Optical Signals from Surface and T System Membranes in Skeletal Muscle Fibers

Experiments with the Potentiometric Dye NK2367

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ABSTRACT Absorbance signals were recorded from cut single skeletal muscle fibers stained with the nonpenetrating potentiometric dye NK2367 and mounted in a three-vaseline-gap voltage clamp. The characteristics of the optical signals recorded under current and voltage-clamp conditions were studied at various wavelengths between 500 and 800 nm using unpolarized light. Our results indicate that the absorbance signals recorded with this dye reflect potential changes across both the surface and T system membranes and that the relative contribution of each of these membrane compartments to the total optical change is strongly wavelength dependent. A peak intensity change was detected at 720 nm for the surface membrane signal and at 670 nm for the T system. Evidence for this wavelength-dependent separation derives from an analysis of the kinetics and voltage dependence of the optical signals at different wavelengths, and results obtained in detubulated fibers. The 670-nm optical signal was used to demonstrate the lack of potential control in the T system by the voltage clamp and the effect of a tetrodotoxin (TTX)-sensitive sodium conductance on tubular depolarization.

INTRODUCTION

The nonpenetrating merocyanine-oxazalone dye NK2367 has been shown to give an absorbance change that is linearly related to membrane potential in stained squid axons (Cohen and Salzberg, 1978; Gupta et al., 1981). In axon studies, this dye is reported to be one of the best available voltage-sensing dyes, with a response time of <30 µs, a large signal-to-noise ratio, and low toxicity. The use of this dye has recently been extended to both skeletal (Nakajima and Gilai, 1980a, b) and cardiac (Morad and Salama, 1979; Fujii et al., 1980) muscle preparations. In skeletal muscle it has been used with the aim of studying potential changes across the transverse tubular membranes.
which are inaccessible to conventional microelectrode measurements (Nakajima and Gilai, 1980a, b).

Nakajima and Gilai (1980a) recorded absorbance changes from isolated single skeletal muscle fibers stained with this dye and stimulated to elicit action potentials. They showed that the optically recorded action potential had a slower time course than the surface action potential, but reached a peak before the onset of fiber shortening, and proposed that this optical signal reported the radially propagating tubular action potential. These authors also investigated the wavelength dependence of the absorbance signal from this dye and found that it was strikingly different from that reported for squid axon. Whereas the action spectrum obtained in axons was monophasic, with a peak intensity increase at 720 nm, the action spectrum in skeletal muscle fibers was triphasic with a peak intensity decrease at 680 nm for unpolarized light. Nakajima and Gilai (1980a) also found that the closely related merocyanine-rhodanine dye WW375 gave an optical signal identical to that recorded with dye NK2367, except that its spectral curve was shifted ~30 nm to the red. They concluded that both dyes monitored potential changes across the tubular membranes. Similar findings with dye WW375 were previously reported by Baylor and Chandler (1978). This interpretation of the WW375 absorbance change, however, differed from that subsequently reported by Vergara and Bezanilla (1981), who studied the optical signal from dye WW375 in cut muscle fibers mounted in a three-vaseline-gap voltage clamp. These investigators showed that dye WW375 at 750 nm gave a very fast signal that they attributed to potential changes across the surface membrane. In another study, Baylor et al. (1981) recorded from isolated single fibers stained with this dye and illuminated with linearly polarized light; they reported that the time course of the optical signal at some wavelengths was not identical for every plane of linear polarization.

The interesting differences reported in the action spectrum of these dyes (NK2367 and WW375) between squid and skeletal muscle preparations (Baylor and Chandler, 1978; Nakajima and Gilai, 1980a), and the findings of Vergara and Bezanilla (1981) and Baylor et al. (1981), suggested that a further investigation of the time course and spectral characteristics of the signals from these merocyanine dyes was needed to clarify the origin of the optical signals in skeletal muscle.

The studies reported in this paper were undertaken to investigate the kinetic and spectral characteristics of the absorbance changes recorded with dye NK2367 in skeletal muscle fibers illuminated with unpolarized light. A vaseline-gap voltage clamp was used so that optical signals elicited in response to current stimulation could be studied across an isopotential patch of surface membrane. In addition, signals could be studied under voltage-clamp conditions in which a simple potential step could be rapidly imposed across the surface membrane. Finally, using voltage-clamped fibers, the speed and linearity of the dye response in skeletal muscle could be compared with that reported for the squid axon preparation.

A preliminary report of this work has been published (Heiny and Vergara, 1981).
METHODS

Voltage Clamp

The voltage-clamp design and method is essentially the same as that reported by Hille and Campbell (1976) and modified by Vergara et al. (1978), with one addition. A passive filtering network with a frequency response matched to the voltage-measuring amplifier (Sigworth, 1980) was added to the series resistance compensation loop in the control circuit. With this network, it was possible to more effectively compensate the series resistance without destabilizing the voltage clamp. The series resistance compensation was adjusted for each fiber by monitoring the membrane current elicited in response to small hyperpolarizing potential steps. The series resistance potentiometer was adjusted until the capacity transient reached the maximum speed and amplitude possible without distortion. Series resistance values of 6.6 + 1.2 Ω·cm² (N = 9) were compensated under these conditions.

The references to pools A, B, C, and E used in this paper follow the nomenclature of Hille and Campbell (1976).

Preparation and Protocols

Single fiber segments, 2-3 cm in length, and 100-200 μm in diameter, including one intact tendon end, were isolated from the dorsal head of the semitendinous muscle of large specimens of the bullfrog Rana catesbiana. Only fiber segments that gave an all-or-nothing fast twitch when stimulated extracellularly were used. The segments were transferred to a small trough containing Ca²⁺-free Ringer's solution (solution A of Table 1, except for the equimolar substitution of Tris-EGTA for CaCl₂). The fiber segment was stretched to ~1.2 times its slack length and fixed at both ends with miniature pins. The solution in the trough was then rapidly exchanged for a depolarizing solution (internal relaxing, Table 1, plus 2 mM TRIS-EGTA). After the solution change, the fiber underwent a brief contracture, then spontaneously relaxed. The above protocol allowed us to use both the fast twitch and the contracture as criteria to select healthy fibers. With this protocol, the contracture was fast (Luttgau, 1963; Caputo, 1972) and approximately isometric resulting in relaxed fibers with a mean sarcomere spacing of 2.08 ± 0.16 μm (N = 16).

The relaxed fiber was then transferred to the voltage-clamp chamber (without passage through an air-water interface) in which all pools and partitions contained depolarizing solutions (solution C, Table I). The fiber segment was mounted across the gaps, which had been covered previously with strings of grease (Glisseal vacuum grease; Atomergic Chemetals Corp., Plainview, NY). The upper seals were applied and the ends of the fiber in the E and C pools were cut. Fiber segments of 200-300 μm were left in pool C and segments of 500-600 μm were left in pool E. The A-pool gap width was 80-150 μm (adjusted for each fiber to be approximately equal to the fiber diameter) and the B-pool width was 250-350 μm. The solution level was then lowered to form the pools and the following solution changes were made immediately. The solution in pool A was exchanged for one of the external Ringer's solutions (Table I); the solution in pools E and C were exchanged for one of the internal solutions listed in Table I. A period of 15-30 min was allowed for equilibration of the solutions and for diffusion of EGTA from the cut ends into the myoplasm, which was necessary to block fiber movement for optical recording. The temperature in the A pool was sensed by a small thermistor mounted close to the muscle fiber and was maintained at 9-11°C by regulated thermoelectric devices (Borg-Warner Thermoelectrics, Des Plaines, IL).

For experiments in glycerol-treated fibers, small bundles of fibers were immersed in
Ringer's solution containing 400 mM glycerol, for 1 h, then washed in a normal isotonic Ringer solution for 1–3 h. Segments of single fibers were isolated from the glycerol-treated bundle, and mounted and voltage-clamped using the protocol described above for intact fibers. Many fibers in the treated bundles were deteriorated and exhibited a large leak current with little or no sodium current. Only fibers that repolarized at least 80 mV under current-clamp conditions after the solution exchange in the A pool from relaxing to one-half Na⁺, one-half TMA⁺ Ringer's, and which had sodium currents under voltage-clamp conditions, were stained.

**Optical Set-Up**

The optical set-up used in these experiments was essentially that described by Vergara et al. (1978). Briefly, the vaseline-gap chamber was modified to allow illumination of the fiber segment in the A pool and the chamber was mounted on the stage of a modified microscope (Nikon Biophot; Nikon, Inc., Garden City, NY) used as a vertical optical bench. The preparation was illuminated with light from a 12-V, 100-W quartz-halogen lamp powered by a highly regulated power supply (JQE-25-20 VP; Kepco, Inc., Flushing, NY). A condensing lens in the microscope lamp housing produced a collimated beam of light that passed through heat absorption (Schott KG-2 Glass; Schott Optical Glass, Inc., Duryea, PA) and narrow-band interference filters (Ditric Optics, Inc., Marlboro, MA; Rolyn Optics, Arcadia, CA; Corion Corp., Halliston, MA; Melles Griot, Irvine, CA; half-bandwidths of 7–14 nm) and an electronic shutter (Vincent Associates, Rochester, NY). The quasi-monochromatic beam was directed to the preparation through the microscope condenser lens, which focused the light onto the end of a tapered fiber optic mounted in the A pool. The tip of the fiber optic rested just below the muscle fiber segment in that pool and had a diameter of 150 μm. The light reaching the muscle fiber was essentially unpolarized, with a polarization factor \( P \) of 0.07, where \( P = (I_1 - I_2)/(I_1 + I_2) \) and \( I_1 \) and \( I_2 \) are the maximum and minimum light intensities, respectively, detected by the photodiode when an analyzer was rotated. Transmitted light from the preparation was collected by an objective (Nikon Fluor 20, N.A. 0.75, Nikon, Inc., or Leitz UMK 50, X32, N.A. 0.4, E. Leitz, Inc., Rockleigh, NY). An \( x-y \) slit mounted in the image plane of the microscope trinocular head was used to mask a rectangular region of the muscle fiber from which light was collected. The size of this rectangle was set to include the width of the muscle fiber and the length of muscle in the A pool, excluding regions beneath the vaseline seals. This image of the fiber was focused by an additional plano-convex lens onto a low noise photodiode (FO 02 E; UDT Technology, Culver City, CA). The light signal was converted to voltage, and the DC signal component caused by the resting light intensity was subtracted by a sample-and-hold circuit before further amplification. The DC input to the sample-and-hold circuit was connected to a digital.
voltmeter and monitored throughout the experiments. The dye NK2367 tended to bleach during the experiments, and this allowed the resting light intensity to be accurately recorded for each measurement.

**Recording Techniques**

Light, current, and voltage traces were fed to one of the inputs of a digital signal averager (Vergara et al., 1978). Data could be acquired at two different sampling rates: the first 512 points were acquired at a fast rate (10–50 μs per point) and the second 512 points were acquired with a sampling interval equal to or greater than the first half of the record. An anti-aliasing filter (six-pole low-pass Bessel filter; Frequency Devices, Haverhill, MA), in front of each input to the signal averager, was set to corner frequencies lower than the Nyquist frequency of the fast sampling interval. The data were stored in digital form on magnetic tape and transferred to a minicomputer (NOVA-3/12, Data General Corp., Westboro, MA) for analysis and graphic display.

**Solutions**

The composition of the solutions used in these experiments is given in Table I. All solutions were adjusted to an osmolarity of 240 mosmol by varying the concentration of the major salt. Osmolarity was determined with a Wescor osmometer (Wescor, Inc., Logan, UT), which was accurate to within 3 mosmol. Thus the millimolar concentrations given in Table I are only approximate for the major ion. The pH of all solutions was adjusted to 7.1. The dye NK2367 (Nippon-Kankoh Shikiso Kenkyusho Co., Ltd., Japan) was mixed fresh before each experiment and kept in the dark on ice during the course of the experiments because the dye in solution loses color after several hours at room temperature. A concentration of 0.5 mg dye/1 ml external solution was used for staining. After the fiber was stained for 15 min with this concentration of dye, a 1:4 or 1:8 dilution of this solution was kept in the A pool throughout the measurements.

**Data Analysis**

Although the potentiometric response of dye NK2367 derives from a dye-related change in absorbance, ΔA (Ross et al., 1977), the optical signals were expressed as the change in intensity ΔI elicited during the test pulse divided by the resting intensity, I. For small intensity changes this fractional transmittance change is linearly related to the absorbance change by the approximate formula, \( \Delta A = -0.43 \frac{\Delta I}{I} \). Our measured \( \Delta I/I \) was generally <5 \( \times \) 10⁻⁴ and therefore the error introduced by using this approximation rather than the exact formula for \( \Delta A \) (Waggoner and Grinvald, 1977) is <1%. Consequently, the optical signals are discussed interchangeably in this paper as intensity or absorbance changes.

The optical signal recorded with dye NK2367 tended to fade during the course of the experiments and the degree of fading varied in different fibers. To correct for this effect, control records at set potentials and wavelengths were repeatedly obtained during each experiment. When the experiments were analyzed, the optical records obtained between controls were proportionally scaled by compensating factors calculated from the amplitude reduction of the control records. This procedure was applied in the analysis of the experiments reported in Figs. 5 and 6 of this paper.

In addition to the progressive reduction of the amplitude of the signals during the course of an experiment, the bleaching of the dye results in an increase in the resting light level during the brief periods of illumination. This is reflected in the optical
records as a sloping baseline instead of a flat reference level; this is illustrated in Fig. 1 for two wavelengths. As shown in the left traces, this drifting baseline was nearly linear during the sweep duration. It could be accurately fit to a straight line and subtracted from each record (right traces) before any analysis was performed. The magnitude of this drift was wavelength dependent. At 720 nm (top left trace), it was relatively small, but it increased at shorter wavelengths. At 550 nm (not shown), the baseline drift during the first 100 ms of sampling could be as large as 2.5 times the voltage-dependent intensity change, but even at these short wavelengths the subtraction procedure yielded corrected records similar to the right traces in Fig. 1. In some experiments, however, control records in which the fibers were illuminated but not stimulated were obtained in order to display the complete time course of the sloping baselines and subtract them from the experimental records.

**Figure 1.** Optical signals at two wavelengths shown before (left traces) and after (right traces) baseline subtraction. The baselines were fit to straight lines having maximum vertical displacements of 26 and 37% of the peak intensity change, at 720 and 670 nm, respectively. The fiber was stepped -100 mV from a holding potential of -100 mV. The external solution contained one-half Na and one-half TMA and the internal solution contained CsASP (solutions B and D, Table I). 16 sweeps averaged per trace. Fiber diameter: 128 μm. Temperature: 9°C. For this and all subsequent figures, the units of the optical calibration bar is given × 10^4.

**RESULTS**

**Recordings of Optical Action Potentials**

Absorbance signals were recorded from fibers stained with dye NK2367 and stimulated under current-clamp conditions in order to compare the time course of the optical signal with the electrically recorded membrane action potential. In similar experiments performed in squid axons, the absorbance change of this dye was shown to follow the membrane action potential faithfully (Gupta et al., 1981).

Fig. 2A shows the membrane action potential (middle trace) and the absorbance signals recorded from a fiber illuminated at 720 (top trace) and 670 nm (lower trace). These wavelengths were chosen because large intensity changes occur at 670 and 720 nm in skeletal muscle fibers stained with this
Figure 2. A. Membrane action potential (solid trace) and absorbance signals at two wavelengths (dotted traces) recorded from a fiber stained with dye NK2367. The internal and external solutions contained the normal concentration of Na and K ions (solutions A and F, Table I). The calibration arrow to the right of each optical trace indicates the direction of a positive intensity change. The sampling interval was 40 µs per point for the first half of each record, and 200 µs per point for the second half. 16 sweeps averaged per trace. Temperature: 11°C. Fiber diameter, 165 µm. B. Optical signal at 720 nm and membrane action potential (records are from Fig. 1) shown superimposed, using an expanded time base. The optical signal was scaled to the voltage trace using the amplitude of the capacity charging transient, which is present in both signals, as a reference. The scaling factor was 0.97.

dye (Nakajima and Gilai, 1980a; see also Fig. 12 of this paper). Both optical signals have been plotted in the same direction as the membrane action potential, for comparison. However, as indicated by the calibration arrows, the intensity change at 720 nm is in a direction opposite to that recorded at 670 nm. For these experiments, the external solution was normal Ringer's and the internal solution contained potassium (solutions A and F, Table I).
The membrane action potential in Fig. 2A was recorded from the potential in the A pool \((V_A, \text{Hille and Campbell, 1976})\) and has a time to peak of 0.7 ms at 11°C, measured from the start of the stimulus pulse. It shows the typical transient caused by the passive charging of the fiber capacity during the brief \((0.5-\text{ms})\) stimulus pulse and has a normal repolarization phase with the characteristic negative after potential. The optical signal recorded at 720 nm shows a fast-rising phase and also reports an inflection that occurs at the same point in time as the charging transient. The time to peak of this signal was 1.0 ms. At 670 nm, however, the rising phase of the absorbance signal does not clearly report this charging transient and rises significantly more slowly than the membrane action potential; the peak of this signal occurs 2.7 ms after the start of the stimulus. In addition, this optical signal returns to baseline with an exaggerated recovery phase.

From the results shown in Fig. 2A, it can be seen that the signals recorded with dye NK2367 from skeletal muscle fibers in response to current stimulation do not show the same time course at every wavelength. There is a suggestion from these records that most of the rising phase of the 720-nm signal closely follows the time course of the membrane action potential. To investigate this more closely, the optical signal at 720 nm and the voltage trace were superimposed (Fig. 2B) using the stimulus-related charging transient, which is reported in both the optical and electrical signals, as the scaling reference. When the traces are scaled in this way, it can be seen that the early part of the optically recorded action potential superimposes on the fast-rising phase of the membrane action potential. However, the optical signal has a second, slower component that reaches a peak later than the electrically recorded membrane action potential. At 670 nm, however, there is no significant part of the optical signal that displays the same time course as the electrically measured potential, and this signal has not been scaled. This latter optical signal does not seem to report potential changes across the space-clamped surface membrane.

A summary of the characteristics of the membrane action potential and the optical signals at both wavelengths is given in Table II, for several fibers. As shown in these data, the mean time to peak of the 670-nm optical signal is significantly longer than that of the membrane action potential; the mean time to peak of the 720-nm signal is also greater than the membrane action potential, which reflects the fact that at 720 nm there is a second slow component in addition to the large early fast phase.

**Experiments in Voltage-clamped Fibers**

To investigate these wavelength-dependent differences in the optical signals in more detail, additional experiments were performed using voltage-clamped fibers. In this way, the optical signals could be recorded in response to a less complex waveform imposed across the surface membrane and the time course of the optical signals could be studied at various potentials. For these measurements, the external solution was replaced with half-sodium, half-TMA Ringer's solution, and the potassium in the internal solution was replaced with cesium (solutions B and E, Table I). The half-sodium Ringer's was used
to ensure a good isopotential condition in the A pool (Vergara, 1981) and the internal cesium eliminated the outward potassium current in order to better resolve the time course of the sodium current (Hille and Campbell, 1976; Vergara and Bezanilla, 1981).

Fig. 3 shows the absorbance signals at 670 nm (top traces), and current

**TABLE II**

| Membrane action potential | Optical signals |
|---------------------------|-----------------|
| $V_A$ | $I_p$ | $\Delta I/I$, 670 nm | $I_p$, 670 nm | $\Delta I/I$, 720 nm | $I_p$, 720 nm |
| mV | ms | $\times 10^{-4}$ | ms | $\times 10^{-4}$ | ms |
| Mean | 139.2 | 0.90 | 5.80 | 2.55 | 5.31 | 1.54 |
| SD | 8.58 | 0.29 | 2.38 | 0.27 | 2.88 | 0.36 |

$\Delta I/I$ is given as the peak intensity change recorded at each wavelength. Time to peak ($I_p$) was measured from the start of the stimulus. Temperature: 10–12°C.

**FIGURE 3.** Absorbance signals at 670 nm (top trace), membrane current (middle trace), and voltage (lower traces) recorded from a fiber stained with dye NK2367. The fiber was voltage-clamped at a holding potential of $-100 \text{ mV}$ and stepped 90 mV in the depolarizing (left) and hyperpolarizing (right) directions. The external solution contained one-half Na and one-half TMA ions, and the internal solution contained Cs-MOPS (solutions B and E, Table I). The direction of the optical calibration arrow indicates the direction of an intensity increase. The peak optical change at 670 nm, for a +90-mV pulse was $-6.9 \times 10^{-4}$. The membrane current shown for the depolarizing pulse is given as net ionic current, after leak and capacity subtraction. 16 sweeps averaged per trace. Fiber diameter: 193 μm. Temperature: 9°C.

(middle traces) in response to potential steps of ±90 mV (lower traces) from a holding potential of $-100 \text{ mV}$. In response to depolarizing potential steps (left traces), the optical signal rises to a peak in ~6 ms; at the off of the pulse, the absorbance signal decays monotonically to the resting level, with a halftime of 6.8 ms. The peak in the optical signal recorded for the depolarizing
pulse occurs significantly later than the peak of the inward sodium current, which occurs in 1.0 ms (left middle trace); it correlates in time, however, with a second slow phase in the inactivation of the sodium current (see below). In response to hyperpolarizing pulses (right traces), the optical signal has a very different time course from that recorded for the same positive potential. This signal is inverted in direction and has a smaller peak amplitude. In addition, it has a monotonic time course at both the onset and decay of the pulse. The half-times of onset and decay of this signal are 4.2 ms. Neither the depolarizing nor the hyperpolarizing optical signal follows the time course of the potential step imposed across the surface membrane (lower traces), which in this fiber was voltage-clamped to a steady level within the first sampling interval of 100 μs.

These findings suggest that the signal from dye NK2367 at 670 nm may arise from the tubular membranes, which are capable of a regenerative sodium response and in which the membrane potential is neither rapidly established nor well controlled by the voltage clamp. They are in agreement with results reported for another potentiometric dye, WW781, which has also been suggested to monitor the tubular membrane potential in this preparation (Ver-gara and Bezanilla, 1981). To investigate this hypothesis further, the optical signal at 670 nm was recorded for a series of test pulses near threshold for activation of the sodium conductance. Fig. 4 shows the 670-nm absorbance change (upper traces of each pair of records) and membrane current (lower traces of each pair) recorded from a fiber stepped to −50, −35, −30, and −20 mV, from a holding potential of −90 mV. For a small depolarizing pulse to −50 mV, the time course of the optical signal is similar to that shown in Fig. 3 for a large hyperpolarizing pulse. At larger depolarizations, a second slowly rising peak begins to appear. The onset of this peak occurs progressively earlier as the fiber is depolarized to −35, −30, and −20 mV, occurring in 9.5, 6.5, and 3.3 ms, respectively, at each of these potentials. This second component in the optical traces closely correlates in time with a slow second component in the inactivation phase of the sodium current, which is most apparent in a narrow range of depolarizing potentials and is sometimes seen as a secondary "notch" (e.g., at −35 mV).

In the records shown in Figs. 3 and 4, the time to peak of the 670-nm signal (3.3–6 ms) was significantly longer than the time to peak of the signal recorded under current-clamp conditions (Fig. 2A) at the same wavelength. As expected, this is related to the fact that the voltage-clamp records were obtained for smaller depolarizations and in a half-Na Ringer’s solution. In records obtained with full sodium Ringer’s under otherwise similar conditions, this peak always occurred within 2.7–3.0 ms, a time to peak comparable to the 670-nm optical action potential.

To study the voltage dependence of the optical signal at 670 nm and compare it with the activation of the sodium conductance, a series of records was taken over a range of potentials from −190 to +50 mV. These data are plotted in Fig. 5 (closed circles), together with the peak sodium current (triangles) recorded simultaneously at each potential. For potential steps
between $-190$ and $-50$ mV, the peak intensity change varied linearly with potential. These data were fit to a straight line with the equation $\Delta I/I \times 10^4 = 0.04 V_m + 3.7$ (correlation coefficient $= 0.99$). At potentials above $-50$ mV, however, the amplitude of the sodium-dependent peak in the optical data was steeply voltage dependent. In contrast, the peak sodium current was activated more gradually above this potential. For example, in the range $-35$ to $-30$ mV, in which only a small fraction of the maximum current has been activated, the optical signal has almost reached a maximum value. This behavior suggests that the 670-nm optical signal reflects potential changes across membranes that are not well controlled by the voltage clamp.

![Figure 4](image)

**Figure 4.** Optical signals at 670 nm (top trace of each pair of records) and net ionic current (lower trace of each pair) recorded from a fiber stained with dye NK2367. The fiber was voltage clamped at a holding potential of $-90$ mV, and stepped to the potential indicated at the left of each pair of traces. The current calibration bars indicate the scaling of left and right sets of traces, respectively; the optical calibration bar is given for all optical traces. Same experimental conditions as Fig. 3. Four sweeps averaged per trace. Fiber diameter: 140 $\mu$m.

In an attempt to demonstrate further that the peak in the optical records observed at large depolarizing potentials was caused by a sodium-related process, additional experiments were performed in which $10^{-6}$ M TTX was added to the external solution. Fig. 6 (left traces) shows the optical signal and sodium current recorded in half-Na, half-TMA Ringer's solution (solution B, Table I), for a $+90$-mV potential step. In this solution, the optical signal shows the typical peak described previously, which in this fiber reached a maximum in 3.5 ms at 9°C. When the external solution was exchanged for the TTX-containing solution (right traces), both this peak in the optical signal
and the sodium current disappeared. The time course of the signal obtained under these conditions is similar to that recorded at small depolarizations or hyperpolarizing potentials (Figs. 3 and 4); it rises and decays with a half-time of 3.9 ms at 9°C.

Fig. 5 (open circles) shows the voltage dependence of the 670-nm optical signal recorded after TTX addition. The large nonlinearity recorded in half-

![Figure 5](image)

**Figure 5.** Voltage dependence of the optical signal at 670 nm. Filled circles: peak intensity change in half-Na Ringer's. Triangles: peak Na current in half-Na Ringer's. Open circles: steady state intensity change after addition of 10⁻⁶ TTX. Ordinate: -ΔI/I × 10⁴, and peak sodium current; abscissa: absolute membrane potential (V_m). Data were obtained from the same fiber as Fig. 4. The dotted curve through the current data was drawn by eye. The solid line through the optical data was drawn from the linear regression given in the text, using data obtained from -190 to -50 mV. No bleaching correction was used for the set of points taken before TTX because from the start to end of this series of traces the deviation in ΔI/I was less than the rms noise (0.25 × 10⁻⁴) of the traces, which in this plot falls within the width of the symbols. Similarly, from the start to end of the series after TTX, the deviation in the optical signals was within the optical noise. However, in the interval between the Na and TTX series, some loss of signal occurred and the points recorded after TTX addition were all scaled × 1.14, using the signal at -80 mV before and after TTX as the scaling reference.

Na Ringer’s disappears and all of the points could be fit to a straight line (not drawn) with the equation ΔI/I × 10⁴ = 0.04 V_m + 3.9 (correlation coefficient = 0.99). The rms noise in these optical records was 2.5 × 10⁻⁵ and therefore this line cannot be distinguished from the line computed before TTX addition using only potentials between -190 and -50 mV. These data, together with the results shown in Figs. 3 and 4, support the proposal that the nonlinearity observed in the 670-nm optical signals recorded with dye NK2367 from non-
TTX-treated fibers reflects a sodium-dependent escape of control from a membrane compartment other than the surface membrane. This effect could occur because of the presence of a voltage-dependent, TTX-sensitive sodium conductance in the T system. If one assumes that the steady state tubular potential after TTX approaches a value equal to the imposed clamp potential, it is possible to estimate from Fig. 5 the extent to which the mean tubular potential deviates from the potential imposed at the surface membrane, when Na is present. For example, for a potential step to -30 mV imposed at the surface, the mean tubular potential approaches a value of +40 mV. The latter value is somewhat less than the sodium equilibrium potential, which can be extrapolated from the Na current data to be about +55 mV in this fiber. At potentials greater than -30 mV, the mean tubular potential (in the absence of TTX) is always brought to about the same constant value, around +40 to +50 mV. Also, the difference between the amplitude of the optical signals in the active and TTX-treated cases becomes less apparent at progressively larger depolarizations.

Because the signals recorded at 720 and 670 nm under current-clamp conditions had different time courses, it was important to also study the 720-
nm optical signals in voltage-clamped fibers. Fig. 7 shows the absorbance signals at 720 nm (top traces) and net ionic currents (middle traces) recorded in response to 100-mV depolarizing and hyperpolarizing pulses (lower traces). The absorbance signals at this wavelength are inverted in direction to those recorded at 670 nm for the same pulse polarities. In addition, these optical signals have different time courses than the 670-nm optical signals at comparable potentials (Fig. 3). In particular, the optical signals at 720 nm are more symmetric than the corresponding 670-nm signals in response to hyperpolarizing and depolarizing pulses. For both pulse polarities, the 720-nm optical signal has an early fast step component that occurs within the first sampling interval of 100 μs. A second slow component is also present and has a similar (but not identical) time course for the two pulse polarities; at large depolarizing potentials it does not report any significant contribution from the sodium-dependent peak. In the hyperpolarizing direction, this component rises to a steady state level with a half-time of 3.6 ms at 10°C, comparable to that recorded at 670 nm for hyperpolarizing and small depolarizing pulses. The presence of a slow signal component in addition to the step intensity change indicates that the 720-nm signal does not reflect potential changes solely across a rapidly voltage-clamped membrane area. This finding is consistent with results reported in Fig. 2, which show that the 720-nm optical action potential also has some contribution from slower intensity changes.

To compare the speed of the early fast component at 720 nm with the speed of the potential imposed by the voltage clamp across the surface membrane,
a series of experiments were made in which a fast sampling rate (20 μs per point) and less filtering (25 kHz) were used. The 670-nm signal was also recorded under the same conditions to determine whether it might also show some contribution from this fast step. Fig. 8 shows the optical signals at 720 (top trace) and 670 nm (middle trace) recorded for a −80-mV potential step. For comparison, these signals have been plotted in the same direction. The potential measured simultaneously at the A pool is shown in the lower trace. This trace is slightly distorted by the positive feedback introduced by the series resistance compensation, which was used to ensure that the potential at the surface membrane was a step change established in <20 μs. The optical signal at 720 nm clearly shows a fast intensity change occurring within this time frame. At 670 nm, however, the optical signal had a much slower time course and did not report this early fast step; in fact, it did not reach a steady state value during the 10-ms pulse.

Because the surface membrane potential can be controlled with the voltage clamp in this preparation, and in view of the previous result which suggested that the 720-nm step intensity change arises at the surface membrane, the voltage dependence of this step was more carefully investigated in this same fiber. Fig. 9 shows a plot of the amplitude of this fast component of the signal vs. the imposed potential. These data show that the step intensity change at 720 nm is linearly related to potential over at least the ±100 mV range, which indicates that it can only arise from the surface membrane. This finding, together with the speed of the step component shown in Fig. 8, indicates that

![Figure 8](image-url)
in this membrane compartment the dye NK2367 responds rapidly and linearly to potential changes.

The contribution of the surface and tubular potential changes to the optical signals recorded with dye NK2367 at these same wavelengths was studied further using fibers treated by the glycerol shock detubulation procedure (Howell and Jenden, 1967; Krolenko, 1969; Eisenberg and Gage, 1967). Records of the absorbance signals at 670 and 720 nm, and membrane current, recorded for a potential step to -20 mV from a glycerol-treated fiber, are shown in Fig. 10. These records differ in several important respects from those obtained in intact fibers. The amplitude of the intensity change at 670 nm (middle trace) is considerably smaller than the amplitude of the 720-nm optical change (top trace). This finding is opposite that usually observed in nondisrupted fibers (see Figs. 3 and 7) for large depolarizing pulses. In addition, the typical sodium-dependent peak is not present in the 670-nm optical signal. The time course of the 670-nm absorbance signal in this fiber was very slow and did not reach a steady state level within the pulse duration. In addition, the decay phase of the sodium current has a single exponential

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**Figure 9.** Voltage dependence of the step component of the 720-nm optical signal. Ordinate: amplitude of the 720-nm step intensity change; abscissa: imposed voltage step ($V$). The amplitude of the step intensity change was determined by computing the difference between the mean of 20 data points at the baseline before stimulation and the mean of data points occurring in a time frame 20–40 μs after the start of the pulse. The fiber was stained with dye NK2367 and stepped to various potentials from a holding potential of -100 mV. The solid line is drawn from the equation $\Delta I/I \times 10^4 = 0.01 V - 0.001$, with a correlation coefficient of 0.99. Same fiber and experimental conditions as Fig. 8.
time course (with a time constant of 1.4 ms) and does not exhibit any second component. The absorbance signal at 720 nm (top trace), on the other hand, has a time course and amplitude very similar to that recorded in intact fibers. The initial step recorded in this fiber rises in <200 μs. The capacitance of this fiber was 5.2 μF/cm². The mean capacitance calculated for glycerol-treated fibers under similar conditions was 3.8 ± 1.7 μF/cm² (N = 5). For comparison, the mean capacitance of nondisrupted fibers, recorded under the same conditions, was 10.6 ± 2.3 μF/cm² (N = 5; mean diameter: 182 ± 43 μm), thus suggesting that the glycerol-treated fibers were at least partially detubulated. The results shown in this figure further support the interpretation that, in intact fibers, the 670-nm signal reflects largely tubular potential changes and that a large part of the 720-nm signal reports the surface membrane potential.

**Spectral Characteristics of the NK2367 Signals**

The results presented in Figs. 2–10 suggest that, in skeletal muscle fibers, the action spectrum of the potential-dependent absorbance changes recorded from different membrane compartments may not be identical. Previously reported action spectra for this dye in skeletal muscle have been determined from the peak intensity change recorded from fibers stimulated to propagate action potentials, without separation of the signals from different membrane com-

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1 The step response described above is less noticeable in this record because the optical traces from this fiber were filtered at 10 kHz to improve the signal-to-noise ratio.
parts (Nakajima and Gilai, 1980a). Because our results show the feasibility of distinguishing these signals on the basis of their time course, it was interesting to investigate further the wavelength dependence of the absorbance change associated with each of these membrane compartments. Fig. 11 shows a series of optical signals recorded in response to 100-mV depolarizing pulses, as the illuminating wavelength was varied from 720 nm to progressively shorter wavelengths. The fast step component (defined as an absorbance change that occurs in <20 μs, or within the time the membrane potential is clamped to a steady level) is significant at 720 nm and is reduced below 700 nm to within the level of the baseline noise. At 720 nm the overall signal is an intensity increase, without a significant contribution from the sodium-depen-

dent peak (as previously shown in another fiber; Fig. 7). This peak begins to appear at 700 nm as an intensity decrease, becomes quite large at 680 nm, and inverts to an intensity increase at 570 nm.

To study the action spectrum of this dye under conditions in which the different optical signals could be referred to the same potential change, a series of records was taken for long hyperpolarizing pulses of -90 mV at wavelengths from 500 to 800 nm, in 10-nm intervals. It was assumed that for long pulse durations the tubular membrane potential would approach a steady state value equal to the imposed clamp potential. Examples of typical absorbance signals recorded under these conditions have been shown in Figs. 3 and 7 (right traces) for 670 and 720 nm. Fig. 12 shows a plot of the steady state
absorbance change (open circles) and the step absorbance change (filled circles) vs. wavelength. At each wavelength, the step absorbance change ($\Delta I/ I_{\text{step}}$) was calculated as described in the legend of Fig. 9 and was subtracted from the total optical change before computing the steady state value at 30 ms ($\Delta I/I_{\text{steady state}}$; see figure insert). These data indicate that for hyperpolarizing pulses the action spectra of these two signal components are clearly different. The fast step change is an intensity decrease at all wavelengths. It has a monophasic action spectrum with a peak at 720 nm, and decreases to below the baseline noise at wavelengths on either side of this peak. In contrast, the steady state intensity change has a triphasic action spectrum; it is an intensity decrease from 500 to 590 nm and 700 to 800 nm, but is an intensity
increase from 600 to 690 nm. Large intensity changes occur at 550, 630, 670, and 720 nm. At three of these peaks it is possible to record a large, nearly pure slow signal, without a detectable contribution from the early fast step. A summary of the mean ΔI/I of the step and steady state signal components recorded from several fibers at these peak wavelengths is given in Table III.

**DISCUSSION**

Identification of Membrane Compartments Giving Rise to the Absorbance Changes Recorded with Dye NK2367

A major finding from these experiments is that the absorbance signals recorded from skeletal muscle fibers stained with dye NK2367 reflect potential changes arising across both the surface and T system membranes, but that the optical changes arising in each of these membrane compartments do not contribute to the total optical change in the same proportion at every wavelength. In optical records obtained from fibers stimulated under both current- and voltage-clamp conditions, significant differences were observed in the characteristics of the optical signals recorded at different wavelengths. From an

| TABLE III | MEAN ΔI/I OF THE STEP AND STEADY STATE OPTICAL SIGNALS RECORDED FOR 90-mV HYPERPOLARIZING PULSES |
|------------|------------------------------------------------------------------------------------------|
| Wavelength (nm) | Steady state ΔI/I | Step ΔI/I |
| Number of Fibers | 550 | 630 | 670 | 720 | 720 |
| Mean ΔI/I (× 10⁻⁵) | 4 | 2 | 7 | 7 | 7 |
| SD | -2.34 | +2.63 | +4.09 | -2.75 | -1.31 |

analysis of the time course, voltage dependence, and spectral properties of these optical signals, it is possible to clearly identify at least two signal components—a slow intensity change at 670 nm and a fast step intensity change at 720 nm—that reflect the surface, and a weighted average tubular potential, respectively.

At 670 nm an intensity change was detected that, under a variety of conditions, had characteristics that suggested that it arises largely in the tubular membranes. In response to current stimulation, the optical signal recorded at this wavelength peaks significantly later than the peak of the membrane action potential and does not report the membrane charging transient. It has a time course similar to that predicted for the tubular action potential from membrane models (Adrian and Peachey, 1973). In addition, its time course is similar to that recorded with fluorescent potentiometric dyes (Vergara and Bezanilla, 1976; Nakajima et al., 1976; Vergara and Bezanilla, 1981) and also with absorbance dyes for some wavelengths and illuminating conditions (Baylor and Chandler, 1978; Nakajima and Gilai, 1981a, b; Baylor et al., 1981), all of which have been proposed to monitor tubular potential changes.
Under voltage-clamp conditions, in response to hyperpolarizing pulses or small depolarizing pulses, the 670-nm optical signal is significantly slower than the potential step imposed across the surface membrane, which suggests that it does not arise from a rapidly voltage-clamped membrane compartment. This is in agreement with the tubular models of Adrian et al. (1969) and Adrian and Peachey (1973), which predict that the potential along a passive, radially distributed cable cannot be voltage clamped instantaneously. These models also predict that the potential change in the inner regions of the T system should have a time course similar to the slow phase of the capacity current transients (Eqs. 17 and 18, Adrian et al., 1969; Fig. 9, Adrian and Peachey, 1973). In qualitative agreement with this prediction, the monotonic, exponential-like time course of this signal parallels the late decay time of the electrically recorded capacity transient (see Fig. 3, right traces). However, to compare the optical signals more accurately with predictions from membrane models, it must be considered that the tubular optical signals represent some weighted average of potential changes occurring along the entire fiber radius. In the simplest case, if the intensity change per millivolt per unit membrane area is assumed to be constant along the radius, then the predicted time course of the tubular optical signal can be calculated from the radial integral of the tubular potential derived from the radial cable equation. When this kind of analysis was applied to the passive optical signal at 670 nm, a unique set of parameters could simultaneously fit the time course of both the optical signals and the membrane currents (unpublished results). A more extensive curve fitting of the tubular optical signals from dye NK2367 to tubular models, including tests of the assumption proposed above, is currently being performed and will be the subject of a future publication.

In response to large depolarizing potential steps, the time course of the 670-nm optical signal is dramatically different from that recorded at small depolarizations, but the characteristics of this signal likewise support the proposal that it originates in an uncontrolled membrane compartment. The sodium conductance of the T system should cause the tubular membranes to generate an active response that should be reflected in the tubular optical signal. Our data show that at depolarizations below threshold for activation of the sodium conductance, the steady state optical change at 670 nm is linear with potential, whereas in the same region of potentials at which the sodium current is activated, the dye signal becomes steeply voltage dependent (Fig. 5). In the presence of TTX, this nonlinearity in the 670-nm optical signal disappears and the optical signal has a time course and voltage dependence similar to the passive membrane.

In glycerol-treated fibers, the sodium-dependent component in the 670-nm optical signal disappears, although a large sodium current is present. This result would be expected if the voltage- and sodium-dependent intensity change were caused by a tubular, rather than surface sodium conductance. The correlation of the slow notch recorded in the inactivation phase of the sodium current with the secondary peak in the optical signal from intact fibers, and the absence of both these phenomena in glycerol-treated fibers, also support this interpretation. Furthermore, both of these phenomena are
predicted from radial cable models that include an activable tubular sodium conductance (Adrian and Peachey, 1973; Hille and Campbell, 1976; Vergara, 1981).

In contrast to the results discussed above for 670 nm, the optical signal at 720 nm had very different characteristics. At this wavelength, one component can be identified as arising from potential changes across the surface membrane. This was observed in fibers stimulated with a brief current pulse in which a large component of the signal closely followed the rising phase of the membrane action potential, including the transient caused by the passive charging of the membrane capacitance (Fig. 2). Under voltage-clamp conditions, the 720-nm signal has an early fast step change that rises with the speed of the potential imposed across the surface membrane and is linearly related to potential. These results indicate that the fast step intensity change of the 720-nm signal reports potential changes across the surface membrane where the potential is controlled by the voltage clamp within 20 μs in this preparation. This finding also demonstrates that dye NK2367 in a skeletal muscle preparation can respond as rapidly and linearly to membrane potential changes as reported for squid axon (Cohen and Salzberg, 1978; Gupta et al., 1981).

At 720 nm, a slower intensity change is also detected that does not seem to arise in the rapidly voltage-clamped surface membrane. Neither does it report potential changes associated with a sodium-dependent escape of control, as can be inferred from a comparison of the hyperpolarizing and depolarizing optical signals recorded at 670 and 720 nm for large potential steps (Figs. 3 and 7). In other experiments in which the 720-nm signal was studied at potentials near threshold, no sodium-dependent peak was seen in the optical records, even though an obvious second component was recorded in the sodium current, and the typical sodium-dependent peak was recorded in the 670-nm optical signal. A possible interpretation of these differences between the 670- and 720-nm slow signals may be that at 720 nm the optical signal preferentially reports potential changes from peripheral regions of the T system in which the potential does not completely escape from voltage-clamp control. In contrast, at 670 nm, the optical signal would better reflect a "weighted average" of potential changes occurring in the entire T system.

Spectral Characteristics of the Absorbance Signals Recorded with Dye NK2367 in Skeletal Muscle

Our studies of the action spectra of dye NK2367 in skeletal muscle (Fig. 12) indicate that, for hyperpolarizing pulses, at least two signal components having different time courses and spectral dependence can be distinguished. A step intensity change, attributed to the surface membrane, has a monophasic action spectrum with a peak at 720 nm. It is interesting to note that this spectrum is very similar to that recorded with this dye in the squid axon using unpolarized light (Ross et al., 1977). A slower intensity change, attributed to potential changes in the tubular membranes, has a different action spectrum than the surface membrane. The spectrum of this component is triphasic, with
a peak at 670 nm. The latter spectrum is similar to that reported by Nakajima and Gilai (1980a) for the spectrum of the “optical action potential” obtained with dye NK2367 in skeletal muscle. However, these authors did not separate intensity changes from the surface and T system contributions. We were able to detect these separate components mainly because of the use of voltage-clamped fibers. An important implication of our findings is that the optical signal recorded with dye NK2367 from skeletal muscle fibers is composed of signals from these different membrane compartments having very different time courses, and which do not add in a simple way (e.g., weighted only by their relative membrane areas) to the total optical signal. Both the particular point in time at which the optical signal is studied and the wavelength used for recording will influence the respective contributions of signals from each membrane. The fact that the spectral dependence of the signals from each membrane compartment is different can explain why we were able to record, at 670 nm, a largely tubular signal, and at 720 nm a signal having a greater proportional surface component. Because the surface signal decreases at wavelengths <700 nm, our results at 670 nm are in agreement with Nakajima and Gilai’s interpretation that their 680-nm optical action potentials reflected largely tubular potential changes.

It should be noted that in our determination of the action spectra from hyperpolarizing pulses, the steady state intensity change was treated as a single signal component at every wavelength. As discussed in the previous Discussion subsection, this assumption may be an oversimplification because there are data (Figs. 3 and 7) to suggest that there are differences between the slow signals at 670 and 720 nm. The absence of a sodium optical peak at 720 nm suggests that the large slow absorbance signal plotted at 720 nm (Fig. 12) may arise in peripheral T system regions that perhaps have the same spectral peak as the surface membrane. These considerations would mean that our proposal that the spectral dependence of the T system optical signals is triphasic may not be strictly correct because more than one tubular region, with different spectral characteristics, may be adding to this spectrum. Further experiments would be needed to clarify these points and separate these possible tubular contributions.

We do not as yet have an explanation for this wavelength-dependent separation of the optical signals from different membranes. It seems unlikely, though, that the wavelength-dependent differences in the optical signals can be explained in terms of dye-related nonlinearities. There are several arguments against the latter interpretation. In the squid axon, a preparation with a single membrane compartment, this dye responds linearly to membrane potential with a single fast time constant of <30 μs and a peak intensity change at 720 nm; it responds only to membrane potential and not to associated current or conductance changes. Our results show that in skeletal muscle this dye at 720 nm similarly responds linearly and rapidly to changes in the surface membrane potential; at 670 nm, the dye response, from a slower membrane compartment, is nevertheless linear with potential under conditions in which nonlinear conductances are blocked (Fig. 5). Further, in squid axon
studies, although the amplitude of the intensity change varies with wavelength, no wavelength-dependent differences in the time course of the optical signal from this dye are reported.

In muscle, there are some obvious differences between the surface and tubular membranes that might account for the different spectral properties of the dye. Differences in the lipid or protein composition of these membranes or perhaps differences in the surface charge near each membrane (Rapoport, 1969) may alter the environment of the membrane-bound dye in such a way as to alter the wavelength dependence of the absorbance change. In support of this proposal differences in the action spectrum of this dye have been reported for different preparations (Ross et al., 1977). These action spectra (obtained with unpolarized light for depolarizing potential changes) seem to fall primarily into two groupings: monophasic and triphasic. Preparations in which a monophasic spectrum is detected, with a peak intensity increase at 720 nm, include squid axon (Cohen and Salzberg, 1978) and barnacle and leech neurons (Ross and Reichardt, 1979). Preparations that show a triphasic spectrum, with a peak intensity decrease at 660–680 nm include: frog sympathetic neurons, chick spinal chord neurons, and rat superior cervical ganglion neurons (Ross and Reichardt, 1979; embryonic chicken heart, Fujii et al., 1980; snail salivary gland, Salzberg and Senseman, 1979). Only one report has been made of an action spectrum showing intensity peaks at two bands; Morad and Salama (1979) report intensity increases at 750 and 490 nm in frog heart muscle. Ross and Reichardt (1979) proposed that the action spectrum of this dye may be monophasic or triphasic because of tissue differences between invertebrates and vertebrates, respectively. However, this hypothesis is not compatible with the result of Salzberg and Senseman (1979), which showed a triphasic spectrum in an invertebrate preparation, or with our finding that both types of spectral curves can be obtained—but from different membrane compartments—in the same vertebrate preparation. Nevertheless, in view of the results reported for different preparations, differences in membrane composition may still account for differences in the spectral response of the dye.

Alternatively, in view of the dichroic spectrum reported for both dye NK2367 and the closely related merocyanine-rhodanine dye, WW375, in squid axons (Ross et al., 1977; Gupta et al., 1981), it is also possible that differences in the geometry and orientation of the surface and tubular membranes may influence the dye absorbance in each compartment in response to polarized light. The experiments reported in this paper have been performed using unpolarized light only and therefore this possibility has not been directly tested. However, it is possible that even under our conditions, oriented membrane compartments within the muscle fiber, stained with highly dichroic dye molecules, could select certain polarization components of a randomly polarized beam and produce cancellations at some wavelengths specific for each compartment. The results of Baylor et al. (1981) with dye WW375 in skeletal muscle fibers suggest that the plane of polarization of the illuminating beam can influence the time course of the optical signals. In addition,
preliminary results with polarized light in our preparation demonstrate a highly dichroic behavior of the optical signals recorded with dye NK2367 (Heiny and Vergara, 1982).

Finally, it should be noted that this spectral separation of signals from different membrane compartments is not found uniquely with dye NK2367 but may be a general property of a class of merocyanine dyes. A fast surface membrane-type signal was detected with the closely related merocyanine-rhodanine dye WW375 at 750 nm (Vergara and Bezanilla, 1981) and we have subsequently detected, at 700 nm, a tubular signal with a time course identical to that shown in Fig. 3 for dye NK2367 (Heiny and Vergara, unpublished results). In our preparation these dyes behave similarly except that the peak intensity changes are shifted ~30 nm in a manner similar to that reported in other muscle (Nakajima and Gilai, 1980a) and nerve preparations (Cohen and Salzberg, 1978).

Importance of the Tubular Sodium Conductance to Tubular Depolarization during Activation

In view of our conclusion that the 670-nm optical signal represents potential changes across the tubular membranes, a most relevant outcome of these studies from the viewpoint of muscle physiology, is the evidence presented regarding the importance of the tubular sodium conductance to tubular depolarization. Previous evidence for the existence of an activable tubular sodium conductance has been derived from experiments in which the tubular depolarization was studied from its effect on later mechanical events (Adrian et al., 1969; Costantin, 1970; Bastian and Nakajima, 1974). Our optical studies give independent evidence for the existence of this conductance by providing a direct measurement of a sodium-dependent tubular potential change. The optical data indicate that the presence of a sodium conductance in the T system has two significant effects on the tubular potential. First, it increases the magnitude of the tubular depolarization. As shown in Fig. 5, at small suprathreshold depolarizations, the amplitude of the 670-nm intensity change recorded in the presence of sodium is more than double that recorded in the presence of TTX. In fact, any potential imposed at the surface membrane above a certain threshold causes the mean tubular potential to approach a value near the sodium equilibrium potential. Second, the tubular sodium conductance has a profound effect on the speed of tubular depolarization. As was shown in Fig. 6, the sodium-dependent peak occurred in 3.4 ms in half-Na Ringer’s, whereas the tubular potential reached a steady state value within 30 ms after TTX addition. In other experiments with full-sodium Ringer’s, this peak occurred in ~2.8 ms. This effect of the tubular sodium conductance on the rate of tubular depolarization may be most important for normal muscle activation. Under physiological conditions, with sodium ions present, the T system would be depolarized more rapidly because of this sodium conductance and would in turn more synchronously excite SR calcium release. Under some conditions, the speed of T system depolarization can limit the rate of calcium release. For example, the time course of the calcium signal
recorded with the calcium indicator dye arsenazo III is slower in TTX-treated fibers than in fibers bathed in sodium-containing Ringer (Palade and Vergara, 1982).

Finally, it is interesting to compare these results obtained from optical studies, with predictions from membrane models that include an activable sodium conductance. The radial cable model of Adrian and Peachey (1973) predicts that the tubular sodium conductance should cause the potential at the center of a voltage-clamped fiber to exhibit a regenerative peak (Fig. 10, Adrian and Peachey, 1973). A similar peak is predicted for the mean tubular potential, when the integral of their equations is computed as described previously, to model the tubular optical signals (Heiny and Vergara, unpublished results), and is similar to that recorded experimentally with dye NK2367 at 670 nm. This model also predicts that the tubular sodium conductance should be manifest as a second component in the measured membrane current, which would be most apparent in a narrow range of suprathreshold depolarizations. Such a second component been experimentally observed in voltage-clamp studies and was well fit by the model (Hille and Campbell, 1976; Vergara, 1981). Our optical studies in voltage-clamped fibers demonstrate that this second component in the sodium current record is closely correlated with a sodium dependent tubular potential change, in agreement with these predictions. A similar finding was also obtained with the potentiometric dye WW781 (Vergara and Bezanilla, 1981).

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