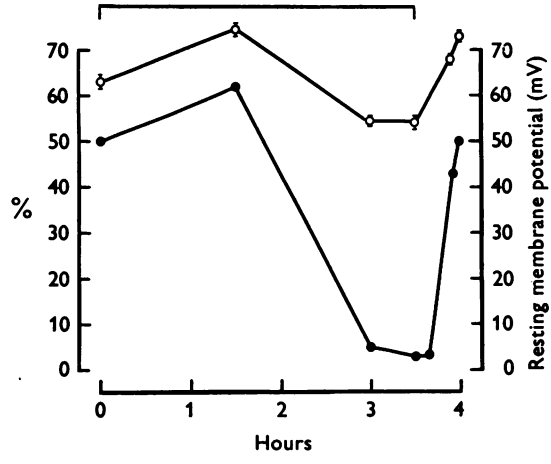

Fig. 9. Effect of K^+-free bathing solution on the amount of spontaneous activity in denervated mouse diaphragm muscles and on the resting membrane potential of the muscle fibres. The horizontal bar indicates the period of perfusion with K^+-free bathing solution. Recovery occurred in 5 mM-K^+ solution. ●, number of fibres with activity expressed as a percentage of 100 fibres (five muscles). ○, mean resting membrane potential ± S.E. from sixty fibres (five muscles).

amount of overshoot of the action potential in denervated muscle. This finding suggested that the blocking effect of ouabain might not be related to its action on Na^+–K^+ exchange *per se*, but due to a decrease in the regenerative Na conductance of the membrane of denervated muscle. To examine this point further, experiments were made with K^+-free bathing solution. Removal of extracellular K^+ increases intracellular Na in skeletal muscle through inhibition of the Na pump (Kernan, 1962) and its reintroduction into the bathing fluid is an effective stimulator of Na^+–K^+ exchange (Kernan, 1962, Cross, Keynes & Rybova, 1965; Adrian & Slayman, 1966). As shown by the graph in Fig. 9 perfusion of denervated muscle with K^+-free bathing solution for 3·5 h caused an initial increase followed by a gradual decrease in the percentage of fibres with spontaneous activity. These modifications of spontaneous activity were accompanied by changes in the resting potential of the muscle fibres. During the first 90 min of exposure to K^+-free solution there was a slight hyperpolarization by about 10 mV. After this period the membrane potential decreased progressively to a value of -54 mV (see Fig. 9). Reintroduction of K^+ (5 mM) to the bathing solution caused a repolarization of the resting membrane potential and a recovery of spontaneous activity which was complete within 30 min. No enhancement of spontaneous activity was, however, observed.

Origin of spontaneous activity

As already mentioned, the discrete depolarizations which were responsible for irregularly firing fibrillation potentials were only observed in the centre part of a muscle fibre and never in regions close to the tendons. The procedures which started and enhanced spontaneous activity in the centre of a fibre, i.e. depolarization by current, low external calcium concentrations or the addition of isoprenaline all failed to induce activity outside that region.

Purves & Sakmann (1974) suggested the possibility that the discrete depolarizations may represent spontaneous regenerative activity within the transverse tubules of the fibre. To test this possibility we examined the muscles for the presence of spontaneous activity before and after procedures known to affect the tubular system.

Treatment with glycerol disrupts the transverse tubules while it leaves action potential generation unimpaired (Zachar, Zacharova & Adrian, 1972). Glycerol treatment irreversibly abolished spontaneous activity in all fibres, reducing the number of active fibres from 128/240 (twelve) to 0/240 (twelve). Low concentrations of Ca or addition of isoprenaline failed to restore activity in glycerol treated muscle fibres.

Hypertonic solutions osmotically affect the transverse tubules, reducing their lumen but leaving the action potential intact (Huxley, Page & Wilkie,

1963; Freygang, Rapaport & Peachey, 1967). Solutions of twice normal tonicity blocked all spontaneous activity in 240 fibres (ten muscles) examined. This effect was reversible, activity reappearing upon immersion of the muscles into isotonic solution. In the presence of the hypertonic media it was impossible to initiate activity by reducing Ca or by adding isoprenaline.

From the results of these experiments it appears that the integrity of the transverse tubular system is a prerequisite for the presence of irregular spontaneous activity and its initiation of fibrillation in chronically denervated muscle.

DISCUSSION

Purves & Sakmann (1974) described depolarizing events of two types associated with spontaneous activity in rat diaphragm muscle strips maintained in organ culture. They observed that spontaneous activity originated from either rhythmical membrane oscillations or from randomly occurring discrete depolarizations. In our experiments, *in vitro*, the origin of fibrillation potentials in 3–12 days denervated diaphragm muscles of the mouse was exclusively of the latter type, i.e. originating from spontaneous discrete depolarizations. In this respect mouse diaphragm muscle appears to be unique since in both denervated mouse soleus and extensor digitorum longus muscles, maintained under similar conditions, spontaneous depolarizations were observed much less frequently and in denervated rat diaphragm muscle fibres they were not encountered. The presence or absence of discrete spontaneous depolarizations in denervated muscle fibres may be dependent upon fibre diameter. Mouse diaphragm muscle fibres are small compared to mouse extensor digitorum longus or soleus muscle fibres or rat diaphragm muscle fibres (Gauthier & Padykula, 1966; Rowe & Goldspink, 1969) and will therefore have a higher input impedance. As a result, the depolarization produced by a given current will be larger and therefore more discernible in these fibres compared to the others (Katz & Thesleff, 1957). The observed occurrence of spontaneous activity in mouse extensor digitorum longus and soleus muscles after longer periods of denervation, i.e. during the period when these muscle fibres are atrophied, is in agreement with this idea.

It is interesting that spontaneous activity was only maintained in denervated mouse diaphragm muscle provided that the muscle was adequately perfused on both sides with bathing fluid. This could indicate that oxygen sensitive metabolic processes were involved in the generation of activity. On the other hand, it was found that spontaneous activity had a low temperature dependence which seemingly refutes such a possibility. An interesting alternative, not examined in the present study, is that a

factor may be released from denervated muscles, which if allowed to accumulate within the tissue, inhibits spontaneous activity.

The mechanism underlying the generation of spontaneous depolarizations is difficult to explain. Purves & Sakmann (1974) showed that they were unaffected by curare but were reversibly abolished by tetrodotoxin or by removal of Na from the bathing fluid and that they were dependent upon membrane potential. They suggested that their mechanism is related to a regenerative Na conductance change similar to that associated with the normal action potential. The observed dependence of these potentials on external calcium ion concentration supports this idea because of the well known effect of Ca on excitability (Frankenhaeuser & Hodgkin, 1957).

The results of the present study show that in denervated mouse diaphragm muscle fibres spontaneous depolarizations are only present and can only be initiated in the centre of the fibre. This is in contrast to the observations of Purves & Sakmann (1974) on rat diaphragm muscle fibres maintained in organ culture who reported a prevalence for sites of origin in the region of the former end-plate but also at the costal insertion. In denervated rat skeletal muscle the maximum rate of rise of the action potential is greater at the former end-plate region than in extrajunctional areas, indicating that this part of the muscle fibre has a higher electric excitability (Thesleff, Vyskocil & Ward, 1974). This could explain why spontaneous activity is preferentially generated in this region.

It has been suggested that since the spontaneous depolarizations are highly localized and are not conducted along the muscle fibre they may represent regenerative activity within the transverse tubules (Purves & Sakmann, 1974). The present finding that spontaneous activity was abolished by tubular disruption or by hypertonic solutions, which cause osmotic shrinkage of the tubules, supports this idea.

In the present study it was found that adrenaline and isoprenaline increased both the total number of denervated muscle fibres with discrete depolarizations and the frequency of these depolarizations as recorded in single fibres of the muscle. These observations would explain the potentiating effect of catecholamines and sympathetic stimulation on fibrillation in denervated skeletal muscle (Luco & Sanchez, 1959; Bowman & Raper, 1965; Luco & Luco, 1971). The results could also explain the increase in tension produced by these drugs in denervated muscle, if, as Bowman & Raper (1965) have suggested, there is a causal relationship between the catecholamine evoked increase in fibrillatory activity and contracture. The basis for the mechanism of action of the catecholamines is unknown but from the present findings it would appear that in denervated muscle isoprenaline enhances the regenerative Na conductance, increasing the rate of rise and amount of overshoot of the action potential. Isoprenaline

is known also to stimulate the Na^+–K^+ pump in mammalian skeletal muscle (Dockry, Kernan & Tangney, 1966; Evans & Smith, 1973) and blocking this exchange mechanism with ouabain or by K^+-free solution abolished spontaneous activity. It is therefore possible that the activity of the Na^+-K^+ pump enhances the excitability of the transverse tubular system.

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