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, $G_{\text{Cl}}$, represents 80% of the total surface and tubular membrane resting conductance, $G_m$; the remainder is largely potassium conductance, $G_{\text{K}}$ (Palade & Barchi, 1977). $G_{\text{Cl}}$ and $G_{\text{K}}$ must be kept at an appropriate level for normal excitability of mammalian muscle fibres. A decreased $G_{\text{Cl}}$ 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 $G_{\text{Cl}}$ and rise of $G_{\text{K}}$ (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 $G_{\text{Cl}}$, and for suppressing $G_{\text{K}}$ 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
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, 1982a). 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. 1982a).

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 ($R_i = 112 \Omega \text{cm}$) we calculated the fibre diameter, $d_{\text{calc}}$, the membrane resistance, $R_m$, and the specific membrane capacitance, $C_m$. Total membrane conductance $G_m = 1/R_m$ 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, 1982b).

In agreement with the results of Bray et al. (1979), the inactivity during reinnervation affected the resting membrane potential, $R_P$, of the EDL fibres (Table
Membrane conductances in reinnervation

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, $R_m$ (measured in a chloride-containing solution), first increased at 10–15 days after the crush, from a control value of $340 \pm 27 \Omega \text{cm}^2$ to $1060 \pm 121 \Omega \text{cm}^2$. At 16–18 days it began to decrease ($750 \pm 89 \Omega \text{cm}^2$) towards control values, even undershooting them at 23–28 days ($268 \pm 11 \Omega \text{cm}^2$), and finally reached original values 30–40 days post-operatively ($328 \pm 15 \Omega \text{cm}^2$). In the reinnervating TTX-treated muscles, $R_m$ began to decrease at 19–22 days after the crush. At 23–25 days $R_m$ ($589 \pm 35 \Omega \text{cm}^2$) 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 $45 \pm 2 \mu m$, $30 \pm 2.5 \mu m$, $40 \pm 4.2 \mu m$ and $33 \pm 4.1 \mu m$ in control, 10–15 day, 23–28 day reinnervating and 23–25 day TTX-treated fibres, respectively. The differences in the values of $C_m$ measured at any time were not significant (McArdle & Albuquerque, 1973). The mean $C_m$ values were $4.3 \pm 0.3 \mu F \text{cm}^2$, $4.8 \pm 0.6 \mu F \text{cm}^2$, and $4.8 \pm 0.6 \mu F \text{cm}^2$ 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

<table>
<thead>
<tr>
<th>Days after nerve crush</th>
<th>Muscle</th>
<th>N</th>
<th>RP (mV)</th>
<th>G_m (μS cm⁻²)</th>
<th>N'</th>
<th>G_K (μS cm⁻²)</th>
<th>G_Cl (μS cm⁻²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-9</td>
<td>Control* (9)</td>
<td>35</td>
<td>78±0.9</td>
<td>3239±161</td>
<td>37</td>
<td>228±51</td>
<td>3011±118</td>
</tr>
<tr>
<td>10-15</td>
<td>Reinnervating (3)</td>
<td>21</td>
<td>68±2.1</td>
<td>3038±201</td>
<td>20</td>
<td>238±58</td>
<td>2800±149</td>
</tr>
<tr>
<td>16-18</td>
<td>Reinnervating (4)</td>
<td>20</td>
<td>61±0.6</td>
<td>1020±85</td>
<td>22</td>
<td>398±48*</td>
<td>622±68*</td>
</tr>
<tr>
<td>16-18</td>
<td>TTX-reinnervating (2)</td>
<td>16</td>
<td>60±1.2</td>
<td>1522±115*</td>
<td>19</td>
<td>408±58*</td>
<td>1114±91*</td>
</tr>
<tr>
<td>19-22</td>
<td>Reinnervating (4)</td>
<td>24</td>
<td>73±0.9</td>
<td>909±115*</td>
<td>15</td>
<td>385±61</td>
<td>524±92*</td>
</tr>
<tr>
<td>19-22</td>
<td>TTX-reinnervating (3)</td>
<td>20</td>
<td>63±0.7</td>
<td>2242±215*</td>
<td>20</td>
<td>358±38</td>
<td>1884±160*</td>
</tr>
<tr>
<td>23-28</td>
<td>Reinnervating (4)</td>
<td>26</td>
<td>79±0.6</td>
<td>1312±205*</td>
<td>21</td>
<td>370±51</td>
<td>942±147*</td>
</tr>
<tr>
<td>23-25</td>
<td>TTX-reinnervating (3)</td>
<td>24</td>
<td>70±0.5</td>
<td>4302±209*</td>
<td>21</td>
<td>309±51</td>
<td>3993±159*</td>
</tr>
<tr>
<td>23-28</td>
<td>TTX-reinnervating (4)</td>
<td>24</td>
<td>70±0.5</td>
<td>2228±195*</td>
<td>23</td>
<td>361±55</td>
<td>1867±144*</td>
</tr>
<tr>
<td>30-40</td>
<td>Reinnervating (4)</td>
<td>30</td>
<td>77±0.9</td>
<td>3429±162</td>
<td>25</td>
<td>295±28</td>
<td>3134±121</td>
</tr>
</tbody>
</table>

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 (*, P<0.05; **, 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 $G_{CI}$ 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


