Nonlinear effects in subthreshold virtual electrode polarization

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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 were paced at 1 Hz by a programmable stimulator. After stabilization, 1-(3-sulfopropyl)hydroxide (di-4-ANEPPS) and pyridinium 4-{2-[6-(dibutylamino)-2-naphthalenyl]-ethenyl}-1-(3-sulfopropyl)hydroxide (di-4-ANEPPS) were perfused into the hearts.

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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, 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 Vm traces (ST-Vm) were recorded from 256 channels of the photodiode array focused on the 5 × 5-mm window around the tip of the test-stimulus electrode. Sampling was performed at a rate of 2,900 frames/s. Therefore, we had a space resolution of 312 μm and a time resolution of 0.3 ms.

For improvement of the signal-to-noise ratio, we averaged 10 consecutive recordings for each stimulus polarity kept at the same duration and current strength. Averaging resulted in a threefold improvement in the signal-to-noise ratios. Vm maps and ST-Vm traces were constructed from these averaged data.

Calibration was performed as previously described (5). Briefly, we assumed resting potential to be -85 mV and action potential amplitude to be 100 mV.

To reach steady state, the heart was paced with 20 basic pulses (with a cycle length of 350 ms) using a bipolar electrode at the base of the left ventricle far from the field of view. After the basic drive, a test-current stimulus was applied from a unipolar electrode (0.12-mm platinum-iridium-Teflon-coated wire) that was positioned in the middle of a 5 × 5-mm field of view at the anterior wall of the left ventricle. The test stimulus was delivered at a coupling interval of 300 ms from a constant-current source (A385; WPI; Sarasota, FL). An ECG was recorded to verify suprathreshold and subthreshold pacing. After determination of the diastolic pacing thresholds for cathodal and anodal pulses, only subthreshold test stimuli were applied. The subthreshold amplitude was kept at 0.1 mA less than the cathodal threshold, and the duration was 20–60 ms. The polarity of the stimuli was reversed each time to ensure a correct comparison between anodal and cathodal stimulations. Basic pacing stimulus strength was adjusted to twice the diastolic threshold of excitation.

Numerical models. We guided our experiments with computer simulations based on the two-dimensional active bidomain model with Luo-Rudy phase I ion channel kinetics (17). For the cases of passive bidomain simulations, the transmembrane current was described by Ohm’s law. The two-dimensional slice of cardiac tissue was assumed to have straight fibers and constant intracellular and extracellular conductivities along and across the direction of the fibers. The parameters for the cardiac tissue were the same as those used by Roth (24). The boundaries were sealed for the intracellular space and grounded (zero potential) for the extracellular space. Different-polarity STSs of 0.8 × threshold strength and 60 ms duration were applied from a point-size source to the center of a 2 × 2-cm square domain. The space 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 Vm values and ionic currents and were plotted and analyzed with MATLAB software (MathWorks; Natick, MA).

RESULTS

ST-Vm 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 Vm 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 Vm that correspond to the ex-
Fig. 1. Experimental virtual electrode polarizations (VEPs) at different times during subthreshold stimulation (STS). Optically recorded and averaged maps of transmembrane potentials ($V_m$) were obtained from a 5 × 5-mm area of anterior epicardium of rabbit heart for unipolar subthreshold current anodal and cathodal stimuli (±0.5 mA, 20 ms). Sepulveda's dog-bone pattern of VEPs for anodal (A) and cathodal (B) stimuli grows in size with time and becomes asymmetric; central depolarized area for cathodal stimulus at 10 ms is larger than the corresponding hyperpolarized area for anodal stimulus. $V_m$ varies from −90 mV (in blue) to −80 mV (in red); −85 mV (in white) represents the resting state.

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.

Fig. 2. VEPs at different times during STS obtained from active two-dimensional bidomain simulations with Luo-Rudy ion channel kinetics. Images for anodal (A) and cathodal (B) stimuli (±60 μA/cm, 20 ms) show the growth of the VEP with time as well as the slight asymmetry of the VEP with respect to the stimulus polarity. $V_m$ variations are within the range of ±1 mV; −84.5 mV represents the resting state (in white).
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
region with perhaps the nonsignificant exception of a
tiny area around the tip of the electrode. This observa-
tion 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 inde-
pendent, 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 depo-
larization, 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

![Fig. 5. Simulated VEPs and ST-$V_m$ for active bidomain
model. A: VEP patterns are illustrated 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 VC (in red) and VA (in blue)
indicated by boxed area in A. VEP patterns are asym-
metric with respect to reversal of the stimulus polarity,
with larger area of depolarization for the cathodal stim-
ulus compared to the corresponding area of hyperpolar-
ization for the anodal stimulus. These results are in
agreement with experimental recordings shown Fig. 4.](image)

![Fig. 6. Ionic currents and inward recti-
tifier potassium current ($I_{K1}$) conduc-
tivities during STS. Changes in major
currents (left) are shown at the two
points of VC (in red) and VA (in blue)
during cathodal (A) and anodal (B)
STS. $I_{K1}$ is the primary current in both
cases and behaves asymmetrically
with respect to the reversal of the stim-
ulus polarity. Time courses of $I_{K1}$ con-
ductivity ($G_{K1}$; right) are provided at
the same two points of VA (in red) and
VA (in blue) (indicated by boxed areas)
and the site of the stimulation (in
black). $G_{K1}$ changes unequally for
cathodal vs. anodal STS.](image)
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 often 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 Vm 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 account 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 pacing, 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 ionic 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.
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 existent ionic 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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