Tension and instantaneous stiffness of single muscle fibers immersed in Ringer solution of decreased tonicity

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ABSTRACT Isometric tension and instantaneous stiffness were measured in frog semitendinosus single muscle fibers in both isotonic and hypotonic Ringer solution. In 0.7 and 0.5 × normal Ringer tension increased 17 and 20%, respectively. There was no corresponding increase in the measured stiffness. The increase in tension in hypotonic Ringer could be reversed by the addition of an osmotic equivalent of sucrose to the bathing solution. These findings suggest that the potentiated tension observed in hypotonic Ringer is due to an increased tension per cross-bridge and not to an increase in the number of attached cross-bridges.

INTRODUCTION

An important element in studies of intact single muscle fibers bathed in solutions of varying tonicity is the effect of changes in the filament lattice on the ability of the actomyosin cross-bridge to undergo a confirmational change probably leading to rotation. Previous studies have shown that the isometric tension of striated muscle is sensitive to changes in the tonicity of the bathing solution. Hypertonic solutions have been reported to decrease tension (Overton, 1902; Ernst, 1926; Howarth, 1958; Bressler, 1977). Vaughan et al. (1983) have shown that the decline in the isometric tension in isolated frog muscle fibers in hypertonic solutions is most likely due to a decreased ability of the actomyosin cross-bridges to generate force rather than a reduction in the total number of cross-bridges formed. With respect to the effects of hypotonic Ringer on tension generation, Okada and Gordon (1972) have reported that the maximum tetanic tension of isolated fiber bundles increases as the tonicity of Ringer solution is decreased. In addition, Edman and Hwang (1977) have shown that both isometric force and maximum velocity of shortening are increased in single frog fibers immersed in hypotonic Ringer solution. The present study was undertaken to attempt to provide additional insight into the mechanism responsible for the contractile change of skeletal muscle bathed in solutions of different tonicities. Our objective was to determine whether the alteration of the filament lattice volume or the decrease in intracellular ionic strength induced by the tonicity of the extracellular fluid was the primary cause of the alteration in tension generating capacity and not the number of attached cross-bridges. It is generally accepted that measurement of instantaneous stiffness is an indication of the total number of active force-producing cross-bridges in striated muscle (Bressler and Clinch, 1974; Ford et al., 1981). Therefore, our investigation focused on correlative changes of isometric tension and instantaneous stiffness in single muscle fibers from frog semitendinosus muscle bathed in solutions of decreased tonicity.

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METHODS

A complete description of the experimental techniques and apparatus has recently been published (Bressler et al., 1988). Briefly, single twitch fibers were dissected from the ventral head of semitendinosus muscle with the aid of a stereomicroscope (Zeiss, West Germany) and dark field illumination. The dissection was carried out in Ringer solution at room temperature using no. 5 stainless steel forceps. After isolation of the fiber, a small hole was made with an insect pin (Carolina Biological Supply Co., Burlington, NC) in each tendon, close to the musculotendinous junction. The fiber was then transferred to the experimental chamber in a small polyethylene boat filled with Ringer solution. The experimental chamber consisted of two plates of hardened aluminum between which were two thermoelectric modules used to control the temperature of the bathing solution. The center of the top plate contained a perspex bath fitted with a double-layered glass floor for illumination of the fiber by a fiber optic probe for photomicroscopy or a laser (He-Ne) to record sarcomere spacing. The sides of the chamber contained two removable platinum plate electrodes running parallel to the fiber over its whole length, and approximately 2 mm away.

The entire chamber assembly was secured on a brass plate which was fitted to the modified stage of a microscope. A servomotor (model 300S, Cambridge Technology, Inc., Cambridge, MA) and a force transducer (model 400, Cambridge Technology, Inc., resonant frequency 2.5 KHz) were mounted on three-way micropositioners fixed to the brass plate. Two pieces of fine stainless steel tubing (OD, 0.01 in; ID, 0.005 in), each containing a beveled hook, and rigidly attached to the level arm of the motor and the output tube of the force transducer, projected into the experimental trough. Under the dissecting microscope, the fiber was suspended between the motor and the force

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transducer by slipping the pieces of wire protruding from these devices into the premade holes in the tendons. Subsequently, the tendons were secured by tying 10–0 monofilament sutures around the tendon and the wire. This procedure reduced the stray series compliance of the preparation.

All experiments were carried out in an amphibian Ringer solution which contained in millimolar: NaCl, 115; KCl, 2.5; $CaCl_2$, 1.8; Na_2HPO_4, 2.15; NaH_2PO_4, 0.85; pH 7.1. Hypotonic Ringer was prepared by simple dilution of 10 × isotonic Ringer and then buffered with 3 mM phosphate to maintain the pH. In several experiments the tonicity of the bathing solution was restored to normal by the addition of sucrose. The temperature of all experiments was 3–4°C.

All experiments were carried out at ℓ_o, the length at which the maximum isometric twitch was recorded. This corresponded to a sarcomere spacing of 2.22 ± 0.047 (n = 25). Stimulation was achieved by supramaximal square pulses of 1 ms duration and all tetani were fused. Throughout an experiment a stimulus regime of three twitches, followed by a tetanus with a 90 s interval between contractions was maintained.

Stiffness values were determined by giving the fiber a rapid shortening step (500 μs duration and 4 nm/half-sarcomere) at the plateau of an isometric tetanus. The ratio of the tension change to the length change was used as a measure of the stiffness for comparison in isotonic and hypotonic Ringer. For all experiments both stiffness and tension changes were expressed relative to the values recorded in isotonic Ringer.

RESULTS

Fig. 1 is a comparison of the maximum isometric tension and instantaneous stiffness changes of isolated muscle fibers over a 40 min time period in a solution diluted to 0.7 × NR. After a 10 min equilibration period, the tension is seen to have increased by an average of 17% compared so that recorded in isotonic Ringer. Moreover this response remained steady for the duration of the recording period which was ~40 min. The instantaneous stiffness during this same time period remained constant relative to that recorded in isotonic Ringer. At a dilution of 0.5 × NR there was a similar response observed (Fig. 2) with the tension showing a slightly greater increase (20%) than at 0.7 × NR.

To establish whether the tension changes observed were due to the change in the lattice volume per se or the decreased intracellular ionic strength experiments were carried in which the cell volume was restored by addition of an osmotic equivalent of sucrose to the bathing solution. The average tension recorded during a 40 min time period in hypotonic solution and hypotonic solution to which sucrose was added is shown in Fig. 3. All values are expressed relative to those recorded in isotonic Ringer. In both 0.7 × NR and 0.5 × NR the isometric tetanus tension returned to levels recorded in isotonic Ringer when sucrose was added to the diluted Ringer. Moreover, in both cases, there was no change in the instantaneous stiffness measured irrespective of whether the bathing solution was hypotonic or diluted but returned to isotonicity by the addition of sucrose (Fig. 4).

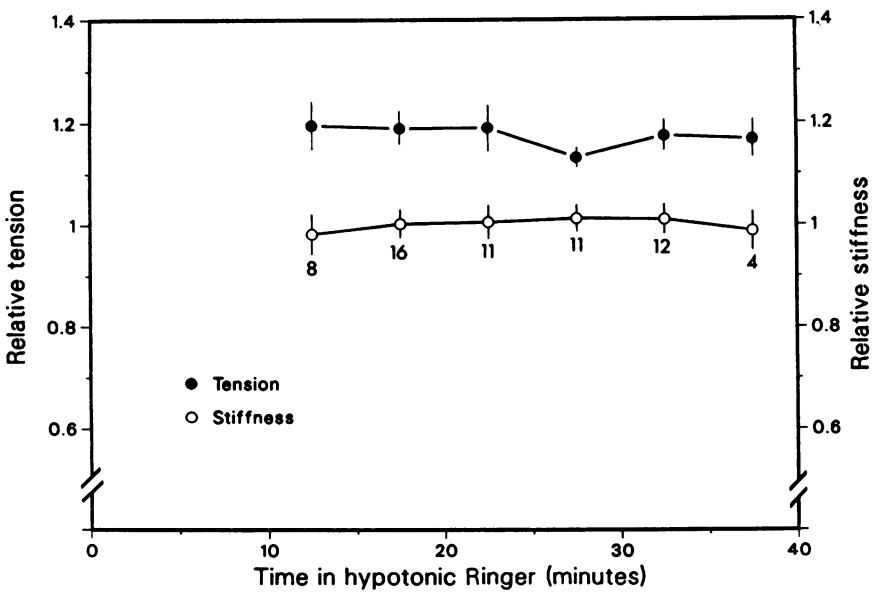

FIGURE 1 Changes in isometric tetanus tension and instantaneous stiffness with time after isolated fibers were placed in hypotonic Ringer (0.7 × NR). Tension and stiffness values are expressed as a fraction of the maximum values recorded in isotonic Ringer. Values are means ± SEM of the average value obtained in 5 min intervals from 10 to 40 min. The number of individual fibers used is indicated. The points are plotted at the midpoint of each 5 min interval.

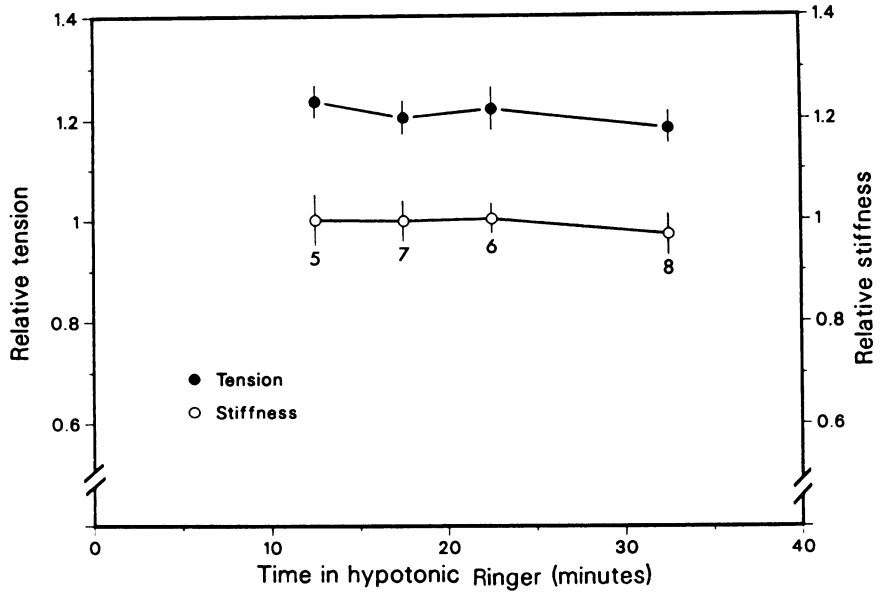

FIGURE 2 Changes in isometric tetanus tension and instantaneous stiffness with time after fiber was placed in hypotonic Ringer (0.5 × NR). Tension and stiffness values are expressed as a fraction of the maximum values recorded in isotonic Ringer. Values are means ± SEM of the average value obtained in 5 min intervals from 10 to 40 min. The number of individual fibers used is indicated. The points are plotted at the midpoint of each 5 min interval.

DISCUSSION

The present study on isolated frog single muscle fibers has shown that a decrease in tonicity of the bathing solution results in an increase in maximum isometric tetanus tension. Moreover, there was no corresponding increase observed in the instantaneous stiffness. This would suggest that the increase in tension recorded under these conditions was not due to an increase in the number of attached cross-bridges, but rather to an increase in the force produced per cross-bridge. A similar conclusion was recently been reported by Bagni et al. (1990) in intact frog muscle fibers. This is based on their observation that in both hypertonic and hypotonic solutions, tension changes are not accompanied by a corresponding change in instantaneous stiffness which is a measure of the number of attached of cross-bridges. Burchfield and Rall (1986) have compared the stable maintenance heat rate, which is a reflection of the ATP hydrolysis in frog semitendinosus muscle in both isotonic Ringer and Ringer diluted to 0.67 × NR. Their data indicated that both force and the rate of ATP hydrolysis increased in hypotonic solution. In addition, the activation heat, a measure of the heat generated by the Ca^{++}-dependent ATPase of the SR decreased in hypotonic solution. Our results, which show no change in the number of attached cross-bridges and those of Burchfield and Rall (1984) are consistent with the conclusion that the increased tension in diluted Ringer is due to an increased tension per cross-bridge.

When an intact muscle fiber is placed in a hypotonic Ringer solution there is an initial swelling of the cell due to the decreased osmolarity of the extracellular fluid. With time the intracellular volume will gradually return to within five percent of the isotonic volume. This is primarily due to the movement of both electrolytes and/or nonelectrolytes out of the cell (Lewis and Donaldson, 1990). The net effect is a cell with a moderate increase in volume and a net decrease in intracellular ionic strength. Complete restoration of the lattice volume by the addition of sucrose to the bathing solution reversed the observed increase in maximum tetanus tension. This would suggest that the increase in tension seen in osmotically swollen intact fibers may be due to either the decrease in intracellular ionic strength or the distance between the thick and thin filaments or a combination of both (Bagni et al., 1990).

Experiments on skinned skeletal muscle fiber allows for investigation of the effect of ionic strength on the contractile process without an alteration in cell volume. Julian and Moss (1981) have reported an increase in force of frog skinned muscle fibers activated in solutions of low ionic strength. Moreover, Brenner et al. (1982)

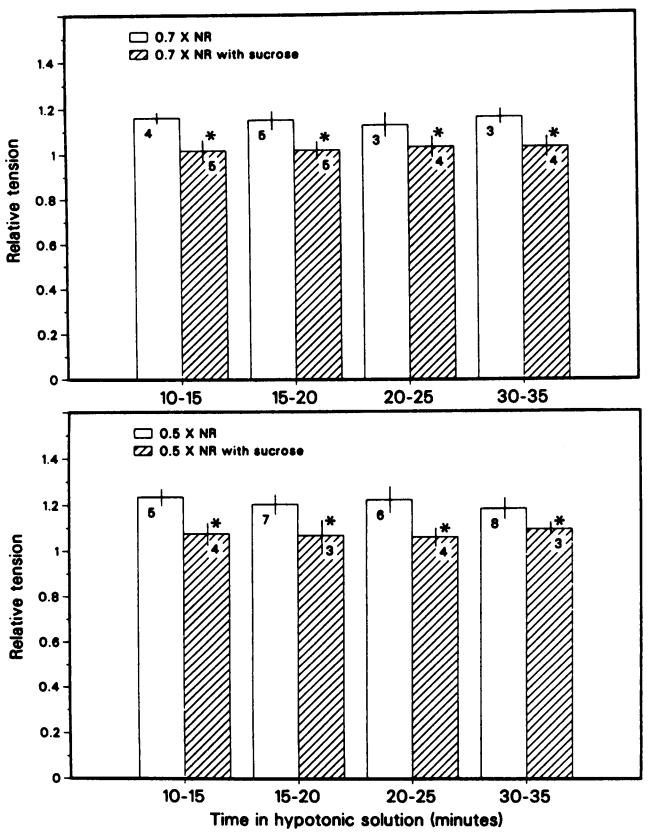

FIGURE 3 Changes in isometric tetanus tension with time after fiber was placed in hypotonic Ringer or diluted Ringer made isotonic by the addition of sucrose. Values are means ± SEM of the average value obtained in 5 min intervals from 10 to 35 min. Asterisks indicate a significant difference at $p \leq 0.05$ using a Student's t test.

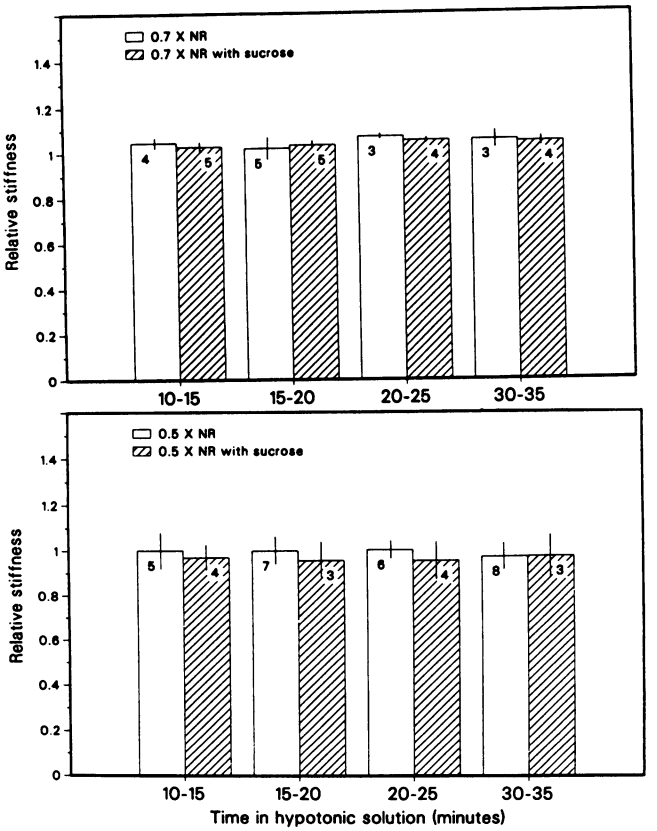

FIGURE 4 Changes in instantaneous stiffness with time after fiber was placed in hypotonic Ringer or diluted Ringer made isotonic by the addition of sucrose. Values are means ± SEM of the average value obtained in 5 min intervals from 10 to 35 min. Asterisks indicate a significant difference at $p < 0.05$ using a Student's t test.

and more recently Jung et al. (1989) have shown that skinned rabbit or frog skeletal muscle fibers at low ionic strength in relaxing solution have higher stiffness values compared to fibers at physiological ionic strength. Their results suggest that there exists attached, but nonforcing producing cross-bridges in the resting state which may be primed to contribute to the increased tension observed on activation of the intact or skinned fiber. Furthermore, based on the observation of an increased intensity of the 1,1 equatorial reflection, Brenner et al. (1984) have proposed that the number of attached cross-bridges at low ionic strength in the relaxed state is almost similar to that in rigor. Xu et al. (1987) have confirmed by x-ray diffraction the existence of actin attached cross-bridges at low ionic strength in skinned frog sartorius muscle in relaxing solution. However, these authors suggest that it is likely that only 15% of the total number of cross-bridges are attached. Whereas there clearly may exist a difference between these two species in response to low ionic strength solutions, the presence of attached cross-bridges under these conditions in the relaxed muscle appears to be substantiated.

In summary, our data has shown that under conditions where the intracellular ionic strength is reduced in the intact fiber, a substantial increase in maximum isometric tension is observed. Moreover the fact that there was not a corresponding change in the measured stiffness would suggest that the increased tension was due to a change in the force per cross-bridge rather than the number of attached cross-bridges. Further experiments, with skinned fibers, are required to establish whether the tension change is due primarily to the decrease in intracellular ionic strength or to the alteration in the interfilament distance.

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REFERENCES

Bagni, M. A., G. Cecchi, and F. Colomo. 1990. Myofilament spacing and force generation in intact frog muscle fibres. *J. Physiol (Lond.)*. 430:61–75.

Brenner, B., M. Schoenberg, J. M. Chalovich, L. E. Greene, and E. Eisenberg. 1982. Evidence for cross-bridge attachment in relaxed muscle at low ionic strength. *Proc. Natl. Acad. Sci. USA*. 79:7288–7291.

Bressler, B. H. 1977. Isometric tension and instantaneous stiffness in amphibian skeletal muscle exposed to solutions of increased tonicity. *Can. J. Physiol. Pharmacol.* 55:1208–1210.

Bressler, B. H., and N. F. Clinch. 1974. The compliance of contracting skeletal muscle. *J. Physiol. (Lond.)*. 237:477–493.

Bressler, B. H., L. A. Dusik, and M. R. Menard. 1988. Tension responses of frog skeletal muscle fibres to rapid shortening and lengthening steps. *J. Physiol. (Lond.)*. 397:631–641.

Burchfield, D. M., and J. A. Rall. 1986. Energetics and mechanics of frog skeletal muscle in hypotonic solution. *Am. J. Physiol.* 251:C66–C77.

Edman, K. A. P., and J. C. Hwang. 1977. The force-velocity relationship in vertebrate muscle fibers at varied tonicity of the extracellular medium. *J. Physiol. (Lond.)*. 269:255–272.

Ernst, E. 1926. Untersuchungen uber Muskelkontraktion III Durchstomungsversuche. *Pfluegers Arch. Gesamte Physiol. Menschen Tiere.* 213:133–143.

Ford, L. E., A. F. Huxley, and R. M. Simmons. 1981. The relationship between stiffness and filament overlap in stimulated frog muscle fibers. *J. Physiol. (Lond.)*. 311:219–249.

Howarth, J. V. 1958. The behaviour of frog muscle in hypertonic solutions. *J. Physiol. (Lond.)*. 144:167–175.

Julian, F., and R. L. Moss. 1981. Effects of calcium and ionic strength on shortening velocity and tension development in frog skinned muscle fibers. *J. Physiol. (Lond.)*. 311:179–199.

Jung, D. W. G., T. Blange, H. de Graaf, and B. W. Treijtel. 1989. Weakly attached cross-bridges in relaxed frog muscle fibers. *Biophys. J.* 55:605–619.

Lewis, S. A., and P. Donaldson. 1990. Ion channels and cell volume regulation: chaos in an organized system. *News Physiol. Sci.* 5:112–119.

Okada, R. D., and A. M. Gordon. 1972. Excitation, contraction, and excitation-contraction coupling of frog muscles in hypotonic solutions. *Life Sci.* 2:449–460.

Overton, E. 1902. Beitrage zur allgemeinen Muskelund Nervenphysiologie. II. Mitteilung. Uber die Unentbhrlichkeit von Natrium-(oder Lithium)-lonen fur den Kontraktionsakt des Muskels. *Pfluegers Arch. Gesamte Physiol. Menschen Tiere.* 92:346–386.

Vaughan, P. C., B. H. Bressler, L. A. Dusik, and M. J. Trotter. 1983. Hypertonicity and force development in frog skeletal muscle fibers. *Can. J. Physiol. Pharmacol.* 61:847–856.

Xu, S., M. Kress, and H. E. Huxley. 1987. X-ray diffraction studies of the structural state of crossbridges in skinned frog sartorius muscle at low ionic strength. *J. Musc. Res. Cell Motil.* 8:39–54.