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THE INFLUENCE OF INACTIVITY ON MEMBRANE RESTING CONDUCTANCES OF RAT SKELETAL MUSCLE FIBRES UNDERGOING REINNERVATION

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SUMMARY

The role of activity in the maintenance of the normal component resting conductances of skeletal muscle fibres has been evaluated *in vitro* in rat extensor digitorum longus muscle during reinnervation from 2 to 40 days (a) after crushing of the peroneus nerve and (b) after local application of tetrodotoxin (TTX) to the crushed nerve. Whereas membrane conductances were regained after crushing alone, they were not completely restored when impulse propagation was blocked with TTX. It is concluded that nerve trophic factors are of primary importance in the control of muscular membrane conductances, and that transmission at the endplate and the muscle usage triggered by it have a minor but significant effect.

INTRODUCTION

In mammalian skeletal muscle, the chloride conductance, G_{Cl}, represents 80% of the total surface and tubular membrane resting conductance, G_m; the remainder is largely potassium conductance, G_K (Palade & Barchi, 1977). G_{Cl} and G_K must be kept at an appropriate level for normal excitability of mammalian muscle fibres. A decreased G_{Cl} has been shown to be the basis of repetitive firing in some forms of hereditary myotonia and in mammalian muscle made myotonic by certain drugs (Bryant & Morales-Aguilera, 1971; Adrian & Bryant, 1974). Denervation of mammalian skeletal muscle has been shown to determine a delayed fall of G_{Cl} and rise of G_K (Bryant & Camerino, 1976; Camerino & Bryant, 1976; Lorkovic & Tomanek, 1977). Thus, the motor nerve exerts control over the resting membrane conductances of skeletal muscles.

In previous studies we gave evidence that factors transported by the microtubular system of the axon are necessary for maintenance of the high G_{Cl} and for suppressing G_K in normal muscles. It was shown that the specific block of axoplasmic transport by chronic application to the nerve of agents known to disassemble microtubules without blocking impulse transmission (colchicine, vinblastine and vincristine) leads to

Key words: Rat, skeletal muscle, membrane conductances, reinnervation, inactivity.

drastic alterations in the membrane conductances (Camerino & Bryant, 1976; Conte-Camerino & Bryant, 1977; Conte-Camerino, 1978). However, we have further shown that there is a partial decrease of G_{Cl} in normal muscles made inactive by local application of tetrodotoxin (TTX) to the motor nerve (Conte-Camerino, Bryant & Mitolo-Chieppa, 1982*a*). To study further the role of muscle activity in the regulation of membrane conductances and other electrical characteristics of skeletal muscles, we compared these parameters in muscles undergoing reinnervation that were spontaneously active, and in muscles reinnervating in the presence of TTX. This toxin has been shown to produce local blockade of nerve impulses without interfering with axoplasmic transport or causing nerve degeneration (Pestronk, Drachman & Griffin, 1976).

RESTING POTENTIAL, CABLE PARAMETERS AND COMPONENT CONDUCTANCES IN ACTIVE REINNERVATING AND INACTIVE REINNERVATING MUSCLES

In one group of rats, the peroneus nerve was crushed 10 mm from its entry into the extensor digitorum longus (EDL) muscle and the animals were allowed to recover. In a second group, the peroneus nerve was crushed and 8–9 days after the crush, a time when reinnervation is known to commence (McArdle & Albuquerque, 1973), the conduction of impulses to the muscle was blocked by surrounding the crushed region and 2–3 mm distally with a loose wrapping of cotton soaked with 10 μg TTX (in citrate-buffer) carefully isolated from the surrounding tissue by a Parafilm cuff (modified technique of Tiedt, Wisler & Younkin, 1977). The cotton soaked with TTX was left in the animals for 7 days and was then replaced with another for an additional 7–8 days. With this procedure the muscles were paralysed continuously from the 8th–9th day to the 23rd–25th day after nerve crush. Paralysis of the limb in all the TTX-treated rats was tested daily by observing the toe-spreading reflex and by stimulating the peroneus nerve immediately before EDL dissection (Bray, Hubbard & Mills, 1979; Conte–Camerino *et al.* 1982*a*).

Between 2 and 40 days after surgery the EDL muscles were removed from the treated and control rats and electrical parameters were measured at 30 °C by intracellular recording. Cable analysis of single muscle fibres was performed by injecting a current pulse through one electrode while recording the change in membrane voltage with another. From the input resistance, space constant, time constant and an assumed myoplasmic resistivity ($Ri = 112\,\Omega cm$) we calculated the fibre diameter, d_{calc}, the membrane resistance, Rm, and the specific membrane capacitance, Cm. Total membrane conductance $G_m = 1/Rm$ is the sum of chloride conductance G_{Cl} and potassium conductance G_K. The large contribution of G_{Cl} to total conductance was determined by taking a second set of measurements in a bathing solution in which all chloride was replaced by an equimolar amount of methylsulphate (see Bryant & Camerino, 1976; Conte-Camerino, Bryant & Mitolo-Chieppa, 1982*b*).

In agreement with the results of Bray *et al.* (1979), the inactivity during reinnervation affected the resting membrane potential, RP, of the EDL fibres (Table

1). It should be mentioned that depolarization of skeletal muscle can lead to block of resting G_K through anomalous rectifying channels (Katz & Lou, 1947) which were not blocked by rubidium (Adrian, 1964) during our experiments. However, as reported below, G_K increased in this study and G_{Cl} does not appear to be sensitive to depolarization over this range of RP (Palade & Barchi, 1977). The membrane resistance, Rm (measured in a chloride-containing solution), first increased at 10–15 days after the crush, from a control value of $340 \pm 27\ \Omega cm^2$ to $1060 \pm 121\ \Omega cm^2$. At 16–18 days it began to decrease ($750 \pm 89\ \Omega cm^2$) towards control values, even undershooting them at 23–28 days ($268 \pm 11\ \Omega cm^2$), and finally reached original values 30–40 days post-operatively ($328 \pm 15\ \Omega cm^2$). In the reinnervating TTX-treated muscles, Rm began to decrease at 19–22 days after the crush. At 23–25 days Rm ($589 \pm 35\ \Omega cm^2$) had not regained the levels of the reinnervating muscles. As found previously (Westgaard, 1975; Cangiano, Lutzemberger & Nicotra, 1977), the inactivity in reinnervating muscles arrested the restoration of normal fibre size. The mean calculated diameters were $45 \pm 2{\cdot}1$, $30 \pm 2{\cdot}5$, $40 \pm 4{\cdot}2$ and $33 \pm 4{\cdot}1\ \mu m$ in control, 10–15 day, 23–28 day reinnervating and 23–25 day TTX-treated fibres, respectively. The differences in the values of Cm measured at any time were not significant (McArdle & Albuquerque, 1973). The mean Cm values were $4{\cdot}3 \pm 0{\cdot}3$, $4{\cdot}8 \pm 0{\cdot}6$, $4{\cdot}5 \pm 0{\cdot}8$ and $4{\cdot}8 \pm 0{\cdot}6\ \mu F\ cm^2$ in control, 10–15 day, 23–28 day reinnervating and 23–25 day TTX-treated fibres, respectively.

The mean resting component conductances were calculated from the membrane resistances (Table 1). In the untreated, reinnervated animals there was first a drastic decrease of G_{Cl} and a significant increase of G_K (days 10–15). G_{Cl} then increased to reach control values (days 30–40) after first overshooting them (days 23–28). In the TTX-treated preparations, the conductances began to approach their original values later, but after 23–25 days of reinnervation they were not completely restored. It was not within the scope of this study to investigate the mechanical properties of muscles during reinnervation (McArdle & Albuquerque, 1973; Bray *et al.* 1979; Herbison, Jaweed & Ditunno, 1980; Taxt, 1983). Nevertheless, in the reinnervating unparalysed rats, 10–11 days after nerve-crush we observed the first weak twitch of the reinnervating muscle in response to nerve stimulation, and about 30 days after nerve lesion the leg movements of the operated rats appeared normal.

CONTROL OF RESTING MEMBRANE CONDUCTANCES

There is little doubt that nerve impulse-related activity plays an important role in the maintenance of the intrinsic properties of skeletal muscle, as direct stimulation can reverse many of the changes associated with denervation (Westgaard, 1975; Lomo & Westgaard, 1975). However, more recently, the exclusive role of impulse-dependent activity in the regulation of muscle properties, such as resting potential and density of extrajunctional acetylcholine receptors, has been questioned (Bray *et al.* 1979). In the present study, we have examined the role of impulse-dependent cholinergic transmission, and the muscle usage triggered by it, in re-establishment of membrane conductances during muscle reinnervation.

Table 1. *Resting potentials and component resting conductances of rat EDL fibres following reinnervation and reinnervation by TTX-inactivated nerves*

Days after nerve crush	Muscle	N	RP (−mV)	G_m ($\mu S\,cm^{-2}$)	N'	G_K ($\mu S\,cm^{-2}$)	G_{Cl} ($\mu S\,cm^{-2}$)
	Control• (9)	35	78±0·9	3239±161	37	228±51	3011±118
5–9	Reinnervating (3)	21	68±2·1[a]	3038±201	20	238±58	2800±149
10–15	Reinnervating (4)	20	61±0·6[a]	1020±85[a]	22	398±48[a']	622±68[a]
16–18	Reinnervating (3)	20	67±1·8[a]	1522±115[a]	19	408±58[a']	1114±91[a]
16–18	TTX-reinnervating (2)	16	60±1·2[a,b]	909±115[a,b]	15	385±61	524±92[a,b]
19–22	Reinnervating (4)	24	73±0·9[a]	2242±215[a]	20	358±38	1884±160[a]
19–22	TTX-reinnervating (3)	20	63±0·7[a,b]	1312±205[a,b]	21	370±51	942±147[a,b]
23–28	Reinnervating (4)	26	79±0·6	4302±209[a]	21	309±51	3993±159[a]
23–25	TTX-reinnervating (3)	24	70±0·5[a,b]	2228±195[a,b]	23	361±55	1867±144[a,b]
30–40	Reinnervating (4)	30	77±0·9	3429±162	25	295±28	3134±121

Values (mean ± S.E.M.) are as follows: days after nerve crush; TTX-block started by the 8th–9th day after crush (see text). Muscle (in parentheses the number of muscles examined): 'Reinnervating', muscles undergoing reinnervation by the crushed nerve. 'TTX-reinnervating', muscles whose nerve was crushed and were in addition exposed to TTX (see text). N and N' number of fibres for G_m and G_K respectively. G_m, total membrane conductance; G_K, potassium conductance and G_{Cl}, chloride conductance. RP, resting membrane potential (referred to N fibres).

• *Control preparations were: contralateral muscles from TTX-reinnervating rats (3); muscles whose nerve received cotton soaked with only citrate buffer* (3); muscles from untreated rats (3). There was no difference among the individual controls, therefore their values were averaged.

[a] Significantly different from mean of control group ([a'], $P<0·05$; [a], $P<0·01$ or less)

[b] Significantly different from mean of corresponding reinnervating muscles ($P<0·01$).

Our experiments show the following: (1) after nerve crush the resting membrane conductances of the corresponding muscle fibres are almost restored to their control values within 30 days; (2) in muscles undergoing reinnervation by a nerve which is crushed and in addition exposed to TTX, the absence of nerve impulse-related activity limits the restoration of membrane conductances. In these muscles 23–25 days after crush G_{Cl} is 38 % lower and G_K 58 % higher than control.

In conclusion the present results together with our previous data (Conte-Camerino, 1978) suggest that the maintenance of mammalian resting membrane conductances is primarily due to substances supplied by axoplasmic flow, while transmission at the end-plate, and the muscle usage triggered by it, contribute to a lesser extent.

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REFERENCES

ADRIAN, R. H. (1964). The rubidium and potassium permeability of frog muscle membrane *J. Physiol, Lond.* **175**, 135–159.

ADRIAN, R. H. & BRYANT, S. H. (1974). On the repetitive discharge in myotonic muscle fibres. *J. Physiol., Lond.* **240**, 505–515.

BRAY, J. J., HUBBARD, J. I. & MILLS, R. G. (1979). The trophic influence of tetrodotoxin-inactive nerves on normal and reinnervated rat skeletal muscles. *J. Physiol., Lond.* **297**, 479–491.

BRYANT, S. H. & CAMERINO, D. (1976). Chloride conductance of denervated gastrocnemius fibers from normal goats. *J. Neurobiol.* **7**, 229–240.

BRYANT, S. H. & MORALES-AGUILERA, A. (1971). Chloride conductance in normal and myotonic muscle fibres and the action of monocarboxylic aromatic acids. *J. Physiol., Lond.* **219**, 367–383.

CAMERINO, D. & BRYANT, S. H. (1976). Effects of denervation and colchicine treatment on the chloride conductance of rat skeletal muscle fibers. *J. Neurobiol.* **7**, 221–228.

CANGIANO, A., LUTZEMBERGER, L. & NICOTRA, L. (1977). Non equivalence of impulse blockade and denervation in the production of membrane changes in rat skeletal muscle. *J. Physiol., Lond.* **273**, 691–706.

CONTE-CAMERINO, D. (1978). Effects of different dosages of vincristine on the component conductances of rat skeletal muscle fibers. *Pharmac. Res. Commun.* **10**, 453–467.

CONTE-CAMERINO, D. & BRYANT, S. H. (1977). Effects of vinblastine on the component conductances of rat skeletal muscle fibers. *Pharmac. Res. Commun.* **9**, 223–233.

CONTE-CAMERINO, D., BRYANT, S. H. & MITOLO-CHIEPPA, D. (1982*a*). Pharmacological block of nerve conduction affects electrical properties of muscle membranes. *Prog. clin. biol. Res.* **91**, 321–327.

CONTE-CAMERINO, D., BRYANT, S. H. & MITOLO-CHIEPPA, D. (1982*b*). Electrical properties of rat extensor digitorum longus muscle after chronic application of emetine to the motor nerve. *Expl Neurol.* **77**, 1–11.

HERBISON, G. J., JAWEED, M. M. & DITUNNO, J. F. (1980). Effect of activity and inactivity on reinnervating rat skeletal muscle contractility. *Expl Neurol.* **70**, 498–506.

KATZ, B. & LOU, C. H. (1947). Electric rectification in frog's muscle. *J. Physiol., Lond.* **106**, 29P–30P.

LOMO, T. & WESTGAARD, R. M. (1975). Further studies on the control of ACh sensitivity by muscle activity in the rat. *J. Physiol., Lond.* **252**, 603–626.

LORKOVIC, H. & TOMANEK, R. J. (1977). Potassium and chloride conductances in normal and denervated rat muscles. *Am. J. Physiol.* **232**, c109–c114.

MCARDLE, J. J. & ALBUQUERQUE, E. X. (1973). A study of the reinnervation of fast and slow mammalian muscles. *J. gen. Physiol.* **61**, 1–23.

PALADE, P. T. & BARCHI, R. I. (1977). Characteristics of chloride conductances in muscle fibers of the rat diaphragm. *J. gen. Physiol.* **69**, 325–342.

PESTRONK, A., DRACHMAN, D. B. & GRIFFIN, J. W. (1976). Effect of muscle disuse on acetylcholine receptors. *Nature, Lond.* **260**, 252–253.

TAXT, T. (1983). Local and systemic effects of tetrodotoxin on the formation and elimination of synapses in reinnervated adult rat muscle. *J. Physiol., Lond.* **340**, 175–194.

TIEDT, T. N., WISLER, L. P. & YOUNKIN, S. G. (1977). Neurotrophic regulation of resting membrane potential and acetylcholine sensitivity in rat extensor digitorum longus muscle. *Expl Neurol.* **57**, 766–791.

WESTGAARD, R. H. (1975). Influence of activity on the passive electrical properties of denervated soleus muscle fibers in the rat. *J. Physiol., Lond.* **251**, 683–697.