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Reversible Cardiac Conduction Block and Defibrillation with High-Frequency Electric Field

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Abstract

Electrical impulse propagation is an essential function in cardiac, skeletal muscle, and nervous tissue. Abnormalities in cardiac impulse propagation underlie lethal reentrant arrhythmias, including ventricular fibrillation. Temporary propagation block throughout the ventricular myocardium could possibly terminate these arrhythmias. Electrical stimulation has been applied to nervous tissue to cause reversible conduction block, but has not been explored sufficiently in cardiac tissue. We show that reversible propagation block can be achieved in cardiac tissue by holding myocardial cells in a refractory state for a designated period of time by applying a sustained sinusoidal high-frequency alternating current (HFAC); in doing so, reentrant arrhythmias are terminated. We demonstrate proof of concept using several models, including optically mapped monolayers of neonatal rat ventricular cardiomyocytes, Langendorff-perfused guinea pig and rabbit hearts, intact anesthetized adult rabbits, and computer simulations of whole-heart impulse propagation. HFAC may be an effective and potentially safer alternative to direct current application, currently used to treat ventricular fibrillation.

INTRODUCTION

Abnormal and chaotic cardiac impulse propagation underlies the pathogenesis of ventricular fibrillation (VF), a leading cause of death in the developed world, accounting for more than 400,000 deaths annually in the United States. Direct current (DC) or biphasic counter shock applied promptly to the heart is the only reliable method to treat VF. Although this is very effective, the use of brief, high-voltage DC shocks to treat malignant cardiac arrhythmias is associated with a host of adverse effects that include cellular injury from electroporation (1, 2), cardiac conduction disturbances (3), mechanical dysfunction (1, 4, 5), and increased mortality in heart failure patients (6). Thus, an alternative method of defibrillation that is devoid of these adverse effects is desirable.

Cardiac muscle tissue and nerve fibers depend on a similar mechanism of impulse propagation to conduct cardiac rhythms and neural signals, respectively. The use of sustained kilohertz-range alternating current (AC) fields to block electrical conduction in nervous tissue has been known for more than a half-century (7). This conduction block is

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instantaneous and completely reversible upon cessation of the stimulus (7, 8). The mechanism of the conduction block is sustained depolarization of the nerve cell membrane under the stimulus electrode (9, 10). AC field-induced neural conduction block has been exploited recently for both diagnostic and therapeutic purposes (8, 11). However, electric field-induced propagation block in myocardial tissue has not yet been described. This is possibly because of the inherent challenge in examining the myocardial cellular response, which is obscured by the electrical stimulus artifact. AC-induced propagation block would be of enormous utility in the clinic, because reversible block would provide a novel and potentially safer means for termination of life-threatening reentrant arrhythmias, such as VF. We therefore hypothesized that electric fields, such as those used for neural block, when applied to cardiac tissue, would similarly produce reversible block of cardiac impulse propagation and lead to successful defibrillation. Here, we provide proof of concept in several cellular and animal models for conduction block in cardiac tissue during high-frequency AC (HFAC) field stimulation, which could be translated to humans for treating cardiac fibrillation.

RESULTS

Reversible cardiac conduction block with HFAC field application

Using optical mapping with a voltage-sensitive dye, we first explored the effect of sinusoidal HFAC electric fields applied for 1 s across confluent monolayers of neonatal rat ventricular cardiomyocytes ($n = 80$ monolayers). During HFAC field application over a broad frequency range (50 to 1000 Hz), the optical fluorescence transmembrane potential (V_f) of cells, normalized to the action potential amplitude, was held at a field-dependent, elevated (partially depolarized) voltage (V_{elev}) throughout the monolayer, and point pacing-initiated waves were completely blocked. The effect of a 1-kHz, 11-V/cm sinusoidal field on V_f of a cell in the cardiomyocyte monolayer is shown in Fig. 1A and movie S1. Immediately after cessation of the HFAC field, V_f returned to the initial resting potential, and subsequent pacing stimuli initiated waves across the monolayer, with a conduction velocity and propagation pattern unchanged from before HFAC field application. Small oscillations in V_f at the HFAC field frequency were sometimes observed ($n = 6$ monolayers, 18 trials) (fig. S1A). V_f oscillations were in-phase (same polarity) and of about uniform amplitude across the monolayer (fig. S1B) and decreased with increasing frequency (fig. S1C).

AC frequencies less than 50 Hz, or field amplitudes below threshold, resulted in repetitive depolarizations of the monolayer, which we term field-evoked activity (FEA). An example of FEA during a 100-Hz AC field at a strength of 4.4 V/cm is shown in Fig. 1B. Frequencies greater than 2 kHz had no effect on the monolayer at the highest field strength tested (11 V/cm) (Fig. 1C). Conduction block was significantly more likely at HFAC frequencies tested between 50 Hz and 1 kHz and field strengths >5 V/cm than at frequencies outside this range or field strengths lower than this range [231 of 305 attempts (76%) versus 6 of 155 attempts (4%), respectively; $P < 10^{-15}$]. Furthermore, the field strength threshold required to achieve conduction block was frequency-dependent (Fig. 1E).

For comparison, we also examined the cardiomyocyte response to application of DC fields of 1-s duration ($n = 10$ monolayers). The DC field depolarized the entire monolayer at the onset (make-excitation); however, V_f rapidly returned to a value near the prestimulus resting potential (Fig. 1D and movie S2). Point pacing during the DC field initiated new propagating waves, indicating a lack of conduction block. Conduction was slower across the monolayer and blocked at regions near the field electrodes (Fig. 1D, double black lines). A second depolarization occurred at field offset (break-excitation). After a 1-s DC field application at 11 V/cm, cardiomyocyte monolayers consistently exhibited aberrant behavior, including heterogeneous propagation ($n = 4$), rapid ectopic activity ($n = 9$), or failure to

excite ($n = 5$)—none of which were observed with 1-s application of HFAC fields of the same amplitude.

HFAC field effect on spiral wave reentry

We evaluated the ability of sustained HFAC field–induced conduction block to terminate stable spiral wave reentry (a wave of excitation propagating around a central defect) in cardiomyocyte monolayers ($n = 22$ monolayers). When HFAC field was applied at a constant amplitude of 11 V/cm, the field onset depolarized the excitable region in front of the wavefront (excitable gap); after this, V_f was held throughout the monolayer at V_{elev} for the entire field duration (870 to 1870 ms), resulting in propagation block followed by complete extinction of the spiral wave (Fig. 2A and movie S3). The transmembrane potential of cells in the wave tail showed an initial graded response that was dependent on the refractory state of the cells at field onset, such that the V_f of cells already depolarized by the spiral wave (before HFAC field onset) progressed directly to V_{elev} without full repolarization (Fig. 2A, locations a and b). Cells partially repolarized at field onset remained depolarized relative to rest, with V_f progressing to the same V_{elev} (Fig. 2A, locations c to e). After HFAC field cessation, V_f at all locations returned to the normal resting potential, followed by quiescence. These findings demonstrate that the applied HFAC field drives V_f to V_{elev} independent of the initial polarization of the cell, resulting in propagation block and termination of spiral wave reentry. Spiral wave reentry termination was significantly more likely at HFAC frequencies tested between 50 Hz and 1 kHz and field strengths >5 V/cm than at frequencies and field strengths outside these ranges [44 of 46 attempts (96%) versus 16 of 57 attempts (28%), respectively; $P < 10^{-12}$]. The minimum field strength required to terminate spiral wave reentry was similar between DC and HFAC fields for frequencies between 50 and 200 Hz (fig. S2). At higher field strengths (>5 V/cm), low-frequency (10 to 20 Hz) AC fields typically reinitiated spiral wave reentry, after terminating reentry at field onset, whereas higher-frequency (>1 kHz) AC fields either reinitiated or had no effect on the spiral wave reentry.

We sought to determine whether HFAC field–induced propagation block and arrhythmia termination depend on make-excitation at the field onset by ramping, rather than stepping, the field amplitude up in time during sustained spiral wave activity ($n = 5$ monolayers, 19 trials). As HFAC field amplitude grew, diastolic transmembrane potential (resting potential between successive action potentials) increased steadily (Fig. 2B and movie S4). Elevated diastolic transmembrane potential has been shown to decrease excitability and slow conduction (12). This resulted in progressive cycle length prolongation before wave extinction (Fig. 2C). Over all trials, average cycle length prolonged significantly from 125 ± 7 to 236 ± 7 ms ($P < 10^{-5}$) (fig. S3). Thus, unlike DC field, HFAC field–induced arrhythmia termination does not depend on make-excitation.

Arrhythmia termination in guinea pig and rabbit hearts

The ability of HFAC fields to block propagation and to terminate arrhythmias was then tested in Langendorff-perfused guinea pig ($n = 15$) and rabbit ($n = 2$) hearts. HFAC fields (25 to 37 V; 50 to 200 Hz) inhibited propagation during epicardial pacing more often than using fields less than 25 V and frequencies between 500 Hz and 10 kHz [53 of 92 attempts (58%) versus 14 of 142 attempts (10%), respectively; $P < 10^{-14}$], with elevation of V_f to V_{elev} (between ~ 30 and 80% action potential amplitude above resting potential) (Fig. 3A). Oscillations at the HFAC field frequency, but of opposite polarity, were observed even within closely spaced regions (Fig. 3A, sites a to c) across the epicardial surface, starting with HFAC field onset and continuing until field cessation (Fig. 3B and movie S5). We did not specifically control the timing of the HFAC field for the phase of the action potential or propagating wave. However, for spontaneous and paced rhythms, irrespective of the timing

of the field, conduction block occurred with HFAC within the optimal frequency range. HFAC fields that blocked conduction, when applied during sustained VF, terminated the arrhythmia (Fig. 3C and movie S6). HFAC fields (25 to 37 V, 50 to 200 Hz) successfully terminated 23 of 28 fibrillation episodes (82%) compared with 26 of 51 episodes (51%) using fields of less than 25 V and frequencies of 500 Hz to 10 kHz ($n = 10$ hearts) ($P < 0.01$). Additionally, across multiple frequencies ($n = 6$ hearts, 15 trials), longer-duration HFAC fields (20 to 1000 ms, average defibrillation field amplitude threshold of 19.8 ± 2.2 V) terminated VF, whereas short, one-cycle duration (2 to 20 ms) fields of equal amplitude failed to terminate fibrillation and required a significantly higher amplitude to defibrillate (25.1 ± 2.3 V) ($P < 10^{-5}$) (fig. S4). These observations demonstrate that longer-duration HFACs block fibrillatory wavefronts, such that a lower-amplitude field is sufficient for successful defibrillation.

When a ramped HFAC field (0 to 25.5 V) was applied to isolated guinea pig hearts ($n = 3$ hearts, 26 trials) in VF, diastolic V_f rose progressively (Fig. 3E), which slowed fibrillatory activity, increased cycle length (Fig. 3F), reduced dominant frequency (DF) from 18 to 4 Hz (Fig. 3, D and G), and diminished the amplitude of fibrillatory activity until VF was extinguished (movie S7). Over all trials, mean DF decreased significantly by the end of the applied ramp compared to initial values (8.7 ± 1.0 versus 17.0 ± 0.2 Hz) ($P < 10^{-7}$) (fig. S5A), and mean percentage of spectral power in the frequency range typically associated with fibrillation (10 to 30 Hz) also decreased significantly from beginning ($84.5 \pm 0.50\%$) to end ($60.8 \pm 1.8\%$) of the AC field ramps ($P < 10^{-12}$) (fig. S5B). These results further demonstrate that HFAC fields terminate fibrillation by a mechanism distinct from that of DC-based defibrillation.

Arrhythmia termination by HFAC fields in intact rabbit hearts

Finally, we tested whether HFAC fields can terminate induced VF in intact rabbit hearts ($n = 3$). VF was induced by a low-voltage (7.5 V), 2-s, 50-Hz field and was the only arrhythmia induced in the intact hearts. Application of the HFAC field (30 V, 200 Hz) for 100 or 200 ms failed to terminate VF in rabbits, whereas a 400-ms application was successful in terminating VF (Fig. 3H). These results were reproducible in all three animals. This suggests that for a given field strength and frequency, there is a requisite field duration for successful defibrillation in an intact animal.

Computational simulation of conduction block

To gain further insight into the mechanism, we examined the effect of HFAC fields in an established computational model of electrical propagation that is based on an anatomically accurate rabbit heart, incorporating realistic geometry and fiber orientation (Fig. 4A) (13). Consistent with our experimental results in Figs. 1 to 3, the model demonstrated that HFAC fields (25 V/cm) elevated simulated transmembrane potential (V_m) (Fig. 4B) and induced oscillations in V_m of opposite polarity at closely spaced sites on the epicardial surface at the HFAC field frequency of 200 Hz (Fig. 4C). This extinguished all preexisting fibrillatory wavefronts, completely blocking propagation in some tissue regions and evoking slow activity in other regions, perhaps because of variable blockade of sodium channels (Fig. 4A). At the cellular level, V_m , ionic calcium and potassium currents (I_{Ca} and I_K), and intracellular calcium (Ca_i) in regions of conduction block approached persistently elevated oscillating levels, whereas the sodium current I_{Na} was completely absent, coincident with the onset of the HFAC field at time 0 (Fig. 4D).

Excitability is a postshock property that depends on the amount of available tissue with V_m below the sodium channel activation threshold. It affects the success of a defibrillation shock (14, 15) and varies greatly during VF. During HFAC field application, the amount of

inexcitable tissue (tissue with V_m elevated above the sodium channel activation threshold) was maintained at an oscillating, elevated steady state (Fig. 4E), thereby reducing the likelihood of reinitiation of fibrillation after field offset. Application of sufficiently strong HFAC fields successfully terminated VF 100% of the time (nine of nine different timings for HFAC field application) (Fig. 4F). Although the phase of application of HFAC was variable, termination was achieved in all the trials using sufficient HFAC field strength, demonstrating that defibrillation success depends on the state of the heart at field offset, regardless of the initial conditions at field onset.

DISCUSSION

These data reveal a previously unrecognized capacity for myocardial cells to be placed in an extended, yet immediately reversible, state of refractoriness by an applied electric field. The imposed refractory state blocked all wave propagation and resulted in termination of reentrant arrhythmias, without impairment of subsequent cellular electrical function or initiation of postshock fibrillatory activity. VF has been shown to be terminable by brief, high-voltage DC or biphasic pulses (16). However, these defibrillation mechanisms rely on evoking a transient response to the external field. In contrast, extended HFAC fields evoke a sustained response, which reaches a quasi-equilibrium, maintains a refractory state for as long as the exogenous field is applied, and results in a state that is insufficient for reinitiation of fibrillatory activity. Our finding that defibrillation is dependent on the duration of applied HFAC field suggests that conduction block must persist long enough for all fibrillatory wavefronts to encounter nonexcitable tissue. Our *in vivo* studies required a field duration in the range of 300 to 400 ms for successful defibrillation, suggesting that the requisite field duration may be related to the duration of the myocyte refractory period or short-term memory. However, the optimal field duration may depend on field strength and frequency, as well as the specific electrophysiological properties of the tissue. Further understanding of the underlying mechanisms for conduction block will lead to more precise optimization of the field duration.

It is somewhat counterintuitive that an AC electric field can suppress cardiac electrical activity, because 50- to 60-Hz AC current causes electrocution death by inducing VF. Historically, 50- to 60-Hz AC waveforms were the first types of electrical therapy used to treat VF, but were abandoned because of the high risk of proarrhythmia (17, 18). However, few studies have evaluated the effects of higher AC frequencies in intact hearts. AC has been used in intact guinea pig hearts, demonstrating a frequency-dependent increase in fibrillation induction threshold (19). The defibrillation efficacy *in vivo* in guinea pig hearts has been evaluated at AC frequencies up to 1 kHz but with a maximum duration of 32 cycles (20). Successful termination of VF *in vivo* required at least 80 cycles (400-ms duration at 200 Hz) in our study. A few studies have also evaluated trains of monophasic DC pulses to terminate arrhythmias (21–23). For example, defibrillation in dogs with monophasic rectangular pulse trains has been demonstrated at frequencies up to 20 kHz (23). However, all of these studies relied on relatively brief waveforms (most less than 30 ms) to achieve defibrillation, and none characterized extended refractoriness or propagation block. Although propagation block might have occurred during AC field application in some of these studies, the biophysical phenomenon would have been obscured by the electrical stimulus itself, thus evading recognition without optical mapping.

To our knowledge, the electrophysiological effects of HFAC field stimulation have not been systematically studied in cardiac muscle. A couple of studies investigated low-frequency (<50 Hz) sinusoidal AC fields (24, 25). Prolongation of action potential duration and V_m oscillations has been shown in simulated cardiac myocytes (24) and a cardiac tissue model (25) subjected to low-frequency AC stimulation. Nevertheless, these studies focused on

entrainment with the low-frequency AC field and did not demonstrate block of pacing-initiated or preexisting electrical propagation. Another way in which AC fields can terminate reentrant arrhythmias is by imposing standing waves of depolarization (26–28), a mechanism that is distinctly different from inducing sustained refractoriness, as we have shown here. The mechanism of refractoriness produced by HFAC fields also differs from that of electrotonic inhibition (29), in which a brief subthreshold pulse applied to a muscle fiber renders the fiber refractory to the next activation stimulus. During HFAC, the transmembrane potential is held at a substantially depolarized state throughout the myocardium, inhibiting sodium channel activation for the duration of field application.

A limitation of our optical mapping approach is the inability to determine absolute transmembrane potential values. However, previous studies have shown a linear relationship between myocyte transmembrane potential and voltage-sensitive dye fluorescence (30). Calibrating V_f from the optical recordings of Fig. 3, B and C, using previously published values for adult guinea pig resting potential and action potential amplitude (31), we can estimate that V_{elev} varied from about -48 to $+15$ mV, which lie above the sodium channel activation threshold even at the lower end of the range and are consistent with the computational simulations and the proposed mechanism of sodium channel inactivation. Calibration of the neonatal rat voltage traces (Figs. 1A and 2A) results in a similar elevated potential range.

In conclusion, the use of HFAC fields to inhibit wavefront propagation might provide an alternative approach for arrhythmia termination, without adverse effects, in future implanted cardiac devices in humans. We have shown proof of this concept across multiple species and models. HFAC fields may be advantageous in conditions resistant to DC shock, such as persistent VF, because HFAC fields actively hold the cell membrane in a refractory state and produce less field-evoked and postshock activity than DC, which are critically important to avoiding reinduction of fibrillation. However, further work on safety and efficacy of HFAC in large mammals is warranted.

MATERIALS AND METHODS

Cell culture

All experiments involving animals or tissues derived from animals were performed in accordance with institutional animal care and use guidelines and approval. Neonatal rat ventricular myocytes were dissociated from 2-day-old Sprague-Dawley rat hearts with trypsin and collagenase, as described previously (32). The resulting cell suspension was plated onto 21-mm-diameter plastic coverslips (10^6 myocytes per coverslip) to form monolayers that became confluent after 3 to 4 days of culture. Experiments were performed on days 6 to 8 after plating. For reentry experiments, before plating, a 4-mm-diameter hole was punched in the coverslip (33).

Cell monolayer electrophysiological recording

The transmembrane voltage of monolayers ($n = 102$ total) was recorded with contact fluorescence imaging, as described previously (34, 35). Briefly, transmembrane potential was recorded by placing the cell monolayer directly on top of a bundle of 253 optical fibers that were 1 mm in diameter, arranged in a tightly packed, 17-mm-diameter hexagonal array. The cell monolayers were stained during the experiment with $10 \mu\text{M}$ di-4-ANEPPS (Molecular Probes), a fluorescent voltage-sensitive dye, and continually superfused with warmed (37°C) Tyrode's solution (135 mM NaCl, 5.4 mM KCl, 1.8 mM CaCl_2 , 1 mM MgCl_2 , 330 μM NaH_2PO_4 , 5 mM Hepes, 5 mM glucose). The excitation source consisted of a custom-built array of high-power green light-emitting diodes placed directly above the

experimental chamber. The fluorescent signal was relayed by the optical fiber bundle to an array of photodetectors and amplifiers and acquired at a rate of 1000 samples per second.

Electric field stimulation was applied across a parallel set of platinum wires that were 2.5 cm long, placed in the bath straddling the monolayer. The field intensity was calibrated from the peak voltage across a pair of silver chloride (AgCl) test electrodes, which were placed at a 1.4-cm spacing in the chamber. DC and sinusoidal HFAC field strengths were expressed as waveform amplitude values.

For conduction experiments, a platinum bipolar point electrode was placed near the edge of the monolayer and used to pace the monolayer at 2 to 6 Hz (10-ms monophasic pulse, $1.5\times$ threshold). For reentry experiments, rapid point pacing was used to induce a stable spiral wave reentry. Stable reentry was considered successful if the wave pinned to the hole for at least 1 min. A 1-s duration AC or DC pulse was applied to the monolayer during each point pacing or reentry episode.

Cell monolayer field response classification

For conduction experiments, monolayer responses to DC and AC field were categorized as no effect, FEA, or conduction block during the field pulse. In “no effect” cases, paced conduction continued at the same pacing rate during the field pulse. In “FEA” cases, rapid activity faster than the point pacing rate was elicited. In “conduction block” cases, no activation occurred during the field pulse. The occurrence of post-pulse ectopic activity was separately identified when multiple spontaneous waves were initiated at a location distinct from the point-pacing site.

Langendorff-perfused guinea pig and rabbit hearts

Hartley guinea pigs ($n = 15$, 500 to 700 g) or New Zealand white rabbits ($n = 2$, 4 to 5 kg) were anesthetized [sodium pentobarbital (100 mg/kg)]. After a midline thoracotomy, the hearts were excised and connected to a Langendorff perfusion system. Warmed (37°C), oxygenated Tyrode's solution was continuously perfused through the aorta.

Whole-heart electrophysiological recording

Hearts were stained with $10\text{ }\mu\text{M}$ di-4-ANEPPS for 10 min, after which they were immersed in a transparent Plexiglas chamber filled with Tyrode's solution. Blebbistatin ($10\text{ }\mu\text{M}$; Sigma-Aldrich) was added to suppress motion artifacts. The left anterior epicardial surface was pressed against the mapping chamber to further suppress motion.

For the optical mapping system, light from two 150-W halogen lamps was collimated and passed through a $520 \pm 20\text{-nm}$ excitation filter. Using a tandem lens assembly, the excitation light reflected off a 560-nm dichroic mirror and focused onto the heart surface. The emitted fluorescence was passed through a 600-nm emission filter and focused on a $100\text{ pixel} \times 100\text{ pixel}$ MiCAM Ultima CMOS camera (SciMedia), acquiring at a frame rate of 500 or 1000 samples per second. Pixel resolution was 0.113 mm with $1\times$ magnification.

For conduction experiments, a platinum bipolar point electrode was placed near the left ventricular base and used to pace the heart at 4 to 6 Hz (10-ms monophasic pulse, $1.5\times$ threshold). HFAC field pulses were applied by platinum field electrodes pressed against the side of the heart during point pacing or induced fibrillation. Field strength was expressed as the amplitude of sinusoidal voltage applied between the electrodes. Fibrillation was induced in each animal by a 1-s, low-voltage amplitude, 50-Hz field and considered stable if lasting longer than 1 min. We only considered termination of stable episodes of VF, although we

did not specifically control for the timing of HFAC field application after VF induction. In control trials, in which VF was not terminated by HFAC, VF episodes always lasted >3 min.

Cell monolayer and whole-heart signal processing and analysis

Individual voltage signals from monolayers were temporally filtered with a five-point median filter. Signals were range-normalized such that the resting potential equaled 0 and the action potential amplitude equaled 1. Baseline drift was corrected for by subtraction of a fitted third-order polynomial at each recording site. Voltage maps from monolayer experiments were created by interpolating the mapped data to a $100\ \mu\text{m} \times 100\ \mu\text{m}$ grid. Recording channels with poor signal were not used for the interpolation. Voltage traces showing oscillations in V_f (fig. S1) were not temporally filtered. The individual voltage signals from whole-heart experiments were baseline-corrected and range-normalized. Activation times were computed as the times of the first derivative maximum, and cycle length was computed as the difference of successive activation times.

Whole-heart frequency analysis

The frequency content of V_f was analyzed when ramped HFAC fields were applied during fibrillation. The DF of fibrillatory activity time series (Fig. 3G) was calculated by first computing the power spectrum at the selected pixel over 1000-ms intervals, with the interval start and endpoints increasing in 100-ms increments. The DF for each interval was calculated by determining the frequency that was less than 30 Hz and had the largest peak in the power spectrum. Spatial DF maps were created after computing DF at each pixel. The mean DF was computed by spatially averaging DF values over all pixels (Fig. 3G, bold trace). Spectral power in the frequency range associated with fibrillation was computed by first determining the total power in the 10- to 30-Hz frequency range, divided by the total power between 0 and 30 Hz, and converted to a percentage, at each pixel over a 1000-ms interval either before or at the end of the applied field. The mean spectral power was computed by spatially averaging spectral power values over all pixels.

Intact rabbit heart preparation

Male New Zealand white rabbits ($n = 3, 4$ to 5 kg) were premedicated intramuscularly with ketamine (22 mg/kg), Sedazine (0.9 mg/kg), and Telazol (1.1 mg/kg). The animals were then intubated and anesthetized with isoflurane [0.5 to 2.0% (v/v) in propylene glycol] and ventilated mechanically with 100% oxygen. Blood pressure and electrocardiogram (ECG) were monitored continuously throughout the procedure.

Rabbits were prepped in a sterile fashion for a catheterization procedure. A single-coil defibrillating lead (Boston Scientific) was advanced through the oropharynx under fluoroscopic guidance into the esophagus and positioned directly posterior to the heart. A dummy titanium can was subcutaneously placed over the heart and served as the second electrode. The titanium can and the esophageal defibrillating electrode were connected to the high-frequency field generator. HFAC field strength was expressed as the amplitude of sinusoidal voltage applied between the electrodes. VF was induced either by rapid pacing or by 50-Hz AC (7.5 V, 2 s) delivered through the same circuit. Surface ECG was continuously recorded by electrodes placed on the forelimb and hindlimb of the animal.

Statistical analysis

Cycle length, defibrillation field amplitude threshold, DF, and spectral power percentage values were expressed as mean $\pm$ SEM, and compared with a paired Student's t test. Statistical significance for instances of conduction block, spiral wave termination, and

fibrillation termination was determined with Fisher's exact test. For all tests, $P < 0.05$ was considered to be significant.

Computational rabbit heart ventricular model

Simulations were performed with a previously validated anatomical model of rabbit ventricles incorporating realistic geometry and fiber orientation (13). Electrical properties of the tissue were modeled with the bidomain representation (36). Membrane kinetics were represented by a modified Luo-Rudy model (37), similar to those used in previous defibrillation studies (38). Additional currents—an electroporation current and a hypothesized outward current that are activated outside the physiological range—were also added for modeling responses to external electric field stimulation (39). Bidomain conductivities and Luo-Rudy model modifications, chosen to produce physiological values for conduction velocity and promote wave breakup and fibrillation, are given in table S1. Numerical techniques for solving the bidomain and ionic equations were described previously (40). To minimize the computational cost associated with the long duration of these simulations, we used a time step of 20 μ s.

VF was induced with a cross-stimulation protocol described previously (13). Briefly, the ventricles were paced from the apex to steady state at a cycle length of 300 ms, and a 10-ms monophasic shock (9.3 V/cm, tilt = 62%) was delivered 200 ms after the last paced beat across left and right plate electrodes. HFAC field pulses of varying field strength were then delivered across the same plate electrodes at nine different timings after the S2 shock (500, 600, 750, 900, 1000, 1100, 1250, 1400, and 1500 ms). Simulations were run for 500 ms after the end of the HFAC pulse.

Field response classification and data analysis

Simulations that had sustained post-pulse activity (≥ 500 ms) were categorized as termination failures. Simulations in which any activating wavefronts terminated within 500 ms were categorized as successes. Defibrillation success rate was then computed as the percentage of successes out of the nine simulations. The percentage of inexcitable tissue was determined by calculating the percentage of nodes in the tissue mesh with V_m above -58.8 mV, the threshold for I_{Na} activation (40).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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REFERENCES AND NOTES

1. Tung L, Tovar O, Neunlist M, Jain SK, O'Neill RJ. Effects of strong electrical shock on cardiac muscle tissue. *Ann N Y Acad Sci.* 1994; 720:160–175. [PubMed: 8010635]
2. Al-Khadra A, Nikolski V, Efimov IR. The role of electroporation in defibrillation. *Circ Res.* 2000; 87:797–804. [PubMed: 11055984]
3. Eysmann SB, Marchlinski FE, Buxton AE, Josephson ME. Electrocardiographic changes after cardioversion of ventricular arrhythmias. *Circulation.* 1986; 73:73–81. [PubMed: 3940671]

4. Tokano T, Bach D, Chang J, Davis J, Souza JJ, Zivin A, Knight BP, Goyal R, Man KC, Morady F, Strickberger SA. Effect of ventricular shock strength on cardiac hemodynamics. *J Cardiovasc Electrophysiol.* 1998; 9:791–797. [PubMed: 9727657]
5. Mollerus M, Naslund L. Myocardial stunning following defibrillation threshold testing. *J Interv Card Electrophysiol.* 2007; 19:213–216. [PubMed: 17846876]
6. Moss AJ, Zareba W, Hall WJ, Klein H, Wilber DJ, Cannom DS, Daubert JP, Higgins SL, Brown MW, Andrews ML. Multicenter Automatic Defibrillator Implantation Trial II Investigators. Prophylactic implantation of a defibrillator in patients with myocardial infarction and reduced ejection fraction. *N Engl J Med.* 2002; 346:877–883. [PubMed: 11907286]
7. Tanner JA. Reversible blocking of nerve conduction by alternating-current excitation. *Nature.* 1962; 195:712–713. [PubMed: 13919574]
8. Ackermann DM Jr, Foldes EL, Bhadra N, Kilgore KL. Conduction block of peripheral nerve using high-frequency alternating currents delivered through an intrafascicular electrode. *Muscle Nerve.* 2010; 41:117–119. [PubMed: 19813186]
9. Bhadra N, Lahowetz EA, Foldes ST, Kilgore KL. Simulation of high-frequency sinusoidal electrical block of mammalian myelinated axons. *J Comput Neurosci.* 2007; 22:313–326. [PubMed: 17200886]
10. Zhang X, Roppolo JR, de Groat WC, Tai C. Mechanism of nerve conduction block induced by high-frequency biphasic electrical currents. *IEEE Trans Biomed Eng.* 2006; 53:2445–2454. [PubMed: 17153201]
11. Boger A, Bhadra N, Gustafson KJ. Bladder voiding by combined high frequency electrical pudendal nerve block and sacral root stimulation. *Neurourol Urodyn.* 2008; 27:435–439. [PubMed: 18041769]
12. Shaw RM, Rudy Y. Ionic mechanisms of propagation in cardiac tissue. Roles of the sodium and L-type calcium currents during reduced excitability and decreased gap junction coupling. *Circ Res.* 1997; 81:727–741. [PubMed: 9351447]
13. Ashihara T, Constantino J, Trayanova NA. Tunnel propagation of postshock activations as a hypothesis for fibrillation induction and isoelectric window. *Circ Res.* 2008; 102:737–745. [PubMed: 18218982]
14. Rodríguez B, Li L, Eason JC, Efimov IR, Trayanova NA. Differences between left and right ventricular chamber geometry affect cardiac vulnerability to electric shocks. *Circ Res.* 2005; 97:168–175. [PubMed: 15976315]
15. Efimov IR, Cheng Y, Yamanouchi Y, Tchou PJ. Direct evidence of the role of virtual electrode-induced phase singularity in success and failure of defibrillation. *J Cardiovasc Electrophysiol.* 2000; 11:861–868. [PubMed: 10969748]
16. Kroll MW, Swardlow CD. Optimizing defibrillation waveforms for ICDs. *J Interv Card Electrophysiol.* 2007; 18:247–263. [PubMed: 17541815]
17. Smith GT, Beeuwkes R, Tomkiewicz M, Abe T, Lown B. Pathological changes in skin and skeletal muscle following alternating current and capacitor discharge. *Am J Pathol.* 1965; 47:1–17. [PubMed: 14308137]
18. Lown B, Neuman J, Amarasingham R, Berkovits BV. Comparison of alternating current with direct electroshock across the closed chest. *Am J Cardiol.* 1962; 10:223–233. [PubMed: 14466975]
19. Weirich J, Hohnloser S, Antoni H. Factors determining the susceptibility of the isolated guinea pig heart to ventricular fibrillation induced by sinusoidal alternating current at frequencies from 1 to 1000 Hz. *Basic Res Cardiol.* 1983; 78:604–616. [PubMed: 6197966]
20. Roberts SJ, Guan D, Malkin R. The defibrillation efficacy of high frequency alternating current sinusoidal waveforms in guinea pigs. *Pacing Clin Electrophysiol.* 2003; 26:599–604. [PubMed: 12710320]
21. Li W, Ripplinger CM, Lou Q, Efimov IR. Multiple monophasic shocks improve electrotherapy of ventricular tachycardia in a rabbit model of chronic infarction. *Heart Rhythm.* 2009; 6:1020–1027. [PubMed: 19560090]

22. Ambrosi CM, Ripplinger CM, Efimov IR, Fedorov VV. Termination of sustained atrial flutter and fibrillation using low-voltage multiple-shock therapy. *Heart Rhythm*. 2011; 8:101–108. [PubMed: 20969974]
23. Sweeney RJ, Gill RM, Jones JL, Reid PR. Defibrillation using a high-frequency series of monophasic rectangular pulses: Observations and model predictions. *J Cardiovasc Electrophysiol*. 1996; 7:134–143. [PubMed: 8853023]
24. Meunier JM, Trayanova NA, Gray RA. Sinusoidal stimulation of myocardial tissue: Effects on single cells. *J Cardiovasc Electrophysiol*. 1999; 10:1619–1630. [PubMed: 10636192]
25. Meunier JM, Trayanova NA, Gray RA. Entrainment by an extracellular AC stimulus in a computational model of cardiac tissue. *J Cardiovasc Electrophysiol*. 2001; 12:1176–1184. [PubMed: 11699528]
26. Aslanidi OV, Benson AP, Boyett MR, Zhang H. Mechanisms of defibrillation by standing waves in the bidomain ventricular tissue with voltage applied in an external bath. *Phys D Nonlinear Phenom*. 2009; 238:984–991.
27. Meunier JM, Eason JC, Trayanova NA. Termination of reentry by a long-lasting AC shock in a slice of canine heart: A computational study. *J Cardiovasc Electrophysiol*. 2002; 13:1253–1261. [PubMed: 12521342]
28. Gray RA, Mornev OA, Jalife J, Aslanidi OV, Pertsov AM. Standing excitation waves in the heart induced by strong alternating electric fields. *Phys Rev Lett*. 2001; 87:168104. [PubMed: 11690250]
29. Davidenko JM, Delmar M, Beaumont J, Michaels DC, Lorente P, Jalife J. Electrotonic inhibition and active facilitation of excitability in ventricular muscle. *J Cardiovasc Electrophysiol*. 1994; 5:945–960. [PubMed: 7889234]
30. Loew LM, Cohen LB, Dix J, Fluhler EN, Montana V, Salama G, Wu JY. A naphthyl analog of the aminostyryl pyridinium class of potentiometric membrane dyes shows consistent sensitivity in a variety of tissue, cell, and model membrane preparations. *J Membr Biol*. 1992; 130:1–10. [PubMed: 1469705]
31. Watanabe T, Rautaharju PM, McDonald TF. Ventricular action potentials, ventricular extracellular potentials, and the ECG of guinea pig. *Circ Res*. 1985; 57:362–373. [PubMed: 4028342]
32. Irvanian S, Nabutovsky Y, Kong CR, Saha S, Bursac N, Tung L. Functional reentry in cultured monolayers of neonatal rat cardiac cells. *Am J Physiol Heart Circ Physiol*. 2003; 285:H449–H456. [PubMed: 12623789]
33. Lim ZY, Maskara B, Aguel F, Emokpae R Jr, Tung L. Spiral wave attachment to millimeter-sized obstacles. *Circulation*. 2006; 114:2113–2121. [PubMed: 17088465]
34. Weinberg S, Lipke EA, Tung L. In vitro electrophysiological mapping of stem cells. *Methods Mol Biol*. 2010; 660:215–237. [PubMed: 20680822]
35. Entcheva E, Lu SN, Troppman RH, Sharma V, Tung L. Contact fluorescence imaging of reentry in monolayers of cultured neonatal rat ventricular myocytes. *J Cardiovasc Electrophysiol*. 2000; 11:665–676. [PubMed: 10868740]
36. Plonsey R. Bioelectric sources arising in excitable fibers (ALZA lecture). *Ann Biomed Eng*. 1988; 16:519–546. [PubMed: 3067629]
37. Luo CH, Rudy Y. A model of the ventricular cardiac action potential. Depolarization, repolarization, and their interaction. *Circ Res*. 1991; 68:1501–1526. [PubMed: 1709839]
38. Trayanova, N.; Eason, J.; Aguel, F. *Computing and Visualization in Science*. Vol. 4. Springer; Berlin/Heidelberg: 2002. p. 259-270.
39. Ashihara T, Trayanova NA. Asymmetry in membrane responses to electric shocks: Insights from bidomain simulations. *Biophys J*. 2004; 87:2271–2282. [PubMed: 15454429]
40. Constantino J, Long Y, Ashihara T, Trayanova NA. Tunnel propagation following defibrillation with ICD shocks: Hidden postshock activations in the left ventricular wall underlie isoelectric window. *Heart Rhythm*. 2010; 7:953–961. [PubMed: 20348028]

Fig. 1.

Effects of field stimulation during point pacing in rat neonatal cardiomyocyte monolayers. (A to D) Top panels show voltage maps before, during (gray boxes), and after field application. Bottom panels show a representative voltage trace near the center of the monolayer at site x. Red regions in voltage maps indicate fully depolarized cells; deep blue indicates fully repolarized cells at rest. Red vertical lines indicate point pacing. White arrows indicate direction of propagation. Double black lines indicate local conduction block. Scale bars, 2 mm. (A) Conduction block at 1-kHz high-strength HFAC field (see movie S1). During the field, transmembrane voltage (V_f) remained at V_{elev} (~50% action potential amplitude above resting potential). (B) Application of low-strength, 100-Hz HFAC field. FEA is indicated by circles in b and c. (C) Application of a 10-kHz HFAC field. (D) Application of DC field during point pacing (see movie S2). (E) Summary of DC and AC field pulse effects on conduction ($n = 80$ monolayers, 460 trials). Response during the pulse was characterized as conduction block (red "X"), no effect (green open circle), FEA (blue triangle), or post-pulse ectopic activity (PPEA; purple diamond).

Fig. 2. HFAC fields terminate spiral wave reentry in rat cardiomyocyte monolayers. **(A)** (Top) Voltage maps before, during (gray boxes), and after a 1-kHz HFAC field lasting 1 s (see movie S3). Dashed lines shown in images between 865 and 880 ms delineate the excitable gap region that was depolarized by the HFAC pulse within the first 5 ms after field onset (at 870 to 875 ms). Red regions in voltage maps indicate fully depolarized cells; deep blue indicates fully repolarized cells at rest. Numbers above indicate time (in milliseconds). Scale bars, 2 mm. (Middle) Representative voltage trace from site a in voltage maps above. (Bottom) Voltage traces at sites a to f (top) before and after the time of field onset (870 ms). At all sites, V_f was held at V_{elev} after the initial response. **(B)** (Top) Voltage maps before, during, and after the 500-Hz HFAC field ramp from 0 to 11 V/cm that lasted 4 s (see movie S4). Scale bars, 2 mm. (Bottom) Representative voltage trace from site x in voltage maps, from 2 to 8 s. **(C)** Spiral wave cycle length plotted as a function of time.

Fig. 3. HFAC fields block propagation and terminate VF in isolated hearts and intact animals. (**A** to **G**) Data are from the same heart, representative of $n = 10$ hearts. (**A**) Voltage maps before, during (gray boxes), and after 200-Hz HFAC field in an isolated guinea pig heart (see movie S5). Regions a and c are close to the field electrodes; region b is in the center of the optical map. Red regions in voltage maps indicate fully depolarized cells; deep blue indicates fully repolarized cells at rest. Numbers above indicate time (in milliseconds). White arrows indicate direction of propagation. Scale bars, 2 mm. (**B**) Voltage traces from sites a to c in (**A**), shown as red, blue, and green lines, respectively. Red vertical lines indicate point pacing times. (**C**) VF was initiated and then terminated by a 200-Hz HFAC field lasting 1 s in isolated guinea pig hearts ($n = 10$ hearts) (see movie S6). (**D**) DF spatial maps of epicardial surface at five time windows, showing a shift during the HFAC field ramp (below) (see movie S7). (**E**) Voltage trace from site b in (**A**) before, during, and after an 8-s linear ramp from 0 to 25.5 V (200-Hz HFAC field). (**F**) Cycle length plotted as a function of time for the individual trace in (**E**). (**G**) DF of individual voltage trace shown in (**E**) (dashed trace) and DF averaged over the entire mapped area on epicardial surface (bold trace). (**H**) ECG recording in an intact anesthetized rabbit. A 50-Hz AC field was used to induce VF. HFAC fields (30 V, 200 Hz) were then applied for varying durations to terminate fibrillation and return the animal to sinus rhythm.

Fig. 4.

Computer simulation of HFAC field application in a bidomain model of a rabbit heart. (A) Simulated transmembrane potential (V_m) map during a 200-Hz HFAC field application at field offset ($t = 1000$ ms). (B) V_m traces before, during (gray bar), and after HFAC field (25 V/cm). (C) V_m traces from (B) at expanded time scale to show oscillations at 200 Hz, with regions of opposite polarization. (D) Sodium (I_{Na}), calcium (I_{Ca}), and delayed rectifier potassium (I_K) currents and intracellular calcium concentration $[Ca^{2+}]_i$ during HFAC field application. (E) Percentage of inexcitable tissue before, during (gray bar), and after HFAC field (red trace). Trace for a VF episode without HFAC field application (black) is also shown. Black and red traces overlap before HFAC field. (F) Defibrillation success rate, plotted as a function of field strength for 200-Hz HFAC fields lasting 1 s.