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Gilmour, Robert F., Jr., Niels F. Otani, and Mari A. Watanabe. Memory and complex dynamics in cardiac Purkinje fibers. Am. J. Physiol. 272 (Heart Circ. Physiol. 41): H1826–H1832, 1997.—The contribution of cumulative changes in action potential duration (memory) to complex cellular electrophysiological behavior was investigated in canine cardiac Purkinje fibers. Complex behavior induced during constant pacing was caused by reciprocal interactions between the time to full repolarization (TFR), where TFR = response duration + latency, and the diastolic interval (DI). The relationship between TFR and the preceding DI during complex behavior differed from that obtained using a standard restitution protocol. In particular, higher-order periodicities and chaos were produced in fibers in which the restitution curve lacked the prerequisites for such behavior. To investigate whether shifts in the restitution curve might be expected during rapid pacing, the relationship between TFR of a test response (TFR), and the immediately preceding response (TFR,) was determined. For any fixed DI, reduction of TFR, from 240 to 130 ms was accompanied by a corresponding reduction of TFR,,+1, whereas as TFR, was reduced further to 190 ms, TFR,,+1 increased. Because of the dependence of TFR,,+1 on TFR, (memory) and on the preceding DI (restitution), the slope of the low-dimensional relationship between TFR,,+1 and DI, at a constant pacing cycle length depended on the slopes of the restitution and memory functions. These results suggest that rapid accumulation and dissipation of memory may contribute importantly to complex electrical behavior in cardiac tissue.

In recent experiments, we have found that higher-order periodicities and chaos can occur in cardiac Purkinje fibers, the restitution relation of which, when determined using standard protocols (1), has a slope < 1. Because the slope of the restitution relation is known to depend on the pacing cycle length and duration of pacing (“memory”) (1, 2, 7, 14), we investigated whether memory might induce dynamic alterations of the relationship between action potential duration and diastolic interval that permit chaotic behavior. The results of the study suggest that rapid accumulation and dissipation of memory contribute importantly to the development of nonlinear electrical behavior in cardiac Purkinje fibers.

MATERIALS AND METHODS

Adult dogs of either sex (n = 18) were anesthetized with pentobarbital sodium (390 mg/ml, 86 mg/kg iv; Fatal-Plus, Vortex Pharmaceuticals), and their hearts were excised rapidly and placed in cool Tyrode solution. Free-running 10- to 20-mm-long and 2- to 4-mm-wide cardiac Purkinje fibers (n = 20) were obtained from either ventricle. The fibers were mounted in a Plexiglas chamber and superfused with normal Tyrode solution at a rate of 15 ml/min. The Tyrode solution was bubbled with 95% O2:5% CO2. The P02 was 400–600 mmHg, the pH was 7.35 ± 0.05, and the temperature was 37.0 ± 0.5°C. The composition of the Tyrode solution (in mM) was 0.5 MgCl2, 0.9 NaHCO3, 4.0 KCl, 137.0 NaCl, 24.0 NaHCO3, 4.0 KCl, and 5.5 glucose. In some experiments the KCl concentration was 2.7 mM. To avoid the possibility that supernormality might contribute to the results when KCl was 2.7 mM (4), the fibers were stimulated at a current intensity of two to three times the diastolic threshold. Stimulus duration was constant at 2 ms. There were no qualitative differences between the results obtained using KCl concentrations of 4.0 and 2.7 mM, and the data were pooled for analysis.

Initially, the fibers were stimulated at a basic cycle length of 500 ms, and transmembrane recordings were obtained using standard techniques, as described previously (9). Microelectrode recordings were obtained from sites located within 1–3 mm of the bipolar stimulating electrode, which was placed near the middle of the fiber. Typically, the site of impalement was midway between the poles of the stimulating electrode (which were 1 mm apart) to minimize the stimulus artifact and the effects of current polarity. If the dynamics were influenced significantly by switching the stimulus polarity, the experiment was discarded.

The steady-state relationships between pacing cycle length and the amplitude and duration of the cellular response to a given stimulus were determined by shortening the pacing cycle length from 500 ms to the cycle length at which 2:1 responses occurred. All responses to stimuli that exceeded 0 mV in amplitude, relative to the take-off potential, were included in the analysis. These responses most likely included nonpropagated active and passive membrane responses in addition to propagated action potentials. Our rationale for including nonpropagated responses in the analysis was that such responses contributed significantly to the dynamics (see RESULTS). At the stimulus intensities used in this study, responses to stimuli occurred only during phases 3...
and 4 of the action potential. At higher stimulus intensities, responses also could be elicited during phase 2 of the action potential. However, our previous studies of responses recorded during ventricular fibrillation indicate that only those responses that occur during phases 3 and 4 of the action potential are physiologically relevant (10).

Response amplitude was measured as the difference between the take-off potential and the peak membrane potential. Because stimulation at short cycle lengths elicited responses during phase 3 of the preceding response, measurements of total response duration required extrapolation of the slope of phase 3 at the time of activation. To avoid potential errors in the estimation of response duration using such a method, the take-off potential, defined as the membrane voltage at the moment of the delivery of the stimulus, was used as an index of the response duration. As validated previously (25), the more negative the take-off potential, the shorter was the previous response duration for diastolic intervals <0.

To determine the time course of restitution of response amplitude, latency, and response duration, the fibers were stimulated using trains of 20 stimuli delivered at a basic cycle length (S1-S1) of 300 ms. This cycle length was used because it was the shortest cycle length at which no alternation of response duration occurred in any of the fibers. Consequently, the effects of memory during rapid pacing could be approximated without the confounding effects of alternating response durations occurring before the delivery of the premature stimulus. After the last stimulus of the train, the diastolic interval was scanned using a single premature stimulus (S2) (9). The duration of the response to S2 was measured at 90% of repolarization and was plotted as a function of the diastolic interval, where the diastolic interval equaled the S1-S2 interval minus the duration of the response to the last S1 stimulus.

To determine the influence of the preceding response duration on the duration of a test response, the restitution of response duration after S2 was determined by fixing the S1-S2 interval (at 180–500 ms for any given trial) and delivering a second premature stimulus (S3) at S2-S3 intervals of 100–300 ms. In addition, the relationship between the diastolic interval after S2 and the duration of the response to S3 was determined by varying the S1-S2 interval from 180 to 300 ms at a constant S1-S1 interval (300 ms) and S2-S3 interval (fixed at 140–500 ms for any given trial). All experimental procedures were approved by the Institutional Animal Care and Use Committee at Cornell University.

The experimental data were imported into the MATLAB analysis package (version 4.0a, MathWorks) running on HP 9000/700 series workstations. The package was used in conjunction with script files written in the MATLAB language to generate, display, and print three-dimensional surface plots of the data and two-dimensional cross-sectional plots of these surfaces, as determined by the mathematical model described below. Surface generation was performed by MATLAB using an inverse distance method. Cross-sectional data were produced via bilinear interpolation.

RESULTS

Period doubling bifurcations and chaos in cardiac Purkinje fibers. In 12 of the 20 fibers, progressive reduction of the pacing cycle length (BCL) was accompanied by a transition from 1:1 stimulus-response locking to 2:1 locking, without intervening patterns. In the remaining eight fibers, period-doubling bifurcations and the induction of irregular dynamics occurred. Stimulus-to-response ratios >4:4 were observed in two of the experiments. In those experiments, 4:4 locking progressed to 8:8 locking and then to variable locking (e.g., 10:10, 18:18), including 16:16 locking. In each experiment, every stimulus elicited a response (as defined above), except during transients, until 2:1 locking occurred (i.e., patterns such as 3:2 and 5:4 were not observed).

An example of period-doubling bifurcations of response amplitude and duration is shown in Fig. 1. During progressive reduction of the BCL from 200 to 111 ms (in 1-ms steps), successive doublings of the stimulus-to-response ratio from 1:1 to 2:2, 4:4, and 8:8 occurred. Further reduction of the BCL to 104 and 95 ms induced 16:16 locking and irregular dynamics, respectively (not shown). Examples of 16:16 locking and irregular dynamics in a different fiber are shown in Fig. 2. At still shorter BCL, aperiodic activity was
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TFR\(_{n+1}\), that is

\[
TFR_{n+1} = f(DI_n, TFR_n)
\]

With memory present, it would not be possible to determine the sequence of TFR during higher-order periodic or aperiodic behavior from a single restitution curve, for if the TFR\(_n\) were different one to the next, different restitution curves would need to be used for each excitation. However, for the case of constant pacing, the problem can be reduced to a unidimensional map, in that constant pacing imposes the condition

\[
DI_n = BCL - TFR_n
\]

in which BCL is a constant. When this relationship is substituted into Eq. 2, TFR\(_{n+1}\) is solely a function of TFR\(_n\). Alternatively, we can cast Eq. 2 in the form of a “generalized” restitution function TFR\(_{n+1}(DI_n)\), appro-

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![Fig. 2](image1.png)

Fig. 2. Higher-order periodic and aperiodic activity in a canine Purkinje fiber. Responses are shown during constant pacing at cycle lengths of 120 ms, where 16:16 stimulus-response locking occurred (A), and 118 ms, where irregular dynamics occurred (B). KCl = 4 mM. Fiber was different from that used in Fig. 1. Vertical calibration = 40 mV; horizontal calibration = 1 s.

replaced by 4:4 and, finally, by 2:1 locking, as shown in Fig. 3, which contains the complete bifurcation diagram and samples of the time series for the fiber shown in Fig. 1.

From previous studies (3, 12) it was expected that the sequence of response durations (RD) during periodic pacing could be approximated from the relationship

\[
BCL = RD + LAT + DI
\]

where LAT is the latency between the delivery of the stimulus and the upstroke of the response and DI is the diastolic interval. The relationship between RD and RD + LAT [the time to full repolarization (TFR)] and the preceding DI (DI\(_n\), as determined using the standard restitution protocol, is shown in Fig. 4. As the DI was shortened from 10 to -55 ms, RD decreased, as did TFR. As the DI was reduced to less than -55 ms, RD continued to decrease, whereas TFR increased, until, at a DI of -80 ms, it decreased precipitously. The very short TFR corresponded to a subthreshold response.

Influence of memory on the restitution of RD. Although the restitution relations for RD and TFR shown in Fig. 4 contained nonmonotonic regions and regions of steep slope, the slopes of the latter were <1. According to previous studies of cardiac Purkinje fibers (3) and other low-dimensional dynamical systems (8, 16), such a relation should not be able to support higher-order periodic or aperiodic behavior.

In order to determine whether a steepening of the TFR-restitution relation might occur secondary to accumulation and dissipation of memory, the influence of the preceding TFR (TFR\(_n\)) on succeeding TFR (TFR\(_{n+1}\)) was determined in six Purkinje fibers. Our hypothesis was that any given TFR was a function of DI\(_n\) and
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Fig. 4. Dependence of time to full repolarization (TFR, ○), response amplitude (+), and response duration (●) on diastolic interval (DI) in a canine Purkinje fiber. Repetition of response amplitude, response duration, and TFR for DI < 20 ms to show steeply ascending and nonmonotonic regions. Fiber was paced at a constant S1-S1 interval of 300 ms, and a single premature stimulus, S2, was delivered at S1-S2 intervals varying from shortest S1-S2 interval that produced a measurable response to an S1-S2 interval of 1,000 ms (inset). Fiber was the same as that used in Fig. 1.

The curve representing the function may be viewed by looking from the right (in from the positive TFRn axis) in Fig. 5. With this perspective, the traces in Fig. 6 were obtained.

For any given BCL, a diagonal cross section of the surface shown in Fig. 5 represents the intersection of the surface with the locus of points that satisfy Eq. 3.
The influence of memory on the low-dimensional map can be seen from Fig. 6. In the range of DI from 10 to 30 ms, the slope of the ascending portion of the map is steeper than the slopes of the restitution curves. This result is expected, given that these relatively long DI were accompanied by short TFR, according to Eq. 3. For short TFR, the slope of the memory function is negative (Fig. 5), which, according to Eq. 4, will increase the slope of the map.

Over a range of shorter DI (from 0 to 10 ms), the slope of the unidimensional map is less steep than that of the restitution curves. The DI were preceded by longer TFR. Given the positive slope of the memory function for this range of TFR (Fig. 5), the slope of the map will be less positive than that predicted from restitution alone (Eq. 4). As the DI is shortened further from -15 to -30 ms, the slope of the map becomes negative. Over this range of DI, restitution has a negative slope and the memory function has a positive slope because of the long preceding TFR. Subtraction of the positive slope of the memory function from the negative slope of the restitution function (Eq. 4) generates a more negative slope of the unidimensional map than would result from restitution alone.

The characteristics of the unidimensional map derived from three-dimensional surfaces of the type shown in Fig. 5 were confirmed experimentally in six Purkinje fibers by varying the S1-S2 interval at a fixed S2-S3 interval, i.e., at a fixed BCL (Eq. 3). As shown in Fig. 7A, the experimentally determined unidimensional map (S3 TFR) and restitution curve (S2 TFR) clearly resembled the unidimensional map and restitution curves derived from the three-dimensional surfaces (Fig. 6, dark and light lines, respectively). In addition, as shown in Fig. 7D, there was not a one-to-one correspondence between the S2 TFR and the S3 TFR, indicating that the S3 TFR was not uniquely determined by the S2 TFR.

Role of memory in the induction of higher-dimensional nonlinear behavior. To determine whether the periodic and aperiodic behavior of the type shown in Fig. 1 derived from a low-dimensional mechanism, first return plots for response amplitude and take-off potential were generated. Return plots for stimulus-to-response ratios of 1:1, 2:2, and 4:4 indicated that response amplitude and RD varied within a fixed set of values along a unidimensional map (not shown). During higher-order periodic behavior (e.g., period 8; Fig. 8, A and B), RD and response amplitude also alternated sequentially within a fixed set of values, but the values did not lie along a unidimensional map. During aperiodic behavior, RD and response amplitude varied over a wider range of values but were confined to attractors having characteristics similar to the attractors for the periodic behavior (Fig. 8, C and D).

DISCUSSION

Period-doubling bifurcations and chaotic dynamics are known to occur in low-dimensional nonlinear dynamical systems (8, 16, 21), including theoretical models and experimental preparations of spontaneously active and paced cardiac tissue (3-5, 11-13, 15, 19, 24, 25). The functions describing such behavior contain a region of steep slope and a critical point. In previous studies of cardiac Purkinje fibers, the steep slope corresponded to the ascending region of restitution of action potential duration, whereas the critical point and associated region of negative slope were caused by the induction of significant latency at short diastolic intervals (3, 5). The results of the present study indicate that these features of restitution are modified importantly by memory. Consequently, the generation of higher-order periodic or aperiodic behavior may be enhanced or suppressed, depending on the nature of the memory effect.
The influence of memory on the low-dimensional map and, therefore, on rate-related alterations of cellular electrical behavior varied as a function of the diastolic interval. Memory induced a steepening of the positive and negative slope regions of restitution at long and at short diastolic intervals, respectively, effects that would be expected to facilitate the development of complex dynamics by creating unstable fixed points. Conversely, memory tended to induce a flattening of restitution at intermediate diastolic intervals and to smooth the normally sharp transition from the negative slope region to the region of positive slope at short diastolic intervals. Such reductions of slope might create stable fixed points that ordinarily would not exist on the restitution curve. In fact, a region of 1:1 locking at very short pacing cycle lengths was observed in three of the preparations.

The development of complex dynamics was contingent on the presence of responses having short time to full repolarizations. In the absence of such responses, the slope of the memory function was uniformly positive, which reduced the slope of the low-dimensional map at long diastolic intervals. In addition, the negatively and positively sloped regions of the restitution curve at short diastolic intervals. Such reductions of slope might create stable fixed points that ordinarily would not exist on the restitution curve. In fact, a region of 1:1 locking at very short pacing cycle lengths was observed in three of the preparations.

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Short-duration responses, when coupled with long latencies, also were at least partly responsible for the lack of a unidimensional attractor during higher-order periodic and aperiodic activity. Because two responses could have the same time to full repolarization but different combinations of response duration and latency, their subsequent memory-mediated effects differed (Fig. 7). Accordingly, the time to full repolarization that incorporated the longer latency was followed by the longer response. The failure of a given time to full repolarization to uniquely determine the next time to full repolarization was reflected by the lack of a unidimensional return map during chaotic dynamics.

Lack of a unidimensional attractor during complex behavior also may have occurred because the influence of memory on the low-dimensional map was not limited to the immediately preceding action potential. Previous studies by Boyett and Jewel (2) and by others (7, 14) have described a long-term memory component in ventricular tissue, having a time constant on the order of seconds. A memory or “fatigue” phenomenon also contributes importantly to the electrical behavior of the atrioventricular node (22, 23). Accumulation of long-term memory has been shown to suppress chaotic behavior in Purkinje fibers, when such behavior is initiated by pacing at relatively long cycle lengths after a period of quiescence (4). However, we studied the dynamics that occurred after sustained periods of continuous pacing and sequential shortening of the pacing cycle length. Consequently, it is likely that accumulation and dissipation of long-term memory stabilized at each cycle length and did not contribute significantly to the rate-dependent changes in dynamics.

The results of a recent modeling study (17) suggest that short-term and intermediate-term memory (having time constants of 50 and 400 ms, respectively) may account for the higher-dimensional complex dynamics observed in our experiments. In the modeling study the
generation of 8:8 and 16:16 locking did not result from a period-doubling cascade but developed from a Hopf bifurcation of a 2:2 pattern. Thus the dynamics changed gradually with decreasing cycle length from being essentially one-dimensional and period doubling to fully two-dimensional, allowing for the sequence of patterns observed in our experiments: 1:1, 2:2, Hopf-bifurcated 2:2, back to a wider 2:2, and then 2:1.

Taken together, the results of this study suggest that memory should limit the development of complex dynamics in the absence of responses having a short duration, whereas memory should facilitate the development of complex dynamics in the presence of such responses. The observation that short time to full repolarization was a prerequisite for the development of complex dynamics provides some support for the idea that prolongation of action potential duration may be antiarrhythmic (6, 18). However, the use of potassium channel blocking agents to create such an effect may be problematic, in that the increase in membrane resistance caused by these agents could facilitate the induction of short-duration electrotonic responses during the action potential plateau or terminal repolarization.

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