Gap Junction Blockers Decrease Defibrillation Thresholds Without Changes in Ventricular Refractoriness in Isolated Rabbit Hearts

Xiangqian Qi, MD, PhD; Pryamvada Varma, MSc; David Newman, MD; Paul Dorian, MD, MSc

Background—The maintenance and termination of reentry arrhythmias are determined by tissue properties such as refractoriness and conduction velocity. Although the effects of Na+ and K+ channel block on electrophysiological properties and defibrillation threshold (DFT) have been studied, little is known about the effect of gap junction blockers on defibrillation and tissue electrophysiological properties.

Methods and Results—Triplicate DFTs (volts) were obtained before and 15 minutes after 4 μmol/L 16-doxyl-stearic acid (16-DSA, n=8), 1 mmol/L 1-heptanol (n=12) (both gap junction blockers), 3 μg/mL lidocaine (a sodium channel blocker) (n=8), and respective controls (n=27) in isolated perfused rabbit hearts. DFT decreased after 16-DSA (23±14%, P<0.01) and 1-heptanol (21±16%, P<0.01) but increased after lidocaine (26±28%, P<0.05). Ventricular fibrillation cycle length (VFCL) and QRS duration increased after all 3 agents, by 36±19% and 44±16% (16-DSA), 87±42% and 49±15% (heptanol), and 57±20% and 43±26% (lidocaine), respectively (all P<0.01). Spatially averaged temporal VFCL dispersion decreased significantly after all 3 agents, by 47±42% (16-DSA, P<0.05), 74±19% (1-heptanol, P<0.01), and 82±13% (lidocaine, P<0.01), respectively. Ventricular effective refractory period and monophasic action potential duration at 90% repolarization were unchanged after 16-DSA and 1-heptanol (P=NS) but increased after lidocaine (16±13%, P<0.01, and 6±5%, P=NS, respectively). There were no significant changes in DFT or any other electrophysiological variable in control hearts.

Conclusions—Electrical uncoupling by 16-DSA and 1-heptanol significantly lowers DFT and dispersion of VF without altering refractoriness; lidocaine, at doses resulting in similar slowing of conduction, increases DFT. (Circulation. 2001; 104:1544-1549.)

Key Words: defibrillation ■ blockers ■ refractoriness ■ dispersion

The concept of action potential and refractoriness prolongation (class III activity) as a useful tool for antiarrhythmic or antifibrillatory drug action was first advanced >3 decades ago.1-2 For example, drugs whose predominant action is to block potassium currents and thereby prolong cardiac action potential reduce the incidence of ventricular tachycardia3 and decrease the energy necessary to defibrillate the heart.4-5 In contrast, slowing of conduction velocity (class I action) increases defibrillation energy requirements and may increase the risk of clinical arrhythmias.1 Conversely, the electrophysiological and potentially antiarrhythmic effects of altering passive membrane properties, which are influenced by cell-to-cell communication, have been examined theoretically but have been little studied experimentally.6-7

Gap junctions are specialized regions of adjoining cell membranes that are composed of intercellular low-resistance channels. In the heart, gap junctions mediate current flow, thereby coordinating the spread of excitation and subsequent contraction throughout the myocardium.8,9 Computer modeling and experimental studies suggest that decreased gap junction conductance may lead to or contribute to arrhythmias.10,11

The effect of alterations in gap junction function on defibrillation threshold (DFT) and electrophysiological variables in vivo during ventricular fibrillation (VF) is not known. We therefore investigated the effect of 16-doxyl-stearic acid (16-DSA) and 1-heptanol, both gap junction blockers,12,13 on defibrillation energy requirements, ventricular refractoriness, and variability of activation-to-activation intervals in VF in Langendorff-perfused isolated rabbit hearts. Because heptanol may also have sodium channel blocking properties,14,15 the sodium channel blocker lidocaine was used as a positive control.

Methods
Fifty-five male New Zealand White rabbits weighing 2.8 to 4.8 kg (mean 4.1±0.5 kg) were studied in 3 groups: 16-DSA (n=16),
1-heptanol (n=23), and lidocaine (n=16). Experiments were performed according to the guiding principles of the Canadian Council on Animal Care and approved by the Animal Care Committee of St Michael’s Hospital.

After the rabbits were heparinized (=1000 IU IV) and anesthetized with pentobarbital 60 mg/kg IV, the heart was quickly removed, and the aorta was connected to a Langendorff perfusion system. The hearts were perfused at 37±1°C with a temperature-controlled circulating water bath with a constant pressure of 68 mm Hg, resulting in a flow of ~126 mL/min, and passed through a 0.45-μm filter (Gelman Sciences) to remove contaminant particles. The composition of the Krebs-Henseleit solution in the 16-DSA group contained (mmol/L): NaCl 118.6, NaHCO3 25, KCl 4.7, KH2SO4 1.18, MgSO4 1.2, CaCl2 2.5, and glucose 11.1. The Tyrode’s solution in the 1-heptanol and lidocaine groups consisted of the following (mmol/L): NaCl 130, KCl 5.6, NaHCO3 24.2, CaCl2 2.2, MgCl2 0.6, NaH2PO4 1.2, and glucose 12. Bovine serum albumin (BSA) (Sigma) was added to both perfusates at a concentration of 4.013×10−3 mol/L (40 mg/L) to improve the stability and performance of isolated perfused rabbit hearts. The solution reservoir was bubbled with a mixture of 95% O2 and 5% CO2 and had a pH of ~7.4.

A 7.6-cm2 rectangular titanium mesh patch was placed directly on the epicardial surface of the left ventricle and used to deliver shocks to the heart, and a coil electrode with a diameter of ~0.3 cm was inserted into the right ventricle and used for inducing VF and defibrillating the heart. A quadripolar 6F contact Ag-AgCl electrode (EP Technologies Inc) was inserted into the right ventricle for pacing and measurement of monophasic action potentials (MAP). A latex balloon was advanced into the left ventricle through an incision in the left atrial appendage. The balloon was filled with distilled water to establish an end-diastolic pressure of 5±1 mm Hg. The intraventricular balloon was attached to a pressure transducer set (P23ID, Statham, Bionetics Inc), and intraventricular pressure during isovolumetric contraction was measured with a pressure amplifier (Hewlett Packard). Two bipolar epicardial electrograms were recorded with 4 plunge electrodes; one pair was inserted into the right ventricle (~1 cm apart), and another pair was connected to the left ventricle and left atrium, respectively. All epicardial electrograms and MAPs were amplified with a custom-made amplifier (Cartesian Laboratories) and recorded with a custom-made computer software program (Electrophysiological Recording System, Acqui2, Cartesian Laboratories).

**Defibrillation Voltage Requirement Determination**

The delayed up-down algorithm was used for the determination of shock strength (voltage) required for a 50% probability of successful defibrillation (V50). VF was induced with 15 V of fully rectified 60Hz current pulse. An external defibrillator (HVS02, Ventrijet Inc) was used to deliver 3 ms monophasic shocks after 15 seconds of VF. If any shock failed to defibrillate, higher-voltage shocks were delivered in successive 10-V increments at 3-second intervals until successful defibrillation; DFT was defined as the lowest successful voltage to defibrillate. After a successful shock, fibration was again induced and the next shock sequence started 10 V lower; if successful, shock strength was decreased in 10-V steps until failure to defibrillate. Fibration and successful defibrillation were identified by means of both the epicardial bipolar electrogram and the left intraventricular pressure recording. The up-down algorithm was applied until 3 DFTs (failure followed by success or vice versa, each success or failure being counted only once) had been measured. Three such DFTs were averaged to estimate V50.

**Electrophysiological Measures**

Pacing was performed with a programmable stimulator (HVS02, Ventrijet Inc) with 2.0-ms pulse width and amplitude of twice diastolic threshold with right ventricular epicardial or endocardial electrodes. MAP detection at 90% repolarization (MAPD90) was measured after 1 minute of ventricular pacing at 400-ms cycle length (CL). Right ventricular effective refractory period (VERP) was determined during continuous ventricular pacing at a CL of 400 ms by the extrastimulus method to 2-ms precision, with incremental extrastimuli introduced every eighth beat. Mean VFCL, defined as the mean interval between discrete electrogram deflections during VF, was determined from the epicardial bipolar electrogram by averaging the last 20 activation-to-activation intervals immediately before defibrillation. QRS duration was measured from the left ventricular–left atrial electrode pair electrogram by averaging 5 QRS intervals after 1 minute of ventricular pacing at 400-ms CL.

Temporal dispersion of VFCL was defined as the difference between the maximum and minimum deflection-to-deflection intervals during the last 20 intervals of each VF episode measured on the left ventricular epicardial lead electrogram. Relative temporal dispersion (%) in VFCL, ie, coefficient of variation of VFCL, was calculated as follows: (SD of VFCL/mean VFCL)×100. Spatially averaged temporal dispersion of VFCL was calculated from the average of 3 temporal dispersion measurements of VFCL in the 3 electrodes. Spatially averaged relative temporal dispersion of VFCL (coefficient of temporal variability in intralead activation-to-activation intervals from 60 intervals over 3 leads) was calculated as the average of 3 relative temporal dispersion (%) measurements in VFCL.

**Experimental Protocol**

The experimental design comprised a baseline and treatment condition and control (no treatment) for the 16-DSA (n=8 treatment, n=8 control), 1-heptanol (n=12 treatment, n=11 control), and lidocaine (n=6 treatment, n=6 control) groups. The hearts in the 16-DSA treatment and 16-DSA control groups were perfused with Krebs-Henseleit buffer, and in the 1-heptanol and lidocaine treatment and 1-heptanol and lidocaine control groups with Tyrode’s buffer. All hearts were perfused for an initial stabilization period of ≥20 minutes before the start of each protocol. After baseline measurements of DFT, VERP, MAP, and QRS duration, drug was added to the perfusate in concentrations of 4 μmol/L 16-DSA12 in the 16-DSA group, 1 mmol/L 1-heptanol13 in the 1-heptanol group, and 3 μg/mL lidocaine16 in the lidocaine group. The drugs were dissolved directly in the perfusate immediately before administration. After a 15-minute equilibration period, DFTs and electrophysiological parameters were repeated for each of the groups. Measurements were repeated at the same time intervals in the control groups.

**Statistical Analysis**

The data are expressed as mean±SD. Differences in DFT and the other electrophysiological parameters before and after drug were analyzed by repeated-measures ANOVA, with significant time-by-treatment interactions denoting a significant drug effect, and Student’s paired t test. Differences were considered significant at a value of P<0.05.

**Results**

There were no significant differences between the 16-DSA, 1-heptanol, lidocaine, and control groups in body or heart weight. Mean DFT, VERP, MAPD90, VFCL, QRS duration, temporal and relative temporal VFCL dispersion, spatially averaged temporal, and spatially averaged coefficient of VFCL dispersion at baseline, repeat (time control) measurements or after each drug for the 3 control groups and the drug groups are shown in the Table. There was no significant difference in any parameter between baseline and repeat measurements for the 3 control groups. No significant difference in any baseline variable was observed between any of the controls and any of drug-treated groups.

Both 16-DSA and 1-heptanol resulted in a significant decrease in DFT (both comparisons, P=0.001, time-by-treatment interactions for DSA and heptanol compared with control), a significant prolongation in both VFCL (P=0.001 and P<0.001 for DSA and heptanol, respectively) and QRS...
duration compared with the control group (both $P<0.001$), and significant decreases in measures of VFCL dispersion (temporal and spatial) (Table). DFT decreased by 23±14% ($P=0.001$) in the 16-DSA group and by 21±16% ($P=0.001$) in the 1-heptanol group. 16-DSA and 1-heptanol prolonged VFCL by 36±19% ($P<0.001$) and by 87±42% ($P<0.001$), respectively, and produced a significant prolongation in QRS duration by 44±16% ($P<0.001$) and 49±15% ($P<0.001$), respectively. Temporal and relative temporal VFCL dispersion decreased by 66±32% ($P=0.028$) and 35±124% ($P=0.120$) in the 16-DSA group and by 64±27% ($P=NS$) and 53±48% ($P=NS$) in the 1-heptanol group, respectively. Spatially averaged temporal and spatially averaged coefficient of variation of VFCL dispersion decreased by 47±42% ($P=0.02$) and 57±32% in the 16-DSA group ($P=0.003$) and by 74±19% ($P=0.008$) and 82±13% ($P<0.001$) in the 1-heptanol group, respectively. After lidocaine, temporal and relative VFCL dispersion decreased by 90±7% ($P<0.001$) and 91±4% ($P=0.001$), and spatially averaged temporal and spatially averaged coefficient of VFCL dispersion decreased by 83±12% ($P<0.001$) and 83±9% ($P<0.001$), respectively. There was no correlation between DFT and VERP, VFCL, QRS duration, or VFCL dispersion before and after each drug.

Neither VERP nor MAPD$_{90}$ was significantly altered by 16-DSA or 1-heptanol treatment (Table). In the 16-DSA group, VERP increased by 11±25% ($P=NS$), and MAPD$_{90}$ increased by 6±17% ($P=NS$). In the 1-heptanol group, VERP increased by 1±12% ($P=NS$), and MAPD$_{90}$ decreased by 2±9% ($P=NS$). There was also no significant change in VERP and MAPD$_{90}$ in any of the control groups.

After lidocaine, DFT increased by 26±28% ($P=0.02$ by paired $t$ test, $P=0.12$ by repeated-measures ANOVA). The relative change in DFT (%) from baseline was significantly different for lidocaine versus DSA (+26±28% versus −23±14%, respectively, $P<0.001$) and similarly for lidocaine versus heptanol (+26±28% versus −21±16%, respectively, $P<0.001$). Unlike 16-DSA and 1-heptanol, lidocaine increased DFT and prolonged VERP (Table), although it also prolonged QRS duration and VFCL and decreased VFCL dispersion and spatially averaged temporal and spatially averaged coefficient VFCL dispersion. VERP was prolonged by 16±13% ($P=0.002$), MAPD$_{90}$ was prolonged by 62.5% ($P=NS$), VFCL increased by 57±20% ($P<0.001$), and QRS duration increased by 43±26% ($P=0.001$), respectively. There was no significant effect on DFT, any VFCL dispersion measure, or any electrophysiological parameters after control buffer in any of the control groups.

The pattern observed on the epicardial electrograms also changed after administration of 16-DSA, 1-heptanol, or lidocaine. The predrug VF morphology consisted of irregular, disorganized, high-frequency deflections in all electrodes (Figure 1), in contrast to the more organized deflections occurring at a slower frequency after 16-DSA, 1-heptanol, or lidocaine (bottom panels of Figures 2 to 4, respectively).

### Discussion

The main findings of the present study are that both 16-DSA and 1-heptanol significantly decrease defibrillation voltage requirements in perfused rabbit hearts. They prolong VFCL and QRS duration, while causing no change in ventricular refractoriness and action potential duration. This suggests that parameters other than ventricular repolarization may play a role in the pattern of VF and its termination.$^{16–18}$

Previous studies demonstrated that increased refractoriness is related to decreased DFT, whereas decreased conduction velocity via inward Na$^+$ current block is related to increased DFT.$^{4,5,19}$ but the effect of gap junction blockers on DFT has not previously been tested. Antiarrhythmic agents, such as lidocaine, increase DFT in animals and humans, whereas potassium channel blockers have been shown to reduce DFT.$^{19–21}$ In other studies of lidocaine, increases in defibrillation voltage requirements similar to those found in this study were observed.$^{22,23}$ Despite small increases in refractoriness, there is generally an inverse correlation between changes in refractoriness and changes in DFT.$^{4,5}$ This has led to the hypothesis that prolonging ventricular refractoriness may prevent wave fronts of activation remaining after a

<table>
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<tr>
<th>Control (n=8)</th>
<th>16-DSA (4 μmol/L, n=8)</th>
<th>Control (n=11)</th>
<th>Heptanol (1 μmol/L, n=12)</th>
<th>Control (n=6)</th>
<th>Lidocaine (3 μmol/L, n=8)</th>
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<tr>
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<td>BL</td>
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<td>DFT, V</td>
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<td>136±35</td>
<td>123±21</td>
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<td>161±22</td>
<td>156±30</td>
<td>169±26</td>
<td>178±21</td>
<td>172±22</td>
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<td>MAPD$_{90}$, ms</td>
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<td>135±21</td>
<td>148±15</td>
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<td>VFCL, %</td>
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<td>118±20</td>
<td>112±12</td>
<td>152±25*</td>
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<td>QRSd, ms</td>
<td>42±5</td>
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<td>T-VFCLdisp, %</td>
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<td>RT-VFCLdisp, %</td>
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<td>SAT-VFCLdisp, %</td>
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<td>58±6</td>
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<tr>
<td>SART-VFCLdisp, %</td>
<td>17±4</td>
<td>16±5</td>
<td>19±4</td>
<td>8±7*</td>
<td>17±8</td>
</tr>
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</table>

BL indicates baseline; QRSd, QRS duration; T-VFCLdisp, temporal dispersion of VFCL; RT-VFCLdisp, relative temporal dispersion of VFCL; SAT-VFCLdisp, spatially averaged temporal VFCL dispersion; and SART-VFCLdisp, spatially averaged relative temporal VFCL dispersion.

*P<0.01 and †P<0.05 vs baseline by repeated-measured ANOVA; ‡P<0.05 by paired t test.
defibrillating shock from propagating throughout the myocardium. This does not explain, however, how adenosine or verapamil can increase DFT with little or no effect on ventricular repolarization.

Cardiac gap junctions contain multiple distinct connexin proteins, each of which forms channels with different electrophysiological and regulatory properties. The function of cardiac gap junction channels is compromised by changes in pH, cGMP, and lipophilic compounds, including fatty acids. Like other short-chain fatty acids, 16-DSA is a selective blocker of gap junctions that induces conformational changes in the channel that result in channel closure by localized disordering of the membrane-channel complex at a depth corresponding to the C-9 to C-18 position of acyl chains. Fatty acids, such as 16-DSA, appear to have little effect on action potentials: palmitoleic acid, which is a potent uncoupler, did not affect action potential shapes in rat heart cells.

Heptanol has direct effects on sodium channel function and depresses the inward Na⁺ current in addition to blocking gap junctions, thus slowing cardiac conduction velocity. It is unlikely, however, that sodium channel blocking (conduction slowing) properties of 16-DSA or heptanol caused a DFT decrease in this model, because slowing of conduction by sodium channel block with lidocaine increased defibrillation voltage requirements. In the present study, 16-DSA and 1-heptanol produced an ∼22% reduction in DFT associated with a 26% to 49% prolongation in QRS duration, a 36% to 87% prolongation in VFCL, and a 56% to 96% reduction in temporal and relative temporal VFCL dispersion, without a change of ventricular repolarization. We cannot, however, rule out the possibility that the decrease in DFT results from a combination of sodium channel and gap junction block. In the absence of detailed epicardial mapping, we cannot be certain that fibrillation is still present after DSA or whether VF is converted to ventricular tachycardia (and presumably fewer or only 1 reentrant circuit), thus accounting for the decrease in defibrillation voltage.

Rohr et al observed paradoxical improvement of impulse conduction in discontinuous cardiac structures exhibiting unidirectional conduction by partial cellular uncoupling with palmitoleic acid. They found that a spatially uniform reduction of electrical coupling induced successful conduction in discontinuous excitable media. Likewise, Spear et al found that heptanol might improve conduction in infarcted myocardium.

Figure 1. Left (LV) and right (RV) ventricular epicardial bipolar electrograms (Bip) and RV endocardial (endo) MAP recording signal during VF in rabbit hearts. Note apparently completely disorganized pattern at baseline (Figures 1 to 4, top), with no obvious periodic component to electrograms. After 4 µmol/L 16-DSA (Figure 2, bottom), 1 µmol/L 1-heptanol (Figure 3, bottom), or 3 µg/mL lidocaine (Figure 4, bottom), signals show increasingly regular, organized patterns, with increasing VFCLs. In control heart, there is no change in patterns after Tyrode’s buffer infusion (Figure 1, bottom). Baseline control (top), buffer control (bottom).

Figure 2. Baseline (top), post-DSA (bottom). Description as in Figure 1.
Conduction in infarcted epicardial tissues was more susceptible to the effects of heptanol than normal myocardium, and some sites that showed no activity during control became active after heptanol. If the same mechanism was present during VF, anisotropic conduction may be accentuated during VF, producing larger reentrant wavelets by favoring longitudinal conduction while slowing or blocking transverse conduction.7,13

In an experimental model of epicardial reentry, Girouard et al29 observed that abrupt changes in electrotonic load during wave front pivoting were major determinants of the wavelength of the reentry circuit. “Turning” of the wavefront in this model was associated with reduced axial resistance, increased current load, and slowed conduction. Cellular uncoupling may indirectly improve conduction in certain orientations, while depressing it in others, by altering axial resistance and modifying source-sink relationships during anisotropic propagation. Girouard et al showed that wavelength was variable during a single rotation of a reentry wave and not simply a product of refractory period and conduction velocity. Changes in passive membrane properties thus may prolong wavelength even though conduction velocity is slowed. These potential effects of cellular uncoupling by exogenous agents are in contrast to the relationship between heterogeneous uncoupling and defibrillation in models of cardiomyopathy30,31 in which DFTs are increased. In the latter models, however, there is fibrosis and cellular disarray, which provide a substrate for disordered conduction independent of alteration in gap junction function.

Limitations
These studies were performed in normal perfused rabbit hearts and may not apply to diseased and/or larger hearts, such as in humans. Defibrillation shocks, however, produce qualitatively similar electrophysiological effects during ischemic versus nonischemic VF in rabbit hearts.32 This isolated heart model can also be used to produce voltage dose-response curves for defibrillation similar to those observed in whole-animal and human studies.33 Our estimate of conduction velocity was not based on absolute measures of conduction velocity or surface QRS but is probably an accurate estimate of relative changes in conduction, provided that activation sequence is not altered. Gap junction blockers, although they do not alter action potential configuration, may also alter nonjunctional currents.8

Conclusions
Electrical uncoupling by 16-DSA and 1-heptanol significantly lower DFT and temporal dispersion of VF activation without affecting ventricular refractoriness. The present study supports the idea that VF organization, as measured by
dispersion of VFCLs, may be a determinant for defibrillation independent of changes in refractoriness. Further studies are necessary to characterize the role of gap junction blockers in arrhythmias.

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References
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