THE EFFECT OF HYPERTONIC SOLUTIONS ON THE RATE OF RELAXATION OF CONTRACTURE TENSION IN *MYTILUS* SMOOTH MUSCLE

By T. TAMEYASU

Department of Physiology, School of Medicine, Teikyo University, Itabashi-ku, Tokyo, Japan

(Received 19 September 1977)

SUMMARY

1. The effect of the bathing solution tonicity on the mechanical properties of the anterior byssal retractor muscle (ABRM) of *Mytilus edulis* was examined.

2. The rate of relaxation of contracture tension produced by acetylcholine (ACh) was greatly reduced when the bathing solution tonicity was increased by adding NaCl, KCl or LiCl after the removal of ACh, whereas a decreased tonicity increased the rate of relaxation.

3. The contracted ABRM in hypertonic solutions showed no active shortening after an isotonic release and barely redeveloped active tension after a quick release.

4. The resistance to stretch increased with increasing tonicity of the bathing solution.

5. The wet weight of the ABRM decreased much more markedly in sucrose-hypertonic solution than in Na-, K- or Li-hypertonic solutions, but the decrease in the rate of relaxation was less marked in the former, indicating that there may be little relation between the rate of relaxation and the degree of osmotic deformation of the ABRM fibres.

6. It is suggested that the elevated ionic strength in the myoplasm may be related to a reduction in the rate of detachment of the cross-linkages between the thick and thin filaments.

INTRODUCTION

Certain molluscan smooth muscles, including the anterior byssal retractor muscle (ABRM) of *Mytilus edulis*, exhibit a special kind of tonic contraction which has been called 'catch'. During the catch, the muscle maintains tension only passively. According to the paramyosin hypothesis, the catch state is due to the interaction between the paramyosin filaments and is independent of the actomyosin system which generates tension actively (for a review, see Johnson, 1962; Rüegg, 1971). Alternatively, the linkage hypothesis supposes that the catch tension is maintained by the cross-linkages between the actin and myosin filaments, a very small rate of detachment of the cross-linkages being assumed in the catch state (Lowy & Millman, 1963). Recent structural, biochemical and physiological studies seem to support the linkage hypothesis (Szent-
Several authors have argued that intracellular Ca ions may be the factor controlling the rate of detachment of the cross-linkages (Twarog & Muneoka, 1973; Baguet & Marchand-Dumont, 1975; Atsumi & Sugi, 1976). Sugi, Yamaguchi & Tanaka (1977) reported that a hypertonic solution made by the addition of NaCl caused much smaller change in the wet weight of the ABRM than did a solution made hypertonic by the addition of non-electrolyte substances. Raising the intracellular ionic strength by changing the concentration of external NaCl appears to cause little osmotic shrinkage of the ABRM fibres. In the present experiments, the effect of hypertonic solutions on the contracted ABRM has been examined to study the effect of increasing the intracellular ionic strength on the rate of relaxation of contracture tension.

**MATERIALS AND METHODS**

**Preparation**

Specimens of *Mytilus edulis* were collected in the vicinity of the Misaki Marine Biological Station and kept in tanks of aerated sea water at 18–20 °C. The ABRM was isolated with a piece of shell at one end and the byssal organ at the other. It was carefully teased under a binocular microscope to obtain a fibre bundle of less than 1.5 mm diameter. The fibre bundle was mounted horizontally in an acrylite chamber (10 ml) filled with the experimental solution. To record tension and length changes of the preparation simultaneously, the shell was securely held by an acrylic holder connected to the tension transducer, while the byssal end was hooked to the lever of the displacement transducer. In some experiments, only the isometric tension was recorded.

**Transducers**

A strain gauge (U-gauge, Shinko Tsushin Co.) was used as the tension transducer. Its compliance was 1 µm/g and its natural frequency of oscillation about 150 Hz.

The length change of the preparation was measured with the displacement transducer with an aluminium lever attached to a rotatory core, the angle of which was sensed by a differential transformer (ME-4012, Medical Electronics Commercial Co.). The angle signal was linearly related to displacement of the lever over a range of 10 mm at the point of attachment of the preparation, which was 3 cm from the centre of the rotatory core. The compliance of the lever at the point of attachment of the preparation was 0.5 µm/g. In some experiments, a light beam–phototube system, which was essentially the same as that used by Tameyasu & Sugi (1976), was used as the displacement transducer with similar results. The changes in both tension and length were recorded simultaneously on an ink-writing oscillograph.

**Solutions**

Table 1 shows the composition and the osmolarity of the major solutions used in the experiments. The solutions are named according to the osmolarity relative to the standard solution and the major constituent; for example, 4·2 TNa represents an osmolarity 4·2 times the standard and its major cation is Na. The osmolarity of solutions was estimated as the sum of the calculated osmolarity of the major con-

---

Györgyi, Cohen & Kendrick-Jones, 1971; Sobieszek, 1973; Nonomura, 1974; Tameyasu & Sugi, 1976). Several authors have argued that intracellular Ca ions may be the factor controlling the rate of detachment of the cross-linkages (Twarog & Muneoka, 1973; Baguet & Marchand-Dumont, 1975; Atsumi & Sugi, 1976).
Tension in Mytilus muscle

Table 1. Composition of solutions

<table>
<thead>
<tr>
<th>Solution</th>
<th>Salts (mm)</th>
<th>Sucrose (mM)</th>
<th>Approximate osmolarity (m-Osmol)</th>
<th>Relative osmolarity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard solution</td>
<td>NaCl 450</td>
<td>KCl 10</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>0.5 T NaCl</td>
<td>175</td>
<td>10</td>
<td>—</td>
<td>1020</td>
</tr>
<tr>
<td>2.0 T NaCl</td>
<td>1000</td>
<td>10</td>
<td>10</td>
<td>2050</td>
</tr>
<tr>
<td>2.1 TS</td>
<td>450</td>
<td>10</td>
<td>10</td>
<td>2110</td>
</tr>
<tr>
<td>3.1 T NaCl</td>
<td>1550</td>
<td>10</td>
<td>10</td>
<td>3140</td>
</tr>
<tr>
<td>4.2 T NaCl</td>
<td>2100</td>
<td>10</td>
<td>10</td>
<td>4310</td>
</tr>
<tr>
<td>4.2 T LiCl</td>
<td>—3270</td>
<td>50</td>
<td>—</td>
<td>4310</td>
</tr>
</tbody>
</table>

To make the ABRM fibres contract maximally, a 10⁻⁴ to 10⁻³ M acetylcholine (ACh) solution was made by dissolving solid ACh-chloride in the standard solution. The solution containing 5-hydroxytryptamine (5-HT) was usually made by adding 10⁻⁸ M stock solution, and in some cases by adding solid 5-HT. The pH of all solutions was adjusted to 6.8–7.6 by the addition of NaHCO₃ less than 0.5 mM.

**Experimental procedures**

The resting length $L_0$ of the preparation, at which the resting tension was just barely detectable in the presence of 10⁻⁸ M 5-HT, was determined prior to the experiments (Lowy & Millman, 1963). The ABRM fibre bundle was made to contract with ACh, and after the contracture tension reached a peak, the bathing solution was changed to solutions of various tonicities. The rate of relaxation in the standard solution was almost constant if the preparation was stimulated at intervals of about 20 min, and the constant rate was taken as the control rate of relaxation. After an exposure to hypertonic solutions for 20–30 min, the preparation was returned to and kept in the standard solution for 1–3 h to restore its mechanical response to ACh before the next experiment was started. When the tonicity of the solution was not more than 3.1 times that of the standard solution, the magnitude of ACh-induced contracture tension recovered completely to the original value within 1–3 h. If the tonicity was 4.2 times the standard, the recovery of ACh-contractures was much slower and often incomplete, whereas the contracture tension produced by 300 mM-K (substituted for Na), which was as great as ACh-induced contracture tension, recovered completely within 3 h, indicating that the hypertonic solution did not damage
the contractile apparatus of the ABRM fibres. The solution in the chamber was changed by injecting a new solution after draining an old one with a water-vacuum suction tube.

The isotonic release experiments were performed as follows: the preparation was first made to contract isometrically by preventing the movement of the lever of the displacement transducer by a stop; at any chosen moment, the lever was released by moving the stop with an electromagnetic device so as to change the load on the preparation quickly from the isometric value to a lower level determined by the loading spring. In the quick release experiments, the lever was released for a distance without the loading spring, the amount of quick release being determined by changing the distance between the stops. The stretch resistance of the preparation was measured by moving the lever slowly for a given distance with an electromagnetic device and by recording the coincident length and tension changes (for details, see Tameyasu & Sugi, 1976).

Unless otherwise stated, the experiments were performed within the range of lengths where the resting tension was negligible. All experiments were made at room temperature (18–25 °C).

RESULTS

Relaxation in solutions of various tonicities

The effect of hypertonic solutions on the rate of relaxation of ACh-induced contracture tension was examined using the hypertonic solutions made by adding NaCl to the standard solution (2·0–4·2 TNa). On exposure to a hypertonic (3·1 TNa) solution, the rate of tension decay decreased markedly and increased again on returning to the standard solution (Fig. 1a, b). The tension decay rate under a given tonicity varied from one preparation to another, though the variation appeared to decrease with increasing tonicity. In the same preparation, the tension decay rate under a given tonicity was almost constant, decreasing with increasing tonicity (Fig. 1c). Alteration of the length of the preparation from 0·4 to 1·0 L0 had no significant effect on the rate of relaxation in the hypertonic solutions.

The hypertonic solutions also markedly reduced the rate of relaxation of ACh-induced contracture tension in the presence of 10–6 M 5-HT and the rate of relaxation of K-contracture tension induced by 300–460 mM-K (substituted for Na). On the other hand, a solution made hypotonic (0·5 T) by subtracting NaCl from the standard solution increased the rate of relaxation of ACh-induced contracture tension.

Relationship between composition of hypertonic solution, change in wet weight and rate of relaxation

Fig. 2a shows the effect of hypertonic solutions of various compositions on the wet weight of the whole ABRM. The wet weight was measured by lightly blotting the preparation with a filter paper after the shell and the byssal organ had been removed. As reported by Sugi et al. (1977), the hypertonic solution made up mostly of NaCl caused much smaller change in the wet weight of the ABRM than did the hypertonic solution made by adding sucrose to the standard solution. They have also observed the change of appearance of individual fibres in thin fibre bundle preparation in response to change of the bathing solution tonicity using a Zeiss Nomarski micro-
Tension in Mytilus muscle

Fig. 1. Effect of hypertonic solutions on the rate of relaxation of ACh-induced contractures. (a) and (b) are examples of experimental records. After contracture tension reached a peak, the bathing solution was changed to the standard solution in (a) and to 3·1 TNa solution in (b). Application and removal of ACh are shown by upward and downward arrows. Horizontal bar in (b) indicates the period of application of 3·1 TNa solution. Note the increased rate of relaxation after returning to the standard solution (S) in (b). (c) The logarithmic plots of contracture tension against time after the change of bathing solution to the standard (●), 2·0 TNa (△), 3·1 TNa (●) and 4·2 TNa solution (○). Tension is expressed as a fraction of the peak contracture tension (P0). Data were obtained from the same preparation.

scope, and found that the degree of the shrinkage of the fibre was much smaller in Na- than sucrose-hypertonic solution. Therefore, the change of the whole muscle wet weight seems to reflect to some extent the volume change of the component muscle cells. Not only the Na-hypertonic solution but also the hypertonic solution made up mostly of KCl or LiCl caused smaller weight change than did the sucrose-hypertonic solution. In Na-, K- and Li-hypertonic solutions, the weight change appeared to be larger in the order of K-, Na- and Li-hypertonic solutions (Fig. 2a). Thus, the weight change caused by 4·2 TK solution was as great as that caused by 2·1 TS solution (Fig. 2a).

On the other hand, the rate of relaxation of contracture tension was much greater in 2·1 TS than in 4·2 TK, 4·2 TNa and 4·2 TLI solutions. As in 4·2 TNa solution (Fig. 5e), the muscle barely redeveloped tension after a quick release in 4·2 TK or 4·2 TLI solution; the tension is maintained only passively in these solutions. As shown
Fig. 2. Effects of various kinds of hypertonic solutions on the wet weight (a) and on the rate of relaxation (b). (a) The bathing solution was changed from the standard to hypertonic solutions, and changed back. Ordinate: wet weight expressed as the percentage of the control value in the standard solution. Abscissa: time. The ABRM was soaked in 4·2 TNa (O), 4·2 TK (△), 4·2 TLi (▼) and 2·1 TS solution (●) during the period indicated by horizontal bar between the upper and lower records. The upper records were obtained from one of a pair of ABRMs from the same animal, and the lower records from the other. (b) The logarithm of tension is plotted against time after the change of bathing solution to 4·2 TNa (O), 4·2 TK (△), 4·2 TLi (▼) and 2·1 TS solution (●). Data were obtained from the same preparation.

in Fig. 2b, there was no significant difference in the rate of relaxation in 4·2 TNa, 4·2 TLi and 4·2 TK solutions.

The wet weight appeared to reach an equilibrium value very slowly when the preparation was soaked in 2·1 TS solution (Fig. 2a). It is therefore difficult to evaluate the effect of osmotic deformation of the muscle fibre on the rate of relaxation by comparing the rate of relaxation in 2·1 TS and in the other hypertonic solutions. Therefore, the contracted muscle was first soaked in 4·2 TNa and subsequently in 2·1 TS solution, the change in tension and wet weight being measured separately using paired muscles
obtained from the same animal. As shown in Fig. 3, the rate of relaxation increased on transfer to 2:1 TS solution in spite of a further decrease in the wet weight. In agreement with this decrease, observations on thin fibre bundle preparations using a phase-contrast microscope (Nikon; objective, 40 x or 100 x) showed that individual fibres shrank in response to the change from 4:2 TNa to 2:1 TS solution. Thus, little relationship was found between the rate of relaxation and the degree of osmotic deformation of the muscle fibre.

Effect of 5-HT on relaxation in hypertonic solutions. It has been shown that 5-HT affects the rate of relaxation of catch contraction (Twarog, 1954; Twarog & Muneoka, 1973). As shown in Fig. 4, the accelerating effect of 5-HT on the rate of relaxation of ACh-contractures decreased with increasing tonicity of the bathing solution. Under the tonicity at and above 3:1 times the standard, 5-HT of a concentration of below $10^{-4}$ M had no appreciable effect on the rate of relaxation.

Redevelopment of tension after a quick release. Fig. 5 shows the results of a typical experiment, in which the contracture tension exerted by the preparation under various conditions was suddenly reduced almost to zero by a quick release and the redevelopment of tension at a new length was examined. As has been shown by Jewell (1959), a marked redevelopment of tension occurred when the quick release was made at a peak of ACh-induced contracture tension (Fig. 5 a), whereas the tension redeveloped to only about 30% of the initial value when the release was made during the phase of relaxation in the standard solution (Fig. 5 b). The behaviour of the fibre bundle after the release in hypertonic solutions resembled that in the standard solution, except that the amount of quick release required to reduce the tension from a given value to zero decreased with increasing tonicity (Fig. 5 c–e).

Shortening after an isotonic release. In the ABRM, the redevelopment of tension
after a quick release has been thought to reflect not only the ability to reform the cross-linkages between the thick and thin filaments but also the effect of the inert elastic material (Jewell, 1959; Lowy & Millman, 1963). Therefore, the isotonic release experiments were made to examine further the ability to form the cross-linkages in hypertonic solutions.

When the isotonic release was made during the early phase of Ach-induced contractures, the fibre bundle shortened rapidly under a light load after the shortening of the series elastic component coincident with the reduction of tension (Fig. 6a). Little shortening occurred when the release was applied during relaxation in the standard solution (Fig. 6b).

The behaviour of the fibre bundle after the release in 2.0 TNa solution closely resembled that in the standard solution (Fig. 6c). In 3.1 TNa and 4.2 TNa solutions, active shortening after the release was virtually absent (Fig. 6d, e). Thus, the ABRM
Fig. 5. Redevelopment of tension after a quick release under various conditions. In this and subsequent figures, the upper and lower traces show the changes in tension and length, respectively. Broken lines indicate the level of zero tension. A quick release was made at a peak of ACh-induced contracture tension (a) and during relaxation at 5–10 min after the change of bathing solution to the standard (b), 2·0 TNa (c), 3·1 TNa (d) and 4·2 TNa solution (e). The amount of the length change was selected so that the tension was reduced almost to zero. Tension and time calibrations are common to all records, but displacement calibration in (a) is different from the others. Records were obtained from the same preparation.

Resistance to stretch under various conditions. The resistance to stretch was examined by stretching the preparation by about 0·28 mm with the velocity of about 0·006 L0/s. The small variation in the velocity had no significant effect on the tension response (Lowy & Millman, 1963). With the velocity employed, the amount of the tension change for a given amount of the length change appeared to depend to some extent on the level of tension at the beginning of the length change. Therefore, the length change was applied at almost the same level of tension under various conditions. As
clearly shown in Fig. 7a–d, the stretch resistance expressed as the amount of tension change caused by a given amount of stretch greatly increased with increasing tonicity of the bathing solution.

**Effect of a hypertonic solution on relaxed ABRM**

As shown in Fig. 8a, the slightly stretched resting preparations (at 1.0–1.2 L₀) showed a tension development when they were transferred from the standard to 3.1 or 4.2 TNa solutions, as has been the case in frog skeletal muscle (e.g. Hill, 1968). On returning to the standard solution with 10⁻⁶ M 5-HT, the tension rapidly fell to the original level. On the other hand, the preparation previously depolarized with 460 mM-K failed to develop tension in response to 4.2 TNa solution (Fig. 8b). Instead, a fall of the resting tension was observed.
Fig. 7. Tension response to a slow stretch under various conditions. Stretches were applied at almost the same level of tension during the phase of relaxation of ACh-induced contractures in the standard (a), in 2·0 TNa (b), in 3·1 TNa (c) and in 4·2 TNa solutions (d). Records were obtained from the same preparation.

Fig. 8. Mechanical response of the relaxed ABRM to 4·2 TNa solution. (a) and (b), tension response of a slightly stretched ABRM in the isometric state. Broken lines indicate the level of zero tension. Change from the standard (a) and the isotonic K (b) to 4·2 TNa solution at ↓, change back at ↑. In (b), 4·2 TNa solution was applied after the isotonic K-induced contracture tension had relaxed completely. The standard solutions contained 10⁻⁶ M 5-HT. (c) and (d), simultaneous recordings of changes in tension (upper trace) and length (lower trace). (c) An afterloaded shortening under 0·3 g load. 4·2 TNa solution was injected gently into the chamber without draining the standard solution in order to avoid the mechanical artifact. Arrow indicates the beginning of injection. In (d), an isotonic release was made at 5 min after the change from the standard to 4·2 TNa solution. The length of preparation was below \( L_0 \) in (d). Records (a) and (b) were obtained from one preparation, and those (c) and (d) from another.
As shown in Fig. 8c, the preparation could shorten several mm under a light load in response to the application of 4.2 TNa solution. When an isotonic release was made about 5 min after the application of 4.2 TNa solution, no active shortening occurred (Fig. 8d).

**DISCUSSION**

The present results have shown that hypertonic solutions made up mostly of NaCl, KCl or LiCl greatly reduced the rate of relaxation of contracture tension, and that the tension was maintained only passively in these hypertonic solutions (Figs. 5, 6). It is thought that there is little formation of new cross-linkages between myosin- and actin-containing filaments in the hypertonic solutions. Though hypertonic solutions caused the decrease of wet weight of the preparation, there is little relationship between the rate of relaxation and the degree of decrease of wet weight (Figs. 2, 3). The change in the wet weight seems to reflect the volume change of the muscle fibres. Therefore, the osmotic deformation of the ABRM fibres by itself may not be a primary cause of the slow relaxation in the hypertonic solutions.

The resting *Mytilus* ABRM responded to the application of 4.2 TNa solution with a tension development in the isometric condition (Fig. 8a) or with an active shortening under a light load (Fig. 8c). Probably, the application of 4.2 TNa solution caused a rise in the myoplasmic Ca ion concentration, resulting in a contraction due to an active process of the contractile apparatus. A change in the membrane potential may be involved in this activation of the contractile apparatus, since the application of 4.2 TNa solution to the ABRM previously depolarized with 460 mM-K failed to cause a contraction (Fig. 8b). Several minutes after the application of hypertonic solutions, the muscle lost the ability to shorten actively and to redevelop active tension, while the contracture tension lasted (Figs. 5c-e, 6c-e, 8d). It may not be possible to explain such a tension maintenance only by a continuous rise in the myoplasmic free Ca ion concentration, though it is not certain whether the myoplasmic Ca ion concentration remains high over the period of the application of hypertonic solutions or not.

Johnson (1962) and Ruegg (1971) have proposed that, in molluscan catch muscle, the tension-holding mechanism is due to the fusion of paramyosin filaments. However, the structural evidence that paramyosin forms a core of the thick filament and is surrounded by myosin makes the direct interaction of paramyosin molecules between different thick filaments unlikely (Lowy & Hanson, 1962; Szent-Györgyi et al. 1971; Sobieszek, 1973; Nonomura, 1974). Moreover, in frog skeletal muscle, which does not contain paramyosin, Hill (1968) reported that the tension developed in response to the application of hypertonic solutions relaxed at a very small rate without any sign of the active state. This behaviour of the frog muscle in response to hypertonic solutions closely resembles that of the *Mytilus* ABRM (Fig. 8). Therefore, the slow relaxation of contracture tension in hypertonic solutions probably results from a small rate of detachment of the cross-linkages between myosin and actin, i.e. between the thick and thin filaments.

The change in the wet weight was much smaller in Li-, Na- and K-hypertonic solutions compared with that in sucrose-hypertonic solution (Fig. 2a; Sugi et al. 1977), suggesting that the membrane permeability to LiCl, NaCl and KCl is high in
the *Mytilus* ABRM. Since there was no significant difference in the rate of relaxation in these hypertonic solutions (Fig. 2b), the increase in the myoplasmic salt concentration is thought to be related to the decrease of the rate of detachment of the cross-linkages. However, it is at present uncertain whether the rate of detachment is reduced by the increased salt concentration directly or by some unknown factor which in turn arises in the myoplasm as a result of the increase in the myoplasmic salt concentration.

During catch contraction, the *Mytilus* ABRM had little ability to redevelop active tension after a quick release and to shorten actively after an isotonic release (e.g. Jewell, 1959; Tameyasu & Sugi, 1976). The response of the ABRM in hypertonic solutions to a quick release and to an isotonic release resembled that during catch contraction, though the stiffness of the series elastic component increased as the bathing solution tonicity increased (Figs. 5, 6), as has been reported previously (Tameyasu & Sugi, 1976).

The molluscan catch muscle contains a large amount of Na ions, and the membrane permeability to Na ions is believed to be high (Potts, 1958; Sugi *et al.* 1977). ACh is shown to increase the membrane permeability to Na and other ions (Hidaka & Goto, 1973). If it is assumed that the intracellular salt concentration changes to some extent as a result of the change in the membrane permeability under physiological conditions, the increased intracellular salt concentration might contribute to the generation of catch contraction by slowing the rate of detachment of the cross-linkages between the thick and thin filaments.

I wish to express my sincere thanks to Professor H. Sugi for his helpful suggestions and for his critical reading of the manuscript.

REFERENCES


