EFFECTS OF THOULET’S REAGENT ON THE GLYCERINATED MUSCLE FIBER\textsuperscript{1,2,3}

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Many theories have been proposed about the actomyosin (AM)-adenosine-triphosphate (ATP) interaction as an essential mechanism of muscular contraction. Weber (1, 2), Bozler (3), and Nagai (4, 5, 6) emphasized that the muscular contraction is an active process utilizing the splitting energy of ATP and that the relaxation is a passive process.

In contrast to this, Szent-Györgyi (7, 8, 9) and Morales (10, 11) stated that the ATP splitting corresponds to the relaxation and that contraction is a spontaneous process.

It was found by Varga (12) and Laki and Bowen (13) that the glycerinated muscle fiber and AM-thread can be shorten intensively by Thoulet’s reagent (14) (HgI\textsubscript{2} in KI solution)(13). Morales (11) considered that these observations raised a real question about the nature of ATP contraction of the glycerinated muscle fiber.

In the present paper, the authors studied the shortening of the glycerinated muscle fiber and glycerinated tendon caused by Thoulet’s reagent, CuCl\textsubscript{2}\textsuperscript{4} and high temperature in expecting to clarify whether the effects of Thoulet’s reagent on the AM system are the same as those of ATP or not.

EXPERIMENTAL

1) Materials: The glycerinated muscle fiber was prepared from rabbit psoas muscle following the method of Szent-Györgyi’s (15). The glycerinated tendon was made from tendon of rabbit dorsal muscle in the same way.

2) Test solutions: Thoulet’s reagent (10\textsuperscript{-4} ~ 5 \times 10\textsuperscript{-1} M),\textsuperscript{4} CuCl\textsubscript{2} (10\textsuperscript{-4} ~ 5 \times 10\textsuperscript{-1} M) and ATP solution\textsuperscript{5} (containing 4 \times 10\textsuperscript{-3} M ATP, 0.16 M KCl and 1.5 \times 10\textsuperscript{-3} M MgCl\textsubscript{2} per liter) were used.

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\textsuperscript{1} Preliminary reports were published on the Sapporo Medical Journal Vol. 9, No. 1, Jan. 1956 and Vol. 9, No. 5/6, May/June, 1956.

\textsuperscript{2} The essential points of this paper were presented at the meeting of Physiological Society of Japan in Okayama, May 1956.

\textsuperscript{3} The effects of CuCl\textsubscript{2} were reported by Varga (12) and he stated that the shortening caused by CuCl\textsubscript{2} resembles that induced by Thoulet’s reagent.

\textsuperscript{4} 0.5 M Thoulet’s reagent is 0.5 M HgI\textsubscript{2} in 1.0 M KI solution.

\textsuperscript{5} ATP-Ba salt, prepared in this laboratory, was converted into K salt and was diluted to the concentration of 4 \times 10\textsuperscript{-3} M.
3) Observations of free contraction: Before experiment, a bundle of glycerinated muscle fibers was washed for an hour in 20% glycerol solution at room temperature. Thereafter, the bundle was divided into fine bundles (0.3 mm in diameter) under the microscopic scale, and they were treated for 3 minutes with 0.16 M KCl solution. Then the length of each of these fiber bundles (original length) was measured and they were immersed in test solutions. After 3-5 minutes the fibers were took out and their length was measured again. The grade of shortening was expressed by the ratio of the length lost by shortening against the original length in per cent. The effects of ions such as K, Mg and Ca were observed by adding them to Thoulet's reagent (0.15 M) in desired concentrations. For studying the effect of salyrgan, the fibers were treated with $4 \times 10^{-3}$ M salyrgan for 4 minutes before the immersion in the test solution.

The influence of temperature was observed by adjusting the temperature of test solution to a desired height within the range of from 0° to 60° C.

For studying the effect of Thoulet's reagent on the ATP contraction of the fiber, the fibers were treated previously with Thoulet's reagent and the grade of shortening by Thoulet's reagent alone was measured. Then, the fibers were immersed in ATP solution.

The measurement of the grade of the shortening of the glycerinated tendon was performed in the same way as that with the glycerinated muscle fiber.

4) Observations of isometric contraction: By binding together both ends of a fine bundle, a ring of the bundle was formed. The fiber ring was connected with an isometric lever. Then the fiber ring was immersed into the test solution after having been washed in 0.16 M KCl solution under the thermostatic condition. The tension developed was plotted against time. The measurement of the shortening induced by high temperature was performed in 0.16 M KCl solution.

RESULTS

When the glycerinated muscle fiber is immersed in Thoulet's reagent, the fiber shortens to about 40-50% of its original length and becomes very elastic. On the other hand, the glycerinated muscle fiber develops tension of about 0.8 g/mm² instantaneously by the immersion in this solution. As shown in fig. 1, the tension falls gradually thereafter. The tension developed by ATP is about 6 g/mm² in this condition.

The shortening of fiber in Thoulet's reagent is prevented by K, Mg and Ca ions coexisted in Thoulet's reagent (fig. 2-4). However, even when 1.9 M KCl is used, the grade of shortening decreases less than 10%. The inhibitory effect of Mg ions is practically equal to that of Ca ions.

As shown in fig. 5, $4 \times 10^{-2}$ M salyrgan gives no marked influence on the Thoulet's reagent shortening of the glycerinated muscle fiber, while ATP contraction suffers notable inhibition by salyrgan so that the grade of shortening which may reach at least 70% without salyrgan, is limited to only 5% with it.

Fig. 6 shows that the grade of the Thoulet's reagent shortening increases with the rise of temperature in the range of 0° to 60° C. The temperature dependency of the shortening becomes less marked with increase of the concentration of the reagent.
Fig. 1. Tension developed in glycerinated muscle fiber by Thoulet's reagent or ATP. Dashed line: 0.5 M Thoulet's reagent. Continued line: \(4 \times 10^{-3}\) ATP (containing 0.16 M KCl, 1.5 \(\times 10^{-3}\) M MgCl\(_2\)). Temperature: 20°C. In the case of ATP, the fiber was loaded with 0.6 g/mm\(^2\) before the experiment.

Fig. 2. Effect of K ions on the shortening of the glycerinated muscle fiber by Thoulet's reagent (0.15 M). Temperature: 15°C. Incubation time: Shortening was measured 4 min after immersion in the reagent.

Fig. 3. Effect of Mg ions on the shortening of the glycerinated muscle fiber by Thoulet's reagent (0.15 M). Temperature: 15°C. Incubation time: 5 min.

Fig. 4. Effect of Ca ions on the shortening of the glycerinated muscle fiber by Thoulet's reagent (0.15 M). Temperature: 15°C. Incubation time: 5 min.
FIG. 5. Effect of salyrgan on the shortening of the glycerinated muscle fiber by Thoulet's reagent.

1. $4 \times 10^{-3}$ M ATP (containing $0.16$ M KCl, $1.5 \times 10^{-3}$ M MgCl$_2$).
2. $0.15$ M Thoulet's reagent.
3. $0.25$ M Thoulet's reagent.
4. $0.35$ M Thoulet's reagent.
5. $0.5$ M Thoulet's reagent.

Full column: Treated for 4 min with $4 \times 10^{-3}$ M salyrgan.
Empty column: Without salyrgan.
Incubation time: ATP, 3 min; Thoulet's reagent, 4 min.
Temperature: $18^\circ$C.

FIG. 6. Effect of temperature on the shortening of the glycerinated muscle fiber by Thoulet's reagent.
Incubation time: 4 min.
No shortening of the glycerinated muscle fiber is practically induced by $10^{-4}$ M Thoulet's reagent (fig. 7). However, the fiber treated with the reagent of this concentration shows an ATP contraction about 20% smaller compared with that of the control. The fiber shows only 1-2% shortening by $5 \times 10^{-4}$ M or $10^{-3}$ M Thoulet's reagent, but ATP contraction is inhibited remarkably by Thoulet's reagent in these concentrations and the grade of contraction remains merely 2-3%.

The glycerinated tendon shortens to 10-20% of its original length by Thoulet's reagent as shown in fig. 8 and becomes somewhat transparent. However, ATP induces no contraction in the glycerinated tendon.

Further, the shortening of glycerinated muscle fiber caused by CuCl$_2$ and high temperature was studied in the same way as with Thoulet's reagent. The results are summarily shown in table 1.
TABLE 1. Effect of 4 Contracting Agents

<table>
<thead>
<tr>
<th>Contracting agent</th>
<th>External appearance of the fiber</th>
<th>Grade of the maximum shortening (%)</th>
<th>Isometric tension (g/mm²)</th>
<th>Effect of ions on the shortening</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thoulet's reagent</td>
<td>Semi-transparent</td>
<td>55</td>
<td>0.8</td>
<td>Slightly prevented (Coexistence of 1.9 M KCl causes only 10% inhibition of the shortening)</td>
</tr>
<tr>
<td>CuCl₂</td>
<td>White turbid</td>
<td>46</td>
<td>0.9</td>
<td>Slightly prevented (Coexistence of 2 M KCl causes 20% inhibition of the shortening)</td>
</tr>
<tr>
<td>High temperature (above 50°C)</td>
<td>White turbid</td>
<td>51</td>
<td>0.56</td>
<td></td>
</tr>
<tr>
<td>ATP</td>
<td>Semi-transparent</td>
<td>60-80</td>
<td>6.0</td>
<td>Notably prevented (The contractility scarcely appears if KCl concentration rises above 0.6 M)¹¹</td>
</tr>
</tbody>
</table>

Note: ¹¹ cited from (6). ²² cited from (18), (19) and (20). ³³ cited from (18), (19)

DISCUSSION

Varga (12) reported that the glycerinated muscle fiber can be shortened to 50% of its original length by Thoulet's reagent. Laki et al. (13) also reported about the Thoulet's reagent shortening of single fiber of glycerinated muscle and that of AM-thread. The present results were the same as those obtained by Varga and Laki et al.

Then the above stated properties of shortening caused by Thoulet's reagent were compared with those caused by ATP.

In ATP solution the glycerinated muscle fiber contracts to 30-40% of its original length (fig. 5) and develops tension of about 6 g/mm². This grade of contraction is the same as that reported by A. G. Szent-Györgyi (16), Maruyama (17) and Nagai et al. (5) who all made observations under almost the same condition. Szent-Györgyi (15) stated that the glycerinated muscle fiber can develop tension of 20 g/mm² by ATP solution. This value is somewhat larger than that found in the present experiment. This inconsistence may be due to some differences in experimental conditions. In the Thoulet's reagent contraction, the tension and the grade of shortening are considerably smaller than those in the ATP contraction.

As to the effects of several ions on ATP contraction of the glycerinated muscle fiber, many authors have already reported that the fiber shows maximum free contraction in the range of 0.1-0.5 M KCl and that when KCl concentration increases over 0.5 M the contraction of the fiber scarcely appears (5, 16, 17). The effects of Mg and Ca ions are very specific and antagonises each other, i.e. Mg ions in a certain concentration accelerate contraction and relaxation of the fiber while Ca ions prevent both contraction and relaxation (18, 19, 20).

On the other hand, the Thoulet's reagent shortening is inhibited by increase in concentration of coexisted ions, and this can be seen in all species of ions.
THOULET’S REAGENT AND MODEL FIBER

on the Glycerinated Muscle Fiber

<table>
<thead>
<tr>
<th>caused by the agents</th>
<th>Shortening inhibition by salyrgan</th>
<th>Effect of temperature on the shortening</th>
<th>Effect of the agent on ATP contraction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mg</td>
<td>Prevented</td>
<td>(-)</td>
<td>The grade of shortening increases with the rise of temperature within the range of 0°-60° C.</td>
</tr>
<tr>
<td>Ca</td>
<td>Prevented</td>
<td>(-)</td>
<td>The grade of shortening increases with the rise of temperature within the range of 0°-60° C.</td>
</tr>
<tr>
<td>Accelerated(^2)</td>
<td>Prevented(^3)</td>
<td>(+)</td>
<td>The shortening shows maximum value at 20°-30° C. The grade of shortening decreases in the both sides of the above range.(^3)</td>
</tr>
</tbody>
</table>

and (20). 4) cited from (22), (23) and (24).

applied. Moreover, the fact that even under 1.9 m KCl in which the AM is considered to be in dissociated state the fiber can be shortened by Thoulet’s reagent suggests the presence of difference in the mode of action between Thoulet’s reagent and ATP.

The inhibition of Thoulet’s reagent shortening caused by increase in concentration of the coexisted ions may be resulted from changes in the dissociation constant of Thoulet’s reagent or from changes in the ratio of binding Thoulet’s reagent to AM.

Salyrgan, a typical SH reagent, prevents ATP contraction of the glycerinated muscle fiber, but it bears no influence on Thoulet’s reagent shortening. The inhibitory action of salyrgan on ATP contraction was formerly observed by Weber (1) and Kuschinsky et al. (21). They interpreted the mechanism of this inhibition as an inhibition of ATPase activity. The fiber in which the ATPase activity of AM is even considerably inhibited by the treatment with salyrgan, can be shortened by Thoulet’s reagent. This fact suggests distinctly that the mechanism of action is different in these two agents, Thoulet’s reagent and ATP.

The Thoulet’s reagent shortening increases in degree with rise in temperature up to 60° C., and the decrease of shortening at high temperatures which was reported already in the ATP contraction of the glycerinated muscle fiber (22, 23, 24) can not be observed. In this point also, one can recognize the difference between the two contractions induced by ATP and Thoulet’s reagent.

Varga (12) observed that the developed tension of the fiber in Thoulet’s reagent is independent of temperature between 0° and 25° C. And, from this data, he considered that internal energy seems to dominate in the Thoulet’s reagent shortening while in the ATP contraction entropy change plays a dominant role. However, these data obtained by Varga differ from the present
results. This difference may be originated from the application of high concentration of Thoulet's reagent by Varga in which the contractility of the fiber shows very low temperature dependency as shown in fig. 6. Standing on the point of view advanced from this laboratory (4, 5, 6) which emphasizes that the ATP contraction is rather due to energy changes, the Thoulet's reagent shortening could be considered to have a entropic nature as stated by Pryor (14, 25) also.

Thoulet's reagent solution, even if its concentration is so low that it induces no shortening, inhibits ATP contraction remarkably. The other substances which prevent ATP contraction of the glycerinated muscle fiber, e.g. salyrgan, pyrophosphate etc., do not induce per se shortening of the fiber (16, 27). Then, the mechanism of action of these agents on the fiber should be distinguished from that of Thoulet's reagent.

Glycerinated tendon can be shortened to 10-20% of its original length by Thoulet's reagent. The Thoulet's reagent shortening of the native tendon was already reported by Pryor (14). On the contrary, ATP does not shorten the glycerinated tendon as shown in the present experiment. These facts suggest that ATP bears specific effects on the AM system and, moreover, that the effects of Thoulet's reagent are entirely unspecific.

As shown in table 1, the shortening of the fiber by CuCl₂ and by high temperature bear a striking resemblance to the Thoulet's reagent shortening except the external appearance, while these shortenings much differ from the ATP contraction in many points. It is known already that Cu and high temperature induce denaturation of AM (8, 15, 22). Accordingly the Thoulet's reagent shortening of the glycerinated muscle fiber may also be considered to be due to the denaturation of AM.

After this paper was completed, the authors received Bowen and Laki's paper (26). They reported that the extent and speed of the shortening of glycerinated muscle fiber in Thoulet's reagent are temperature dependent and that the mechanism of the shortening is probably different from that brought about by ATP.

SUMMARY

1) The glycerinated muscle fiber develops a tension of about 0.8 g/mm² by 0.5 M Thoulet's reagent.

2) The shortening of the glycerinated muscle fiber by Thoulet's reagent is prevented by K, Mg and Ca ions coexisted in Thoulet's reagent. However, even with 1.9 M KCl, the grade of shortening decreases less than 10%.

3) Salyrgan bears no marked influence on the Thoulet's reagent shortening of the glycerinated muscle fiber.

4) The Thoulet's reagent shortening of the glycerinated muscle fiber increases in degree with rise of temperature in the range of 0° to 60° C.

5) Even in low concentration (10⁻⁴ M), Thoulet's reagent shows a marked inhibition on the ATP contraction of the glycerinated muscle fiber.

6) Glycerinated tendon is shortened to about 10-20% of its original length by Thoulet's reagent.
7) Thoulet’s reagent shortening of the glycerinated muscle fiber bears a striking resemblance to the shortening caused by CuCl₂ and that by high temperature, but differs in many points from ATP contraction.

REFERENCES