

Changes in force and stiffness during stretch of skeletal muscle fibers, effects of hypertonicity

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ABSTRACT Slow stretch ramps (velocity: $0.17 \text{ fiber lengths s}^{-1}$) were imposed during fused tetanic contractions of intact muscle fibers of the frog ($1.4\text{--}3.0^\circ\text{C}$; sarcomere length: $2.12\text{--}2.21 \mu\text{m}$). Instantaneous force-extension relations were derived both under isometric conditions and during slow stretch by applying fast (0.2 ms) length steps to the fiber. An increase in tonicity (98 mM sucrose added to control Ringer solution) led to significant reduction of the maximum isometric tension but at the same time to marked increase in the force enhancement dur-

ing slow stretch. The maximum force level reached during the stretch was affected very little. Experiments on relaxed fibers showed that recruitment of passive parallel elastic components were of no relevance for these effects. Hypertonicity slightly increased the instantaneous stiffness of the active fiber both in the presence and in the absence of stretch. The total extension of the undamped fiber elasticity was considerably reduced by increased tonicity under isometric conditions but was only slightly affected during slow stretch. The change in length of the

undamped cross-bridge elasticity upon stretch was thus greater in the hypertonic than in the normotonic solution suggesting a greater increase in force per cross-bridge in the hypertonic medium. The contractile effects are consistent with the assumptions that hypertonicity reduces the capability of the individual cross-bridge to produce active force and, furthermore, that hypertonicity has only minor effects on the number of attached cross-bridges and the maximum load-bearing capacity of the individual bridge.

INTRODUCTION

The contractile properties of skeletal muscle are greatly affected by raised tonicity of the extracellular medium. The maximum isometric tension (Howarth, 1958; Edman and Andersson, 1968; Gordon and Godt, 1970; Edman and Hwang, 1977) and the maximum velocity of shortening (Edman and Hwang, 1977) are both reduced in response to increased tonicity and these effects are associated with a marked reduction in curvature of the force-velocity relation (Edman 1988). The results suggest strongly (Huxley, 1957) that the performance of the myosin cross-bridges is altered as the tonicity is increased. A straightforward explanation of the reduction in maximum tetanic tension would be that the number of force producing cross-bridges is reduced by hypertonicity due to changes in the rate constants for attachment and detachment of the bridges (cf. Huxley, 1957). However, in apparent conflict with this idea the instantaneous tetanic stiffness of the fiber (an index of the number of attached cross-bridges; Ford et al., 1981) is much less affected by increased tonicity than is the isometric force (Bressler, 1977; Månsson and Mörner, 1988). The effects of increased tonicity on crossbridge performance are

obviously complex and further characterization of these effects is needed.

In the present investigation the effect of hypertonicity on the tension response to slow stretch was investigated in tetanically activated single muscle fibers of the frog. An interesting observation was made in this study. The force enhancement during slow stretch was markedly increased by hypertonicity despite significant reduction of the maximum isometric tension under these conditions. In order to explore the mechanism behind this effect the stretch induced changes of the instantaneous force-extension relations of the fiber were compared in isotonic and hypertonic solutions. The results suggest that slow stretch produces a greater increase in the force per cross-bridge in the hypertonic medium than in the normotonic solution. This effect can largely account for the effect of increased tonicity on the force enhancement during stretch. Preliminary reports of some of the results have been given earlier. (Månsson, 1986; Månsson and Mörner, 1988).

METHODS

Single fibers from the tibialis anterior muscle of *Rana temporaria* were mounted horizontally in a thermostatically controlled bath ($1.4\text{--}3.0^\circ\text{C}$)

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between a force transducer (modified from Edman [1979]; resonance frequency [in water]: 20 kHz) and an electromagnetic puller. The experiments were carried out at a resting sarcomere length of 2.12–2.21 μm . The tonicity of the bathing fluid was increased to a value 44% above the control by addition of 98 mmol sucrose per liter of the isotonic Ringer solution (Edman and Hwang, 1977). Length changes were imposed on the fiber by means of a servo-controlled electromagnetic puller. The puller and servo-system used in the majority of the experiments were similar to those described by Månsson and Edman (1985). The puller used in experiments designed for measurements of instantaneous stiffness generated small length changes ($<70 \mu\text{m}$) complete in $\sim 0.2 \text{ ms}$. The position of the puller arm was detected by means of a photoelectric transducer (rise time $10 \mu\text{s}$). Further details of the techniques (composition of the normal Ringer solution, massive field stimulation, determination of sarcomere length by laser diffraction, and recording and measurement of responses) have been given earlier (Månsson and Edman, 1985).

Statistical significance was determined by Student's *t*-test. Data are given as mean \pm standard error of the mean.

Instantaneous stiffness and extension of the undamped fiber elasticity

During the tetanus plateau instantaneous stiffness was determined as the peak change in tension in response to fast (0.2 ms) stretches and releases divided by the amplitude of length change (0.1–0.2% of the fiber length). The same approach was used for measuring stiffness during slow stretch of active fibers but in this case only fast releases were used. Assuming that the fiber elasticity is linear, or nearly linear (Ford et al., 1977), the extension (Y_0) of the undamped fiber compliance was estimated from the length intercept of a straight line (cf. *dashed lines* in Fig. 2) extrapolated from the instantaneous force-extension curve for small length changes (0.1–0.2% of the fiber length). The instantaneous stiffness (S) is taken as a measure of the number of attached cross-bridges and the quantity Y_0 is assumed to represent the average extension of the undamped cross-bridge elasticity (see Ford et al., 1981; Tawada and Kimura, 1986). The presence of tendon compliance in series with the sarcomeres will to some extent affect the absolute values of Y_0 and S . However, the changes in fiber stiffness and in Y_0 produced by increased tonicity cannot be accounted for on this basis. This is evident from the finding that isometric stiffness was slightly increased by hypertonicity (see Results and Discussion) whereas tendon stiffness would be reduced under these conditions due to the reduction in tension (Cleworth and Edman, 1972; Ford et al., 1985). That the presence of tendon compliance had little effect on the results is also supported by a preliminary experiment in which a modified version of the surface marker technique (Edman and Reggiani, 1984) was used to detect length changes of a central fiber segment. This experiment suggested that the tonicity induced changes in stiffness were the same whether measured from the whole fiber or from a tendon-free segment.

RESULTS AND DISCUSSION

Fig. 1 illustrates the effect of increased tonicity on the tension response to slow stretch of a single muscle fiber (velocity of stretch: $0.17 \text{ fiber lengths s}^{-1}$; amplitude: $0.03 \text{ fiber lengths}$). It can be seen (Fig. 1 *A*) that, whereas the plateau tetanic tension (T_0) was reduced by raised tonicity (mean reduction of force: $20.6 \pm 0.9\%$, $n = 14$,

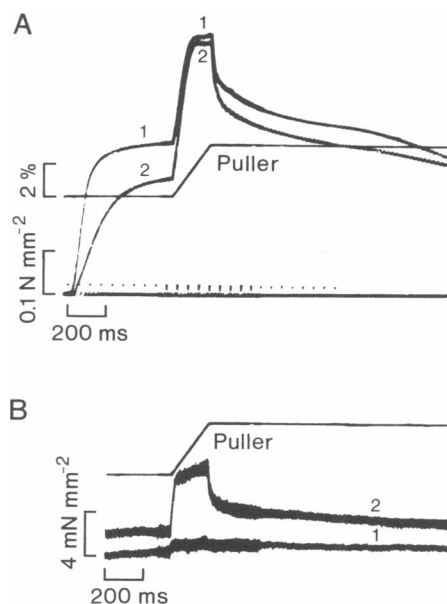


FIGURE 1 Photographically superimposed records showing the force response of a single muscle fiber to slow stretch (velocity: $0.17 \text{ fiber lengths s}^{-1}$) during the tetanus plateau (*A*) and at rest (*B*). Records 1. control Ringer solution. Records 2. hypertonic solution (normal Ringer + 98 mM sucrose). Stimulation markers above tension base line in *A*. Puller calibration (same in *A* and *B*) in percent of fiber length. Note different force scales in *A* and *B*. Sarcomere length: $2.13 \mu\text{m}$. Fiber length: 7.75 mm . Cross-sectional area: $14.7 \times 10^{-3} \text{ mm}^2$. Temperature: 1.8°C .

$p < 0.001$), the force enhancement (E_f) during stretch was markedly increased. The maximum force during stretch was thus virtually unaffected by the tonicity change. The mean increase of E_f corresponded to $16.8 \pm 0.7\%$ of the control isometric tension (T_0^c) and the reduction of the maximum force was only $2.2 \pm 0.7\%$ of the control value ($n = 14$, $p < 0.01$).

The force enhancement during slow stretch of relaxed fibers also increased in response to raised tonicity (Fig. 1 *B*, Table 1). This change (mean: $1.1 \pm 0.2\%$ of T_0^c , $n = 10$, $p < 0.001$) was, however, considerably smaller than that recorded during activity. Thus, recruitment of passive parallel elastic components would not seem to play a significant part in the effects of increased tonicity on the stretch response of the active fiber. The effects of hypertonicity in the active fiber were therefore not corrected for any possible contribution from passive elastic structures.

The reduction of the maximum isometric tension caused by increased tonicity was associated with specific changes of the instantaneous force-extension relation of the fiber. Thus, as demonstrated by one experiment in Fig. 2, the slope of the isometric force-extension curve was

TABLE 1 Force enhancement during slow stretch—effects of hypertonicity (98 mM sucrose)*

	Force enhancement, % T_0^c		Change, % T_0^c	n
	Control	98 mM sucrose		
Active fiber	65.6 ± 1.6	82.4 ± 2.0	+16.8 ± 0.7 [†]	14
Relaxed fiber	0.19 ± 0.01	1.31 ± 0.16	+1.12 ± 0.16 [‡]	10

*Data expressed as Mean ± SE.

[‡]Statistically significant (paired observations; $p < 0.001$).

slightly increased and the intersection of the curve with the abscissa was shifted towards smaller release amplitudes in the hypertonic medium. The latter result indicates that Y_0 (representing the total average extension of the undamped cross-bridge elasticity; see Methods) was markedly reduced by increased tonicity during isometric contraction (mean reduction: 1.7 ± 0.1 nm half-sarcomere⁻¹, $24.1 \pm 1.2\%$ of control). The increase in slope of the force-extension relation is an indication that the stiffness of the fiber was increased by raised tonicity. The increase ($2.9 \pm 1.9\%$) was not statistically significant but appeared in 5 out of 6 fibers tested. The fact that the fiber stiffness changed very little is consistent with the idea that the number of attached cross-bridges was virtually unaffected by increased tonicity.

Changes of the instantaneous force-extension relations produced by slow stretch (amplitude: 1.5% of the fiber length; 16.3 nm half-sarcomere⁻¹)¹ are also illustrated in Fig. 2. It can be seen that the force enhancement during stretch in both normotonic and hypertonic solution was associated with (a) increase in slope of the instantaneous force-extension curve and (b) a shift of the length intercept of the curve towards length changes of greater amplitude. Similar effects of stretch have previously been reported by Colomo et al. (1986) and by Sugi and Tsuchiya (1988). The most straightforward interpretation of these results (cf. Colomo et al., 1986) is that the number of attached cross-bridges and the average extension of the undamped cross-bridge elasticity increase upon stretch.

The force-extension relations derived during slow stretch differed only slightly in hypertonic and normotonic solutions (Fig. 2). Similar to the finding in the absence of slow stretch, the slope of the force-extension curve (the instantaneous stiffness) was slightly increased by hypertonicity (mean change: $4.9 \pm 1.6\%$, $n = 6$, $p < 0.05$). The length intercept, however, was much less affected by raised tonicity during stretch than under pure

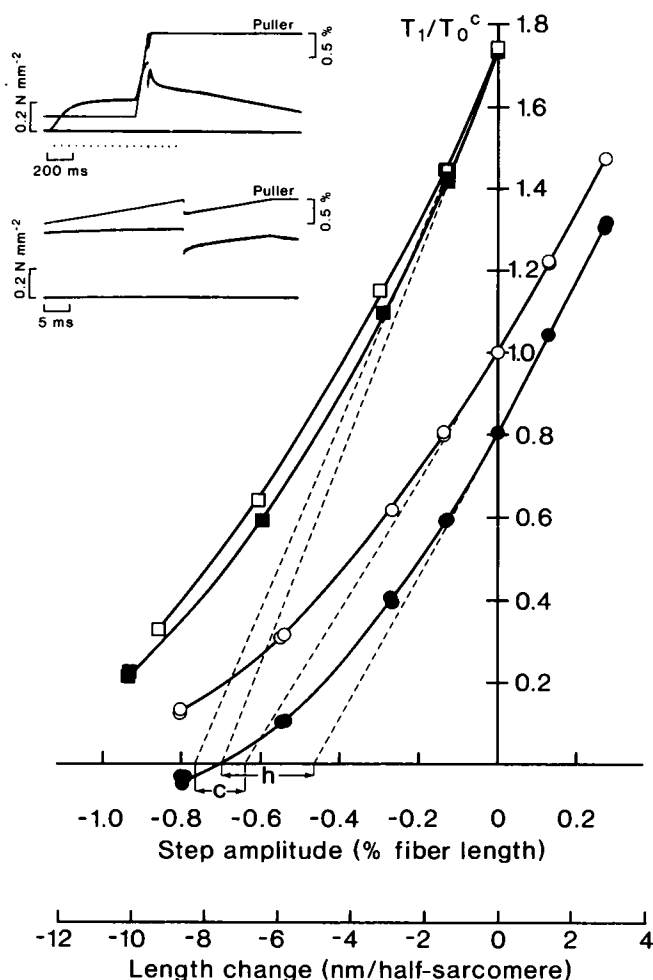


FIGURE 2 Effect of hypertonic solution (normal Ringer + 98 mM sucrose) on instantaneous force-extension relations obtained during fused tetanus, under isometric conditions (circles) and during slow stretch (squares), (amplitude of stretch: 16.3 nm half-sarcomere⁻¹). (Open symbols) Control Ringer solution. (Filled symbols) Hypertonic solution. The tension existing immediately before the length step and the peak tension, T_1 , reached in response to fast (0.2 ms) releases (—) or stretches (+) normalized with respect to plateau tetanic tension in the control solution (T_0^c). Lower scale of the abscissa gives the length change per half-sarcomere under the assumption of zero tendon compliance (see Methods). (Full lines) Force-extension relations fitted by eye to the experimental data. (Dashed lines) Extrapolation of the force-extension curves for small length changes ($\pm 0.13\%$ of fiber length). The slope of the dashed lines is given by the instantaneous stiffness of the fiber (see Methods). Quantities c and h , marked by arrows at the abscissa, denote changes in Y_0 upon stretch in the control solution (c) and in hypertonic solution (h), (see text). (Insert) Experimental procedure for determination of the instantaneous force-extension diagram during a stretch ramp. Tension response to length step during a slow stretch shown on both slow (upper row) and fast (lower row) time base. The calibration of the superimposed tension and puller signals are given in $N\text{ mm}^{-2}$ and percent of fiber length respectively. Sarcomere length: $2.15\text{ }\mu\text{m}$. Fiber length: 7.75 mm . Cross-sectional area: $7.1 \times 10^{-3}\text{ mm}^2$. Temperature: 2.0°C .

¹The amount of stretch is close to the critical sarcomere extension (16.6 nm half-sarcomere⁻¹; Edman et al., 1981) needed to bring tension to a breakpoint after which it stays at a relatively stable level for the remainder of the stretch.

TABLE 2 Effects of hypertonicity (98 mM sucrose) on extension of undamped fiber elasticity in isometric contraction and during slow stretch*

	Y_0		Mean change
	Isometric	Stretch	
Control	7.0 ± 0.1	9.2 ± 0.4	+2.2 ± 0.3 [‡]
98 mM sucrose	5.3 ± 0.1	8.7 ± 0.4	+3.4 ± 0.4 [‡]
Mean change (nm)	-1.7 ± 0.09 [‡]	-0.50 ± 0.13 [‡]	+1.2 ± 0.1 [‡]
Mean change (%)	-24.1 ± 1.2 [‡]	-5.4 ± 1.3 [‡]	

*Data from six experiments. Extension of undamped fiber elasticity (Y_0 ; see Methods) given in nanometers per half-sarcomere. Mean ± SE. Increase: +, Decrease: -.

[‡]Statistically significant (paired observations; $p < 0.001$).

[‡]Statistically significant (paired observations; $p < 0.01$).

[‡]Statistically significant (paired observations; $p < 0.05$).

[‡]Difference between 98 mM sucrose and the control solution.

isometric conditions (mean displacement of Y_0 towards smaller release amplitude: 0.5 ± 0.1 nm half-sarcomere⁻¹, $5.4 \pm 1.3\%$ of control). This finding has the interesting implication that slow stretch produced a significantly greater horizontal shift of the force-extension relation in the hypertonic medium than in ordinary Ringer solution. The mean enhancement of Y_0 in response to slow stretch (amplitude 16–22 nm half-sarcomere⁻¹) thus increased from 2.2 ± 0.3 nm half-sarcomere⁻¹ under normotonic conditions (range “c” in Fig. 2) to 3.4 ± 0.4 nm half-sarcomere⁻¹ in hypertonic solution (range “h” in Fig. 2). The difference between these changes (mean: 1.2 ± 0.1 nm half-sarcomere⁻¹) was statistically significant at the 0.1% level. The effects are summarized in Table 2.

In conclusion then, the results show that the increase of E_f in hypertonic solution was associated with a greater length change of the undamped cross-bridge elasticity upon stretch. That is, the increase of E_f was to a great extent based on increased force enhancement per cross-bridge. On the assumption that cross-bridge stiffness was unaffected by increased tonicity, the changes in Y_0 (see above) thus suggest that the average force per bridge in hypertonic solution was 95% of the control value during stretch compared with only 76% of the control value during isometric contraction. However, hypertonicity also produced a slight increase of the instantaneous fiber stiffness, both under isometric conditions and during stretch. This effect could mean that the change of E_f also involved a small increase in the number of attached cross-bridges in the hypertonic medium.

The contractile effects of increased tonicity are consistent with a model based on the following assumptions: (a) the capacity of the individual cross-bridge to produce active force is markedly reduced by hypertonicity. This would account for the decrease of T_0 . (b) The number of

attached cross-bridges and the maximum load bearing capacity of each bridge is little affected. The latter point is consistent with the finding that the maximum force attained during slow stretch and the instantaneous stiffness were only slightly changed by hypertonicity. According to the present model increased tonicity may thus be thought to greatly reduce the force per cross-bridge during active force-generation suggesting a failure of the energy-transducing mechanism. When energy is supplied by slowly stretching the fiber the cross-bridge may hold almost the same maximum force as in the ordinary Ringer solution.

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