The role of dynamic instability and wavelength in arrhythmia maintenance as revealed by panoramic imaging with blebbistatin vs. 2,3-butanedione monoxime

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Lou Q, Li W, Efimov IR. The role of dynamic instability and wavelength in arrhythmia maintenance as revealed by panoramic imaging with blebbistatin vs. 2,3-butanedione monoxime. Am J Physiol Heart Circ Physiol 302: H262–H269, 2012. First published October 28, 2011; doi:10.1152/ajpheart.00711.2011.—Unlike other excitation-contraction uncouplers, blebbistatin has few electrophysiological side effects and has gained increasing acceptance as an excitation-contraction uncoupler in optical mapping experiments. However, the possible role of blebbistatin in ventricular arrhythmia has hitherto been unknown. Furthermore, experiments with blebbistatin and 2,3-butanedione monoxime (BDM) offer an opportunity to assess the contribution of dynamic instability and wavelength of impulse propagation to the induction and maintenance of ventricular arrhythmias. Recordings of monophasic action potentials were used to assess effects of blebbistatin in Langendorff-perfused rabbit hearts (n = 5). Additionally, panoramic optical mapping experiments were conducted in rabbit hearts (n = 7) that were sequentially perfused with BDM, then washed out, and subsequently perfused with blebbistatin. The susceptibility to arrhythmia was investigated using a shock-on-T protocol. We found that 1) application of blebbistatin did not change action potential duration (APD) restitution; 2) in contrast to blebbistatin, BDM flattened APD restitution curve and reduced the wavelength; and 3) incidence of sustained arrhythmia was much lower under blebbistatin than under BDM (2/123 vs. 23/99). While arrhythmias under BDM were able to stabilize, the arrhythmias under blebbistatin were unstable and terminated spontaneously. In conclusion, the lower susceptibility to arrhythmia under blebbistatin than under BDM indicates that blebbistatin has less effects on arrhythmia dynamics. A steep restitution slope under blebbistatin is associated with higher dynamic instability, manifested by the higher incidence of not only wave breaks but also wave extinctions. This relatively high dynamic instability leads to the self-termination of arrhythmia because of the sufficiently long wavelength under blebbistatin.

blebbistatin; 2,3-butanedione monoxime; action potential duration restitution; wavelength; reentry

OPTICAL IMAGING TECHNIQUES have been widely used to study the cardiac electrophysiology in species ranging from embryonic zebrafish to human beings (13, 23, 29). Optical mapping offers several important advantages over alternative mapping techniques: easily adjustable high spatial resolution, no physical contact with the tissue, absence of stimulation artifacts, and the capacity to simultaneously map different physiological parameters (13). Despite these advantages, optical mapping suffers from the need to utilize excitation-contraction (EC) uncouplers to remove motion artifacts from the optically recorded signals. Unfortunately, most of these uncouplers have electrophysiological side effects. For example, the popular inexpensive uncoupler 2,3-butanedione monoxime (BDM) results in changes of the action potential (AP) and conduction velocity (CV) in a species-dependent manner by affecting the ion channels, Ca2+ handling, and gap junctional coupling (8, 33, 53, 55, 56). Another EC uncoupler, cytochalasin D, also changes AP morphology (1, 4, 7) by affecting various ion channels (38, 41, 50, 54).

Our group has previously shown that blebbistatin could completely eliminate mechanical contraction without appreciable electrophysiological effects on AP morphology, electrocardial (ECG) parameters, conduction, and refractoriness in the rabbit heart (17). Following this introduction of blebbistatin as an EC uncoupler (17), it has increasingly gained popularity in cardiac electrophysiological studies. As of today, it was successfully applied in optical mapping studies of the hearts from embryonic zebrafish (27), mouse (9), rabbit (17, 40, 42), dog (18, 30), horse (19), and human beings (15, 16, 23, 34).

Both BDM and cytochalasin D were found to affect the vulnerability to ventricular arrhythmias (7) and the pattern of ventricular fibrillation (25, 31, 51). However, the effect of blebbistatin on ventricular arrhythmia is unknown. The aim of this study was to determine the vulnerability and characteristics of shock-on-T-induced arrhythmias under blebbistatin compared with BDM. Because shock-induced arrhythmia typically self-terminates in normal rabbit hearts in vivo (37) and in vitro (7) in the absence of EC uncoupler, we hypothesized that sustainability of shock-induced arrhythmia will be lower under blebbistatin vs. BDM.

METHODS

Experimental protocols. Animal protocol approved by the Washington University Institutional Animal Care and Use Committee. New Zealand White rabbit hearts (n = 12, 4 mo old) were Langendorff-perfused with oxygenated 37°C Tyrode solution as previously described (12, 35). In the first set of experiments (n = 5), we recorded monophasic action potentials (MAP) using a MAP electrode (Harvard Apparatus, Holliston, MA) under control conditions (i.e., no drug) and under 10 μM blebbistatin. We applied the S1S2 ventricular pacing protocol consisting of an initial train of 15 S1 stimuli with an S1–S1 interval of 300 ms followed by a single S2 stimulus. We gradually decreased the S1–S2 interval from 400 ms to the refractory period. Pacing current was adjusted to two times the pacing threshold. Continuous MAP recordings were maintained under both conditions. No voltage-sensitive dye was used in this part of the study. Quasi-ECG Lead I was measured by two Ag/AgCl electrodes (9 mm diameter).

In the second set of experiments (n = 7), the heart was stained with di-4-ANEPPS (20–40 μl of 1.25 mg/ml; Invitrogen, Carlsbad, CA). The heart was perfused in the following sequence: 1) Tyrode solution with 15 mM BDM (Fisher Scientific, Hampton, NH); 2) Tyrode solution alone to wash out the BDM; and 3) Tyrode solution with 10 μM blebbistatin (Tocris Bioscience, Elllisville, MO). The heart was perfused with BDM first because BDM can be completely washed out...
more than six beats but no shock. The arrhythmia was categorized as "nonsustained" if it lasted 1 min after the shock. The arrhythmia was characterized as "sustained" if it lasted for ≥1 min, which always required a defibrillation shock for termination. To avoid excessive shocks applied to the heart, the vulnerability grid was tested for just one polarity configuration where the mesh electrode facing the right ventricle (RV) was the cathode and the mesh facing the left ventricle (LV) was the anode.

**Optical imaging system.** The panoramic optical imaging system could record the optical AP from almost the entire ventricular epicardium of the rabbit heart (52). Details of the panoramic imaging system and corresponding data analysis methods have been described earlier (36, 45). This system, including three photo-diode arrays (PDAs), optically maps the APs at three different angles (120° apart). To combine the data from three PDAs into one unified surface, the epicardial three-dimensional surface of every experimented rabbit heart was first reconstructed from 36 silhouette images of that heart. The panoramic optical imaging system optically maps the APs at three different angles (120° apart). To combine the data from three PDAs into one unified surface, the epicardial three-dimensional surface of every experimented rabbit heart was first reconstructed from 36 silhouette images of that heart. The panoramic optical imaging system could record the optical AP from almost the entire ventricular epicardium of the rabbit heart (52). Details of the panoramic imaging system and corresponding data analysis methods have been described earlier (36, 45). This system, including three photo-diode arrays (PDAs), optically maps the APs at three different angles (120° apart). To combine the data from three PDAs into one unified surface, the epicardial three-dimensional surface of every experimented rabbit heart was first reconstructed from 36 silhouette images of that heart. The optical signals were then registered onto the reconstructed surface. After that, various parameters, including APD, CV, wavelength (APD × CV), and wave propagation, were quantified, and can be visualized on the reconstructed surface. To characterize Winfree’s "elbow room" needed for reentry to sustain, we introduce a new factor, wavelength surface area, computed as the product of longitudinal and transverse wavelengths.

**Data analysis.** The APD was measured at 80% repolarization. The longitudinal and transverse CVs were measured near the pacing site at the epicardial surface. The phase was calculated by Bray and Wikswo’s (5) method and was used to visualize the wavefronts and phase singularities during reentrant arrhythmias.

**Statistical analysis.** Averaged data were presented as means ± SE. Comparisons were made between results under control conditions and blebbistatin, between results under blebbistatin and BDM when they were sequentially perfused in the same hearts, and between results under blebbistatin with and without previous perfusion of BDM. To determine the level of statistical significance, two-way repeated-measures ANOVA was used for Figs. 1 and 2, and the two-tailed paired t-test was used for Fig. 3. Differences were considered significant when \( P < 0.05 \).

**RESULTS**

**Monophasic AP with and without blebbistatin.** To determine the effects of blebbistatin on the AP morphology and APD, the MAP were recorded before and after the application of blebbistatin under multiple pacing rates. We did not detect significant differences between the control and blebbistatin in the measurement of APD. Figure 1 shows representative MAP recordings (Fig. 1A) and the statistical summary (Fig. 1B). The overlap between the MAP recordings and ECG for the control (blue) and blebbistatin (red) in Fig. 1A indicates no change of

(4, 31) while it is very difficult to wash out blebbistatin (17). The average duration of experiments under BDM was 69 min. To determine whether the AP duration (APD) and CV under blebbistatin was affected by the preceding perfusion of BDM, we conducted another five experiments without BDM, where APD and CV were measured when blebbistatin was not preceded by BDM.

The S1S2 pacing protocol was applied for the quantification of APD restitution and CV restitution. The bipolar pacing electrode was placed epicardially in the center of the anterior view of the heart to allow the measurement of the CV in both longitudinal and transverse directions of the fiber orientation, as described earlier (36, 45).

The vulnerability to shock-induced arrhythmia was quantified using the vulnerability grid (14, 32), in which inducibility of arrhythmia was determined for varying shock strengths (2–14 V/cm) and varying coupling intervals spanning the T wave (equivalently, 70–140% of the averaged APD). Uniform far-field square monophasic shocks (10 ms in duration) were delivered via two mesh electrodes on opposite sides of the heart (10, 32) after a train of 15 stimuli with S1–S1 interval of 300 ms and varying coupling interval between the last S1 and the shock. The arrhythmia was categorized as “nonsustained” if it lasted more than six beats but ≤1 min after the shock. The arrhythmia was defined as “sustained” if it lasted for >1 min, which always required a defibrillation shock for termination. To avoid excessive shocks applied to the heart, the vulnerability grid was tested for just one polarity configuration where the mesh electrode facing the right ventricle (RV) was the cathode and the mesh facing the left ventricle (LV) was the anode.

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**Fig. 1.** Monophasic action potential (MAP) recordings. A: representative MAP and electrocardial (ECG) recordings for S1S2 = 300 and 200 ms. B: summary of MAP durations at 80% repolarization (MAPD80) at various S1S2 values. MAPD80 was not significantly changed under blebbistatin.

**Fig. 2.** Comparison of action potential duration (APD) restitution, conduction velocity (CV) restitution, and wavelength (APD × CV) between blebbistatin and BDM (blue squares) and blebbistatin (red circles) from the same hearts. Green triangles (noted as blebbistatin-2 in C) indicate the values when hearts were perfused with blebbistatin without previous perfusion of BDM.
AP morphology after the application of blebbistatin. This is further demonstrated in the summary in Fig. 1B, which showed no significant effects of blebbistatin on APD.

APD and CV restitution and wavelength. Figure 2A shows representative optical AP recordings, and Fig. 2C, top, summarizes APD restitution curves under blebbistatin and BDM. Figure 2B shows representative activation maps, and Fig. 2C, middle, shows CV restitution curves along both longitudinal and transverse directions under blebbistatin and BDM. As expected, the APD was shorter and the APD restitution curve was flatter under BDM; the CV was slower under BDM in both directions. Wavelength was computed for longitudinal and transverse conduction and was significantly longer under blebbistatin than BDM (Fig. 2C, bottom). At different cycle lengths, the wavelength surface area, which is a simple estimation of area needed for sustaining reentry, ranged from 19~34 cm² under BDM and 39~60 cm² under blebbistatin. The average ventricular epicardial surface area was 39.4 ± 1.8 cm² (n = 7), which was well above the wavelength surface area of BDM but close to or below the wavelength surface area of blebbistatin.

Figure 2C shows the APD, CV, and wavelength under BDM (blue squares) and two conditions of blebbistatin perfusion, when blebbistatin was preceded by BDM and washout (red circles) and when BDM was not used before blebbistatin (green triangles). We did not observe any statistically significant differences between blebbistatin with and without preceding BDM, suggesting that the effects of preceding perfusion of BDM on the APD and CV under blebbistatin were minimal.

Vulnerability to shock-induced ventricular tachyarrhythmia. Figure 3 shows the arrhythmic incidence at different shock strengths and various coupling intervals under BDM (Fig. 3A, left) and blebbistatin (Fig. 3A, right). In total, 99 shocks were applied under BDM, and 123 shocks were applied under blebbistatin in all hearts. The overall incidence is the ratio between the number of all arrhythmia events and the number of all shocks from seven experiments. In the present study, the overall incidence of arrhythmia (including both nonsustained and sustained arrhythmia) was 38/99 under BDM and 33/123 under blebbistatin (Fig. 3A, row on top). The average incidence is the mean of individual incidence, calculated from each individual experiment. This average incidence was significantly lower under blebbistatin (27 ± 5% under blebbistatin vs. 51 ± 8% under BDM, P = 0.046, Fig. 3B). Furthermore, the total incidence of sustained arrhythmia is 23/99 under BDM and 2/123 under blebbistatin (Fig. 3A, row on bottom), that is, the average probability of sustained arrhythmia under blebbistatin was drastically lower than under BDM (1 ± 1% under blebbistatin vs. 30 ± 5% under BDM, P = 0.001, Fig. 3B).

Induction of arrhythmia. The induction of arrhythmias was similar under both conditions and was due to a well-established mechanism termed virtual electrode-induced phase singularity (11). Figure 4 shows a representative example of arrhythmia induction (under BDM). Essentially, the far-field shocks produced spatially heterogeneous virtual electrode polarizations at different regions of the heart (Fig. 4, A and B), consisting of a virtual anode (blue) and a virtual cathode (red). Figure 4C shows a sequence of snapshots of activation wavefronts (dark red) over the course of arrhythmia induction, starting immediately after the shock. A wavefront of excitation arises at the boundary between the virtual cathode and the virtual anode via the break-excitation mechanism (see emerging red area in the snapshots for 10 and 30 ms in Fig. 4C). Next, it spreads to the de-excited region of the virtual anode, leading to the initiation of reentrant arrhythmia maintained by two phase singularities with opposite topological charges. Phase singularities are seen in Fig. 4C as points where all phases, represented by different colors, converge.

Stabilization of arrhythmia under BDM. The arrhythmia under BDM was able to stabilize even when the arrhythmia started in a meandering and unstable form. Figure 5A shows an example, with the snapshots of wavefronts (dark red) showing the steps of the stabilization of the reentry under BDM. In the first beat, there are multiple lines of conduction blocks, indicated by the crowded isochrones. The number of conduction blocks is reduced to one in the second beat since we know that the left side connects with the right side in that unwrapped map. After that, this line of conduction block starts to shrink until the reentry has stabilized (3rd to 8th beats).

Fig. 3. Vulnerability grid and incidence of shock-induced arrhythmia. A: vulnerability grid with the inducibility of arrhythmia shown for various shock strengths and coupling intervals for both BDM (left, n = 7) and blebbistatin (right, n = 7). The row on top shows the incidence of nonsustained and sustained arrhythmia combined. The row on bottom shows the incidence of sustained arrhythmia only. It can be seen that the inducibility of sustained arrhythmia is significantly lower under blebbistatin (23/99 under BDM vs. 2/123 under blebbistatin). B: summary of the incidence of shock-induced arrhythmia.
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Fig. 4. Induction of arrhythmia by a T wave shock. A: representative action potential recordings with shock marked by the orange stripe. B: the virtual electrode polarization pattern induced by a 10-V/cm shock at a coupling interval of 160 ms. The red color indicates the positive polarity (virtual cathode); the blue color indicates the negative polarity (virtual anode). C: postshock activation snapshots with the end of shock indicated by 0 ms. The shock-induced wavefront initiates from the border between positive and negative virtual electrode polarization and propagates in the form of figure-of-8 reentry. This figure shows the data under BDM. The data for blebbistatin is not shown because the arrhythmia induction under blebbistatin was similar to that under BDM shown here.

We observed 17 morphologies of stable reentries, with each having a single or double phase singularity (or singularities) at the epicardium under BDM. The locations of all phase singularities from seven hearts are summarized in Fig. 5B at three different views (anterior, RV, and LV). It can be seen that the anchoring of reentry was geometrically asymmetrical, with the highest probability at the gray area in Fig. 5B, where RV inserts into the septum and LV. Less frequently, the reentry anchored at the apex or at the RV free wall (Fig. 5B). Interestingly, no epicardial anchoring at the LV was observed.

Unstable maintenance and self-termination of arrhythmia under blebbistatin. Despite a similar mechanism of arrhythmogenesis, the dynamic maintenance of shock-induced arrhythmia was different under blebbistatin. Similar to the in vivo observations (37), >98% of the shock-induced arrhythmia self-terminated under blebbistatin within 1 min. Wave breaks and wave collisions were common, and the wave propagation was unstable with beat-to-beat variations under blebbistatin. Figure 6 shows snapshots of wave propagations under BDM (Fig. 6A) and blebbistatin (Fig. 6B) in the same heart. It can be seen that the reentry under both conditions anchors at the apex of the heart. Despite the similarity in the anchoring site, the regularity or stability of reentry differs under these two conditions. The plot on the bottom right in each panel shows the activation sequence of all the recorded epicardial sites over the course of three consecutive beats, that is, each green dot in this panel indicates the activation of a particular mesh element (y-axis) at a particular time (x-axis). The repeatability of the activation pattern for every beat shown in Fig. 6A, bottom right, indicates the regular and stable conduction under BDM. On the other hand, small local conduction blocks (arrows in Fig. 6B) were frequent under blebbistatin. Figure 6B, bottom right, shows the beat-to-beat variation in propagation. The holes within the green stripes in Fig. 6B, bottom right, indicate the local wave breaks and subsequent wave extinctions (or collisions). Whereas the stable reentry under BDM was terminated by a defibrillation shock, the unstable reentry under blebbistatin self-terminated during the intermission between two consecutive data acquisitions.

Figure 7 is an example of self-termination of an unstable figure-of-eight reentry under blebbistatin, which was anchored at two phase singularities (white dots). Figure 7A is an AP recording over the course of pacing, shock, arrhythmia, and self-termination. Figure 7B shows phase maps (in polar view with the apex in the center) indicating the two phase singularities with opposite topological charges induced by the shock. It is clear that the locations of phase singularities gradually changed over time. The reentry self-terminated due to the collision and annihilation of two phase singularities in the last beat. Figure 7, C–E, is several other snapshots of activation in the same recording and shows two characteristics of reentry

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frequently observed under blebbistatin but not under BDM. One characteristic is the “stop-sign phenomenon,” during which the wavefront appears to stop for a while and then continues from where it stopped (Fig. 7C). This behavior is also evident from the temporal gaps between activations from all mapped areas on the epicardium (Fig. 7D). Another characteristic is that a breakthrough often appears ahead of the wavefront (Fig. 7E). Both characteristics suggest the presence of transmural activations.

DISCUSSION

In this study, we investigated the effect of blebbistatin on the vulnerability to shock-induced ventricular arrhythmias in the normal rabbit heart. We found that 1) APD restitution under blebbistatin was not significantly different from the control condition, 2) BDM significantly reduced APD, CV, and wavelength, and flattened APD restitution, and 3) the sustainability to shock-induced arrhythmia was much lower under blebbistatin (2/123) than under BDM (23/99). These results showed that blebbistatin did not affect the vulnerability to shock-induced arrhythmia and suggested blebbistatin as a superior EC uncoupler to BDM in the arrhythmia studies using optical mapping. In addition, both dynamic instability and wavelength appear to contribute to the self-termination of arrhythmia under blebbistatin and therefore explain the lower susceptibility to sustaining arrhythmia under blebbistatin than under BDM.

Effect of blebbistatin on electrophysiology. It has been previously shown that the ventricular arrhythmia in the normal rabbit heart is prone to self-termination (3, 7, 37). The resistance to sustained arrhythmia under blebbistatin observed in this study suggests the preservation of normal electrophysiology by blebbistatin. We did not observe any changes of ventricular AP morphology under blebbistatin, which is consistent with other studies in the rabbit heart (17, 28), mouse heart (9), embryonic zebrafish heart (27), and equine heart (19). Taken together, these results suggest insignificant electrophysiological side effects of blebbistatin.

While blebbistatin appears to be a “clean” drug for the normal heart, whether blebbistatin changes the electrophysiology in the diseased heart remains to be determined. Blebbistatin inhibits myosin ATPase and thus prevents a significant amount of ATP from being consumed by mechanical contractions, making it available to electrogenic pumps. This effect could enhance the metabolic state of excitation and Ca2+ handling in a diseased heart. Baudenbacher et al. (2) showed that Ca2+ desensitization of myofilaments by blebbistatin could reduce the arrhythmia in transgenic mice expressing troponin-T mutations. By inhibiting the contraction, blebbistatin could also prevent sarcolemmal rupture and cell death by reducing the mechanical stress occurring at the onset of reperfusion after ischemia (20, 26). These protective effects of blebbistatin suggest that caution should be taken in applying blebbistatin when studying diseased heart.

Functional reentry in the rabbit heart. Because there is no structural obstacle (such as myocardial infarction scar) in these normal rabbit hearts, the anchoring of reentry under BDM is achieved at the normal anatomical structures. This observation suggests that an abnormal structure is not necessary for sustaining the arrhythmia. The observed asymmetrical distribution of anchoring points of stable reentrant arrhythmia (Fig. 5B) indicates that the anterior RV insertion area (gray area in Fig. 5B) is the most favorable area for anchoring. A computer simulation study by Park et al. (44) also identified the anterior RV insertion area as a distinct substrate for arrhythmia. In their whole rabbit ventricular model with global reduction of Na+ currents (44), they found that the RV insertion area was more susceptible to arrhythmia because of a source-sink mismatch in the RV insertion area (44). These results suggest that global electrical remodeling does not necessarily lead to a spatially homogeneous increase in the arrhythmia susceptibility. Washout of BDM and application of blebbistatin in our study almost completely abolished the maintenance of ventricular arrhythmia, suggesting that idiopathic ventricular tachycardia with normal heart structure.
might respond well with pharmacological intervention, despite the presence of several preferential anchoring points of reentry.

**Dynamic instability under blebbistatin.** According to the computer simulations, steep APD restitution is associated with enhanced dynamic instability (6, 43, 47, 49). This association could partially explain the more unstable wave dynamics under blebbistatin than under BDM. The instability of wave propagation under blebbistatin was reflected by frequent wave breaks and wave extinctions. The dynamic instability is further reflected by the frequent occurrence of breakthrough excitation ahead of the wavefront (Fig. 7E). This type of breakthrough excitation on the surface of three-dimensional (3D) tissue has been previously observed in an elegant computer simulation study of scroll wave dynamics by Qu et al. (48), who demonstrated correlation between the breakthrough activity with the strong dynamic instability. In a 3D tissue like the rabbit heart in this study, a scroll wave faces excitable tissue not only in its own layer but also in the neighboring layers. According to the simulation (48), the strong dynamic instability and fiber orientations could result in different speed of spiral waves at different layers of the tissue. When the spiral wave at a depth (or intramural conduction) is faster than the spiral wave on the surface, an upward propagation toward the epicardial surface could occur and thus produce the breakthrough excitation on the epicardial surface. The “first stop, and then continue” wavefront (Fig. 7C) ob-

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**Fig. 7.** Self-termination of reentry under blebbistatin. A: a representative action potential recording. Shock is indicated by the orange stripe. B: phase maps from 12 consecutive beats (time points indicated by the asterisks in A) with phase singularities indicated by white dots. The map is shown in polar form with the apex in the center. It is evident that the phase singularities of figure-of-8 reentry meander over time. The annihilation of the two phase singularities with opposite topological charge led to the termination of arrhythmia. C: wave front and tail collision. Two phase maps show that the wavefront stops for 45 ms before it continues. The arrows indicate where the wavefront stops. The activation map is shown on right. D: the activation plot with each dot corresponding to an activation of one pixel. Each pixel is coded with a unique color. The y-axis is the cycle length. The gray stripes indicate the gap where no epicardial activation is observed and correspond to the wave front and tail collision seen in C. E: breakthrough excitation ahead of the wavefront. Three consecutive phase maps are shown with the breakthrough excitation (indicated by the white asterisk) visible on the second one.
served under blebbistatin also suggests the presence of intracardiac conduction connecting the epicardial activation gaps (Fig. 7D).

Dynamic instability and self-termination of arrhythmia. The dynamic instability associated with steep APD restitution was often considered to promote ventricular fibrillation (6, 21, 49, 51, 57). However, this dynamic instability could also lead to the spontaneous termination of arrhythmia in a finite-sized tissue (46). The computer simulation study by Qu (46) demonstrated that “dynamic instability promotes wave breaks, maintaining ventricular fibrillation, but it also causes the waves to extinguish, facilitating spontaneous termination of fibrillation.” This hypothesis seems to correlate well with our observations under blebbistatin. This hypothesis is also supported by the experimental findings of Harada et al. (24). They found that mild hypothermia did not alter the wavelength, but it did increase the dynamic instability, significantly reducing the incidence of sustained arrhythmia (24). Mines (39) and Garrey (22) postulated that the wavelength compared with the tissue size is the major determinant of reentry maintenance. Qu’s (46) simulation indicated that whether dynamic instability promotes or prevents the maintenance of arrhythmia depends on the effective tissue size, which is determined by the wavelength and the actual tissue size. The usefulness of wavelength surface area in predicting the sustainability of arrhythmia in this study suggests the important role of wavelength in determining the arrhythmia maintenance.

It is difficult to quantify the individual contribution of dynamic instability and wavelength in the maintenance of arrhythmia, because the experimental approach does not have the luxury to finely tweak the dynamic instability and effective tissue size in three dimensions as the computer simulations so elegantly do. Nevertheless, our results indicate the beneficial role of dynamic instability and wavelength combined in preventing the maintenance of arrhythmia.

There are limitations in this study. First, we could not measure the true wavelength during the arrhythmia, because the apparent epicardial CV might not reflect the true CV and the baseline (minimum of the signal) might not represent the resting state. Nevertheless, the wavelength surface area, which was calculated from wavelengths during pacing, works surprisingly well in predicting the sustainability of arrhythmia in this study. The second limitation is that reentry dynamics are only studied in the normal heart in this study. Caution is needed to extrapolate the results to diseased hearts. Third, the vulnerability of the heart to arrhythmia between the control condition and under blebbistatin is not compared in this study. However, low arrhythmia vulnerability is expected in the normal heart and has been demonstrated previously both in vivo and ex vivo (7, 14, 37). Finally, we cannot exclude the effects of edema, which might develop in the mechanically silent preparations.

In conclusion, the low incidence of sustained arrhythmia and the preserved APD restitution under blebbistatin suggest blebbistatin as a superior EC uncoupler to BDM. The combination of dynamic instability and wavelength facilitates the spontaneous termination of arrhythmia.

DISCLOSURES

No conflicts of interest are declared by the authors.

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GRANTS

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