Interstitial potential during propagation in bathed ventricular muscle

Stephen B. Knisley, Toru Maruyama, and Jack W. Buchanan, Jr.
Division of Cardiology, The University of North Carolina, Chapel Hill, North Carolina, USA

ABSTRACT Theoretical simulations have suggested that interstitial potential (V_{is}) during action potential propagation affects measurements of the transmembrane action potential in bathed ventricular muscle. To evaluate the V_{is} experimentally, we obtained V_{is} and intracellular action potential (V_{ic}) recordings at various depths in paced guinea pig papillary muscles bathed in oxygenated Tyrode's solution. The peak-to-peak amplitude and the maximum dV/dt (dV/dt_{max}) of the intrinsic downward deflection of the V_{is} recordings were determined. The transmembrane action potential (*TM*) was obtained by subtracting each V_{is} from the corresponding V_{ic} recording, and measurements for the phase zero depolarization and action potential foot of the V_{ic} were compared with the measurements for the *TM*. At penetration depths of ~54 μm, the amplitude and dV/dt_{max} of the V_{is} were 13 mV and −38 V/s. When the depth was increased to 200 μm, these parameters increased to 24 mV and −59 V/s ($P < 0.005$), and when the depth was further increased to 390 μm, the parameters decreased to 16 mV and −38 V/s. Because of the V_{is} at the various depths, the V_{ic} underestimated dV/dt_{max} of phase zero of the *TM* by 20–31%, which would reduce estimates of Na^+ current obtained from dV/dt. Also, the V_{ic} overestimated the time constant of the 2–8 mV foot of the action potential by 48–82%, which would reduce estimates of the "effective" membrane capacitance by 33–45%. These influences of the V_{is} on measurements may affect results of quantitative studies of the ventricular action potential.

INTRODUCTION

Most of the myocardial space is intracellular (Page, 1962; Polimeni, 1974). Because the myocardial interstitial space is restricted, the interstitial resistance is high in comparison with the resistance of a saline bathing solution. Therefore, local circuit current during action potential propagation in a bathed cardiac muscle may produce a larger potential in the interstitium than in the bathing solution. Simulations that used a bidomain model of a cardiac muscle in a saline bath have indicated that an interstitial potential (V_{is}) is present at the leading edge of the propagated action potential (Plonsey and Barr, 1987; Roth, 1988). At interstitial sites ~0.5 mm below the muscle surface, the peak-to-peak amplitude of the V_{is} obtained in the simulations was ~11 mV for transverse propagation (Plonsey and Barr, 1987) and ~20–25 mV for longitudinal propagation (Roth, 1988; Henriquez et al., 1988). For interstitial sites near the surface of the muscle, the V_{is} in the simulations was smaller and more sensitive to the depth of the site than for interstitial sites deep in the muscle (Plonsey and Barr, 1987; Roth, 1988; Geselowitz et al., 1982).

Intracellular potentials (V_{ic}) recorded near the surface of a ventricular muscle are often interpreted as transmembrane potentials (*TM*) when the intra- and extracellular electrode pair are positioned close together or when the extracellular electrode is located in a low-resistance solution bath surrounding the muscle. If ventricular muscle has a V_{is} that is sensitive to the depth of the recording, as predicted in the simulations (Roth, 1988), then the V_{ic} action potential may differ from the *TM*. For example, a V_{ic} recording includes any potential difference that is present between the site of the extracellular reference electrode and the interstitium outside of the impaled cell, though this potential difference is not part of the true *TM*. Because of the difficulty in positioning microelectrodes precisely on either side of the same area of a cell membrane in ventricular muscle, some error due to this potential difference may be unavoidable. If the V_{is} does affect *TM* measurements, it would influence electrical parameters determined from phase zero of the action potential. Therefore, it is important to know how strong an influence the V_{is} has on *TM* measurements.

To determine the magnitude of the V_{is} in ventricular muscle, and to evaluate the importance of the V_{is} to *TM* measurements, V_{ic} and V_{is} were recorded from a single microelectrode at varying depth in bathed guinea pig papillary muscles. The *TM* was obtained using a method which allowed a comparison of action potential measurements for V_{ic} and *TM* at each depth.

Dr. Stephen B. Knisley's present address is Department of Biomedical Engineering, P. O. Box 3140, Duke University Medical Center, Durham, NC 27710.

METHODS

Papillary muscles were removed from the hearts of six methoxyflurane-anesthetized 200–250 g guinea pigs. The muscles were 0.8–1.1 mm in diameter and each muscle was ~5 diam long when gently stretched ~15% of its slack length. The muscles were placed in a tissue bath and superfused at a rate of 3–4 ml/min with a solution containing (in millimolar) 5.0 glucose, 1.8 $CaCl_2$, 125 NaCl, 5.4 KCl, 1.05 $MgCl_2$, 24 $NaHCO_3$, and 0.42 NaH_2PO_4. The solution reservoir was bubbled with a mixture of 95% O_2, and 5% CO_2, and had a pH of 7.4, and a temperature of 36°C.

Recordings were obtained for stimulation at either of two multipolar stimulation electrodes located at the ends of the muscle. The stimulation voltage pulses were 1.5 ms in duration and two times the diastolic threshold strength. The V_{ic} and V_{is} were recorded differentially between a 10–20 MΩ 3 M KCl-filled glass microelectrode and a stationary Ag/AgCl electrode, ~7 mm long, positioned at one side of the bath parallel to the muscle. Before penetrating the muscle, the microelectrode was positioned near the center of the muscle and oriented perpendicular to the muscle surface. It was then lowered in steps to obtain a series of recordings, beginning at the muscle surface, and then inside cells and in the interstitium. The amount of lowering that was performed between each of the intracellular impalements in a series did not vary greatly. Only the fine vertical position control of the micromanipulator (Prior Scientific Instruments, Ltd., Fulbourn, Cambridge, England) was turned when lowering the microelectrode between recordings. The V_{ic} impalements were counted by assigning each a number in order of increasing depth. After obtaining the deepest impalement, the displacement necessary to withdraw the microelectrode to the muscle surface was determined and used to estimate the total penetration depth. The mean depth increase per counted V_{ic} impalement was calculated for each series of recordings, and the depth of each recording was estimated by assuming that all counted impalements for that series had the same depth increase (i.e., the estimated depth of a recording = the mean depth increase per counted impalement × the impalement number of the recording). The amplitude of the signal was monitored continually on a digitizing oscilloscope. The signals obtained from the V_{ic} impalements were recorded only if the V_{ic} amplitude, measured from the preactivation baseline potential to the action potential plateau, exceeded 105 mV. All V_{ic} impalements were counted in estimating the penetration depth per V_{ic} impalement. After each V_{ic} recording, the microelectrode was lowered by the smallest amount necessary for it to come out of the cell. The signal obtained after the microelectrode was outside of a cell was recorded as a V_{is} only if the potential 10 ms after the intrinsic deflection was within 2 mV of the preactivation baseline potential. The monitored signals frequently met this criteria within the 3–5 min after the microelectrode came out of a cell. During that period, the intrinsic deflection which had been an upward deflection, became a downward deflection. Also the potential ~10 ms after the intrinsic deflection, which had been positive with respect to the preactivation baseline potential, became similar to the baseline.

The potentials were amplified at a gain of 10 and a passband of dc to 8,000 Hz. The capacitance compensation of the V_{ic} and V_{is} recording amplifier was adjusted at the beginning of each experiment to produce an ~10% overshoot in the step potential when a rectangular 4 nA current pulse was passed through the microelectrode tip in the bathing solution. Before the muscle penetration began, the amplifier dc offset was adjusted to cancel the microelectrode tip offset potential. No further dc offset adjustment was performed. All recordings were digitized at a sampling rate of 100 KHz and a voltage resolution of 0.04 mV (12 bit). A programmable digitizing oscilloscope (Norland Corporation, Fort Atkinson, WI) was used to store the recordings on magnetic discs and analyze them after the experiment. Each of the V_{is} recordings was numerically subtracted from the corresponding V_{ic} recording to obtain the *TM*. Before each subtraction was performed, it was necessary to ensure that the activation underlying the V_{is} and V_{ic} occurred at the same time in the two recordings. This was accomplished by recording, simultaneously with each V_{ic} and V_{is}, the potential at another glass microelectrode that remained at a constant position on the muscle surface near the epicenter of the impaling microelectrode. The activation time obtained from the maximum slope of the intrinsic deflection of the surface recording verified the uniformity of the activation time for each V_{ic} and V_{is} recording pair.

The peak-to-peak amplitude and dV/dt_{max} were determined using digital measurement cursors and digitally-produced time derivatives of the recordings. The time constants of the foot (τ_f) of the V_{ic} and *TM* were measured using linear regression of the logarithms of the data points of depolarization between 2 and 8 mV. This method gave τ_f as the reciprocal of the regression slope. Statistical significance was determined using Student's t test for paired data. Values are given as the mean ± 1 SD.

RESULTS

Fig. 1 shows the V_{ic} and V_{is} recordings obtained at increasing penetration depth in a representative guinea pig papillary muscle. The V_{ic} impalements were numbered ordinally for identification and for estimating the penetration depth. The V_{is} recordings obtained after the microelectrode came out of a cell were assigned the

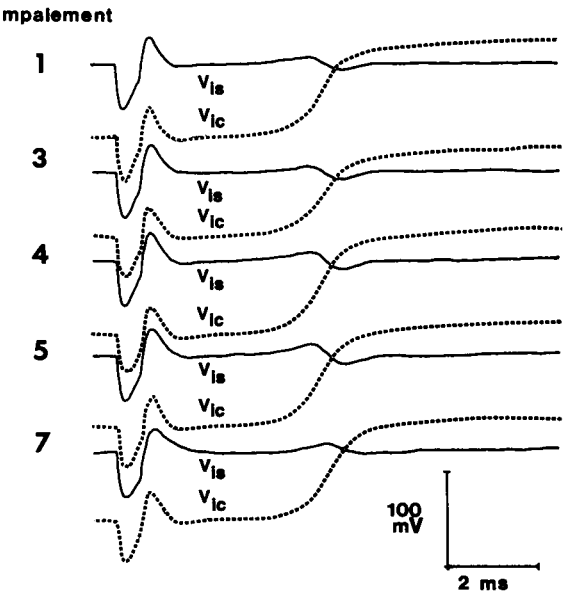

FIGURE 1 Interstitial potential (V_{is}, *solid lines*) and intracellular potential (V_{ic}, *dashed lines*). The impalement numbers are approximately proportional to the depths at which the recordings were obtained. The biphasic deflections at the beginning of each trace are stimulation artifacts. The negative intrinsic deflection of the V_{is} occurred during the rapid phase zero depolarization of the V_{ic} action potential. The V_{is} potentials were biphasic and returned to their preactivation baseline.

same number as the corresponding V_{ic} recordings. Impalements 2 and 6 in this experiment did not satisfy the criteria for V_{is} and V_{ic} records.

The surface and V_{is} recordings were biphasic, first increasing gradually, then decreasing rapidly to potentials below the preactivation baseline potential (intrinsic negative deflection), and finally returning to the baseline potential. The peak-to-peak amplitude and dV/dt_{max} of the extracellular potential at the muscle surface in this experiment were 7.2 mV and −25.3 V/s. These values are smaller than the corresponding values that were obtained at the most superficial V_{is} recording site, ~50 μm deep. The intrinsic negative deflection of the V_{is} occurred at the same time as phase zero of the corresponding V_{ic} action potential.

Fig. 2 illustrates the dependence of the V_{is} peak-to-peak amplitude and dV/dt_{max} on the penetration depth for the recordings shown in Fig. 1. The most superficial V_{is} recording (Fig. 1, *impalement 1*) had an amplitude and dV/dt_{max} of 12.1 mV and −34.0 V/s. The greatest V_{is} amplitude was observed after increasing the penetration depth to the fifth V_{is} impalement, which had an amplitude and dV/dt_{max} of 21.2 mV and −55 V/s. A further increase in the penetration depth to the seventh V_{is} impalement resulted in a decrease of the V_{is} amplitude and dV/dt_{max} to 10.5 mV and −26 V/s. In the experiment shown, the mean depth increase per counted V_{ic} impalement was 54 μm, and the estimated depth where the V_{is} was greatest was 270 μm.

Table 1 shows the amplitude and dV/dt_{max} of the V_{is} from all experiments. The most superficial impalements that gave signals that satisfied the criteria for V_{is} and V_{ic} recordings (Methods) had a mean penetration depth of

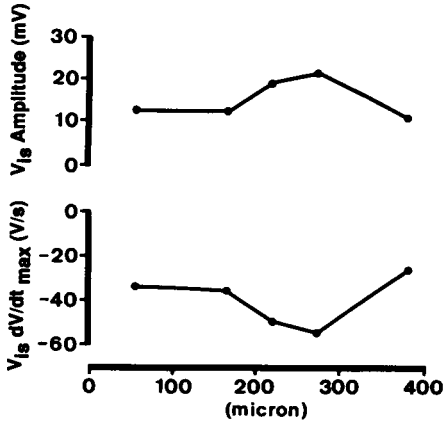

FIGURE 2 Measurements for the V_{is} recordings shown in Fig. 1. The peak-to-peak amplitude (*upper*) and dV/dt_{max} of the intrinsic deflection (*lower*) of the interstitial recordings became greater when the penetration depth was increased to ~270 μm, and then became smaller when the penetration depth was further increased.

TABLE 1 **The peak-to-peak amplitude and dV/dt_{max} of the intrinsic deflection of the interstitial potential (V_{is}) at various penetration depths**

Impalement number	Penetration depth	Amplitude	dV/dt_{max}
	μm	*mV*	*V/s*
1.2 ± 0.4	54 ± 29	13.2 ± 10.1	−37.6 ± 29.2
5.3 ± 1.9	200 ± 40	23.7 ± 13.2*	−58.7 ± 33.8*
9.2 ± 3.2	390 ± 151	15.9 ± 12.9‡	−37.8 ± 31.4‡

* = $p < 0.005$ compared with value at a penetration depth of 54 μm; ‡ = $p < 0.005$ compared with value at a penetration depth of 200 μm. Values are given as mean ± SD ($n = 12$).

54 μm. The largest V_{is} amplitude and dV/dt_{max} were observed at a mean penetration depth of 200 μm, and were significantly larger than these parameters at a penetration depth of 54 μm. The deepest V_{is} recordings were obtained at a mean penetration depth of 390 μm. The amplitude and dV/dt_{max} of the V_{is} recordings obtained at 390 μm were significantly smaller than the corresponding parameters at a penetration depth of 200 μm.

Recordings obtained from the extracellular microelectrode that remained at a fixed location on the muscle surface are shown superimposed in Fig. 3 *a*. Each of the 10 recordings shown in Fig. 3 *a* was obtained simultaneously with one of the V_{ic} or V_{is} recordings shown in Fig. 1. The intrinsic negative deflections in the superimposed recordings in Fig. 3 *a* occur at nearly the same time, indicating that the activation time was uniform for the V_{is} and V_{ic} recordings. Fig. 3 *b* shows the *TM* obtained by subtracting each of the V_{is} recordings in Fig. 1 from the corresponding V_{ic} recording. The amplitude of each *TM* action potential plateau was not different from that of the corresponding V_{ic}. This is because the V_{is} returned close to its preactivation baseline potential after phase zero of the V_{ic} (Fig. 1). However, because the intrinsic deflection of the V_{is} had a considerable amplitude and dV/dt_{max} (Table 1), phase zero of the *TM* was different from that of the V_{ic}. This difference was seen in visual inspection of the recordings and in the measured parameters in Table 2.

Fig. 4 shows the V_{ic} and V_{is} recordings for the impalement which had the largest V_{is} peak-to-peak amplitude seen in the study. The positive baseline potential that is present in the V_{is} recording seen in Fig. 4 *a* was also present when the microelectrode was withdrawn to the superfusing solution after all the recordings were obtained. Thus, the positive baseline is an artifact due to a change in the microelectrode tip offset potential that occurred before obtaining the recordings shown in Fig. 4 *a*. The experimental protocol did not allow repeated adjustment of the recording amplifier dc offset to

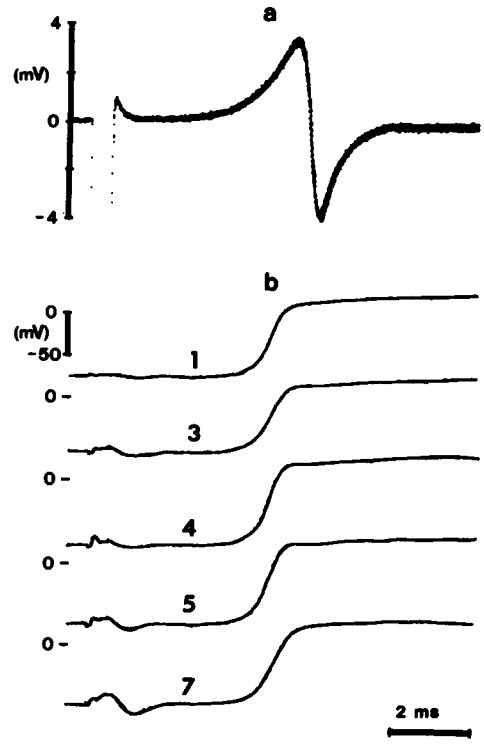

FIGURE 3 10 superimposed recordings of the potential at the muscle's surface (*a*), and the transmembrane action potential (*TM*) (*b*) obtained by subtracting the interstitial potential (V_{is}) from the intracellular potential (V_{ic}). Each of the surface recordings in *a* was obtained simultaneously with one of the V_{is} or V_{ic} recordings shown in Fig. 1. The surface recordings indicate that the activation time after the stimulus was uniform for the V_{is} and V_{ic} recordings. Phase zero of the *TM* action potential depolarized more rapidly and had a shorter foot potential compared with the V_{ic} shown in Fig. 1.

cancel changes in the microelectrode tip potential. Because of the change in the microelectrode tip potential in this experiment, the baseline potential of the V_{ic} is not the true resting membrane potential. However, because V_{is} and V_{ic} were recorded from the same microelectrode, and the V_{is} shown in Fig. 4 was recorded soon after the V_{ic}, the tip potential was probably similar for the V_{is} and V_{ic} recordings shown. The subtraction of the V_{is} from the V_{ic}, therefore, canceled the tip potential giving the resting membrane potential for the *TM* which is shown in Fig. 4 *a*. The subtraction shows that the *TM* depolarization during the positive phase of the V_{is}, i.e., before the V_{is} intrinsic negative deflection, was smaller than the V_{ic} depolarization (Fig. 4 *b*, *upper*). Also, during the negative phase of the V_{is}, i.e., after the V_{is} intrinsic negative deflection, the *TM* depolarization was greater than the V_{ic} depolarization. As a result of these differences in the depolarization of the *TM* compared with the V_{ic}, the action potential foot and overshoot were visibly different for the V_{ic} compared with the *TM*. The subtraction of the V_{is} from the V_{ic} produced a *TM* phase zero that had a larger dV/dt_{max} than the V_{ic} (Fig. 4 *b*, *center*). The semilogarithmic plots of the depolarization of the *TM* and V_{ic} indicated that τ_f of the *TM* was smaller than τ_f of the V_{ic} (Fig. 4 *b*, *lower*).

The combined results for the measurements of the *TM* compared with the V_{ic} are summarized in Table 2. At all penetration depths examined, dV/dt_{max} was larger for the *TM* compared with the V_{ic}. The difference between dV/dt_{max} for the *TM* compared with the V_{ic} was greatest at a depth of ~200 μm, which was the same depth that had the largest V_{is}. At all penetration depths, τ_f of the *TM* was smaller than τ_f of the V_{ic}. Thus, the action potential obtained from V_{ic} recordings in bathed ventricular muscle gives an underestimation of dV/dt_{max} of phase zero of the transmembrane action potential, and an overestimation of τ_f of the transmembrane action potential foot. The measurement errors that are introduced under the experimental conditions when the V_{ic} is assumed to represent the *TM* are included in Table 2. The errors are given as the difference between the corresponding V_{ic} and *TM* measurements, divided by the *TM* measurement. The measurement error for dV/dt_{max} of phase zero was −20 to −31%. The measurement error for τ_f was 48–82%.

TABLE 2 Measurements for phase zero of the intracellular (V_{ic}) and transmembrane potentials (*TM*) and the percent error of the V_{ic} measurements

Mean depth	dV/dt_{max}			τ_f		
μm	*V/s*		*% error*	μs		*% error*
	V_{ic}	*TM*		V_{ic}	*TM*	
54	145 ± 37	183 ± 51*	−20 ± 11	442 ± 118	306 ± 87*	48 ± 37
200	118 ± 27	175 ± 38*	−31 ± 14	469 ± 171	292 ± 83‡	75 ± 130
390	119 ± 23	154 ± 28*	−21 ± 16	506 ± 231	309 ± 102‡	82 ± 101
all ≤390	122 ± 16	167 ± 27*	−27 ± 14	496 ± 94	285 ± 28*	74 ± 40

* = $p < 0.005$, and ‡ = $p < 0.01$, *TM* compared with V_{ic}. % error was calculated from the individual paired samples as the difference between the V_{ic} and *TM* measurements divided by the *TM* measurement. Values are given as mean ± SD (n = 12).

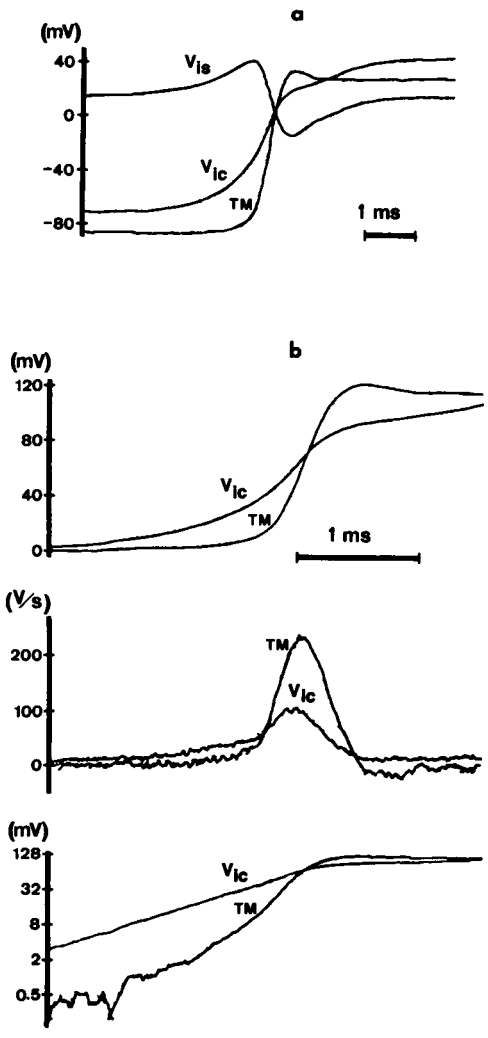

FIGURE 4 The interstitial recording (V_{is}) that had the largest peak-to-peak amplitude observed during the study, superimposed with the corresponding intracellular recording (V_{ic}) and transmembrane potential (*TM*) (*a*), and the V_{ic} and *TM* depolarization (*b, upper*), time derivative (*center*) and semilogarithmic plot (*lower*). The *TM* had a larger overshoot than the V_{ic}. The *TM* did not exhibit the ~15 mV microelectrode tip offset potential that was present in the V_{ic} and V_{is} recordings shown in *a*. The dV/dt_{max} of phase zero of the *TM* exceeded that of the V_{ic}, and the time constant of the 2–8 mV foot of the *TM* was smaller than that of the V_{ic}. The estimated penetration depth for the recordings was 174 μm.

DISCUSSION

The increase is the V_{is} amplitude when the penetration depth was increased from 54 to 200 μm agrees qualitatively with previous simulation results for cardiac tissue bathed in saline solution (Plonsey and Barr, 1987; Roth, 1988). The mean of the maximum amplitude of the V_{is}, 23.7 mV (Table 1), is similar to values obtained from simulations (Roth, 1988; Plonsey and Barr, 1987; Henriquez et al., 1988). Both the mean results and the largest V_{is} that was observed, 54 mV (Fig. 4), indicate that the V_{is} amplitude during action potential propagation in ventricular muscle can be a considerable fraction of the transmembrane action potential amplitude.

When the penetration depth was increased from ~200 to 390 μm, the V_{is} amplitude decreased to 16 mV. This finding differs from the previous simulation results in which the V_{is} did not decrease when the depth was increased, but became constant at the greater depths (Plonsey and Barr, 1987; Roth, 1988). If the downward movement of the microelectrode had brought the tip closer to the superfusing solution which was present on all sides of the muscle, the decreased V_{is} would agree with the simulations. However, the decrease in V_{is} at 390 μm was not due to a decrease in the distance between the microelectrode tip and the superfusing solution because 390 μm is smaller than the muscle radius and the microelectrode was approximately centered over the muscle.

The decrease in the V_{is} at the large depth could be due to decreased interstitial current or resistance. The decrease in the mean dV/dt_{max} of phase zero of the *TM* from 175 to 154 V/s when the penetration depth was increased from 200 to 390 μm (Table 2) suggests less rapid inward membrane current at the deeper penetration sites. It is not definitely known why the mean dV/dt_{max} of the *TM* decreased as the depth was increased. It is possible that a partially ischemic core affected dV/dt_{max} even though the superfusate pO_2 was held much higher than atmospheric pO_2 to aid the delivery of oxygen to the core of the muscle. Without arterial perfusion, transport of metabolites between the core and the superfusing solution may be diffusion limited. A theoretical analysis of the diffusion of tracer ions in cylindrical muscle bundles (Macdonald et al., 1974) may be helpful in evaluating diffusion transport in experiments with superfused muscles. Experimental conditions that simulate ischemia are capable of decreasing dV/dt_{max} of cells near the surface of guinea pig papillary muscles (Hiramatsu et al., 1989). Thus, an ischemic core may have contributed to the reduction in the mean dV/dt_{max} of phase zero of the *TM* at 390 μm. This, in turn, may have contributed to the decreased V_{is} at a depth of 390 μm by reducing the local circuit current. On the other hand, an ischemic core may decrease the V_{is} by osmotically altering the interstitial volume (Tranum-Jensen et al., 1981; Riegger et al., 1989) and, in effect, decreasing the interstitial resistance.

The result that τ_f was different for the V_{ic} compared with the *TM* (Table 2) implies that experimental determinations of the "effective" membrane capacitance calculated from τ_f (C_{eff}) (Tasaki and Hagiwara, 1957) could

have a considerable error. Because C_{eff} is inversely proportional to τ_f (Tasaki and Hagiwara, 1957), the overestimation of τ_f when the V_{ic} was used (Table 2), would produce an underestimation of C_{eff} by 33 and 45% at impalement depths of 54 and 390 μm, respectively. An underestimation of C_{eff} determined from V_{ic} recordings would be qualitatively consistent with the low value of C_{eff} (2.4 μF/cm^2) in comparison with the membrane capacitance determined with constant current pulses (12.8 μF/cm^2) in Purkinje fibers (Fozzard, 1966), although other factors may account for the reported low value of C_{eff}.

Both the V_{is} obtained in simulations (Geselowitz et al., 1982; Plonsey and Barr, 1987) and the experimentally-recorded extracellular potential at the ventricular muscle surface (Spach et al., 1979) are larger when propagation is in the direction of fastest velocity compared with the direction of slowest velocity. The reasons for the direction-dependence of extracellular or interstitial potential, though not definitely known, probably include the anisotropic resistance of ventricular muscle and other factors that vary along and across fibers such as propagation velocity, the dimension of cells, and the distribution of discrete cellular junctions. If the V_{is} in ventricular muscle is indeed larger for propagation in the direction of fastest velocity compared with the direction of slowest velocity as the previous simulations suggest, then the V_{is} may contribute to the unexpected direction-dependence of τ_f and dV/dt_{max} of phase zero of the action potential that was reported in bathed cardiac muscle (Spach et al., 1981). The present results indicate that phase zero of the action potential depolarizes more slowly for the V_{ic} than the *TM* (Table 2) and that this difference arises because the method of recording the V_{ic} does not incorporate the V_{is}. Because a larger V_{is} produces a greater difference between the V_{ic} and *TM*, a larger V_{is} for propagation in the direction of the fastest propagation velocity implies a greater difference between V_{ic} and *TM* for propagation in that direction. This hypothesis is consistent with the previous results that dV/dt_{max} of phase zero is smallest for action potential propagation in the direction of the fastest propagation velocity (Spach et al., 1981), though other factors have been proposed to account for the previous results. The membrane potential in that study (Spach et al., 1981, Table 1) was obtained by subtracting the extracellular potential at the muscle surface from the intracellular potential. However, the extracellular potential at the surface does not fully incorporate the V_{is} because the V_{is} at superficial impalement sites is larger than the potential at the muscle surface and the V_{is} increases when the impalement depth is slightly increased.

Previous experimental results have indicated differences between phase zero of the ventricular action potential obtained from microelectrode recordings compared with optical recordings. The phase zero dV/dt_{max} for optical recordings in ventricular fibers was 261 V/s (Windisch et al., 1985). However, for the corresponding microelectrode recordings, dV/dt_{max} was 189 V/s, which is 28% smaller than the value obtained optically. This difference is similar to the −27% error in dV/dt_{max} for the V_{ic} recordings obtained in the present study (Table 2). The present results suggest that this difference is due to the V_{is} outside of the cell. The optical recordings (Windisch et al., 1985) may give the true transmembrane phase zero including the contribution of the V_{is} that is present outside of the cells. Therefore, the reported difference between the optical and microelectrode recordings is consistent with the present results.

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