Conditioning Prepulse of Biphasic Defibrillator Waveforms Enhances Refractoriness to Fibrillation Wavefronts

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The mechanism of biphasic waveform defibrillation threshold reduction is unknown. We tested the hypothesis that, during refractory period stimulation, sarcolemmal hyperpolarization by the first pulse of biphasic waveforms facilitates excitation channel recovery, which enhances graded responses produced by the second depolarizing pulse. This prolongs cellular refractoriness to fibrillation wavefronts when compared with a monophasic depolarizing stimulus. Monophasic (10 msec, rectangular wave) or symmetrical biphasic (10 msec, each pulse) current injection S1 stimuli at 1.5 and two times S1 threshold were used to scan the S1 action potential refractory period (S1 cycle length, 600 msec) in myocardial cell aggregates. S2 waveforms were delivered with normal and reversed polarity to test the hyperpolarizing action of biphasic waveforms. Responses to an S3 stimulus, which simulated a potential incoming fibrillation wavefront, were also determined. Results showed that biphasic S2 waveforms produced longer graded responses during and immediately after the S1 refractory period than did corresponding monophasic S2 waveforms. The maximum difference in response duration produced by the biphasic and monophasic waveforms was 58.6±10.0 msec (p<0.001). This maximum difference occurred 10 msec before the end of the S1 refractory period. The longer response durations produced by biphasic S2 also produced longer refractoriness to the S1 stimulus. The maximum difference in total refractoriness to S1 of 51.8±2.8 msec (p<0.002) occurred at the same S3 coupling interval as the maximum difference in S1 response duration. Prolonged refractoriness may protect ventricular cells from refibrillation wavefronts and act as the cellular basis for greater biphasic waveform defibrillation efficacy. (Circulation Research 1991;68:438–449)

Defibrillation threshold for biphasic waveforms is significantly lower than that for corresponding monophasic waveforms both clinically and in animal models. However, the mechanism by which biphasic waveforms reduce defibrillation threshold remains unknown. In vitro studies with the cultured cell model suggest that improved excitation of refractory cells may underlie the enhanced effectiveness of biphasic waveforms. In these studies, biphasic waveform external field stimulation lowered excitation threshold compared with monophasic waveform stimulation under conditions simulating fibrillation. Because postrepolarization refractoriness increased when potassium-depolarized cells were stimulated at short cycle length, cells were stimulated during the relative refractory period resulting in a K+-dependent and cycle length–dependent increase of monophasic waveform threshold. As monophasic threshold increased, the ratio between biphasic and monophasic waveform thresholds decreased linearly, showing that biphasic waveforms become more efficacious under conditions that increase monophasic waveform threshold. Excitation strength–duration curves also showed that relative biphasic waveform efficacy increased with increasing duration of the first pulse with a time constant suggestive of excitation channel recovery. These results suggested the hypothesis that the first pulse of the biphasic waveform acts as a “conditioning prepulse,” which allows time- and voltage-dependent recovery of excitation channels in those portions of...
the sarcolemma that are transiently hyperpolarized by the external field stimulus.

This study tests the conditioning prepulse hypothesis by using intracellular current injection, which hyperpolarizes or depolarizes all regions of the sarcolemma, to separate the hyperpolarizing and depolarizing effects of the biphasic waveform on excitation channel recovery and activation. The results show that the hyperpolarizing prepulse lengthens the total duration of the shock-induced response and prolongs refractoriness to a premature stimulus that simulates an incoming fibrillatory wavefront. The prolonged refractoriness to potential fibrillating wavefronts may explain the enhanced effectiveness of biphasic waveforms at low shock intensities.

Materials and Methods

Techniques for culturing myocardial cell aggregates have been previously described. In brief, hearts were aseptically harvested from 9–11-day-old chicken embryos. Atria and great vessels were removed, and ventricular cells were dispersed with 0.05% trypsin in Ca²⁺- and Mg²⁺-free phosphate buffered saline. After enzymatic dispersion, cells were resuspended and placed in L-15 medium (GIBCO, Grand Island, N.Y.) with 10% fetal calf serum at a concentration of 10⁶/ml. Cell aggregates 100–185 μm in diameter were formed by incubating cells for 24–48 hours in a gyromatic water bath at 37°C. Thirty minutes before experimentation, aggregates were plated onto 60-mm tissue culture dishes.

In vitro electrophysiological studies were performed at 37°C. Cells were superfused at 10 ml/min with modified Krebs’ solution (110 mM NaCl, 32 mM NaHCO₃, 4 mM KCl, 5.5 mM dextrose, 1.2 mM MgSO₄, 1.2 mM Na₂HPO₄, 2.5 mM CaCl₂, and 10 IU/l regular insulin). Recording electrodes were made from 1.0-mm o.d. capillary tubing (FHC, Inc., Brunswick, Me.) by using a two-stage gravity pull and filled with solution consisting of 140 mM KCl, 1.0 mM CaCl₂, 11 mM EGTA, 2 mM MgCl₂, and 10 mM HEPES adjusted to pH 7.2 with KOH. Electrodes had tip resistances of 8–12 MΩ and were coupled to a current-passing bridge electrometer (model 9200, Dagan Corp., Minneapolis, Minn.) by using a sintered Ag-AgCl half cell (WPI, New Haven, Conn.). Transmembrane potentials were recorded by placing the electrode tip onto a cell surface and gradually applying low-level suction (–2 cm H₂O) to the pipette interior to form a gigohm seal. The cell interior was accessed with a brief application of higher-level suction (up to –8 cm H₂O).

The experimental protocol is shown in Figure 1. Intracellular current injection was used to deliver basic trains of eight monophasic, rectangular waveform S₁ stimuli of 10-msec duration with a basic cycle length of 600 msec. Excitation threshold ranged from 5 to 20 nA depending on aggregate size (11.0±1.9 nA). The constant current source was adjusted to 1.5 times S₁ diastolic threshold. S₂ stimuli waveforms were generated with a random function generator (Wavetek, San Diego). The monophasic S₁ waveform was a 10-msec rectangular wave. The second phase of the biphasic waveform was identical to the monophasic waveform; the first phase was a conditioning prepulse equal in amplitude and opposite in polarity to the second phase. Monophasic and biphasic S₂ stimuli were delivered with intracellular current injection at 1.5 and two times S₁ diastolic threshold. Biphasic S₂ stimulus coupling intervals were adjusted so that the second depolarizing phase of the biphasic stimulus occurred at the same coupling interval as the monophasic S₂ waveform to which it was compared, as shown in Figure 1. S₁ refractory period and

Figure 1. Diagram illustrating experimental protocol. See text for detailed description.
duration of the $S_2$ response for each aggregate were determined by scanning the eighth $S_1$ action potential of the basic train with an $S_2$ that decreased by 10 msec with each basic train.

To confirm that increased efficacy of the biphasic waveform was due to membrane hyperpolarization, the effect of waveform polarity on the $S_2$ graded response was determined by delivering the $S_2$ monophasic waveform in both the normal depolarizing and the reverse hyperpolarizing morphology in five experiments. The biphasic waveform was also delivered in both the normal hyperpolarizing/depolarizing configuration and the reverse depolarizing/hyperpolarizing configuration.

To confirm that longer $S_2$ response duration increased refractoriness to stimulation by a potential incoming fibrillatory wavefront, correlation between $S_2$ response repolarization and cellular refractoriness was tested in five aggregates. $S_2$ was fixed at four different coupling intervals that spanned $S_1$ action potential repolarization, and a 10-msec $S_2$ was used to define the duration of refractoriness established by each $S_2$ response. $S_1$ was identical in morphology and amplitude to $S_1$. At the end of each experiment, which included the $S_1$ protocol, the aggregate’s stability was confirmed by repetition of the original $S_1S_2$ protocol.

All recordings were displayed on an oscilloscope (Tektronix, Beaverton, Ore.) and photographed on Polaroid film. Data were extracted from the photographic records. The end of the $S_2$ response at 100% repolarization was defined as the intersection between a line drawn from the linearized phase 3 of that response to a line in the midpoint of the white band representing a linearized phase 4 resting potential. This method allowed graphic, rather than mathematical, analysis of each photograph. Total response duration was measured from the beginning of the $S_1$ stimulus response to 100% $S_2$ repolarization as defined using this linearized phase 3, phase 4 technique. Results were reported as mean±SEM. Differences were determined with Student’s paired $t$ test and were considered significant at $p<0.05$. Regression analysis was performed using SIGMAPLOT (Jandel Scientific, Corte Madera, Calif.).

**Results**

**Monophasic and Biphasic $S_2$ Response Durations**

The duration of responses produced by depolarizing monophasic and hyperpolarizing/depolarizing symmetrical biphasic $S_2$ were compared in a paired study of 484 $S_2S_2$ events in 11 aggregates. Action potential recordings from a single experiment are shown in Figure 2. Each oscillograph shows 11 superimposed tracings illustrating the response to different $S_1S_2$ coupling intervals, each separated by 10 msec. The reported $S_2S_2$ cycle length was adjusted so that the depolarizing portion of the biphasic waveform coincided with that of the monophasic waveform as described in “Materials and Methods.” The upper tracing shows intracellular current injection waveform morphology and amplitude as well as the 0-mV reference. The lower tracing shows membrane potential responses to the eighth $S_1$ and each $S_2$ stimulus. Because of the large transmembrane voltage artifact associated with current injection, the action potential recordings are visible only after the current injection pulse. This is most easily observed in the relation between the $S_1$ current injection tracing and the $S_1$ action potential tracing. A vertical line showing the 190-msec $S_2S_2$ coupling interval and the resulting total $S_1+S_2$ response duration are marked on each panel as reference points. Figure 2A shows a monophasic $S_2$ delivered at 1.5 times the $S_1$ threshold with coupling intervals of 120–220 msec. The first response that showed a plateau phase was produced at an $S_1S_2$ coupling interval of 200 msec, which was 20 msec before 100% $S_1$ repolarization. (Because of the 10-msec current injection artifact, this response appears to begin at 210 msec.) At an $S_1S_2$ coupling interval of 190 msec, just before the first monophasic response, the $S_2$ stimulus prolonged $S_1$ action potential duration but did not produce a response with a plateau. Total $S_1S_2$ response duration was 240 msec. Figure 2B shows responses produced by a biphasic $S_2$ delivered at the same amplitude with coupling intervals of 130–230 msec (as measured to the beginning of the depolarizing portion of the waveform). The first response with a plateau phase was produced 40 msec before 100% $S_1$ repolarization. This was 20 msec earlier than the first response produced by the 1.5 times threshold monophasic $S_2$. Because the biphasic waveform produced a response with a plateau at a coupling interval of 190 msec, the total response duration was increased to 320 msec. This was 80 msec longer than the response produced by the corresponding monophasic $S_2$.

Figures 2C and 2D show the responses of the same cell to an $S_2$ stimulus that was twice the $S_1$ threshold. For monophasic $S_2$, the first response with a plateau was produced 20 msec before $S_1$ repolarization. Total $S_1S_2$ response duration at the reference coupling interval of 190 msec was 260 msec (Figure 2C), 20 msec longer than the response obtained at 1.5 times threshold. In contrast, Figure 2D shows that biphasic $S_2$ at twice threshold produced the first response with a plateau 50 msec before $S_1$ 100% repolarization, 30 msec before that produced by the corresponding monophasic waveform. At an $S_1S_2$ of 190 msec, total $S_1S_2$ response duration was 320 msec. This was 60 msec longer than the corresponding monophasic $S_2$ stimulus response. These results show that, in this aggregate, biphasic waveforms of 1.5 to two times $S_1$ threshold produce a response with a plateau up to 30 msec earlier than the corresponding monophasic waveform and that the response is up to 80 msec longer.

Results from another experiment with monophasic and biphasic $S_2$ stimuli with amplitudes of 1.5 and two times $S_1$ threshold are summarized in Figure 3 (left panel). Total duration of the $S_1S_2$ response at
100% S₂ repolarization is shown as a function of the S₁S₂ coupling interval normalized to 100% S₁ repolarization. This morphologically normalized coupling interval (MNICI) is equal to the difference between time of S₁ delivery and S₁ action potential duration. Therefore, MNICI of 0 msec defines the end of the S₁ action potential. At MNICI shorter than -50 msec, no response with a plateau occurs for either waveform. At both S₂ stimulus intensities, the first biphasic S₂ graded response having a plateau is produced at -30 msec, which is 20 msec before the first monophasic response. Total response duration for biphasic S₁ is longer than that for the monophasic S₁ at both stimulus intensities for all MNICI between -45 and +20 msec. The maximum difference in total S₁S₂ response duration of 70 msec occurs 20 msec before 100% S₁ repolarization. In Figure 3 (right panel), the difference between total S₁S₂ response duration produced by monophasic and biphasic S₂ stimuli at the same coupling interval is illustrated for the data shown in the left panel. A difference greater than 0 msec indicates a longer response to biphasic than to monophasic S₂. This format was used to statistically identify differences in biphasic and monophasic total response durations from different cells as shown in Figure 4.

Figure 4 shows the mean difference in total response duration produced by biphasic and monophasic waveforms (B-M) as a function of the S₁S₂ coupling interval in 10 aggregates. In the left panel, the mean response duration difference is normalized to S₁ action potential duration as described in Figure 3.
Figure 3. Results from a single experiment using monophasic (M) and biphasic (B) S2 stimuli with amplitudes of 1.5 (open symbols) and two (closed symbols) times S1 threshold. Left panel: Total S1+S2 response duration at 100% S1 repolarization is shown as a function of the morphologically normalized S1S2 coupling interval referenced to S1 action potential at 100% repolarization. Right panel: The difference in total response duration produced by monophasic and biphasic S2 stimuli for the data in the left panel is shown as a function of the morphologically normalized S1S2 coupling interval. A difference greater than zero indicates that the biphasic S2 stimulus produces a longer response than the monophasic S2.

(right panel). Although responses similar to those shown in Figure 3 were obtained from each aggregate, the relation between maximal B-M to morphologically normalized coupling interval varied between experiments. Variable timing of maximum B-M is reflected as multiple peaks in both curves and an overall reduction in mean peak B-M. In spite of these variations, biphasic S2 stimulation produced a longer mean response duration throughout phase 3 and early phase 4 of the S1 action potential.

Because the traditional morphometric approach to action potential analysis resulted in multiple peaks, data were also analyzed by referencing S1S2 coupling intervals to the monophasic waveform absolute refractory period. These functionally normalized coupling intervals (FNCI) were obtained by subtracting the monophasic waveform absolute refractory period from the S1S2 interval. A functionally normalized coupling interval of 0 msec defines the end of S1 action potential absolute refractory period to monophasic S2 of a given stimulus intensity (1.5 or two times S1 threshold). Figure 4 (right panel) shows that, regardless of S2 intensity, maximum B-M occurred at FNCI=0 msec. At 1.5 times threshold, B-M was positive for functionally normalized coupling intervals between −20 and 10 msec. At two times threshold, the curve was broader so that the range of positive B-M began earlier and persisted longer (FNCI=−40 to +30 msec). At very short coupling intervals, including phase 2 and very early phase 3 of

Figure 4. Combined results from 10 aggregates showing the mean difference in total response duration produced by the monophasic and biphasic S2 stimuli. Left panel: The difference in total response duration is shown as a function of morphologically normalized S1S2 coupling interval referenced to the end of S1 repolarization. Right panel: The difference in total response duration is shown as a function of functionally normalized S1S2 coupling interval referenced to the end of the S1 refractory period.
the $S_1$ action potential, depolarizing monophasic $S_2$ current injection caused a slight delay in repolarization. This was reflected as a small negative B-M between $-60$ and $-50$ msec. As normal action potentials developed at $S_1S_2$ coupling intervals longer than 20 msec past the end of the $S_1$ refractory period, monophasic and biphasic $S_2$ waveforms produced responses of equal duration.

**Effect of Waveform Polarity Reversal**

To determine that improved responses produced by biphasic $S_2$ were due to a specific action of the hyperpolarizing/depolarizing configuration on membrane excitability characteristics, we carried out experiments with waveforms of opposite polarity. In five aggregates, we evaluated responses to hyperpolarizing monophasic and depolarizing/hyperpolarizing biphasic stimuli. As a control, we also reevaluated responses to depolarizing monophasic and hyperpolarizing/depolarizing biphasic waveforms in the same aggregate. Results from one aggregate with $S_2$ stimuli of 1.5 times threshold are shown in Figure 5. Panels A (depolarizing monophasic $S_2$) and B (hyperpolarizing/depolarizing biphasic $S_2$) show typical responses similar to those shown in Figure 2. With the hyperpolarizing/depolarizing biphasic $S_2$, the first graded response with a plateau phase occurred 30 msec earlier than with the corresponding depolarizing monophasic stimulus. The maximum difference in response duration of 60 msec occurred at $S_1S_2=190$ msec.

In contrast to the depolarizing monophasic $S_2$, the hyperpolarizing monophasic $S_2$ (Figure 5C) caused early repolarization, bringing the membrane potential to near resting value 30 msec earlier than normal. However, no anodal break excitation was observed. Because of this early repolarization, the membrane...
potential encountered by the depolarizing portion of a biphasic waveform is more negative than that encountered by a corresponding depolarizing monophasic stimulus. This more negative potential may increase the number of excitation channels available for the second depolarizing portion of biphasic S2 stimulation.

With a reversed (depolarizing/hyperpolarizing) biphasic waveform (Figure 5D), the shortest coupling interval that produced a response with a plateau was 10 msec longer than that seen in panel A for a simple depolarizing monophasic waveform. Furthermore, the response for any given S1S2 interval was shorter and of lower amplitude than that produced by monophasic S2. The aborted graded responses are attributed to the second hyperpolarizing portion of the reversed biphasic S2.

**S3 Stimulation**

The results described above show that hyperpolarizing/depolarizing biphasic stimuli delivered during the S1 relative refractory period produce graded responses earlier than comparable depolarizing monophasic stimuli and that, for a given coupling interval, the response is of longer duration. Because S2 simulates a defibrillating pulse, we determined whether these prolonged responses enhanced refractoriness to a potential refribrillating wavefront. The re-fibrillating wavefront was simulated with a monophasic S1 that was identical to S1. If S3 and S1 are considered as fibrillation action potentials, then our hypothesis suggested that a biphasic “defibrillating” S2 would be more effective in extending S1 refractory period and preventing formation of an S3 action potential capable of propagation.

To determine the effect of monophasic and biphasic S2 on refractoriness to S3 stimulation, curves similar to those shown in Figure 3B were obtained for each aggregate. Then four S1S2 coupling intervals from different portions of the curve were selected, and an S1 stimulus was used to scan each S2 response.

Figure 6A shows results from a single experiment with a monophasic S2 at an S1S2 coupling interval of 180 msec. The figure shows the end of the last S1 stimulus, followed by a monophasic S2, and 11 superimposed S3 tracings representing 10 different S1S3 coupling intervals. The figure shows that, in this aggregate, an S3 response having a plateau occurred for all but the shortest S2S3 coupling interval. The first S3 response was observed at an S1S2 coupling interval of 50 msec.

Figure 6B shows the same aggregate with the same S2S3 coupling interval range after a biphasic S2. The biphasic S2 produced a graded response that was 60 msec longer than the monophasic S2 response at the same S1S2 coupling interval. The longer biphasic S2 response prevented an S3 response having a plateau for the first six coupling intervals. The first graded S3 response occurred at an S1S2 coupling interval of 100 msec, 50 msec later than the first S3 response after the monophasic S2.

Figure 7 shows S3 refractory periods after biphasic and monophasic S2 stimulation, as indicated by duration of S3 responses. The S3 response duration is shown as a function of S1S2 coupling interval. Initial S3 action potentials after the S2 refractory period were identified by a sharp transition in the slope of each curve. In each case, the first action potential had a duration equal to or greater than 100 msec. The S2 refractory period was defined as the longest S1S3 coupling interval that did not produce an S3 action potential having a duration equal to or greater than 100 msec. This figure shows that, for an S1S2 coupling

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**Figure 6.** Action potential recordings from a single experiment showing superimposed membrane responses to S1 stimuli delivered at 10 different S1S2 coupling intervals when the 1.5 times threshold S2 was delivered at an S1S2 coupling interval of 180 msec. Each panel shows the terminal portion of the S1 response, the monophasic or biphasic S2 response, and 11 superimposed S3 responses. The vertical voltage scale, as well as the horizontal time scale, is shown by the length of the calibration lines between the panels. Panel A: Monophasic S2 with S1S2 coupling intervals of 40–140 msec. Panel B: Biphasic S2 with S1S2 coupling intervals of 40–140 msec. TRD, total response duration.
interval of 180 msec, the refractory period after the two times biphasic S$_2$ persisted to S$_2$S$_3$ = 100 msec, while the refractory period after two times monophasic S$_2$ ended 50 msec earlier.

Results from 880 observations in five aggregates, shown in Figure 8, demonstrate that the difference in total refractoriness to S$_2$ produced by monophasic versus biphasic S$_2$ stimuli was a function of the functionally normalized S$_2$S$_3$ coupling interval. Biphasic S$_2$ extended refractoriness to S$_1$ when delivered with a functionally normalized S$_2$S$_3$ coupling interval ranging from −40 to +40 msec. The mean maximum difference of 51 ± 2.8 msec (p < 0.002) occurred at the end of the S$_1$ refractory period (FNCI = 0). This relation, which is similar to that observed between the S$_2$ response duration and functionally normalized coupling interval in Figure 4 (right panel), suggests that prolonging S$_2$ response duration extends refractoriness to an S$_3$ stimulus.

**Discussion**

**Improved Defibrillation Efficacy With Biphasic Waveforms**

Clinical and animal studies have consistently demonstrated that specifically shaped symmetrical and asymmetrical biphasic waveforms decrease defibrillation threshold compared with corresponding monophasic waveforms. For example, with nonthoracotomy internal defibrillation in the canine model, threshold for a specifically shaped biphasic waveform with total duration of 10 msec was 361 V (9.7 J), while that for the corresponding 10-msec monophasic waveform was 584 V (19.5 J). Similar results were obtained in another canine study with large contoured epicardial patches. Threshold for a 5-msec monophasic waveform was 191 V (3.0 J) and that for a 10-msec monophasic waveform was 159 V (3.2 J), while that for an asymmetrical biphasic waveform with the first pulse larger than the second and total duration of 10 msec was only 106 V (1.3 J).

Because these in vivo studies used biphasic truncated exponential waveforms having polarity reversed at various fractions of total duration, the percent tilt or droop of the first and second pulses, as well as the transition voltage between positive and negative polarities, varied with resistance and capacitance. Therefore, determination of underlying mechanisms from these studies is difficult.

Results from two studies in the transthoracic calf model are of special interest because they use rectangular monophasic and symmetrical biphasic waveforms to create actual strength–duration curves. In these studies, threshold can be compared for biphasic waveforms having individual pulse durations equal to those of the comparable monophasic waveforms as well as biphasic waveforms having a total duration equal to that of the monophasic waveform. Therefore, these studies provide a link between clinical studies for which biphasic waveform duration was defined as total duration for both pulses and cellular studies for which biphasic waveform duration was defined as that of the second defibrillating pulse. This definition allowed the effect of the first conditioning prepulse on the efficacy of the defibrillating pulse to be examined. Results from the calf studies show that, at low shock intensities (35 A), defibrillation success for the monophasic rectangular waveform increases with duration as predicted by traditional strength–duration relations. Probability of success for monophasic waveforms was 11% at 4 msec, 28% at 8 msec, and 41% at 16 msec. With
symmetrical biphasic waveforms, probability of success was 82% for the 4+4 waveform (4 msec each pulse or 8 msec total) and 95% for the 8+8 waveform. At the same shock intensity, the symmetrical biphasic waveform efficacy was superior to that of monophasic waveforms having durations identical to either total biphasic waveform duration or individual pulse duration. These results confirm that biphasic waveform efficacy is not due to increased total pulse duration or to total pulse energy, but to a specific action of the biphasic polarity.

With increasing fibrillation duration, defibrillation threshold for a monophasic waveform increases in the canine model. This increase in monophasic threshold parallels the increase for monophasic waveform excitation threshold that occurs in the cultured cell model under simulated fibrillation conditions. In both cases, high thresholds may be due to increasing postrepolarization refractoriness that results from developing ischemia, short cycle length stimulation, and increased K. Under these conditions, biphasic waveforms lower excitation threshold so that the ratio of biphasic waveform to monophasic waveform thresholds (B/M) is inversely proportional to monophasic threshold, resulting in stabilization of biphasic waveform threshold. If the defibrillating effectiveness of biphasic waveforms increases as monophasic waveform threshold increases in the intact heart as it does in the cultured cell model, then biphasic waveforms should stabilize threshold with increasing fibrillation duration. In our laboratory, studies with an isolated right- and left-sided working rabbit heart model confirmed the stability of biphasic waveform threshold as monophasic threshold increased with fibrillation duration. The defibrillation threshold for a 5-msec monophasic waveform increased from 51.7±4.4 V (0.18 J) after 5 seconds of fibrillation to 72.1±3.9 V (0.33 J) after 30 seconds of fibrillation. In contrast, threshold for a 5-msec biphasic waveform having 50% undershoot was 38.2±2.2 V (0.12 J) at 5 seconds and increased to only 46.6±3.2 V (0.17 J) after 30 seconds of fibrillation. Again, these results suggest a specific action of polarity reversal to lower threshold that becomes more effective as monophasic waveform threshold increases.

Relevance of S1S2 Stimulation to Defibrillation

During fibrillation, each ventricular cell undergoes an excitation/recovery cycle similar to that which occurs during normal beating. However, because each cell is excited soon after the end of its refractory period, cycle length is very short and excitation occurs from a depolarized membrane potential, In addition, because activation fronts during fibrillation are slow conducting and nonuniform, individual cells of the ventricle are found in all phases of the action potential at any given time. Therefore, most cells will be in some phase of their refractory period at the time of the defibrillating shock.

One current hypothesis suggests that defibrillation is achieved by depolarizing each cell to produce refractoriness in a “crirical mass” of myocardium, causing fibrillation wavefronts to cease. However, because the defibrillating pulse must interact with individual cells that are at all phases of the action potential, but primarily with cells in the refractory period, the depolarization hypothesis must be extended from cellular activation to include any action of the shock that extends the refractory period of each ventricular cell. If the fibrillation action potential is defined by S1, and the defibrillatory shock-induced response by S2, then shock-induced prolongation of refractoriness could be produced not only by production of shock-induced S2 action potentials, but also by production of S2 graded responses, or even by nonregenerative prolongation of S1 action potential duration by stimuli delivered early in phase 2. Therefore, we used the working hypothesis that any characteristic of the shock, such as intensity or waveform, that improves its ability to prolong refractoriness to incoming fibrillatory wavefronts, defined by S2, over a wide range of S1S2 coupling intervals, improves defibrillation success. This working hypothesis was tested by examining responses to two S2 waveforms, 10-msec duration monophasic and biphasic rectangular waves, whose relative effects on defibrillation threshold were well known.

Because defibrillation is produced by an external field, the shock depolarizes portions of the sarcolemma at one end of the cell and hyperpolarizes the opposite end. Therefore, refractory period extension and possible effects of the conditioning prepulse during biphasic waveform defibrillation may be dependent on complex interactions between the hyperpolarizing and depolarizing effects. To separate possible interactions, we used intracellular current injection so that the independent effects of hyperpolarization and depolarization on the induced response could be examined.

Our results showing the effects of S2 stimulus intensity on S2 response duration are consistent with the working hypothesis described above. Monophasic S2 stimulation in late phase 3 produced a short, but normal, response similar to action potentials produced by phase 4 stimulation. Late phase 2 or early phase 3 S2 stimulation produced a response that prolonged S1 action potential duration. Duration of the S2 response produced at any specific S1S2 coupling interval depended on shock intensity. At two times threshold, the monophasic S2 stimulus produced a response at shorter S1S2 coupling intervals than at 1.5 times threshold. S2 response duration was longer after two times threshold stimulation than after 1.5 times threshold for coupling intervals beyond 60% S1 repolarization. For example, as shown by Figures 2A and 2C, responses at 190-msec cycle length are 240 msec at 1.5 times threshold and 260 at two times threshold. The longer S2 response produced at the higher intensity in turn prolonged refractoriness to S1 (Figure 7).

During fibrillation in intact hearts, longer S2 responses produced during the S1 refractory period by
higher shock intensities would produce longer refractoriness in a larger mass of myocardium and increase probability of successful interruption of multiple asynchronous fibrillation wavefronts. Therefore, probability of success would increase with greater shock intensity. In agreement with this hypothesis, the well-known improvement in defibrillation success produced at higher shock intensities leads to the probability of success versus intensity curves used to obtain “defibrillation threshold” in many experimental studies.4,5,14

Similarly, our results comparing monophasic and biphasic waveform S2 stimulation are also consistent with the working hypothesis that refractory period extension improves defibrillation success. During the S1 refractory period, biphasic waveforms of the same intensity as monophasic waveforms produced S2 responses of greater duration. This effect significantly prolonged total refractory period to an S2 fibrillating stimulus over a wide range of S1S2 coupling intervals (Figure 6). Again, these results are consistent with the greatly enhanced defibrillation ability of biphasic waveforms of same shock intensity as comparable monophasic waveforms. Possible cellular mechanisms underlying enhanced prolongation of refractoriness by biphasic S2 stimuli are discussed below.

Role of the Conditioning Prepulse in Threshold Reduction With Biphasic Waveforms

Excitation strength–duration curves using external field stimulation with monophasic and biphasic rectangular waveforms under “fibrillation” conditions in the cultured cell model suggested a working hypothesis for biphasic waveform threshold reduction.10. As described above, this hypothesis suggested that the first pulse of the biphasic waveform acts as a conditioning prepulse to hyperpolarize the sarcolemma, which allows time- and voltage-dependent recovery of inactivated excitation channels toward the closed or resting state. These channels then become more available for activation by the subsequent depolarizing excitation or defibrillating pulse. Increased availability of excitation channels would permit the second pulse to generate a larger response than would be produced by a monophasic pulse of the same amplitude. If defibrillation is achieved by extending cellular refractoriness and prevention of incoming wavefronts from continuing the “circus movement,” then those waveforms that produce the longest, most uniform refractoriness after the shock will defibrillate with the highest efficacy at a given shock intensity. By producing a longer graded response in a greater percentage of cells, biphasic waveforms reduce the probability of refribrillating wavefront propagation, thereby enhancing defibrillation efficacy compared with corresponding monophasic waveforms.

Our experiments with monophasic and biphasic waveforms having reversed polarity confirmed that the initial hyperpolarizing portion of a biphasic waveform is responsible for production of earlier and longer graded responses during the S1 refractory period. When polarity of the biphasic waveform was reversed, it failed to produce prolonged S2 responses over most S1S2 coupling intervals (Figure 5D).

At short coupling intervals, that is, 50 msec before the end of the S1 action potential, hyperpolarizing monophasic S2 stimulation shortens S1 action potential duration (Figure 5C), causing early repolarization, while depolarizing S2 stimulation slightly lengthens S1 duration (Figure 5A). The depolarizing/hyperpolarizing biphasic waveform appears to cancel these nonregenerative responses (Figure 5D). In contrast, the hyperpolarizing/depolarizing biphasic waveform produced action potential responses suggestive of excitation channel recovery at most short coupling intervals (Figure 5B). Early repolarization caused by the monophasic hyperpolarizing pulse is thought to allow excitation channel recovery and enhance the response to the second depolarizing pulse.

At S1S2 coupling intervals beyond the S1 refractory period, both the normal and reversed polarity biphasic S2 caused action potential formation. In contrast, only the depolarizing monophasic waveform was able to produce an S2 action potential. At the S2 intensities examined, 1.5 and two times diastolic threshold, the hyperpolarizing monophasic waveform, when applied during the S1 refractory period, shortened action potential duration without producing break excitation.

In addition to providing insight into the sarcolemmal responses of individual cells to electric field stimulation, our data may also provide new insights into defibrillation mechanisms. Mathematical models of the distribution of transmembrane potential in a periodic strand of cardiac muscle suggest that, during defibrillation, the periodic component of transmembrane potential predominates in regions far from the stimulating electrodes.22 This produces depolarization of the part of the cell proximal to the cathode and hyperpolarization of the distal part. However, in regions within approximately three space constants of the electrodes, the aperiodic term dominates and whole cells may be depolarized or hyperpolarized producing responses similar to those observed with intracellular current injection. Because the hyperpolarizing waveform actually shortened the S1 action potential rather than producing an S2 response, this waveform would be expected to increase both temporal and spatial dispersion of refractoriness compared with the biphasic waveform. Therefore, at low intensities, it would fail to defibrillate effectively, in agreement with observed results in the transthoracic calf model. In contrast, regions of the heart that are near defibrillation electrodes would be excited by either polarity of biphasic waveform. In regions of the heart far from the electrodes, opposite poles of the sarcolemma of each cell would also be excited. This would produce a relatively uniform refractoriness throughout much of the ventricle, consistent with the increased probability of successful defibrillation produced with biphasic waveforms.
Comparison With Results From Others

In contrast to our results, $S_2$ field stimulation during the $S_1$ refractory period with short duration waveforms produced longer responses with monophasic than with biphasic stimuli.23 In these experiments, the initial pulse of the biphasic $S_2$ waveform was only 2.5 msec in duration. Our previous work showed that excitation threshold for a 2.5 msec/2.5 msec symmetrical biphasic waveform was actually higher than that for the 5-msec monophasic waveform, presumably because, while gradual membrane hyperpolarization takes place during the initial pulse, insufficient time elapses for significant channel recovery.10

Consistent with the findings of this study, we observed greater action potential prolongation during refractory period extracellul Ar field stimulation in myocardial cell aggregates with biphasic rectangular waveforms (10 msec, each pulse) when compared with 20-msec monophasic waveforms.24 Similar action potential prolongation was also obtained with refractory period field stimulation in the isolated rabbit heart during normal sinus rhythm. Transmembrane potential recordings with voltage-sensitive dyes showed that the degree of action potential prolongation depended on both stimulus intensity and coupling interval.25,26

Limitations of the Study

This study, which was designed to examine mechanisms of threshold reduction by biphasic defibrillator waveforms, differs in several respects from clinical in situ defibrillation. First, constant-current square-wave stimuli, rather than truncated exponentials, were used. Second, electrophysiological characteristics may differ between isolated cells and those in the in situ fibrillating heart. Finally, although clinical defibrillation is produced by application of a field stimulus throughout most of the myocardium, current injection rather than field stimulation was chosen to isolate the conditioning prepulse effects. However, because qualitatively similar $S_2$ responses are produced by field stimulation in the same model,24 the results of this study with intracellular current injection are applicable to field stimulation.

In summary, this study showed that 1) biphasic stimuli, delivered during the $S_1$ refractory period, produce graded responses earlier than comparable monophasic waveforms; 2) at coupling intervals ranging from 60% to 100% $S_1$ repolarization, $S_2$ responses produced by biphasic stimuli are significantly longer than those produced by comparable monophasic stimuli; 3) the longer biphasic $S_2$ response increases the refractory period to an $S_1$ stimulus; and 4) the initial hyperpolarizing portion of the biphasic waveform is responsible for the earlier and longer $S_2$ responses, possibly through shock-induced time- and voltage-dependent excitation channel recovery.

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References


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