SHORTENING VELOCITY IN SKINNED SINGLE MUSCLE FIBERS

Influence of Filament Lattice Spacing

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ABSTRACT In this study maximum shortening velocity (V_{max}) and isometric tension (P_0) in skinned single fibers from rat slow soleus (SOL) and fast superficial vastus lateralis (SVL) muscles were examined after varying degrees of filament lattice compression with dextran. In both fiber types V_{max} was greatest in the absence of dextran and decreased as the concentration of dextran was increased between 2.5 and 10 g/100 ml. At 10% dextran, which compressed fiber width by 31–38%, V_{max} relative to the initial 0% dextran value was 0.28 ± 0.03 (mean ± SE) and 0.26 ± 0.02 in SVL and SOL fibers, respectively. The effect of compression to depress V_{max} was reversed completely by returning the fiber to 0% dextran. The force-generating capability of skinned fibers was not as sensitive to variations in cell width. In both the SOL and SVL fibers P_0 increased by 3–7% when the concentration of dextran was increased from 0 to 5%. Further compression of lattice volume with 10% dextran resulted in a 8–13% decline in P_0 relative to the initial value. While the precise mechanism by which filament lattice spacing modulates contractile function is not known, our results suggest that the major effect is upon the rate constant for cross-bridge detachment.

INTRODUCTION

The diameters of single skeletal muscle fibers swell by ~20% after removal of the sarcolemma by chemical or mechanical methods (Godt and Maughan, 1981). The skinned fiber can be manipulated back to its in situ diameter or less by adding to the bathing solution osmotically active long-chain polymers such as dextran, which because of their size cannot penetrate the filament lattice. Within the past few years a number of studies have used these polymers to examine the effect of osmotic compression on the force generating capacity of skinned fibers. For example, it has been shown that with moderate amounts of compression, Ca^{2+}-activated force in skinned fibers increases slightly; however, force progressively declines with further compression (Godt and Maughan, 1981; Krasner and Maughan, 1984). In addition, osmotic compression has been reported to increase fiber stiffness, suggesting that cross-bridge attachments to the thin filament have increased (Berman and Maughan, 1982; Kawai and Schulman, 1985). Such studies have demonstrated that variations in the lateral spacing of the filament lattice can affect the likelihood of cross-bridge attachment; however, little information is available concerning the effect of osmotic compression on indices of rates of cross-bridge cycling. Recently, Kawai and Schulman (1985) demonstrated a significant alteration in oscillatory work and rate constants of cross-bridge cycling in skinned fibers after osmotic compression with dextran. In addition, Krasner and Maughan (1984) reported a decline in the rate of ATP hydrolysis during isometric contraction in compressed skinned fibers, suggesting that the frequency of cross-bridge cycling was reduced under these conditions. It is presently unknown whether ATP hydrolysis and/or shortening velocity are reduced at zero load in compressed skinned fibers, so that the effect of filament lattice spacing on cross-bridge detachment rates remains to be determined.

The primary purpose of this study was to examine the maximum velocity of shortening at various degrees of osmotic compression in skinned skeletal muscle fibers. In an attempt to establish a relationship between shortening velocity and physiologically relevant cell widths, we have utilized dextran concentrations of 2.5, 5, and 10 g/100 ml, since a 5% solution reduces cell width to that of the intact cell (Godt and Maughan, 1981).

MATERIALS AND METHODS

Female Sprague-Dawley rats (average weight 270 g) were injected with Nembutal (50 mg/kg body wt, i.p.) and the soleus (SOL; 84% type I, 16% type IIa, Ariano et al., 1973) and the superficial region of the vastus lateralis (SVL; 100% type IIb, Baldwin et al., 1972) hind limb muscles

were removed and placed in cold relaxing solution (see below). Bundles of ~50 fibers were dissected from each muscle and tied with surgical silk to glass capillary tubes. Bundles were stored at −22°C in a relaxing solution containing 50% (vol/vol) glycerol for 2–20 d before use. Before each experiment bundles were first placed for 30 min in relaxing solution containing 0.5% (wt/vol) Brij-58 (Moss, 1979), after which individual fibers were pulled free from one end of the bundle. While in relaxing solution, the fiber was mounted between a force transducer (model 403, Cambridge Technology Inc., Cambridge MA; sensitivity 20 mV/mg) and a DC torque motor (model 300s, Cambridge Technology, Inc.). Complete details of the connectors, mounting procedures, and experimental apparatus have been reported previously (Moss, 1979; Moss et al., 1983). As shown in Fig. 2, the connectors provided for low fiber end compliance, which is of critical importance when making repeated mechanical measurements on skinned single fibers. The overall length of each fiber was adjusted while relaxed to yield a sarcomere length of ~2.60 μm. During maximal activation, sarcomere length was 0.03–0.1 μm less than that measured in relaxing solution. The average fiber length in this study was 2.35 mm. Fiber cross-sectional area was calculated by equating fiber width to fiber diameter, which was measured while the fiber was briefly suspended in air.

Relaxing and activating solutions contained 7.0 mM EGTA, 1.0 mM free Mg^{2+}, 4.42 mM total ATP, 14.5 mM creatine phosphate, 20 mM imidazole, and sufficient KCl to yield a total ionic strength of 180 mM. Solution pH was adjusted to 7.00 with KOH. Relaxing solution had a pCa ($-\log [Ca^{2+}]$) of 9.0, while the pCa required for maximal activation was 4.5. The computer program of Fabiato and Fabiato (1979) was used to calculate the final concentrations of each metal, ligand, and metal-ligand complex, based upon the stability constants reported by Godt and Lindley (1982). The apparent stability constant for Ca^{2+}-EGTA was corrected for temperature (15°C), pH, and ionic strength (Fabiato and Fabiato, 1979).

Fibers were osmotically compressed by adding dextran (531,000 mol wt; Sigma Chemical Co., St. Louis, MO) to relaxing and activating solutions which had previously been prepared as described above. As indicated earlier (Godt and Maughan, 1977) this polymer is large enough to be excluded from the myofilament lattice, thereby eliciting nontransitory osmotic compression. To avoid the possibility of mixing errors between groups, concentrated stocks of pCa 9.0 and 4.5 were divided into four equal parts, and before dilution to final volume, 2.5, 5, and 10 g/100 ml dextran were added to three of them (Godt and Maughan, 1981).

Maximum shortening velocity was assessed using the slack test method (Edman, 1979; Moss et al., 1982). Briefly stated, a fiber was maximally activated to allow steady tension to develop; subsequently, slack was rapidly (within 1 ms) imposed at one end of the fiber and tension fell to zero. The fiber shortened for a period of time (dt) while taking up the slack, after which force began to redevelop. The amplitude of the slack step, dL, was plotted as a function of the duration of unloaded shortening (dt, i.e., the time from imposition of zero tension to the start of tension redevelopment). In this study six different length steps were used on each preparation to determine V_{max}. V_{max} was expressed in muscle lengths (ML)/s, where ML was the end-to-end segment length when sarcomere length was 2.5–2.6 μm. The slope of the best fit straight line through these data ($r > 0.98$) was taken as V_{max}. Five separate slack tests were performed on each fiber beginning with 0% dextran followed by 5, 10, 2.5%, and then back to 0% dextran. Experiments were conducted at 15°C. After each experiment a portion of the fiber was placed in a 0.5-ml microfuge tube containing SDS buffer (10 μl/mm fiber length), and stored at −22°C for subsequent analysis of contractile and regulatory protein content by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE), as described previously (Giulian et al., 1983; Moss et al., 1985).

A two-way analysis of variance (ANOVA) was used to determined (a) whether V_{max} was significantly altered by osmotic compression, (b) if differences existed between SOL and SVL, and (c) if there was an interaction between effects due to compression and muscle type. A probability level of $P < 0.05$ was selected as indicating significance.

RESULTS

The effect of dextran concentration on maximum shortening velocity (V_{max}) is shown in Figs. 1 and 2. Clearly, as the concentration of dextran was increased from 0 to 10%, V_{max} in both the SOL and SVL fibers progressively declined. At 5% dextran, V_{max} was significantly reduced relative to the value in 0% dextran in the SOL (0.67 ± 0.02, mean ± SE) and SVL (0.65 ± 0.04) fibers. Increas-

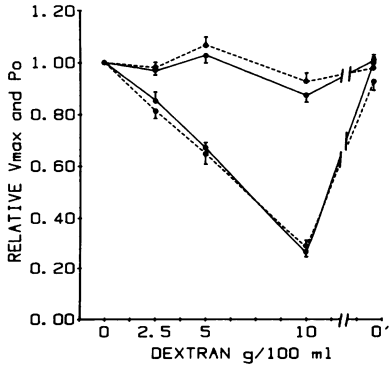

FIGURE 1 Variations in V_{max} (lower two lines) and P_0 (upper two lines) due to changes in dextran concentration in SOL *(solid lines)* and SVL *(dashed lines)* single skinned fibers. Values are mean ± SE, $n = 6$ and are scaled to initial values of V_{max} (average initial values for SOL and SVL were 1.46 ± 0.11 and 5.31 ± 0.51 ML/s) and P_0 (1,401 ± 56 and 1,193 ± 95 g · cm^{-2}) in the absence of dextran.

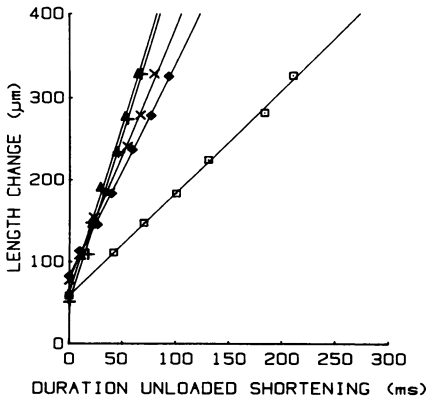

FIGURE 2 Representative slack test data from a single SOL fiber obtained in the following order (+) 0%; (□) 10%; (♦) 5%; (×) 2.5%; and back to (▲) 0% dextran. The slope of the best fit regression line was taken as V_{max}. V_{max} values, expressed in muscle lengths/second, were 1.31 dextran (0%), 0.40 (10%), 0.82 (5%), 0.98 (2.5%), and 1.32 (0%). Overall fiber length was 3.10 mm. Sarcomere length was 2.64 μm in relax and 2.52 μm during peak activation. Fiber compliance was 1.6% L_0, the length of the fiber segment end-to-end. Correlation coefficient (r) for each linear regression was >0.99.

ing dextran to 10% further reduced V_{max} in SOL (0.26 ± 0.02) and SVL (0.28 ± 0.03) fibers. The effect of increased dextran was reversible, since relative V_{max} increased to ~0.83 and 1.00 when the concentration of dextran was subsequently lowered to 2.5 and 0%, respectively. As shown in Fig. 1, peak force generating capacity (P_0) was not as sensitive to manipulations in dextran concentration. In 2.5% dextran maximum isometric tension was generally unchanged with respect to initial values at 0% dextran. Increasing dextran to 5% resulted in a 3–7% increase in P_0, while at 10% dextran P_0 was depressed by 8–13% of the initial value at 0% dextran.

The relationship between V_{max} and relative fiber width due to osmotic compression is illustrated in Fig. 3. In agreement with earlier reports, dextran concentrations in the range 2.5–10 g/100 ml significantly reduced cell width

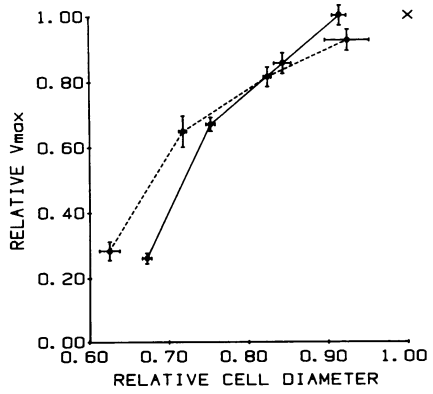

FIGURE 3 Variations in V_{max} due to changes in cell diameter in SOL *(solid line)* and SVL *(dashed line)* single skinned fibers. All values are mean ± SE, n = 6 and are scaled relative to the initial value in the absence of dextran (shown as an X).

(Godt and Maughan, 1981). In 5% dextran, fiber width was reduced by 23–29% of the initial value in the absence of dextran (i.e., W_0). Increasing the concentration of dextran to 10%, however, only compressed cell width by an additional 8–9% W_0. Osmotic compression had no effect on sarcomere spacing.

Maximum shortening velocity was highly sensitive to alterations in cell width (Fig. 3). In all fibers, V_{max} was greatest in the noncompressed state (i.e., 0% dextran), with average values of 1.46 ± 0.11 (mean ± SE) and 5.31 ± 0.51 ML/s for SOL and SVL fibers, respectively. In the range of relative widths between 0.95 and 0.75 W_0, V_{max} declined approximately linearly with decreasing width. However, when the fibers were compressed below 0.75 W_0, V_{max} declined precipitously. In addition, in the range 0.65–0.75 W_0, SOL fibers underwent a greater decline in V_{max} compared with SVL fibers.

In this study all SOL fibers were found to contain solely the slow isoforms of contractile (myosin heavy and light chains) and regulatory proteins (troponin and tropomyosin subunits), while SVL fibers contained exclusively the fast isoforms. Representative gels of SOL and SVL fibers have been published elsewhere (Metzger and Moss, 1987).

DISCUSSION

The primary finding of this study was a reduction in maximum shortening velocity in skinned muscle fibers that were osmotically compressed with dextran. Recently, Gulati and Babu (1984, 1986) reported a decrease in maximum shortening velocity in intact single frog fibers osmotically compressed with sucrose. However, as a consequence of their experimental design, osmotic compression of cell width was associated with a marked increase in intracellular ionic strength (from 190 to 265 mM). This, together with the finding that ionic strength in itself may modulate shortening velocity in living fibers (Edman and Hwang, 1977), may make a straightforward interpretation of their data difficult. This problem was circumvented in the present study by using the skinned fiber preparation. In this way cell width could be manipulated independently of changes in ionic strength within the filament lattice (April and Maughan, 1986). Thus, the reduction in V_{max} observed in the present study can be solely attributed to osmotic compression of interfilament spacing.

The precise mechanism by which variations in cell width modulates V_{max} is currently unknown. One possibility, as yet untested, may involve steric hinderance of cross-bridge cycling as filament spacing becomes compressed. For example, Matsubara et al. (1984) have shown in frog skinned muscle fibers compressed with 10% polyvinylpyrrolidone that center-to-center spacing of the thick filament (d_m)[1] decreases from 47 nm to ~31 nm at a sarcomere

[1]Calculated from the data of Matsubara et al. (1984) as $d_m = d$ 1,0/sin 60°.

length of 2.20 μm. This reduction in lattice spacing, coupled with estimates of cross-bridge (i.e., myosin S-1) length (19 nm, Elliot and Offer, 1978) and the diameters of the thick (12 nm) and thin (8 nm; Hanson, 1968) filaments, certainly suggests that cross-bridge movement may be impaired in a compressed fiber. Similar schemes have been proposed by others in attempt to explain reduced force production in osmotically compressed fibers (Maughan and Godt, 1981; Krasner and Maughan, 1984; Gulati and Babu, 1985; April and Maughan, 1986).

In agreement with previous work (Godt and Maughan, 1981; Krasner and Maughan, 1984) force was found to increase slightly when cell width was compressed by 20–25%. The basis for this increase is not known but does not appear to involve augmented force per individual cross-bridge. Using single plane geometry it has been argued that alterations in the angle that S_2 subtends with respect to the myosin backbone as a result of reduced filament separation will have virtually no affect on the axial component of the force vector (Julian et al., 1978). Thus, from strictly a geometrical perspective, force per cross-bridge is essentially independent of radial separation of filaments within the physiologic range of filament lattice volume. Alternatively, increased force may be related to a greater statistical probability of myosin-actin interactions as a result of reduced filament separation (April and Maughan, 1986). In the present study force declined in fibers compressed with 10% dextran. Reduced force in highly compressed fibers has been demonstrated previously (Godt and Maughan, 1981; Krasner and Maughan, 1984) and has been explained in terms of cross-bridges having difficulty completing a power stroke (Kawai and Schulman, 1985). With osmotic compression fiber stiffness is elevated, suggesting an increase in the number of cross-bridge attachments to the thin filament (Kawai and Schulman, 1985; Berman and Maughan, 1982). Kawai and Schulman (1985) concluded that these attached cross-bridges were in a pretension state and thus did not contribute to force. They further proposed that these cross-bridges were capable of cycling, albeit at a reduced rate, while Berman and Maughan (1982) described such cross-bridges as rigor-like.

The observations of the present study can be adequately described in terms of an accepted model of cross-bridge cycling in which the rates of attachment and detachment are made to depend upon the axial position (x) of a cross-bridge with respect to its binding site on actin (Huxley, 1957). A progressive decline in V_{max} with compression can be accommodated in the model by a reduction in g_{+2}, the rate constant for the detachment of cross-bridges at negative values of x, where attached cross-bridges would oppose contraction. While g_{+2} is important in determining V_{max}, alterations in this rate constant should have no impact on isometric force production. The initial rise in force for small degrees of compression may best be related to an increase in f_1, the rate constant for cross-bridge attachment due to the closer proximity of the thick and thin filaments. However, the decline in P_0 with further compression may reflect a reduction in f_1 (Gulati and Babu, 1986) due possibly to restricted access to cross-bridge binding sites on actin.

The results of the present investigation also provide useful information about contractile properties of skinned versus intact skeletal muscle fibers. It is well known that fibers swell by 20–25% after removal of the sarcolemma (Godt and Maughan, 1981). In agreement with earlier work we found a dextran concentration of 5% was necessary to compress cell width by 23–29%, thus reestablishing in situ dimensions. With this degree of compression the force generating capacity of the skinned fibers was maximal. In addition, it has recently been demonstrated that the characteristic steep and shallow portions of the ascending limb of the length-tension relationship found in living mammalian muscles is not fully expressed in mammalian skinned muscle fibers until interfilament lattice spacing is compressed with 5% dextran (Allen and Moss, 1987). These two results provide evidence to suggest that the filament lattice spacing in living cells is optimal in terms of tension generation. Interestingly, our present results indicate that myofilament lattice spacing in the intact fiber is less than optimal for maximum shortening velocity. The basis for this conclusion is that V_{max} in skinned fibers is markedly reduced after compression with 5% dextran, to a value that approximates V_{max} in the living cell. In an attempt to address the second aspect of this hypothesis we have plotted our SOL V_{max} data along with values from living whole SOL muscles obtained by Ranatunga (1982) using temperatures between 35 and 20°C (Fig. 4). From this analysis, it is readily seen that in the presence of 5% dextran, V_{max} in skinned fibers is close to the value predicted on the basis of linear extrapolation from the living muscle data.

In summary, the results of this study show that V_{max} was

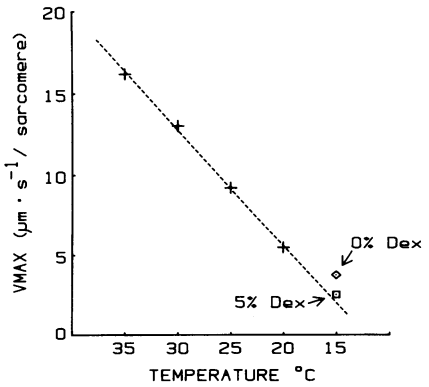

FIGURE 4 Relationship between V_{max} in intact rat whole SOL muscles (+, data from Ranatunga [1982], assuming a sarcomere length of 2.50 μm and fiber/muscle length ratio of 0.72) and that of skinned SOL fibers from the present study with and without compression using 5% dextran. Dotted line represents best fit straight line of Ranatunga's data.

significantly reduced after osmotic compression. Force production initially increased but then decreased as cell width was progressively compressed. While the precise mechanism by which filament lattice spacing modulates contractile function is not known, a model involving spacing-dependent changes in the rate constants for attachment and detachment and, at greater degrees of compression, a steric hinderance of cross-bridge movement seems most reasonable; however, the possibility that compression acts at additional sites in the sarcomere cannot at present be ruled out.

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