Mechanisms of make and break excitation revisited: paradoxical break excitation during diastolic stimulation

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The ability of electric point stimulation to produce a response in excitable tissues (11, 28) and induce (12) or terminate (17) arrhythmia in the heart is well known. However, the exact mechanisms of electric stimulation have been obscure until the recent discovery of virtual electrode polarization (VEP) produced by point stimulation (13, 15, 24, 30, 31). It results in a characteristic “dogbone” pattern of positive and negative polarizations. These polarizations of opposite sign are thought to be induced by so-called virtual cathode and virtual anode. These virtual electrodes represent the driving force, which can be mathematically expressed as an activating function (19, 27), which is also referred to as secondary sources (10). The activating function is governed by two major parameters: the gradient of extracellular electric field and structural heterogeneity of the heart, contributing to the polarization of the cellular membrane during stimulus. Active ionic properties of the heart, particularly calcium channels, modulate these polarizations (2, 18, 20, 22).

Dekker (4) demonstrated that both the onset (make) and termination (break) of stimulation of appropriate intensity and duration could produce a propagated response. Roth (20) and Wikswo et al. (30) provided the first mechanistic explanation of the “make” and “break” stimulation based on the VEP phenomenon.

According to their theory, clinically relevant make stimulation produces initial depolarization at sites of positive polarization or virtual cathodes. Such stimulus-induced depolarization results in the opening of activation, or the m gates of sodium channels. Sodium entering the cell through the opened channels completes the depolarization. Adjacent areas of hyperpolarization (virtual anode) are subsequently driven to depolarization by the diffusion of transmembrane potential (V_m) through electrotonic coupling. Thus, during make stimulation, the heartbeat originates at the virtual cathode and is delayed at the virtual anode.

Break stimulation represents an entirely different mechanism. Break excitation usually is induced by a termination (break) of a very long pulse, usually lasting >100 ms. Such duration is required to avoid or separate make stimulation. For example, Wikswo et al. (30) used 150- and 180-ms pulses, which started during the refractory period to avoid make stimulation and then terminated after the recovery of excitability, producing break excitation. In this case, stimulus-induced positive polarization does not evoke an active response, because sodium channels in this area of virtual cathode remain inactivated. In contrast, virtual anode forces accelerated the recovery from inactivation (opening of h gates) of sodium channels, providing an excitable substrate for the depolarization propagation at the cessation of the stimulus. Subsequently, this break excitation of hyperpolarized areas of virtual anode spreads to the rest of the tissue, which is completely recovered by that time. Thus, during break stimulat-
tion, the heartbeat originates at virtual anode and is delayed at the virtual cathode. Alternatively, it is possible to induce break excitation by a stimulus as short as 5 ms applied during a relative refractory period (6, 7, 14, 21); however, this type of stimulation was not considered in our study.

The goal of our study was to investigate mechanisms of stimulation with clinically relevant short stimuli of near-threshold intensities delivered from unipolar and bipolar electrodes during diastole.

METHODS

Experimental preparation. This study conformed to the guidelines of the American Heart Association. The experiments were performed on perfused cardiac preparations obtained from New Zealand White rabbits (n = 12). The heart was placed horizontally onto a Langendorff apparatus, where it was retrogradely perfused with oxygenated modified Tyrode solution (Fig. 1) as previously described (6). The saline level above the heart was 5–7 mm.

The heart was paced at a steady-state cycle length of 400 ms at the base of left ventricle with a basic pacing electrode (Fig. 1). Test stimuli were applied with a test stimulus electrode in the middle of the field of view (yellow box in Fig. 1) at a coupling interval of 350 ms with an amplitude of 0.2–60 mA and a duration of 2 ms from a constant current source (A385, WPI). A recording electrode was used to detect a propagated response to confirm suprathreshold pacing. The basic pacing stimulus strength was adjusted to twice the diastolic threshold of excitation.

Optical mapping techniques. Figure 2 illustrates the high-resolution epifluorescence optical mapping setup used in our experiments. The light produced by a 250-W quartz tungsten halogen direct current power source passed through a 520 ± 45-nm excitation filter, was reflected by a 585-nm dichroic mirror, passed through a 50-mm lens, and illuminated the field of view at the anterior epicardium of the rabbit heart. The fluorescence emitted from the heart was collected by the same lens, passed through the dichroic mirror (>585 nm), and was additionally filtered by a long-pass filter (>610 nm). It was then focused on the sensing area of a 16 × 16 photodiode array (C4675, Hamamatsu). The magnification was adjusted to 250 × 250 μm per diode, such that the entire array covered a field of view of 4 × 4 mm. The current produced by each of the 256 photodiodes passed through a separate current-to-voltage converter integrated within the same head stage (C4675, Hamamatsu). The outputs of the first-stage amplifiers were connected to 256 second-stage amplifiers, alternating current coupled with a time constant of 30 s (32). Resetting of the second-stage amplifiers before data acquisition was used to remove the direct current offset of the optical signals. Sampling was performed at a rate of 2,900 frames/s. Each frame included 256 optical channels and 8 instrumentation channels and was stored for off-line analysis. Instrumentation channels recorded electrocardiograms, stimulation and defibrillation triggers, and perfusion pressure. Custom-developed data acquisition and analysis software was used as previously described (5).

Data analysis and signal calibration. Photodiode fluorescence signals recorded from the 4 × 4-mm area around the test stimulus electrode were normalized on changes during the last basic beat, assuming the resting potential and action potential amplitude were −85 and 100 mV, respectively (Fig. 3, left) (3). Our normalization was based on the optical signal collected during the last basic beat propagating from the basic pacing electrode (see Fig. 1 for location of the electrode) because 1) basic beat provides a nearly linear wavefront moving across the field of view, and 2) normalization on the signals obtained during clearly nonuniform propagation after a test stimulus, which was applied in the center of the field of view, could be dependent from the test stimulus strength. The intensity graphs (Fig. 3, right), representing the surface
distribution of VEP, were plotted from the data collected in the middle of a 2-ms stimulus (VEP maps in Fig. 3). We resampled the initial 16 × 16 data array to a 256 × 256 matrix by cubic spline interpolation. The color scale (Fig. 3) reflects the change of the fluorescence caused by the voltage-sensitive dye di-4-ANEPPS, which corresponds to the changes in \( V_m \) from −95 to −75 mV. The circles in Fig. 3 mark the electrode positions, and the + and − symbols show.

Fig. 2. Experimental setup. Fluorescence was excited by light produced by a 250-W quartz tungsten halogen direct current (DC) power light source. The excitation light passed through a 520 ± 45-nm excitation filter and was then reflected by a 585-nm dichroic mirror to the surface of the preparation. The fluorescence emitted from the heart was collected by a 50-mm lens, passed through a long-pass filter (>610 nm), and was focused on the sensing area of the 16 × 16 photodiode array. The 256 signals were amplified, filtered, and then digitized for off-line analysis.

Fig. 3. Data analysis. This figure illustrates the technique used for calibrating virtual electrode (VE) patterns during epicardial stimulation. The traces on the left show the fluorescence signals from 4 different areas located near the electrode tips (circles with + and − symbols) 10 ms before and 60 ms after stimulus. The signals shown were recorded by 4 photodiodes from 250 × 250-mm areas each. Similar traces were obtained from all 256 photodiodes. We assumed that 1) the changes in fluorescence are proportional to the changes in transmembrane potential, 2) the baseline fluorescence before the test stimulus corresponds to −85 mV, and 3) the fluorescence during the full depolarization corresponds to +15 mV. After such recalibration, we plotted the values from all traces in the middle of a 2-ms DC diastolic stimulus as an intensity graph (right) with a −95-mV (red) to −75-mV (blue) color scale. For the purpose of display, a 16 × 16 matrix was interpolated to a 256 × 256 element grid by the cubic spline method. Top and bottom areas show the calibration technique for different bipolar electrode orientations.
the stimulus polarities. The orientation of the myocardial fibers is indicated by the arrow.

Numerical model. We guided our experiments with theoretical predictions derived from the bidomain model. It is based on the representation of the tissue as two interpenetrating extracellular and intracellular domains, each having different conductivities along and across the direction of the fibers (24, 26). The state variables describing the system are intracellular (Φi) and extracellular potentials (Φe) defined everywhere in the domain of interest (Ω). The variable of physiological importance is \( V_m \), defined as the difference (\( V_m = \Phi_i - \Phi_e \)). The following coupled reaction-diffusion equations constitute the bidomain model

\[
\nabla \cdot (\sigma_i \nabla \Phi_i) - I_m = 0 \quad (1)
\]

\[
\nabla \cdot (\sigma_e \nabla \Phi_e) = -I_m - I_0 \quad \text{in } \Omega \quad (2)
\]

where \( \sigma_i \) and \( \sigma_e \) are intracellular conductivity, respectively, \( I_m \) is the volume density of the transmembrane current; and \( I_0 \) is the volume density of the stimulation (shock) current.

The stimulus current density from the point-size electrode placed at a point \((x_1,y_1)\) can be described in terms of B-function as \( I_{sd}(x - x_1)\delta(y - y_1) \).

The transmembrane current \( (I_m) \) is generally represented as the sum of capacitive, ionic, and electroporation currents (26)

\[
I_m = \beta \left[ C_m \frac{dV_m}{dt} + I_{ion}(V_m, t) + G(V_m, t) \times V_m \right] \quad (3)
\]

where \( \beta \) is the surface-to-volume ratio (total membrane area divided by total tissue volume), \( t \) is time, \( C_m \) is the specific membrane capacitance, \( I_{ion}(V_m, t) \) is the ionic current, and \( G(V_m, t) \) is the electroporation conductance.

The last is described by empirical equations (26), e.g.

\[
\frac{dG}{dt} = \alpha \exp[\beta(V_m - V_{rest})^2](1 - \exp[-\gamma(V_m - V_{rest})^2])
\]

\[
G(0) = G_0 \quad (4)
\]

where \( \alpha, \beta, \) and \( \gamma \) are electroporative coefficients and \( V_{rest} \) is the resting \( V_m \). The values of all the parameters are given in Tables 1 and 2.

For the case of the passive bidomain model, which does not consider active ion channel kinetics, when the main point of interest is the steady-state \( V_m \) distribution, the expression for \( I_m \) simplifies to Ohm’s law

\[
I_m = (\beta/R_m) V_m \quad (5)
\]

where \( R_m \) is the membrane resistance times unit area.

For the case of the active bidomain as a model of cardiac myocytes, we used the Drouhard-Roberge version of the Beuler-Reuter model (1), modified by Skoubye et al. (26), to extend the model for strong electric fields.

We used the following boundary conditions

\[
\frac{\partial \Phi_i}{\partial n} = 0 \quad \Phi_e = 0 \quad \text{on } \partial \Omega \quad (6)
\]

For numerical solution of the bidomain system, we used a square \( 8 \times 8 \) mm grid with the space step \( hx = h_2 = 0.2 \) mm, and the time step was \( \Delta t = 0.005 \) ms. To invert the sparse matrix, we employed the generalized minimum residual method (16, 23) as the most robust and fast for our case. We used diagonal preconditioning for this method. Calculations were performed on a Dell Pentium III PC.

### RESULTS

#### Virtual electrode polarization pattern.

Numerical simulations predicted that during unipolar anodal stimulus the VEP pattern represents a “dogbone”-shaped hyperpolarized area with two positive polarization zones at both sides (Fig. 4A), as previously shown by Sepulveda et al. (24). Simulated cathodal stimulus produced the same VEP but with inverted polarities. Modeling of the bipolar pacing revealed a more complex pattern of VEP, dependent on the location of the stimulating dipole with respect to the fibers (Fig. 4, B and C), as was previously reported by Trayanova and Pilkington (29) and Sepulveda and Wikswo (25).

Experimental data for the virtual electrode polarization patterns during unipolar anodal and cathodal stimuli (Fig. 5, A and D) are in excellent agreement with the results of Sepulveda et al. (24) and our own numerical simulations. Results of the bipolar stimulation of either polarity are shown in Fig. 5, B, C, E, and F. The bipolar electrode was oriented perpendicularly and along the epicardial fibers. The interelectrode distance was 0.8 mm. One can see how dramatically the VEP is affected by the pacing dipole orientation as it was predicted by theory (25, 29). Figures 6 and 7 show

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### Table 1. Two-dimensional passive model parameters

<table>
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<tr>
<td>( \sigma_{i,x} ), mS/cm</td>
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<td>( \beta ), cm⁻¹</td>
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<td>( I_0 ), mA/cm²</td>
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<td>Electrodes across the fibers, ((x_1, y_1)) and ((x_2, y_2))</td>
<td>(0, -0.8) and (0, 0.8)</td>
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<tr>
<td>Electrodes along the fibers, ((x_1, y_1)) and ((x_2, y_2))</td>
<td>(-0.8, 0) and (0, 0.8)</td>
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</tbody>
</table>

\( \sigma_{i,x} \) and \( \sigma_{i,y} \) intracellular potentials along the \( x \)- and \( y \)-axes, respectively; \( \sigma_{e,x} \) and \( \sigma_{e,y} \) extracellular potentials along the \( x \)- and \( y \)-axes, respectively; \( R_m \), membrane resistance times unit area; \( \beta \), surface-to-volume ratio; \( I_0 \), volume density of the stimulation (shock) current.

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### Table 2. 2D active model parameters

<table>
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<tr>
<td>( C_m ), ( \mu )F/cm²</td>
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<td>( \beta ), cm⁻¹</td>
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<td>( \alpha ), mS·cm⁻²</td>
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<tr>
<td>( \beta ), mV⁻²</td>
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<tr>
<td>( I_0 ), mA/cm²</td>
<td>50</td>
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\( C_m \), specific membrane capacitance.

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how the VEP changes in response to the increase in the interelectrode distance from 0.5 to 1.5 mm. The VEP observed during unipolar stimulation using one of two of the same bipolar electrode leads with different polarity are also presented for comparison. As it is shown, the greater the electrode dipole separation, the more closer the VEP pattern corresponds to simple superimposition of individual VEP from single electrode leads.

Fig. 4. Numerically simulated virtual electrode patterns for an interelectrode distance of 0.8 mm. Results were obtained from the passive two-dimensional bidomain model with unequal anisotropy ratios. A: 40 mA/cm anodal current applied to the center of the 8 × 8-mm square sheet; B: bipolar stimulation with the two electrodes located perpendicular to the direction of the fibers; C: bipolar stimulation with the electrodes located along the direction of the fibers. Zero level corresponds to the resting transmembrane potential (white).

Fig. 5. Virtual electrode patterns optically recorded from a 4 × 4-mm area of the rabbit anterior epicardium during unipolar and bipolar stimulation. A and D: conventional "dogbone"-shaped virtual electrode polarizations (VEP) during unipolar cathodal and anodal stimulation. B and E: VEP during bipolar stimulation with a pacing dipole placed perpendicular to myocardial fibers. C and F: results of bipolar stimulation with electrodes along the fibers. The interelectrode distance was 0.8 mm. Images were collected in the middle of a 2-ms diastolic stimulus.
Closer placement of the two electrodes leads to distortion due to the electrotonic interaction between the two patterns produced by individual electrodes.

**Origin of wavefront of activation during unipolar stimulation.** To visualize how excitation originates, we low-pass filtered the data in each optical trace at 250 Hz, differentiated [change in fluorescence over time (dF/dt)] by subtraction two sequential frames (31), and normalized to the last basic beat recorded from the same channel [(dF/dt)/(dF/dt)_{max}] for all of the 256 optical channels. When we suppressed the motion artifacts with BDM, the derivative normalization gave an analogous result to raw signal normalization because the action potential shapes in all channels were similar during the last basic beat. Without BDM the derivative normalization method was the only one possible. The two-dimensional intensity graphs of normalized derivative values [(dF/dt)/(dF/dt)_{max}] for each time frame were used to construct an animation of a wavefront. We set “0” at +20% of (dF/dt)_{max} during basic beat action potential and “1” at 100% of the same value.

Figure 8 shows patterns of VEP and snapshots of wavefronts of excitation for different electrode configurations and pacing current strengths. Maps of VEP were recorded in the middle of a 2-ms stimulus. The first frames in each row (0 ms) in Fig. 8 correspond to the middle of the stimulus and resemble the stimulus-induced VEP, because the positive dF/dt corresponds to depolarization, whereas the negative dF/dt corresponds to hyperpolarization. The rest of the frames in Fig. 8 show how the wave of activation spreads from the pacing site.

Figure 8, A and B, illustrates the case of anodal excitation by strong far-from-threshold (×3) +15-mA and threshold +5-mA stimuli. Threshold was determined with a 1-mA step. These results appear to agree with the results of Wikswo et al. (30). Comparison of maps of VEP shows a predictable difference in the amplitude of positive and negative polarizations. As previously described by Wikswo et al. (30), make excitation in both cases started at areas of positive polarization (virtual cathode; Fig. 8A, 0 ms) and spread outward in all directions. As seen from the comparison of Fig. 8B at 0 and 5 ms, the near-threshold stimulus initially failed to produce a wavefront of excitation from one of two virtual cathodes, which was located in the lower right corner. Therefore, the heartbeat originated only from the upper left virtual cathode. As a result of this failure, a prominent delay in the formation of the wavefront of excitation was observed compared with the +15-mA stimulus, which was able to produce wavefronts from both virtual cathodes.

Cathodal stimulation resulted in a different pattern of activation for far-from-threshold (Fig. 8C, −15 mA) versus near-threshold (Fig. 8D, −5 mA) stimuli. Maps of VEP appeared similar with an expected difference in the amplitude of both positive and negative polariza-
tions, produced by the virtual cathode and virtual anode, respectively. The far-from-threshold (Fig. 8C) stimulus induced a wavefront via the make excitation mechanism. Indeed, at 0 ms, the area of virtual cathode produced a prominent wavefront of excitation in the shape of a dogbone. This wavefront spread outward, initially forming a square (Fig. 8C, 5 ms) due to some delay at areas of hyperpolarization (virtual anode). Later, the wavefront took an elliptical shape with the center coinciding with the stimulation site (not shown). In contrast, near-threshold stimulation (Fig. 8D) resulted in activation via the break excitation mechanism. Indeed, the dogbone-shaped wavefront of excitation (Fig. 8D, 0 ms) formed by the virtual cathode failed to excite all surrounding tissue. Only the hyperpolarized area of virtual anode was excited (Fig. 8D, 5 ms). Furthermore, the two areas of virtual cathodes were excited unequally, such that only the upper left virtual anode area produced a propagated response (asterisk in Fig. 8D, 5 ms). The resulting elliptical pattern was centered on this virtual anode instead of the stimulation electrode.

We observed breaklike excitation during cathodal stimulation at intensities of ×1 through ×2 threshold of excitation in all 12 experiments. In each experiment, we made 60–80 measurements to scan the different current strengths. Those of them (5–8) that were in the mentioned range induced a breaklike activation pattern. In these cases, the locations of the excitation corresponded to the virtual anode areas, which in rabbit hearts are 0.8–1.4 mm from the electrode tip in the longitudinal to cell fiber direction. We did not observe such phenomenon during anodal stimulation at any strength.

**Origin of wavefront of activation during bipolar stimulation.** Similar break excitation was observed during bipolar near-threshold stimulation. Figure 8E illustrates this case. A bipolar stimulation electrode was oriented perpendicular to the fibers. Initial activation (Fig. 8E, 0 ms) again corresponded to the virtual cathode (compare with VEP map). However, this activation failed to produce a propagated response in all directions except the one area of virtual anode in the left lower corner of the field of view. A wavefront of excitation formed in this area at 5 ms (Fig. 8E, 5 ms) and spread outward in all directions. An ellipse was centered on this virtual anode rather than the site of stimulation.

**Control measurements in the absence of BDM.** The observed difference in a wavefront formation between
Fig. 8. Activation wavefront propagation during near- and far-from-threshold pacing stimuli. The orange-black intensity plots of normalized optical signal derivatives (change in fluorescence over time /\[(dF/dt)\]/maximum change in fluorescence over time /\[(dF/dt)_{\text{max}}]\] at different time frames visualize the propagation of the excitation wavefront resulting from unipolar and bipolar pacing. The black color corresponds to the resting potential and the white color corresponds to the wavefront of excitation, which is defined as the maximum rate of rise of the transmembrane potential. Left: corresponding VEP patterns (red-blue plots) at 0 ms (in the middle of a 2-ms stimulus).

Fig. 9. Activation wavefront propagation during near- and far-from-threshold cathodal pacing stimuli without 2,3-butanedione monoxime (BDM). The orange-black intensity plots of normalized optical signal derivatives /\[(dF/dt)/(dF/dt)_{\text{max}}]\] at different time frames visualize the propagation of the excitation wavefront resulting from unipolar cathodal pacing of the heart without BDM. The black color corresponds to the resting potential and white color corresponds to the wavefront of excitation, which is defined as the maximum rate of rise of the transmembrane potential.
near-threshold and far-above-threshold stimulation was preserved in the absence of BDM (Fig. 9). The pacing threshold was significantly smaller when the heart was perfused without BDM (0.3 ± 0.2 vs. 2.7 ± 0.8 mA). Therefore, it was difficult to resolve the VEP pattern for the near-threshold stimulus (Fig. 9B, 0 ms).

Nevertheless, the comparison of the initial positions of wavefronts of excitation (Fig. 9, A, 2.4 ms, and B, 2.5 ms) clearly shows that during the near-threshold cathodal stimulation the wavefront also starts from the hyperpolarized areas of VEP (asterisks in Fig. 9B, 2.5 ms) as it did in the BDM experiment (Fig. 8D). Furthermore, the left virtual anode produced a stronger wavelet compared with the right wavelet.

Biphasic action potential upstroke. Figure 10A shows VEP and the optical traces from the virtual anode (blue) and cathode (red) regions during cathodal stimulus of a near-threshold strength. The traces demonstrate that the virtual cathode produces an initial depolarization of transmembrane potential. However, this depolarization does not succeed in depolarizing neighboring regions. This is perhaps due to source-sink mismatch. As a result, this region is partially repolarized upon stimulus withdrawal due to the diffusion of $V_m$ into neighboring hyperpolarized regions. This biphasic morphology of the upstroke of the response (Fig. 10A, right, red trace) corresponds to the decay of the wavefront at the virtual cathode area shown in Fig. 8D. Simultaneously, the virtual anode produces transient hyperpolarization, which rapidly reverses upon stimulus withdrawal. This reversal overshoots the resting potential and produces an active response, which initiates the heartbeat, driving areas of virtual cathode (Fig. 10A, left, blue trace) after a delay. Optical recordings often are distorted due to depth averaging. Therefore, the observed phenomenon could be explained by averaging of superficial regions of virtual cathode and a slightly deeper region of virtual anode, which could come close in the z-direction due to the rotation of fiber orientation (9). To rule out this hypothesis, we conducted numerical simulations in an active two-dimensional bidomain model.

Computer simulations confirmed our experimental observations (Fig. 10B). Near-threshold stimulus applied to the center of the square sheet of the bidomain model resulted in VEP, which is shown at the end of a

Fig. 10. Biphasic upstrokes of action potentials produced by near-threshold stimulation. VEP (left) and optical signal traces (right) from hyperpolarized and depolarized areas for near-threshold cathodal stimulus are shown. A: experimental data; B: data from the two-dimensional active numerical model.
2-ms pulse. After termination of the stimulus, the wavefront started at the virtual anode areas (hyperpolarized regions), driving the areas of virtual cathode (depolarized regions).

**DISCUSSION**

Break excitation is believed to occur when areas depolarized by the external field cannot develop an active potential due to sodium channel inactivation (30). In this case, their depolarization diffuses to the adjacent hypopolarized regions, which start the break excitation wavefront. A similar mechanism plays a role on a spatially larger scale in shock-induced vulnerability and defibrillation failure (8).

We present evidence suggesting that similar effects take place during near-threshold cathodal stimulation, when the pacing current strength is insufficient to start an active potential in the depolarized area of VEP. Then, as in the case of classic break excitation, the depolarization electrotonically diffuses into initially hypopolarized regions, where it is able to initiate an active potential. But the initially depolarized tissue remains excitable compared with the classic break excitation because the pacing stimulus is short and the state of inactivation is not reached.

Our data suggest that both make and break excitation mechanisms play roles during clinically relevant short diastolic cathodal stimuli. Make excitation is the predominant mechanism for strong stimuli with strength far-above-threshold of excitation. In contrast, weak near-threshold stimuli appear to be driven by a break excitation mechanism. A future careful experimental and theoretical examination of the transition from the break mechanism to the make mechanism with an increasing stimulus strength may have important implications because it takes place at clinically relevant intensities.

**Limitations.** The major part of our study was accomplished with the excitation-contraction uncoupler BDM, which is known to affect ionic channel conductivities. This can amplify the observed effect of break excitation by enhancing the role of initial hypopolarization, which can decrease the number of blocked sodium channels and promote an active response to depolarization from adjacent VEP regions. Yet, as demonstrated by our control study, a similar break excitation phenomenon is observed during near-threshold stimuli in hearts perfused without BDM.

Our numerical model and experimental interpretations ignored the three-dimensional nature of stimulus-induced VEP. The data recorded from the heart surface may represent an average from cellular layers at different depths with different polarizations. This can explain the quantitative discrepancy between the numerical simulation and experimental data. However, qualitatively these two approaches do demonstrate the break excitation at near-threshold stimuli with characteristic biphasic morphology of the upstroke.

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