ELECTRO-MECHANICAL COUPLING IN CRAYFISH MUSCLE FIBERS EXAMINED BY THE VOLTAGE CLAMP METHOD

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Summary 1. Electro-mechanical coupling in the crayfish short muscle fibers was investigated by the voltage clamp method using intracellular microelectrodes.
2. The change in membrane potential was uniformly distributed when depolarization was more negative than $-20 \text{ mV}$ and longer than 100 msec.
3. The mechanical threshold potential was $-50$ to $-58 \text{ mV}$. The curve relating the tension to the membrane potential was nearly linear in the range between $-45$ and $-20 \text{ mV}$ for every duration of depolarization. The slope of the curve was 0.3 of the tension at 0 mV for 10 mV change in potential. The tension-membrane potential curve obtained in the potassium contracture experiments was quite similar to that in the voltage clamp experiments.
4. The rising phase of isometric contraction, except for the initial phase within 0.1 sec, was explained by two exponential components. The time constants were 0.4–0.6 sec and 0.08–0.12 sec at 20–25°C, and they were independent of the membrane potential.
5. The relaxation phase after the end of depolarization was also explained by three exponential components. The time constants were 0.4–0.65 sec, 0.10–0.12 sec, and 0.05–0.08 sec at 20–27°C, and they were independent of the level and duration of the clamped membrane potential.
6. The mechanical threshold potential was unaffected by the change in the speed of depolarization. The maximum rate of tension rise did not decrease until the speed of depolarization was reduced to the critical value of 40–80 mV/sec.

In the process of the electro-mechanical coupling of the striated muscle, the first step is membrane depolarization, which spreads inwardly along the transverse
tubular system and induces the release of calcium ions which activate the contractile machinery (SANDOW, 1965, 1970).

In the short muscle fiber of the crayfish, ORKAND (1962a) showed that the depolarization caused by the current flow from the intracellular microelectrode was nearly uniform along the longitudinal axis of the muscle fiber. DUDEL et al. (1968) used this short muscle fiber and examined the relationship of the peak tension or the maximum rate of tension rise to the membrane potential by the voltage clamp method. ORKAND and DUDEL et al. showed that the tension in the crayfish muscle depended both on the level and on the duration of depolarization. However, it is not clear quantitatively how the tension is related to these two factors. HAGIWARA et al. (1968) described the time course of contraction in the barnacle muscle by two exponential components, using the voltage clamp method. This explanation is useful because it provides information about the transient change of each process involved in the link between depolarization and contraction.

One aim of the present study is to describe the tension of the crayfish muscle as a function of the duration and level of the clamped membrane potential. The second aim is to describe the time course of tension not only during contraction but also during relaxation and to elucidate the time relationship among the steps in the electro-mechanical coupling process. Some of the results have already been reported (MATSUMURA, 1968).

METHODS

The material used for the voltage clamp experiment was the epimeralis contractor muscle of the crayfish, Cambrus clarkii. The preparation was a bundle of 5–10 muscle fibers. Each fiber was 1.7–2.5 mm in length and 100–200 μ in diameter. This preparation was placed horizontally in a 1.25-ml chamber. Both ends of these muscle fibers were attached directly to the cartilaginous tissue. Thus, one end of the bundle preparation was tied tightly to the hook in the chamber and the other end was penetrated by a silver wire of 100 μ in diameter and tied to the mechano-electric transducer (RCA 5734).

The van Harreveld solution contained 200 mM NaCl, 5 mM KCl, 14 mM CaCl₂, 2.1 mM MgCl₂, and 5 mM tris-maleic acid buffer (pH 7.2). The intracellular microelectrode for recording the membrane potential was filled with 3 M KCl and the microelectrode for current feeding was filled with 2 M potassium citrate. The indifferent electrode was also a microelectrode filled with 3 M KCl, with a resistance of 1–3 MΩ. The voltage clamp method with the intracellular microelectrode was similar in principle to that described by TAKEUCHI and TAKEUCHI (1957). The experimental set-up is illustrated in Fig. 1. The potential difference between intracellular and extracellular microelectrodes was led through a cathode-follower amplifier, CF (MZ 3B, Nihon Kohden), to one beam of an oscilloscope (VC 7A, Nihon Kohden). The CF output was fed to one of the input terminals of
the differential amplifier, detonated A (Burr Brown 3308/12C, push-pull), the gain of which was 100. The commanding voltage, G, was induced to another input of amplifier A and the output of this amplifier was connected to the main amplifier, B (Burr Brown 1542/25), the gain of which was 30. Thus the total gain of the feedback amplifier was 3,000. The output of the feedback amplifier was out phase with the membrane potential change and in phase with the commanding signal. The membrane current was recorded by current-to-voltage converting circuit with the operational amplifier, C (Burr Brown 3112/12C), placed between the indifferent electrode and the ground. To avoid distortion of the potential change due to stray capacity, capacitors of less than ±0.002 μF were placed at the input of amplifier A. Care was taken to minimize the volume of the solution in the chamber above the muscle fibers.

For the potassium contracture experiments, a single muscle fiber was prepared from the depressor basipodite muscle (PILGRIM and WIERSMAR, 1963). The fibers were 150–300 μ in diameter and 6–8 mm in length. A single muscle fiber was mounted in a 0.3-ml chamber and the external solution was quickly exchanged. The potassium concentration was changed so that the product of [K+] and [Cl–] might be constantly 1,200 (mM)², the chloride being replaced with equimolar propionate. The intracellular microelectrodes filled with 3 m KCl were usually
made flexible, after the method of FREYGANG et al. (1964), by covering with Estane 5701, which was kindly supplied by Japan Geon Company, Tokyo.

RESULTS

1. Electrical properties of the membrane

The resting membrane potentials were between $-70$ and $-85$ mV. When a small depolarizing or hyperpolarizing current of about 0.5 sec in duration was supplied from the intracellular microelectrode, the membrane potential changed exponentially with a time constant of 60–80 msec. In most preparations the action potentials were not observed, but in some preparations a short abortive spike was elicited and on very rare occasions a spike potential was elicited. Fibers that responded with both abortive and ordinary spikes were excluded from the voltage clamp experiments.

The relationship between the magnitude of membrane potential change and applied current was linear in the range between $-60$ and $-90$ mV, and the slope was $0.7–2.0 \times 10^5 \Omega$. When the membrane was depolarized to $-20$ mV, the input resistance decreased to $1/6–1/8$ of that of the resting membrane.

2. Potential distribution along the muscle fiber

The distribution of the membrane potential along the longitudinal axis of the short muscle fibers has been analyzed by ORKAND (1962a, b) and by HEISTRACHER and HUNT (1969). It was calculated that, if the length constant of the resting muscle membrane was taken as 2.7 mm (ORKAND, 1962a), the length constant decreased to about 1.0 mm when the input resistance at $-20$ mV decreased to $1/6–1/8$ of the control. Since the average muscle length from center to end was 1.1 mm, the potential distribution was still nearly uniform. At $-10$ mV, however, the electrotonic decay along the longitudinal direction of the fiber was not negligible. Figures 2A and B show the records of the membrane potential changes at the center, $V_1$, and at one end, $V_2$, of the muscle fiber when $V_1$ was clamped at various potential levels for 0.48 sec. In Fig. 2A, $V_2$ changed from $-70$ to $-33$ mV while $V_1$ changed from $-70$ to $-32$ mV. The clamping was complete. As shown in Fig. 2B, however, a change of $V_1$ from $-70$ to $-18$ mV caused $V_2$ to change from $-70$ to $-21$ mV. The difference is not negligible in such a large step of clamping. The time course of $V_2$ was not the same as that of $V_1$, but showed overshoot at first, gradual depolarization during clamping, and residual depolarization after controlled repolarization. This time course of $V_2$ can probably be explained by the accumulation of potassium ions in the transverse tubular lumen during depolarization, as proposed by HEISTRACHER and HUNT (1969).

When the pulse duration was shorter than 100 msec, the capacitative distortion was no longer negligible. Figure 2C shows the membrane potential changes recorded at both ends of the muscle fiber. A current electrode and one potential-
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recording electrode were inserted at one end, and the other potential electrode at the other end. The membrane potential at the distant end from the current electrode, $V_3$, showed a slow rise with a half time of 3–6 msec, as compared with the potential change close to the current electrode, $V_2$. It is therefore concluded that, as long as the depolarization is more negative than $-20$ mV and longer than 100 msec, the potential distribution along the fiber is uniform during the clamping, but, it is not uniform if the clamping voltage is higher than $-10$ mV or shorter than 20 msec.

![Fig. 2. Potential distribution along the longitudinal axis of the muscle fiber. In A and B, the current electrode is inserted at the center of the fiber. Membrane potentials are recorded at the point close to the current electrode ($V_1$, trace 1) and at the end of the muscle fiber ($V_2$, trace 2); $V_2$ is the same level and the same gain as $V_1$ but it is displayed separately from $V_1$ so that it may be seen clearly. In C, current electrode is inserted at one end of the muscle fiber. Membrane potentials are recorded at this point ($V_2$, trace 2) and at the other end of the muscle fiber ($V_3$, trace 3). The lowermost trace shows the membrane current. Holding potential: $-70$ mV; 20°C.](image)

3. The relation between tension and membrane potential

In most preparations no tension development was observed on depolarization shorter than 5 msec. On microscopic observation, however, the minimum pulse duration necessary for the initiation of local contraction was found to be 0.5 msec.

When the fiber was depolarized by a square pulse with a duration of less than 0.5 sec, the tension started to rise with some latent period after the beginning of depolarization and also started to fall with a certain delay after the end of depolarization. For longer pulses, tension developed quickly at first but rather slowly afterward, showing a creep phenomenon. Spontaneous relaxation during depolarization was not observed, so the tension produced by a 10 sec pulse was greater than that produced by a 2 sec pulse.

It was not easy to repeat the experiments at a clamped potential of around 0 mV, because the electrode was destroyed due to use of such heavy currents as 10 $\mu$A. In Fig. 3, two examples from different preparations are illustrated, showing the relationship between tension and membrane potential at different durations. The mechanical threshold potential for pulses longer than 0.5 sec was
$-50$ to $-58$ mV, which coincided with the results of potassium contracture experiments described in section 8. The tension increased almost linearly when the membrane was further depolarized in the range between $-45$ and $-20$ mV. In the linear part of the curve, the slope was 0.2–0.4 of $\bar{P}$ for 10 mV change in potential, where $\bar{P}$ was the tension extrapolated to 0 mV in Fig. 3. On further depolarization beyond 0 mV the tension continued to increase, indicating that the maximum tension was attained at an inside positive potential.

![Graph showing the relationship between tension and membrane potential at different pulse durations.](image)

**Fig. 3.** Relationship between tension and membrane potential at different pulse durations. Examples from two different preparations are shown, one being represented by filled circles and continuous lines (24°C) and the other by open circles and broken lines (25°C). Figures at the right end of each curve show the pulse duration in sec.

4. **Time course of tension rise during contraction**

Figure 4 shows the records of tension produced by a membrane potential change from $-80$ to $-35$ mV or $-23$ mV with different durations. The rate of tension rise reached its maximum 0.15–0.20 sec after the start of depolarization. Both the peak and the rate of tension rise increased with increasing size of the step depolarization. Prolongation of depolarization beyond 0.2 sec caused an increase in peak tension but no change in the rate of tension rise. The time of the peak tension showed an apparent delay from the end of depolarization, as observed also by DUDEL et al. (1968), and this delay became shorter with prolongation of the pulse duration.

According to HAGIWARA et al. (1968), the time course of tension rise in the barnacle muscle can be described as two exponential components. Similar calculations were made in the present work. The tension, $P(t)$, at a given time, $t$,
after the start of depolarization increased with time until it reached a final value. The final tension, \( P_\infty \), was graphically determined. When \( P_\infty - P(t) \) was plotted on a logarithmic scale against \( t \), as shown in Fig. 5A, the plots after 0.4 sec were on a straight line. This exponential component was denoted by \( P_1 = Ae^{-t/\tau_1} \).

\[ \text{Fig. 4. Superimposed tracings of tension produced by controlled depolarization of different durations. Potential changes are from } -80 \text{ to } -35 \text{ mV in A and from } -80 \text{ to } -23 \text{ mV in B. The upstroke and downstroke in the records of membrane potential are retouched.} \]

Before 0.4 sec, \( P_\infty - P(t) \) deviated from \( P_1 \). However, the difference, \( P_2 \), between \( P_1 \) and \( P_\infty - P(t) \) plotted on a logarithmic scale against time produced almost a straight line, except for a slight deviation before 0.1 sec (Fig. 5B). Thus, the following equation was obtained:

\[ P_2 = P_1 - \{P_\infty - P(t)\} = Be^{-t/\tau_2}, \quad \text{or} \]

\[ P(t) = P_\infty - Ae^{-t/\tau_1} + Be^{-t/\tau_2}, \quad (1) \]

where \( \tau_1 \) and \( \tau_2 \) are the time constants. In the example shown in Fig. 4A, \( P_\infty \) is 1.04 g, and in Fig. 4B \( P_\infty \) is 1.55 g. As for the time constants, \( \tau_1 \) is 0.52 sec and \( \tau_2 \) is 0.11 sec, and they are independent of the membrane potential or the duration of depolarization. In five experiments at more negative potentials than \(-20 \text{ mV}, \tau_1 \) was 0.4–0.6 sec and \( \tau_2 \) was 0.08–0.12 sec, at 20–25°C. The time constant \( \tau_1 \) did not change even at a clamping potential of \(-10 \text{ mV}, \) but \( \tau_2 \) was slightly shortened at \(-10 \text{ mV}. \) It is not certain, however, whether or not \( \tau_2 \) is affected by clamped potentials above \(-20 \text{ mV,\) because when the potential is more negative than \(-20 \text{ mV the membrane potential change is not uniformly distributed, as shown in Fig. 2B.}\)

In Eq. (1), \( P_\infty, A, \) and \( B \) are constants with time, and they are functions of the membrane potential, \( V. \) However, when the ratios \( A: P_\infty \) and \( B: P_\infty \) are calculated, it is found that the ratios are independent of \( V. \) The results of two
The membrane potential changes are from $-80$ to $-35$ mV (filled circles) and from $-80$ mV to $-23$ mV (open circles). In A, continuous line shows $P_\infty - P(t)$. Broken line is drawn by extrapolating the linear part of $P_\infty - P(t)$ curve to $t = 0$, indicating $P_1$. In B, continuous line shows the difference between $P_1$ and $P_\infty - P(t)$, and broken line is drawn by extrapolating the linear part of continuous line. $P_2$ is indicated by straight representing.

Table 1. The ratios of $A$ and $B$ to $P_\infty$ in Eq. (1).

<table>
<thead>
<tr>
<th>Membrane potential change (mV)</th>
<th>Temp. (°C)</th>
<th>$P_\infty$ (g)</th>
<th>$A/P_\infty$</th>
<th>$B/P_\infty$</th>
</tr>
</thead>
<tbody>
<tr>
<td>from to</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$-80$ to $-23$</td>
<td>25</td>
<td>1.55</td>
<td>1.34</td>
<td>0.43</td>
</tr>
<tr>
<td>$-80$ to $-35$</td>
<td>25</td>
<td>1.04</td>
<td>1.34</td>
<td>0.50</td>
</tr>
<tr>
<td>$-80$ to $-42$</td>
<td>25</td>
<td>0.57</td>
<td>1.35</td>
<td>0.40</td>
</tr>
<tr>
<td>$-80$ to $-18$</td>
<td>22</td>
<td>2.00</td>
<td>1.27</td>
<td>0.33</td>
</tr>
<tr>
<td>$-80$ to $-28$</td>
<td>22</td>
<td>1.38</td>
<td>1.36</td>
<td>0.44</td>
</tr>
<tr>
<td>$-80$ to $-43$</td>
<td>22</td>
<td>0.50</td>
<td>1.33</td>
<td>0.48</td>
</tr>
</tbody>
</table>
experiments are shown in Table 1. The average values of $A/P_\infty$ and $B/P_\infty$ are 1.3 and 0.4, respectively. It seems better to take the tension under 2 sec pulse rather than the tension under 10 sec pulse or the tension in potassium contracture as the actual $P_\infty$, because the latter two contain the slow tension rise like a creep phenomenon. If so, the $P_\infty - V$ relation is almost linear in the range between $-45$ and $-20$ mV with the slope of $0.03 \tilde{P}_\infty$ for 1 mV change in membrane potential (Fig. 3). Based on this approximation, the tension as a function of $V$ and $t$, $P(V, t)$, at 20–25°C, is represented by the equation

$$P(V, t) = 0.03\tilde{P}_\infty(1 - 1.3e^{-t/0.8} + 0.4e^{-t/0.11})(V - V_{th}),$$

where $\tilde{P}_\infty$ is the tension at $V = 0$ and $t = \infty$, and $V_{th}$ is the mechanical threshold potential. The tension at $t = 0$, that is, $P(V, 0)$, becomes negative because of the slight deviation between Eq. (1) and the experimental tension, as seen in Fig. 5B. Studies on the initial deviation will be described later.

5. Time course of tension fall during relaxation

Calculations similar to those performed in section 4 were applied to the relaxation phase. The tension during relaxation, $R(s)$, in the example illustrated in Fig. 4 was taken on a logarithmic scale against the time, $s$, after the end of depolarization (Fig. 6A). In the tail of relaxation, the tension decreased in an exponential manner. Thus, we obtain

$$R_1 = Ce^{-s/\sigma_1},$$

where $R_1$ is the tension indicative of $R(s)$ after 0.6 sec. Before 0.6 sec after the end of depolarization, $R(s)$ deviates from $R_1$, and the difference between them, $R_2$, is also exponential (Fig. 6B). Thus, we obtain

$$R_2 = De^{-s/\sigma_2}.$$

However, the tension curve still deviates from $R_2$ when $s$ is shorter than 0.2 sec. The difference between $R(s)$ and $(R_1 - R_2)$, $R_3$, is again exponential (Fig. 6C). Thus, we obtain

$$R_3 = Ee^{-s/\sigma_3}.$$

Finally, the tension during relaxation is described as follows:

$$R(s) = Ce^{-s/\sigma_1} - De^{-s/\sigma_2} + Ee^{-s/\sigma_3}.$$  \hspace{1cm} (3)
Fig. 6. The time course of tension fall in relaxation, redrawn from the example in Fig. 4. The membrane potential is changed from $-80$ to $-35$ mV for 0.52 sec (filled circles) and from $-80$ to $-23$ mV for 0.58 sec (open circles). In A, tension in relaxation is plotted against time from the end of depolarization. Continuous line shows tension in relaxation, $R(s)$, and broken line is drawn by extrapolating the tail of relaxation. The straight representing indicates $R_1$. In B, the difference between $R_1$ and $R(s)$ is shown by continuous line, the straight representing giving $R_2$. In C, again the difference between $R_2$ and $(R_1 - R(s))$ is plotted against time. See text for further explanation.

Table 2. Time constants indicating the time course of relaxation.

<table>
<thead>
<tr>
<th>Membrane potential change (mV)</th>
<th>Duration of depolarization (sec)</th>
<th>Temp. ($^\circ$C)</th>
<th>$\alpha_1$ (sec)</th>
<th>$\alpha_2$ (sec)</th>
<th>$\beta$ (sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>from $-80$ to $-23$</td>
<td>0.4 (min) 0.9 (max)</td>
<td>25</td>
<td>0.56</td>
<td>0.11 - 0.12</td>
<td>0.08</td>
</tr>
<tr>
<td>from $-80$ to $-35$</td>
<td>0.5 (min) 0.9 (max)</td>
<td>25</td>
<td>0.56</td>
<td>0.12</td>
<td>0.08</td>
</tr>
<tr>
<td>from $-80$ to $-16$</td>
<td>0.3 (min) 0.9 (max)</td>
<td>22</td>
<td>0.46</td>
<td>0.11</td>
<td>0.07</td>
</tr>
<tr>
<td>from $-80$ to $-28$</td>
<td>0.2 (min) 0.5 (max)</td>
<td>22</td>
<td>0.40</td>
<td>0.12</td>
<td>0.08</td>
</tr>
</tbody>
</table>
the observed range. In most experiments, $\sigma_1$ is nearly equal to $r_1$.

The constants $C$, $D$, and $E$ in Eq. (3) are the functions of $V$ and the duration of depolarization, $X$. From the results of two experiments, it was ascertained that $C$ is proportional to $V$, namely, $C = C'(V - V_{th})$, where $C'$ is a function of $X$. At the same clamped potential, $C'$ was increased exponentially with increasing $X$; thus, $C' = C''(1 - e^{-x/a})$, where $C''$ was a constant that was independent of $V$ and $X$. From the example shown in Fig. 4, $\alpha$ was calculated to be 0.4 sec, which was independent of $V$ and $X$. Similar calculations were attempted for $D$ and $E$, but it was impossible to determine these constants accurately, because of variability in the curve of tension fall.

6. Time course of tension rise at the start of contraction

Equation (1) shows that the tension starts to rise convexly after depolarization, but the actual tension rise is always concave. This difference is shown as the deviation of $P_1 - \{P_\infty - P(t)\}$ from $P_2$ in Fig. 5B. For the purpose of studying in more detail the time course of the tension rise in the early part of contraction, the membrane was slowly depolarized by supplying a sawtooth pulse to amplifier $A$ in Fig. 1. Figures 7A and B show the record of tension when the membrane potential was shifted with a constant speed varying from 75 to 400 mV/sec. The peak tension was small when the speed of depolarization, $dV/dt$, was high, because the duration of depolarization was shortened at the same time. In Fig. 7C, the tension was plotted on a logarithmic scale against the time when the depolarization exceeded the mechanical threshold potential. At the foot of contraction, the relation was linear. Thus, we obtain

$$P(t) = e^{(t-d)/f},$$

where $f$ is the time constant of tension rise at the start of contraction and $d$ is the latent period. The time constant, $f$, was dependent on $dV/dt$ and it decreased when $dV/dt$ decreased. The latent period seemed to be prolonged with decreasing $dV/dt$. The term in this early phase of contraction may be written to be proportional to the square of time, or $P(t) = f'(t - d)^2$, as HAGIWARA et al. suggested (1968). However, the exponential relation fits the actual time course of tension better than the parabolic relation. In any case, $f$ or $f'$ depends on $dV/dt$. Therefore, it is concluded that the latent period and the early phase of contraction are influenced by the speed of the membrane potential change.

7. Relation between the maximum rate of tension rise and the speed of depolarization

Equations (1) and (2) show that the rate of tension rise, $dP/dt$, depends on $V$ and $dV/dt$. In order to elucidate whether the slow tension rise of the crayfish muscle was due to the slow spread of depolarization of the surface membrane toward the interior of the fiber or to the slow processes occurring after the depolarization of the internal membrane system, the membrane was depolarized by a ramp-
Fig. 7. A and B show superimposed tracings of contraction produced by depolarization with a sawtooth pulse. The speed of depolarization is 790, 350, or 240 mV/sec in A and 150, 95, or 74 mV/sec in B. Holding potential is -70 mV. Note the difference in calibrations for tension and time between in A and B. 22°C. In C, the initial phase of contraction is shown on a logarithmic scale against time. The abscissa is measured after the time when the sawtooth depolarization exceeds the mechanical threshold potential of -55 mV.

shaped pulse which was a sawtooth pulse immediately followed by a square pulse. Figures 8A and B show the early part of contraction produced by the ramp depolarization from -80 to -23 mV. The mechanical threshold potential was not changed with the speed of depolarization, similar to the finding of Sugi (1968) on frog muscle. If $dV/dt$ was below 180 mV/sec, $dP/dt$ attained a maximum value within the gradual depolarization phase. Thus, it was possible to determine the relationship between the maximum $dP/dt$ and $dV/dt$ values. The maximum $dP/dt$ was constant, unless $dV/dt$ was less than the critical value of 40–60 mV/sec, as illustrated in Fig. 8C. This critical value was so small that it took as long as 0.5–0.8 sec for the membrane potential to increase from $V_{th}$ to -23 mV at the critical speed. These results indicate that the origin of the slow tension rise in crayfish muscle consists in the contractile process rather than in the spread of membrane depolarization toward the interior of the muscle fiber. For depolarization
slower than the critical speed, \( \frac{dP}{dt} \) was decreased. For example, the change in \( \frac{dV}{dt} \) from 80 to 10 mV/sec decreased the maximum \( \frac{dP}{dt} \) to about half.

Fig. 8. A and B show superimposed tracings of contraction produced by ramp-shaped depolarization. Upper trace (trace 2) and lower trace (trace 1) at the start of recording show potential and tension, respectively. Rates of depolarization are 900, 102, 52, and 31 mV/sec in A, and 23, 18.5, and 13.5 mV/sec in B. Holding potential is \(-80\) mV. 20°C. C shows the relationship between the maximum rate of tension rise and the speed of depolarization, \( \frac{dV}{dt} \). Two examples are shown by filled and open circles. 18–20°C.

8. Potassium contracture

Figure 9 shows the membrane potential change and contracture tension caused by potassium-rich solution. The concentration of potassium ions that produced a just-detectable tension was 20 mM. On application of 50–100 mM potassium, the tension increased rapidly and was maintained for more than 20 sec (Figs. 9A, B). Spontaneous relaxation was seen in the isosmotic potassium propionate solution containing 14 mM CaCl\(_2\) and 2.8 mM MgCl\(_2\) (Fig. 9C).

The relationship between peak tension and potassium concentration or membrane potential is illustrated in Fig. 9D. The relative tension in isosmotic potassium propionate solution was taken as 1.0. This tension-membrane potential relation was quite similar to that obtained by the voltage clamp experiment (Fig. 3). The mechanical threshold potential was \(-53\) mV. A nearly linear relationship was maintained between tension and membrane potential or logarithm of potas-
sium concentration in the range between $-50$ and $-27$ mV or $20-60$ mM, and the slope of the line was $0.3 P_0$ for $10$ mV change in membrane potential, where $P_0$ was the tension in isosmotic potassium propionate solution. The tension in $205$ mM potassium solution was slightly greater than that in $100$ mM potassium solution; therefore, it was difficult to determine the real maximum tension.

Fig. 9. A, B, and C: membrane potential (upper trace) and tension (lower trace) in potassium-rich solutions. Potassium concentrations are $30$ mM in A, $60$ mM in B, and $205$ mM in C. The bars at the bottom indicate the period during which the muscle fiber was exposed to potassium-rich solution. 18°C. D: relation between tension and potassium concentrations or membrane potentials. Tension is represented as the ratio to that in $205$ mM potassium propionate. Circles indicate the maximum and minimum tension at the given potassium concentration; inserted figures show the numbers of experiments. Filled circles indicate tension in the experiment shown in A, B, and C and refer to the membrane potential scale. 18-23°C.

DISCUSSION

In the studies on the time course of tension changes in contraction and relaxation, it was necessary for the spatial and temporal distributions of the membrane potential along the longitudinal axis of the muscle fiber to be uniform. This condition was satisfied if the clamped potential was more negative than $-20$ mV and longer than $100$ msec.

The tension produced at a given membrane potential and at a given time after the beginning of depolarization was described by Eq. (2). Namely, the tension was proportional to the membrane potential and to the second order of the exponential relation with time.
The concavely rising initial phase of tension was altered by the speed of depolarization. According to DUDEL et al. (1968), only 5–10 msec was sufficient for the depolarization of the surface membrane to spread toward the interior of the muscle fiber. Also, RÜDEL and TAYLOR (1970) showed that the just suprathreshold depolarization activated all of the myofibrils at the center of the fiber. Therefore, the rate of rise of initial tension can not be determined simply by the speed of inward spread of activation. Probably, the rate of Ca-release or Ca-influx into sarcoplasm depends on the speed of depolarization and limits the rate of tension rise at the start of contraction.

The time constant $\tau_2$ was about 0.11 sec at 20–25°C, and it was comparable to the time required for the rate of tension rise to reach the maximum. The ramp-pulse experiments indicated that the slow tension rise in the crayfish muscle was due to the properties of the contractile machinery. Therefore, $\tau_2$ will indicate the time between the beginning of depolarization and the point at which the full active state is reached. According to HILL (1949), the vertebrate skeletal muscle became fully active almost immediately after stimulation, but MASHIMA (1967) investigated the rising phase of the active state of frog muscle and showed a definite delay of 20 msec at 10°C between stimulus and the peak of the active state. Also, it took more than 0.2 sec at 30°C for the heart muscle to reach peak activation (SONNENBLICK, 1967). Therefore, it is likely that the active state of the crayfish muscle develops very slowly, probably within 0.15 or 0.20 sec at 20–25°C.

Another time constant, $\tau_1$, is related to the viscoelastic properties of muscle fiber during contraction. The tension rises depending on the elastic and viscous components after the active state has fully developed, and $\tau_1$ indicates the retardation time of the viscous and elastic components.

MITTENTHAL and CARLSON (1971) investigated the time course of exponential rise of tension in frog sartorius muscle and suggested that the rate constant would be related to the time course of the activity at the crossbridges between myosin and actin filaments. Their rate constant, $\alpha$, corresponds to the reciprocal of $\tau_1$ in the present study. Apart from the active state theory, it may be stated that the time constant $\tau_1$ is related to the reaction at the crossbridges and the time constant $\tau_2$ is related to the reaction involved in calcium movement.

JEWELL and WILKIE (1960) reported that the tail of the isometric twitch tension of frog sartorius muscle decreased with an exponential time course. The exponential part described by JEWELL and WILKIE seems to correspond to the first exponential part, $Ce^{-t/\tau_1}$, in Eq. (3). This term is positive and $\tau_1$ will be related to the viscoelastic property of muscle during relaxation.

The second term, $De^{-t/\tau_2}$, is negative. The negative term is necessary because the tension decreases more slowly at first than the exponential decrease of $Ce^{-t/\alpha}$. The second and third terms, $(-De^{-t/\tau_2} + Ee^{-t/\alpha})$, indicate the processes opposite to that represented by the term $Be^{-t/\tau_2}$, namely, the process of the decay of the active state. EBASHI and ENDO (1968) suggested that the falling phase of the
active state was determined by the Ca-uptake of the sarcoplasmic reticulum and the release of Ca ions from the contractile protein. If the latter is a slow process (EBASHI et al., 1969), the time course of Ca-removal from the myofilaments will be included in the term $De^{-\tau_2}$. It is also thought that the tension curve will be deformed due to the unequal length of sarcomeres, if present. Since the tension in a long sarcomere is stronger than the tension in a short sarcomere (HUXLEY, 1971), the former stretches the latter during contraction. This effect will continue until an equal tension within each sarcomere is formed at the later part of relaxation. The time constant $\sigma_2$ may include the time course of the relaxation phase with different tensions within sarcomeres.

ASHLEY and RIDGWAY (1970) showed that calcium-mediated light emission decreased exponentially with a time constant of 50–60 msec at 11°C and fell to a very low level at the time of tension peak. The time constant $\sigma_3$ obtained in the present study is 0.05–0.08 sec at 20–27°C, which is larger than the value presented by ASHLEY and RIDGWAY. However, the third term, $Ec^{-\tau_3}$, becomes negligible when the tension reaches its peak 0.1–0.15 sec after the end of depolarization. Therefore, $\sigma_3$ will represent the time constant of Ca uptake by the sarcoplasmic reticulum. MITTENTHAL and CARLSON (1971) indicated that the rate of tension fall in frog sartorius muscle was gradually decreased by increasing the duration of tetanic stimulation. On the contrary, the time course of relaxation of the crayfish muscle is independent of the duration of square-pulse depolarization. Contraction and relaxation are much slower in the crayfish muscle than in the frog muscle, and the rate-limiting factor in relaxation may not be the same for the two kinds of muscles.

As shown in Fig. 9, repolarization after washing in a potassium-rich solution was quite slow. The time course of repolarization shows the diffusion of potassium out of the tubular lumen, as described by NAKAJIMA et al. (1969). The tension in relaxation phase falls to the resting level even when the membrane is still depolarized above the mechanical threshold potential. Similar records were presented by ZACHAR and ZACHAROVA (1966), and by Hodgkin and Horowicz (1960) on frog muscle fibers. Probably, the process necessary for contraction is inactivated during depolarization lasting for 20 sec or more in potassium-rich solution.

The author expresses his sincere thanks to Professor Hidenobu Mashima for helpful discussions and for reading the manuscript. This work was partly supported by a grant from the Ministry of Education of Japan.

REFERENCES

CONTRACTION IN CRAYFISH MUSCLE


