Interaction of Sequential Stimuli Applied during the Relative Refractory Period in Relation to Determination of Fibrillation Threshold in the Canine Ventricle

By Juan Tamargo, Bruce Moe, and Gordon K. Moe

ABSTRACT

An ineffective stimulus applied to cardiac tissue within the relative refractory period can alter the response to an immediately subsequent stimulus. We observed three response patterns that can coexist at different sites of stimulation in the same heart. In the first pattern, a stimulus of two to ten times diastolic threshold, applied too early to elicit a propagated response, becomes effective when a stimulus of equal strength is delivered 10 msec earlier. In the second pattern, a stimulus applied just late enough to evoke a response fails to do so when a stimulus of equal strength precedes it by as much as 30 msec. Finally, in the third pattern, two stimuli, separated by 10 msec, both of which are late enough to be effective when they are given alone, fail to yield a propagated response when they are applied together. These results have a bearing on the use of trains of stimuli to assess the ventricular fibrillation threshold. Possible interpretations are based on the temporal dispersion of recovery from the refractory state.

Nearly 50 years ago, Drury and Love (1, 2) demonstrated that an apparently ineffective stimulus delivered during the refractory period of the frog or turtle ventricle can prevent a propagated response to a subsequent stimulus. This result was obtained only under the influence of certain drugs. Lewis and Drury (3) made similar observations in the dog atrium under the influence of quinidine. These authors postulated that the earlier test stimulus excited the muscle locally but that the response failed to propagate far enough to be detected at the recording electrode. Local refractoriness then prevented the response to the later stimulus.

A possibly related interaction between sequential stimuli has been discussed in brief communications by Lown and his associates (4, 5). They have described a "protective zone" following the ventricular vulnerable period, during which a second stimulus prevents the initiation of ventricular fibrillation by the first, perhaps by occluding a reentrant pathway.

These observations have an obvious bearing on the estimation of ventricular fibrillation thresholds with trains of pulses that span the vulnerable period. In a study of the stimulus strength necessary to induce multiple responses and fibrillation, we observe several response patterns that indicate complex interactions between pairs or trains of stimuli applied to the ventricle during the early stage of recovery of excitability. Both facilitatory and inhibitory events were recorded. In light of these observations, we also compared the fibrillation threshold for single stimuli and trains of stimuli.

Methods

The experiments were done on dogs anesthetized with sodium pentobarbital (30 mg/kg, iv) supplemented as needed. Bipolar stimulating and recording electrodes were attached to the epicardial surface of the exposed ventricles. The heart was driven by rhythmic stimuli applied near the pulmonary conus. After ten or more driving stimuli (S1), one or more test stimuli were delivered through a separate pair of electrodes at another site. After the response patterns had been recorded, the test stimuli were applied at a different site, and the observations were repeated.

In some experiments, fibrillation thresholds were determined using single pulses 5 msec in duration or trains of ten pulses, each 5 msec in duration, delivered at a frequency of 100 Hz. The stimuli were isolated from ground and delivered through bipolar platinum electrodes 1 mm in diameter, embedded 5 mm apart in a plastic plaque stitched to the ventral surface of each ventricle. The current source, an Argonaut constant-current generator, was triggered at appropriate delay intervals from the basic driving stimulator; stimulus amplitude was monitored as the voltage drop across a 100-ohm resistor in series with the stimulating electrodes. Twelve or more driving pulses, obtained from a Tektronix pulse generator and passed through an isolation transformer, were delivered through the same platinum electrodes before each test sequence. Once the peak of the vulnera-

From the Masonic Medical Research Laboratory, Utica, New York 13503.

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Dr. Tamargo is a Royal Arch Masons Fellow on leave from the Department of Pharmacology, University of Madrid, Spain.

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ble period had been defined by a single test pulse, the timing of the train was adjusted to begin 50 msec before and to extend 50 msec after that peak. Defibrillation was accomplished with a d-c pulse (condenser discharge) obtained from an American Optical Company defibrillator set to deliver approximately 100 w-sec. In all trials, at least 15 minutes was allowed to elapse after each fibrillation before another test.

Results

INHIBITION

In one set of studies, a 4-msec test stimulus at two to eight times the late diastolic threshold was delivered through bipolar electrodes at the earliest moment in the cycle when a propagated response could be regularly elicited. The interval preceding the test stimulus was then scanned by an interpolated stimulus of equal strength. The stimulus pattern was S₁ delivered at a remote site, S₂ delivered at the test site too early to cause a response by itself, and S₃ delivered at the same site at a time when it would always succeed in the absence of S₂.

The response pattern is illustrated in Figure 1. The ventricular responses at a remote recording site are shown on the bottom trace in each section, and the test stimuli are indicated on the top trace. The first ventricular complex in each trace is the response to the last of the series of driving stimuli. S₃ was regularly effective by itself, but when it was preceded by S₂, in this case 10 msec earlier, no response occurred. In a number of trials at different test sites and in different experiments, the period within which S₂ could prevent or significantly delay the response to S₃ ranged from 10 to 30 msec.

The pattern of Figure 1 is comparable to the observations from Lewis’ laboratory (1–3) and can be explained similarly, as postulated diagrammatically in Figure 2. S₂ is assumed to excite a local volume of tissue before adjacent fibers have recovered sufficiently to permit the local response to emerge (Fig. 2A). S₃, delivered alone and later, clears the refractory period of the surrounding tissue (Fig. 2B). When both stimuli are applied, S₃ fails because the available pathway has been occluded (Fig. 2C).

A further conjecture is necessary, as shown in Figure 2D. When the combination of S₂ and S₃ fails to yield a response, a fourth stimulus, applied 10 msec after S₃, will often succeed. We suggest that an alternate escape route, recovering too late to be excited by S₂ or S₃, is available by the time S₄ is applied. Similar results were obtained when the test stimuli were unipolar, either anodal or cathodal, although the temporal position of the stimuli in the cardiac cycle was slightly different in the three modes.

SUMMATION

A second pattern was observed in another series of observations (Fig. 3). In this series, the test stimulus was applied too early to initiate a propagated response, i.e., the test stimulus, S₃, failed. However, when S₃ was preceded by S₂, given 10 msec earlier, a propagated response was regularly observed; in other words temporal summation occurred. On occasion, three successive stimuli were necessary to demonstrate summation (Fig. 4).

Assuming, as in the first pattern, that the tissue exhibits some dispersion of recovery times, we can postulate two convergent pathways, neither one of which by itself can successfully activate a still refractory barrier. The diagram of Figure 5 suggests a possible explanation. Two fiber bundles, a and b, one of them longer than the other, are both
FIGURE 2

Schematic representation of the pattern shown in Figure 1. The stippled areas denote refractoriness in the tissue under the stimulating electrodes. A: Premature stimulus, 2, excites immediately subjacent tissue, but the response is blocked by persistence of refractoriness in neighboring elements. B: Response to a later stimulus, 3, clears the refractory period of adjacent tissue. C: S3 blocks the response to S2. D: S4 successfully engages an escape route not available to S2 or S3.

MUTUAL INHIBITION

A third pattern was demonstrated on many occasions. In the example shown in Figure 6, S2 and S3 were applied at times when each stimulus by itself resulted in a propagated response, as shown in Figure 6A and B. When they were applied together, no response occurred (Fig. 6C). In the diagram of Figure 7, the stippled areas represent the duration of refractoriness in the escape route. The local responses simulated on the right of the figure are assumed to occur where two pathway converge in tissue just proximal to the escape route. The curved broken line indicates the recovery of excitability in that proximal element. Whether either S2 or S3 is delivered alone, the response in the proximal element occurs late enough to clear the refractory period of the escape route (Fig. 7A and B). When both stimuli are applied in sequence, summation of converging wave fronts excites the proximal element too early, and block occurs at the refractory barrier (Fig. 7C). This interpretation is conceptually similar to the events described at the neuromuscular junctions in 1923 by Forbes et al. (6).

FIGURE 3

Summation of two stimuli. Neither S2, delivered 134 msec after the last basic response, nor S3, delivered at 144 msec, evoked response when they were given alone.
Fig. 4  Summation of three stimuli: $S_1$, $S_2$, and $S_3$ given alone or in any combination of two failed to generate a propagated response, but the sequence of three was regularly effective. Numbers refer to the time interval in milliseconds after the last basic response.

**The Protective Zone**

In several experiments, an early premature stimulus ($S_3$) was applied at an intensity sufficient to evoke two closely coupled ventricular responses, i.e., just short of the threshold for ventricular fibrillation (Fig. 8A). When a stimulus of equal strength was applied 10 msec earlier ($S_2S_3$), the double response still occurred (Fig. 8B), but when $S_3$ was followed by $S_4$, applied 10 msec later, the second ventricular response was regularly abolished (Fig. 8C). As in the experiments of Lown and his colleagues (4, 5), $S_4$ may have occluded a potential reentrant pathway.

**Fibrillation Threshold: A Single Stimulus vs. Trains of Stimuli**

Because various combinations of stimuli can cause either facilitation or inhibition, it is difficult to predict whether a train of stimuli spanning the vulnerable period will be more or less effective than a single pulse in evoking ventricular fibrillation. In six experiments, we compared the fibrillation thresholds for both methods. Because of the possibility that sequential stimuli at one site might inhibit and at another site facilitate the development of fibrillation, electrode plaques were stitched to the surface of both the right and the left ventricle, and comparisons of stimulus strength were made at each site in four of these experiments.

In each experiment, the vulnerable period was scanned by a rectangular constant-current pulse 5 msec in duration at increasing intensity until...
In all of the experiments, the fibrillation threshold was significantly higher for a single pulse than it was for the train of ten (Fig. 9). In most experiments, the threshold was lower at the right ventricular stimulating site than it was at the left, in confirmation of the findings of Shumway et al. (7).

Because the first few stimuli in a train might excite adrenergic fibers within the range of the electrodes and thereby alter the excitable field for the subsequent “trigger” stimulus, several trials were made to assess the diastolic threshold for a train and for single pulses before and after the injection of propranolol (0.25–0.4 mg/kg). No significant differences were noted in either case.

Propranolol was also used to determine whether the fibrillation threshold for the train of pulses could be elevated relative to that for a single pulse. Propranolol did not alter the ratios.
INTERACTION OF SEQUENTIAL STIMULI

FIGURE 8
The protective zone. A: S3 (130 msec after the basic response) is delivered at sufficient strength to evoke a double response of the ventricle. B: S2 fails to prevent the double response. C: The combination of S3 and S4 yields only a single response.

Discussion
The constructions developed to explain the several patterns of inhibition and summation are simplistic. They are all based on the assumption that temporal dispersion of the recovery process exists within the zone of tissue accessible to the stimulating electrodes and do not consider what might be called “side effects” of the test stimuli. Temporal dispersion has been demonstrated in ventricular muscle (8); there is no reason to doubt that some fibers recover from the refractory state earlier than do others within the effective current field of the stimulating electrodes. The possibilities displayed in Figures 2, 5, and 7 must therefore be real.

Two side effects of the testing procedure should be considered. One of these is the well-known abbreviation of action potential and refractory period duration induced by anodal stimulation during repolarization (9). The test stimuli, delivered through bipolar electrodes at two to eight times the diastolic threshold, should accelerate repolarization near the anode. This acceleration would, of course, enhance the preexisting dispersion of recovery. This situation does not introduce any conceptual complications in our interpretation of the results, with the possible exception of the schema in Figure 5. Here we might assume that the early stimulus, S2, induces some abbreviation of the refractory period under the anode but fails to excite. The later stimulus, S3, could do the same. When the two are applied in sequence, the acceleration of repolarization induced by S2 could permit excitation of the underlying tissue by S3. Summation was, however, also demonstrable for unipolar cathodal stimulation.

A second possible side effect is the stimulation of adrenergic fibers terminating within the excitation zone. This possibility is perhaps more tenuous than the anodal effect on refractory period duration; the latency for the development of an adrenergic effect is probably longer than the 10-msec interval between the test stimuli in the response patterns we have described, and the quantitative effect of a single conditioning pulse on the response to a subsequent stimulus is probably small. Nevertheless, the expected effect of adrenergic activation is a slight enhancement of excitability; abbreviation of the refractory period of the prior response could hardly be a factor. This effect, too, could conceivably explain the summation pattern, but it does not conceptually alter the explanation of the other phenomena observed.

FIGURE 9
Fibrillation thresholds (ma) for single stimuli (s) and for trains of stimuli (t) delivered to right (RV) and left (LV) ventricular surfaces in four experiments. Means ± se are shown.
Summation and inhibition in special preparations of cardiac tissue have been studied recently by Cranefield and Hoffman (10). The circumstances under which these phenomena were demonstrated may or may not be relevant to the observations reported in the present paper. In their experiments, both summation and inhibition were elicited in branched preparations of false tendons, in which the excitability at the junction of two branches was depressed. Stimuli applied individually to each branch failed to excite the junction, but properly timed stimuli applied conjointly evoked a propagated response, i.e., summation. In our experiments, both stimuli were applied to the same point. Inhibition in their experiments was somewhat more complex, but it appears to be more or less comparable to the schemata in Figures 2 and 7, again with the caveat that in our experiments there was only one stimulated site.

It may be asked whether the present results are not examples of Wedensky facilitation and inhibition. Wedensky facilitation, as demonstrated in nerve fibers beyond a region of block, has also been shown in cardiac Purkinje fibers (11). In those experiments, it was found that the threshold of a fiber, beyond a site of conduction failure, is lowered during the subthreshold depolarization induced electrotonically by a prior blocked response. This situation is not quite comparable to that in the present experiments, but a similar event may be in part responsible and is in fact apparent in the postulated summation of local responses depicted in Figure 5.

Wedensky inhibition has never been clearly defined, apart from humorally mediated synaptic events, but the construction of Figure 7 may be an example of the heterogeneous phenomena loosely called Wedensky inhibition (12). Little is gained by applying a name that does not define the mechanism.

Many of the interactions discussed so far must apply to the determination of fibrillation thresholds with trains of stimuli. This technique has been used because it saves time in the testing procedure (13). It is tacitly assumed that one of the ten or more pulses falls near the peak of the vulnerable period and that the threshold recorded is the same as, or at least constantly related to, the value that would have been obtained with the more time-consuming scanning procedure used in Wiggers’ laboratory years ago (14). Not surprisingly, the threshold values recorded using these two methods are not the same. One might expect that at some stimulated sites the threshold would be higher for the train, i.e., stimuli subsequent to the “trigger” stimulus would fall in the protective zone. That this phenomenon might happen on occasion has not been excluded by the present experiments, but it is clearly not a frequent occurrence. In the experiments by Lown’s group (4, 5), a single discharge was used to induce fibrillation, and a subsequent single stimulus was used to define the protective zone. This phenomenon can probably be demonstrated at any site of stimulation and in any heart; the similar event shown in Figure 8 was by no means difficult to demonstrate. But the protective zone might be “deprotected” by prior or subsequent stimuli in the train of pulses—the rules need not be the same. In any event, we found that the threshold for a train was regularly less than that for a single pulse and that the ratio of the two thresholds varied widely at various sites and in different experiments. These findings strongly suggest that the two methods do not measure precisely the same properties of the heart. Indeed, the question may be raised whether the determination has any real physiological significance. It does not follow, of course, that an agency that alters the fibrillation threshold would not operate in the same direction for both test procedures.

Locally induced adrenergic discharge, which probably has little influence on the interaction of two stimuli 10 msec apart, could conceivably play a role in the train of ten pulses. Initial pulses in the train might alter the excitability of the tissue in a nonuniform pattern, early enough to affect the response to later pulses. Adrenergic effects could also abbreviate the refractory period in a potential reentrant pathway, thereby facilitating the induction of fibrillation. The fact that propranolol did not reduce the discrepancy between the two methods is probably not definitive evidence that adrenergic effects are not involved.

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J Tamargo, B Moe and G K Moe

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