A Gap Isolation Method to Investigate Electrical and Mechanical Properties of Fully Contracting Skeletal Muscle Fibers

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ABSTRACT We describe here a single-gap isolation method that allows the simultaneous measurement of electrical activity and tension output from fully contracting segments of frog skeletal muscle fibers. By using single pulses and pulse trains of varying frequency (5–100 Hz), records obtained for both electrical and mechanical fiber response demonstrate that the physiological properties of the fiber segments have been preserved. Action potentials could be recorded free of movement artifacts, even while segments were in fused tetani and developing maximum tensions of more than 600 kN/m^2. Single current pulses evoked action potentials that averaged 144 ± 16 mV (mean ± SD, n = 8) in amplitude and twitches that averaged 285 ± 66 kN/m^2 and 55 ± 5 ms (mean ± SD, n = 20) in magnitude and time to peak, respectively. Trains of action potentials elicited patterns of tension development that exhibited summation, unfused tetani, and fused tetani in a frequency-dependent manner. The AC and DC electrical properties of the single grease gap were modeled with a simple Thévenin equivalent circuit, which satisfactorily predicted the experimental results. Our methodology is easily implemented and potentially applicable to any muscle preparation in which fiber segments with an intact end attached to a piece of tendon can be dissected.

INTRODUCTION

The mechanical output of fully contracting single skeletal muscle fibers has thus far been accurately measured in tendon-to-tendon preparations (Lüttgau, 1965; Grabowsky et al., 1972; Vergara and Rapoport, 1974; Vergara et al., 1977; Nassar-Gentina et al., 1976; Lännergren and Westerblad, 1986). The use of intracellular microelectrodes to study the cause-effect relationship between the electrical activation (seen as an action potential, AP) and the mechanical response in these preparations is hampered by technical problems such as fiber injury due to impaling or contraction, microelectrode dislodgment during mechanical activation, and microelectrode alteration of the mechanical response (Stefani and Schmidt, 1972; Oz and Frank, 1994). Thus, concurrent measurements of electrical and mechanical behavior under physiological conditions have been achieved with only marginal success. Indeed, analyses of electrical properties in intact muscle fibers have been restricted primarily to highly stretched, mechanically paralyzed, or resting fibers (Fatt and Katz, 1951; Hodgkin and Horowicz, 1957; Gordon and Godt, 1970; Hanson and Persson, 1971; Stefani and Schmidt, 1972; Lännergren and North, 1973; Oz and Frank, 1994).

Alternative methodologies circumvent some of the constraints imposed by the use of standard intracellular microelectrodes, but each has its own limitations for physiological investigation. For example, it has been demonstrated that movable (Vaughan Williams, 1959) or flexible (Howell and Snowdowne, 1981) microelectrodes can be used to record single APs during twitch responses. These techniques, however, record the electrical behavior of single muscle fibers during tetanic stimulation less reliably. Furthermore, transmembrane potential can be measured in segments of fibers mounted in gap isolation chambers (Nakajima and Bastian, 1974; Hille and Campbell, 1976; Caille et al., 1978; Kovacs and Schneider, 1978; Vergara et al., 1991; Sanchez and Vergara, 1994), but until now, these methods have been used primarily with mechanically arrested fibers. Attempts to measure tension development in combination with gap isolation techniques have failed to report tension measurements beyond a fraction of those recorded in intact fibers (Bastian and Nakajima, 1974; Nakajima and Bastian, 1974; Caille et al., 1978; Duval and Leoty, 1978; Potreau and Raymond, 1980).

The aim of this paper is to present a straightforward, practical technique to simultaneously record single APs (or trains of APs at tetanic frequencies) and the resulting isometric tension generated in fully contracting fibers. Our methodology exploits the advantages of gap isolation for electrical recording while preserving the fibers' in vivo mechanical properties.

MATERIALS AND METHODS

Dissection and mounting of fiber segments in the single-gap chamber

Single muscle fiber segments, with an intact end attached to a piece of the tendon, were dissected free in Ringer's solution (in mM: 114 NaCl, 2.5 KCl, 10 3-(*N*-morpholino)propanesulfonic acid-Na (MOPS-Na), 1.8 $CaCl_2$, 10 dextrose; pH 7.0) from the dorsal head of the semitendinosus muscle of *Rana catesbeiana*.

Segments of fiber 80–120 μm in diameter and 10–20 mm in length were transferred to a "high-K^+" depolarizing solution (in mM: 80 K_2SO_4,

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10 MOPS-K), which induced a contracture. After spontaneous relaxation, the tendon was gripped with an aluminum foil clip. This clip was used to guide the transfer of the muscle fiber segment to a single partition experimental chamber (Fig. 1 *A*) while under the solution. The chamber was prepared by flooding its pools with the high-K^+ solution and laying down a 2-mm-wide grease preseal (Glisseal; Borer Chemie, Zuchwil, Switzerland) on the groove of the chamber's partition (Fig. 1 *A*). The aluminum clip at one end of the fiber was fixed to the tension transducer's hook, the fiber was extended across a groove in the chamber's partition, and the cut end was secured to the bottom of pool 2 with stainless steel pins. Typically,

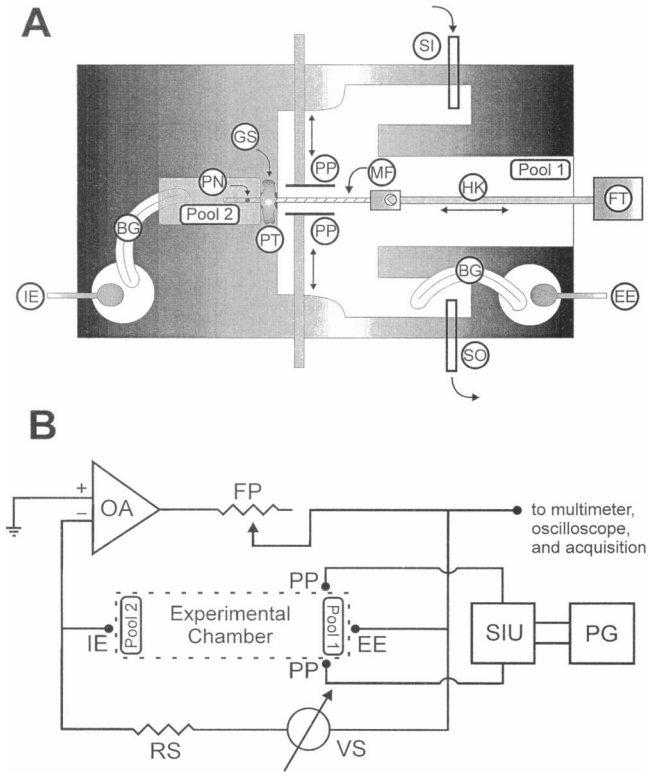

FIGURE 1 (*A*) Schematic diagram of experimental chamber. Two pools (pool 1 and pool 2), separated by a 2-mm-wide partition, were milled in a plexiglass block (5 × 3 × 0.5 cm). A 0.2-mm-deep groove to hold the muscle fiber (MF) was machined into the middle of the partition. The bottom of pool 2 was lined with a 0.5-mm-deep layer of an elastomeric resin (Sylgard; Dow Corning no. 182), allowing the insertion of stainless steel pins (PN) to secure the cut end of the muscle fiber. A 1-cm-long stainless steel hook (HK), glued to the force transducer (FT), was oriented horizontally in pool 1. The force transducer was positioned with the aid of a three-axis micromanipulator. Two movable, platinized-platinum plates (PP) were connected to the output of a stimulus-isolation unit (SIU) (Grass Instruments). Pools 1 and 2 were connected to Ag-AgCl pellet electrodes (EE and IE, respectively) through agar bridges (BG), which contained floating Pt wires. The flow rate of Ringer's solution into pool 1 was selected by adjusting the head pressure at the solution inlet (SI), while the solution outlet (SO) was directly coupled to a vacuum trap. (*B*) Schematic diagram of the electrical circuit. The output of an uncompensated operational amplifier (OA) (LF357; National Semiconductors Corp., Santa Clara, CA) was connected to the pool 1 electrodes (EE) and a variable voltage source (VS) through a feedback potentiometer (FP; 10 MΩ). The pool 2 electrodes (IE) and a resistor (RS; 21.1 MΩ) in series with the variable voltage source were connected to the negative input of OA. The positive input of OA was grounded. Stimulating pulses generated by a pulse programmer (PG) (WPI) were applied to the muscle fiber preparation through the SIU and Pt plates (PP).

one or two pins inserted through the fiber perpendicular to the floor of the chamber adequately immobilized the cut end. The length of the segment was adjusted until a mean striation spacing of 2.4–2.6 μm (measured with a 40× objective; ULWD, Nikon, Garden City, NY) was attained. Under these conditions, the segments showed virtually no measurable resting tension, and the length of muscle fiber segments in pool 1 ranged between 5 and 10 mm. The grease seal was then completed by laying another layer of grease on top of the muscle fiber at the partition, and the solution level was lowered to isolate both pools.

The cut end of the muscle fiber, in pool 2, was washed with an internal relaxing solution (aspartate-K, 110 mM; MOPS-K, 20 mM; ATP-K_2, 5 mM; phosphocreatine-Na_2, 5 mM; creatine phosphokinase, 0.1 mg/ml; $MgSO_4$, 2 mM; pH 7.0), permeabilized with a saponin-containing solution (internal plus 80 μg/ml saponin) for 2 min, and then thoroughly washed with internal solution. Free [Mg^{2+}] in the internal solution was ~100 μM (calculated with Maxchelator software, v. 6.51; Pacific Grove, CA). Finally, to further secure the cut end, the solution in pool 2 was replaced with internal solution containing 2% (w/w) of a "low melting temperature" Agarose (International Biotechnologies, New Haven, CT) at 26–30°C.

Resting potential polarization

Once this solution gelled, Ringer's solution was admitted into pool 1 to replace the high-K^+, causing the fiber segment to repolarize. After 20–30 min, the fibers attained measured resting potentials in the range of −40 to −65 mV. The measured potential was then "set" to values between −90 and −100 mV by injecting current; the circuit is described below (Fig. 1 *B*). The experiments were performed at temperatures between 15°C and 18°C.

Electrical circuit

The circuit used for electrical stimulation of the muscle fiber segment and for detection of APs is shown in Fig. 1 *B*. Pool 1 was connected to the output terminal of an operational amplifier through a salt bridge (2% agar in Ringer's solution, including a platinum wire to reduce high-frequency impedance) in series with a Ag-AgCl pellet electrode immersed in 120 mM KCl. Pool 2 was similarly connected to the noninverting terminal of the operational amplifier and thus was held at virtual ground. Accordingly, the potential in pool 1 corresponded to approximately $-V_m$. This potential (which was monitored with a voltmeter) was adjusted to +90 mV by varying the voltage of the source within a range of 0.1–3.0 V. A 21.1 MΩ resistor, placed in series with the voltage source, served as a current limiter.

Single-pulse and tetanic stimulation protocols

Single pulses or trains of pulses, 0.5–0.7 ms in duration, were generated by a digital pulse generator (Pulsemaster 2000; WPI). In some experiments, the pulses were differentiated with a passive network to generate on and off transients with relaxation time constants of about 0.1 ms. Pulses were amplified with a high-voltage amplifier (model 3582J; Burr-Brown, Tucson, AZ) and fed to a stimulus isolation unit (model SIU 5; Grass Instruments, Quincy, MA). The output of the isolation unit was connected to platinized-platinum plates flanking the fiber segment in pool 1 (Fig. 1 *A*). The distance between the plates could be varied and was minimized for each experiment. For a typical experiment, this distance was approximately 1 mm.

Tension measurement

Isometric tension was recorded using a capacitative force transducer (Photocon Dynagauge, Los Angeles, CA). The transducer was calibrated by reading its output in response to known masses attached to the transducer's hook. For these measurements, the body and hook of the transducer were

oriented vertically. Linear response occurred between 0.1 and 1 mN, a range encompassing that of the active tension produced by the fibers.

Action potential recordings using the triple vaseline gap method

In control experiments, APs from cut muscle fibers were recorded under current-clamp conditions using the triple vaseline gap method (Hille and Campbell, 1976; Vergara et al., 1978, 1991; Sanchez and Vergara, 1994). The internal solution used in these experiments was the same as above, but with 0.5–1.0 mM EGTA-K added to reduce mechanical activation of the fiber. Furthermore, muscle fibers were stretched to striation spacings ranging between 3.3 and 3.8 μm.

Data acquisition and analysis

APs and tension signals were filtered at 5 kHz by using 6- and 8-pole Bessel filters (Frequency Devices, Locust, MA), respectively, and acquired at 10 kHz with a 12-bit data acquisition board (TL-125 Labmaster; Scientific Solutions, Solon, OH). Signals were displayed and analyzed with a commercial software package (Axotape 2.0.2; Axon Instruments, Foster City, CA), running on a PC-compatible 486 computer. The signal-to-noise of some of the experimental traces (Figs. 2*A* and *B*, and 3 *B*) was enhanced for final presentation by smoothing them with Adjacent Average and FFT algorithms (Origin 4.0; Microcal Software, Northampton, MA). In each case, care was taken to ensure that fidelity to the original trace was not compromised.

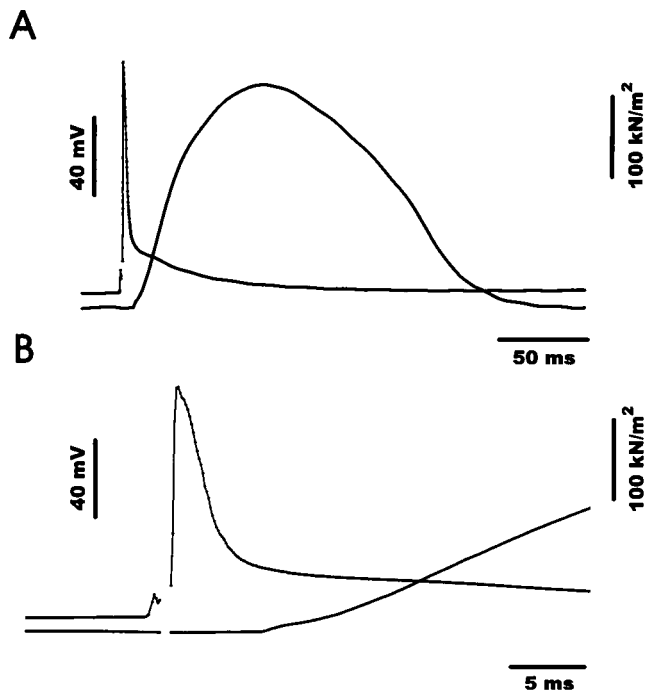

FIGURE 2 Electrical and mechanical response of a muscle fiber to a single stimulus pulse. (*A*) A single action potential (AP) and the resulting isometric tension twitch. AP: time to peak = 0.6 ms; peak amplitude = +30 mV; holding potential set at −90 mV. Isometric tension: time to peak = 57 ms; peak tension = 262 kN/m^2; 90% duration = 150 ms. (*B*) Expanded time scale of the same traces reveals that isometric tension development begins 5 ms after the onset of AP rapid depolarization. The membrane potential recording shows a stimulus artifact preceding the AP. The discontinuity in the traces represents the hyperpolarizing points of the stimulus artifact, which were not plotted for viewing convenience. Fiber diameter = 120 μm.

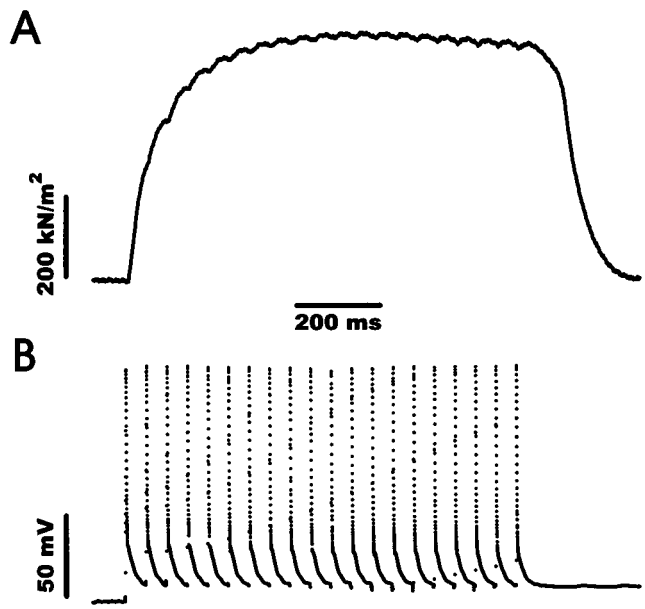

FIGURE 3 Electrical and mechanical responses to tetanic trains of pulses. (*A*) Isometric tension development in response to a 1-s, 20-Hz stimulus train. Maximum tension attained during unfused tetanus was 574 kN/m^2. (*B*) Electrical response of the same fiber to a 1-s, 20-Hz current pulse train. Holding potential was adjusted to −100 mV. Fiber diameter = 100 μm.

RESULTS

Single action potential and twitch

Stimulation of the fiber segment in pool 1 with a single suprathreshold current pulse elicited an AP followed by an isometric twitch (Fig. 2 *A*). In this particular fiber, the resting potential was adjusted to −90 mV. Subthreshold stimuli elicited only electrotonic responses that did not produce any detectable mechanical activation (results not shown). The AP spike in Fig. 2 *A* reaches a peak at +30 mV in 0.6 ms and is followed by a fast repolarization and an early after potential (EAP), features that characterize skeletal muscle APs (Hodgkin and Horowicz, 1957; Adrian and Freygang, 1962; Persson, 1963; Gage and Eisenberg, 1969; Adrian et al., 1970; Almers, 1980).

The characteristic parameters of APs recorded from eight fibers using the single-gap technique are reported in Table 1, together with those obtained in control fibers mounted in a triple vaseline gap chamber (Hille and Campbell, 1976; Vergara et al., 1978, 1991). The purpose of these comparisons is to gauge the validity of our single-gap electrical recordings. Whereas amplitude, EAP, and $\dot{V}_{EAP}$ show only slight differences between the two preparations, $\dot{V}_{dep}$ and $\dot{V}_{rep}$ from the triple-gap method are approximately 2 times and 1.5 times larger, respectively, than those from the single-gap method. We believe that the reductions in $\dot{V}_{dep}$ and $\dot{V}_{rep}$ result mainly from a frequency response limitation

TABLE 1 Properties of action potentials detected with single- and triple-gap isolation methods

	Amplitude (mV)	$\dot{V}_{dep}$ (V/s)	$-\dot{V}_{rep}$ (V/s)	EAP (mV)	$-\dot{V}_{EAP}$ (V/s)
Single gap (n = 8)	144 ± 16	327 ± 40	63 ± 11	37 ± 8.0	0.33 ± 0.06
Triple gap (n = 8)	149 ± 2.4	642 ± 198	93 ± 14	31 ± 3.6	0.48 ± 0.13

Values are expressed as mean ± SD. EAP denotes early afterpotential; $\dot{V}_{dep}$ is the maximum rate of depolarization; $\dot{V}_{rep}$ is the maximum rate of repolarization; $\dot{V}_{EAP}$ is the rate of repolarization of the EAP.

intrinsic to the passive recording of V_m across the single grease seal (see Discussion).

Fig. 2 *A* demonstrates that the segment of fiber in pool 1 is able to develop normal isometric twitch tension. During the twitch, observation under the microscope indicated that the entire fiber segment underwent a synchronized, transient mechanical response. It is important to note that the active tension developed by this fully contracting fiber does not affect the electrical recording in any way. For the twitch in Fig. 2 *A*, tension rises to a peak of 262 kN/m^2 in 57 ms (maximum rate of tension development = 10 kN/m^2·ms), and then relaxes at a slower rate. The duration of the twitch, as measured from the onset of tension development to 90% relaxation, is 150 ms. From single-twitch pooled data, values of 55 ± 5 ms (n = 20) and 285 ± 66 kN/m^2 (n = 20) were obtained for the time to peak and peak tension, respectively.

On an expanded time scale, it can be seen that tension development starts about 5 ms after the onset of the AP (Fig. 2 *B*). This temporal relationship between electrical and mechanical activation (Hodgkin and Horowicz, 1957; Lännergren and Westerblad, 1986; Claflin et al., 1994) is another characteristic of intact fibers that has been preserved in the cut segment.

Electrical and mechanical responses to repetitive stimulation

To further characterize the physiological responses of fiber segments, repetitive stimulation protocols of varying frequencies (5–100 Hz) were utilized. A fiber's electrical response to a 20-Hz, 1-s stimulus train is shown in Fig. 3 *B*. Each current pulse elicits an AP, the amplitude and time course of which are conserved throughout the train. The average peak potential during the train is +53 ± 0.9 mV (20 APs), and the holding potential was adjusted to −100 mV. AP repolarizations during the train are not complete, resulting in a slightly depolarized baseline potential 8–11 mV above the prestimulus level. Electrical recordings at higher frequencies (not shown) evidenced similar sustained levels of depolarization, the magnitudes of which increased in a frequency-dependent manner. As observed with single pulse stimulation, no signs of movement artifacts are evident in the electrical record (Fig. 3 *B*).

Fig. 3 *A* shows the mechanical response associated with a 20-Hz, 1-s train of APs (Fig. 3 *B*). For each AP, there is a corresponding inflection in the tension trace. Tension rises to 257 kN/m^2 in response to the first AP of the train, and mechanical responses to subsequent APs summate and evolve into an unfused tetanus, reaching a maximum tension of 574 kN/m^2.

When pulse trains of 10, 33, and 50 Hz were applied, fibers responded by developing tension as exhibited in Fig. 4, *A–C*. At 10 Hz (Fig. 4 *A*), tension shows a three-inflection staircase, which leads to an unfused tetanus (542 kN/m^2); at 33 Hz (Fig. 4 *B*), there is a faster summation and rise to a quasifused tetanus (614 kN/m^2); finally, at 50 Hz, a rapid rise to a completely fused tetanus (633 kN/m^2) is observed.

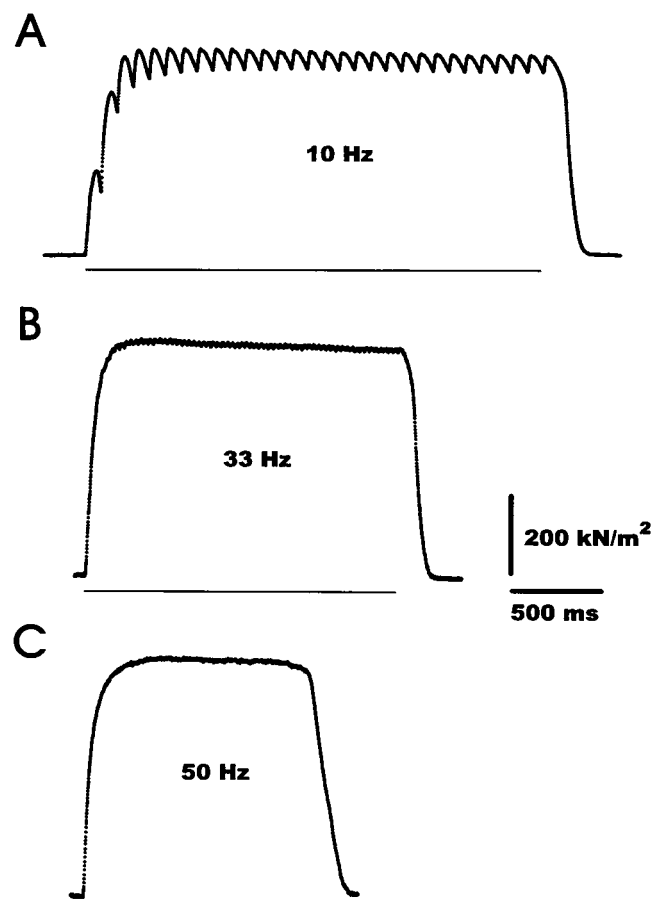

FIGURE 4 Mechanical response at three different frequencies of stimulation. (*A*) Tension developed in response to a 10-Hz, 3-s stimulus train. The fiber segment responds to the first three APs with a three-level staircase of tension, progressing to an unfused tetanus. (*B*) A 33-Hz, 2-s train gives rise to a quasifused tetanus. (*C*) A 50-Hz, 1.5-s train elicits a rapid rise to a completely fused tetanus. Horizontal bars show the period of stimulus applied.

It is important to note that the maximum tension developed during each train was sustained through the end of the stimulus period. The tetanic tension level (at 33 and 50 Hz) did not evidence droops or drifts during these extended periods of stimulation. At every frequency, full height APs (not shown) of the type shown in Fig. 3 *B* were recorded simultaneously with the tetanic tension traces. Altogether, Figs. 3 and 4 confirm that the fiber segments mounted in this single-gap chamber exhibit the frequency-dependent phenomena of tension summation and generation of unfused tetanus and fused tetanus, which have been well documented for intact fibers.

Table 2 shows pooled data from tetanic trains of varying frequency. Average peak tension values were not significantly different for frequencies from 20 to 100 Hz. Using an average value of maximum tension for the 50–100 Hz data (643 kN/m^2), and the average peak tension for single twitches (Table 2), a tetanus/twitch ratio of 2.3 was obtained. Our values for these measurements are similar to those previously reported (Grabowsky et al., 1972; Lännergren and Westerblad, 1986; Westerblad et al., 1990).

DISCUSSION

We introduce here a useful methodology for investigating the cause-effect relationship between electrical and mechanical properties of fully contracting skeletal muscle fibers. Our results illustrate that, when mounted in a single-gap isolation chamber, segments of fibers with an intact end can recover their electrical properties and develop full twitch and tetanic tension. A critical manipulation for promoting this recovery is the injection of small, sustained hyperpolarizing currents through the cut end, as explained in Materials and Methods. Moreover, the single-gap isolation method provides adequate recording of action potentials (elicited in response to current pulses) while the fiber contracts. This was demonstrated not only for single twitches, but also for long trains of action potentials at various tetanic frequencies.

Electrical recording of action potentials

When bathed in Ringer's solution, cut fiber segments could spontaneously repolarize to resting potential levels in the range of −40 to −65 mV. At these potentials, the fibers were too depolarized to give APs in response to current pulse stimulation. The low V_m values suggested to us that the single grease seal employed to electrically isolate pool 1 from pool 2 was imperfect; its relatively low resistance shunts current between the pools, thus "loading" the fiber segment and attenuating transmembrane voltage measurement (see Stämpfli and Hille, 1976; Bezanilla et al., 1982, for discussion about imperfect seal shunting effects). To compensate for this leak of current across the seal, we added a current source (represented by the variable voltage source VS, in series with a large resistor, RS, Fig. 1 *B*) in parallel with the seal.

A simplified equivalent circuit of the single-gap preparation (Fig. 5) illustrates the principle by which the current injection method may operate. In this circuit, the electrical properties of the fiber segment have been lumped into the equivalent elements R_{in} and E_m (Rall, 1977). R_{in} represents the segment's input resistance which, in a finite-length cable model, incorporates the following parameters: fiber segment radius, r; segment length, l; resting membrane resistance, R_m; space constant, λ; internal resistivity of the fiber, ρ; E_m represents the equivalent internal battery (see Fig. 5 legend). From the model, an R_{in} of 1 MΩ predicts that a fiber's "true" $E_m = -90$ mV would be seen as an attenuated

TABLE 2 Maximum tension developed during stimulus trains of varying frequencies

Frequency (Hz)	Maximum tension (kN/m^2)	No. of observations
Single	285 ± 66	20
20	600 ± 65	18
33	657 ± 57	10
50	634 ± 57	10
80	658 ± 30	3
100	638 ± 16	3

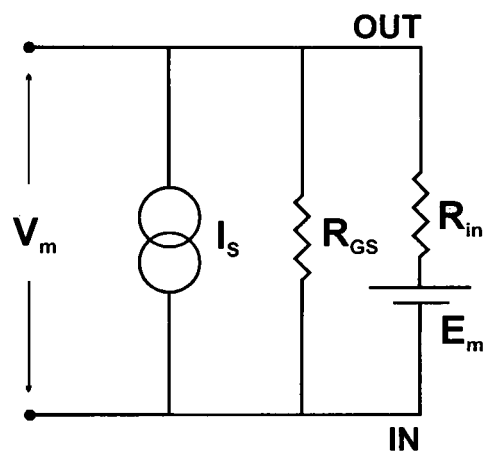

FIGURE 5 Thévenin equivalent circuit of the muscle fiber in the single-gap preparation. We used a Thévenin equivalent of the muscle fiber segment in pool 1, in which R_{in} is the input resistance of the fiber's cable; E_m is the fiber's membrane potential; R_{GS} is the effective resistance of the grease seal; I_S represents the variable current source; and V_m represents the experimentally measured voltage. R_{in} of the segment at steady state was calculated using the sealed end cable equation (Rall, 1977):

$$R_{in} = \left(\frac{\rho R_m}{2\pi^2 r^3}\right)^{1/2} \cdot \coth(L),$$

where $L = l/\lambda$, l is the length of the fiber segment in pool 1, r is the radius, ρ is the internal resistivity, R_m is the membrane resistance, and λ is the space constant, defined as

$$\lambda = \left(\frac{rR_m}{2\rho}\right)^{1/2}$$

measurement of $V_m = -63$ mV due to the current shunt across a 3 MΩ seal resistance. For a typical fiber with $r = 45$ μm and $l = 0.7$ cm, resting values for cable parameters compatible with an R_{in} of 1 MΩ are $\lambda = 0.26$ cm; $R_m = 7.5$ kΩ·cm^2; $\rho = 250$ Ω·cm, which are comparable to those published (Fatt and Katz, 1951; Falk and Fatt, 1964; Eisenberg and Gage, 1967; Adrian et al., 1970; Hodgkin and Nakajima, 1972; Hille and Campbell, 1976). The value of the seal resistance calculated, on the other hand, is slightly higher than the 1.8 MΩ reported for a vaseline seal in a muscle fiber preparation (Hille and Campbell, 1976), but lower than the ~10 MΩ in myelinated nerve fibers (Stämpfli and Hille, 1976). Using the same model parameters as above, the model predicts that an injection of ~30 nA of current compensates for the shunted current and polarizes the seal to a measured voltage, $V_m = -91$ mV. The experimental observations concurred with this prediction, as a current of 35 nA gave a V_m of −90 mV; moreover, at −90 mV, the capability of the fiber segment in pool 1 to generate APs and to contract was restored.

The conclusion reached from the theoretical and experimental analysis is that current injection at the cut end establishes a membrane potential at the boundary (vaseline seal), which matches that of the remaining fiber segment. This compensates for the "loading" imposed by the imperfect vaseline seal and restores a uniform level of polarization in the fiber segment (isopotentiality). An experimental result consistent with this explication is that the length of the segment in pool 1 did not affect either excitability or contractility along the entire span of the segment. It is important to note that the injected current becomes negligible during an AP. For example, using the same model parameters as above, but with $R_m = 100$ Ω·cm^2 (to represent the active membrane), the calculated R_{in} falls to 118 kΩ. Injecting 30 nA of current under these conditions would result in a minimal 3.5-mV reduction in the peak of the AP.

In addition to the steady-state attenuation effect of the seal, which can be compensated as described above, the seal introduces bandwidth limitation in the AP recordings as well. As noted in the Results, the APs recorded in our single-gap preparation compared quite favorably with those recorded in the triple vaseline gap preparation, except for a reduction in the maximum rate of depolarization and repolarization, $\dot{V}_{dep}$, and $\dot{V}_{rep}$, respectively (Table 1). This discrepancy is most likely due to the low-pass filtering effects of passive recording across a single grease seal. As has been discussed elsewhere (Stämpfli and Hille, 1976; Bezanilla et al., 1982), the physical separation of charges across a grease seal gives rise to a capacitative element in parallel with the membrane and the seal's effective resistance. The resulting low-pass RC filter is expected to most prominently alter the recording of high-frequency events, such as the AP rising and repolarization phases. We verified this effect by passing "ideal" APs recorded in the triple-gap preparation (e.g., $\dot{V}_{dep} = 625$ V/s, $\dot{V}_{rep} = -109$ V/s; see Table 1) through a single-pole digital low-pass filter. We found that a corner frequency of 1.5 kHz (time constant, $\tau = 105$ μs) yields values for $\dot{V}_{dep}$ and $\dot{V}_{rep}$ of 312 V/s and −92 V/s, respectively, which are close to the experimental averages obtained with the single gap (see Table 1). An estimated resistance of 3 MΩ and a capacitance of about 35 pF associated with the grease seal could mimic the filter above. Despite this filtering effect, APs recorded from the single-gap preparation exhibit relatively good fidelity to those from the optimized triple-gap methodology. Our single-gap APs show values of $\dot{V}_{dep}$ and $\dot{V}_{rep}$, which are similar to those reported for microelectrodes (Lüttgau, 1965; Nakajima and Bastian, 1974), but smaller than those obtained with other gap isolation methods (Nakajima and Bastian, 1974; Hille and Campbell, 1976; this work).

Mechanical stability and tension development

Current pulse excitation can cause mechanical activation of not only the fiber segment in Pool 1, but also part of the short segment under the grease seal. This movement compromises the integrity of the grease seal and poses a major threat for electrical recording during repetitive stimulation of the fiber. We addressed this problem by embedding the pinned segment of fiber in pool 2 in a 2% agarose internal solution, which vastly improved the mechanical stability of the preparation. The gel firmly anchors the cut end and minimizes potential movement of the fiber segment covered by grease, thus preserving the ability to reliably record APs, even during prolonged periods of tetanic stimulation.

It is important to note that our records report full-scale twitch tension on the same order of magnitude as those reported in tendon-to-tendon fibers. Our average values for maximum twitch tension (285 kN/m^2) and time to peak (55 ms) were in the higher range of those reported previously for intact fibers in different frog species (Hodgkin and Horowicz, 1957; Lüttgau, 1965; Grabowsky et al., 1972; Nakajima et al., 1975; Vergara et al., 1977; Lännergren and Westerblad, 1986; Caputo and Bolaños, 1994). Although these comparisons attest to the physiological integrity of the fiber segments in our single-gap preparation, they should be used only qualitatively because the numbers reported in the literature refer to fibers from different frog species that were tested under slightly different experimental conditions.

Our tension values for tetanic stimulation also compare well with those in the literature. The obtained tetanus-to-twitch ratio of 2.3 was in the lower range of those reported, whereas our average value of maximum tetanic tension (Table 2) was larger than those previously reported (Grabowsky et al., 1972; Lännergren and Westerblad, 1986; Westerblad et al., 1990).

Few studies on the mechanical activity of fibers mounted in gap isolation preparations have been published. For example, Kovacs and Schneider (1978) documented only the visual shortening at threshold potentials without measuring

tension; using the sucrose gap method, Caille et al. (1978) measured twitch tensions of about 12–18 kN/m^2. Hence, the method described here is the first that successfully combines gap isolation methods with quantitative tension measurements, while preserving the physiological properties of the fiber.

In the use of this single-gap methodology, we have overcome many of the practical difficulties that impede the facile use of intact fibers and microelectrodes. For example, the dissection of tendon-to-tendon intact fibers is a time-consuming and dexterity-demanding method. Furthermore, this dissection is not applicable to preparations possessing abundant connective tissue or long fiber lengths (e.g., human and many other types of mammalian muscle). As mentioned earlier, impaling microelectrodes in single fibers innately fosters membrane damage and requires experience for efficient implementation while minimizing structural injury. In contrast, the current methodology requires minimal experience in an uncomplicated procedure and is broadly applicable to the study of the excitation-tension development relationship in muscle fiber specimens previously believed inaccessible or impractical to investigate. Pertinent examples include human muscle, from which even small biopsy sections would likely suffice, and fibers from diseased (e.g., dystrophic) or long muscle, which have traditionally been nearly impossible to dissect tendon to tendon.

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