Isometric Force Development, Isotonic Shortening, and Elasticity Measurements from Ca\textsuperscript{2+}-Activated Ventricular Muscle of the Guinea Pig

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ABSTRACT Isometric tension and isotonic shortening were measured at constant levels of calcium activation of varying magnitude in mechanically disrupted EGTA-treated ventricular bundles from guinea pigs. The results were as follows: (a) The effect of creatine phosphate (CP) on peak tension and rate of shortening saturated at a CP concentration > 10 mM; below that level tension was increased and shortening velocity decreased. We interpreted this to mean that CP above 10 mM was sufficient to buffer MgATP intracellularly. (b) The activated bundles exhibited an exponential stress-strain relationship, and the series elastic properties did not vary appreciably with degree of activation or creatine phosphate level. (c) At a muscle length 20% beyond just taut, peak tension increased with Ca\textsuperscript{2+} concentration over the range slightly below 10\textsuperscript{-6} to slightly above 10\textsuperscript{-4} M. (d) By releasing the muscle to different loads and measuring the length at which shortening stopped, muscle length-active tension curves were constructed. Force declined to 20% peak tension with a decrease in muscle length (after the recoil) of only 11% at 10\textsuperscript{-4} M Ca\textsuperscript{2+} and 6% at 4 \times 10\textsuperscript{-6} M Ca\textsuperscript{2+}. (e) The rate of shortening after a release was greater at lower loads. At identical loads (relative to maximum force at a given Ca\textsuperscript{2+} level), velocity at a given time after the release was less at lower Ca\textsuperscript{2+} concentrations; at 10 M\textsuperscript{-3}, velocity was 72% of that at 10\textsuperscript{-4} M, and at 4 \times 10\textsuperscript{-6} M, active shortening was usually delayed and was 40% of the velocity at 10\textsuperscript{-4} M. Thus, under the conditions of these experiments, both velocity and peak tension depend on the level of Ca\textsuperscript{2+} activation over a similar range of Ca\textsuperscript{2+} concentration.

INTRODUCTION

Intracellular free calcium plays a role in the activation and regulation of cardiac muscle contraction (Ebashi, 1974). Model systems have become increasingly useful for studying the effects of Ca\textsuperscript{2+} on force generation under controlled ionic conditions. In the model systems, the plasma membrane properties (and thus the diffusion barrier to small ions) are destroyed by mechanically removing the membrane (i.e., "skinning" a single cell; Fabiato and Fabiato, 1973), by chemical treatment (Winegrad, 1971; Henry et al., 1972), or by a combination of
chemical treatment and mechanical disruption of muscle tissue (Bloom, 1970; Kerrick and Best, 1974) so that the concentration of \( \text{Ca}^{2+} \) and other important ions surrounding the myofilaments may be directly altered and controlled. Isometric tension is generally found to increase with \( \text{Ca}^{2+} \) concentration in the range \( 10^{-6} \text{--} 10^{-4} \text{ M} \) (e.g., Best et al., 1977), the degree of activation depending on the concentrations of other ions (e.g., MgATP\( ^{2-} \) and Mg\( ^{2+} \)) (Fabiato and Fabiato, 1975b; Best et al., 1977).

In addition, cardiac muscle model systems promise to be useful in studying force-velocity relationships at controlled intracellular \( \text{Ca}^{2+} \) levels of varying magnitude. We describe here an initial set of quick-release experiments using mechanically disrupted, EGTA-treated preparations from the guinea pig ventricle. These multicellular fragments have an advantage over single skinned cells in that they develop enough force to overcome the inertia and static friction of the lever in a conventional quick-release system. The experiments yielded information on isometric force development, isotonic shortening, and the elastic properties of the muscle fragments at various levels of \( \text{Ca}^{2+} \). The fragments were activated in the presence of an ATP-regenerating system (creatine phosphate and creatine phosphokinase) in order to overcome the diffusion limitation and consequent depletion of ATP complexes in the bundle core during contraction.

**METHODS**

**Apparatus**

The experimental apparatus, based on the design of Podolsky and Teichholz (1970), is illustrated schematically in Fig. 1. The muscle bundle was gripped at either end by tweezers; one tweezer was attached to a force transducer (left side), the other to a lever which could be released quickly (right side). The characteristics of the force transducer were: linear range, 0.8 g; sensitivity, \( \pm 7.5 \text{ mV/mg} \); resonant frequency, 70 Hz; overall compliance, 0.2 \( \mu \text{m/mg} \), measured at the end of the tweezer. The equivalent mass of the tweezer and lever assembly was 27 mg, measured by analyzing the acceleration of the lever to a step increase in force. The overall compliance of the restrained lever was \( < 0.1 \text{ \mu m/mg} \). The displacement transducer was linear over a range of 2 mm, and had a sensitivity of \( \pm 2 \text{ mV/mm} \).

The solution changer consisted of a series of 1.5-ml wells milled into a plexiglas plate. To transfer the bundle from one solution well to another, the spring loaded plate was pulled down from beneath the bundle, quickly moved until the next well was beneath the tweezers, and then released upwards, immersing the bundle and tweezers in the new solution. Glass slips covered all unused wells to prevent solution evaporation.

Force, length, and velocity were recorded on a Beckman-Offner curvilinear oscillograph (frequency response, 130 Hz center 1 cm of scale; 70 Hz, full scale; Beckman Instruments, Inc., Fullerton, Calif.). Velocity was obtained by electronic differentiation of the displacement signal (time constant, 2 ms). Tension and length were also recorded on a Tektronix 564 storage oscilloscope (Tektronix, Inc., Beaverton, Ore.); photographs of these traces were taken with a Polaroid camera (Polaroid Corp., Cambridge, Mass.).

The length and diameter of the muscle bundles were estimated by using a graticule with a Bausch & Lomb stereomicroscope at \( \times 45 \) (Bausch & Lomb, Scientific Optical Products Div., Rochester, N.Y.). Muscle length was measured from the end of one tweezer to the other. Diameters were measured at the mid-section in air, where the surface tension of water forced irregularly shaped bundles to assume a cylindrical shape.
Solutions

The compositions of the relaxing solution and other buffered Ca\(^{2+}\) solutions are given in Table I. Total concentrations of salts were determined with the aid of a computer by solving a set of reaction equations which relate various ions to their complex products (Godt, 1974). The stability constant for CaEGTA\(^{2-}\) was taken to be \(2.62 \times 10^{10}\) M\(^{-1}\) (Godt, 1974). Other stability constants and association products are given by Godt (1974).

Total concentrations of salts presented in Table I were calculated to achieve the
following concentrations of ions: \([\text{Mg}^{2+}] = 0.5 \text{ mM}, \text{pH} = 7.0, \text{ionic strength} = 0.15 \text{ M}; [\text{Ca}^{2+}], [\text{MgATP}^2^-], \) and creatine phosphate concentrations as indicated. Inasmuch as tension and shortening rates in \(\text{Ca}^{2+}\)-activated muscle preparations are known to depend markedly on the concentrations of the above ions, the concentrations listed were those appropriate for comparing our results with those of other studies. The concentrations of \(\text{Mg}^{2+}, \text{H}^+, \) and total ionic strength given above also approximate free intracellular levels under physiological conditions (Polimeni and Page, 1973; Gordon et al., 1973; Fabiato and Fabiato, 1975a, b). The total concentration of intracellular MgATP²⁻ is 5-6 mM,

### Table I

**Composition of Relaxing and Activating Solutions**

\([[\text{Mg}^{2+}] = 0.5 \text{ mM}]\)

| \(\frac{[\text{Ca}^{2+}]}{[\text{MgATP}^2-]}\) | \(\text{Na}_2\text{ATP}\) | \(\text{CaCl}_2\) | \(\text{MgCl}_2\) | \(\text{Na}_2\text{CP}\) | \(\text{KCl}\) |
| --- | --- | --- | --- | --- | --- |
| 10⁻⁻⁻ | 2 | 15 | - | 2.80 | 2.14 | 54 |
| 10⁻⁻ | 2 | 15 | 3.99 | 2.73 | 2.15 | 62 |
| 4 \(\times\) 10⁻⁻ | 2 | 15 | 5.90 | 2.69 | 2.15 | 60 |
| 10⁻⁻ | 2 | 15 | 6.54 | 2.68 | 2.16 | 58 |
| 10⁻⁻⁻ | 2 | 15 | 7.24 | 2.67 | 2.51 | 56 |
| 2.5 \(\times\) 10⁻⁻ | 2 | 15 | 7.71 | 2.67 | 2.57 | 54 |
| 10⁻⁻ | 2 | 25 | 7.25 | 2.77 | 2.31 | 26 |
| 10⁻⁻ | 2 | 10 | 7.28 | 2.62 | 2.31 | 72 |
| 10⁻⁻ | 2 | 10 | 7.22 | 2.57 | 2.31 | 86 |
| 10⁻⁻ | 2 | 10 | 7.22 | 2.52 | 2.31 | 101 |
| 10⁻⁻ | 5 | 15 | - | 5.83 | 5.36 | 51 |
| 10⁻⁻ | 5 | 15 | 12.41 | 5.69 | 9.57 | 75 |
| 10⁻⁻ | 5 | 0 | 12.21 | 5.55 | 9.57 | 58 |

Total \([\text{EGTA}] = 7 \text{ mM}\).

Solutions were titrated to \(\text{pH} = 7.0\) by adding 0.1 N KOH or HCl, \([\text{H}^+]\) was buffered by 20 mM imidazole. CPK, 0.5 mg/ml, was added to the solutions before each experiment. The CPK activity was 150 U, where 1 U phosphorylates 1 nmol of creatine per minute at 25°C. EGTA, imidazole, \(\text{Na}_2\text{ATP}\), \(\text{MgCl}_2\)-6\(\text{H}_2\text{O}\), \(\text{Na}_2\text{CP}\), and CPK were obtained from Sigma Chemical Co., St. Louis, Mo. KCl, KOH, and KCl (for pH and ionic strength adjustments) were reagent grade (Fisher Scientific Co., Pittsburgh, Pa.). \(\text{CaCl}_2\) was diluted from an analytical concentrate (24.95 ± 0.05 mM; J. T. Baker Chemical Co., Phillipsburg, N. J.). Solution and rinse water was de-ionized (Hydro Service and Supplies, Inc., Durham, N.C.; specific resistance, 18 m\(\Omega\)-cm.)

* Relaxing solution.

assuming that the total intracellular \([\text{ATP}]\) is 24 mol/g dry wt (Hearse et al., 1976), the dry to total weight ratio is 0.178 (Dobson, 1974), the intracellular to extracellular space ratio is 0.19 (Polimeni, 1974) and that most of the ATP is bound to \(\text{Mg}^{2+}\) (Polimeni and Page, 1975). Therefore the concentration of unbound interfilament \(\text{MgATP}^2-\) is probably < 5 mM because much of the intracellular \(\text{Mg}^{2+}\) and \(\text{ATP}^4-\) is located in the mitochondria (which occupy approximately a third of the cell volume [Smith and Page, 1976]). Accordingly, some experiments were carried out at \([\text{MgATP}^2-] = 5 \text{ mM}\) while others were carried out at 2 mM to cover the range of probable \([\text{MgATP}^2-]\) and to compare our data with those of others who used intermediate values (e.g., Kerrick and Donaldson, 1975; Fabiato and Fabiato, 1975a, b; Best et al., 1977). Finally, the total concentration of endogenous creatine phosphate in guinea pig heart is 6-7 mM/kg wet wt (Dobson et al., 1974). Taking into account the extracellular space, the concentration of interfilament
creatine phosphate (CP) is probably > 10 mM (cf. also Feinstein, 1962). A range of 0–25 mM was used to determine an optimal level of CP under the conditions of these experiments.

ATP and CP were added fresh each time solutions were made. Other stock solutions were kept in a refrigerator between experiments. CP and creatine phosphokinase (CPK) were stored in a desiccator in the freezer compartment. Final solutions were divided into 5–10-ml aliquots and kept frozen (at −30°C) in plastic or glass jars between uses. Stocks were analyzed for Ca²⁺ and Mg²⁺ contamination with an atomic absorption spectrophotometer (model 303, Perkin Elmer Corp., Norwalk, Conn.).

All experiments were carried out at room temperature (20.2–23.5°C). During each experiment, temperature varied < 0.5°C.

Preparation

Guinea pigs (ca. 0.8 kg) were sacrificed by a blow to the head, and a small piece of tissue, cut from the apex of the left ventricle, was blotted and fragmented by grinding the tissue in EGTA-buffered relaxing solution. This disruption in EGTA destroys the membrane properties so that ions in the bathing solution are allowed to equilibrate freely with the intracellular fluid (Winegrad, 1971; Kerrick and Best, 1974; Best et al., 1977). Fragments of tissue used in these experiments were ribbon-like or cylindrical, 0.07–0.2 mm in diameter, and 0.8–2.0 mm in length mounted (Fig. 2a). Microscopic inspection shows that the longitudinal integrity of the muscle was little affected (Fig. 2b). The appearance of individual sarcomeres in electron micrographs (unpublished) does not differ significantly from those in nondisrupted guinea pig ventricular tissue (Hearse et al., 1976). The cellular membranes and mitochondria, however, are disrupted extensively.

At the beginning of each of the following experiments, the muscle bundle (which had been mounted slack) was pulled until just taut (L₀, i.e., the point at which an increase in tension was first noticed) and then stretched an additional 20% (to 1.2L₀) to compensate for internal shortening during activation (cf. Discussion). Quickly stretching the muscle to 1.2L₀ produced an abrupt rise and then an exponential decline in resting tension. After a 1/2–1-min period of stress relaxation, the final tension level was < 5% of the maximal active force which the muscle was capable of producing.

If no appreciable stress is borne by the relaxed contractile elements after the stress relaxation at 1.2L₀, the external force borne by the elastic elements in parallel with the contractile elements constitutes no more than 5% of the total force. For lengths below 1.2L₀ (including all those lengths to which the muscles were released), the percentage is even smaller in that passive force drops with muscle length. Therefore, in the range of loads above 5% of the maximal active force, the effect on force-velocity relations of elastic elements in parallel with the contractile elements was neglected.

From light micrographs taken in a preliminary set of experiments at L₀, 10 consecutive sarcomeres were measured in at least five regions of the central two-thirds of each bundle. The variation of sarcomere lengths in each bundle was typically 0.11 µm (SD) around the mean. The (grand) mean sarcomere length, taken from the means of eight guinea pig ventricular bundles held just taut at rest, was 2.19 ± 0.09 µm (SD). Stretching each bundle by 20% produced an 8–10% lengthening of the mean sarcomere spacing.

As above, all statistics in the text will be given as mean ± standard deviation. Student's t test (for paired or unpaired variables) was used in the data analysis.

Experimental Protocol

At 1.2L₀, the muscle was first transferred from relaxing solution to a series of solutions containing higher Ca²⁺ concentrations (Table I), and then returned to relaxing solution.
In each solution, force was allowed to reach a plateau (signaling the equilibration of Ca\(^{2+}\) within the muscle). At each [Ca\(^{2+}\)], maximal (plateau) force \(F_p\), was measured with respect to the passive resting force (dotted line, Fig. 3a). An example of a record obtained during the course of solution changes is given in Fig. 4c.

During subsequent activations, the muscles were quickly released at the peak of activation to constant loads, \(P\), of varying magnitude (Fig. 3A-C). The muscle actively shortened after an initial rapid recoil. The bundle was then again quickly transferred back to the relaxing solution and reextended to its initial length, and the procedure was repeated with a new afterload. Muscle velocity was measured 80 ms after the onset of the release after the length oscillations had nearly or completely abated (cf. Fig. 10A inset for oscilloscope trace of initial shortening time-course).

To reduce the number of relaxation/activation/relaxation cycles required for a given set of force-velocity relations, we explored the possibility of repeatedly releasing a muscle to various loads while the muscle was continuously activated (re-extending the muscle before each new release). Multiple releases and re-extensions during the peak of

**Figure 2.** Mechanically disrupted guinea pig ventricular bundles bathed in relaxing solution ([Ca\(^{2+}\) = 10^{-8} M). (a) Muscle bundle inserted into tweezers in the experimental chamber. Scale: 0.2 mm. (b) High-power photograph of bundle, pretreated with 0.5% wt/wt Brij-58 (polyoxyethylene 20 cetyl ether, Sigma Chemical Co.) for 30 min to improve the clarity of the sarcomere patterns. The increased clarity results from the breakdown of membranous light-scattering elements such as mitochondria. Scale: 40 \(\mu\)m.
activation were found to have little or no deleterious effects on either subsequent tension redevelopment or shortening (Fig. 4A–C). Consequently, we used the multiple release method, in addition to the single release method, to collect the following force-velocity data.

Peak tension and the shortening time-course did not depend on the particular protocol of solution changes used to activate the muscle. Peak tension and shortening rates from muscles directly switched to 10^{-4} M Ca^{2+}-activating solutions (Fig. 4B) differed by no more than 5% from those obtained from muscles which were first activated to intermediate Ca^{2+} levels (Fig. 4C).

**Figure 3.** Maximal isometric tension development and isotonic shortening after a quick release to different afterloads in guinea pig ventricular bundles. In each frame the timing marks are indicated above the record; the top trace is muscle length; the middle trace, force; and the bottom trace, velocity. Muscle shortening and tension decreases are downward deflections. The bundle, initially stretched by 20% (not shown) in relaxing solution, was transferred from the relaxing solution to [Ca^{2+}] = 10^{-8} M solution, and then (after a period of equilibration) switched to a 10^{-4} M Ca^{2+} solution. After the tension reached P_o, the muscle was quickly released and allowed to shorten against a load of \( P = 0.75 P_o(A) \), 0.42 \( P_o(B) \), or 0.27 \( P_o(C) \). After each period of shortening, the muscle was quickly transferred back to the relaxing solution and restretched to 1.2\( L_o \). Bundle no. 10/5/76B: 1.2\( L_o \) = 0.81 mm; diameter = 0.10 mm. Temperature = 20.2°C.

**Results**

**Effect of [Creatine Phosphate], [Creatine Phosphokinase], and [MgATP^{2-}] on Isometric and Isotonic Responses**

The effect of the concentration of creatine phosphate on maximal isometric tension and isotonic shortening was studied in solutions containing 0–25 mM CP. In 2 mM MgATP^{2-}-activating solutions containing no CP, muscle tension was considerably greater and velocity (at a given load) less than at 15 mM CP (Fig. 5). Moreover, the stiffness of the muscle was greater in CP-free solution, indicated by the increased amplitude of the rapid rise in tension upon re-extending the bundle (compare Fig. 5A and B). When the muscle was re-activated at 15 mM (Fig. 5C), there was some recovery of the ability of the
muscle to shorten rapidly, but tension was depressed. Muscle stiffness remained high, even after prolonged (2 h) soaking in 15 mM CP relaxing solution. In solutions containing 10–25 mM CP, the bundles could be repeatedly and reversibly activated without appreciably altering the mechanical characteristics of the muscle (Fig. 7 and 10 C).

Mean isometric tension and velocity (at $P/P_o = 0.2$), expressed as percentages of their values at CP = 15 mM, are plotted as a function of CP concentration at 2 mM MgATP$^{2-}$ (Fig. 6). As CP concentration is increased from 0–10 mM, tension decreases and velocity increases; above 10 mM CP, the effect of CP on force and velocity is comparatively small, although there is a significant depression of velocity at 25 mM.
Elevating the [MgATP$^{2-}$] from 2 to 5 mM had no significant affect on either force or velocity at 15 mM CP (at 2 mM MgATP$^{2-}$, $P_o = 0.57 \pm 0.20$ kg/cm$^2$, $V(P/P_o = 0.2) = 0.40 \pm 0.11$ muscle lengths/s; at 5 mM MgATP$^{2-}$, $P_o = 0.55 \pm 0.18$ kg/cm$^2$, $V = 0.45 \pm 0.18$ muscle lengths/s; $P > 0.5$, paired $t$ test of results from four bundles). However, at 0 mM CP, values obtained at 5 mM MgATP$^{2-}$ showed that force was not nearly so elevated or velocity so depressed as those values obtained at 2 mM MgATP$^{2-}$, although the effect of CP on force and velocity was still significant (at 0 mM CP, $P_o = 0.54 \pm 0.13$ kg/cm$^2$, $V = 0.37 \pm 0.13$ muscle lengths/s).

**Figure 5.** Force development and shortening at two levels of creatine phosphate in a guinea pig ventricular bundle. The bundle is activated at 10$^{-4}$ M Ca$^{2+}$ with creatine phosphate concentration 15 mM (A), 0 mM (B), and 15 mM (C). $P/P_o$ and velocity measurements were taken 80 ms after onset of quick release. In (C), the gain was increased 21/2 times in the force record (at heavy arrow) during the development of tension; final calibration given at left. Bundle no. 8/11/76B; 1.2$L_o$ = 1.33 mm; diameter = 0.07 mm. Temperature, 23.5°C.
0.11 muscle lengths/s; at 15 mM CP, \( P_o = 0.47 \pm 0.13 \text{ kg/cm}^2, V = 0.45 \pm 0.13 \) muscle lengths/s; \( P < 0.1 \), paired t test of results from five bundles at 5 mM MgATP²⁻.

Varying the CPK concentration between 0.1 and 1.0 mg/ml (CP 15 mM, MgATP²⁻ 2 mM) had little or no affect on muscle tension, velocity, or stiffness. Therefore, we used 0.5 mg/ml CPK at 15 mM CP and 2 mM MgATP²⁻ as a standard operating level in the remaining experiments.

**Effect of \([\text{Ca}^{2+}]\) on Developed Force**

Developed force \( P_o \) increased with \([\text{Ca}^{2+}]\) over a range slightly below \( 10^{-6} \) M to slightly above \( 10^{-4} \) M (Table II). For purposes of comparison, the force developed at \( 10^{-4} \) M \( \text{Ca}^{2+} \) (nearly sufficient for maximal activation) was defined as 100% in each bundle. In this set of experiments mean absolute force at \( 10^{-4} \) M \( \text{Ca}^{2+} \) was \( 0.83 \pm 0.24 \text{ kg/cm}^2 \), comparable to that obtained from maximally activated single cardiac cells (Fabiato and Fabiato, 1975a). (At \( L_o \), peak developed force at \( 10^{-4} \) M \( \text{Ca}^{2+} \) was about 80% of that at \( 1.2L_o \)).

![Figure 6](http://rupress.org/jgp/article-pdf/71/4/431/1246860/431.pdf)

**CREATINE PHOSPHATE CONCENTRATION**

Figure 6. Effect of creatine phosphate concentration on isometric tension and isotonic shortening rate. Bundles activated by \( 10^{-4} \) M \( \text{Ca}^{2+} \). Mean isometric tension (○) and velocity at \( P/P_o = 0.2 \) (△) expressed as percentages (±SD) of their values at 15 mM creatine phosphate. Measurements at other [CP] were taken immediately after, and compared to, measurements at 15 mm CP.

For a typical bundle activated at \( 10^{-4} \) M \( \text{Ca}^{2+} \), shortening velocity characteristic increased as the load was decreased (Fig. 7). The order in which the loads were presented had an insignificant effect on the force-velocity relationship.

Comparable force-velocity relationships were obtained in most bundles. At a given load, however, absolute shortening rates varied appreciably from bundle to bundle. For example, at \( P/P_o = 0.21 \pm 0.03 \), the variation in velocity in 19 bundles was ± 0.11 muscle lengths/s (SD), about a third of the mean of 0.32 muscle lengths/s.
Effect of $[Ca^{2+}]$ on Isometric Force and Isotonic Velocity

AT $P/P_o = 0.2$ RELATIVE TO THE FORCE AND VELOCITY DEVELOPED AT $10^{-4}$ M $Ca^{2+}$ IN GUINEA PIG VENTRICULAR BUNDLES

| $[Ca^{2+}]$, M | Force ($P_o$), % | Velocity, % |
|---------------|----------------|-------------|
| $2.5 \times 10^{-4}$ | 106 ± 3 | 105 ± 2* |
| $10^{-4}$     | 87 ± 6 | 72 ± 7* |
| $10^{-5}$     | 64 ± 8 | 40 ± 11‡ |
| $4 \times 10^{-6}$ | 5 ± 5 | — |

$n = 5, P < 0.01$  $n = 12, P < 0.01$  $n = 9, P < 0.01$  $n = 12, P < 0.01$

Force and velocity values (means ± standard deviations) are expressed as percentages of their maximal values at $[Ca^{2+}] = 10^{-4}$ M. No velocity measurements were made at $10^{-4}$ M $Ca^{2+}$, in that muscle forces were generally insufficient to overcome the inertial forces of the lever in the quick release. Absolute mean force and velocity values at $[Ca^{2+}] = 10^{-4}$ M are 0.85 ± 0.24 kg/cm² of bundle cross-section and 0.30 ± 0.11 muscle lengths/s in 12 bundles from eight hearts. Paired $t$ tests were used to determine the above $P$ values, which show significant differences from the values in $[Ca^{2+}] = 10^{-4}$ M solution in every case. For the velocity measurements, $P/P_o = 0.21 ± 0.03$, where $P_o$ refers to the force developed at the specific $[Ca^{2+}]$ studied. Temperature, 22 ± 1°C; $[Mg^{2+}] = 0.5$ mM; $[MgATP^2-] = 2$ mM; $[CPK] = 15$ mM; $[CPK] = 0.5$ mg/ml.

* Taken at 80 ms after onset on release.
‡ Peak shortening rate after period of no shortening (Fig. 8C).

**Figure 7.** Force-velocity relationship in $Ca^{2+}$-activated guinea pig ventricular bundle. (O) Single releases, ($\Delta$, $\triangle$) multiple releases; pointing right, in order of ascending loads; pointing left, descending loads. ($\triangle$) Releases carried out in 15 mM creatine phosphate activating solution ($[Ca^{2+}] = 10^{-4}$ M); ($\Delta$) in 25 mM creatine phosphate-activating solution. Same bundle as in Fig. 5.

Effect of $[Ca^{2+}]$ on Rate of Shortening

Quick releases also were carried out at $2.5 \times 10^{-4}$, $10^{-5}$, and $4 \times 10^{-6}$ M $Ca^{2+}$ where $P_o$ was approximately 1.06, 0.9, and 0.6 of that in muscle activated at $10^{-4}$ M.
At identical relative loads, the rate of shortening at a given time after the release was less at 10^{-5} M Ca^{2+} (Fig. 8B) than at 10^{-4} M (Fig. 8A). In a study of 12 bundles (6 hearts) in which velocities were compared at 80 ms after a release to \( P/P_0 = 0.2 \), the velocity at 10^{-5} M Ca^{2+} was 28% less than that at 10^{-4} M (Table II). At 2.5 \times 10^{-4} M Ca^{2+}, velocity was slightly greater (5%) than that at 10^{-4} M (Table II).

At 4 \times 10^{-6} M Ca^{2+}, there was generally a period of little or no shortening after the elastic recoil (Fig. 8C). A delayed shortening phase then developed during which the muscle achieved a peak velocity which was always a fraction of that recorded at the higher Ca^{2+} concentrations for comparable relative loads (Table II). Occasionally there was a rapid shortening phase of brief duration and small amplitude which preceded the events described above. This latter phenomenon has not been systematically studied at this time.

**Effect of \([Ca^{2+}]\) on the Active Length-Tension Relationship**

The quick release experiments described above also provide information about the relationship between muscle force and length at varying levels of activation. If, at each Ca^{2+} concentration, the muscle is allowed to shorten maximally against a specific load, the point at which the muscle ceases to shorten is that point on the length-tension relationship where the internal force developed by the muscle equals the external load. Therefore, by releasing the muscle to different loads and measuring the muscle length at which shortening stops, muscle length-tension curves for varying Ca^{2+} concentrations can be constructed.
An example of this is seen in Fig. 3 and 9. At high loads, shortening ceases within a few seconds after the release (Fig. 3 A). At low loads, shortening is generally more prolonged (Fig. 3 C). Muscle force (expressed as a fraction of the total force developed at 1.2L₀; i.e., \( P/P₀ \)), plotted as a function of muscle displacement (expressed as a fraction of the total muscle length at 1.2L₀) indicates that force decreases rather abruptly with length, falling to 0.2 \( P₀ \) with a drop in muscle length of only 11% (Fig. 9). At \( 4 \times 10^{-6} \) M Ca²⁺, the decline is even more abrupt, falling to 0.2 \( P \) with a drop in length of 6%.

![Figure 9](image_url)

**Figure 9.** Effect of \([\text{Ca}^{2+}]\) on the active length-tension relationship. \([\text{Ca}^{2+}] = 10^{-4} \) M (○), \( 4 \times 10^{-6} \) M (●). The variables used to construct the plots are defined in the text, where \( \Delta l₁ \) is taken at the point where shortening ceases. 1.2L₀ = 1.61 mm; diameter = 0.10 mm. Temperature, 23.5°C.

**Series Elasticity Measurements**

The elasticity of the elements in series with the contractile apparatus was evaluated by the quick release technique of Jewell and Wilkie (1958). The extent of shortening during the recoil was estimated by extrapolating the active shortening phase through the length oscillations to the rapid shortening phase and the muscle displacement \( \Delta l₁ \) measured at the point where these two lines intersect (inset Fig. 10 A). (Contractile element shortening during the 5-10 ms long recoil was estimated to be < 0.5% of the initial muscle length.) The load \( P \) was estimated by extrapolating through the initial force oscillations to the rapid fall in tension (see inset); \( P \) was measured at that point with respect to \( P₀ \).

Relative stress, \( P/P₀ \), is plotted for a single bundle as a function of relative
strain, \( \Delta l / 1.2L_o \) (Fig. 10 A–C). The stress-strain relationships are fit satisfactorily over the range \( P/P_o = 0.2-1.0 \) with an exponential function of the type
\[
P/P_o = a \exp \left( \frac{\Delta l / 1.2L_o}{b} \right) + c,
\]
where \( a, b, \) and \( c \) are constants given in the legend of Fig. 10.

The derivative of muscle force with respect to length in the above expression is a measure of the stiffness of the preparation and can be written as
\[
\frac{dP}{dL} = kP + C,
\]
where \( L = \Delta l \), \( k = -1/1.2L_o b \) and \( C = P_{o}c/1.2L_o b \).
In 14 bundles activated at $10^{-4}$ M Ca$^{2+}$, \( k = -25.35 \pm 9.41 \text{ mm}^{-1} \), and \( C = 0.193 \pm 0.196 \text{ g/mm} \). Mean cross-sectional area was \( 0.0091 \pm 0.0046 \text{ mm}^2 \); \( 1.2L_0 \) was \( 1.25 \pm 0.28 \text{ mm} \). A paired comparison of six bundles did not reveal any significant difference in the stiffness constant \( k \) and \( C \) between maximal or submaximal activation (at \( 10^{-4} \text{ M Ca}^{2+} \), \( k = -24.29 \pm 6.41 \text{ mm}^{-1} \), \( C = 0.108 \pm 0.087 \text{ g/mm} \); at \( 4 \times 10^{-4} \text{ M Ca}^{2+} \), \( k = -28.42 \pm 9.13 \text{ mm}^{-1} \), \( C = 0.072 \pm 0.05 \text{ g/mm} \), \( P > 0.05 \)). The series elastic properties also appear to be indifferent to varying creatine phosphate levels (Fig. 10C).

**DISCUSSION**

**Bundle Condition and the Role of an ATP Regenerating System**

Part of the resiliency of the preparation to repeated Ca$^{2+}$ activations, quick releases, and stretches may be due to collagenous supporting structures which, in spite of the disruption procedure, remain intact. Another factor may be that the creatine phosphate/creatine phosphokinase ATP regenerating system used in the bathing solutions prevents deleterious rigor bonds from forming during contraction.

Godt (1974) showed that, at room temperature, an ATP regenerating system (creatine phosphate/creatine phosphokinase) was necessary for maintaining intracellular MgATP$^{2-}$ levels during Ca$^{2+}$ activation of skinned skeletal muscle cells of the frog. In the absence of an ATP-regenerating system, the rate of splitting of ATP during contraction in the core was greater than the rate at which ATP was replenished by diffusion of ATP complexes from the bathing medium surrounding the skinned cell, thus depleting the core of ATP. Under low MgATP$^{2-}$ conditions (less than 0.1 mM) stiff rigor bonds form between the myofilaments (Bremel and Weber, 1972) which alter the mechanical properties of the activated preparation (Godt, 1974; Best et al., 1977).

In the experiments reported here at low creatine phosphate concentrations (0–5 mM; 2 mM MgATP$^{2-}$), muscle tension and stiffness were greater and muscle velocity was less than at higher CP concentrations. These differences diminished at 5 mM MgATP. These results are consistent with the idea that stiff rigor complexes form in the core as a result of MgATP$^{2-}$ depletion (Godt, 1974). Increasing CP concentration between 10 and 25 mM had little effect on force, velocity, or stiffness, suggesting that 10 mM is sufficient to offset the limited diffusion of ATP complexes from the bath into the muscle core. At 15 mM CP probably few, if any, rigor links are present since elevating the [MgATP$^{2-}$] from 2 to 5 mM had no significant effect on the results.

**Muscle Compliance**

Stretching the (just taut) bundles by 20% increased the mean sarcomere spacing by \( \approx 10\% \) (cf. Methods), indicating that there is appreciable compliance in series with the sarcomeres. Most of this compliance is thought to be located in the damaged ends where the tweezers are directly attached to the tissue (Kreuger and Pollack, 1975). Cellular branching (Muir, 1965) and shearing through the Z-lines and intercalated disks (Abbott and Gordon, 1975) may also contribute to the compliance. This summed compliance allows an obligatory amount of
internal shortening to occur during the development of isometric tension preceding the final measurements of force and velocity. The degree to which the sarcomeres shorten depends on the stress-strain characteristics of the series elastic elements and the amount of force which the sarcomeres develop at a given length and Ca\(^{2+}\) activation.

The isolated attached muscle exhibited an exponential stress-strain relationship (Fig. 10), where the muscle stiffness increased with applied (= developed) force. The reciprocal of the stiffness constant \(k\) in Eq. 2 (a measure of the series elastic compliance) was \(3.15 \pm 0.09\%\) of \(1.2L_o\). This value is 0.8–1.7 times that for isolated papillary muscle (cf. Table I of McLaughlin and Sonnenblick, 1974). This wide range of compliance values can probably be accounted for by differences in the extent of damaged tissue at the ends and differences in the extrapolation or measurement procedures (McLaughlin and Sonnenblick, 1974).

Unfortunately, these experiments do not enable us to separate the component of muscle end compliance from that of intercellular connections or even the cross-bridges themselves. It is apparent, however, that the compliance of the cross-bridges is comparatively low since the series elasticity has little or no dependency on either [Ca\(^{2+}\)] (Fig. 10 B) or creatine phosphate level (Fig. 10 C), factors which would be expected to have an effect on compliance through their influence on the number of activated cross-bridges.

Calcium-Tension and Length-Tension Relationships

The dependency of maximal tension on [Ca\(^{2+}\)] was similar to that of other cardiac preparations which are also directly activated by EGTA-buffered Ca\(^{2+}\) solutions. Activation was primarily in the range 10\(^{-6}\)–10\(^{-4}\) M Ca\(^{2+}\), similar to the range reported in mechanically disrupted, EGTA-treated rat ventricular preparations at 20°C (Kerrick and Donaldson, 1975; Best et al., 1977). If allowances are made for differences in assumed CaEGTA association constants and ionic composition, similar activation ranges are also reported for EGTA-treated bundles from the ventricle of the cat (Winegrad, 1971) and dog (Salaro et al., 1974), glycerinated rabbit ventricular bundles (Henry et al., 1972) and skinned cells from the rat ventricle (Fabiato and Fabiato, 1975a, b).

Because of the series elasticity, however, the \(P/P_o\) vs. [Ca\(^{2+}\)] relationship is derived under conditions in which the sarcomeres are not held isometrically, even though the muscle is (neglecting equipment compliance). In fact, tension appears to depend on Ca\(^{2+}\) over a narrower range than it would under truly isometric conditions. A simple example will illustrate this point. Assume that at a muscle length of 1.2L\(_o\), the mean sarcomere length is 2.36 \(\mu\)m (Methods). Further assume that the series compliance is stretched by 17% of 1.2L\(_o\) during full “isometric” tension development at 10\(^{-4}\) M Ca\(^{2+}\) (extrapolating by eye the data of Fig. 10 A). Therefore the sarcomere spacing will be 2.36 \(\mu\)m – (0.18 \times 2.36 \(\mu\)m) = 1.94 \(\mu\)m when full tension is evaluated. At lower Ca\(^{2+}\) concentrations the sarcomere spacing will be greater because the series elastic component is not stretched so much. Thus, the \(P/P_o\) vs. [Ca\(^{2+}\)] relationship at 1.2L\(_o\) covers a sarcomere range of 1.94–2.36 \(\mu\)m. If tension declines above 2.25 \(\mu\)m and below 2.05 \(\mu\)m as it does in frog skeletal muscle (Gordon et al., 1966), the upper and lower tension points will be underestimated. If, as other data suggests, tension
declines monotonically with length over the range 2.3–2.0 μm (Julian et al., 1976) and below (Fig. 9), all tension points will be underestimated, lower tension points least. Both cases, however, will cause \(P/P_0\) to appear to depend on Ca\(^{2+}\) over a narrower range than it actually does.

The abrupt fall in developed tension with length reported here (Fig. 9) may be due to opposing internal forces which resist shortening. If so, structures which produce this force may be located in the sarcolemma because the Ca\(^{2+}\)-activated tension declines with length far less abruptly in single cardiac cells which have been stripped of their sarcolemmas (Fabiato and Fabiato, 1976). It may be that structures which resist compression are removed during the stripping process.

Tension decreases with muscle length even more rapidly at lower Ca\(^{2+}\) levels than at higher levels (Fig. 9). In interpreting this effect, we found it helpful to express tension as a function of estimated sarcomere length (Fig. 11). Using the method of estimating sarcomere spacing presented earlier, the muscle before release will be at a mean sarcomere length of 1.95 μm at \(10^{-4}\) M Ca\(^{2+}\). At \(4 \times 10^{-6}\) M Ca\(^{2+}\), the series elastic elements will be less stretched and consequently the sarcomere spacing will be > 1.95 μm (we estimate 2.08 μm, extrapolating by eye the data of Fig. 10 B). Using these estimates of the mean sarcomere spacing at the time of release, the data of Fig. 9 is replotted in Fig. 11, where tension is expressed as a fraction of \(P_o\) at \(10^{-4}\) M Ca\(^{2+}\) and length as final sarcomere spacing.

At least two mechanisms could produce the difference between the two curves. One possibility is that at a given sarcomere length, the restoring force which is presumed to have a fixed value may have a relatively greater effect on tension at lower levels of activation where less active tension is developed. For example, at 1.95 μm, a restoring force of 0.1 \(P_o\) at \(10^{-4}\) M Ca\(^{2+}\) would diminish active tension by 10%, whereas at \(4 \times 10^{-6}\) M Ca\(^{2+}\) (identical sarcomere length), the restoring force would effectively eliminate the active force. Note that in both cases the rate of tension decline (as a function of change in sarcomere length) is similar, a result which is consistent with the above explanation. Given the data of Fig. 11, however, one cannot rule out the possibility that the sensitivity of the Ca\(^{2+}\)-binding troponin-tropomyosin regulatory system is altered by sarcomere length. In fact, there is other evidence that the activation process (and hence muscle force) depends on resting sarcomere length (Nilsson, 1972; Parmely and Chuck, 1973; Julian et al., 1976; Fabiato and Fabiato, 1976; Lakatta and Jewell, 1977), although most of these reports favor a length dependence mediated at the level of the cellular or sarcoplasmic reticular membranes.

**Force-Velocity Relationship and Dependency on [Ca\(^{2+}\)]**

The rate of isotonic shortening at maximal activation and \(P/P_o = 0.2\) was 0.30 ± 0.11 muscle lengths/s, measured when the length oscillations had abated at 80 ms after the release. The measurements were probably carried out on the ascending limb of the muscle length-velocity relation (Krueger and Pollack, 1975; Pollack and Krueger, 1976) indicated by the continuous decrease in active shortening rate immediately after the release (cf. Fig. 3). Thus, our velocity values are less than they would be had initial internal shortening been prevented.
during isometric tension development, and the measurements carried out at longer initial sarcomere lengths immediately following the release. (The standard deviation in velocity measurements, about a third of the mean, is probably due in large measure to variations in initial sarcomere length.) It should be noted that velocity per active muscle length is greater than velocity per total muscle length, inasmuch as the clamped ends consist of some damaged cells that probably do not shorten (up to 8.5% of the muscle length at each end).

The Ca\(^{2+}\)-dependent differences in shortening rates given in Table II cannot be accounted for by the fact that sarcomeres were longer at submaximal [Ca\(^{2+}\)] than at maximal Ca\(^{2+}\) levels. In all cases muscles were probably released at or below ca. 2.3 \(\mu\)m, i.e., sarcomere lengths where velocity remains constant or drops with length (as mentioned above). Thus, the velocity differences given in Table II are probably underestimates of the differences which would have occurred had the releases been carried out at the same sarcomere length.

Edman (1975) and Bodem and Sonnenblick (1974) showed that length perturbations produce deactivation of contraction which diminishes subsequent tension redevelopment and rate of shortening. Deactivation is particularly evident at low loads where the perturbations are greatest. Bodem and Sonnenblick (1974) showed, however, that both tetanization (maximal activation) and lowered temperatures (to 22\(^{\circ}\)C) eliminated or substantially reduced deactivation in twitched (submaximally activated) rabbit papillary muscle. Thus, we expected little or no deactivation in the experiments of Fig. 7, but guinea pig ventricular

![Figure 11](image-url)

**Figure 11.** Active tension as a function of estimated mean sarcomere length at 10\(^{-4}\) M Ca\(^{2+}\) (○) and 4 \(\times\) 10\(^{-6}\) M Ca\(^{2+}\) (●). Data are taken from Fig. 9. See text for explanation.
muscle may differ from rabbit papillary muscle in this respect. It is possible, therefore, that deactivation at low loads could be present, an effect which would be even more evident in submaximally activated preparations, as it is in untetanized muscle (Bodem and Sonnenblick, 1974). The delayed shortening at $4 \times 10^{-6}$ M Ca$^{2+}$ may in fact reflect an underlying reactivation time-course (Fig. 8C).

Recently, De Clerck et al. (1977) studied contraction in single rat ventricular cells activated by Ca$^{2+}$ released by iontophoresis in an EGTA-buffered solution. Although the Ca$^{2+}$ concentration could not be directly measured or controlled, the sensitivity to Ca$^{2+}$ of the mechanical variables could be demonstrated. Peak force, shortening, and velocity of shortening were found to decrease with a decline in the amount of Ca$^{2+}$ ejected from the microelectrode near the cell. Our results, given in Table II and Fig. 9., generally agree with theirs; i.e., at submaximal activation, the preparations develop less force, shorten less, and shorten less rapidly than at maximal activation.

To account for the analogous dependence of velocity on Ca$^{2+}$ in skeletal muscle, Julian (1971) and others (Wise et al., 1971) propose that Ca$^{2+}$ may have a direct affection on myosin cross-bridge kinetics. Alternatively, Thames et al. (1974) suggest that, under certain intracellular conditions which depend on ionic strength, some "abnormal" cross-bridges linking myosin to actin (present even in the absence of Ca$^{2+}$) retard the sliding of filaments produced by "normally" cycling (Ca$^{2+}$-activated) cross-bridges, thereby reducing the contraction velocity. Differences in velocity at different Ca$^{2+}$ levels could thus arise because, at submaximal levels of activation, i.e., at low Ca$^{2+}$ levels, there are relatively fewer normally cycling cross-bridges. A similar argument holds for the restoring forces mentioned earlier in that a given force may have a relatively greater effect on velocity at lower Ca$^{2+}$ levels where fewer "normal" cross-bridges are cycling. In general, however, the internal load due to abnormal cross-bridges or restoring forces would have to be large ($0.4-0.8 P/P_o$) in order to account for differences in velocity between [Ca$^{2+}$] = $4 \times 10^{-6}$ and $10^{-4}$ M in our preparation.

At present it is not clear which, if any, of these mechanisms account for the Ca$^{2+}$ effect on velocity of muscle shortening. The physiological significance is clear, however, in that the concentration range over which we found velocity to depend on intracellular Ca$^{2+}$ is, based on the tension data, that range over which intracellular Ca$^{2+}$ levels are likely to change during activation and contraction of the heart.

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