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Smooth Muscle Myosin: A High Force-Generating Molecular Motor

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ABSTRACT Smooth muscle generates as much force per cross sectional area of muscle as skeletal muscle with only one-fifth the myosin content. Although this apparent difference could be explained at the tissue or cellular level, it is possible that at the molecular level smooth muscle cross-bridges generate greater average force than skeletal muscle cross-bridges. To test this hypothesis, we used an in vitro motility assay (VanBuren et al., 1994) in which either chicken thiophosphorylated gizzard smooth or pectoralis skeletal muscle monomeric myosin is adhered to a nitrocellulose surface. A fluorescently labeled actin filament, attached to an ultracompliant (50–200 nm/pN) glass microneedle, is brought in contact with the myosin surface. Isometric force, being generated by myosin cross-bridges pulling on the attached actin filament, is calculated from the extent to which the calibrated microneedle is deflected. By measuring the density of myosin adhered to the surface, we estimated the number of myosin cross-bridges that are able to interact with a length of actin filament in contact with the myosin surface. In a direct comparison between smooth and skeletal muscle myosin, the average force per cross-bridge was 0.8 and 0.2 pN, respectively. Surprisingly, smooth muscle myosin generates approximately 4 times greater average force per cross-bridge head than skeletal muscle myosin. Because average isometric force is the product of the cross-bridge unitary force and duty cycle, we are presently using a laser optical trap in an attempt to measure unitary events from single myosin molecules. This approach should allow us to determine whether an increase in unitary force, duty cycle, or both contribute to smooth muscle myosin's enhanced force-generating capacity compared with skeletal muscle myosin.

Smooth muscle generates as much force per cross sectional area as skeletal muscle with only one-fifth the myosin content (Murphy et al., 1974). If these data are interpreted at the molecular level, then smooth muscle cross-bridges may generate greater average force than skeletal muscle cross-bridges. To test this hypothesis, we have adopted the approach first described by Kishino and Yanagida (1988) for measuring force in the in vitro motility assay. Using this technique (Fig. 1), we have directly measured both smooth and skeletal muscle myosin's force-generating capabilities (VanBuren et al., 1994). Surprisingly, smooth muscle myosin generates approximately 4 times greater average force per cross-bridge head than skeletal muscle myosin.

To measure myosin's force, we attached a single fluorescently labeled actin filament to an ultracompliant glass microneedle. The free end of the actin filament was allowed to interact with the myosin-coated surface (Fig. 1), causing the microneedle to deflect. By measuring the extent of microneedle deflection and knowing the stiffness of the microneedle (Fig. 1), we can determine myosin's force (Fig. 2).

The time to steady-state force for smooth muscle myosin is an order of magnitude longer than that for skeletal muscle myosin (Fig. 2). This is not surprising given that a similar difference in velocity is seen with freely moving actin filaments over these two myosin surfaces (Warshaw et al., 1990). The steady-state force generated by both smooth and skeletal muscle myosin is plotted against the length of actin in contact with the surface (Fig. 3). The slope of this relationship is 4 times greater for smooth muscle myosin (46.2 pN/μm) than for skeletal muscle myosin (12.2 pN/μm). These data can be normalized for the estimated maximum number of cross-bridge heads that can interact with a unit length of actin. From both myosin NH_4-EDTA ATPase measurements on the coverslip (Harris and Warshaw, 1993) and protein-binding assays (Warshaw et al., 1991), we estimate that approximately 50–60 cross-bridge heads, per micron of actin filament length, can potentially interact. By using this estimate, and the force per unit length actin data (Fig. 3), we calculate the average force per cross-bridge head to be approximately 0.8 pN for smooth muscle myosin and 0.2 pN for skeletal muscle myosin. The average force per skeletal muscle cross-bridge head that we predict is similar to that which Yanagida and co-workers (Kishino and Yanagida, 1988; Ishijima et al., 1991) obtained in a similar assay. We believe that the absolute force values we report here and those of Yanagida and co-workers are assuredly underestimates, given that the myosin is randomly oriented on the coverslip. For example, improperly oriented heads may not contribute to force (Ishijima et al., 1994) but are a part of the myosin density estimates used to calculate the average forces in our study.

Regardless of the absolute values, the more salient fact is that smooth muscle cross-bridge heads appear to generate 4 times more average force than skeletal muscle cross-bridge heads under identical experimental conditions. A similar conclusion was drawn from motility studies in which smooth and skeletal myosin were either mixed together (Sellers et al.,

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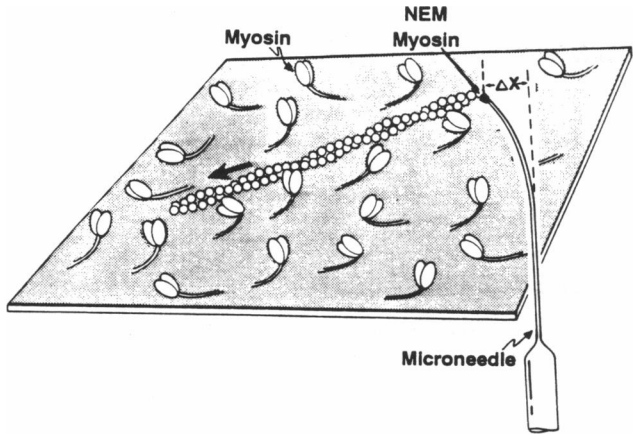

FIGURE 1 An actin filament attached to a microneedle interacts with a myosin coated surface. The microneedle is deflected (X) by the force generated in the actin-myosin interaction. NEM, N-ethylmaleimide.

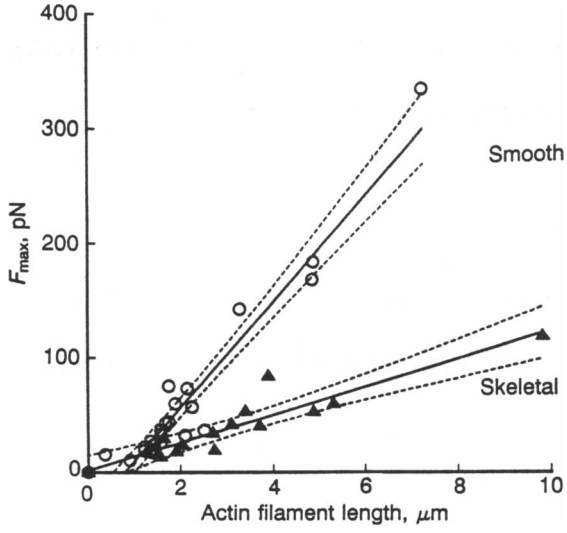

FIGURE 3 The steady-state force per actin filament length in contact with the smooth and skeletal muscle myosin surface. Each data point is the result of an individual actin filament measurement. The linear regressions (——) and the 95% confidence limits (- - - -) are displayed. The slopes of the regressions (i.e., force/μm actin) are listed in the text. F_{max} is the maximal steady-state force value.

1985; Harris et al., 1994) or independently with N,N′-p-phenylenedimaleimide skeletal muscle myosin (Warshaw et al., 1990).

Can alterations in the kinetics of the cross-bridge cycle or the manner in which chemical energy is converted to mechanical work explain smooth muscle myosin's greater force production? If we assume a simple two-state cross-bridge model, then the cross-bridge first attaches weakly to actin and then more strongly as it undergoes the powerstroke to generate its unitary force (F_{uni}). The fraction of the cross-bridge cycle in which myosin is strongly bound to actin and generating force is termed the duty cycle (f). The average cross-bridge force, measured in the microneedle assay, is the resultant product of the unitary force and duty cycle ($F_{avg} = F_{uni} \cdot f$). Therefore, the greater average force per cross-bridge for smooth muscle myosin could be explained by a greater unitary force, an increased duty cycle, or a combination of the two. To distinguish among these possibilities, we have begun to measure unitary cross-bridge events in the motility assay.

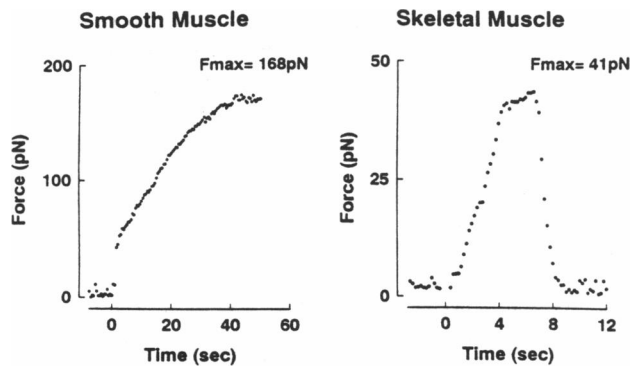

FIGURE 2 Force generation over time for two individual force measurements. Microneedle position was determined from digitized video images every 0.5 and 0.2 s for the smooth and skeletal muscle myosins, respectively. The decline in force, seen in the skeletal muscle myosin force measurement, is the result of actin filament release from the microneedle. Note that the microneedle force returns to baseline. The length of actin filament in contact with the myosin surface was 4.9 μm for smooth muscle myosin and 3.7 μm for skeletal muscle myosin. F_{max} is the maximal steady-state force.

Recently, Finer et al. (1994) have described an elegant approach using the laser optical trap to record both the myosin step size and the unitary force from a single heavy meromyosin molecule. We have adapted this technique as follows. A single fluorescently labeled actin filament is attached at both ends to NEM-coated fluorescent polystyrene beads. The beads, and thus the actin filament, are held in solution at the focal point of a microscope objective by two separate laser optical traps. The taut actin filament is then lowered onto the surface of a myosin-coated 2.0-μm glass bead, which itself is adhered to a glass coverslip. The myosin density is such that optimally one, or at most several, myosin molecules can interact with the actin filament. The brightfield image of one of the trapped beads is projected onto a quadrant photodiode detector so that small displacements of the actin filament can be recorded with nanometer and millisecond resolution. In Fig. 4 are preliminary data showing small displacements of an actin filament as it interacts with skeletal muscle myosin. Notice that single-step displacements as well as staircase displacements (presumably from multiple cross-bridge events) are clearly detected. It appears that for skeletal muscle myosin the average unitary displacement is approximately 10 nm, similar to that reported by both Finer et al. (1994) and Ishijima et al. (1994).

To address whether differences in the smooth and skeletal muscle unitary force and/or duty cycle exist, we have used the bead's position in feedback mode to effectively stiffen the optical trap and thereby create an isometric force transducer capable of measuring molecular forces from a single myosin

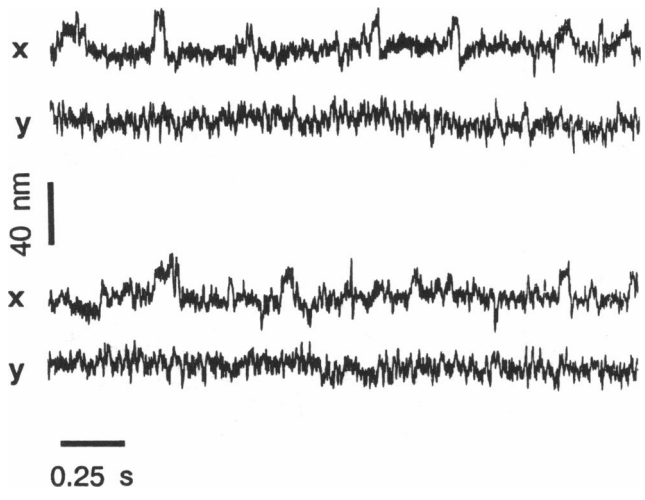

FIGURE 4 Bead position over time as recorded by a quadrant photodiode detector. An actin filament, attached to two NEM-myosin-coated beads, is held in solution by two optical traps. The data were recorded when the actin filament was lowered onto the skeletal muscle myosin surface (25 μg/ml introduced into the 10-μl chamber). Displacements in the x (i.e., along actin filament's long axis) and y directions (i.e., perpendicular to filament's long axis) are displayed in the upper and lower traces, respectively. Note that no displacements are observed in the y direction that correlate with events recorded along the actin filament's long axis. In addition, no displacements were observed in either direction before contact with the myosin surface. Assay conditions were as described previously (Warshaw et al., 1990), i.e., low salt (25 mM KCl) and 0.5 mM MgATP at room temperature, trap stiffness of each trap at 0.06 pN/nm. Data, sampled at 4 kHz, as presented are raw and unfiltered.

molecule (Finer et al., 1994). Although data are not yet available from these optical trap studies, evidence for an increased duty cycle in smooth muscle has been obtained from mechanical measurements of single muscle cells (Warshaw, 1987; Yamakawa et al., 1990). In skeletal muscle, duty cycle estimates are as high as 75% (Goldman and Simmons, 1977) under isometric conditions. Therefore, it is likely that both unitary force and duty cycle are increased to contribute to the fourfold higher average force per smooth muscle cross-bridge.

Clues to how this unique functional difference between smooth and skeletal muscle myosin has evolved may reside in the primary amino acid sequence differences that exist between these two myosin species and its effect on smooth muscle myosin's molecular structure.

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DISCUSSION

Session Chairperson: Margaret A. Titus
Scribe: Jeremy Gollub

SAMUEL CHAKO: Your measurements for smooth muscle are made using skeletal muscle actin. In view of the fact that the actin types in various smooth muscles are different, do you think it will make a difference if you use smooth muscle actin for measuring the force?

DAVID WARSHAW: In fact, we thought that maybe all the difference was in the actin itself. Dave Harris in my laboratory has actually done an actin-to-actin comparison, where we swap the actins and keep the myosins constant, and under all the conditions that we do the experiment, we see no difference whatsoever. So the argument would be that the enhanced force generation has to be in the myosin.

STEVE BLOCK: One of the differences between kinesin and myosin heads, which we've discussed alot at this meeting, has been this business about duty cycle: that kinesin has such