Nonlinear effects in subthreshold virtual electrode polarization

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Sambelashvili, Aleksandre T., Vladimir P. Nikolski, and Igor R. Efimov. Nonlinear effects in subthreshold virtual electrode polarization. Am J Physiol Heart Circ Physiol 284: H2368–H2374, 2003; 10.1152/ajpheart.00988.2002.—Introduction of the virtual electrode polarization (VEP) theory suggested solutions to several century-old puzzles of heart electrophysiology including explanation of the mechanisms of stimulation and defibrillation. Bidomain theory predicts that VEPs should exist at any stimulus strength. Although the presence of VEPs for strong suprathreshold pulses has been well documented, their existence at subthreshold strengths during diastole remains controversial. We studied cardiac membrane polarization produced by subthreshold stimuli in 1) rabbit ventricular muscle using high-resolution fluorescent imaging with the voltage-sensitive dye pyridinium 4-{2-[6-(dibutylamino)-2-naphthalenyl]-ethenyl}-1-(3-sulfopropyl)hydroxide (di-4-ANEPPS) and 2) an active bidomain model with Luo-Rudy ion channel kinetics. Both in vitro and in numero models show that the common dog-bone-shaped VEP is present at any stimulus strength during both systole and diastole. Diastolic subthreshold VEPs exhibited nonlinear properties that were expressed in time-dependent asymmetric reversal of membrane polarization with respect to stimulus polarity. The bidomain model reveals that this asymmetry is due to nonlinear properties of the inward rectifier potassium current. Our results suggest that active ion channel kinetics modulate the transmembrane polarization pattern that is predicted by the linear bidomain model of cardiac syncytium.

electrophysiology; stimulation; excitation; ion channels

RECENT INVESTIGATION OF ELECTRICAL stimulation of the heart revealed an important role of tissue heterogeneity in the generation and propagation of muscle excitation. One of the important intrinsic forms of the heterogeneity consists of inequality of anisotropic properties of the extracellular and intracellular spaces of the cardiac syncytium. This inequality was first theoretically described within the framework of the bidomain model (9, 18, 29), which represents cardiac tissue as two interpenetrating extra- and intracellular domains with different conductivities along and across the direction of the fibers, which are coupled via membrane resistance. Using the bidomain model, Sepulveda et al. (26) predicted the existence of adjacent areas of hyperpolarization and depolarization during unipolar stimulation of the tissue. These areas were termed virtual electrode polarizations (VEPs). For a point-size cathodal stimulus, the transmembrane potential (\(V_m\)) distribution pattern had a central depolarized virtual cathode (VC) region with a characteristic dog-bone shape and two elongated hyperpolarized virtual anode (VA) regions on the sides that were parallel to the direction of the fibers. In the linear bidomain model, for the opposite polarity of the stimulus, the VEP pattern was symmetrically reversed.

The dog-bone VEP pattern induced by point stimulation was experimentally confirmed using electrode mapping (31) and optical imaging (12, 19, 30). This virtual electrode phenomenon resulted in the formulation of a unified theory of stimulation and suggested solutions to the old puzzles of break-excitation and anodal stimulation (24, 30). The concept of activating function generalized VEP theory to electric fields of any geometrical configuration (27). In particular, strong electric shocks applied during defibrillation also induce areas of adjacent positive and negative polarization, which can be considered VEPs (8). This concept allowed the explanation of shock-induced arrhythmias and defibrillation failure via virtual electrode-induced phase singularities (7, 15, 16).

Despite the impressive success of VEP theory in a wide range of investigations, the very existence of VEPs remains controversial as it pertains to subthreshold stimulation (STS) (1). Measurements of subthreshold VEPs represent a significant experimental challenge (1). Nevertheless, STS plays a crucial role in the generation and propagation of electric pulses. STS of Purkinje fibers was shown to interrupt ventricular tachycardia by synchronization of ventricular excitation (25). Preconditioning by STS inhibited the excitability of Purkinje fibers, which is in contrast to the facilitation of muscle cell fiber excitability (6). Finally, STS is used in the assessment of tissue coupling (1, 2, 14, 22).

Using optical imaging and the active bidomain model, we sought to determine the existence and dynamics of VEPs during unipolar STS.

METHODS

Experimental preparation, optical mapping setup, and protocol. This study conformed to the guidelines of the American Heart Association. The experiments were performed in vitro on hearts obtained from New Zealand White rabbits (\(n = 4\)). In two additional experiments, guinea pig hearts (\(n = 2\)) were used to check for possible interspecies differences. The hearts

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were placed onto a Langendorff apparatus where they were retrogradely perfused with oxygenated modified Tyrode solution as previously described (21). Motion artifacts in optical recordings were suppressed by 15 mM 2,3-butanedione monoxime (BDM). The voltage-sensitive dye pyridinium 4-([6-(dibutylamino)-2-naphthalenyl]-ethenyl)-1-(3-sulfopropyl)-hydroxide (di-4-ANEPPS; 0.5 μM; Molecular Probes; Eugene, OR) was added to the circulating solution within 5 min. Hearts were stained for 40 min before the recordings were started.

The optical mapping setup used for acquisition of the optical signals was described previously (20, 21). Briefly, the light produced by a 250-W quartz-tungsten-halogen DC-powered light source passed through a 520 ± 45-nm excitation filter, was reflected by a 585-nm dichroic mirror, and passed through a 50-mm lens before it illuminated a rabbit heart. The fluorescence emitted from the heart was collected by the same lens, passed through the dichroic mirror, and filtered by a long-pass filter (>610 nm). The light was then detected by a 16 × 16 photodiode array (C4675; Hamamatsu).

For each subthreshold test, stimulus \( V_m \) traces (ST-\( V_m \)) were recorded from 256 channel-tissue pixels at the same time steps in the directions both across and along the fibers were chosen to be 0.1 mm, which was less than half of the smallest space constant. The time step was 10 μs. To solve for the extracellular potential, the fast Fourier transform method (28) was employed at each step as the most robust and the fastest for our case. Repeating the simulations for the twice-smaller space and time steps confirmed the stability and accuracy of our calculations to <0.001 mV. All calculations were performed on a Pentium 800 MHz personal computer. The results were obtained in terms of \( V_m \) values and ionic currents and were plotted and analyzed with MATLAB software (MathWorks; Natick, MA).

RESULTS

ST-\( V_m \) distribution exhibits classical dog-bone-shaped pattern. PACing thresholds determined in our experiments ranged from −0.15 to −0.60 mA for cathodal stimulation and from +0.85 to +1.76 mA for anodal stimulation. A representative example of optical maps of the \( V_m \) recorded at different times (2, 4, and 10 ms) during 20-ms cathodal and anodal STS is shown in Fig. 1. Even for subthreshold stimuli, the VEP pattern is dynamic. Small VEPs are created around the electrode tip almost instantaneously (faster than our temporal resolution) after the application of the stimulus, and the pattern then rapidly spreads out with a simultaneous increase in magnitude of both depolarization and hyperpolarization. The most pronounced VEP pattern is observed at ~4 ms (13), which is determined by the time constant for the passive cardiac tissue resistor-capacitor network. Later VEPs continue to grow slowly; in the case of the cathodal stimulus, this occurs by spreading of the two regions of hyperpolarization, and in the case of the anodal stimulus, it occurs by spreading of the two regions of depolarization. In both cases, the maximum polarization at these two regions diminishes, which makes it more challenging to detect the characteristic VEP pattern at times >10 ms after the onset of the stimulus.

Resting tissue at diastole cannot be considered totally passive. A remarkable asymmetry exists in the VEPs for anodal and cathodal stimuli at 10 ms in Fig. 1. First, the central hyperpolarized area for the anodal pulse is substantially smaller than the corresponding depolarized area for the cathodal pulse. The distance from the electrode tip to the closest point with no deviation from the resting potential (white area) is 0.7 mm in the first case, whereas in the second case it is 1.4 mm, which is twice as large. The average ratio of these distances for cathodal vs. anodal stimuli obtained from all animals (\( n = 4 \)) was 1.9 ± 0.3. Second, VC areas for the anodal stimulus have a maximum depolarization of +1.9 mV and therefore, in absolute terms, are 2.1 times more pronounced than the VA areas for the cathodal stimulus, where the maximum hyperpolarization is only ~0.9 mV. The average ratio (\( n = 4 \)) of the maximum polarizations of the side virtual electrodes for anodal vs. cathodal stimulation was 2.3 ± 0.2.

To understand the reason for the asymmetry described, we conducted numerical simulations on the basis of the active bidomain model. Figure 2 illustrates simulated maps of the \( V_m \) that correspond to the ex-
Experimental maps from Fig. 1. Similar dynamics of growing VEPs can be observed. Transmembrane VEPs reach maximum values at nearly 4 ms after the stimulus application. At 10 ms, the pattern is asymmetric with respect to the stimulus polarity, which is similar to the experimental observations: namely, the central hyperpolarized area for the anodal pulse is slightly smaller than the depolarized area for the cathodal pulse, and the smallest distance from the pacing point to the zero-deflection line is 0.6 mm in the first case and 0.8 mm in the second case. In addition, the maximum depolarization at the VCs for the anodal stimulation is almost twice as high (≈0.34 mV) compared with the maximum hyperpolarization at the VAs for the cathodal stimulation (≈0.18 mV).

To analyze the asymmetry in detail, we considered ST-V_m traces from two different spots of oppositely polarized areas of VEP for both anodal and cathodal stimuli. Figure 3 demonstrates such traces for bidomain simulations assuming a passive and linear tissue response. As one might expect, the ST-V_m curves from the two sites are symmetric with respect to the stimulus polarity. They reflect the passive VEP dynamics. First the transmembrane VEPs in the central and the side areas grow rapidly, reaching a peak by 4–5 ms after the application of the pulse. Later, as the side VEP areas move away from the center, the corresponding polarizations taken from the sites at some distance along the direction of the fibers decrease to a lower value (in the example shown, to zero). The second peak of the voltage traces after the withdrawal of the pulse indicates the charge diffusion and the quick elimination of the dog-bone pattern.

As mentioned, if the tissue were completely passive and linear, then the reversal of the stimulus polarity would simply invert the VEP pattern, and depolarized areas and responses would become hyperpolarized and vice versa, which is shown in Fig. 3. However, experimental traces of ST-V_m on Fig. 4 clearly demonstrate that for the cathodal stimulus, the central VC area grows to a larger extent than the corresponding VA.
area for the anodal stimulus thus suppressing the opposite negative response from the two neighboring VA regions and creating the ST-$V_m$ asymmetry. Active bidomain simulations of the Luo-Rudy model (Fig. 5) are in good agreement with the experiment. One can see a substantially higher level of the absolute polarization in the VC area for the anodal stimulus compared to the VA area for the cathodal stimulus. From these observations, we can conclude that cardiac tissue at diastole cannot be considered completely passive. Nonlinear ionic mechanisms significantly modulate VEP responses to STSs in a stimulus polarity-dependent manner.

Nonlinearity in inward rectifier current causes dog-bone-shaped asymmetry. We analyzed the contributions of different ionic currents into the formation of the ST-$V_m$ traces at the two sites of interest. Figure 6A shows the time course of the changes of the major currents during stimulation. This image illustrates that the total transmembrane current is affected primarily by the inward rectifier potassium current ($I_{K1}$), which is known to be responsible for the maintenance of the resting potential. According to our simulations, for cathodal STS, due to rather small deviations of $V_m$ from the resting value, the $I_{K1}$ plays the major role for almost the entire central depolarized dog-bone-shaped

**Fig. 3.** Simulated VEP and subthreshold stimulation induced by $V_m$ (ST-$V_m$) for the passive bidomain model. A: VEP patterns for cathodal and anodal stimuli of the same amplitude at 2 ms after the stimulus application. B: time courses of the $V_m$ changes from the two points of virtual cathode (VC; in red) and virtual anode (VA; in blue) indicated by boxed areas in A. For the passive model, the VEP at any time is reversed with the reversal of the stimulus polarity.

**Fig. 4.** Experimental VEP and ST-$V_m$. A: optical maps of VEP patterns for cathodal and anodal stimuli of the same amplitude at 2 ms after the stimulus application. An example of an optical recording of $V_m$ is also shown (bottom). B: time courses of the $V_m$ changes from the two points of VC (in red) and VA (in blue) indicated by boxed area in A. VEP patterns are asymmetric with respect to reversal of the stimulus polarity, with a larger area of depolarization for the cathodal stimulus compared with the corresponding area of hyperpolarization for the anodal stimulus. A slight negative trend in the resting potential is due to slow residual repolarization from the previous beat.
region with perhaps the nonsignificant exception of a tiny area around the tip of the electrode. This observation excludes a possible explanation of the asymmetry as being created by the influence of the undeveloped excitation in the case of cathodal stimulation.

Because the $I_{K1}$ in the Luo-Rudy model is time independent, i.e., its strength is determined solely by the $V_m$, the discrepancies between cathodal and anodal stimulation must be explained by the dependence of the $I_{K1}$ conductance on $V_m$.

Figure 6B shows that $I_{K1}$ conductance is in general increased by hyperpolarization and decreased by depolarization, although not by the same absolute value. Because the length constant of the tissue is inversely proportional to the square root of the transmembrane conductance (23), it must be larger for depolarized regions compared with hyperpolarized regions. So the larger central depolarized area for the cathodal pulse in comparison with the central hyperpolarized area for the anodal pulse can be explained by the changing of the space constant during polarization of the tissue. The same effect of the $I_{K1}$ conductance alterations over the region of the VEP leads to stronger VC areas for anodal stimulation compared with the VA areas for cathodal stimulation.

The results obtained in our experiments with guinea pig hearts were similar to those obtained for rabbit hearts; i.e., we observed the same dynamics of VEP...
development and polarity-dependent asymmetry of the STS response. However, the small number of experiments with guinea pigs did not allow us to make any significant comparisons between the species.

DISCUSSION

Conditions for detection of VEP patterns. Advances in voltage-sensitive dye techniques have enabled us to detect VEP patterns on the surface of the heart during stimulation. However, in most of the reported results, the stimuli have been either very strong (suprathreshold) or they have been applied during the plateau phase of the action potential. In the first case, stronger shocks produce higher VEP magnitudes and therefore require less voltage resolution. If a strong shock is delivered during diastole, the resulting VEP pattern becomes overlapped by excitation within several milliseconds, thus preventing one from observing possible VEP dynamics at later times. We show here that for STS, the VEP pattern is most pronounced at 4–5 ms from the moment of the stimulus application, which is the time that is comparable with the resistance-capacitance time of the tissue. Then, because of charge diffusion, the pattern slowly becomes less spatially concentrated with the diminishing of the $V_m$ differences. The STS VEP is asymmetrical with respect to the polarity of the stimulus. We explained the modulation of the VEP pattern by the nonlinear influence of the transmembrane currents, primarily the $I_{K1}$. These facts should be taken into consideration when one is trying to experimentally obtain the ST-$V_m$ distribution as well as in attempts to make certain conclusions regarding the tissue properties, for instance, such as intracellular conductance (1), from the STS data.

In the second case of stimulation during the plateau phase, larger and stronger VEPs are produced than if the same amplitude of current stimulus were applied during the resting state (Fig. 7). As we see it, the reason for this lies in a seemingly paradoxical fact that the total transmembrane conductance of the ion channels for the most part of the plateau phase is smaller than that of the diastolic period (32). According to theory, a bigger transmembrane resistance allows for stronger expression of the differences in the anisotropies of the intracellular and extracellular spaces and therefore causes a more pronounced VEP pattern. This explains why it has been easier to detect the VEPs for shocks of high magnitude or shocks applied during the plateau phase of the action potential.

Nonlinearity of $V_m$ response to electric shock. The $V_m$ response to external shock plays a critical role in pac- ing, defibrillation, and cardioversion. It is generally a function of the strength of the external field, the time of its application, its polarity, and possibly other factors. Many studies investigating the effect of stimulus polarity on $V_m$ changes ($\Delta V_m$) have appeared recently. In experiments on cardiac cell strands, Cheek et al. (3) demonstrated that negative $\Delta V_m$ values for a strong anodal stimulus applied during the plateau phase are bigger than positive $\Delta V_m$ values for the same-strength cathodal shocks. This asymmetry was attributed to nonlinearity in $Ca^{2+}$ channel kinetics. Similar results have been obtained from microelectrode recordings of guinea pig papillary muscle plateau-phase $\Delta V_m$ values (33) and from isolated ventricular myocytes (4). The main idea of these studies consists of emphasizing the crucial role of active ion channel properties in generation of the $V_m$ response to electric stimulation.

In this work, we show that even at the resting state for a weak STS, the membrane response cannot be accurately represented by a simple, passive resistance-capacitance model. Ionic modulation can lead to small but experimentally detectable asymmetry of the response, which may be quite important if one takes into account the all-or-none nature of the action potential generation process.

Limitations. An optical mapping setup collects averaged fluorescence not only from the surface of the epicardium, but also from a thin layer beneath that surface (10, 11). Therefore, without knowing the exact three-dimensional pattern of the $V_m$ distribution, it is hard to make conclusions about the reasons for the observed asymmetry in response to stimuli of different polarities. Aside from that, the experimental part of our study was accomplished with the excitation-contraction uncoupler BDM, which is known to affect ion channel properties. Nevertheless, we believe that the results of our computer simulations, which show good qualitative agreement with experimentally observed ST-$V_m$ patterns, alleviate these limitations.

Fig. 7. VEP for weak cathodal stimulus applied during plateau phase of the action potential: experiment and active bidomain simulation. A: optical maps of VEP at 10 ms of the −0.5-mA, 15-ms stimulus that was applied at a 50-ms coupling interval. B: same VEP obtained from active bidomain simulations is shown. For the same stimulus strength, the resulting VEP for plateau-phase stimulation was larger compared to the stimulation at resting state (see Fig. 1). $V_m$ values are shown as deviations from normal action potential values.

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The bidomain model we used was two dimensional and assumed the simple geometry of straight fibers. In addition, the Luo-Rudy phase I model of the cardiac myocyte ignored some existing ion channels as well as the role of certain cellular structures such as sarcoplasmic reticulum. These and other factors might have caused the discrepancies in the values of the \( \Delta V_m \) for the simulations and the experiments. Despite these limitations, we think that for our case, the chosen model captured all the substantial features and was sufficient for a qualitative description of electrophysiological responses of the heart tissue to STS.

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REFERENCES


