# FLUORESCENCE MONITORING OF RAPID CHANGES IN MEMBRANE POTENTIAL IN HEART MUSCLE

H. WINDISCH, W. MÜLLER, AND H. A. TRITTHART
*Univ.-Institut für Medizinische Physik und Biophysik, Universität Graz, Harrachgasse 21, A-8010 Graz, Austria*

ABSTRACT The rising phase of rat cardiac action potentials was measured in physiological solutions using the voltage-sensitive dye RH 237. A newly designed optical system and an argon ion laser for excitation allowed measurements without averaging over small areas (20–90 μm diameter) with high time resolution (response time 10–90%, 0.12 ms). The mean value of the fractional change in the fluorescence signal was ~3%/100 mV. The signal-to-noise ratio was ~60 rms (spot diameter 70 μm) allowing signal differentiation after digital filtering. Multiple measurements within the same spot showed a decrease in the fractional fluorescence change of 20 to 25% after 45 min without changes in the shape of the rising phase and with no measureable phototoxic effects. The optically measured rising phases showed rise times significantly ($P < 0.01$) shorter and maximum upstroke velocities equal to or most often greater than those obtained with microelectrode techniques. Comparing simultaneous optical and electrical measurements within the same spot the microelectrode signal was often slightly delayed. This refined system seems well suited to detect fast cellular electrical activities with time and space resolutions comparable or even superior to those obtained using microelectrode techniques.

## INTRODUCTION

Fluorescence signals from fast (i.e., rapidly-responding) voltage-sensitive dyes have provided a valuable method for measuring changes in the membrane potential of various cells and tissues (Davila et al., 1973; Salzberg et al., 1973; Salama and Morad, 1976; Ross et al., 1977; Morad and Salama, 1979; Dillon and Morad, 1981; Gupta et al., 1981; Grinvald et al., 1982; Hill and Courtney, 1982; and Grinvald et al., 1983; for reviews see Cohen and Salzberg, 1978 and Waggoner, 1979). The method is particularly useful in systems of complex structure and function and/or when electrode damage, e.g., by cell membrane penetration, is critical for the problem under study.

Action potentials in cardiac tissues and cells are usually measured using conventional microelectrode techniques. This well-established method is, however, hampered by difficulties arising in connection with the impalement itself, which are largely related to the tissue structure and to movement of cardiac muscle. Precisely placed and stable, long-lasting impalements are difficult to obtain and the feasibility of multi-electrode techniques is drastically limited. Therefore, optical probes to measure membrane potentials in heart muscle without cell impalement seem advantageous and have been applied successfully.

Spontaneous action potentials have been monitored in early embryonic chick hearts in normal solution (Hirota et al., 1979; Fujii et al., 1980, 1981) and in frog hearts exposed to low $Ca^{++}$- or $Ca^{++}$-free solution and absorption signals were detected that resembled the normal action potential (Morad and Salama, 1979; Sawanobori et al., 1981). The optical probe signals were, however, inferior to the microelectrode signals with regard to time and space resolution. In addition, low $Ca^{++}$-solutions introduce unphysiological conditions causing serious deterioration of membrane function and structure (Tritthart et al., 1973; Frank et al., 1982).

It was the aim of the present study to perform repetitive measurements of cardiac action potentials of mammalian heart fibers superfused with physiological solutions with a time and spatial resolution comparable to that commonly obtained in microelectrode measurements. Preliminary results were presented at the 8th International Biophysics Congress, Bristol, England, 1984.

## METHODS

### Preparations

Rats were killed by a blow to the neck. The hearts were quickly removed and placed in $O_2$-saturated and pH-balanced Tyrode's solution. Ventricular flaps or atrial sheets from either side were excised and transferred to the staining solution or the superfusion chamber.

### Solutions

In all experiments the preparations were superfused with dye-free Tyrode's solutions at 36.5 ± 0.5°C. The normal Tyrode's solution had the following composition: 132.1 mM NaCl, 2.7 mM KCl, 2.5 mM $CaCl_2$, 1.15 mM $MgCl_2$, 24 mM $NaHCO_3$, 0.42 mM $NaH_2PO_4$, and 5.6 mM D-glucose saturated with 95% $O_2$ and 5% $CO_2$. The pH was balanced at 7.2. KCl-enriched Tyrode's solutions for partial depolarization and

sodium inactivation were of identical composition but contained excess KCl up to 7.3 mM.

## Staining Procedure

Staining solutions were freshly prepared for each experiment from a stock solution of ethanol saturated with the fast voltage-sensitive dye RH 237 (4-[6-(4-dibutylaminophenyl)-1,3,5-hexatrienyl] 1-(4'sulfobutyl)pyridinium), first synthesized by Grinvald et al. (1982). The stock solution was diluted in $O_2$-saturated Tyrode's solution by 100- to 200-fold (~25–50 $\mu$M) and immediately thereafter the preparations were stained for ~8–12 min at room temperature.

## Lack of Toxicity of the Dye

The staining of cells can lead to toxic effects dependent on both the dye and the preparation (Cohen and Salzberg, 1978; Waggoner, 1979; Windisch and Müller, 1983). We performed experiments monitoring action potentials from atrial fibers via persistent microelectrode impalement during our entire staining procedure. Maximum upstroke velocity and action potential shape remained essentially unchanged. This indicates that dye molecules binding to the membrane have negligible effects upon excitation and relevant membrane properties.

## Apparatus

Optical measurements of the change in membrane potential were performed with a newly designed apparatus using the "Mikrobank-System" from Spindler and Hoyer, (Göttingen, W. Germany). The apparatus (Fig. 1) was mounted on a vibration isolation table. An argon ion laser (Coherent Innova 90-4, Neu Isenburg, W. Germany) in single-line mode with a wavelength of 514.5 nm and 2–2.5 W output power was used as the excitation source. The beam, after passing a Pockel's cell (Laser Optronic, München, W. Germany) or an electrically-driven mechanical shutter was expanded and adjusted by a telecentric system and then partially reflected (~10%) by a normal microscope slide placed at 45° to the beam direction. The reflected laser light illuminated an aperture that was located in the second image plane of a microscope system consisting of a 40-fold water-immersion objective (Zeiss achromat 40/0.75, NA 0.75, Carl Zeiss, Oberkochen, W. Germany) and an 80-mm achromatic lens. The objective, with a working distance of 1.6 mm, dipped into the Tyrode's solution. The overall magnification of this microscope system was 140-fold. By means of this system a reduced image of the illuminated aperture was formed on the stained preparation surface with a spot diameter varying from 5 $\mu$m (aperture essentially closed) up to 90 $\mu$m (aperture fully opened). The same system collected the light emerging from the illuminated spot and formed its magnified image on the aperture. The reflected and backscattered light passing the aperture was stopped by the barrier filter (KV 550, Schott Glaswerke, Mainz, W. Germany) whereas the fluorescence light, passing through this filter, was detected by a photodiode (RCA C30822, ENA Technik, Quickborn, W. Germany). The photo current signal, after amplification by a Keithley 427 current amplifier (Keithley Instrument Incorporation, Cleveland, OH), was fed to the first channel of a storage oscilloscope (Nicolet, series 2090, Nicolet Instrument Corporation, Madison, WI). To eliminate the effects of fluctuations of the exciting laser light a simultaneous reference measurement was performed with a second photodiode (RCA C30808). In the electrical path from the reference diode to the storage oscilloscope an RC-filter was inserted, producing essentially the same time response as the main signal path. The overall response time (10–90%) of the whole optical-electrical detection system was 0.12 ms. The usual sample rate of the oscilloscope was 100 kHz. The average intensity of the excitation light at the preparation surface was in the range of 90–200 W/cm$^2$. In spite of these high light intensities no bleaching correction was necessary due to the short exposure times of ~40 ms and the fact that the rising phase of an action potential lasts ~1 ms. The stored samples were passed to an HP 1000 MXE series computer (Hewlett Packard Company, Palo Alto, CA) for further analysis and disc storage.

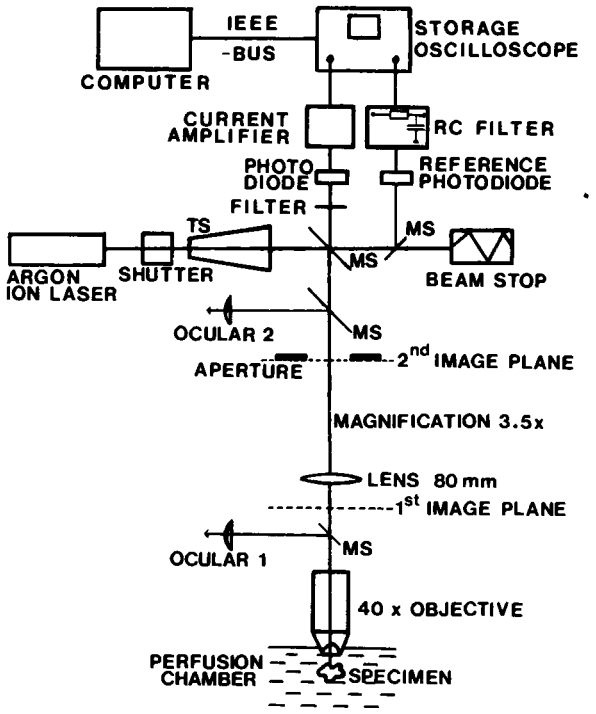


FIGURE 1 Schematic diagram of the apparatus used for fluorescence measurements of membrane potential changes. Fluorescence was excited with an argon ion laser and detected with a photodiode. The laser beam (2–2.5 W, 514.5 nm) was expanded and adjusted by the telecentric system TS. Conventional microscope slides (MS) provided, via partial reflectance: (*a*) incoupling of excitation light; (*b*) outcoupling of laser light to derive the reference signal; and (*c*) outcoupling of backscattered light to supply the oculars. Ocular 1 was used to select the measuring spot and ocular 2 to set the aperture diameter. The microscopic system consisted of a 40-× water immersion objective and an 80 mm achromatic lens providing an overall magnification of 140. The variable aperture in the second image plane was used to define an identical spot for both excitation by the laser light and collection of the fluorescence light. The spot diameter could be adjusted between 50 and 90 $\mu$m. The response time of the whole optical-electrical system was 0.12 ms (10–90%).

## Optical Measurements

Immediately after the staining procedure the preparations were mounted on the rubber floor of a superfusion bath which was moveable in all directions and located under the objective. At low light intensities a measuring spot was selected on the surface of the muscle and the spot diameter adjusted. In simultaneous optical and microelectrode measurements a microelectrode was impaled into the optically measured spot with a very flat angle through the gap between objective and preparation surface (1.6 mm). Usually, just before impalement of the electrode, an optical measurement was also taken. The standard microelectrode techniques applied are described earlier (Windisch and Tritthart, 1981).

## Signal Processing

Usually the following procedure was applied: To eliminate the noise of the exciting laser light the fluorescence signal was divided by the reference light signal. In a second step this signal was digitally low-pass filtered. The filtered signal was visually compared to the unfiltered on an overlay plot to determine the optimal filter parameters for each recording. For most measurements a cut-off frequency of 4 kHz and a filter order of 40 was chosen for the nonrecursive digital filter. The filtered signal was then differentiated digitally. For comparison with microelectrode measure-

ments the amplitude of the fluorescence change was normalized to the mean value of action potential amplitude obtained using a microelectrode. In this way an approximate value of the maximum rate of rise of the optically measured action potential was also obtained.

## RESULTS

### Fluorescence Signal

Fig. 2 *A* illustrates an optical single sweep measurement of the rising phase of a rat ventricular action potential. In this experiment the fractional change of fluorescence was −3.8% and the signal-to-noise ("typical" peak-to-peak noise) ratio was ~20:1 (rms signal-to-noise ratio ~60). The fast part of the rising phase was shorter than 0.5 ms. The peak value of the differentiated signal (Fig. 2 *B*) was 14%/ms. Assuming an action potential amplitude of 100 mV (typical for rat atrial and ventricular myocardium) this value for maximum differential amplitude would correspond to a maximum upstroke velocity of 371 V/s. A phaseplane plot (Fig. 2 *C*) of the same rising phase is well shaped, as estimated for a traveling wave front in an undisturbed transmission linelike structure (Paes de Carvalho, 1976; Campos de Carvalho and Paes de Carvalho, 1980). The fluorescence signal of the action potential plateau phase and repolarization is markedly disturbed by mechanical contraction artefacts (not shown). However, the electromechanical coupling latency allows an undisturbed measurement of the excitation onset, i.e., the rising phase of the cardiac action potential.

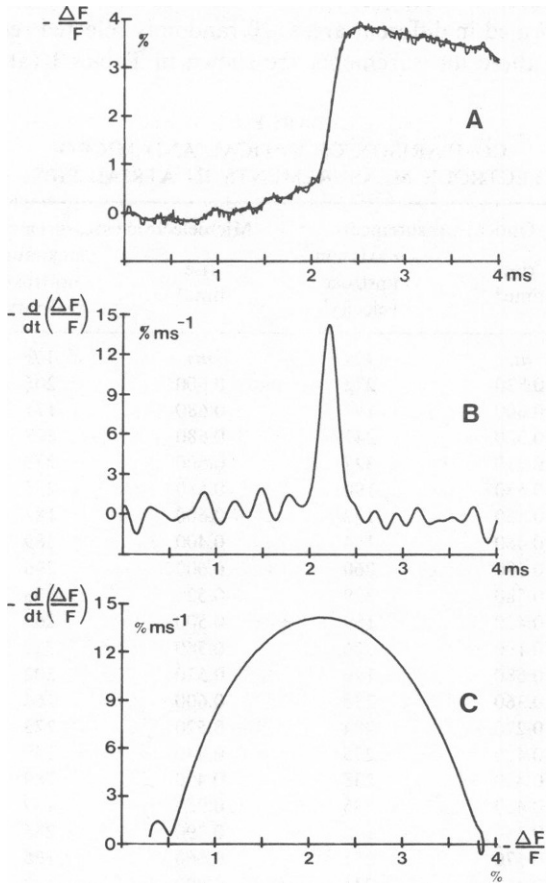


FIGURE 2 Example of fluorescence measurement of a cardiac action potential rising phase obtained from rat ventricle. The preparation was stained with the voltage sensitive dye RH 237. The diameter of the measuring spot was 70 μm. Averaging was not used. (*A*) Time course of the fractional fluorescence change ($\Delta F/F$). The superimposed smooth trace was obtained by digital filtering. (*B*) First time derivative of the fractional fluorescence change. Assuming an action potential amplitude of 100 mV the peak value would correspond to an upstroke velocity of 371 V/s. (*C*) Phase plane plot of the rising phase.

### Influence of Spot Size

Electrically driven atrial and ventricular preparations show spread of excitation with tissue type-dependent velocities in the range of 0.3 to ~2 m/s. To avoid the integration of fluorescence signals obtained from distant locations with their associated time lags, the measuring spot should be as small as possible. We have performed measurements of the rise time of excitation with spot diameters in the range of 20–90 μm. Meaningful single sweep measurements, using diameters below 20 μm, though possible by the optical system (see Methods), were limited by the chosen light intensity. The exciting light distribution within a 90 μm spot was fairly homogeneous as was the distribution of the fluorescence signals within the whole illuminated area. We have compared rise times (10–90% of the rising phase amplitude) and upstroke velocities of spots with diameters of 50 μm or less to those of 70 to 90 μm. Decrease in the spot size below 50 μm did not lead to any significant changes in the rise time.

Experiments on six different spots using 41 single sweep measurements indicate prolongation of the rise time by ~25% and a diminution of the maximum upstroke velocity of ~12% when the spot size was increased from 50 μm or below to 70–90 μm. Using a spot diameter of 90 μm, the measured rise times and upstroke velocities were still as fast as or even faster than those measured using microelectrode impalement (see also Fig. 4). An increase above 90 μm would lead to a better signal-to-noise ratio but also to a further decrease in the maximum upstroke velocity and prolongation of the rise time (Morad and Salama, 1979; Sawanobori et al., 1981).

### Reproducibility of Optical Measurements

We have performed a series of optical measurements of the rising phase of rat ventricular action potentials within the same measuring spot. The preparation was stimulated continuously at 0.7 Hz and optical measurements were repeated at 2–4 min intervals. The excitation wave passed the measuring spot with a constant time delay between stimulus and roughly constant maximum rate of rise. An evaluation of 10 measurements at a particular 50 μm spot produced an absolute mean value of $\Delta F/F$ of $-4.11 \pm 0.35\%$ and a maximum rate of rise of the normalized fluorescence signal of $3.95 \pm 0.47$ ms$^{-1}$. This would

correspond to 395 ± 47 V/s for 100 mV action potential amplitude. Reproducibility increases with spot size due to the improvement in signal-to-noise ratio.

Over a longer time range (30–60 min) reproducibility is influenced by dye washout and bleaching. A typical decrease in $\Delta F/F$ for the action potential amplitude was ~20–25% over a time span of 45 min of continuous superfusion with ~20 measurements at 40 ms duration each for a total light exposure of ~800 ms. Superfusion duration rather than the number of optical measurements appeared to be the important factor in signal attenuation. In further experiments the extracellular potassium concentrations were raised in order to study the effects of a decrease in resting potentials upon the rate and amplitude of the rising phase of the corresponding action potentials. Fig. 3 shows the effects of an increase in the potassium concentration from 2.7 to 8 or 10 mM. Action potential amplitudes are not normalized here and the superimposed curves of the rising phases show a decrease in maximum rate of rise (Fig. 3 *B*) and in rate of depolarization when approaching the overshoot at both 8 and 10 mM potassium concentrations as well as a decrease in amplitude at 10 mM potassium concentration. These curves were measured 10 min after changes in the concentrations and subsequent washout effects by 2.7 mM potassium concentration were measured 45 min after the control measurement. $\Delta F/F$ decreased by 25% during the 45 min, but the original control curve and the normalized washout signal (dotted lines) show almost identical time courses (Fig. 3 *A*) and first derivatives (Fig. 3 *B*). These findings also indicate that after >20 exposures to laser light (40 ms each) there were no measurable phototoxic effects. Although in all these experiments no absolute values of the action potential amplitudes could be obtained, characteristic changes in the slope of the rising phase (e.g., Windisch and Tritthart, 1981) could be detected by optical measurements.

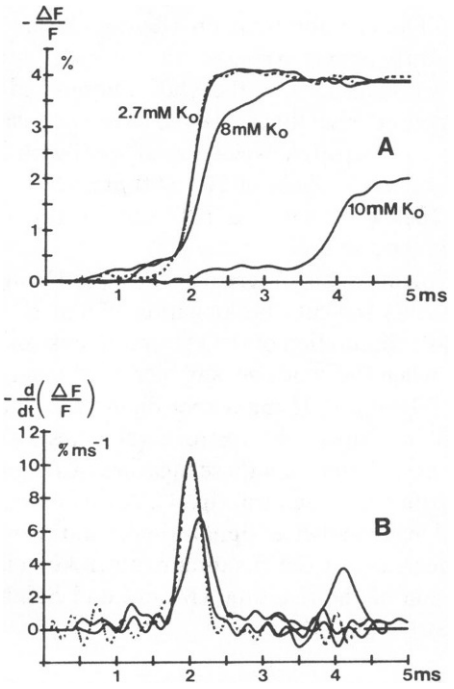


FIGURE 3 Optically measured rising phases of rat ventricle action potentials obtained after superfusion for 10–12 min with solutions of different potassium concentrations ($K_o$). The diameter of the measuring spot was 70 μm. Digital filtering without averaging was performed. (*A*) Time courses of the fractional fluorescence changes. (*B*) First time derivatives. In solutions containing higher potassium concentrations (8 and 10 mM) the rising phases are prolonged and the peak values of the differentiation signals are diminished. The onset of excitation at higher potassium concentrations is delayed due to a decrease in conduction velocity. The dotted lines represent the rising phase and its time derivative obtained after 12 min wash in normal potassium concentration (2.7 mM), 45 min after taking the control measurement in the same solution. For comparison these curves are normalized to the amplitude of the control curves.

## Fluorescence vs. Microelectrode Measurements

In atrial and ventricular fibers, three to five fluorescence measurements using spot diameters from 50 to 90 μm were performed in different areas. 20 randomly selected results from these measurements are shown in Tables I (atrial)

TABLE I
COMPARISON OF OPTICAL AND MICROELECTRODE MEASUREMENTS IN ATRIAL FIBERS

| Optical measurements | | Microelectrode measurements | |
|---|---|---|---|
| rise time* | maximum upstroke velocity‡ | rise time* | maximum upstroke velocity |
| *ms* | *V/s* | *ms* | *V/s* |
| 0.830 | 272 | 0.600 | 206 |
| 0.600 | 197 | 0.680 | 171 |
| 0.320 | 247 | 0.680 | 308 |
| 0.210 | 327 | 0.600 | 270 |
| 0.530 | 190 | 0.580 | 236 |
| 0.550 | 172 | 0.600 | 187 |
| 0.460 | 164 | 0.400 | 289 |
| 0.590 | 260 | 0.600 | 296 |
| 0.580 | 299 | 0.520 | 233 |
| 0.420 | 189 | 0.500 | 269 |
| 0.190 | 326 | 0.340 | 370 |
| 0.580 | 196 | 0.520 | 302 |
| 0.360 | 235 | 0.600 | 164 |
| 0.270 | 284 | 0.520 | 273 |
| 0.420 | 295 | 0.840 | 149 |
| 0.420 | 238 | 0.490 | 289 |
| 0.450 | 235 | 0.950 | 159 |
| 0.350 | 146 | 0.390 | 285 |
| 0.570 | 251 | 0.560 | 126 |
| 0.410 | 231 | 0.900 | 123 |
| 0.455 ± 0.15 ms | 238 ± 52.9 V/s | 0.592 ± 0.16 ms | 235 ± 70 V/s |
| $\Delta F/F$ = 2.5 ± 0.54%§ | | APA = 98 ± 7 mV‖ | |

*The rise time was measured 10 to 90% of action potential amplitude.
‡Maximal upstroke velocity, adjusted according to the individual factor used to normalize the fluorescence change to the average action potential amplitude.
§$\Delta F/F$ is the average fractional fluorescence change.
‖APA is the average action potential amplitude.

and II (ventricular). Duration of the rise time from 10 to 90% and rate of rise of the normalized curves are given and compared with corresponding microelectrode measurements. In atrial fibers values for maximum rate of rise measured by microelectrodes and via the fluorescence signals were almost identical but rise time was significantly shorter ($P < 0.01$) in the fluorescence measurements. However, in rat ventricular fibers (Table II), maximum rate of rise of action potentials measured by microelectrode was significantly lower ($P < 0.005$) than that derived from the normalized fluorescence signal. As in atrial fibers duration of rise time was also significantly shorter in ventricular fluorescence measurements ($P < 0.01$). The mean values of $\Delta F/F$ as well as action potential amplitude are also indicated in Tables I and II. Using identical staining procedures $\Delta F/F$ was in most experiments greater in ventricular than in atrial fibers.

In a series of experiments simultaneous optical and microelectrode measurements were performed in rat atrial and ventricular fibers within the same spot (90 $\mu$m). Fig. 4 shows one example of this type of experiment for an atrial preparation. In Fig. 4 *A* the normalized fluorescence signal before impalement of the microelectrode and the ensuing normalized electrical microelectrode signal are superimposed. These signals are very similar in shape and the fluorescence signal is as fast as or even faster than the electrical signal but slightly disturbed by noise. In Fig. 4 *B* the optical and electrical signals were obtained simultaneously from the same excitation, and the rising phases are compared after normalization. Again, a nearly identical slope is evident. Evaluation of 12 similar measurements indicated that although the microelectrode was impaled within the optically defined spot, time differences often occurred between the onset of the fluorescence and that of the electrical signal. In 7 out of 12 measurements the fluorescence signal occurred earlier (up to 0.35 ms), in four measurements both signals occurred almost simultaneously (time differences <0.05 ms, e.g., Fig. 4), and in one experiment the electrical microelectrode signal actually started ~0.1 ms earlier than the optical signal. In a few cases striking differences in shape and upstroke velocities

TABLE II
COMPARISON OF OPTICAL AND MICROELECTRODE MEASUREMENTS IN VENTRICULAR FIBERS

| Optical measurements | | Microelectrode measurements | |
|---|---|---|---|
| rise time* | maximum upstroke velocity‡ | rise time* | maximum upstroke velocity |
| *ms* | *V/s* | *ms* | *V/s* |
| 0.310 | 152 | 0.620 | 320 |
| 0.330 | 360 | 0.680 | 189 |
| 0.340 | 273 | 0.540 | 207 |
| 0.700 | 272 | 0.640 | 230 |
| 0.510 | 243 | 0.500 | 246 |
| 0.520 | 237 | 0.350 | 303 |
| 0.700 | 253 | 0.260 | 117 |
| 0.770 | 194 | 0.900 | 145 |
| 0.690 | 320 | 0.910 | 137 |
| 0.530 | 245 | 0.940 | 146 |
| 0.550 | 225 | 0.986 | 117 |
| 0.640 | 294 | 0.820 | 127 |
| 0.560 | 202 | 0.810 | 174 |
| 0.250 | 328 | 0.700 | 170 |
| 0.350 | 321 | 0.720 | 179 |
| 0.460 | 394 | 0.440 | 278 |
| 0.760 | 239 | 0.760 | 175 |
| 0.470 | 265 | 0.660 | 173 |
| 0.530 | 196 | 0.600 | 208 |
| 0.540 | 199 | 0.860 | 133 |
| 0.525 ± 0.15 ms | 261 ± 61 V/s | 0.685 ± 0.2 ms | 189 ± 60 V/s |
| $\Delta F/F$ – 2.9 ± 1.08%§ | | APA – 99 ± 4 mV‖ | |

*The rise time was measured 10 to 90% of action potential amplitude.
‡Maximal upstroke velocity, adjusted according to the individual factor used to normalize the fluorescence change to the average action potential amplitude.
§$\Delta F/F$ is the average fractional fluorescence change.
‖APA is the average action potential amplitude.

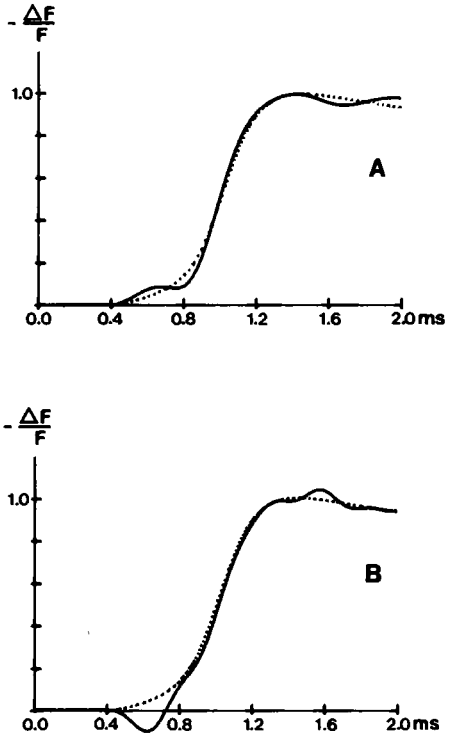


FIGURE 4 Comparisons of optical (solid line) and microelectrode (dotted line) measurement within the same measuring spot in a rat atrial preparation. The spot size was 90 $\mu$m and the signals were digitally filtered. Averaging was not used. For comparison all signal amplitudes are normalized to 1. In *A* the time course of the fractional fluorescence change before impalement and an ensuing microelectrode measurement are compared. The signals are very similar in shape and the fluorescence signal is as fast as or even faster than the electrical signal. In *B* optical and microelectrode measurement were performed simultanously. Again the signal shapes are nearly identical. In *B* the optical signal noise is greater than in *A*. Microelectrode impalement in the measuring spot attenuates the optical signal amplitude (not detectable in the normalized curves) and leads to a decrease in the signal-to-noise ratio.

were found. Though the microelectrode was located superficially in the area of the optical measuring spot, the possibility of impaling structures of different electrical features cannot be excluded.

A general finding in this type of experiment was the presence of microelectrode-induced disturbance of the fluorescence signal. After impalement by the microelectrode the fluorescence signal amplitude was diminished and the signal-to-noise ratio reduced. In one measurement following impalement in a central area, the fluorescence signal was even attenuated to noise level. In this experiment the very tip of the microelectrode remained sharply focused, indicating an impalement in the uppermost cell layer. The normal finding was marked attenuation of the fluorescence signal and a slight reduction in the rate of rise after microelectrode impalement.

## DISCUSSION

We have developed a new optical system to measure the rising phase of cardiac action potentials in mammalian heart tissue using a fast fluorescence probe. The apparatus resembles that described by Grinvald et al. (1982) but uses an argon ion laser instead of a conventional light source for excitation. The use of a He-Ne laser in connection with other dyes (e.g., WW 781 as used by Dillon and Morad, 1981) is also possible with our system. However, with this laser and our staining procedure the fractional change obtained with WW 781 in guinea pig preparations was found to be slightly smaller than that with RH 237 excited by an argon ion laser. The powerful argon ion laser itself is superior for simultaneous multipoint measurements and also offers a number of different wavelengths. An advantageous feature of our system is the definition of the size of the exciting light spot by passing the excitation light through the same aperture that also determines the optically measured area. This arrangement assures an exactly defined measuring spot as well as convenient visually controlled selection of both its size and position. Rising phases of cardiac action potentials are of short duration with high upstroke velocities. Therefore, the response time (10–90%) of the whole measuring system was held to within 0.12 ms, considerably shorter than the more usual millisecond range (Davila et al., 1973; Salzberg et al., 1973; Sawanobori et al., 1981; Grinvald et al., 1982). Even faster responding systems with a time resolution down to 2 $\mu$s have been used in measurements of other preparations, e.g., squid axons (Cohen et al., 1974; Ross et al., 1977; Loew et al., 1985).

Average values of $\Delta F/F$, obtained from rat atrium and rat ventricle were $-2.5\%$ and $-2.9\%$, respectively. Every single value obtained was much lower than those found by Grinvald et al. (1982) for neuroblastoma cells in cell culture using the same dye ($-14\%$). The difference is probably due to a much larger background fluorescence in cardiac multicellular preparations because of dye binding to nonmyocardial cells, particularly to the endocardium.

The first time derivative of the filtered signals (e.g., Figs. 2 *B* and 3 *B*) provides information about the depolarizing current through its peak value (Walton and Fozzard, 1979) as well as the possibility of exact measurement of time lags between such peaks (see Fig. 3 *B*). A comparison of optical and electrical measurements of rapid potential changes in various cells and tissues has typically been done to verify the optical measurement technique (see also Davila et al., 1973; Salzberg et al., 1973; Ross et al., 1974; Cohen et al., 1974; Salama and Morad, 1976; Salzberg et al., 1977; Morad et al., 1979; Sawanobori et al., 1981; Grinvald et al., 1982). We have also performed such experiments and, as shown in Fig. 4 *B*, within the noise deviation of the optical signal there are negligible differences in the shapes of the rising phase. However, doing a series of such experiments with simultaneous optical and electrical measurements within the same spot showed that often time lags of up to ~0.35 ms were observed between the optical and microelectrode signals, most often with the optical signal leading. As indicated by experiments in cardiac tissue performed by Tranum-Jensen and Janse (1982), at stable microelectrode impalements the very tip of the microelectrode was nearly always found in deeper cell layers (100 to >200 $\mu$m from the surface). In contrast, the following findings support the assumption that we obtained fluorescence signals from the surface cell layers:

(*a*) Even a slight touch of the muscle surface (e.g., by the objective) was sufficient to suppress the optical signal although the preparation showed normal mechanical activity.

(*b*) Rising phases, measured either by fluorescence techniques or by microelectrode impalement in the inner ventricular surface, showed that usually the upstroke velocities obtained by optical technique were significantly higher (Table II). The same series of experiments performed in atrial tissue led to similar upstroke velocities by both techniques. Fast conducting cell systems with higher rate of rise are typically found in the inner ventricular surface but are rarely found in atrial surfaces (Myerburg et al., 1978).

(*c*) The time lags between optical and microelectrode signals within the same measuring spot mentioned above likely reflect differences in the spread of excitation between the surface layer and deeper regions.

An optical signal, integrating the fluorescent light in the radial direction can only show a prolongation of the "true" rising phase together with a reduction in the maximum rate of rise when time lags are present. This effect is described by Morad and Salama (1979), who stained not only the surface of the muscle but the whole preparation. The same integration occurs when optical absorption techniques are used to detect the excitation onset in multicellular preparations, thus limiting drastically the real spatial-temporal resolution of the measurement (Sawanobori et al., 1981). The average values of our optically measured rise times in both atrial and ventricular preparations were

shorter than those obtained by microelectrode measurements (Tables I and II). These findings indicate that the space integrating property of the microelectrode is at least equal to that of our optical techniques. Though the very tip of a usual microelectrode is geometrically localized within <0.5 μm (the tip diameter), the impaled cell together with neighboring cells lying within the path of the impaling tip form a small group of cells with various degrees of damage which are in electrical contact with the muscle mass (Tranum-Jensen and Janse, 1982). Thus, the space resolution of the microelectrode is likely to be multicellular and does include some effects of cell damage. This suggestion is supported by our results indicating that average values of rate of rise of microelectrode action potentials are not higher than those optically measured in spots with 70–90 μm diam. In addition, microelectrode impalement itself often led to a decrease in the optical signal and maximal upstroke velocity, compared with those obtained optically before impalement. The diminution of the optical signal by microelectrode impalement may be partially due to disturbances of the light path by the microelectrode. It is tempting to speculate that the observed shorter rise times (10–90%) of optical measurements are due to the ability of optical measurements to detect the signal without cell contact and damage. In summary, the results show the feasibility of optical measurements of the rising phase of the cardiac action potential with time and spatial resolution comparable, or in some respects superior, to that of a standard microelectrode technique. Several of the improvements incorporated in our measuring system (e.g., restriction of the illuminated area to the measuring spot and optically controlled selection of its size and position) could certainly be applied advantageously to optical measurements in other tissues, cells, and organelles.

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