VOLTAGE-SENSITIVE DYES

Discerning Contraction and Electrical Signals in Myocardium

BRUCE C. HILL AND KENNETH R. COURTNEY
Research Institute of the Palo Alto Medical Foundation, Palo Alto, California 94301

ABSTRACT Muscle contraction can introduce artifact in attempted optical measurements of the action potential in heart tissue stained with voltage-sensitive dyes. Using rabbit sinus node and atrial tissue in vitro, we found that the voltage-sensitive part of the optical signal remains relatively unchanged by variations in the rate of external stimulation or by the application of transmural stimulation (TS), while the contraction-related component can be significantly increased by these same interventions. The relative contributions of membrane voltage and contraction to the optical signal can thus be determined. In particular, the rapid upstroke component of the action potential can be easily identified using this technique.

INTRODUCTION

Dyes that bind to excitable membranes and change their optical properties in response to a change in membrane potential have been used to observe action potentials in both nerve and muscle (Cohen and Salzberg, 1978; Waggoner, 1979; Gupta et al., 1981). The stained tissue is illuminated with quasi-monochromatic or laser light, and the intensity of transmitted, reflected, or fluorescent light (depending on the type of dye being used) from one or more areas of interest is monitored by a photodetector system. Recent work has indicated that such dyes can be used to study the propagation of the action potential in frog heart (Sawanobori et al., 1981) and in mammalian heart (Dillon and Morad, 1981). The application to mammalian cardiac tissue holds considerable promise in studies of propagation disturbances that may underlie arrhythmogenesis. Such studies depend upon unambiguous identification of the rapid upstroke of the action potential to measure its propagation latency at various points in the heart. But, because the action potential is accompanied by contraction, it is necessary to distinguish between the optical signal resulting from the voltage sensitivity of the dye and the signal produced by the effects of contraction.[1]

To this end, we have exploited the fact that the contraction-related portion of the signal is increased by either lengthening the stimulation interval in tissue that is electrically driven (atrium) or transmurally stimulating (Amory and West, 1962; Vincenzi and West, 1963; Malik et al., 1979) spontaneously active tissue (sinus node). The action potential upstroke is not increased by these interventions, and thus there is no increase in the portion of the optical signal that represents the dye's voltage-sensitive response to the upstroke.

METHODS

Hearts were excised from New Zealand white rabbits (1.5–2 kg) after they were killed by cervical dislocation. Sinus node, right atrial, or combined sinoatrial (SA) tissue was excised and attached with several minutae pins along each tissue edge to a Sylgard (Dow Corning Corp., Midland, MI) surface in a 5 ml tissue bath. The use of many pins along the edges substantially reduces but does not eliminate contraction effects. The bath was perfused with a Tyrode's solution of the following composition (in millimoles per liter): NaCl, 137; KCl, 4.5; $NaHCO_3$, 12; $MgCl_2$, 1.1; $CaCl_2$, 1.8; and glucose, 5.5. The solution was gassed with 95% O_2–5% CO_2 and was passed through the bath at a rate of 2 to 3 ml/min at a temperature of 34°–35°C. Tissues were stained by voltage-sensitive dyes dissolved in the bath. Dye NK2761 (Nippon Kankoh-Shikiso Kenkyusho Co., Ltd., Okayama, Japan) was applied for 30 min at 2 mg/ml; dye WW781(supplied by Alan Waggoner, Dept. of Chemistry, Amherst College, Amherst, MA 01002) was applied for 5 min at 1 mg/ml.

[1]Included among the effects of contraction upon the optical signal in both stained and unstained tissue are movement-related contributions as seen in cultured heart tissue (Bucher, 1957; Sinclair, Miller and Harrison, 1970; Boder, Harley, and Johnson, 1971; Clusin, 1980) and in isolated cardiac Purkinje fibers (Kass, 1981). Other contraction related events such as changes in tissue thickness, slope, and contractile proteins could also contribute to the signal, which we found to be wavelength and location dependent in sinoatrial tissue. In tissue stained with a voltage-sensitive dye, the effect of contraction on the optical signal depends upon the dye being used and its modality (fluorescence or absorption). The fluorescent dyes tend to be less sensitive to contraction effects due, in part, to their relatively greater voltage sensitivity compared to absorption dyes.

For transmission measurements, light from a DC-powered 100-W tungsten-halogen lamp was collimated, passed through a narrow-band interference filter, and focused onto the tissue with a spot size of 2 mm. Fluorescence measurements were made by obliquely illuminating the tissue with 633 nm red light from a 5 mW HeNe laser (Spectra-Physics, Inc., Mountain View, CA, model 120) focused to a 100 μm spot on the tissue. In both modalities, light was collected by a 50-mm focal length lens and projected onto an image plane. The intensity from the illuminated area was detected by an avalanche photodiode (Texas Instruments, Inc., Houston, TX, model TIXL 69) with an area that corresponded to 90 μm diameter in the object plane. In the fluorescence mode, excitation light was blocked with a 645-nm long-pass filter (Schott Optical Glass, Inc., Duryea, PA, RG645) placed before the photodetector. Optical recordings were displayed as fractional changes in transmitted ($\Delta T/T$) or fluorescence ($\Delta F/F$) light intensity.

Intracellular recording techniques were used to monitor transmembrane potentials of the myocardium. Machine-pulled glass pipettes filled with 3 M KCl were attached to the headstage of a high-impedance preamplifier with a fine chloridized silver wire and thereby "floated" on the contracting tissue. Atrial flaps were driven with bipolar stimulating electrodes at a cut edge of the preparation using 3 ms pulses of 10 V amplitude. The transmural stimulation scheme (TS, also called electrorelease) of Amory and West (1962), which allows stimulation of the autonomic nerve endings in the SA node, without excitation of the myocardial fibers per se, was used to produce a poststimulation beat of greatly enhanced contraction (Vincenzi and West, 1963) without concommitant enhancement of the action potential upstroke (Amory and West, 1962). Bipolar stimulation was delivered via two minutae pins placed on opposite sides of the tissue; 50 pulses (70–100 V amplitude, 300 μs duration, 50 Hz frequency) were applied to these pins (F. Haer and Co., Brunswick, ME, Pulsar 6i stimulator). The large voltage was required to overcome current shunting by the bathing solution. The optical and microelectrode signals were displayed on a storage oscilloscope (Tektronix, Inc., Beaverton, OR, model 5111).

RESULTS AND DISCUSSION

The effect of TS in producing an optical signal with potentiated mechanical component is seen in Fig. 1 *A*. Intensity of light transmitted through unstained SA tissue was monitored by a photodetector while TS was applied. Because this tissue is unstained, the optical signal would not be expected to have a voltage-dependent component. A cessation of pacemaker activity caused by the cholinergic release (Malik et al., 1979) during TS is followed by a beat of greatly enhanced contraction that is clearly seen in the optical record. The effect of TS on action potentials is seen in Fig. 1 *B*. After the cholinergic rate pause, action potentials are usually smaller in both amplitude and upstroke velocity and are certainly never enhanced as is the first contraction after TS. Other studies have reported similar observations (Amory and West, 1962; Toda and West, 1967; Courtney et al., 1979).

The ability to differentiate voltage-sensitive and mechanical components in optical recordings using TS is seen in the faster sweeps of Figs. 2 and 3, which are typical recordings for two different dyes. Fig. 2 shows the action potential upstroke in atrial tissue stained with NK2761 and illuminated with 710 nm light. This dye is an analogue of dye XVII (Ross et al., 1977) and dye XXIII (Gupta et al., 1981) and has been used to record conducted action potentials in bullfrog atrium (Sawanabori et al., 1981) and

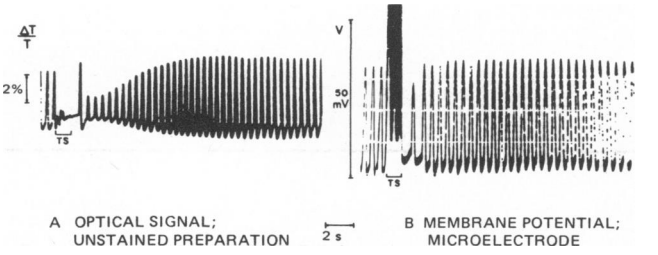

FIGURE 1 *A*, potentiation of contraction-related optical signal following transmural stimulation (TS). Recording is fractional intensity of 730 nm (50 nm half-power bandwidth) transmitted light passing through unstained sinus node tissue. Note cessation of contractile activity immediately following TS, which represents response to release of acetylcholine. This cholinergic pause is followed by an enhanced contraction. (The delayed response to adrenergic release is seen several beats later as an increase in both beat rate and size of the contraction signal.) *B*, lack of potentiation of electrical signal following TS. Intracellular microelectrode recording from a different sinus node preparation shows somewhat diminished action potential response immediately following TS.

to locate pacemaker activity in embryonic chick heart (Kamino et al., 1981). The upstroke is seen as an initial rapid decrease in the intensity of transmitted light followed by a later contraction-dependent signal. Application of TS results in a much larger contraction signal while the early upstroke-dependent (voltage sensitive) component remains relatively unchanged. A more striking distinction is seen in Fig. 3, a recording from sinoatrial tissue stained with the fluorescent dye WW781. This is dye XXV of Gupta et al. (1981) and has been used by Dillon and Morad (1981) to map propagated cardiac action potentials in frog and mammalian hearts. TS causes a severalfold increase in the contraction component, but the initial upstroke component is unchanged. In both preparations, TS allows unambigu-

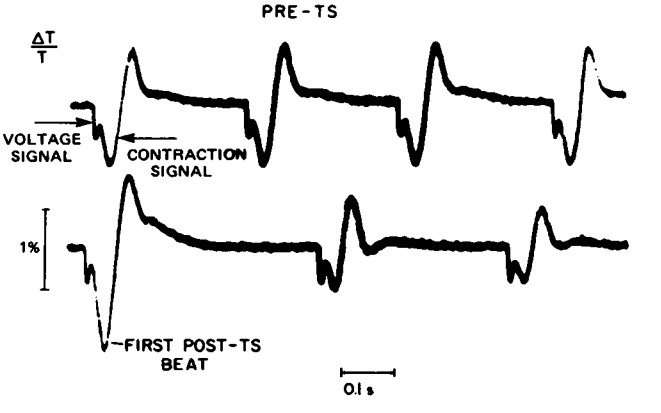

FIGURE 2 Effect of TS on optical recording from atrial tissue (with attached sinus pacemaker) stained with voltage-sensitive dye NK2761. Recording is transmitted intensity of 700 nm (9 nm bandwidth) light transmitted through atrial flap. Upper trace represents four pre-TS beats; lower trace begins with first beat following TS. Initial rapid decrease in transmitted light seen in each beat is voltage-sensitive response to action potential upstroke and is unchanged by TS; slower contraction-related component is greatly enhanced in first post-TS beat. Note the contraction signals in subsequent beats are reduced as in Fig. 1 *A*, although electrical component remains unaffected.

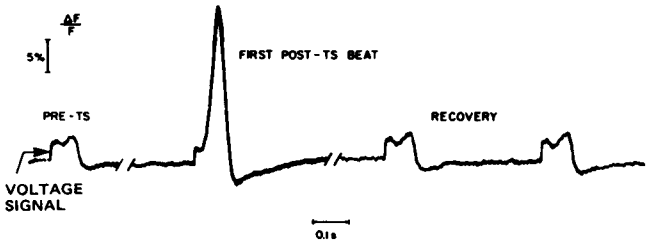

FIGURE 3 Effect of TS on optical recording from atrial tissue (with attached sinus pacemaker) stained with voltage-sensitive dye WW781. Tissue was illuminated with 633-nm laser light and intensity of fluorescent light of wavelength longer than 645 nm was recorded. First beat immediately precedes TS, then first post-TS beat, finally normal beats seen ~10 s following TS. Voltage-sensitive component is seen as initial rapid increase in intensity and is unchanged following TS while slower contraction-related component is enhanced severalfold.

ous identification of the rapid upstroke (voltage) component and thus permits use of the optical signal for latency measurements of the cardiac action potential at the observed point. If recordings are made from a preparation that is not spontaneously active, such as atria without attached pacemaker tissue, then varying the stimulation rate can significantly alter the contractile strength without affecting the rapid upstroke of the action potential. Fig. 4 shows optical recordings from NK2761 stained atria externally driven at two different rates. The slower rate results in a larger and somewhat longer contraction signal, but the initial upstroke component (seen as a transient decrease in intensity with this dye) is the same for both rates. In summary, simple schemes involving either changes in drive rate or release of autonomic transmitters can be used to modify the contraction of cardiac tissue without concomitant changes in the rapid upstroke of action potentials. Such a manipulation allows one to discern which components of optical signals observed during experiments with voltage-sensitive dyes really represent electrical event markers that will be useful for mapping electrical signal propagation in heart. This distinction is especially crucial in sinus pacemaker tissue, where the relatively slow action potential upstroke (compared with atrial tissue) is easily confused with contraction events.

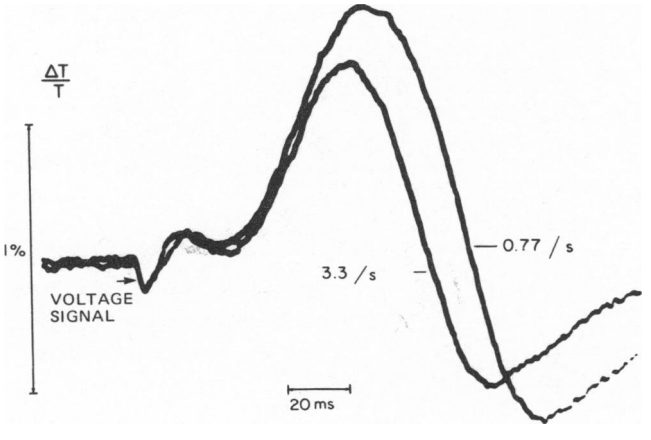

FIGURE 4 Effect of stimulation rate on optical signals from NK2761-stained atria. Transmitted light intensity at 520 nm (10 nm bandwidth) recorded with oscilloscope trace triggered before stimulus. Slower rate (0.77/s) produced a larger contraction component than faster rate (3.3/s), but initial voltage-dependent response (fast decrease in light intensity) was unchanged.

We thank Elaine Etter for her assistance with the experimental work and Carol Mead for her preparation of the figures.

This research was supported by grants from the National Institutes of Health (BRSG S07 RRO 5513 and HL 25489).

Received for publication 22 June 1982 and in revised form 10 August 1982.

REFERENCES

Amory D. W., and T. C. West. 1962. Chronotropic response following direct electrical stimulation of the isolated sinoatrial node: a pharmacologic evaluation. *J. Pharmacol. Exp. Ther.* 137:14–23.

Boder, G. B., R. J. Harley, and I. S. Johnson. 1971. Recording system for monitoring automaticity of heart cells in culture. *Nature (Lond.).* 231:531–532.

Bucher, O. M. 1957. A photoelectric recording set for pulsation curves of heart muscle cultures in vitro. *Exp. Cell Res.* 13:109–115.

Clusin, W. T. 1980. Correlation between relaxation and automaticity in embryonic heart cell aggregates. *Proc. Natl. Acad. Sci. U. S. A.* 77:679–683.

Cohen, L. B., and B. M. Salzberg. 1978. Optical measurements of membrane potential. *Rev. Physiol. Biochem. Pharmacol.* 83:35–88.

Courtney, K. R., R. A. Jensen, and E. E. Davis. 1979. Sodium ions affect adrenergic control of sinoatrial rate. *J. Mol. Cell. Cardiol.* 11:237–244.

Dillon S., and M. Morad. 1981. A new laser scanning system for measuring action potential propagation in the heart. *Science (Wash., D. C.).* 214:453–455.

Gupta, R. K., B. M. Salzberg, A. Grinvald, L. B. Cohen, K. Kamino, S. Lesher, M. B. Boyle, A. S. Waggoner, and C. H. Wang. 1981. Improvements in optical methods for measuring rapid changes in membrane potential. *J. Membr. Biol.* 58:123–137.

Kamino, K., A. Hirota, and S. Fujii. 1981. Localization of pacemaking activity in early embryonic heart monitored using voltage-sensitive dye. *Nature (Lond.).* 290:595–597.

Kass, R. S. 1981. An optical monitor of tension for small cardiac preparations. *Biophys. J.* 34:165–170.

Malik, R., K. R. Courtney, and R. A. Jensen. 1979. Imipramine affects autonomic control of sinoatrial rate in isolated right atrial preparations. *J. Pharmacol. Exp. Ther.* 211:284–289.

Ross, W. N., B. M. Salzberg, L. B. Cohen, A. Grinvald, H. V. Davila, A. S. Waggoner, and C. H. Wang. 1977. Changes in absorption, fluorescence, dichroism, and birefringence in stained giant axons: optical measurement of membrane potential. *J. Membr. Biol.* 33:141–183.

Sawanobori, T., A. Hirota, S. Fujii, and K. Kamino. 1981. Optical recording of conducted action potential in heart muscle using a voltage-sensitive dye. *Jap. J. Physiol.* 31:369–380.

Sinclair, A. J., H. A. Miller, and D. C. Harrison. 1970. An electrooptical monitoring technique for heart muscle cells in culture. *J. Appl. Physiol.* 29:747–749.

Toda, N., and T. C. West. 1967. Interactions of K, Na, and vagal stimulation in the S-A node of the rabbit. *Am. J. Physiol.* 212:416–423.

Vincenzi, F. F., and T. C. West. 1963. Release of autonomic mediators in cardiac tissue by direct subthreshold electrical stimulation. *J. Pharmacol. (Paris).* 141:185–194.

Waggoner, A. S. 1979. Dye indicators of membrane potential. *Annu. Rev. Biophys. Bioeng.* 8:47–68.