DISSOCIATION OF ELECTRICAL AND MECHANICAL EVENTS IN DENERVATED FROG SKELETAL MUSCLE

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Several conditions to depress the activity of the linking process between the excitation of plasma membrane and the contraction of muscle (the E-C coupling process), namely, the hypertonicity, the treatment of some drugs (2,4-dinitrophenol and azide), fatigue and survival in Ringer solution for long time, have been reported. Up to date, however, the reasonable explanation about the mechanism of this linking process has not yet been discovered.

Recent studies on the changes in the sensitivity to acetylcholine, the electrical excitability, the mechanical behaviors and the subcellular structure, have led to the view that the effects of denervation are quite different from those of disuse in many respects. In these studies, however, no observation has been done on the relationship between the E-C coupling process and denervation. In the present experiment, special attention was paid to this point, and the dissociation of the electrical and the mechanical events in denervated frog skeletal muscle was found.

MATERIALS AND METHODS

1. Materials. Rana japonica and R. nigromaculata from September to the next April were used. One of the pair sartorius muscles of frog was denervated, and another was served as the control. Prior to the experiments, isolated muscles were always immersed in Ringer solution for 1 hour.

2. Procedure of denervation. Some frogs were anaesthetized with 1-2 ml of 8% solution of urethane injected intraperitoneally, but in most cases, anaesthesia was not used, since no essential trouble occurred at the time of operation. About 10 mm of sciatic nerve and all its branches were removed through the small incision which was made at the back of thigh root. The operated frogs were kept from 8 to 41 days at room temperature.

Received for publication June 6, 1964

The synopsis of the present report was announced in the 41st General Congress of the Physiological Society of Japan in 1964 (Chiba City).

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In order to confirm whether the denervation had been perfectly accomplished, the mechanical responses of the muscles to indirect electrical stimulations and to acetylcholine were tested. All experiments were performed on the perfectly denervated muscles.

3. Recording of electrical phenomena. The resting and the action potentials of muscle fibers were determined using the intracellular microelectrode technique with the method described previously. In the present experiments, however, the stimulation electrodes consisted of two Ag-AgCl wire electrodes. The stimuli were applied to the muscle transversely.

4. Recording of mechanical phenomena. The isotonic contraction evoked by direct electrical stimulation was recorded on the smoked drum with the isotonic lever loaded with 1.5 g weight as previously reported.

The mechano-electronic transducer RCA-5734 tube was employed to pick up the tension of the isometric contraction of muscle and its signal was recorded photographically on the cathode ray oscilloscope. The muscles were stimulated directly and transversely by rectangular pulses through the massive Ag-AgCl plate electrodes. The stimulating current was always selected to be about 2 times of the maximal stimulus, and its duration was 0.5 and 5 msec.

5. Duration of the plateau of active state. The plateau duration of active state was determined according to Macpherson et al.; namely, the duration between the time point of stimulation and the moment, at which the tension curve for twitch deviates from that for tetanus, was measured (Fig. 1). The stimulation was given through the massive electrodes, and its frequency in the case of tetanus was 100 cycle per sec.

![Fig. 1](image)

**Fig. 1.** An illustration for the determination of the plateau duration of active state in frog sartorius muscle. The period of denervation was 30 days. The vertical bar refers to 5 g and each mark indicates 20 msec. Curves refer to isometric contraction.

D: twitch in denervated muscle; DT: tetanus in denervated muscle; I: twitch in contralateral innervated muscle; and IT: tetanus in contralateral innervated muscle.

6. ATP-induced contraction of glycerol-extracted muscle fibers. The glycerol-extracted muscles were prepared from the denervated and the contralateral innervated sartorii of frog according to the method of Szent-Györgyi. These muscles were kept at 0°C in 50% glycerol solution for 2 weeks prior to experiments. The preparation of single muscle fibers and the determination of the developed tension in
ATP-induced contraction of them were according to Nagai et al.^{25}

7. Experimental environments. Ringer solution consisted of 112 mM NaCl, 2.0 mM KH$_2$PO$_4$ and 1.3 mM CaCl$_2$. pH of the solution was adjusted to 7.0-7.2 by addition of M/8 NaHCO$_3$. Caffeine and acetylcholine chloride were dissolved in Ringer solution. The composition of the ATP solution used was 2.0 mM MgCl$_2$, 82.4 mM KCl, 2.0 mM ATP and 66.7 mM Tris-acetate buffer of pH 7.0. All experiments were performed at room temperature from 17 to 20°C.

RESULTS

I. Mechanical responses of denervated muscles to electrical stimulations.

Figs. 2 and 3a illustrate that the size of isotonic and isometric twitches evoked by direct electrical stimulations were reduced gradually with the lapse of time after denervation. At the 30th day after denervation the size of contraction was below 50% of the control. Also in the case of isometric tetanus the tension development was reduced in denervated muscles (Figs. 3a and 3b).

![Graph](image1)

**Fig. 2.** The change in the isotonic twitch height of frog sartorius muscle after denervation.

Ordinate: relative isotonic twitch height of denervated muscles to that (i.e. 100%) of the contralateral innervated ones. Each dot refers to one case.

Abscissa: days after denervation.

2. Resting and action potentials of denervated muscles. As shown in Table 1 and Fig. 4 there was no recognizable difference between the resting potentials of denervated and contralateral innervated muscle fibers of sartorii. Also in the shape and size of action potentials, which were recorded in the vicinity of stimulating site, no significant difference was observed between the dener-
E-C COUPLING PROCESS IN DENERVATED MUSCLE

403

Fig. 3a. The change in tension of isometric contraction of frog sartorius muscle after denervation.

Ordinate: relative tension development of twitch and tetanus in denervated muscles to that (i.e. 100%) of the contralateral innervated ones. The marks ○, × refer to twitch and tetanus, respectively. For the other explanations see in Fig. 2.

Fig. 3b. The illustration of an experiment in Fig. 3a.
A: the innervated muscle.
B: the contralateral muscle at 28 days after denervation.
The lightly curved vertical lines indicate twitch responses.
The frequency of tetanic stimuli was 100 cycle per sec.

vated muscle and the control. These results agree with those of NICHOLLS13), LEVINE14) and Li et al.27). GUTMAN20) and others15, 27, 28) have pointed out that the fibrillation was observed in denervated muscles. Also in the present experiments the same phenomenon was observed, but soon disappeared. The determinations in TABLE 1 were made after the disappearance of fibrillation.
The size of resting and action potentials in the denervated and contralateral innervated fibers of frog sartorius muscles. The period of denervation was 21-30 days.

<table>
<thead>
<tr>
<th>Denervated fibers</th>
<th>Resting Potentials (mV±SD)</th>
<th>Action Potentials (mV±SD)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>84±0.8 (in 50 fibers of 10 cases)</td>
<td>113±1.6 (in 50 fibers of 10 cases)</td>
</tr>
<tr>
<td>Contralateral innervated fibers</td>
<td>83±0.6 (in 60 fibers of 10 cases)</td>
<td>118±1.2 (in 56 fibers of 10 cases)</td>
</tr>
</tbody>
</table>

In denervated muscles, the conduction velocity of action potentials decreases, and the action potential deforms with its conduction. These alterations involve the possibility that, if the electrical stimulation is given incautiously at random on denervated muscles, the magnitude of evoked contraction decreases owing to the insufficient spatial and temporal summation of contractions in each part of muscle fibers and to the insufficient activation of contractile apparatus due to the deformed action potentials. In the present experiments, however, all these possible disturbances could be excluded by means of employing the massive electrodes, which were able to stimulate all points of muscle simultaneously. It is already known that the action potentials evoked by massive stimulations are the same as those caused by ordinary point stimulations. It is, thus, indicated that the results of Figs. 1 and 2 were not due to the changes in the electrical activity of these denervated muscle fibers.

3. ATP contraction in glycerol-extracted fibers of denervated muscles. The ATP-induced contraction in glycerol-extracted muscle fibers at the 3rd and 7th week after denervation was not different from the control, regardless of
the duration of denervation (Table 2). It was recently reported, in the denervated skeletal muscle of rat, that the amount of contractile protein was reduced with the lapse of time after denervation\(^{30,31}\). According to our photomicroscopic observations, however, no difference in the range of fiber diameter (50-70\(\mu\)) was recognized between the denervated and the contralateral innervated muscle fibers. Therefore, the present results indicate that in frog muscles, the contractile element remains unchanged at least for 7 weeks after denervation.

**Table 2**

<table>
<thead>
<tr>
<th></th>
<th>Developed maximal tension ((\mu/cm^2))</th>
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<tbody>
<tr>
<td></td>
<td>3 weeks</td>
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<tr>
<td>Denervated muscle</td>
<td></td>
</tr>
<tr>
<td>2164</td>
<td>2014</td>
</tr>
<tr>
<td>1829</td>
<td>1930</td>
</tr>
<tr>
<td>1987</td>
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<td>1676</td>
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<td></td>
<td>1877</td>
</tr>
<tr>
<td>Mean</td>
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</tr>
<tr>
<td>Innervated muscle</td>
<td></td>
</tr>
<tr>
<td>1976</td>
<td>1684</td>
</tr>
<tr>
<td>2047</td>
<td>1866</td>
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<td>1759</td>
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</tr>
<tr>
<td>2087</td>
<td>1780</td>
</tr>
<tr>
<td>Mean</td>
<td>1927</td>
</tr>
</tbody>
</table>

4. **Caffeine-induced contracture in denervated muscles.** It has been pointed out that caffeine produces the contractile response in muscles by activating the contractile apparatus through a similar process as in the case of electrical stimulation but showing no membrane potential changes\(^{33,34,47}\). Fig. 5 illustrates a pattern of caffeine-induced contracture in frog sartorius muscles at the 30th day after denervation. The size and shape of the responses in the denervated muscle were not different from the control. From these results and those in glycerol-extracted muscle fibers mentioned above, it is deduced that the function of contractile apparatus to contract and the direct energy source for contraction remain intact at least 30 days after denervation.

5. **Duration of the plateau of active state.** The effective stimulus to contraction is followed by a sudden change in the mechanical properties of muscle, that is, the development of active state\(^{23,55,56}\). According to Hill et al.\(^{57}\), this state in stimulated muscle is already full activated during early
stage of contraction. As shown in Table 3 the plateau duration of the active state in denervated frog muscles was the same as in the control, and the values were not different between 3 and 7 weeks after denervation. These findings are considered to exclude the possibility that a change in the plateau duration of the active state may contribute to the decline in twitch size due to denervation.

### Table 3

The plateau duration of active state of denervated and innervated muscles at 18 to 20°C.

<table>
<thead>
<tr>
<th>Case</th>
<th>Denervated muscle (msec±SD)</th>
<th>Contralateral innervated muscle (msec±SD)</th>
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<tbody>
<tr>
<td>1</td>
<td>29.2±1.8</td>
<td>28.8±1.7</td>
</tr>
<tr>
<td>2</td>
<td>22.6±3.1</td>
<td>22.1±3.1</td>
</tr>
<tr>
<td>3</td>
<td>27.2±5.0</td>
<td>27.3±4.9</td>
</tr>
<tr>
<td>4</td>
<td>27.0±2.2</td>
<td>27.4±3.0</td>
</tr>
</tbody>
</table>

Days after denervation: case 1 and 2, 21 days; case 3 and 4, 30 days. Each value represents the mean obtained from 5-7 measurements in one muscle.
The results in Figs. 1, 2a and 2b show that in frog skeletal muscles, the mechanical responses evoked by direct electrical stimulations remarkably decrease with lapse of time after denervation. In spite of the reduction in the mechanical responses, the resting and the action potentials of these muscles were not changed (Fig. 4 and Table 1). And the size of caffeine-induced contracture (Fig. 3) and the ATP-induced contraction in glycerol-extracted fibers (Table 2) of denervated muscles were not different from those in the control. It is considered that the contractile element and the energy source for contraction were not affected by denervation at least in this experimental period. Therefore, it is concluded that the reduction in the mechanical responses of denervated muscles to electrical stimulations is due to gradual impairment of the E-C coupling process.

It is noticeable that the twitch size of denervated muscles reduces without any change in the plateau duration of the active state. It is well known that the E-C coupling process correlates together with the active state, and some changes in the coupling process can influence the active state\(^{50,51,52}\); e.g. the enhancing action of the monovalent anion series\(^{53,54}\) upon the mechanical responses has been explained as a result of the prolongation in the plateau duration of the active state under the influence of these anions on the coupling process. However, the present results in denervated muscles showed that no change in the plateau duration of the active state and, in addition, the mechanical responses to electrical stimulations reduced remarkably even in the case of complete tetanus. These facts suggest strongly that the reduction of the mechanical responses may originate in the decrease in the intensity of the active state of denervated muscles. From these considerations, it may be pointed out that the E-C coupling process can affect not only the plateau duration of the active state, but also the intensity of the state.

Recently, the sarcoplasmic reticulum system within muscle cells is noticed as the structural basis for the intracellular impulse conduction\(^{38,39,40,41,42}\). Krüger and Günther\(^{18}\) observed photomicroscopically in mammalian skeletal muscle fibers that the denervation produces an interesting structural change in the reticulum system previously to all other alteration of the muscle fibers. In regard to this respect, electron microscopic study on the subcellular structures of denervated muscle fibers is very interesting.

Many reports\(^{43,44,45}\) have presented the view that Ca ion may be a linking agent between the excitation of the plasma membrane and the contraction of muscle. It would be thus interesting to examine whether the linking process is depressed selectively by a possible decrement of Ca metabolism in denervated muscles. Concerning this problem, however, our unpublished data show that the increment of Ca ion concentration up to 2.6 mM in bathing solution
has not brought about any sign of increase in twitch tension of denervated muscles. Of course, this result itself would not exclude the emphasized role of Ca ion in the linking process.

In view of many reports and the present results about the effects of denervation on the function of muscles, it would be very interesting to note that, in the entire processes of the propagation of impulses of excitation from motor nerve fibers to muscle ones and, furthermore, to the contractile elements within them, there are at least two sites, which are easily affected by denervation, namely, the neuromuscular junction\textsuperscript{10, 22} and the E-C coupling process.

**SUMMARY**

In chronically denervated sartorius muscles of frog, the mechanical responses to direct electrical stimulations, caffeine-induced contracture, and the duration of plateau of the active state have been studied. The ATP-induced contraction of glycerol-extracted muscle fibers, which were prepared from denervated and contralateral innervated muscles, have been also observed.

1. The mechanical responses to direct electrical stimulations were gradually decreased with the lapse of time after denervation.
2. Even at the end of 30 days after denervation the resting and the action potentials of denervated muscle fibers were normal.
3. The developed tension due to 2 mM ATP in glycerol-extracted muscle fibers prepared from the 7 weeks-denervated muscles was not different from that in contralateral innervated muscle fibers.
4. Both the shape and the size of caffeine-induced contracture in denervated muscles were the same as those in the contralateral innervated ones.
5. The duration of plateau of the active state in denervated muscles was ranged from 22.6 to 29.2 msec. at about 18°C. These results were not different from those of the contralateral innervated ones.
6. Based on the above-mentioned results it is concluded that the E-C coupling process of frog sartorius muscle is depressed due to denervation.

The author is deeply indebted to Professor T. NAGAI, assistant Professor M. FUJINO and Dr. T. YAMAGUCHI for their valuable advices and encouragement throughout this study.

This work was supported in part by a grant in Aid for Fundamental Scientific Research from the Ministry of Education.

**REFERENCES**

E-C COUPLING PROCESS IN DENERVATED MUSCLE