Force: Velocity Relationship in Single Isolated Toad Stomach Smooth Muscle Cells

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ABSTRACT The relationship between force and shortening velocity (F:V) in muscle is believed to reflect both the mechanics of the myosin cross-bridge and the kinetics of its interaction with actin. To date, the F:V for smooth muscle cells has been inferred from F:V data obtained in multicellular tissue preparations. Therefore, to determine F:V in an intact single smooth muscle cell, cells were isolated from the toad (Bufo marinus) stomach muscularis and attached to a force transducer and length displacement device. Cells were electrically stimulated at 20°C and generated 143 mN/mm² of active force per muscle cross-sectional area. At the peak of contraction, cells were subjected to sudden changes in force (dF = 0.10–0.90 F₀) and then maintained at the new force level. The force change resulted in a length response in which the cell length (L₉₀) rapidly decreased during the force step and then decreased monotonically with a time constant between 75 and 600 ms. The initial length change that coincided with the force step was analyzed and an active cellular compliance of 1.9% cell length was estimated. The maintained force and resultant shortening velocity (V) were fitted to the Hill hyperbola with constants a/F₀ of 0.268 and b of 0.163 L₉₀/s. From the F:V, the maximum shortening velocity (V₉₀) was estimated as 0.608 L₉₀/s. V₉₀ was also determined by a procedure in which the cell length was slackened and the time of unloaded shortening was recorded (slack test). From the slack test, V₉₀ was estimated as 0.583 L₉₀/s, in agreement with the F:V data. The F:V data were analyzed within the framework of the Huxley model (Huxley, 1957. Progress in Biophysics and Biophysical Chemistry. 7:255–318) for contraction and interpreted to indicate that in smooth muscle, as compared with fast striated muscle, there may exist a greater percentage of attached force-generating cross-bridges.

INTRODUCTION

Active cell shortening and force production in smooth muscle reflects the cyclic interaction of the myosin cross-bridge with actin (Murphy, 1980; Fay et al., 1981; Hellstrand and Paul, 1982). In comparison with fast striated muscle, the velocity of shortening in smooth muscle is considerably slower. In addition, smooth muscle can generate a maximum force per muscle cross-sectional area that is equal to or greater than that in striated muscle, but with much less myosin.
Since the cross-bridge is the most basic contractile unit in smooth muscle, possible differences in the cross-bridge mechanics (i.e., compliance) and the kinetics of its interaction with actin may account for smooth muscle's unique contractile characteristics. Therefore, analysis of the active force and velocity of shortening may provide insight into possible alterations in cross-bridge mechanics and the kinetics of the cross-bridge cycle in smooth muscle as compared with those in fast striated muscle.

In 1938, A. V. Hill presented the relationship of active force production with shortening velocity in whole skeletal muscle as a rectangular hyperbola. In smooth muscle, a similar force vs. velocity relationship ($F:V$) has been described (Murphy, 1980; Fay et al., 1981; Hellstrand and Paul, 1982). It was this similarity in $F:V$ shape between the two muscle types that served as evidence for a qualitatively similar cross-bridge mechanism in smooth and skeletal muscle. However, to date, all studies of smooth muscle $F:V$ have been limited to multicellular tissue preparations, in which it is difficult to assess the dependence of the $F:V$ on possible inhomogeneities in cellular activation and mechanical responses. Since the mechanical behavior of multicellular preparations must be a mechanical average of the individual cell responses, the similarity between the $F:V$ shape observed in smooth muscle tissue preparations and that seen in striated muscle may have been coincidental. With the ability to isolate single smooth muscle cells and measure their mechanical responses (Warshaw and Fay, 1983b; Warshaw et al., 1986), one can now address the question of whether or not the hyperbolic $F:V$ in smooth muscle is truly a characteristic property of the individual smooth muscle cells.

In this study, single smooth muscle cell velocity data were obtained by: (a) imposing a sudden change in cell force and analyzing the resultant length response as force was maintained at its new level (i.e., isotonic release), and (b) releasing the cell to its slack length and determining the time required for the cell to take up its slack (i.e., slack test procedure). Isotonic release data were used to construct $F:V$ in single smooth muscle cells. The maximum shortening velocity estimated from $F:V$ data compared favorably with values obtained from the slack test procedure. Since $F:V$ data reflect cross-bridge kinetics (Huxley, 1957; Eisenberg et al., 1980), these data were used to predict that in smooth muscle as compared with fast striated muscle: (a) cross-bridge detachment is slower, and (b) the percentage of attached cross-bridges is greater. Therefore, smooth muscle's slower shortening velocity and force-generating abilities may reflect alterations in specific steps within the cross-bridge cycle. An initial account of these data was presented previously (Warshaw, 1985).

**METHODS**

*Single Cell Isolation and Preparation*

The procedures for isolation and preparation of single smooth muscle cells from the toad (*Bufo marinus*) stomach muscularis have been previously described in detail (Fay et al., 1982; Warshaw and Fay, 1983b). In brief, single smooth muscle cells were obtained by enzymatically (i.e., trypsin and collagenase) digesting tissue slices of stomach muscularis.
Cells were then transferred to an inverted microscope on a glass slide containing amphibian physiological saline (Warshaw and Fay, 1983b) with 10 μM isoproterenol added to the solution. The isoproterenol served to relax cells during their attachment to the mechanical recording device. Cells were attached at one end to a force transducer (200 Hz natural frequency; 0.01 μN resolution, 0.1 nm/μN compliance) and at the other end to a piezoelectric length displacement device (1 kHz natural frequency; ±10 μm displacement) by means of specially designed glass micropipettes. The cells were electrostatically attached to anionic exchange resin beads, which were glued at the micropipette tips. This initial attachment allowed the cell to be knotted at both ends using micromanipulators while being viewed under the microscope at 400X. Cells were then stretched to a length (Lmax) at which a transient passive force of 0.2 N was recorded in response to the stretch. This ensured that the knots were tightened sufficiently to prevent cell slippage upon contraction. Previous studies (Warshaw and Fay, 1983b) indicated that the method of attachment did not introduce a significant end compliance in the region of the knots. Although to date there are no steady state active tension:length relationships for these single cells, the knot-tightening procedure that is uniformly done to all cells may set cells at approximately the same length on their length:tension relationship. The assumptions are that if cells having similar lengths (100 μm) are chosen for ease in knotting and if all cells possess similar length:tension relationships, then stretching cells until a given passive force is recorded should place cells at a relatively constant point on their length:tension relationships. Cells were stimulated by transverse electric field stimulation through platinum paddles (cross-sectional area, 0.2 mm²). A sequence (1 Hz) of single electrical shocks (100 mA, 0.1 ms duration) produced maximal isometric force for these stimulus parameters at 20°C. This mode of supramaximal stimulation elicited contractions that were sustained even when the stimulus was removed and, after 1 min of contraction, became rigor-like. Although these cells are capable of multiple contractions when the stimulus parameters are submaximal, the single sustained contractions were chosen to avoid complications associated with submaximal activation. Once stimulated to contract, cells generated active force isometrically. At the peak of contraction, cells were subjected to experimental protocols in which either muscle force (i.e., isotonic release) or cell length (i.e., isometric release for slack test procedure) was controlled (see Figs. 1 and 2).

The force and length (detected by an eddy current displacement sensor) signals were stored on FM tape and later digitized on a 12-bit analog-to-digital converter at 1 kHz sampling rate for analysis on an IBM PC-XT personal computer.

**Force Feedback Control**

The ability to measure isotonic shortening was accomplished using a computerized force feedback circuit that adjusted cell length so that cell force was controlled. The basic operating principles were as follows. The cell force signal was used as an input to the feedback circuit. This force signal was then compared with a computer-generated reference signal that equaled the desired muscle force. When cell force differed from the desired force reference signal, the feedback circuit generated a voltage control signal that was amplified and fed into the piezoelectric displacement device. Depending on the polarity of the piezoelectric control signal, the displacement device would either increase or decrease cell length (at a maximum rate of 10 μm/s) in order to match cell force with the desired force level.

To control for the existence of any system-related shortening, the following experiment was performed. A cell that had been attached to the recording system was fixed in place by glutaraldehyde fixation (2.5%) (Warshaw and Fay, 1983b). Once fixed (~20 s), the cell was stretched to a force level comparable to the active force normally observed (2.5 μN).
The force reference level was then abruptly changed so that the fixed cell force was maintained by feedback control at 10% of its original value. Since the glutaraldehyde-fixed muscle was unable to shorten actively, the observed shortening rate (0.002 \( L_{\text{cell}}/s \)) was attributed to the feedback system and viscoelasticity within the fixed cell. This system drift was 300 times less than the cells' maximum shortening velocity and was therefore not accounted for in any of the active cellular records.

**Protocols**

*Isotonic releases.* In order to determine the \( F:V \) in a single smooth muscle cell, it was necessary to measure cell shortening velocity at various force levels. Therefore, the following computer-controlled protocol was used. Before stimulation, the resting tension signal was digitized for 0.5 s at 500 Hz and the average value was calculated. The cell was then electrically stimulated to contract and, at the peak of isometric contraction, cell force was once again digitized for 0.5 s at 500 Hz and the average value was calculated. Since the time to reach peak force can vary from cell to cell (i.e., 13 ± 2 s; \( n = 10 \)), the time at which maximum force was obtained was determined by eye and the computer was then signaled through a keyboard entry. By subtracting the resting force value from that at the peak of contraction, a value for maximum active force \( (F_{\text{max}}) \) was determined. \( F_{\text{max}} \) was then used to calculate the necessary reference signals for a series of five isotonic releases. Once the reference signals were calculated \(<1 \text{ s}\), the computer signaled the feedback circuit to begin force control. At this point, the cell was subjected to a series of isotonic releases (each release complete in 30–65 ms) of varying magnitudes \( (0.10–0.90 \ F_{\text{max}}) \). The usual series of force steps maintained cell force at the following levels: 0.20, 0.35, 0.50, and 0.60 \( F_{\text{max}} \) (see Fig. 1). Each force step was maintained for 500 ms, after which time the force was returned gradually to \( F_{\text{max}} \) over a 1-s period. The cell was maintained at \( F_{\text{max}} \) for 1 s before the next force step. Thus, the entire isotonic release protocol lasted 12.5 s.

![Figure 1. Isotonic releases and resultant length responses. Four isotonic releases to various force levels are superimposed and presented on a shorter time scale. The time between points is 4 ms. Note the immediate length change that coincides with the force step. Following the immediate length response when force is maintained constant, \( L_{\text{cell}} \) decreases for the entire period of the maintained step (500 ms). At that time, force is returned to \( F_{\text{max}} \) over a 1-s period. \( F_{\text{max}} = 2.15 \mu N; \ L_{\text{cell}} = 76 \mu m; \) cross-sectional area = 20.7 \( \mu m^2 \).](image-url)
Since the entire isotonic release protocol was completed within a single contraction, it was necessary to control for any slowing in shortening velocity that may have occurred during the protocol. Therefore, an isotonic release protocol similar to that described above was performed; however, the magnitude of the isotonic releases was held constant at 0.5 \( F_{\text{max}} \). By imposing isotonic releases to the same force level, any variations in the observed shortening velocity would thus characterize any dependence of shortening velocity on the duration of the contraction.

**Maximum shortening velocity: slack test method.** In addition to determining the maximum shortening velocity \( (V_{\text{max}}) \) from the \( F-V \), \( V_{\text{max}} \) can also be estimated from the slack test procedure as described by Edman (1979). This procedure is based upon a cell's ability to take up its slack immediately after a length change that is sufficient to make the cell go slack. The slack test protocol, as seen in Fig. 2, was as follows. At peak isometric force, a series of two to three length changes between 0.10 and 0.30 \( L_{\text{cell}} \) was imposed. Each length change was complete in 5 ms, after which the length was maintained constant for 1 s and then returned to the original length over a 1-s period. Once the cell was returned to its initial length, it was maintained for 1 s before the next release. The magnitude of these cell length changes was sufficient to drop force to zero and to make the cell go slack as visualized through the microscope. The entire length change protocol lasted 6–9 s.

**Data Analysis**

**Shortening velocity.** A detailed mathematical analysis of the length response after completion of the force step was performed to determine an appropriate mathematical description. The length response was fitted to the following functions using various least-squares regression analysis subroutines in the BMDP statistical software package (Dixon, 1981) on our university DEC-20 computer: (a) linear (BMDP5R); (b) polynomial

![Figure 2. Protocol for estimating \( V_{\text{max}} \) by the slack test procedure. Cell length (upper trace) and force (lower trace) were digitized at 1 kHz. The computer plot of the data in this figure is that of every 50th point. Force data were digitally filtered using a second-order differential equation to account for the force transducer's inertia and viscoelastic properties (Warshaw and Fay, 1983b). At the peak of isometric contraction, a series of computer-generated length steps was imposed so as to make the cell go slack and thus drop force to zero. In this experiment, the magnitude of the length steps was varied between 20 and 30% \( L_{\text{cell}} \). \( F_{\text{max}} = 2.64 \mu \text{N} \); \( L_{\text{cell}} = 96 \mu \text{m} \); cell cross-sectional area = 20.7 \( \mu \text{m}^2 \).
(BMDP5R); (c) single exponential (BMDP3R); (d) two exponentials (BMDP5R). Goodness of fit was evaluated by minimizing the residual sum of squares and calculating an $R^2$ value (i.e., coefficient of determination) for the fit. The $R^2$ is an estimate for the extent of variation in the observed data that can be explained by the fitted curve. Therefore, a value $>0.85$ was judged a good fit. The best fit was the single-exponential function with $R^2 = 0.94$, as graphically illustrated in Fig. 3.

The isotonic release protocol provided a series of active muscle force steps and their resultant length changes, which were used to construct the $F:V$ in a single cell. Since cell length changes following the completion of the force step were described by a single-exponential function, a value for the velocity of shortening normalized to cell length was determined from the derivative of the exponential fit of cell length and time. Since the

![Figure 3](image-url)

**Figure 3.** Exponential fit of length responses. The slowing of velocity observed in all shortening responses in Fig. 1 is characterized by a single exponential, as seen in the plot of the logarithm of $(1 -$ fractional response) vs. time (solid lines fitted by eye). The fractional response is the change in cell length ($dL_{cell}$) normalized to the total length change that occurred during the 500 ms of maintained force ($dL_{tot}$) with $dL_{cell}$ beginning at the completion of the length step. The length transients ($r$) for different final force levels and associated time constants (determined as the time to reach 37% of total response); dashed lines are 185 ms for 0.2 $F_{max}$, 171 ms for 0.3 $F_{max}$, 76 ms for 0.5 $F_{max}$, and 92 ms for 0.8 $F_{max}$. $F_{max}$, $L_{cell}$, and cross-sectional area are equal to those given in the legend to Fig. 2.

shortening velocity varied with time when force was maintained constant at its new level, the velocity was determined immediately after completion of the isotonic release.

**Force-velocity relationship.** Hill (1938) described the dependence of shortening velocity on load as a rectangular hyperbola of the form:

$$ (F/F_{max} + a/F_{max})(V + b) = (1 + a/F_{max})b, \quad (1) $$

with constants $a/F_{max}$ and $b$, which related to the $F:V$ curvature and intrinsic shortening velocity, respectively. In addition to the usual hyperbolic description, Aubert (1956) described the $F:V$ as an exponential of the form:

$$ F = (F_{max} + c)[exp(-V/d)] - c, \quad (2) $$

with $c$ and $d$ being constants. Therefore, without any a priori assumptions, the data were
fitted by a nonlinear regression analysis (Dixon, 1981) to both the hyperbolic and exponential forms. \(F:V\) curves were fitted for both the individual cells and combined data from all cells. Goodness of fit was judged by the coefficient of determination \(R^2\), as described above.

All data are presented as a mean ± 1 SE unless otherwise noted.

**Slack Test.** The slack test data used to estimate \(V_{\text{max}}\) were analyzed in the following manner. Once the cell length was released to a slack length (length change = 0.1–0.3 \(L_{\text{cell}}\)), thus dropping force to zero, the time to take up this slack was determined as the time from the beginning of the release to the time when the redevelopment of force was just detected (see Fig. 7A). Since the load on the cell is zero while slack, the amount of slack cell length taken up by the cell divided by the time to take up this slack is thus an estimate of unloaded shortening velocity. To be certain that the amount of slack to be taken up by the cell had no effect on unloaded shortening, the magnitudes of various length changes were plotted against the time to take up different amounts of slack, as described above (see Fig. 7A). The slope of the line fitted by linear regression to these points was then taken as an estimate of \(V_{\text{max}}\). In addition to \(V_{\text{max}}\), an estimate of the cell’s series compliance can be obtained from the y-intercept of the regression line (Edman, 1979). The assumption is that the length change that is just sufficient to make the cell go slack equals the extension of the cell’s series elastic element at peak isometric force. Thus, a cell shortening under zero load would instantly redevelop force only if the series elastic element’s length went slack because of the length change.

**RESULTS**

**Isotonic Releases**

A single smooth muscle cell’s ability to shorten under various loads was studied after a sudden change in force. The time course of the length change in response to a force step (see Figs. 1 and 3) was biphasic. The initial phase is a rapid decrease in cell length that is coincident with the sudden drop in force and reflects the cell’s elastic response. After the force step, for the entire period (500 ms) that cell force was maintained at a new level, cell length decreased with a single time constant (see Fig. 3).

An estimate of the active cellular elasticity can be obtained by analyzing the initial length change, which coincides with the sudden drop in force. Thus, by plotting force vs. length during the force step, a length:force characteristic \((L:F)\) is obtained (see Fig. 4) with the slope equal to cell stiffness \((dF/dL_{\text{cell}})\). The shape of the \(L:F\) appears linear for force changes up to 0.6 \(F_{\text{max}}\). However, for force changes below 0.6 \(F_{\text{max}}\), the \(L:F\) begins to deviate from the initial linear response. A similar deviation was observed in the \(L:F\) obtained in previous isometric transient studies on the same preparation (Warshaw and Fay, 1983a). It was shown that in the \(L:F\) from isometric transients, the deviation could be accounted for by the recovery of force that occurred during the length step. Therefore, it is possible that because of the relatively slow force changes in this study, there may be sufficient time for cell shortening to occur. Thus, any shortening during the force change would tend to cause an overestimate of the length change. If the deviation from linearity within the \(L:F\) is in fact due to cell shortening, then the best estimate of cell compliance (i.e., the inverse of stiffness) would be obtained from the initial linear portion of the \(L:F\), where little time is available.
for cell shortening. By extrapolating the $L:F$ to zero force, active cell compliance is $1.9\% L_{cel}$, which agrees with the $1.5\%$ estimate from isometric transient studies (Warshaw and Fay, 1983b).

Upon completion of the force change, $L_{cell}$ decreased exponentially with a time constant ranging between 75 and 600 ms. Although it appeared initially that the time constant for the length change may have been related to the magnitude of the force step (see Fig. 3), the apparent difference in time constants may relate in part to the time within the contractions at which the measurements were made.

![Figure 4](image-url)

**Figure 4.** Length vs. force relationship in single smooth muscle cell. At the peak of force production, isotonic releases to $0.3 F_{max}$ (□) and $0.5 F_{max}$ (○) were imposed. The normalized cell length ($dL/L_{cel}$) is plotted against normalized active force ($F/F_{max}$) during the force step. Cell compliance was determined by drawing a tangent to the $L:F$ at the linear portion of the curve. It appears that a $1.9\%$ change in $L_{cell}$ would drop force to zero in these cells. Notice the deviation from linearity at lower force levels, which may be due to cell shortening that occurs during the force step.

$F_{max} = 2.58 \mu N; L_{cel} = 154 \mu m; \text{cell cross-sectional area} = 11.7 \mu m^2$.

The velocity of shortening equals the slope of the length response at any point in time; thus, it is apparent that an estimate of shortening velocity could vary by a factor of 2.0 owing to curvature in the length response. Therefore, to avoid variations in velocity estimates, velocity was determined at the moment the isotonic release was complete. However, no attempt was made to extrapolate back to the moment of the release for an estimate of velocity before any slowing.

The velocities of shortening estimated from the isotonic release protocol were used to construct the relationship between active cell force and shortening velocity. Since these isotonic releases were obtained in a single contraction, it
was important to control for any variation in shortening velocity with duration of contraction. Fig. 5 shows the lack of any such dependence of shortening velocity at a constant load (0.5 $F_{\text{max}}$) since the slope of the regression line through the data on either a single experiment or grouped basis was not significantly different than zero ($p > 0.20$). Thus, the estimates for shortening velocity at various loads were used without correction to construct the $F:V$ in single smooth muscle cells (see Fig. 6).

**Force:Velocity Relationship**

The relationship between active cell force and shortening velocity was best fitted by the Hill hyperbola ($R^2 = 0.94$) rather than the Aubert exponential form ($R^2 = 0.77$). From the hyperbolic fit, values for the hyperbolic constants from individual experiments ranged between 0.137 and 0.611 for $a/F_{\text{max}}$ and between 0.100 and 0.354 $L_{\text{cell}}$/s for $b$. The force and velocity data from four experiments were grouped and the resultant hyperbolic constants were 0.268 for $a/F_{\text{max}}$ and 0.163 $L_{\text{cell}}$/s for $b$ (see Fig. 6 and Table I). From the fitted equations for individual cells, an estimate for the maximum velocity of shortening was determined at zero force and ranged between 0.396 and 1.090 $L_{\text{cell}}$/s. The average maximum shortening velocity from individual experiments (0.627 $L_{\text{cell}}$/s) compared favorably with the predicted maximum shortening velocity from grouped data (0.608 $L_{\text{cell}}$/s).

**$V_{\text{max}}$: Slack Test**

When cells were subjected to large rapid releases to slack lengths, cell force dropped to zero (see Fig. 7A). The time required for the cell to take up this slack could be easily detected as the time when the cell begins to redevelop force. The time to take up the slack was dependent upon the magnitude of the length
change and thus was longer for longer releases (see Fig. 7A). These data were then used to estimate $V_{\max}$ as described in the Data Analysis section. From six experiments, the average slope of the regression line (i.e., $V_{\max}$) through the slack test data equaled 0.583 $L_{\text{cell}}$/s (see Fig. 7B), with individual cells having a $V_{\max}$ that ranged between 0.26 and 0.89 $L_{\text{cell}}$/s. The slack test estimate of $V_{\max}$ thus agrees with the $V_{\max}$ estimate obtained from the $F:V$. However, note that in two experiments in which three releases were performed, the relationship between

![Figure 6](image.png)

**Figure 6.** Force:velocity relationship in single smooth muscle cells. Isotonic releases to new force levels at the peak of isometric force ($F_{\text{max}}$) result in cell shortening, as seen in Fig. 2. The velocity of shortening normalized to cell length ($L_{\text{cell}}$/s) is plotted vs. the maintained force level normalized to $F_{\text{max}}$. Data from four cells are presented as different symbols. The data points from all cells were fitted to the Hill hyperbola, with the best fit indicated as a solid line. The hyperbolic constants for the grouped data are $a/F_{\text{max}} = 0.268$ and $b = 0.163 L_{\text{cell}}$/s. The estimated maximum velocity at zero force is 0.608 $L_{\text{cell}}$/s. From four experiments, $F_{\text{max}} = 2.13 \pm 0.16 \mu$N; $L_{\text{cell}} = 100 \pm 13 \mu$m; cross-sectional area = 21.1 $\pm 9.1 \mu$m$^2$.

the length change and the time to take up the slack became nonlinear for larger releases. If this trend existed for all length changes, then the slack test may slightly underestimate $V_{\max}$.

In addition to $L:F$ data from isotonic releases, an estimate of the active cellular compliance was obtained from the y-intercept of the regression line through the slack test data (see Fig. 7B). The average y-intercept was 0.047 $\pm 0.027$ ($n = 6$) $L_{\text{cell}}$. This value of cell compliance is greater than that estimated from the $L:F$ values of isotonic releases. The difference in the estimated compliance obtained from the slack test data suggests that the slack test analysis may overestimate cell
compliance. This overestimation could result, as stated above, from the apparent tendency for deviation from linearity in the relationship between the magnitude of release and the time to take up the slack for large releases, as reported recently by Gunst (1986).

**FIGURE 7.** Slack test procedure and data analysis for estimating $V_{\text{max}}$. (A) Force responses (upper trace) resulting from sudden step decreases in cell length (lower trace) are analyzed to determine $V_{\text{max}}$ in single smooth muscle cells. Releases in cell length of varying magnitude result in cell force dropping to zero while the cell goes slack. Note that once the cell has taken up the slack, force begins to redevelop (arrows) and that the greater the release, the longer the time to redevelop force. The force traces are normalized to the change in force that occurs in response to the length change. (B) $V_{\text{max}}$ is estimated from data in A that are plotted as the magnitude of the length change to slack length vs. the time to redevelop force. The solid line represents the average slope (i.e., $V_{\text{max}}$) and $y$-intercept (i.e., series compliance) from the individual experiments. The different symbols indicate data from different experiments. $L_{\text{cell}} = 100 \pm 8 \mu m$ ($n = 6$).
DISCUSSION

Since Hill (1938) first described the hyperbolic shape of the $F:V$ in whole skeletal muscle, similar hyperbolic functions have been reported in single skeletal muscle fibers (Julian, 1971; Edman, 1979) and cardiac (Meiss and Sonnenblick, 1974) and smooth muscle tissue preparations (Murphy, 1980; Fay et al., 1981; Hellstrand and Paul, 1982). Since smooth muscle $F:V$'s have only been obtained in multicellular tissue preparations, it may be difficult to assess the extent to which the $F:V$ shape is dependent upon inhomogeneities in the individual cellular responses. In the present study, the ability to obtain $F:V$ in a single smooth muscle cell has been demonstrated. Since single smooth muscle cell $F:V$'s are also hyperbolic in shape, these data support the idea that a qualitatively similar cross-bridge mechanism exists in smooth muscle as compared with that in striated muscle. In addition, the similarity in hyperbolic constants that describe $F:V$ from smooth muscle tissue preparations and single smooth muscle cells supports what to date has been a crucial assumption, that tissue $F:V$'s are a reasonable estimate of their constituent cells.

### TABLE I

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<th>Parameter</th>
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<td>Maximum shortening velocity $V_{max}$</td>
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<td>Maximum shortening velocity $V_{max}$</td>
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The numbers in parentheses equal the number of cells.

* These are the mean and asymptotic standard deviation of the parameter estimates determined by the nonlinear regression analysis.

† From the hyperbola fit to the grouped $F:V$ data, the 95% confidence limits were estimated for $V_{max}$ (Afifi and Azen, 1979).

Isotonic Releases

$F:V$'s in single smooth muscle cells were constructed from individual length responses after a sudden change in cell force. In the past, investigators studying the time course of these length responses (i.e., isotonic transients) in single skeletal muscle fibers (Civan and Podolsky, 1966; Huxley, 1974) have interpreted
the multiphasic length responses as reflecting the cross-bridge mechanical response (i.e., the initial shortening phase that coincides with the force step), followed by the transitions of a relatively synchronized population of cross-bridges passing through a series of steps in the cross-bridge cycle before reaching steady state shortening. The length transients upon completion of the force step in skeletal muscle are characterized by an initial rapid shortening that lasts for 2–5 ms, which is followed by a slowing or hesitation in shortening lasting 50 ms, and a third and final phase of steady state shortening (Civan and Podolsky, 1966; Huxley, 1974). The length responses from single smooth muscle cells are also characterized by an initial length change that coincides with the force change. Estimates of cell compliance (~1.9% \( L_{cell} \)) from this initial length response agree with previous estimates from single toad stomach smooth muscle cells (Warshaw and Fay, 1983a), as well as estimates from other smooth muscle tissue preparations (Pfizer et al., 1982).

In skeletal muscle, the compliance is believed to originate within the cross-bridge (Ford et al., 1977), but the exact location in smooth muscle is still speculative. In smooth muscle tissue preparations, the connective tissue matrix must account for a fraction of the compliance (Mulvany and Warshaw, 1981). Even in single smooth muscle cells free of their connective tissue matrix, as much as 50% of the cellular compliance resides in intracellular structures other than the cross-bridge (Fay and Warshaw, 1984). If a compliant non-cross-bridge site exists in smooth muscle cells (e.g., dense bodies), then 0.75–0.9% \( L_{cell} \) compliance would be cross-bridge related. In comparison with fast skeletal muscle, which requires only 0.5% of muscle length to drop force to zero, smooth muscle cross-bridges appear inherently more compliant and thus may underlie the contractile capabilities of smooth muscles, as originally suggested by Warshaw and Fay (1983a, b).

During the 500 ms following the sudden change in force, cell shortening does not exhibit a multiphasic length response that is characteristic of fast skeletal muscle as described above. The smooth muscle cell shortening velocity is monophasic and slows with a time constant of 75–600 ms. The apparent lack of an initial rapid velocity transient (observed in smooth muscle tissue preparations by Hellstrand and Johannson, 1979; Mulvany, 1979; Dillon and Murphy, 1982) and the subsequent hesitation of shortening in the single cell are probably related to the amount of time required to impose the change in force (i.e., 65 ms). These relatively slow force changes were necessary to prevent resonant oscillations in the force recording and feedback control system. The probability that an initial velocity transient occurs during the force step is supported by the results of isometric transient studies in smooth muscle cells (Warshaw and Fay, 1983a, b). In these studies, rapidly imposed length changes (e.g., 1.5 ms) resulted in tension responses characterized by an initial rapid force recovery (\( \tau = 5–20 \) ms) followed by a slower recovery (\( \tau = 50–200 \) ms). If the isometric and isotonic transients reflect similar underlying cross-bridge events, then an initial rapid velocity transient would not be recorded, as a result of the slow force steps. Therefore, any attempt to analyze the kinetics of the length responses from smooth muscle cells in terms of specific steps in the cross-bridge cycle would be premature at this time.
After an isotonic release, the slowing of the cell shortening velocity observed in single smooth muscle cells is similar to that reported in smooth muscle tissue preparations (Hellstrand and Johansson, 1979; Mulvany, 1979; Dillon and Murphy, 1982). The slowing of velocity during the maintained force step could be explained by internal compressive forces that resist further cell shortening and/or an ongoing process that slows cross-bridge cycling as a function of time. Evidence for internal compressive forces is supported by the ability of single toad stomach smooth muscle cells to re-extend fully immediately after a contraction to 30% of the resting cell length (Fay and Delise, 1973). Although the structures or processes responsible for cell re-extension are unknown, their compression during shortening would act as an internal load to further shortening and possibly result in the observed slowing of shortening velocity in single smooth muscle cells. Another possibility is the existence of some process that results in a continual slowing of cross-bridge cycling with time of contraction. Possible processes are the appearance during a contraction of noncycling or slowly cycling cross-bridges (i.e., latch-bridges; Dillon and Murphy, 1982), which act as an internal load to the normally cycling bridges or actin-binding proteins that may hinder the cross-bridge interaction with actin (e.g., caldesmon; Nagai and Walsh, 1985). Although these explanations are all possible, the exact cause of the slowing during individual length responses remains to be investigated.

Form of Single Smooth Muscle Cell \( F:V \)

As a means of comparing \( F:V \)'s between preparations, as well as \( F:V \)'s from different muscle types, investigators in general have adopted the Hill hyperbola. This choice was initially based upon the assumption that the hyperbolic constants \( a/F_{max} \) and \( b \) related to thermal measurements of contraction (Hill, 1938). This view thus placed a physiological basis on the \( F:V \)'s hyperbolic shape. Although Hill (1970) proved this not to be the case, the hyperbolic form of the \( F:V \) remains a useful standard for comparison.

The \( F:V \)'s from single smooth muscle cells in normal extracellular calcium (1.8 mM) were best fitted by the Hill hyperbola. Using this fit, \( V_{max} \) (0.61 \( L_{cell}/s \)) for single toad stomach smooth muscle cells was calculated at zero force. This value agrees with the \( V_{max} \) determined by the slack test procedure in this laboratory and recently in a preliminary report by Yagi and Fay (1985) in the same preparation. No \( F:V \) data are available for the tissue from which these cells are derived. Given that cells within the toad stomach are not uniformly oriented along the axis of force measurement (unpublished data), mechanical measurements would be difficult to interpret, and thus any comparisons with the single cell data are precluded. However, comparisons with other smooth muscle tissue preparations indicate that the cells' average hyperbolic constants and \( V_{max} \) are in the range of values reported for tissue whose cellular orientations are well defined (Hellstrand and Paul, 1982). Thus, by mathematically averaging individual cell \( F:V \)'s, the resultant cell \( F:V \) is similar to the tissue \( F:V \) that arises from mechanically averaging individual cell \( F:V \)'s.

It is interesting to note that individual cell \( F:V \)'s and slack test results exhibit a range of hyperbolic constants and estimated \( V_{max} \) values (see Results and Table
Before interpreting this finding as the result of true variations in the $F:V$ and $V_{\text{max}}$ values among different cells, possible sources for the observed variability in the data should be addressed. With regard to variations in $F:V$, more data points per experiment are probably needed to minimize variations of fit (Podolin and Ford, 1983). In addition to a greater number of data points, velocities should be obtained at forces <0.80 $F_{\text{max}}$ to avoid possible nonhyperbolic regions of the $F:V$ as reported by Edman et al. (1976) for skeletal muscle. Another concern is that the range of smooth muscle cell $F:V$'s may simply be explained as differences resulting from the isolation procedure. Regardless of the source of variability in the shape of $F:V$ and the $V_{\text{max}}$ values, it should be emphasized that single smooth muscle cells do possess a hyperbolic $F:V$, which until now was only an assumption. However, it is more intriguing to think that different $F:V$'s reflect the possible existence of myosin isozymes, as reported recently in whole smooth muscle (Paganini et al., 1985) and in cultured vascular smooth muscle cells (Owens et al., 1986). The coexistence of fibers having different myosin isozymes with inherently different speeds of shortening has been observed in whole striated muscle preparations (Gauthier et al., 1982).

**Cross-Bridge Cycle Kinetics**

Since the $F:V$ is a basic property of muscle, investigators have attempted to correlate the $F:V$ shape with specific events in the cross-bridge cycle. Huxley (1957) proposed a model of muscle contraction that could account for the dependence of both heat production and shortening velocity on load. From this model, Huxley proposed that rate constants for "side piece" (i.e., cross-bridge) attachment and detachment could be derived from the $F:V$ constants. In a more recent model, Eisenberg et al. (1980) proposed that $V_{\text{max}}$ was correlated with cross-bridge detachment, whereas the shape of the curve between 0.25 $F_{\text{max}}$ and $F_{\text{max}}$ was dependent upon the rate of cross-bridge attachment. Using these models, two aspects of the cross-bridge cycle in smooth muscle, as compared with fast striated muscle, can be predicted: (a) a slower rate of cross-bridge detachment, and (b) a greater percentage of attached cross-bridges. The cross-bridge models proposed by Huxley (1957) and Eisenberg et al. (1980) predict that as the muscle shortens, an attached population of cross-bridges shift to positions relative to the actin-binding site that results in these bridges producing negative force. These cross-bridges would oppose further shortening and thus decrease $V_{\text{max}}$. In smooth muscle, then, it is possible that the detachment of these bridges is slower than in fast skeletal muscle and would retard shortening velocity to a greater extent, accounting for the slower $V_{\text{max}}$. With regard to the percentage of attached cross-bridges, the Huxley (1957) model predicts that multiplying the hyperbolic constants (i.e., $ab$) equals the ratio $g_1/f_1$, where $g_1$ and $f_1$ are the cross-bridge detachment and attachment rates, respectively, in the positional region where myosin cross-bridges will attach to actin. Since the Huxley model consists of only one attached and one detached cross-bridge state, the ratio $f_1/g_1$ [i.e., 1/(ab)] is an estimate of the equilibrium constant between detached and attached states or the relative proportion of attached to detached cross-bridges. Using this as a first approximation, $f_1/g_1$ for smooth muscle cells ranges between 12 and
23, as compared with 12 for fast frog skeletal muscle at 0°C (Hill, 1938). Therefore, smooth muscle cells may have a greater percentage of attached cross-bridges, which is twice that in fast striated muscle during isometric steady state. Evidence to support this prediction can be obtained from biochemical data describing the interaction of actin and myosin in smooth muscle. Krisanda and Murphy (1980) have demonstrated that once smooth muscle myosin binds to skeletal muscle actin, it will remain attached much longer than would skeletal muscle myosin. Since estimates for the numbers of attached cross-bridges in skeletal muscle vary between 20 and 50%, depending on the experimental techniques used (Squire, 1981), there is enough leeway in the number of attached bridges to accommodate the predicted increase in smooth muscle. Although an increased percentage of attached cross-bridges is predicted for smooth muscle owing to differences in the hyperbolic constants between smooth and fast skeletal muscle (Hill, 1938), it is important to note that the value of \( ab \) can vary substantially among fast skeletal muscles (Homsher and Kean, 1978) and thus affect the comparison of \( ab \) values between muscle types presented here. Although variability exists between fast skeletal muscles, it is interesting to note that both slow amphibian (Lannergren, 1978) and tortoise (Woledge, 1968) skeletal muscles have \( a \) and \( b \) values that would also predict increased percentages of attached cross-bridges. It is possible that all slowly contracting muscles have cross-bridge cycle kinetics that result in a greater percentage of attached bridges relative to fast striated muscle.

An increased percentage of attached cross-bridges may help to explain smooth muscle's ability to generate comparable or greater force per cross-sectional area as in striated muscle, with far less of the contractile protein myosin (Cohen and Murphy, 1979). Alternative explanations for smooth muscle myosin's enhanced force-generating capability have been an increased contractile unit length, side-polar myosin filament, and obliquely oriented contractile protein filaments (Fay et al., 1981). However, a major determinant of force generation is the fraction of the cross-bridge cycle that is spent in a force-generating state (duty cycle). Since Huxley's 1957 model, at least one additional attached, non-force-producing cross-bridge state has been proposed (Ford et al., 1977). Therefore, the percentage of the cross-bridge cycle spent in the attached force-producing state would be the more important parameter for comparison between the two muscle types. Warshaw and Fay (1983a, b) have obtained tension transient data from toad stomach smooth muscle cells and concluded that a greater percentage of attached cross-bridges are in the force-producing state in smooth muscle as compared with fast striated muscle. Therefore, although the \( F:V \) data suggest an increased percentage of attached cross-bridges in smooth as compared with fast skeletal muscle, the enhanced force-generating ability of smooth muscle may in part result from a greater percentage of the attached cross-bridges being in a force-producing state.

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**REFERENCES**


