STUDIES ON THE ORIGIN OF RESTING TENSION OF SKELETAL MUSCLE

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Summary: Glycerol extracted frog skeletal muscle fibres at 2.2 µm sarcomere length (in situ-length) in a solution free of Ca^{++} and Mg^{++} but containing ATP, show a decrease in both their resting tension and their elastic modulus, if the ionic strength of the bathing solution is increased. This finding is compared with the behaviour of intact skeletal muscle fibres in hypertonic solution. It is concluded that the resting tension of intact skeletal muscle fibres at in situ-length is caused by the longitudinal sarcoplasmic reticulum as well as by interactions between the contractile filaments.

In a hypertonic solution skeletal muscles and single fibres of skeletal muscle at in situ-length show an increase in both resting tension (1, 2, 3, 4,) and elastic modulus (1, 4). An increase in the salt concentration and thus in the ionic strength of the bathing solution causes a decrease in the resting tension of both glycerol extracted muscle fibres in ATP free solution (rigor tension (5)) and skinned skeletal muscle fibres at in situ-length (6).

As the sarcoplasmic salt concentration of the intact muscle fibre increases in hypertonic solution (7), the results cited above seem to be contradictory. We have, therefore, investigated the effect of the ionic strength of the bathing solution on the resting tension as well as on the elastic modulus of glycerol extracted muscle fibres at in situ-length. Our results have made it easier to exclude any such contradiction in the dis-

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cussion of the findings cited above concerning the origin of the resting tension of skeletal muscle.

Method: The experiments are performed on single fibres or on bundles of 2 - 3 fibres from the M. semitendinosus of Rana esculenta, glycerol extracted in accordance to (8) at pH = 7.0. Small pieces of the fibres (6 - 8 mm length) are glued at one end to the lever of an isometric force transducer (Shinkoh U-Gage) and at the other end to a piezoxide device (PXE Multimorf, Valvo). In order to alter the length of the fibre, the piezoxide device may be moved along the long axis of the fibre by means of a micrometer screw driven by a motor with a constant velocity. The piezoxide is supplied by a sinusoidal voltage (function generator PM 5168 with amplifier PM 5170, Philips) so that sinusoidal length changes of constant amplitude are imposed on the fibre. The length changes are measured by a transducer (Shinkoh U-Gage) with its lever attached to the piezoxide. The output of the force transducer and of the length transducer is displayed on an XY-oscilloscope.

After being placed in the experimental chamber (bath volume: 2 ml) the fibre is held in a solution (100 mM KCl, 20 mM TRIS*, pH = 7.0) for 15 min, in order to wash out the glycerol. After this, the fibre is allowed to equilibrate for two hours in a solution composed of 15 mM $ATPNa_2$, 5 mM EDTA, 20 mM TRIS (pH = 7.0, osmolarity= 65 mosmol, ionic strength= 0.02).The experiments are then performed in solutions of this composition, the ionic strength being varied by adding appropriate amounts of KCl to the solution.The changing of the solutions of different ionic strength is completed within about 15 s. Bath temperature is set at 20° C.
During the experiment, the sarcomere length of the fibre is

* Tris(hydroxymethyl)aminomethane

measured by light diffraction (9, 10). From the length tension diagrams (Lissajous loops) which result from the sinusoidal length changes, both the difference of force between maximum and minimum length (in phase tension) and the quadrature tension are measured, and from these measurements are calculated the elastic (E) and the viscous component of the dynamic Young's modulus respectively (11).

Results: At slack length of the fibres (L_o) the sarcomere length is 2.0 - 2.3 µm. The shape of the length tension curves which result from stretching the fibres (beginning at L_o) at a stretching velocity of 88 µm/s depends on the ionic strength of the bathing solution (fig. 1). Increasing ionic

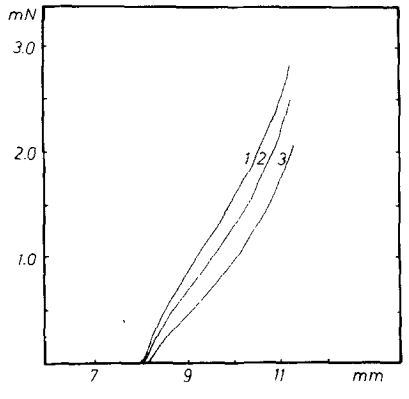

Fig. 1
Length tension relation of glycerol extracted muscles fibres at different ionic strengths (original drawing)
ordinate: tension in mN, abszissa: length in mm
ionic strengths: 1 = 0.02, 2 = 0.07, 3 = 0.17

strength shifts the curves to the right, i.e. with constant lengthening above L_o the force decreases. This decrease in force is reversible. In fig. 2 the results of several experiments are shown. Here the lengthening is expressed relative to L_o, and the corresponding force relative to the force at ionic strength 0.02 of the bathing solution.

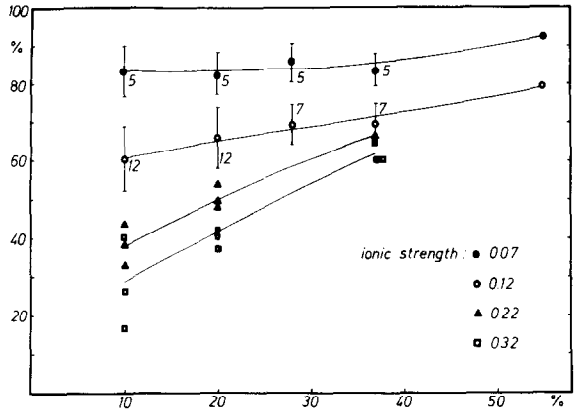

Fig. 2
Length tension relation of glycerol extracted muscle fibres at different ionic strengths
ordinate: Relative force, the force at ionic strength 0.02 is taken as 100 %. abscissa: Relative stretching above L_o, L_o is taken as 100 %. Symbols with vertical bar represent mean values (± S.D., n = 5). The remaining symbols are single values.

E is measured on single fibres. The fibres are given an initial length of 1.02 L_o and a sinusoidal length change of ± $1.5 \cdot 10^{-3}$ L_o at various frequencies is then superimposed. Fig. 3 shows

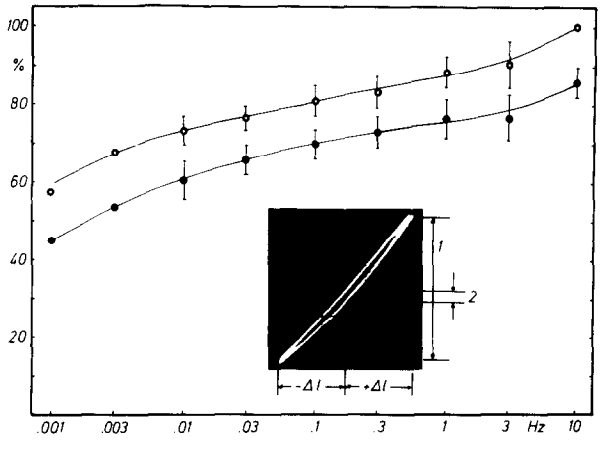

Fig 3
Relation between E and frequency of the sinusoidal length changes at different ionic strengths
ordinate: Relative E, E at a frequency of 10 Hz and an ionic strength of 0.02 is taken as 100 %. abscissa: frequency in Hz
ionic strengths: O 0.02, ● 0.22
Symbols with vertical bar represent mean values (±S.D., n = 5)
Inset: Original photograph of a Lissajous loop
1: in phase tension, 2: quadrature tension, $2\Delta l$ = total length change

that increasing the frequency causes an increase in E, whereas increasing the ionic strength causes a decrease. In the fig. E is expressed relative to its value at a frequency of 10 Hz and an ionic strength of 0.02. If one calculates the mean of the differences in E at the ionic strengths 0.02 and 0.22 for any particular frequency - each time taking 5 measurements at 7 frequencies within the range of 0.01 - 10 Hz (fig. 3)- the result one obtaines when changing the ionic strength from 0.02 to 0.22 is a decrease of 15.5 ± 7.2 % S.D. (n: 35). The corresponding value for the viscous component of the dynamic Young's modulus is 4.2 ± 15.3 % S.D. (n: 35) which is not significantly different from zero value.

Discussion: Our results show that the resting tension of glycerol extracted skeletal muscle fibres decreases with increasing ionic strength. This finding is in accordance with investigations of Gordon et al. (6), who have demonstrated that elevated ionic strength decreases the resting tension of skinned frog skeletal muscle fibres at in situ-length. At the case of glycerol extracted fibres in rigor, too, tension decreases as the salt concentration and thus the ionic strength of the bathing solution are increased (5). The tension decrease due to the elevated ionic strength of the preparations mentioned here originates in a change in the interaction between the contractile filaments (5, 6) which, in our opinion and according to (5), is caused by a change in electrostatic repulsive forces between the backbone of the contractile filaments (12, 13, 14, 15) and/or between the S_1 heads of the myosin cross bridges and the actin filaments (16, 17).

In the case of the intact fibre an elevated extracellular osmolarity (produced by addition of sucrose) causes an increase

in sarcoplasmic salt concentration and thus ionic strength (7). Hypertonic solutions, however, induce a tension increase (1, 2, 3, 4) which is graded with tonicity and consists of a transient and a maintained phase. The transient phase, which is induced at tonicities above 1.7 T (1 T = isotonic), may be suppressed by tetracaine. The maintained phase is not affected by tetracaine. The possibility may therefore be excluded that it is caused by a Ca^{++} activation of the contractile apparatus (4). The increase in resting tension of the intact fibre in hypertonic solution is associated with an increase in the elastic modulus - measured at a constant stretching velocity of 25 um/s - which increases tenfold in response to an increase in tonicity to 2 T (4). This finding is contradictory to the behaviour of the glycerol extracted fibre, because here E decreases with increasing ionic strength. We suggest therefore that the maintained increase in the resting tension and the increase in the elastic modulus of the intact fibre in hypertonic solution are not due to a change in the interaction between the contractile filaments.

The sarcolemma does not contribute to intact fibre tension at in situ-length (see for example 18, 19, 20). Moreover, the existence of S-filaments, postulated by (21), which might contribute to the resting tension, has never been proved. So we conclude that the changes in resting tension and elastic modulus of tetracainized skeletal muscle fibres in hypertonic solution are caused by the longitudinal sarcoplasmic reticulum (LSR). This conclusion is in accordance with the finding that the volume - and, in our opinion, thus the tension - of the LSR increase in hypertonic solution (22). The tension of the LSR must contribute to the resting tension of intact muscle fibres

in isotonic solution, too. This is concluded from investigations concerning the origin of the latency relaxation of skeletal muscle (23, 24, 25), the results of which suggest that the latency relaxation of skeletal muscle is due to a change in the tension of the LSR. However, the resting tension of intact skeletal muscle fibres at in situ-length is not due to the LSR alone. This is shown in experiments on the 'elastic limit' of skeletal muscle fibres (1, 2, 4), the existence of which, in both isotonic and hypertonic bathing solutions, is regarded as evidence that cross bridges between the filaments act even in resting muscle.

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