Shock-induced arrhythmogenesis is enhanced by 2,3-butanedione monoxime compared with cytochalasin D

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Cheng, Yuanna, Li Li, Vladimir Nikolski, Don W. Wallick, and Igor R. Efimov. Shock-induced arrhythmogenesis is enhanced by 2,3-butanedione monoxime compared with cytochalasin D. Am J Physiol Heart Circ Physiol 286: H310–H318, 2004. —Investigation of the mechanisms of arrhythmia genesis and maintenance has benefited from the use of optical mapping techniques that employ excitation-contraction uncouplers. We investigated the effects of the excitation-contraction uncouplers 2,3-butanedione monoxime (BDM) and cytochalasin D (Cyto D) on the induction and maintenance of arrhythmia by electric shocks. Electrical activity was optically mapped from the anterior epicardium of rabbit hearts (n = 9) during shocks (−100 V, 8 ms) applied from a ventricular lead at various phases of action potential duration (APD). Restitution curves are obtained by using S1-S2 protocol and measurement of APD values at 70% of repolarization. Compared with Cyto D, BDM significantly shortened APD at 90% of repolarization, although no significant difference in dispersion of repolarization was observed. Wavelength was also shortened with BDM. In general, shock-induced arrhythmias with BDM and Cyto D were ventricular tachyarrhythmias of nature. With respect to shock-induced sustained arrhythmias, the vulnerable window was wider and the incidence was higher with BDM than with Cyto D. There was also a difference in the morphology of ventricular tachycardia (VT) between the two agents. The arrhythmias with BDM usually resembled polymorphic VT, especially those that lasted >30 s. In contrast, arrhythmias with Cyto D more resembled polymorphic VT. However, the average number of phase singularities increased with Cyto D vs. BDM, whereas no significant difference in the dominant frequency of shock-induced sustained arrhythmias was observed. BDM reduced the slope of the restitution curve compared with Cyto D, but duration of arrhythmia under BDM was significantly increased compared with Cyto D. In conclusion, BDM increased arrhythmia genesis and maintenance relative to Cyto D.

Optical imaging of electrical activity with voltage-sensitive dyes is commonly used in studies of arrhythmia induction, maintenance, and termination. The advantages of these techniques compared with conventionally used electrode-based mapping include the ability to map entire action potentials from hundreds or thousands of sites; immunity from electrical stimulus-induced artifacts, and adjustable spatial and temporal resolutions (18).

However, these advantages come at a price. The major difficulty in applying optical mapping techniques is the motion artifact, which is caused by vigorous contraction of the myocardium. One way to overcome motion artifacts is to use excitation-contraction uncouplers such as 2,3-butanedione monoxime (BDM; Refs. 5, 15, 19, 25, 28) to abolish the myocardial contraction. However, BDM is known to inhibit many ionic currents at concentrations that are effective at uncoupling contractile activity, and it causes a significant shortening of action potential duration (APD) in canine (7, 42), sheep (32), guinea pig (32), and swine (31) hearts. Cytochalasin D (Cyto D) was recently proposed as a novel excitation-contraction uncoupler for optical mapping studies (7, 42). Cyto D has been shown to block contractions without affecting action potential shape or duration in rat (39, 44), canine (7, 42), and swine (31) hearts. Cyto D is becoming an excitation-contraction uncoupling agent of choice in optical mapping studies despite its effects on mouse action potentials (26) and its significant cost and toxicity. Thus additional characterization of this agent is warranted to evaluate its wider applicability in the field.

The recently formulated restitution hypothesis suggests that BDM has an antifibrillatory effect. Riccio et al. (37) and Lee et al. (31) showed that BDM prevented the induction of ventricular fibrillation (VF), caused less dynamic complexity of fibrillation, and converted existing VF into ventricular tachycardia (VT). They also suggested that this conversion of VF to VT was due to a reduction of the slope of the APD restitution curve by BDM. On the other hand, the slope of the restitution curve with Cyto D has been shown to be steeper than that of BDM (1, 31). Therefore, investigation of the effects of the two agents on the restitution curve with Cyto D has been shown to be steeper than that of BDM (1, 31). Therefore, investigation of the effects of the two agents on the restitution curve was used to elucidate mechanisms of arrhythmia maintenance. However, a recent report by Banville and Gray (2) illustrates that alternans and arrhythmia dynamics in rabbit hearts are affected by the spatial dispersion of APD restitution as well as conduction velocity (CV) restitution and not simply by the slope of APD restitution.

Qin et al. (36) recently reported the effects of major interventions of heart isolation, voltage-sensitive dye, and excitation-contraction uncouplers used during optical mapping on VF in pig hearts. Despite the importance of optical mapping in vulnerability/defibrillation research, the comparative effects of BDM and Cyto D on shock-induced arrhythmia genesis and maintenance have not yet been systematically evaluated. The purpose of this study was 1) to compare the effects of BDM and Cyto D on optically recorded action potentials in Langendorff-perfused rabbit hearts at the effective concentrations routinely used to immobilize the heart, 2) to examine the effects of BDM and Cyto D on control monophasic action potential duration restitution; optical mapping

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potentials under the same conditions as the optical mapping, 3) to assess the effects of BDM and Cyto D on shock-induced vulnerability and the duration of induced arrhythmia, 4) to compare the dynamics and characteristics of shock-induced arrhythmias between BDM and Cyto D, and 5) to determine whether these changes occur in parallel with changes in the slopes of APD restitution curves and wavelengths.

METHODS

Our experimental protocol was approved by the Animal Research Committee of the Cleveland Clinic Foundation. All animals used in this study received humane care in compliance with the National Institutes of Health’s Guide for the Care and Use of Laboratory Animals.

Experimental preparation. Initially, nine Langendorff-perfused hearts from young rabbits (60–110 days old) were used in the study. Details of the experimental preparation have been described in our previous publications (13, 14, 17, 19, 20–22). Briefly, after administration of anesthesia, the heart was removed and placed on a Langendorff apparatus, where it was retrogradely perfused with oxygenated (95% O2–5% CO2) modified Tyrode solution with the following composition (in mM): 128.2 NaCl, 1.3 CaCl2, 4.7 KCl, 1.05 MgCl2, 1.19 NaH2PO4, 25 NaHCO3, and 11 glucose. Temperature and pH were continuously maintained at 36°C ± 0.5°C and 7.30 ± 0.05, respectively.

A custom-made 10-mm platinum coil electrode (Guidant) was inserted into the right ventricular cavity through the pulmonary artery. A second similar electrode was positioned in the bath 1 to 2 cm above and 1 to 2 cm behind the heart. The heart was stained with 10 μM 4-[β-[2-(di-n-butylamino)-6-naphthyl]vinyl]pyridinium (di-4-ANEPPS) (Molecular Probes) over 5–10 min. In each experiment, the heart was perfused in the following sequence: 1) control: modified Tyrode’s solution; 2) BDM: modified Tyrode solution plus 15 mM BDM (Fisher Scientific); 3) BDM washout: modified Tyrode solution without BDM; and 4) Cyto D: modified Tyrode solution plus 20 μM Cyto D (Sigma). We added BDM first in the perfusate because its effects can be fully washed out (7, 31), unlike the effects of Cyto D (7). However, in two separate experiments, the effects of Cyto D were tested without prior perfusion of BDM. We found no significant difference in the slopes of restitution curves or other characteristics of the restitution properties obtained in the presence of Cyto D after washout of BDM versus those obtained without prior perfusion of BDM. Thus the experimental protocol described (see Experimental protocol) was first executed with BDM and then with Cyto D. In each experiment, the heart was equilibrated in the chamber for ~30 min before the start of the experiment. The experimental protocol was started 10–15 min after administration of either BDM or Cyto D. The BDM washout period was usually 30 min. In general, the entire study lasted ~3 h, which is well within the normal physiological survival range of Langendorff-perfused hearts.

Optical mapping. Di-4-ANEPPS fluorescence was excited by a direct current-powered light source at a wavelength (λ) of 520 ± 45 nm (Oriel). The emission was collected above 610 nm by a 16 × 16-element square matrix of photodiodes (Hamamatsu) coupled to a computerized data-conditioning and acquisition system (Microstar Laboratories). Data were filtered at 1 kHz and sampled at a rate of 1,894 frames/s to yield a temporal resolution of 528 μs. The field of

Fig. 1. Representative traces of optical transmembrane action potential (AP), left ventricular (LV) pressure (LVP), and electrocardiograms (EKG) in controls (A) and during perfusion with 2,3-butanedione monoxime (BDM; B), during BDM washout (C), and with administration of three concentrations of cytochalasin D (Cyto D; D–F). Three consecutive optical action potentials were taken from 1 of the 256 channels recorded during steady-state ventricular pacing at a cycle length of 300 ms. LV pressure traces were measured with a latex balloon in the LV cavity.
view was 17.5 × 17.5 mm in all experiments. Optical action potentials were recorded before, during, and after the application of shocks.

Experimental protocol. The heart was positioned in a temperature-controlled glass chamber with the anterior wall facing the optical apparatus and was paced at a basic cycle length of 300 ms from the apex of the heart. To obtain the shock-induced vulnerability in BDM and Cyto D, truncated exponential cathodal monophasic shocks (intensity, 100 V; duration, 8 ms) were delivered at various phases of APD from a defibrillator (HVS-02, Ventritex) between the two electrodes described. We chose to use a −100-V shock, because in our previous study (43), we found that the arrhythmia incidence induced by a −100-V shock reached 100% when applied at the vulnerable phase of APD.

Standard APD restitution curves (30) for each rabbit heart in the presence of BDM and Cyto D were measured using an S1-S2 stimulation protocol, and APD values at 70% of repolarization (APD70) were averaged from all 256 optical channels. Single test pulses (S2) were delivered after every 20th basic pulse (S1) at a basic cycle length (S1-S1) of 300 ms, and the stimulus protocol was repeated with progressively shorter S1-S2 intervals. We began with an S1-S2 interval of 500 ms, and then we tested intervals of 400, 350, 300, 250, 220, and 200 ms. After that, the S1-S2 value was decreased in 10-ms steps until the premature pulse was blocked. The S1-S2 coupling interval was then increased by 5–10 ms to restore capture and was subsequently shortened in 5-ms increments until S2 was blocked. The duration of the response to S2 was measured at APD70 and plotted as a function of the preceding diastolic interval (DI). We used the following formula to calculate the DI: DI = TI2 − TI1 − APD70, where TI2 is the time interval from the last S1 stimulus to the peak upstroke of the response induced by S2, TI1 is the time interval from the last S1-stimulus to the peak upstroke of the response induced by the last S1, and APD70 is the APD of the second-to-last S1-induced action potential. We measured DI using APD70 induced by the second-to-last S1 stimulus instead of the last S1 stimulus because this gave us more accurate and reliable DI measurements. We assumed that APD was stabilized under steady-state pacing of the heart. The parameters of APD70 and DI were automatically calculated using a custom-built data analysis program based on LabView (National Instruments).

Monophasic action potential recordings. Because control action potentials cannot be faithfully recorded in the absence of excitation-contraction uncouplers during optical mapping, we used eight additional Langendorff-perfused rabbit hearts to record control monophasic action potentials (MAP) in the absence of voltage-sensitive dye. They were compared with those under 15 mM BDM and in the presence of 20 μM Cyto D. In each heart, a pressure MAP catheter (model 1675PS, EP Technologies) was inserted into the left ventricular cavity via a cut in the left atria. The heart was kept at the same conditions as those described in Optical mapping. Because Cyto D is not completely washable and because we aimed to limit the duration of each experiment to exclude any adverse effects of prolonged MAP recordings, we divided eight rabbits into two groups of four rabbits. One group was used to compare MAP values between control solutions and in the presence of BDM, whereas the other group was used to compare MAP values between control measurements and in the presence of Cyto D.

Classification of shock-induced arrhythmia. VT/VF is not readily sustainable in normal young rabbit hearts either in vivo (34) or in vitro (6, 33, 35) and spontaneously terminates, perhaps due to the small heart size compared with the wavelength and/or due to the lack of fibrosis. In some cases, no extra beats resulted from the shock. Occurrence of one or more extra beats was defined as shock-induced arrhythmia, which can be further divided into sustained or nonsustained arrhythmas. In accordance with Fabritz et al. (23), we defined shock-induced arrhythmias as sustained if a sequence of ≥6 regular or irregular fast (cycle length <160 ms) responses (extra beats) were present. Induction of 1–5 repetitive responses (extra beats) was regarded as nonsustained arrhythmia. We also measured a number of arrhythmias that lasted >5 s under BDM and Cyto D conditions.

Table 1. Optical action potential recordings during steady-state ventricular pacing at cycle length of 300 ms

<table>
<thead>
<tr>
<th></th>
<th>APD90, ms</th>
<th>APD50, ms</th>
<th>APD30, ms</th>
<th>CV, m/s</th>
<th>λ, cm</th>
</tr>
</thead>
<tbody>
<tr>
<td>BDM (15 mM)</td>
<td>123.0±10.3</td>
<td>143.1±11.4</td>
<td>164.6±11.7</td>
<td>0.54±0.11</td>
<td>9.0±1.9</td>
</tr>
<tr>
<td>Cyto D (20 μM)</td>
<td>160.3±9.7*</td>
<td>178.0±10.7*</td>
<td>197.2±12.3*</td>
<td>0.63±0.13†</td>
<td>12.5±2.6*</td>
</tr>
<tr>
<td>Reduction in BDM vs. Cyto D, %</td>
<td>23</td>
<td>20</td>
<td>17</td>
<td>14</td>
<td>28</td>
</tr>
</tbody>
</table>

Values are means ± SD; n = 9 hearts. APD90, APD50, and APD30, action potential duration at 90, 50, and 30% repolarization, respectively; CV, conduction velocity across the field of view; λ, wavelength; BDM, 2,3-butanedione monoxime; Cyto D, cytochalasin D. *P < 0.001; †P < 0.05.
Table 2. Monophasic action potential recordings during steady-state ventricular pacing at 300-ms cycle length

<table>
<thead>
<tr>
<th></th>
<th>APD_{50} ms</th>
<th>APD_{70} ms</th>
<th>APD_{90} ms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>136±4</td>
<td>155±5</td>
<td>176±5</td>
</tr>
<tr>
<td>15 mM</td>
<td>111±2*</td>
<td>132±5b</td>
<td>151±8*</td>
</tr>
<tr>
<td>Cyto D</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>132±7</td>
<td>149±7</td>
<td>166±9</td>
</tr>
<tr>
<td>20 μM</td>
<td>147±10*</td>
<td>164±9d</td>
<td>180±7*</td>
</tr>
</tbody>
</table>

Reduction in BDM
vs. control, %       18 15 14
Increase in Cyto D  vs. control, % 11 10 8
Reduction in BDM  vs. Cyto D, % 24 20 16

Values are means ± SD; n = 4 hearts/control group. *P < 0.01; bP < 0.001; cP = 0.12; dP = 0.06; eP < 0.05.

Characterization of shock-induced arrhythmias under BDM and Cyto D. We applied fast Fourier transform (FFT) analysis (38) to study the dynamics of the shock-induced sustained arrhythmias (≥6 extra beats) with BDM and Cyto D. Depending on the length of the recorded arrhythmias, the length of the data analyzed was either 750 or 1,500 ms. For each arrhythmia instance, FFT of the optical data was calculated for all 256 channels. The FFT values at 0-Hz frequency were set to 0. The sum of the FFT curves of all the channels was called the composite FFT. In the composite FFT, the frequency value that had the highest amplitude was considered the dominant frequency. For the single-channel FFT, the frequency value that had the highest amplitude was considered the local dominant frequency. The standard deviations of the local dominant frequencies for all channels were calculated for each arrhythmia instance and were named the dominant frequency standard deviation. We also defined the monomorphic or polymorphic nature of VT using visual inspection of optical data that was performed independently by two investigators based on morphology and periodicity of recordings (cf. Fig. 5, A–C).

Electrical activity was analyzed in the phase space using Bray-Wikswo algorithms (9–11). Using phase representation, we identified phase singularities and measured the dynamics and average numbers in the field of view. For each arrhythmia data set, we averaged the number of phase singularities over time.

Data analysis and visualization. The signal analysis software programs used in this study were previously described (13, 19, 20). These programs automatically calculated maps of activation, repolarization, and APD from all 256 optical recordings. Activation time (AT) was subtracted from coupling interval (CI), which is defined as the time difference between the stimulus and the shock application to calculate fractional (percent) APD in each channel in which the shock was applied according to the following formula: %APD = (CI – AT)/APD × 100. The dispersion of repolarization was defined as the difference between the shortest and longest repolarization times across the field of view (6). The vulnerable window was defined by excluding the CI at which arrhythmia incidence was <50% (12, 43).

Statistical analysis. Group data were expressed as means ± SDs. Statistical comparisons were performed using the paired or unpaired t-test. Differences were considered significant when P < 0.05.

RESULTS

Effects of BDM and Cyto D on optically recorded APDs. In the present study, we tested BDM and Cyto D at concentrations that effectively abolish contractions in the intact rabbit heart. In the past, we routinely used BDM at a concentration of 15 mM to immobilize the heart (13, 14, 17, 19, 20–22). To determine the effective concentration of Cyto D, we conducted a preliminary study in two hearts. The effects of 10, 20, and 30 μM Cyto D on contractility of intact hearts were assessed by measuring left ventricular pressure with a latex balloon and comparing the responses to those in the presence of 15 mM BDM. Figure 1 shows a representative result from one heart. Addition of 15 mM BDM abolished most of the contraction, making the optical action potential recording possible. Upon washout of BDM, contractility of the heart returned to the control level. Subsequent addition of 10 μM Cyto D largely eliminated contraction, whereas 20 and 30 μM Cyto D almost completely abolished contraction. Thus we chose 20 μM Cyto D in this study, because 10 μM Cyto D left significant motion artifacts at the edges of the field of view in some hearts.

Figure 2A shows an example of superimposed optical action potentials recorded with 15 mM BDM and 20 μM Cyto D from two different optical channels in the same heart. APDs in the presence of BDM were consistently shorter than those recorded in the presence of Cyto D. In all hearts (n = 9), we found that 15 mM BDM significantly (P < 0.001) shortened the APDs at 50, 70, and 90% of repolarization (APD_{50}, APD_{70}, and APD_{90}, respectively) compared with 20 μM Cyto D at the basic cycle length of 300 ms. On average, the reduction in APD_{50} was 23%, APD_{70} was 20%, and APD_{90} was 17%. See Table 1 for details. Accordingly, the average repolarization time across the field of view defined at APD_{90} was also significantly reduced by BDM compared with Cyto D (208.8 ± 9.9 vs. 235 ± 13.6...
ms; \( P < 0.001 \). However, no significant difference in dispersion of repolarization was observed between the two groups (53.7 ± 23.1 in BDM vs. 48.5 ± 55.5 ms in Cyto D; \( P = 0.68 \)). Furthermore, the average conduction times across the field of view (minimum AT – maximum AT) were 33.2 ± 7.3 and 28.3 ± 5.8 ms (\( P < 0.05; n = 9 \) hearts) in BDM and Cyto D, respectively. CV values across the field of view (field of view divided by conduction time) were reduced in BDM relative to Cyto D. However, the activation patterns for both agents remained similar. The wavelength (\( \lambda = \text{APD}_{90} \times \text{CV} \)) was significantly shorter with BDM compared with Cyto D (9.0 ± 1.9 vs. 12.5 ± 2.6 cm; \( P < 0.01 \)). See Table 1 for details.

These optical action potential recordings lacked controls, because we were unable to faithfully record them in the absence of excitation-contraction uncouplers. Therefore, in an additional study that included eight Langendorff-perfused rabbit hearts, we recorded control MAPs and compared them with those in the presence of 15 mM BDM (\( n = 4 \)) or 20 \( \mu \)M Cyto D (\( n = 4 \)). Compared with control, 15 mM BDM significantly shortened \( \text{APD}_{50} \), \( \text{APD}_{70} \), and \( \text{APD}_{90} \) by 18, 15, and 14%, respectively. In contrast, 20 \( \mu \)M Cyto D slightly but significantly prolonged \( \text{APD}_{90} \) by 8%. The percentage reductions of \( \text{APD} \) in BDM compared with Cyto D are shown in Fig. 2B. Summarized data are shown in Table 2. Typical examples of MAP recordings are shown in Fig. 2B. Summarized data are shown in Table 2.

**Comparison of characteristics of shock-induced arrhythmias under BDM and Cyto D.** We performed FFT analysis of all shock-induced sustained arrhythmias in BDM or Cyto D from nine hearts, which we found to be tachycardic in nature, demonstrated by the fact that most of the frequency components were \(< 10 \) Hz (longer than the 100-ms cycle length) as shown in Table 4. We further characterized the type of shock-induced VT under BDM and Cyto D. As summarized in Fig. 4, the shock-induced arrhythmias in BDM usually resembled monomorphic VT; this was especially true of those \( > 30 \) s. In contrast, shock-induced arrhythmias in Cyto D more resembled a polymorphic tachyarrhythmia. Among the 37 shock-induced sustained arrhythmias under BDM, 23 were monomorphic VT and 14 were polymorphic VT. In comparison, among the 20 shock-induced sustained arrhythmias under Cyto D, the numbers of mono- and polymorphic VTs were 1 and 19, respectively (see Fig. 4A). This difference was especially pronounced.

### Table 3. Effects of BDM and Cyto D on arrhythmia genesis and maintenance

<table>
<thead>
<tr>
<th>Incidence, %</th>
<th>Arrhythmia</th>
<th>Sustained arrhythmia</th>
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</thead>
<tbody>
<tr>
<td>BDM (15 mM)</td>
<td>72</td>
<td>37</td>
</tr>
<tr>
<td>Cyto D (20 ( \mu )M)</td>
<td>50</td>
<td>16</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Range of Vulnerable Window, %APD</th>
<th>Arrhythmia</th>
<th>Sustained arrhythmia</th>
</tr>
</thead>
<tbody>
<tr>
<td>BDM</td>
<td>30–80</td>
<td>40–70</td>
</tr>
<tr>
<td>Cyto D</td>
<td>40–80</td>
<td>60–70</td>
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<table>
<thead>
<tr>
<th>Incidence of Arrhythmia Lasting &gt;5 s, %</th>
<th>Arrhythmia</th>
<th>Sustained arrhythmia</th>
</tr>
</thead>
<tbody>
<tr>
<td>BDM</td>
<td>18</td>
<td>5</td>
</tr>
<tr>
<td>Cyto D</td>
<td>2</td>
<td>0</td>
</tr>
</tbody>
</table>

Values are means ± SD; *\( P = 0.16 \); †\( P < 0.01 \); ‡\( P < 0.05 \).

### Table 4. Characteristics of shock-induced arrhythmias with BDM and Cyto D

<table>
<thead>
<tr>
<th></th>
<th>Dominant Frequency, Hz</th>
<th>Dominant Frequency SD, Hz</th>
<th>Phase Singularities, Average No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>BDM (15 mM)</td>
<td>7.8 ± 0.9*</td>
<td>0.34 ± 0.13</td>
<td>1.5 ± 0.5</td>
</tr>
<tr>
<td>Cyto D (20 ( \mu )M)</td>
<td>7.0 ± 0.5†</td>
<td>1.12 ± 0.28†</td>
<td>2.2 ± 0.4†</td>
</tr>
</tbody>
</table>

Values are means ± SD; *\( P = 0.16 \); †\( P < 0.01 \); ‡\( P < 0.05 \).
Fig. 4. Number of monomorphic (Mono) and polymorphic (Poly) sustained arrhythmias under BDM and Cyto D. Numbers of sustained arrhythmias (≥6 extra beats) from 9 hearts (A) are compared with numbers of sustained arrhythmias that lasted >30 s, also from 9 hearts (B).

Fig. 5. Representative examples of sustained arrhythmia under BDM and Cyto D. Shown are a sustained arrhythmia that lasted >30 s under BDM (A), a sustained arrhythmia that self-terminated after 11 s under BDM (B), and a sustained arrhythmia that lasted >30 s under Cyto D (C) conditions. Snapshots of transmembrane voltage selected at one-fourth and three-fourths duration of the data set are represented (top). Phase singularity with clockwise reentry around it (●) and phase singularity with counterclockwise reentry around it (○) are indicated in corresponding phase maps. Optical traces were picked from the center of the fields of view with data length of 1.57 s. Number of phase singularity (PS) vs. time (T) is shown. Composite fast Fourier transforms for the shown data set are illustrated (bottom).
after 30 s. Arrhythmias lasting >30 s were all monomorphic under BDM and all polymorphic under Cyto D (see Fig. 4B). This indicates that initially unstable polymorphic arrhythmia under BDM stabilizes during this 30-s period if it survives. In contrast, it always remains polymorphic under Cyto D. Typical examples of sustained arrhythmias under BDM and Cyto D conditions are shown in Fig. 5. Figure 5A represents the dynamics of a sustained arrhythmia lasting >30 s in the presence of BDM. The optical trace picked from the center of the field of view was regular and repeated exactly from beat to beat. The average number of phase singularities was 0 over the 1.57-s time interval. Dominant frequency was 5.33 Hz, which corresponds to a cycle length of 188 ms. It was a typical monomorphic VT. Figure 5B illustrates a sustained polymorphic arrhythmia that self-terminated after 11 s under BDM conditions. The average number of phase singularities was 1.88 over the 1.57-s time interval. Dominant frequency was 7.33 Hz with a larger dispersion than that in Fig. 5A. Figure 5C shows a sustained arrhythmia that lasted >30 s under Cyto D conditions. It was polymorphic with an average of 2.47 phase singularities. Its dominant frequency was 7.33 Hz, and it had the largest dispersion among all the examples. As summarized in Table 4, the average number of phase singularities under Cyto D conditions was greater than under BDM (2.2 ± 0.4 vs. 1.5 ± 0.5; P < 0.05; Cyto D vs. BDM). No statistically significant differences were found in dominant frequencies under BDM and Cyto D conditions (7.8 ± 0.9 vs. 7.0 ± 0.5 Hz; P = 0.16; BDM vs. Cyto D). However, the standard deviation of dominant frequency under Cyto D was significantly larger than that under BDM (1.12 ± 0.28 vs. 0.34 ± 0.13 Hz; P < 0.01; Cyto D vs. BDM). Both the increased average number of phase singularities and the dispersion of dominant frequencies were consistent with increased fractionation of arrhythmia wave fronts under Cyto D compared with BDM conditions.

Effects of BDM and Cyto D on APD restitution curves. Finally, we constructed APD restitution curves from nine hearts in the presence of BDM and Cyto D. Figure 6A shows a representative curve from one heart and an averaged curve from nine hearts (Fig. 6B). The average values for maximum slope from nine hearts were 0.91 ± 0.38 under BDM and 1.51 ± 0.18 under Cyto D (P < 0.05). From this result, it is apparent that BDM flattened the restitution slope compared with Cyto D as was reported by others (1, 2, 31).

DISCUSSION

In this study, we showed that BDM (15 mM) produced a significant reduction of APD50, APD70, and APD90 when compared with the effects of 20 μM Cyto D in the whole rabbit heart, which is in agreement with findings from others (7, 31). Furthermore, APD50 in control MAP recording was slightly yet significantly shorter than that under Cyto D but was significantly longer than that for BDM, which is also in agreement with a recent report (2). We demonstrated that BDM resulted in a significant increase of vulnerability to shock-induced arrhythmias compared with Cyto D. This was evident from a widening of the vulnerable window and increased propensity to initiate arrhythmias. We did not observe any significant increase of ventricular repolarization heterogeneity between the two agents, yet we observed that BDM shortened the wavelength compared with Cyto D with a mean of 9 vs. 12.5 cm. Also CV across the field of view was slower in BDM. We found that shock-induced arrhythmias in BDM and Cyto D were tachycardic in nature. In addition to a different propensity to induce arrhythmias between these two agents, there was a difference in morphology in shock-induced VT. Namely, the arrhythmias in BDM usually resembled monomorphic VTs; this was especially true of those >30 s. In contrast, arrhythmias in Cyto D more resembled polymorphic tachyarrhythmias. In agreement with the results of others, we confirm that BDM flattened the restitution curve (1, 31, 41), which also agrees with the restitution theory that suggests reduced propensity to polymorphic tachyarrhythmia. We also confirm that in agreement with the restitution hypothesis, BDM reduced fractionation of wave fronts and average numbers of phase singularities compared with Cyto D. However, such reduction was accompanied by both increased vulnerability to shock-induced arrhythmias and increased duration of arrhythmia in BDM compared with Cyto D. This poses a question regarding usefulness of flattening the restitution curve as an antiarrhythmic drug-development strategy. Our point of view is in agreement with a recent report by Gray et al. (2).

The recently formulated restitution hypothesis states that the slope of the restitution curve is an important factor in the development of wave breaks (dynamic wave-front fractionation), which are suggested to be a primary mechanism of the transition from VT to VF as well as the mechanism responsible for the maintenance of VF (41). Furthermore, the restitution
hypothesis suggests that strategies for antiarrhythmic drug development should be based on targeting the slope of the restitution curve and the range of diastolic intervals at which this slope exceeds the critical value of 45° (8, 24, 27, 29, 30, 40).

On the other hand, such flattening can be achieved by shortening the APD at long coupling intervals or by prolonging the APD at short coupling intervals. The former preserves the wavelength, whereas the latter shortens the wavelength. We suggest that the two possibilities will have different impacts on arrhythmia maintenance despite similar effects on the slope of the restitution curve. Indeed, our results show that despite the flattened restitution curve under BDM, arrhythmia duration was increased.

Shock-induced arrhythmogenesis is governed by reentrant mechanisms. We have previously demonstrated that such arrhythmias are induced by virtual electrode-induced phase-singularity mechanisms (13, 19, 20). Phase singularities are observed in shock-induced arrhythmias under both BDM and Cyto D, which suggests that the mechanism for maintenance of the arrhythmias is also reentry. Decreased dispersion of the standard deviation of the dominant frequency and decreased average numbers of phase singularities in BDM compared with Cyto D indicate that BDM caused more regular patterns of activation in optical recordings during arrhythmia and decreased fractionation of wave fronts. This is in agreement with the restitution hypothesis (slope <1), which suggests less-frequent fractionation. Yet a more-regular reentrant pattern (monomorph VT) under BDM conditions was maintained longer. This could result from significant shortening of the wavelength and slowing of CV by BDM.

Furthermore, our study demonstrates that BDM enhanced vulnerability to arrhythmia induced by shock compared with Cyto D. We believe that this might be due to suppression of several ionic currents, including 1) the Ca 2+ current, which was suggested as a target for flattening of the restitution slope (41); and 2) the Na+ current. BDM produces a significant reduction of APD 50, APD 70, and APD 90 compared with Cyto D. Cyto D is known to have no significant effect on the APD morphology in various species (7, 31, 39, 42). However, compared with controls, Cyto D slightly but significantly prolonged APD 90 in rabbit hearts as reported in this and another study (2). The ionic basis for this prolongation needs to be further investigated. Considering that there is no significant increase of ventricular repolarization heterogeneity between BDM and Cyto D, we speculate that an enhanced susceptibility to deexcitation due to shortening of APD by BDM might contribute to the difference in vulnerability between BDM and Cyto D that was observed in this study. Shock-induced arrhythmias are critically dependent on the ability of the shock to deexcite tissue. We have recently demonstrated that deexcitation during the early plateau phase is prevented by Ca 2+ current (43). Therefore, suppression of Ca 2+ current could potentially enhance deexcitation and vulnerability. Although additional studies are required to explore this at the cellular level, we suggest that targeting Ca 2+ channels for restitution flattening has to be approached with care. In addition, suppression of Na+ channels results in slowing of conduction, which enhances arrhythmogenesis.

It is noteworthy that in our study we used young rabbit hearts (average age of rabbit, 2 mo) as in most of our previous studies (13, 14, 17, 19–22). We observed that the majority of shock-induced arrhythmias in these young rabbits with BDM and Cyto D were transient. For example, 82% of shock-induced arrhythmias in BDM and 95% of arrhythmias in Cyto D lasted <5 s. This is consistent with reports of others without BDM or Cyto D (6, 33–35). On the other hand, Manoach et al. (34) demonstrated that verapamil converted transient fibrillation into sustained fibrillation in young rabbits. Similarly, verapamil exerted a proarrhythmic effect in the goat model of atrial fibrillation (16).

Finally, both BDM and Cyto D are frequently used to suppress motion artifacts in optical mapping studies. Our results indicate that although both agents have effects on control MAPs in rabbit heart, the relative prolongation of MAP duration with Cyto D is less pronounced than the shortening and triangulation of the MAP associated with BDM (see Table 2 and Fig. 2). The number and characteristics of shock-induced arrhythmias under Cyto D are also closer to those of the control hearts. Thus for studies on rabbit hearts, Cyto D more closely approximates the control conditions. In view of already-published reports (36), it is apparent that this result may not be generalizable from species to species or perhaps even from ventricular to atrial preparations within the same species.

In conclusion, BDM shortens APD and thus flattens the restitution curve. In contrast, Cyto D slightly but significantly prolongs APD. In addition, BDM results in slowing of conduction compared with Cyto D. As a result, wavelength is significantly shorter under BDM than under Cyto D. In agreement with the restitution hypothesis, flattening of the restitution curve by BDM is associated with decreased fractionation of arrhythmia wave fronts and the more monomorphic nature of arrhythmia compared with polymorphic arrhythmias under Cyto D. However, our findings indicate that BDM enhances vulnerability to shock-induced arrhythmia and increases its duration compared with Cyto D. This could be a result of the reduction of wavelength. Thus flattening of the restitution curve as an antiarrhythmic drug-development strategy did not work in this model.

Study limitations. Because the main focus of this study was to compare the shock-induced arrhythmias under BDM and Cyto D, we did not evaluate the dynamic restitution relations in the present study, which other studies have already reported (Refs. 1, 31).

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AJP-Heart Circ Physiol • VOL 286 • JANUARY 2004 • www.ajpheart.org


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