BRIEF COMMUNICATION

AN OPTICAL MONITOR OF TENSION FOR SMALL CARDIAC PREPARATIONS

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ABSTRACT When a light beam is focused on a muscle preparation which is allowed to contract, large changes in the intensity of the emerging light accompany the contraction. These movement-related optical signals were studied and compared to simultaneous measurements of force in isolated cardiac Purkinje fibers. The two signals were compared in response to action potentials and to graded changes in membrane potential controlled under voltage clamp. These experiments indicate that the optical signal is a sensitive monitor of tension development under these conditions. This technique is particularly well-suited to force measurements in smaller preparations in which direct mechanical techniques are not feasible.

Simultaneous measurement of tension and membrane current has proven to be an important combination in voltage clamp studies of excitation-contraction (E-C) coupling in a variety of cardiac muscle preparations (Gibbons and Fozzard, 1975; Beeler and Reuter, 1970; Trautwein et al., 1975). In addition to E-C coupling studies, this combination has also been very useful to our understanding of calcium-sensitive currents in the heart (Kass et al., 1978; Siegelbaum et al., 1977). In each of these previous investigations, tension was measured with one of a variety of force transducers mechanically attached to the preparation under investigation.

Recently, a series of cardiac preparations has been introduced which offers several advantages for voltage clamp studies. These new preparations include rabbit Purkinje fibers (Colatsky and Tsien, 1979), dog Purkinje fibers (Gadsby and Cranefield, 1979) and isolated myocardial cells (Lee et al., 1979). Because of their smaller size and/or larger intracellular clefts, these fibers afford better voltage uniformity and fewer complications due to ion depletion and accumulation. However, because of their smaller size and delicate nature they are not well-suited for direct mechanical measurement of tension.

This paper describes an alternate, optical method of monitoring tension. This approach does not require direct mechanical intervention and is thus better suited for studies in these smaller preparations.

When a light beam is focused onto a small region of a muscle preparation which is free to contract, it is well known that large changes in the intensity of the emerging light accompany the contraction (see Baylor and Oetliker, 1977). This intensity change, presumably due to the scattering of light out of the solid angle subtended by the detector, in many cases obscures

smaller optical signals of interest. On the other hand, particularly in work on cultured heart cells, these movement-related optical signals have been found to be useful as indices of contraction (Bucher, 1957; Sinclair, Miller and Harrison, 1970; Boder et al., 1971; Clusin, 1980).

This earlier work has suggested that light scattering due to movement might be used to measure tension, but previous studies have not tested the accuracy of this signal or its sensitivity to small, graded changes in contractile activation. In the present study, optical signals are compared to force signals measured simultaneously with a piezoelectric transducer under conditions of controlled tension development. This is thus the first comparison of these two measurements, and the results indicate that the optical signal is a simple and accurate monitor of tension.

The apparatus used in this study is illustrated in Fig. 1 *A*. The experiments were carried out in short (1.5–2.5 mm) segments of calf cardiac Purkinje fibers, and tension was measured as previously described with a piezoelectric force transducer (Kass et al., 1978). The experimental chamber was mounted on an optical rail and micro-optical bench components (Klinger Scientific Corp., Jamaica, N.Y.) were used to assemble a simple optical system. Light from a tungsten-halogen lamp was collimated with two slits using Köhler illumination. The collimated beam was focused on the preparation with a long working distance ×20 microscope objective (Nikon Inc., Instrument Div., Garden City, N.Y.). The incident beam was adjustable in three planes to permit focusing at different locations along a preparation. Light was collected by a short piece of ⅛-in diam optical clad rod (American Optical Corp., Buffalo, N.Y.) which was permanently mounted in the chamber. A photo diode/operational amplifier (EG&G, Inc., Electro-Optics Div., Salem, Mass.) was used as a detector. Voltage clamp experiments were carried out with a conventional two-microelectrode technique. The optical records shown in this paper are representative of signals obtained in a total of five action potential and seven voltage-clamp experiments. In each of these experiments, the signal to noise ratio of the optical trace was similar to that of the data shown in these figures.

Fig. 1 *B* illustrates the simplest test of the optical technique. The figure compares mechanical measurement of twitch tension and the optical signal in response to externally stimulated action potentials. The figure shows simultaneous chart recordings of membrane potential (*a*) and mechanically measured tension (*b*) obtained from a Purkinje fiber which was being stimulated at 0.5 Hz. In addition to these records, the figure also shows an optical trace (*c*). This signal monitors changes in light which passes through a region of the preparation directly under the voltage electrode. Comparison of the optical and mechanical records indicates that the changes in light intensity are a good monitor of twitch tension under these conditions. In addition, no damaging side effects were observed on either the action potential or the twitch upon prolonged exposure (15–30 ft) to the light beam.

This experiment suggests that the optical signal is a reliable measure of the twitch, but it does not test the sensitivity of this technique to graded changes in tension. To test this, optical and mechanical signals were compared in voltage clamped preparations. In this manner, voltage, and thus the degree of contractile activation, could be selectively controlled.

The results of a voltage clamp experiment are shown in Fig. 2, which shows the voltage dependence of tension measured independently with each of these techniques. Panel *A* compares records of tension measured with the force transducer (*b*) with changes in light

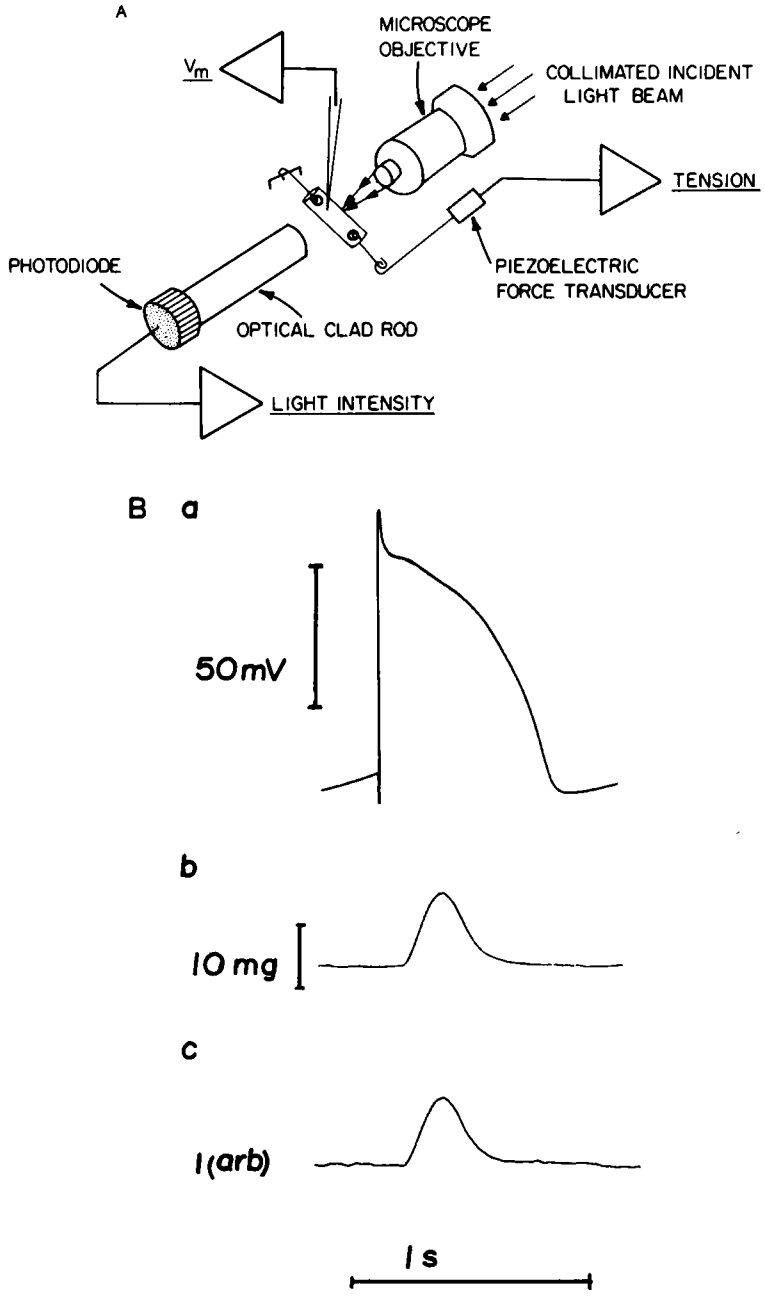
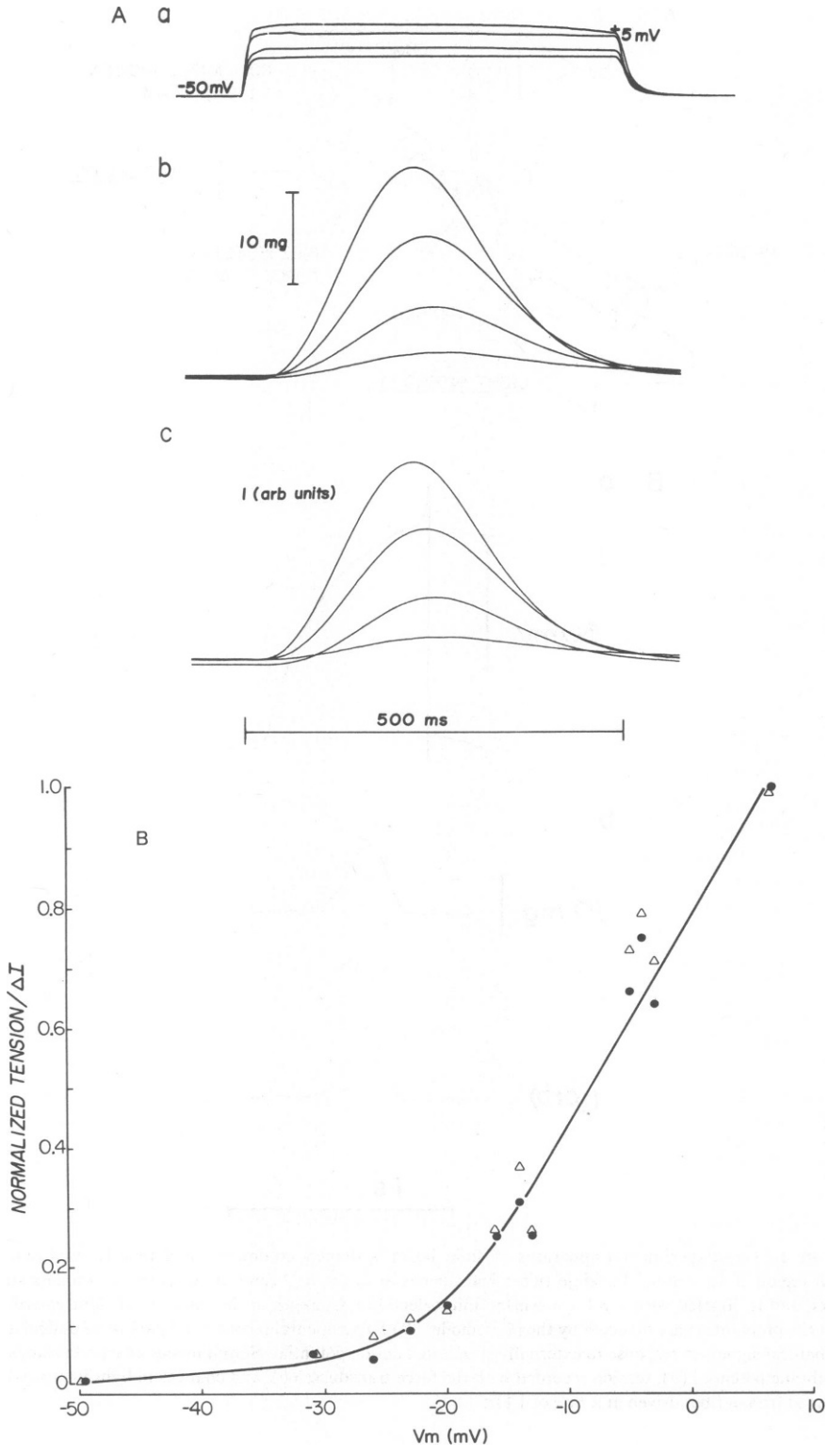


FIGURE 1 (*A*) Experimental apparatus. A light beam is shaped, collimated, and then focused onto a small region of an isolated Purkinje fiber. The fiber is attached to a force transducer via stainless steel hooks, and is impaled with a voltage-sensing microelectrode. Changes in the intensity of light emerging from the preparation are detected by the photodiode. (*B*) Experimental records: comparison of optical and mechanical signals in response to externally stimulated action potential. Simultaneous chart recordings of membrane potential (*a*), tension recorded with the force transducer (*b*), and changes in light intensity (*c*) obtained from a fiber driven at a rate of 1 Hz.


A
a
+5 mV
-50 mV
b
10 mg
c
I (arb units)
500 ms
B
NORMALIZED TENSION/ΔI
1.0
0.8
0.6
0.4
0.2
0
-50
-40
-30
-20
-10
0
10
Vm (mV)

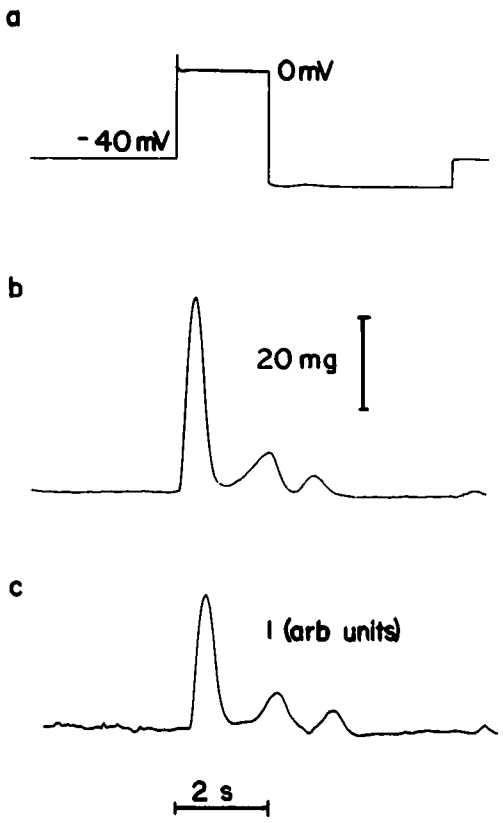


FIGURE 3 Comparison of measurement of tonic tension and after contraction with force and optical signals. Chart records of membrane potential (*a*), force (*b*), and light intensity (*c*) obtained from a preparation which had been treated with 2 μM strophanthidin (see text). Under these conditions a component of tonic tension develops over the duration of the voltage pulse in both the optical and force records. In addition, following the end of this pulse, an after-contraction is apparent in both traces.

intensity (*c*) recorded simultaneously during the four voltage steps shown. Each of these records is the average of four sweeps of data filtered at 50 Hz. The two measurements appear similar in their sensitivity to different degrees of contractile activation.

Panel *B* compares optical and mechanical measurements of movement over a more complete voltage range. The data shown have been normalized to the maximum signal for each technique for sake of comparison. The plot confirms the agreement between the two procedures over this voltage range.

Fig. 3 illustrates the final test of the optical technique which was a comparison of optical and mechanical resolution of tonic and slowly time-dependent components of tension. The

FIGURE 2 Comparison of optical and force signals under voltage clamp. (*A*) Force (*b*) and changes in detected light intensity (*c*) in response to four 500-ms voltage steps from a −50 mV holding potential. Records are *X*–*Y* plots of digitalized data which had been averaged over four sweeps at each potential. Optical and mechanical signals were filtered at 50 Hz. (*B*) Normalized force and optical signals plotted against test potential over a wider range of potentials. Δ, force records normalized to the signal at +4 mV; ●, optical signal normalized to record at +4 mV. Curve is hand drawn.

figure shows voltage clamp records obtained from a Purkinje fiber which had been exposed to 2 μM of strophanthidin for 20 min. This dose of strophanthidin was chosen as it often produces a toxic state which is characterized by enhanced tonic tension as well as slow, oscillatory after-contractions (Kass et al., 1978). These force components are evident in the records obtained with the force transducer in this experiment (*b*). This trace shows a phasic tension component in response to the voltage depolarization and, in addition, indicates a component of tension which increases monotonically over the duration of the test pulse. The termination of the test pulse is accompanied by an after contraction which is roughly one-fourth the magnitude of the preceding twitch. All of these tension components are clearly resolved in the optical signal (*c*). Thus this technique appears suitable for detecting these smaller, slower, and graded components of mechanical activity.

In conclusion, the optical measurement of movement described in this paper provides a simple technique for monitoring tension in preparations in which size restricts the use of direct mechanical techniques. Since the optical signal does not require mechanical intervention and does not appear to have harmful side effects, it is particularly well-suited for tension measurement in small preparations and thus should be useful in studies in a variety of recently introduced cardiac preparations.

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