Regional gap junction inhibition increases defibrillation thresholds

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Sims, J. Jason, Kell L. Schoff, Jennifer M. Loeb, and Nicholas A. Wiegert. Regional gap junction inhibition increases defibrillation thresholds. Am J Physiol Heart Circ Physiol 285: H10–H16, 2003. First published March 6, 2003; 10.1152/ajpheart.01074.2002.—It is clear that ischemia inhibits successful defibrillation by altering regional electrophysiology. However, the exact mechanisms are unclear. This study investigated whether regional gap junction inhibition increases biphasic shock defibrillation thresholds (DFT). Sixteen swine were instrumented with a mid-left anterior descending (LAD) perfusion catheter for regional infusion of 0.5 mM/h heptanol (n = 8) or saline (n = 8). DFT values and effective refractory periods (ERP) at five myocardial sites were determined. Regional conduction velocity (CV) was determined in an LAD drug-perfused and nondrug-perfused region in an additional seven swine. Regional heptanol infusion increased 50% DFT values by 33% (P = 0.01) and slowed CV by 42–59% (P < 0.01) but did not affect ERP. Regional heptanol also increased CV dispersion by ~270% (P < 0.05) but did not change ERP dispersion. Regional placebo did not alter any of these parameters. Furthermore, regional heptanol infusion induced spontaneous ventricular fibrillation in eight of eight animals. Increasing spatial conduction velocity dispersion by impairing regional gap junction conductance increased DFT values. Dispersion in conduction velocity slowing during regional ischemia may be an important determinant of defibrillation efficacy.

conduction velocity; electric countershock; ventricular arrhythmias

DESPITE ADVANCES in implantable cardioverter-defibrillator (ICD) therapy, sudden cardiac arrest due to ventricular fibrillation (VF) remains a significant cause of death (44, 47). This may be due to the fact that up to 70% of ICD patients have ischemic coronary artery disease that impairs the ability of a shock to defibrillate (16, 46). Recent studies indicate that VF associated with regional myocardial ischemia requires higher defibrillation shock energy to successfully defibrillate (2, 3, 28, 35, 40). However, the exact mechanisms by which ischemia impairs electrical defibrillation are unknown.

Electrical and optical mapping studies demonstrate that nonuniform activation patterns occur following a defibrillation shock and are responsible for failed defibrillation (21, 45). Furthermore, previous studies indicate that postshock nonuniform propagation is likely due to spatial myocardial electrical heterogeneity (30, 38, 43). Regional myocardial ischemia can increase myocardial electrical heterogeneity by locally altering a number of factors, including increasing extracellular potassium concentrations, decreasing pH, increasing adenosine levels, and increasing passive tissue resistance (7, 17). The net effect is increased electrical heterogeneity because of a regional slowing in conduction velocity and/or a regional change in refractoriness. We have previously shown that an increase in spatial conduction velocity dispersion via regional sodium channel blockade impairs defibrillation (38). Thus homogeneity of conduction velocity appears to be an important determinant of defibrillation efficacy. Regional myocardial ischemia may increase conduction velocity heterogeneity by impairing regional gap junction channel activity.

Gap junctions are composed of connexins, predominantly connexin 43, in the human ventricle, and provide aqueous conduits between cells for electrical current flow (7, 39). After ~15 min of ischemia, gap junctions “uncouple,” which results in impaired cell-to-cell communication, increased passive tissue resistance, and decreased myocardial conduction (4, 22). Thus regional ischemia may uncouple gap junctions, increase conduction velocity heterogeneity, and impair defibrillation. The current study assessed the effects of regional gap junction inhibition on defibrillation efficacy.

METHODS

Animal preparation and surgical instrumentation. The University of Wisconsin Research Animal Resource Center and Animal Care Unit approved the following procedures. Domestic farm swine (25–30 kg) were premedicated with ketamine (15 mg/kg), anesthetized with pentobarbital (15 mg·kg⁻¹·h⁻¹), and mechanically ventilated. With the use of fluoroscopy, monophasic action potential catheters (EP Technologies; Mountain View, CA) were placed into the right ventricular apex and against the left ventricle lateral wall. Defibrillation leads (Sprint, Medtronic; Minneapolis, MN) were placed into the right ventricular apex (anode) and superior vena cavae (cathode). Subcutaneous wire arrays (cathode) were placed within the lateral anterior chest wall. The defibrillation leads were interfaced with an external biphasic defibrillator using a truncated waveform (65% fixed duration).

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till) and pulse duration between 8 and 14 ms. Device output was determined by preset voltage adjustments (Ventak ECD, Guidant; St. Paul, MN).

As previously illustrated, following a sternotomy, a small area (3–5 mm) on the left anterior descending (LAD), just past the second diagonal, was isolated from the surrounding myocardium (30, 38). A 24-gauge plastic catheter was inserted into the LAD and affixed to adjacent epicardium. A 2-mm ultrasonic flow probe (VF-1, Crystal Biotech) was placed distal to the catheter, which demonstrated that coronary flow was unaffected by the catheter. At this point, the perfusion solution only contained normal saline, 100 U/ml heparin, and 0.5 mg/ml nitroglycerin infused at 10 ml/h to prevent clotting and vasospasm. After 30 min, the coronary infusion consisted of saline and heparin. Quadrupolar (5 mm interpole distance) Ag-AgCl electrodes (In Vivo Metrics, Healdsburg, CA) were placed onto the epicardium of the left ventricular apex (LAD catheter-perfused area), left ventricular base, and right ventricular outflow tract. Monophasic action potential probes were placed adjacent to these electrodes. An intramural temperature probe (Yellow Springs) was inserted proximal to a nonperfused area. Myocardial temperature consistently remained 0.5°C above rectal temperature. The chest was draped to retain moisture and heat. Arterial blood gases and sodium and potassium concentrations were measured every 20–30 min and maintained at physiological values (Rapidlab 348, Chiron Diagnostics; Medfield, MA) (31).

Study design. Electrophysiology and defibrillation threshold (DFT) values were measured at baseline. Subsequently, the coronary perfusion (10 ml/h) was changed to the randomly determined treatment group. The control group (n = 8) received heparinized normal saline. The heptanol group (n = 8) received 0.5 mM/h heptanol in heparinized saline. The dose of heptanol was chosen to impair gap junction activity. Previous studies indicate that heptanol (0.5 mmol/l) will inhibit gap junction conductance without significantly altering sodium channel activity. Previous studies indicate that heptanol (0.5 mmol/l) will inhibit gap junction conductance without significantly altering sodium channel activity (1, 25, 33). Thus we chose to infuse heptanol at 0.5 mM/h into the LAD. A 30-min period separated baseline and treatment periods to completely clear the infusion line.

DFT testing. Defibrillation shocks were applied ~8 s after VF induction (21). Defibrillation trials were repeated every 4 min but not until arterial blood pressure was within 10% of preshock value. DFT values were measured using a step-down, step-up method that incorporates 12 fibrillation-defibrillation trials per study phase (36). This estimates the energy-response curve (20–80% successful response) that achieves 20% (ED20), 50% (ED50), and 80% (ED80) successful responses using an iterative computer program (MERFFIT, Guidant; St. Paul, MN) (36).

Conduction velocity. Regional changes in conduction velocity were evaluated in seven additional animals. The animals were instrumented as described above except that two 16-pole electrode arrays were affixed to the epicardium. One array was located within the LAD-perfused region (left ventricular apex) while the other was affixed to a nonperfused region located at the right ventricular base. As previously illustrated, the electrodes were arranged in bipolar pairs in two perpendicular rows with each row containing four bipolar Ag-AgCl electrodes (2 mm interbipole spacing) (19, 30, 38). Conduction velocity was assessed by local cathode pacing at a 300-ms cycle length for 20 s. The anode was located on the right ventricular outflow tract. Fiber orientation was determined during cathode pacing and rotation of the electrode array until the differences in conduction times between the fourth and D electrode were maximal. The signals were processed with a high-gain amplifier and filtered with a bandwidth of 0.1–1 kHz (Universal amplifier, Gould Instruments; Valley View, OH). Conduction velocity was the time required for the impulse from the first electrode of a row to reach the distal electrodes of the same row divided by distance traveled. Arrival time was the point of maximum change in voltage over change in time (dV/dt) at each bipolar. After baseline conduction velocity was recorded, heptanol (0.5 mM/h) was continuously infused into the LAD. Conduction velocity was assessed 45 min after initiation of the heptanol infusion to mimic the time needed to conduct DFT testing.

Electrophysiological parameters. The electrophysiological parameters (paced QRS duration and action potential duration) were measured during right ventricular pacing at a 300-ms cycle length and averaged from five consecutive beats. Ventricular pacing was continued for 15–20 s before these parameters were measured to assure a near steady-state level of channel conductance and channel block. Pacing threshold was measured at each stimulation site using an isolated constant current unit (Bloom Associates, Fisher Imaging; Denver, CO) and delivered current was measured with a digital multimeter (Fluke 867, Fluke Instruments; Everett, WA).

Myocardial repolarization was assessed from the monophasic action potential duration at 90% complete repolarization. Local effective refractory period (ERP) was determined at a 300-ms cycle length using a premature stimulus method as previously described (29). Each ventricular recording site was tested in random order. VF cycle length (VFCL) was measured before the defibrillation shock. VFCL was measured from monophasic action potentials recorded from five ventricular sites as previously described (37). Spatial heterogeneity (dispersion) between recording sites was evaluated for the following electrophysiological parameters: action potential duration, ventricular refractoriness, pacing threshold, VFCL, and ventricular conduction velocity. Dispersion was calculated as the difference between the maximum and minimum value of the five recording sites (two sites for conduction velocity). All electrophysiological measurements were obtained at the start and end of the DFT protocol at baseline and drug treatment phases. These values were averaged for each study phase. Electrophysiological and hemodynamic signals were processed with Gould Universal Amplifiers, digitally acquired at 3,000 Hz, and stored for off-line analysis (Datawave; Boulder, CO). Monophasic action potentials were DC coupled, filtered at 300 Hz, amplified, and stored.

Data analysis. Paired t-test or two-way analysis of variance evaluated differences between parameters at baseline and treatment within group (animal as its own control). One-way analysis of variance tested differences between groups. Fisher’s exact test compared frequency of VF induction during electrical pacing between treatment groups. All data and statistical analysis were performed with a personal computer using SigmaStat 2.0 (SPSS Science; Chicago, IL). Statistical significance was a P value < 0.05 using a two-tail test. Data are presented as means ± SE.

RESULTS

Defibrillation thresholds. Mean baseline DFT values between the two groups were not different (Fig. 1). DFT values for the control group during saline treatment did not differ from baseline. However, regional heptanol greatly increased baseline DFT20, DFT50, and DFT80 values (Fig. 1: DFT50 = 16.2 ± 1.6 to 21.5 ± 2.8 joules, P = 0.01). The DFT values increased in a relatively linear manner such that 20% (DFT20), 50%
(DFT$_{50}$), and 80% (DFT$_{80}$) values increased by 22%, 31%, and 46%, respectively. Lead impedance did not differ between the two groups. However, in both groups there was a slight drop in impedance over time, a common finding in this model (41). Thus the above findings cannot be attributed to changes in lead impedance.

**Conduction velocity.** Regional heptanol significantly slowed longitudinal and transverse conduction velocity within the perfused region by 59% and 40%, respectively (Fig. 2). However, regional heptanol did not affect conduction velocity at the nonperfused right ventricular site. Hence, regional heptanol increased dispersion in conduction velocity between the left ventricular apex and right ventricular base (Fig. 3) and, likely, other non-LAD-perfused regions. Moreover, regional heptanol inhibited the anisotropic conduction properties of the myocardium at the left ventricular apex such that the ratio of longitudinal to transverse conduction velocity was significantly decreased (2.6:1, baseline vs. 1.8:1, heptanol). Also, QRS duration, a marker of global ventricular conduction velocity, was unaffected by regional heptanol (baseline = 76 ± 3.2 ms vs. heptanol = 77 ± 4.5 ms), demonstrating the local effect of regional heptanol on conduction.

**Refractoriness and excitability.** Similar to regional placebo, regional heptanol did not affect ERP at any site and thus did not increase dispersion in refractoriness (Table 1). However, regional heptanol significantly increased the pacing threshold (decreased excitability) at the left ventricular apex from 0.16 ± 0.03 to 0.32 ± 0.05 mA ($P < 0.05$). Regional heptanol did not affect pacing thresholds at the four nonperfused pacing sites, indicating this effect was local. Thus regional heptanol increased dispersion in pacing thresholds by 38% (0.21 ± 0.04 to 0.29 ± 0.06 mA, $P = 0.06$) but did not reach statistical significance. Pacing thresholds in
DISCUSSION

Ventricular arrhythmias and sudden cardiac death are common complications in patients with coronary artery disease. Rapid defibrillation by an ICD is a major advancement in preventing mortality associated with these sequelae (23). Unfortunately, these patients frequently have periods of myocardial ischemia, which may alter the efficacy of these devices by increasing spatial electrical heterogeneity (2, 3, 28, 35, 40). The current study assessed the effects of regional gap junction uncoupling, which occurs during prolonged ischemia, on defibrillation efficacy. We found that regional gap junction inhibition created by a local infusion of heptanol markedly increased biphasic DFT values. The increase in DFT values occurred in tandem with increased myocardial electrical heterogeneity, evidenced by regional conduction velocity slowing, increased conduction velocity dispersion, a regional increase in pacing threshold, and increased dispersion in VFCL. The finding that regional gap junction inhibition increases DFT values, whereas global gap junction inhibition decreases DFT values (27), strongly indicates the importance of regional changes in electrical activity on defibrillation efficacy.

Significant increases in passive tissue resistance begin to occur after 15 min of ischemia and reach peak at around 30 min (4). This increase in passive tissue resistance has been linked with the Ib phase of ventricular arrhythmias (11, 26, 32). Moreover, specific regional gap junction uncoupling without ischemia produces similar ventricular arrhythmias (5). Thus it is clear that cell-to-cell communication, passive tissue resistance, and gap junction conductance play an important role in the induction of ventricular arrhythmias. In the current study, regional gap junction inhibition was shown to significantly increase the energy needed to successfully defibrillate. Therefore, it appears that gap junction conductance plays an important role in ischemia-induced changes in defibrillation efficacy.

There are several proposed mechanisms of ventricular defibrillation, such as VF organization (12, 13, 34, 37), critical mass (42, 45), virtual electrode polarization (14), and the upper limit of vulnerability (8, 9). It is unlikely that the first two hypotheses account for the findings of the current study. The VF organization hypothesis states that the more organized fibrillation is, the fewer number of wavefronts there are propagat-

Table 1. Electrophysiological parameters

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VFCL, ms

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Values are means ± SE. ERP, effective refractory period; VFCL, ventricular fibrillation cycle length; RV, right ventricular endocardium; LV, left ventricular endocardium; LVA, left ventricular epicardial apex; LVB, left ventricular epicardial base; RVB, right ventricular epicardial base. *P < 0.05, baseline vs. heptanol.

the placebo group remained similar to baseline during the treatment phase at all pacing sites.

Ventricular fibrillation. Neither continuous rapid pacing nor a single premature stimulus could induce VF at baseline or during regional saline. However, regional heptanol induced VF in eight of eight animals (P < 0.01 vs. control) with either continuous rapid pacing or a single premature stimulus. Regional heptanol infusion increased VFCL by 28% (P = 0.02) in the heptanol-perfused region, whereas VFCL was not altered in the nonperfused regions (Table 1). Thus spatial VFCL dispersion was increased by 153% (P < 0.05). Furthermore, during VF regional heptanol significantly altered epicardial action potential morphology (Fig. 4) at the left ventricular apex. Specifically, fibrillation action potentials in the control group (regional saline) were fractionated, had double potentials, and rarely reached diastolic potentials. In comparison, regional heptanol decreased the number of action potentials at the perfused recording site, widened the action potential duration, and qualitatively changed the action potential appearance such that fewer double and fractionated potentials were observed. However, no such changes were observed at other recording sites. This suggests that the myocardium in the heptanol-perfused region was less likely to be excited by colliding wavefronts and/or there was less block of wavefront propagation in this region (12).

![Fig. 4. Monophasic action potentials from LV apex epicardium (LV Apex), LV base epicardium (LV Base), and RV base epicardium (RV Base) recorded during VF and treatment at baseline with placebo (left) and during regional heptanol (right) in a representative animal.](http://ajpheart.physiology.org/)

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ing in the myocardium, thus making it easier to defibrillate (13). This hypothesis explains how interventions decrease DFT values and is the proposed mechanism by which global gap junction inhibition lowers DFT values (27). Although regional heptanol made VF less organized as measured by VFCL dispersion, recent data indicate that the VFCL dispersion may not accurately predict changes in DFT values (38). Therefore, we believe that regional heptanol increased DFT values by another mechanism.

The second mechanism, critical mass, relates to cellular excitability and depolarization, such that when a critical mass of myocardium is depolarized VF is extinguished. On the basis of our measurements of pacing threshold, regional heptanol decreased cellular excitability in the drug-perfused region. Although not reported (27), it is likely that global gap junction inhibition increases pacing thresholds but is associated with decreasing DFT values. Moreover, we have previously shown that very high doses of lidocaine drastically decreased excitability but decreased DFT values (37). Thus we do not believe that altering the critical mass was responsible for the results seen in the current study.

Although separate hypotheses, the virtual electrode polarization and the upper limit of vulnerability hypotheses relate to how defibrillation fails and importantly share a common theme (5–10, 14). Both hypotheses suppose that failed defibrillation results from the shock itself, inducing an electrophysiological state immediately after the shock that promotes reentry and fibrillation, even if a critical mass of myocardium is depolarized. We believe that this may be the mechanism by which regional gap junction inhibition impeded defibrillation. Specifically, regional gap junction inhibition likely increased electrical heterogeneity via conduction velocity dispersion immediately after the shock. Subsequently, this increase in conduction velocity dispersion promoted the propagation of early postshock activations, leading to fibrillation and inhibited successful defibrillation.

Conduction velocity is normally homogeneous across the myocardium, albeit anisotropic, resulting in uniform propagation of an activation front. However, regional ischemia slows regional conduction velocity increasing spatial conduction velocity dispersion, which is highly arrhythmogenic, thus increasing the probability of ventricular arrhythmias (5, 11, 26, 32). Consistent with these reports, the present study demonstrated that regional conduction velocity slowing by local heptanol was highly arrhythmogenic to point stimulation, similar to what we have previously reported with regional lidocaine (38). Thus it appears that the conduction velocity dispersion created by regional heptanol increased myocardial vulnerability to VF. In fact, previous computational and mapping studies have shown that VF induction is closely associated with regions of slow conduction velocity and that spatial conduction velocity dispersion can be highly arrhythmogenic by causing conduction block, wavefront instability, and wave break (6, 10, 15).

Regional changes in conduction velocity may produce a situation where the propagation of an impulse is fast in one myocardial region while slow in another resulting in conduction block and wave break. This will cause the action potentials in the slow conduction region to be out of phase with action potentials in other myocardial regions. Because the slowly conducting tissue activates later in time compared with tissue in fast conduction region, repolarization and refactoriness will be out of phase even if the action potential duration is similar across the myocardium (15). The resultant dispersion of activation and repolarization between the slow and the normal conduction regions may disrupt uniform propagation (20), creating postshock arrhythmias. However, because of the complexity of defibrillation, it is not likely that postshock conduction velocity dispersion is the sole mechanism.

Limitations. Gap junction inhibition occurred in a single myocardial region. We cannot speculate whether numerous regions of electrical heterogeneity would affect defibrillation in a different manner, or if increasing dispersion by altering gap junction conductance in a myocardial region other than the middistal LAD would behave differently. Second, although all precautions were taken to ensure that heptanol only altered gap junction conductance, we cannot absolutely sure regional sodium channel blockade did not contribute to effects on DFT values. However, regional heptanol in the current study produced significantly different effects on conduction velocity than previous results with regional lidocaine, indicating a different affect on conduction velocity than sodium channel blockade (38). Nonetheless, it is not wise to make assumptions that the gap junction uncoupling produced in this model provide a direct mechanism for ischemia-induced increases in DFT values. Finally, we did not document that regional heptanol increased conduction velocity dispersion postshock. However, drug-induced electrophysiological changes that occur during pacing and fibrillation are similar to changes that occur immediately after a biphasic shock (18, 24, 29).

Clinical implications. Mortality from sudden cardiac death can be prevented by rapid defibrillation. However, high DFT values limit these devices. Previous studies indicate that myocardial ischemia, similar to what occurs in patients with coronary artery disease, causes transient increases in DFT values (3, 28, 40). The current study found that regional gap junction inhibition, such as what occurs during prolonged ischemia, increases DFT values. This suggests that regional gap junction uncoupling may be a mechanism by which myocardial ischemia impairs defibrillation. If future studies that assess other components of myocardial ischemia continue to indicate that regional gap junction uncoupling is the predominate mechanism of ischemia-induced elevation in DFT values, then gap junctions may become a therapeutic target so that device or drug therapies can be developed that limit gap junction uncoupling and postshock conduction heterogeneity.
GAP JUNCTION INHIBITION AND DEFIBRILLATION

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