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Research Article

Acute effects of adrenergic agents on post-defibrillation arrest time in a cultured heart model

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Abstract. Possible drug interactions with electrical defibrillation were examined. We tested the hypothesis that adrenergic agents (epinephrine, norepinephrine, isoproterenol) and a calcium channel blocker (verapamil), when applied acutely, alter the duration of arrest following a defibrillator shock. A secondary hypothesis (based on observations) was that the drugs alter the occurrence of changes to normal rhythms following the shock. Dissociated heart cells from 10-day chicken embryos were cultured to form spherical aggregates and plated in petri dishes. In the experiments, the spheres were paced at 0.75 V/cm above contraction threshold, and a biphasic defibrillator shock was applied for 1 ms at 46 V/cm. The arrest time and occurrence of rhythm changes were recorded. The adrenergic agents shortened the duration of arrest following a defibrillator shock, while the calcium channel blocker lengthened the arrest time. Comparisons with the control proportion of double beats showed no significant change with the adrenergic agents and a decrease with verapamil.

Key words. Electroporation; asystole; bigeminy; epinephrine; norepinephrine; isoproterenol; verapamil.

Both electrical stimulation of the heart and cardiac pharmacological agents exert physiological effects by acting either directly or indirectly upon ion channels. Electrical stimulation is capable of either opening ion channels or inactivating them. Electrical stimulation can also alter intracellular calcium ion concentrations which would produce secondary effects [1]. Similarly, pharmacological agents produce direct or indirect effects upon channels. For example, the drug verapamil directly blocks conductance of the L-type calcium channel, and adrenergic agents modulate adenylate cyclase activity to indirectly alter calcium conductances in heart cells. Because both electrical stimulation and pharmacological agents use the same substrate for their physiological effect, one would expect these modalities to interact.

Drugs and electrical stimulation are known to have interactive effects on the defibrillation threshold. For example, various potassium channel blockers lower the defibrillation threshold [2]. In the clinic, a beneficial interaction between electric shock and medication is obtained when intravenous amiodarone increases the percentage of successful defibrillations compared to a lidocaine [3], which raises the defibrillation threshold [4]. Another example of pharmacological-like effects of electrical stimulation is the use of pulsed shocks to increase inotropicity during congestive heart failure. The device [5] applies an initial shock to elevate cytosolic calcium, and this increases contractile force. None of these studies of drug-defibrillator interactions examined effects related to sec-

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ondary dysfunction caused by strong electric shocks. Failure to defibrillate and defibrillation to asystole are problems that can occur when strong electric shocks are applied. Whether drugs can modify such potentially harmful effects of defibrillator shocks is the subject of this study.

Cardiac dysfunction can occur secondary to defibrillation when certain electric shocks are applied to defibrillate a heart [6, 7]. The desired outcome is resumption of a functional rhythm after the fibrillation is arrested. The arrest time between defibrillation and the resumption of a normal rhythm should be short so that circulation will resume as quickly as possible, thus a long arrest time is undesirable. Ideally, the arrest time lasts less than 1 s, and this time without activity is known as the isoelectric window [8]. Other undesirable outcomes following a defibrillation shock are asystole (sustained arrest), generation of a secondary dysrhythmia, or secondary fibrillation. In in vitro models of defibrillation, measurement of the arrest period is used as an indicator of successful defibrillation. A long arrest period is indicative of electroporation and cellular damage [9]. During the arrest period, the membrane potential is depolarized [10, 11] and cytosolic free calcium is elevated [1, 12]. In the whole heart, electroporation from internal shocks tends to affect the bundles and papillary muscles [13] and cause increased pacing thresholds [14]. In this study, we used the arrest period in cultured cell aggregates to examine the interaction between certain drugs and defibrillation shocks. The drugs chosen for this initial study are adrenergic agents with well-characterized effects and common usage in cardiology. Our results show an expected shortening of the arrest period with adrenergic agonists.

Materials and methods

Cell culture

Isolated cells cultured from chicken embryo hearts were prepared as described previously [1, 7]. Briefly, whole hearts from the embryo (CBT Farms, Chestertown, Md.) were removed at incubation day 10, and dissociated in 0.05% trypsin solution (GIBCO/BRL, Grand Island, N. Y.) in Dulbecco's phosphate-buffered saline solution without calcium or magnesium (GIBCO/BRL). After centrifuging, the cells were moved to a 50-ml conical flask, incubated in Leibovitz L-15 (GIBCO/BRL) tissue culture medium with 10 µM cytosine arabinofuranoside (Sigma, Fountain Valley, Calif.), 10% fetal calf serum, L-glutamine and penicillin-streptomycin (GIBCO). The flask was placed in a water shaker (WB-10/SC, ELMECO, Rockville, Md.) at 37 °C with a rotation speed of 68–70 rpm/min for 24 h. Shaker bath agitation forced the cells to form aggregate spheres. Each day for 1 week, the aggregate spheres were plated in the center of 35-mm polystyrene culture dishes (Corning, New York, N. Y.) and incubated at 37 °C with 100% relative humidity for 4 h to allow time for cells to adhere to the dishes. After initial incubation, 1.5 ml Leibovitz tissue culture medium was added and the cultures were used for experiments 20 h later.

Drugs and solutions

Single doses for each agent were used in these experiments. All of the agents were used at suprathreshold doses. Adrenergic agents were diluted in the L-15 tissue culture medium for acute delivery to the cell aggregates during the experiments. The epinephrine concentration of 0.9 µM was selected because it is a suprathreshold level for altering fibrillation [15]. Norepinephrine was applied at a similar concentration of 1.0 µM because of its similar dose-response characteristics for changing cardiac contraction. Isoproterenol was applied at a concentration of 1.0 µM. The dose-response characteristics of isoproterenol for cardiac contraction are an order of magnitude to the left of norepinephrine and epinephrine, with a maximal effect on cardiac contraction. Verapamil was applied at 2.0 µM because this concentration produced a maximum reduction in the calcium action potential in the heart cell cultures (data not shown). The cationic concentrations in the L-15 solution, as supplied by GIBCO/BRL, were: $[Na^+]$ = 140 mM, $[K^+]$ = 5.8 mM, $[Ca^{2+}]$ = 1.3 mM, $[Mg^{2+}]$ = 1.8 mM.

Apparatus

A Zeiss (model IM 35; Thronwood, N. Y.) phase contrast microscope with a ×25 objective was used in these experiments. Optical viewing was done through a Hamamatsu C2400 and recorded by VHS video cassette recorder. Electric shocks were applied through a parallel pair of 1-mm diameter, 4-cm-long, platinum-iridium (10:1) rods (Goodfellow, Berwyn, Pa.) separated by 1 cm and submerged in the petri dish. The waveforms were produced by using a defibrillator (Ventritex model HVC-02; St Judes Medical, St. Paul, Minn.) with a 100-Ohm resistor in parallel with the electrodes. Between the shock electrodes spaced 1 cm apart, a second pair of platinum-iridium (10:1) wires (100 µM diameter, 1.5 cm long, 1 mm apart) was submerged in the culture dish for measuring the field generated by the other electrodes. These wires measured the field in the petri dish which was amplified with a Tektronix differential amplifier. This output was displayed on a Hewlett Packard 54501A 100-MHz oscilloscope (Palo Alto, Calif.). The field strengths of shocks were measured and recorded during the experiments.

Experimental procedure

Cells plated in petri dishes were placed in a heated microscope stage to keep them at a temperature of 34 °C (Bipolar Temperature Controller Medical System Corp., Green-

vale, N. Y.). The 34 °C temperature is considered normothermic for the heart, and temperature-related functional changes to adrenergic agents are seen with temperatures at 28 °C [16]. The cells had either 2 ml L15 or L15 with drug solution added to the culture dishes. The pacing threshold for beating at 1 Hz (with a fixed 3-ms pulse duration) was found. The amplitude of the pacing pulse was then increased by 0.75 V/cm in the dish. The cells were allowed to stabilize for 20 min with pacing. The cells were then shocked with a 46 V/cm, 1.0-ms-duration, asymmetric biphasic defibrillation shock (with the phase durations divided equally, and the leading-edge voltage of the second phase being one-half of the residual value of the first phase. The arrest time was measured along with the behavior of the aggregate sphere after the return of paced mechanical activity. Whether single mechanical beats occurred or double beating (bigeminy) was recorded.

Each set of drug-treated cells was compared to a set of controls from the same culture. Thus each experimental effect was observed relative to a co-cultured control. The experiments were performed with the sequential use of different drugs over a period of months. Depending upon the individual culture over that period of time, differences in arrest time occurred (see Results).

Results

Aggregate spheres

Following 24 h of incubation in a shaker bath, aggregate spheres (30–200 μm) formed from the dissociated heart cells. These spheres contracted spontaneously. Figure 1 A is a photomicrograph of a section through the central plane of one of the spheres, 100 μm in diameter. The section contains approximately 300 cells (determined by counting nuclei). These aggregate spheres contracted spontaneously and with extracellular pacing. Action potentials were recorded from the cells. The upper trace in

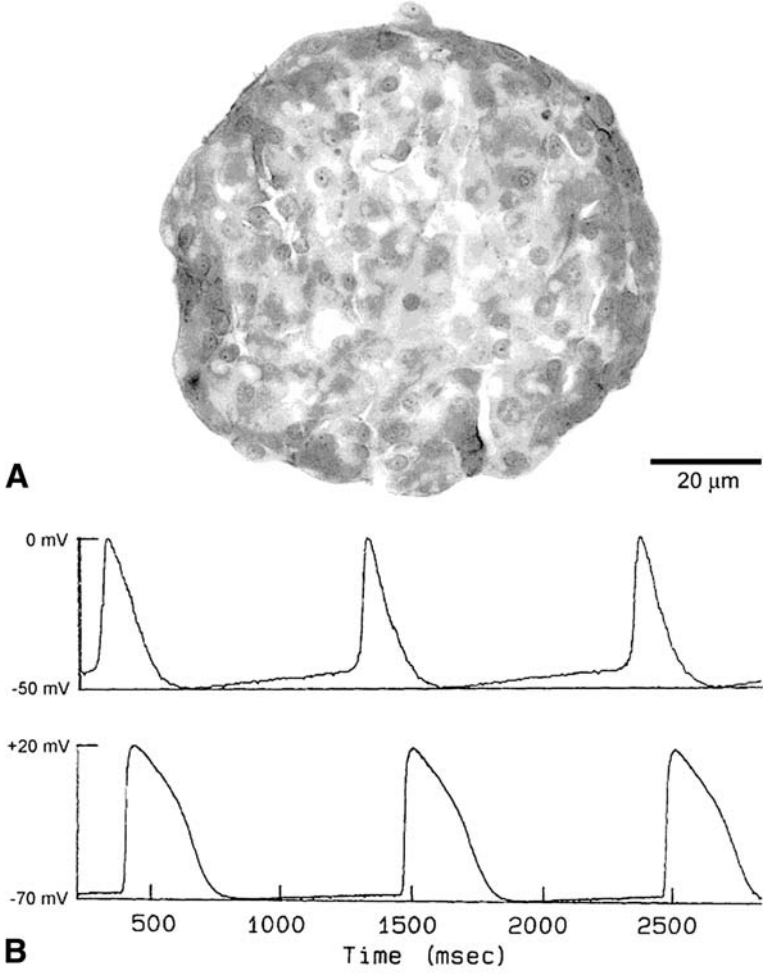

Figure 1. Characteristics of cultured heart cells. (*A*) Section through an aggregate sphere of heart cells. The sphere was cultured in a shaker bath for 24 h; formalin fixation and methyl-green pyronin-G stain. (*B*) Spontaneous action potentials, recorded intracellularly, from two different cells. Standard glass microelectrodes were used to obtain the recordings.

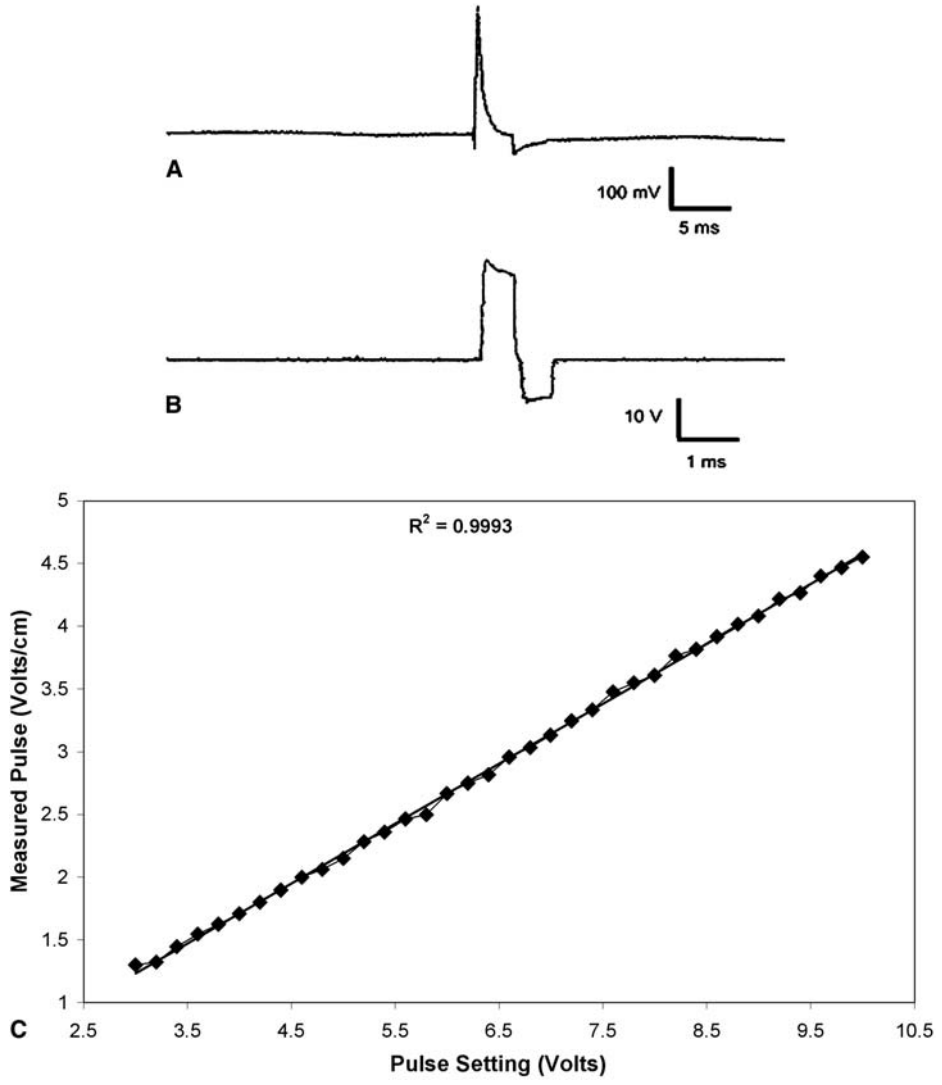

Figure 2. Electrical stimulation pulses and magnitude. Pacing pulse waveform (*A*) and defibrillation waveform (*B*); note the time scale difference. The lower graph (*C*) shows the electric field magnitude measured in the culture dish for the applied voltage from the HVS-02 device.

figure 1B is characteristic of a pacemaker cell because it has a short-duration action potential, without a plateau, and a sloping resting potential; the lower trace displays action potentials characteristic of an atrial cell [17]. Overall, the cells in the spheres produced cardiac action potentials that were 87.3 ± 13.5 mV in amplitude, 242 ± 69 ms in duration at 10% amplitude, with a 49.5 ± 11.3 mV (n = 15 cells, ± SD).

Electric field measurements

During every experiment, both the pacing and defibrillation waveforms were recorded by a pair of differential electrodes in the dish (fig. 2A, B), and calibrated according to field magnitude at the location of the aggregate spheres (fig. 2C). Figure 2A represents a pacing pulse recorded in the tissue bath, and figure 2B represents the biphasic defibrillation pulse, also recorded in proximity to the tissue. Thus for every experiment, the waveform and electric field magnitude were known. In these experiments, cells were paced at 0.75 V/cm above capture threshold, which had a mean of 3.6 ± 0.6 V/cm. When a 100-V, 1-ms duration biphasic shock was set on the defibrillator, a 46 V/cm field was delivered to the heart cell aggregates.

Mechanical responses to shocks

For each test, after pacing had been established for 20 min, the aggregates were given the defibrillation shock. Following the shock, one of three results occurred: (i) a return to regular beating with the pacing pulse after a period of time; (ii) complete arrest for at least 2 min (even with continued pacing); (iii) a return with double

Table 1. Summary of data of arrest time from drug-exposed cultures and co-cultured controls.

Agent	n	Arrest time (±SE)	Percent of control arrest	t test result	Percent with bigeminy (± 90% CI)	Bigeminy change
Control for epinephrine	55	18.2 ±2.6 s			27.3 ± 9.9	
epinephrine (0.9 μM)	54	11.8 ±1.6 s	65 ± 9	p = 0.02	29.6 ± 10.2	+2.3%
Control for norepinephrine	107	13.1 ±1.1 s			65.4 ± 6.5	
norepinephrine (1.0 μM)	133	6.2 ±0.5 s	47 ± 4	p < 0.001	71.4 ± 6.5	+6.0%
Control for isoproterenol	56	11.5 ±1.5 s			76.8 ± 9.2	
isoproterenol (1.0 μM)	90	3.0 ±0.4 s	26 ± 4	p < 0.001	75.5 ± 7.5	–1.3%
Control for verapamil	37	13.8 ±2.5 s			32.1 ±13.3	
verapamil (2.0 μM)	28	19.8 ±2.8 s	143 ± 20	p = 0.065	59.4 ±14.5	+27.5%

contractions for a short period followed by a return to regular beating with the pacing pulse. All sessions were videotaped through the microscope, and synchronous simultaneous recordings of voltage from the bath were made. A summary of the control responses and drug effects can be seen in table 1. All tests between control aggregates and the drug experimental ones were performed in paired cultures; i.e., culture dishes from the same time and prepared identically were paired into experimental and control groups. This was done to avoid the differences in shock effects seen between aggregates cultured at different times and from different embryos.

The main results of the study are illustrated in figure 3. The adrenergic agents epinephrine, norepinephrine and isoproterenol all significantly decreased the arrest time following a defibrillator shock, whereas the calcium channel blocker verapamil tended to increase the arrest time. The details of the drug effects are as follows.

Epinephrine (0.9 μM) was dissolved in the tissue culture medium and allowed to incubate with the cell aggregates for the 20-min pacing period. For epinephrine, the mean arrest period following the shock decreased from a control mean of 18.2 ± 2.6 s (n = 55) to 11.8 ± 1.6 s (n = 54) (p = 0.02, t test). The epinephrine-treated spheres therefore had a 35% reduction in arrest time (table 1, fig. 3) and resumed paced contractions sooner than the control. The return rhythm, following the defibrillation shock, was noted. With epinephrine present, the proportion with double contractions (bigeminy) was 29.6 ± 10.2% (90% confidence interval) compared to 27.3 ± 9.9% (90% confidence interval) for the control.

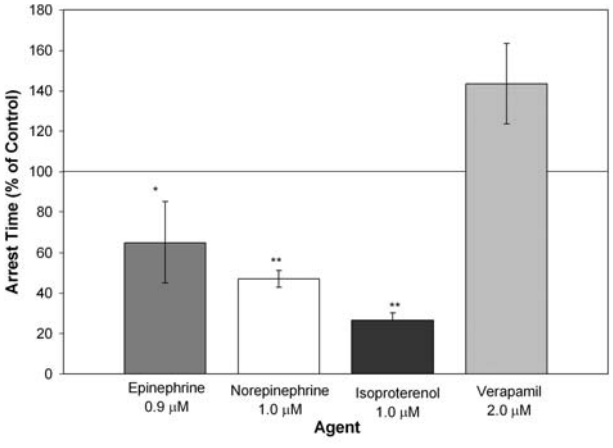

Figure 3. Graphical representation of the arrest time (relative to control) for the various drugs. * $p < 0.05$, ** $p < 0.01$. See text for details.

When the same experiment was performed on cells with a norepinephrine (1.0 μM) solution, the mean arrest time decreased from 13.1 ± 1.1 s (n = 107) to 6.2 ± 0.5 s (n = 133) ($p = 3.5 \times 10^{-8}$, t test). With norepinephrine present, the proportion of bigeminy was 71.4 ± 6.5% (90% confidence interval) compared to 65.4 ± 6.5% (90% confidence interval) for concurrent control cultures.

With 1 μM isoproterenol, the mean arrest time decreased from 11.5 ± 1.5 s (n = 56) to 3.0 ± 0.4 s (n = 90) ($p = 3.6 \times 10^{-7}$, t test). The proportion of cellular aggregates with bigeminy was 75.5 ± 7.5% (90% confidence interval) for the cells in the isoproterenol solution and 76.8 ± 9.2% (90% confidence interval) for the cells in the control solution.

Finally, the same protocol was followed for comparing arrest time of heart cell aggregates in a 2.0 μM verapamil solution to those in a control solution. With verapamil present, the mean arrest time increased from 13.8 ± 2.5 s (n = 37) to 19.8 ± 2.8 s (n = 28) (p = 0.065, t test). The proportion of cellular aggregates with bigeminy was 32.1 ± 13.3% (90% confidence interval) for the cells in the verapamil solution and 59.4 ± 14.5% (90% confidence interval) for the cells in the control solution. The decrease in the proportion of spheres exhibiting post-shock bigeminy was reduced by verapamil, but not with a 95% confidence interval.

Discussion

Heart cells cultured from chicken embryo were used as a cellular model for examining possible acute interactions

between certain drugs and electrical defibrillation. The arrest time following a defibrillator shock was employed as a biomarker for defibrillator secondary effects. In the clinic, a short arrest time following a defibrillator shock would be more desirable than a prolonged arrest period, and would be associated with a better outcome. Our overall findings are that adrenergic agonists (both alpha and beta) decrease the arrest time following a uniform defibrillation shock to cultured heart cell aggregates, and that the L-type calcium channel blocker verapamil tends to prolong the arrest time. In addition, for bigeminy (a possible biomarker for secondary arrhythmia), only verapamil showed a tendency toward a significant effect, with a 90% confidence interval.

Previous authors have hypothesized that post-shock arrest is due to electroporation [7, 18]. The findings in this work demonstrate that pharmacological agents affect post-shock arrest time. Our previous work [1] demonstrated two phases to the calcium change following the shock: a rapid calcium rise followed by a prolonged calcium plateau. Cheek and Fast [19], by blocking potassium channels in cultured myocytes after a shock applied during the action potential plateau, concluded that a rapid hyperpolarization was caused directly by electroporation. In the present study, we examined the effects of such shocks on longer-term events. The effects that Cheek and Fast [19] examined occurred within a millisecond; we examined effects that occurred over several seconds. The pharmacological effects we observed may be secondary to immediate electroporation effects. The prolonged calcium plateau we reported in a previous work [1] may also be a manifestation of secondary effects of such electroporation effects.

Our findings of pharmacological interactions with shock arrest time imply that the effect of the electric shock is not solely attributable to electroporation of the membrane even though electroporation appears to be involved in producing post-shock arrest. Pharmacological agents could modulate the electroporation effects by (i) direct alteration of the electroporation event itself, (ii) by altering the secondary effects that produce the arrest, or (iii) by direct alteration of the ionic channel. For example, drugs that alter membrane fluidity or the voltage gradient profile of the membrane could alter the size and duration of the pore. Alternatively, drugs that alter channel permeability, channel kinetics, intracellular ion dynamics or cell metabolism would secondarily affect the duration of the arrest.

The recently described phenomenon of the 'isoelectric window' may be related to the 'post-shock arrest' described in the results. The isoelectric window is the period between electric shock application and the defibrillated cardiac action potential. This period typically lasts less than a second [20, 21]. The nature of this arrest period is controversial [8, 20, 21], and whether the isoelectric window is due to cellular events or is a phenomenon related to shock interactions with networks of action potential wavefronts remains unclear. However, the isoelectric period and the post-shock arrest from stronger shocks are likely to be related. The isoelectric window may be an abbreviated post-shock arrest that occurs with lower-strength shocks.

In the present study, the pharmacological shortening of post-defibrillation arrest time was mediated by both alpha and beta adrenergic receptors because both norepinephrine (a predominant alpha agonist) and isoproterenol (a purely beta agonist) decreased the arrest time. In contrast, verapamil increased the duration of the arrest following the shock. The opposite effects of isoproterenol and verapamil correlate with the opposite effects of these two drugs on intracellular free calcium concentration. Isoproterenol, through adenylate cyclase activation, increases the release of sarcoplasmic reticulum (SR) calcium, whereas verapamil blocks calcium entry, and decreases release of SR calcium. This plausible effect of these drugs on the arrest time is in contrast to previous findings that certain defibrillation shocks cause arrest and an elevation of intracellular calcium (possibly by electroporation) [1, 12]. A possible explanation of this paradox is that the arrest period following the shock is not caused by the increased calcium, and the increase in calcium activates the cellular machinery that resolves the shock effect. An important next step in this work will be to ascertain the dose-response characteristics for the drugs. This would permit comparisons between drugs and a more detailed dissection of the mechanism by which electric shocks arrest heart cells.

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