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# STUDIES ON THE MECHANISM OF FIBRILLATION POTENTIALS IN DENERVATED MUSCLE

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SUMMARY

1. Intracellular electrodes were used to study the origin of fibrillation potentials in chronically denervated rat muscle.

2. Fibrillation potentials were observed to start from spontaneous biphasic membrane potential oscillations. Each action potential was followed by an after-hyperpolarization which in turn served as a pre-potential for the next spike. The critical level (threshold) for the initiation of the first spike in a train was lower than that of the next and subsequent spikes.

3. A correlation was found between the level of membrane potential and the critical level for action potential generation. This relation was most marked around the resting membrane potential (−60 to −80 mV) where 10 mV hyperpolarization caused a 9 mV increase in the critical potential level. At higher membrane potentials the correlation was less pronounced. In innervated muscles a similar correlation existed but it was less marked and was present only at membrane polarizations below the resting potential.

4. Increasing the external calcium concentration from 2 to 8 mM reduced the membrane potential – critical level relationship in denervated fibres towards that of innervated ones.

5. As critical level changed with membrane hyperpolarization, the rate of rise of the action potential increased, suggesting a progressive removal of sodium inactivation.

6. It is suggested that a mechanism similar to anode break excitation is important for the induction and maintenance of fibrillation potentials.

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## INTRODUCTION

It is well known that following denervation, spontaneous repetitive action potentials (fibrillation potentials) appear in mammalian skeletal muscle. The origin and membrane mechanism underlying this activity are, however, poorly understood. In the present investigation we have tried to study some of the alterations in membrane properties which may be responsible for the initiation and maintenance of repetitive spontaneous action potentials.

## METHODS

Experiments were carried out on extensor digitorum longus (EDL) muscles of male Wistar rats weighing 200–250 g. The muscles were denervated by sectioning the peroneal nerve at the knee under ether anaesthesia. Rats used for *in vivo* studies 6–12 days after denervation were anaesthetized with urethane (1·75 g/kg I.P.). The anterior surface of the EDL muscle was exposed by reflecting the tibialis anterior muscle and prevented from drying out by covering the muscle with a pool of liquid paraffin saturated with water. Under these conditions, blood supply and fibrillatory activity was maintained for a period of several hours. The temperature of the muscle was kept at 37° C.

For *in vitro* studies, the EDL muscle was removed and placed in a constant temperature bath (37° C) perfused with an oxygenated bathing fluid (Liley, 1956).

Conventional 3 M potassium chloride micro-electrodes were used for intracellular recording. Action potentials were generated by the introduction of a second intracellular micro-electrode filled with 2 M potassium citrate. The inter-electrode distance was kept less than 100 $\mu$m. The citrate electrode was also used for polarization of the muscle fibres, allowing the potential to be locally set to levels between resting potential and $-150$ mV (Redfern & Thesleff, 1971). Action potentials were elicited by 5 msec cathodal pulses adjusted to produce an action potential with 1–3 msec latency. The maximum rate of rise and fall of action potentials was obtained by use of an RC differentiating circuit. The critical level of membrane potential for an action potential (i.e. threshold) was taken as the level at which a regenerative response was observed to be initiated. It should be noted that the length constant of muscle fibres is short compared with the total length of the fibre, and so the membrane potential may only be set locally. The potential will then decay exponentially with the length constant to either side of the current-passing electrode, whether a depolarizing or hyperpolarizing current is applied. Provided the two micro-electrodes are inserted close together, it seems reasonable to assume that action potentials will be triggered at the site where depolarization is maximal, i.e. at the stimulating and recording electrode site.

When the calcium concentration of the bathing fluid was changed, isotonicity was maintained by the adjustment of the sodium chloride concentration.

In some experiments, muscles were detubulated according to the method of Eisenberg & Eisenberg (1968). Isolated muscles were soaked in oxygenated bathing fluid containing 4 mM calcium and 600 mM glycerol for 1 hr, after which they were returned to isotonic bathing fluid containing 4 mM calcium for 4 hr before the experiment commenced. After such treatment, muscles maintained their resting potential and were able to generate action potentials without a mechanical twitch.

## RESULTS

### *Experiments in vivo*

In muscles denervated for 6 days or longer, fibrillatory activity was present in a large number of superficial muscle fibres. The resting membrane potential of relatively quiescent muscle fibres was $-62.4 \pm 0.83$ (mean $\pm$ S.E., $n = 30$). The type of spontaneous activity recorded in many fibres is shown in Fig. 1. Action potentials were present in fibres with varying levels of membrane potential, from $-60$ to $-80$ mV, indicating that the resting potential is not critical for the presence of fibrillation potentials. Some spontaneous action potentials arose from a pre-potential whilst others took off abruptly suggesting that they were conducted from sites of origin more than a length constant from the recording site. Action potentials following a pre-potential were more frequently observed in the centre of a muscle fibre, indicating that they originated from that part of the fibre (Belmar & Eyzaguirre, 1966). Each action potential was followed by an after-hyperpolarization which in turn could serve as a pre-potential for a subsequent spike (Fig. 1*a*). In a few fibres, small rhythmic subthreshold potentials were observed in addition to spontaneous action potentials (Fig. 1*b*). These potentials were similar to those described by Muchnik, Ruarte & Kotsias (1973) but we failed to observe that the potentials acted as a trigger for action potentials. The origin of these potentials was not studied.

In many instances, it was possible to record the initiation of repetitive activity in a single fibre as shown in Fig. 1*c*. On such occasions, a train of action potentials was always preceded by rhythmic biphasic membrane potential oscillations increasing in amplitude. When these oscillations reached a certain level, they triggered an action potential which initiated repetitive firing. In some instances, membrane oscillations spontaneously subsided before they triggered an action potential. After the cessation of a train of action potentials, damped oscillations were observed (Fig. 1*d*), and after a period of quiescence, oscillations of increasing amplitude could reappear to restart the cycle.

It was striking that the critical level of the membrane potential for the initiation of the second action potential in a train was higher (i.e. more negative) than that for the first one, and that the amount of overshoot was greater in the second and subsequent spikes, as shown in Fig. 1*c*.

Marshall & Ward (1974) have reported that the critical level for action potential generation is related to membrane polarization in denervated muscle fibres, but not in innervated fibres. Raising the membrane potential of denervated fibres shifted the critical level to a more negative value. This observation could be the basis for the effect of oscillation or

after-hyperpolarizations in the initiation and maintenance of repetitive activity and was therefore examined quantitatively.

The membrane of non-fibrillating fibres was locally polarized by introducing a second current-passing micro-electrode intracellularly, and the

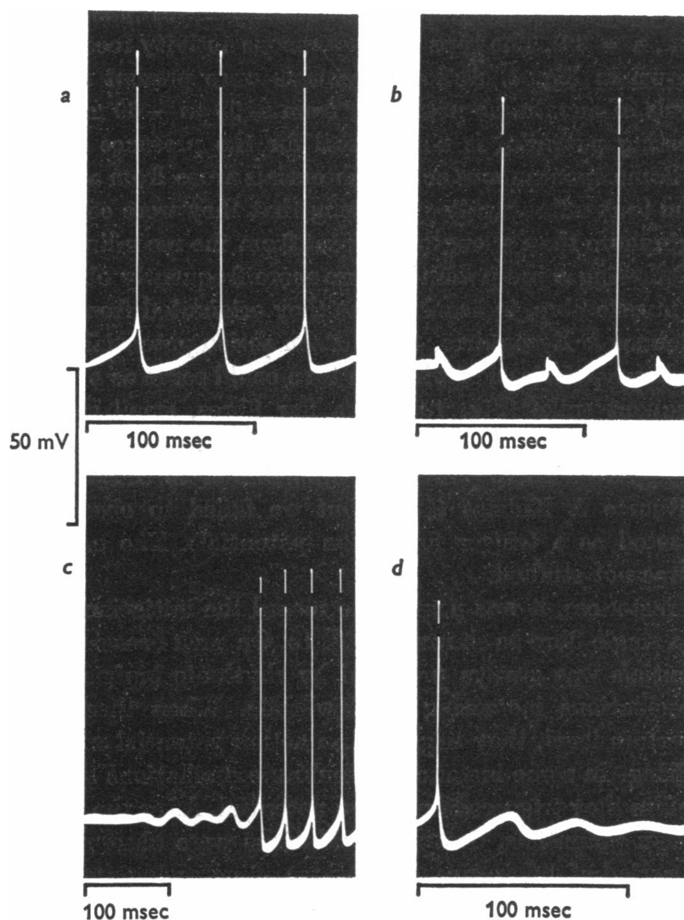


Fig. 1. Representative spontaneous fibrillation potentials recorded in denervated EDL muscles *in vivo*. The break in the trace indicates the zero potential. Time calibrations 100 msec, voltage calibration 50 mV (records retouched for greater contrast).

critical level was determined at potentials between $-60$ and $-150$ mV. Generally, only one action potential was fired in each fibre, and all determinations were made in the centre of the muscle fibre, since fibrillatory activity seems to be more frequently initiated in this part.

The results from such experiments are shown in Fig. 2, from which it is evident that raising the membrane potential markedly increased the critical level. The shape of the graph prompted us to arbitrarily group the results into three ranges of membrane potentials, i.e. −60 to −79, −80 to −114, and −115 to −150 mV. Regression lines were calculated for

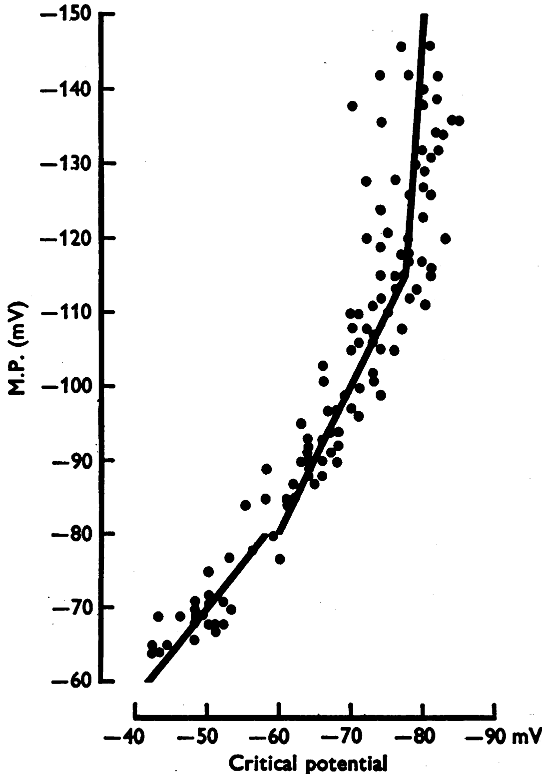


Fig. 2. The influence of the level of membrane hyperpolarization (M.P.) on the critical level for action potential generation in denervated muscle *in vivo*. Each point shows the value obtained in a single fibre. Regression lines were calculated for the membrane potential ranges, −60 to −79 mV; −80 to −114 mV; −115 to −150 mV and were inserted into the Figure. The correlation coefficient for the first two ranges was greater than 0·75 and for the highest range 0·20.

each group and inserted in the Figure. The slope of these lines enabled us to calculate the change in critical level resultant from a 10 mV change in membrane potential. The influence of membrane potential on critical level is most marked between −60 to −79 mV; raising the membrane potential by 10 mV produced a 9 mV shift in critical level. Between −80 and −114 mV, the shift in critical level was 5 mV per 10 mV change of

membrane potential. At higher membrane potentials, the influence of membrane potential on critical level was less pronounced.

*Experiments in vitro*

To investigate in more detail the membrane mechanisms underlying the membrane potential–critical level relationship, experiments were done on isolated muscles *in vitro*, since under such conditions, spontaneous activity quickly ceases, facilitating double micro-electrode impalements.

As shown by the graphs of Fig. 3, the relationship between membrane potential and critical level was in denervated muscles *in vitro* in 2 mM calcium in close agreement with that obtained *in vivo*.

In innervated muscle fibres *in vitro*, a 10 mV increase in membrane potential in the range between $-60$ and $-79$ mV gave a 5 mV increase in critical level, one half of the value obtained in denervated muscle. At levels higher than $-80$ mV, changing the membrane potential had no effect on critical level in innervated fibres.

The observed correlation between critical level and membrane potential could be explained in many ways, some of which are: (i) an alteration in the calcium binding capacity of the denervated muscle membrane, thereby affecting the electric field of the membrane (Frankenhaeuser & Hodgkin, 1957), (ii) an alteration in the activation–inactivation characteristics for sodium conductance, and (iii) changes in potassium conductance mechanisms.

As shown in Fig. 3, increasing the external calcium concentration to 8 mM reduced the effect of membrane potential on critical level in denervated fibres, shifting the relationship towards that found in innervated muscle in 2 mM calcium. In addition, the well known membrane stabilizing effect of calcium was observed, in that not only was the slope of the membrane potential–critical level relationship shifted, but critical levels attained a less negative value when external calcium was increased (see Fig. 4). This decrease in absolute values for critical potentials was approximately 8 mV/fivefold increase in calcium concentration. When external calcium was reduced to 1 mM, the membrane potential–critical level relationship of denervated fibres was greater than *in vivo* or in 2 mM calcium *in vitro*. In this lowered calcium concentration, an action potential usually gave rise to a train of action potentials similar to that observed in fibrillating denervated muscle.

To investigate the possibility that the membrane hyperpolarization was acting on the sodium-carrying system (Hodgkin & Huxley, 1952) it was desirable to estimate the extent of sodium activation at various membrane potentials. In the absence of voltage-clamp conditions, the maximum rate of rise of the action potential was taken as an index of sodium conduc-

tance. It was also desirable to correlate rates of rise with critical level as determined at various levels of membrane potential in a single fibre. This is possible in detubulated preparations since the abolition of excitation–contraction coupling enables the recording of several action potentials without the risk of mechanical twitch damage to the inserted electrodes and the muscle membrane.

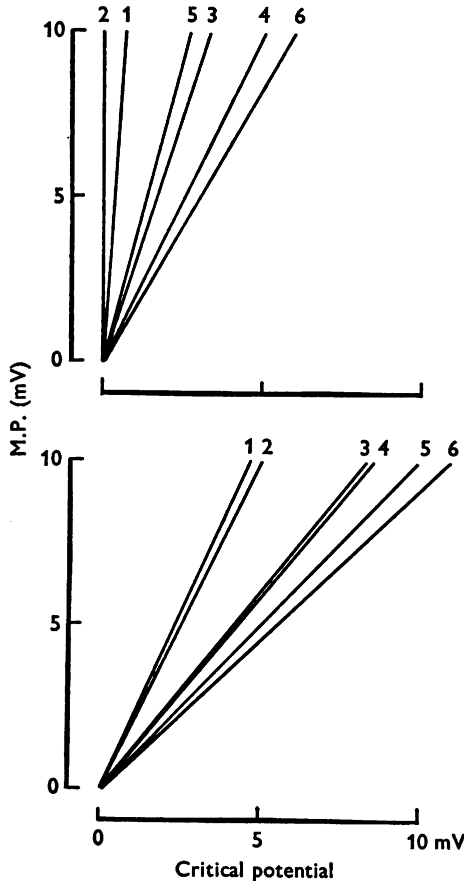


Fig. 3. The graph shows the slope of the regression lines of the relationship between membrane potential and critical level for action potential generation expressed as the change in critical level resultant from a 10 mV membrane hyperpolarization. Lower graph in the membrane potential range −60 to −79 mV and upper graph −80 to −114 mV. The results were obtained from experiments similar to those illustrated in Fig. 2. The numbers marking the lines refer to different experimental conditions: (1) denervated muscle *in vitro* 8 mM calcium; (2) innervated muscle *in vitro* 2 mM calcium; (3) denervated muscle *in vitro* 2 mM calcium and 5 mM-TEA; (4) denervated muscle *in vivo*; (5) denervated muscle *in vitro* 2 mM calcium; (6) denervated muscle *in vitro* 1 mM calcium.

Fig. 4 shows superimposed action potentials at various levels of membrane potential in detubulated fibres. In denervated fibres (Fig. 4*a*), in contrast to innervated fibres (Fig. 4*c*), changes in critical level accompany changes in membrane potential, the relationship being of the same order

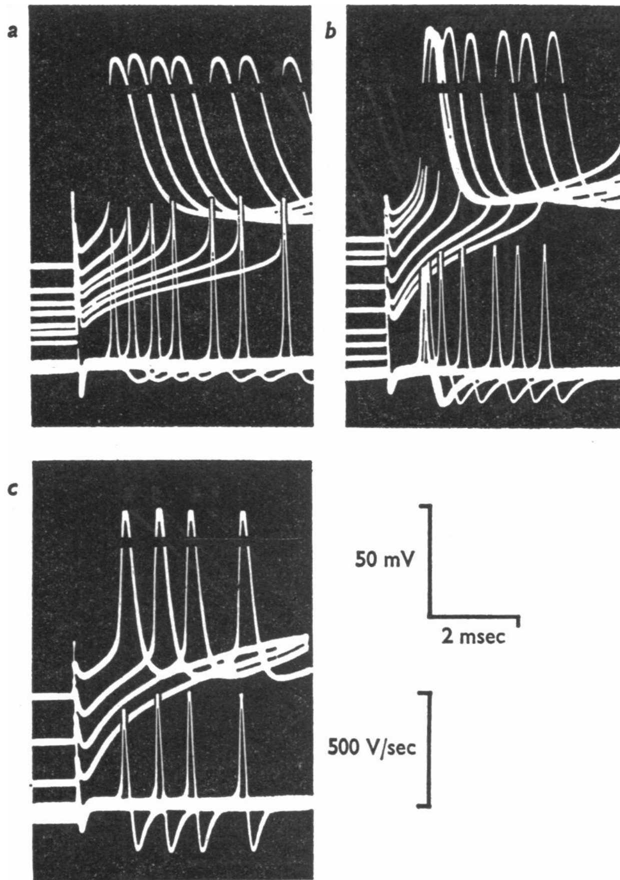


Fig. 4. Superimposed action potentials and their first derivative (lower trace) at various levels of membrane polarization in detubulated fibres, (*a*) in a denervated fibre in 2 mM calcium; (*b*) in a similar fibre in 8 mM calcium and (*c*) in an innervated fibre (2 mM calcium). The break in the upper trace indicates the zero potential (records retouched for greater contrast).

as that observed in intact fibres. The sarcotubular system is not apparently implicated in the mechanism of the critical level shift. In denervated fibres in 2 mM calcium (Fig. 4*a*), the shift in critical level is accompanied by a change in the maximum rate of rise of the action potential. As the critical

level increases with raised membrane potential, the rate of rise of the action potential also increases, and becomes constant at about the point at which the critical level is not further changed by hyperpolarization. In innervated muscle fibres (Fig. 4*c*) or in denervated fibres in 8 mM calcium (Fig. 4*b*), the maximum rate of rise becomes constant at much lower levels of membrane potential.

The possibility that changes in the potassium conductance of the active membrane were responsible for the observed membrane potential–critical level relationship was examined by the use of tetraethylammonium chloride (TEA), an agent which selectively depresses potassium movements across the membrane (see Hille, 1970). TEA (5–10 mM) failed to markedly affect the critical level shift in denervated fibres (Fig. 3).

## DISCUSSION

The recordings from denervated muscles *in vivo* showed that spontaneous action potentials in denervated muscles were initiated by biphasic membrane oscillations of increasing amplitude. Spontaneous electrical activity seemed to be maintained by the prepotential resultant from the after-hyperpolarization of the preceding action potential. This indicated that membrane hyperpolarization was important for the initiation and maintenance of fibrillatory activity and that the action potentials were possibly triggered by a mechanism analogous to that of anodal break excitation. The results of the present study show that a correlation existed between membrane potential and the critical level for action potential generation. This relationship was most marked in denervated muscle at levels around the resting membrane potential (i.e. in the range $-60$ to $-79$ mV), there being an almost one to one relationship between the two parameters. A temporary membrane hyperpolarization would therefore raise the critical level above that of the resting potential, and as the membrane potential returns towards the resting level, an action potential would be triggered. Even if such a relationship holds for innervated muscle, it is only half that of denervated fibres at these levels of membrane potential, and at the resting membrane potential of innervated muscle fibres (about $-80$ mV) it is practically non-existent, and anode break excitation cannot occur (Marshall & Ward, 1974). The membrane potential–critical level relationship became less marked as denervated fibres were hyperpolarized. The rate of rise of action potentials attained their maximal value at the membrane potential at which the critical level became constant, suggesting that the membrane potential–critical level relationship was the result of a progressive removal of sodium inactivation. It would appear that in denervated muscle, the sodium inactivation

($h$) curve is altered as compared to innervated fibres, so that sodium inactivation is only removed at higher levels of membrane potential; the exact conformation of the shift must await voltage-clamp analysis. A simultaneous change in the sodium activation ($m$) curve is not excluded.

As shown by Frankenhaeuser & Hodgkin (1957), the sodium-carrying system of squid axons is more inactivated in low calcium concentrations than in high; reducing calcium has an effect similar to membrane depolarization, whilst increasing calcium reduces inactivation in a way similar to membrane hyperpolarization. Our observation that 8 mM calcium to a large extent removed the correlation between membrane potential and critical level in denervated muscle suggests that calcium was exerting its effect by reducing the proportion of the sodium-carrying system which was in an inactive state.

It could be that physiological levels of calcium ions in denervated muscle are less effective in their membrane stabilizing action, since it is only in increased calcium concentrations that the excitability characteristics of denervated fibres approach that of innervated muscle. In this context, it is of interest to note that decalcification produces membrane oscillation and anode break excitation in nerve fibres (Frankenhaeuser & Hodgkin, 1957; Frankenhaeuser, 1957).

The basis for the initiation of fibrillation potentials would be biphasic membrane potential oscillations of increasing amplitude, the hyperpolarizing phase removing sodium inactivation, thereby increasing the critical level for excitation, allowing the depolarizing phase to exceed threshold. The after-hyperpolarization following each action potential would have a similar influence on membrane excitability and help in maintaining repetitive firing. This mechanism is facilitated when (i) the resting potassium conductance is decreased, as observed for denervated muscle by Klaus, Lüllmann & Muscholl (1960) and Kernan & McCharty (1972) and/or (ii) when the potassium conductance increase with depolarization is delayed with respect to the sodium conductance, as the presence of after-hyperpolarization indicates (Thesleff, 1963). In denervated muscle, the critical level for action potential generation at a given membrane potential is more negative in end-plate regions than in non-junctional zones (Thesleff, Vyskočil & Ward, 1974) and this would explain why fibrillation potentials are preferentially generated in the former end-plate region of a denervated muscle fibre.

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*Note added in proof:* Purves & Sakman (*J. Physiol.* (1974) **239**, 125–153) have described the membrane properties underlying spontaneous activity of rat muscle maintained in organ culture. They observed that the activity originated from rhythmical membrane oscillations and after longer periods in culture from randomly occurring discrete polarizations. In our experiments *in vivo*, the origin of fibrillation potentials in 6–12 days denervated muscles was, in most fibres, of the former type, i.e. originating from membrane potential oscillations.

## REFERENCES

BELMAR, J. & EYZAGUIRRE, C. (1966). Pacemaker site of fibrillation potentials in denervated muscle. *J. Neurophysiol.* **29**, 425–441.

EISENBERG, B. & EISENBERG, R. S. (1968). Selective disruption of the sarcotubular system in frog sartorius muscle. A quantitative study with exogenous peroxidase as a marker. *J. cell Biol.* **39**, 451–467.

FRANKENHAEUSER, B. (1957). The effect of calcium on the myelinated nerve fibre. *J. Physiol.* **137**, 245–260.

FRANKENHAEUSER, B. & HODGKIN, A. L. (1957). The action of calcium on the electrical properties of squid axons. *J. Physiol.* **137**, 218–244.

HODGKIN, A. L. & HUXLEY, A. F. (1952). The dual effect of membrane potential on sodium conductance in the giant axon of *Loligo*. *J. Physiol.* **116**, 497–506.

HILLE, B. (1970). Ionic channels in nerve membranes. *Prog. Biophys. biophys. Chem.* **21**, 3–32.

KERNAN, R. P. & MCCHARTY, I. (1972). Effects of denervation on $^{42}$K influx and membrane potential of rat soleus muscles measured *in vivo*. *J. Physiol.* **226**, 62–63*P*.

KLAUS, W., LÜLLMANN, H. & MUSCHOLL, E. (1960). Der Kalium-Efflux beim normalen und denervierten Rattenzwerchfell. *Pflügers Arch. ges. Physiol.* **271**, 761–775.

LILEY, A. W. (1956). An investigation of spontaneous activity at the neuromuscular junction of the rat. *J. Physiol.* **132**, 650–666.

MARSHALL, M. W. & WARD, M. R. (1974). Anode break excitation in denervated rat skeletal muscle fibres. *J. Physiol.* **236**, 413–420.

MUCHNIK, S., RUARTE, A. C. & KOTSIAS, B. A. (1973). On the mechanism of denervatory electrical activity of single muscle fibers as tested *in vivo* with tetrodotoxin. *Acta physiol. latinoam.* **23**, 24–28.

REDFERN, P. & THESLEFF, S. (1971). Action potential generation in denervated rat skeletal muscle. I. Quantitative aspects. *Acta physiol. scand.* **81**, 557–564.

THESLEFF, S. (1963). Spontaneous electrical activity in denervated rat skeletal muscle. In *The Effect of Use and Disuse on Neuromuscular Functions*, ed. GUTMANN, E. & HNIK, P., pp. 41–51. Prague: Czechoslovak Academy of Sciences.

THESLEFF, S., VYSKOČIL, F. & WARD, M. R. (1974). The action potential in end-plate and extrajunctional regions of rat skeletal muscle. *Acta physiol. scand.* **91**, 196–202.