CONDUCTION AND REFLECTION IN PURKINJE FIBERS/Jalife and Moe

Excitation, Conduction, and Reflection of Impulses in Isolated Bovine and Canine Cardiac Purkinje Fibers

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SUMMARY When an impulse arrives at an area of impaired conductivity, a slowly rising electrotonic potential may bring the distal tissue to threshold after a delay imposed by the passive electrical properties of the system and by the time-dependent changes of these properties during diastole. This phenomenon can be demonstrated in Purkinje strands in which an area of depressed conductivity has been induced by the impedance of a sucrose gap and can be mimicked by the application of relatively long current pulses of low amplitude. The functional refractory period, defined as the shortest interval between two distal responses both propagated across the gap, was determined by the application of premature stimuli at progressively earlier intervals. The time course of the recovery of excitability as well as the conduction interval could be varied almost at will by manipulating the electrical impedance between proximal and distal ends of the fiber. When the time of activation of the distal end across the gap exceeded the absolute refractory period of the proximal segment, the impulse reflected back as a closely coupled premature beat. Time-dependent changes in the passive electrical properties of the depressed segment may set the conditions for reflection. The results suggest the possibility of reflection as a mechanism for premature beats and demonstrate obligatory shifts in the patterns of premature reentrant activity accompanying changes in basic cycle length. These experiments provide important clues for the distinction between reentrant and parasystolic mechanisms. Circ Res 49: 233-247, 1981

IN previous publications we have proposed the Purkinje fiber-sucrose gap preparation as a model for ventricular parasystole (Jalife and Moe, 1976; Moe et al., 1977; Jalife and Moe, 1979). In those studies, the dynamic behavior of a spontaneous pacemaker in response to electrotonic depolarizations across an area of impaired conductivity was described by a biphasic phase response curve (PRC). Frequency-dependent changes in the pattern of ectopic activity that occur in various experimental and clinical situations could be predicted by the PRC and by the entrainment characteristics of the pacemaker. More recently, Antzelvitch et al. (1980) demonstrated in a similar preparation that conditions can exist in which premature reentrant activity (reflection) can be generated without the need of a circuitous pathway. A driven impulse on the proximal end of the fiber can be transmitted electrotonically across the sucrose gap and can excite the distal segment after a delay imposed by the passive electrical properties of the system. If the delay is sufficiently long, the impulse can be reflected to the proximal segment. Frequency-dependent altera-
tions in the arrhythmic patterns could be demonstrated also in the reflection model; the alterations were qualitatively similar to those of the parasystole model. It was concluded that parasystole and reflection are part of a continuous spectrum that extends from purely parasystolic to purely reentrant mechanisms. We undertook the present study to extend our observations on the reflection model.

Methods

Determination of Input-Output Characteristics

The preparation has been described in detail in earlier publications (Jalife and Moe, 1976; Jalife and Moe, 1979; Antzelevitch et al., 1980). Free-running false tendons were dissected from calf hearts obtained at slaughter, and from the hearts of anesthetized (sodium pentobarbital, 35 mg/kg iv) dogs. Unbranched preparations (500–800 μm in diameter; 6–12 mm long) were placed in a three-chambered tissue bath and superfused with Tyrode’s solution containing 4.0 mM KCl.

Following an equilibration period of 1 hour, the preparation was driven at various frequencies by stimuli delivered through one of two pairs of thin bipolar electrodes applied close to the cut ends of the fiber in the outer chambers. Premature responses were induced at various test intervals after each 10th basic stimulus. Transmembrane potentials from the two outer chambers were recorded simultaneously as previously described (Jalife and Moe, 1976).

After pertinent data had been obtained from the preparation under control conditions, the central chamber (2 mm long) was perfused with isotonic sucrose solution. The extracellular impedance, and thus the degree of block across the sucrose gap, was controlled by connecting the two outer chambers by means of an external shunt circuit. The conduction properties of the system were studied again at various cycle lengths and shunt resistance values, at least 1 hour after sucrose superfusion had started.

Determination of Diastolic Threshold

The current necessary to bring the membrane potential to threshold (Ith) and the potential at which the cells in the test compartment of the sucrose gap produced a regenerative response (Vth) were determined in five experiments in which depolarizing current pulses of various durations were used to scan the diastolic interval after every 10th basic stimulus.

The test compartment was perfused with Tyrode’s solution containing 4 mM KCl, whereas the opposite chamber was perfused with 10 mM KCl Tyrode’s. The central compartment was perfused with isotonic sucrose solution. Transmembrane potentials were recorded differentially from the test segment with two conventional microelectrodes, one intracellular and the other extracellular, placed closely together.

Intracellular stimulation was accomplished in all five experiments by passing depolarizing current pulses across the sucrose gap (Jalife and Moe, 1976). In three of these experiments, current pulses were also applied through a second intracellular microelectrode located in the test compartment. This current electrode could be connected either to a single-ended microelectrode amplifier (designed and constructed by W.J. Mueller) or to a DC current source (P6i, Frederick Haer) by means of a toggle switch. With the system connected in the recording mode, the tip of the microelectrode was positioned intracellularly within less than 50 μm from the differential recording electrodes. Once a stable impalement had been obtained the system was switched to the stimulation mode, while the input stage of the amplifier was automatically connected to ground. This permitted the application of current pulses through one electrode while their effects were being monitored constantly through the closely located recording electrodes. Current pulses were applied at various test intervals, after every 10th basic beat, and the stability of the impalement could be checked periodically by switching back and forth to the recording mode after every stimulus. Current was measured as the voltage drop across a series 1-MΩ resistor.

Definitions

As in a previous study (Antzelevitch et al., 1980), the terms proximal (P) and distal (D) indicate the respective fiber segments in relation to the area of block in the sucrose gap (the distal segment was also used as test segment when current pulses were applied). Basic proximal responses (P1) induced distal discharges (D1) across the gap. Premature proximal discharges (P2) were followed by D2 responses after a delay imposed by the inactive segment in the gap. Proximal responses reflected from the distal segment are designated as P3 or as Pref. The effective refractory period (ERP) of the system is defined in this study as the longest P1-P2 interval at which P2 failed to generate a distal response. The functional refractory period (FRP) of the system is the shortest attainable D1-D2 interval generated across the sucrose gap. Intervals were measured at the midportion of the action potential upstroke ("phase 0").

Results

Conduction and Shunt Resistance

During continuous propagation of an action potential in a homogeneous cable, the impulse may be considered to move from active to resting tissue at a uniform velocity. If, on the other hand, the impulse travels over a serial arrangement of non-homogeneous elements (each of which can be treated
as internally homogeneous), finite interruptions of transmission may occur at one or more junctional sites (Forbes et al., 1923; Rosenblueth, 1958a). This can be demonstrated in Purkinje strands in which an area of depressed conductivity has been induced by the impedance of a sucrose gap. When an impulse arrives at the area of impaired conductivity, it will stop at the junction between the active segment and the inexcitable element in the gap. The impulse may become extinguished, or it may stop and renew its journey only after the delay imposed by the passive electrical properties of the system.

Because a low extracellular impedance is essential for local circuit current propagation in cardiac tissues, it is possible to vary the conduction intervals across the tissue by manipulating the conductivity of the extracellular milieu. The results in Figure 1 demonstrate the critical dependence of impulse conduction on the external impedance between proximal (P) and distal (D) segments of a calf Purkinje fiber mounted in a sucrose gap. P-D conduction times were determined during continuous superfusion of the central segment with sucrose solution, and rhythmic stimulation of the proximal segment at a basic cycle length (BCL) of 2000 msec or 1000 msec. P and D were connected through an external shunt pathway that controlled the extracellular resistance across the gap. The data points were obtained under steady state conditions at each BCL and shunt resistance (SR) value. Figure 1 demonstrates that there is a linear relationship between the conduction interval across the gap and the value of the SR, and that the slope of this relation becomes steeper when the BCL is abbreviated.

The inset in Figure 1 shows superimposed analog records from the same preparation. The upper recordings are transmembrane potentials from the proximal (stimulated) segment and the lower are from the distal segment. Each of the four superimposed traces represents the 10th beat after the value of the SR had been adjusted to 20 KΩ (trace 1); 40 KΩ (trace 2); 50 KΩ (trace 3); and 60 KΩ (trace 4), during continuous stimulation of the proximal segment at a BCL of 1000 msec. When the SR was at 60 KΩ, action potentials initiated in P were blocked.

**Figure 1** Dependence of conduction on the external impedance between proximal and distal segments of a calf Purkinje fiber in the sucrose gap. Ordinate: P-D conduction time expressed in msec. Abscissa: shunt resistance value expressed in KΩ. Triangles: P-D conduction time at a basic cycle length (BCL) of 2000 msec. Circles: data at BCL = 1000 msec. The black lines were drawn by eye. The dotted line extending the upper curve (BCL = 1000 msec) indicates the development of Wenckebach periods with variable conduction times and followed by complete block. Inset: upper traces are transmembrane potentials from P; lower traces are from D. BCL = 1000 msec. Four superimposed traces are shown at shunt resistance values of 20 KΩ (trace 1); 40 KΩ (trace 2); 50 KΩ (trace 3); and 60 KΩ (trace 4). Calibrations: 300 msec and 50 mV.
in the sucrose gap, but their electrotonic effects caused subthreshold depolarizations in the distal segment. A decrease of SR to 50 KΩ caused an increase in the amplitude of the electrotonic depolarizations (trace 3, inset) and in some cases (dotted line, Fig. 1) action potentials were activated in D at a ratio of 2:1 or with Wenckebach cycles at ratios of 4:3 and 3:2. When the SR was decreased further to 40 KΩ, action potentials initiated in the proximal segment produced electrotonic depolarizations with enough amplitude to bring the distal segment to threshold (trace 2) after a delay of 190 msec, imposed by the resistive-capacitive (RC) properties of the system. When the value of the SR was decreased further to 20 KΩ, the rate of rise of the electrotonic depolarization was increased (trace 1) and the P-D latency was decreased to 110 msec.

Conduction Intervals and Prematurity of an Impulse

The frequency-dependent changes in P-D conduction demonstrated in Figure 1 suggest that, especially at high SR values, premature action potentials may not propagate to the distal segment if initiated early in diastole. A detailed analysis of the dependence of the P-D conduction times on the prematurity of the impulse at various SR values is presented in Figures 2-4. In these illustrations, all obtained from the same experiment, the proximal (top) and distal (bottom) activities were recorded from opposite ends of a calf Purkinje fiber in the sucrose gap. The initial response in each panel is the last of a series of 10, evoked by rhythmic stimulation of the proximal segment (P1) at a BCL of 2000 msec. In Figure 2, premature stimuli (P2) were applied at progressively earlier intervals every 10 basic beats. The value of the shunt resistance was set at 20 KΩ. Under these conditions the P1-D1 intervals was 60 msec. Panels A and B show that abbreviation of the P1-P2 intervals from 800 msec to 550 msec prolonged the P2-D2 interval from 93 msec to 105 msec. In panel C, a premature response evoked in the proximal segment at an interval of 470 msec initiated excitation in the distal segment before full repolarization. This discharge elicited an electrotonic depolarization that brought the membrane potential of the impaled cell rapidly to threshold. Even though the amplitude and duration of the D2 action potential in panel C were reduced significantly, the P2-D2 conduction time now was only 83 msec, indicating the presence of a relatively “supernormal” phase. In D, the test interval was 460 msec, P2 was still effective in bringing the distal segment to threshold, and D2 was manifest after an interval of 95 msec which was also “supernormal” with respect to that shown in panel B. In panel E of Figure 2, the premature stimulus, initiated in the proximal segment at 430 msec, failed to excite the distal segment; only a subthreshold depolarization was recorded. The superimposed traces in panel F of Figure 2 show that, under the conditions of impaired conductivity produced by sucrose superfusion of the central segment, but with a low ohmic resistance bridging this segment, an impulse, P2, initiated late during the repolarization phase can induce distal responses at briefer P-D conduction times than P1 responses initiated at earlier intervals. Proximal discharges, induced by premature stimulation at P1-P2 intervals of 440-480 msec, elicited distal action potentials with upstrokes that clustered at a constant D1-D2 of 480 msec. This interval defines the end of the functional refractory period of the system, under the specific conditions imposed on the preparation (see also Figs. 5 and 12).

Activation of the distal segment by electrotonic depolarization across the sucrose gap is dependent not only on the value of the shunt resistance bridging the gap, but also on the basic cycle length (Fig. 1). Successful activation must depend on the passive electrical properties of the system (as modified by changes of SR) and also on the time-dependent changes of these properties (Fig. 3 and 4). In Figure 3, the preparation was maintained under the same conditions as in Figure 2, with the exception of the SR value, which was increased to 35 KΩ. With this maneuver, the basic cycle was scanned with test stimuli at progressively shorter P1-P2 intervals, progressively longer P2-D2 intervals ensued. This was accompanied by a gradual increase in the duration.
Dependence of conduction on prematurity and on the electrical impedance across the gap. Same experiment as in Figure 2 (10/3/78). The preparation was maintained under similar conditions but the shunt resistance was increased to 35 kΩ. The duration of the functional refractory period (854 msec) now outlasted the recovery of the resting membrane potential by about 350 msec.

of the electrotonic “foot” that preceded the action potential, and by a progressive shift of the “take-off” potential to less negative levels. Furthermore, and in contrast with Figure 2 (panels C and D), premature impulses initiated in the proximal segment at relatively long P1-P2 intervals activated the distal segment earlier than impulses at earlier intervals. This is apparent when the records of Figure 3C are compared with those of 3D. In C, the input P1-P2 interval was 700 msec; P2-D2 was 247 msec, and thus the output D1-D2 interval was 854 msec. In panel D, however, abbreviation of P1-P2 to 680 msec produced a large delay in the activation of the distal segment, and the output D1-D2 interval was increased to 900 msec. Stimuli applied to P at intervals earlier than 675 msec (the effective refractory period) were blocked at the gap, and they induced only subthreshold depolarizations in the distal segment (Fig. 3E). These depolarizations decreased in amplitude as the test stimulus was applied at progressively earlier intervals (panel F).

In Figure 4, the value of the shunt resistance was increased further to 50 kΩ, while the BCL was maintained at 2000 msec. Under these circumstances, stimuli at P1-P2 intervals shorter than 915 msec did not excite the distal segment, and the briefest (successful) P1-P2 interval (panel D) yielded an output D1-D2 interval of 1075 msec. In panel F, 30 traces were superimposed to illustrate the progressive increase in the amplitude of the electrotonic depolarizations induced in the distal segment as P2 was initiated at progressively later intervals, reflecting the time-dependent changes of membrane resistance and space constant during diastole.

The Functional Refractory Period

The functional refractory period (FRP) of the system, defined as the shortest attainable interval between two distal responses propagated across the sucrose gap, may long outlast the duration of the repolarization phase (Figs. 2-4). When a high degree of block exists between proximal and distal segments, the FRP is not a function of the “true” refractory period (the distal segment was clearly not “refractory” to stimuli applied directly to it at times earlier than FRP), but it is related to the time-dependent changes in the passive membrane properties during phase 4. This suggests that the duration of the FRP can be changed in a predictable manner by manipulating the electrical impedance across the sucrose gap. Figure 5 summarizes the results of the complete experiment from which the records in Figures 2-4 were taken. In both panels, the D1-D2 intervals are plotted on the ordinates against the corresponding P1-P2 intervals (abscissas), both expressed in msec. Panel A shows the relationships at BCL = 2000 msec. The data ob-
The influence of cycle length and shunt resistance on conduction across the sucrose gap. The values obtained before sucrose served as the control (dotted lines in A and B). A relatively supernormal phase of conduction is apparent at both cycle lengths when the value of the shunt resistance was low (10 KΩ or less).

An increase of the SR to 30 KΩ resulted in a completely different pattern (Fig 5A; unfilled triangles). P1-P2 intervals as long as 900 msec yielded D1-D2 intervals longer than the control; i.e., conduction of the premature response was already compromised. With shorter P1-P2 intervals, D1-D2 increased sharply, producing a "hook" in the curve relating distal and proximal responses. With further increase of the SR to 35, 40, and 50 KΩ, the shape of the curve remained similar but it was deviated to the right and upward. Figure 5A shows that, when the driven cycle is relatively long, the conduction time of a premature beat across the gap, as well as the duration of the FRP, can be varied gradually by manipulating the electrical impedance between the proximal and distal ends of the fiber. In contrast, as shown in Figure 5B, due to the frequency-dependent nature of propagation across the gap (Fig 1), when the basic cycle length was decreased to 1000 msec, graded changes in shunt resistance resulted in abrupt variations in the shape of the input-output relation. This clearly was apparent when the SR was increased from 20 KΩ (filled triangles) to 30 KΩ (unfilled triangles). This manipulation alone was sufficient to increase the duration of the FRP from 450 to 870 msec; i.e., at this point, the duration of the functional refractory period outlasted the duration of the repolarization phase in the distal segment by more than 450 msec.

The influence of shunt resistance on the conductive and refractory periods of the gap preparation was studied in the five experiments summarized in Table 1. The control data were obtained during 4 mM KCl Tyrode's perfusion in all three chambers, whereas all experimental results were obtained at least 1 hour after sucrose perfusion was started in the central chamber. There were large differences in the absolute values of P1-D1 conduction time, ERP, and FRP from one preparation to another, and particularly between the two species represented in the table. However, it is apparent that the degree of conduction impairment in each preparation at a given cycle length is a direct function of the extracellular impedance across the gap.

Excitability as Measured by Current Pulses

Previous studies have demonstrated that the electrical threshold of excitability and the conduction velocity of isolated Purkinje fibers, as studied by the application of brief intracellular current pulses, are fully restored at the moment of complete repolarization and remain relatively constant throughout diastole (Weidmann, 1955; Spear and Moore, 1974a, 1974b). However, as Figures 3 and 4 show, under the conditions of high-degree block in the sucrose gap, a premature stimulus (P2), presented at progressively earlier intervals, activates D2 at gradually longer intervals as a result of an increasingly delayed and less negative "take-off" potential. This is to be expected because a slowly rising depolarization of the membrane can lead to a partial inactivation of the sodium inward current (accommodation) and thus to a gradual shift of the threshold to less negative levels of membrane potential. When the behavior of the preparation is studied by its input-output relations across the gap, the recovery of excitability and conduction velocity can greatly outlast the duration of the action potential (Figs. 4-5). Under these circumstances, excitability, conduction, and functional refractory period depend critically on the characteristics of the input (the electrotonic depolarization), on the passive electrical properties of the system, and on the time-dependent changes of these properties.

We tested this hypothesis in five experiments in which we studied the effects of subthreshold depolarizing current pulses applied either across the sucrose gap, or through an intracellular microelectrode (see Methods). The results, illustrated in Figure 6, obtained from a calf Purkinje fiber-sucrose gap preparation, are representative of this group. The current necessary to bring the membrane po-
tential to threshold (I th) and the potential at which excitation occurs Vth were determined by scanning the diastolic interval with pulses of various durations. As in previous experiments, the test (D) compartment was perfused with 4 mM KCl Tyrode's solution, and the opposite chamber (P) was perfused with 10 mM KCl Tyrode's solution. Under these circumstances, when current was applied across the gap, it was possible to prevent active responses (anodal break excitation) in the proximal segment.

The upper panel of Figure 6 shows two superimposed recordings from a cell in the test compartment, as well as the “threshold potential-interval” curves obtained during the application of depolarizing pulses with durations of 200 msec (filled circles); 100 msec (unfilled squares); and 75 msec (filled triangles). The amplitude of the current flowing across the gap and the “strength-interval curves” obtained in this experiment are presented in the lower panel. The initial action potential is the last of a series of ten evoked by biphasic stimulation of the test segment at a basic cycle length of 2000 msec. In the example shown, a 200-msec, 12.5-μA pulse, beginning 2580 msec after the driven pulse, reached the membrane potential, indicating a lag in the recovery of excitability. When a 200-msec current pulse was delivered at progressively earlier intervals, both Vth and Ith increased gradually until, as shown by the encircled dots in both panels, at 1320 msec (measured at the break of the pulse), current amplitudes of as much as 24 μA induced large depolarizations of the membrane, but failed to initiate an active response. This moment was more than 100 msec later than the full repolarization of the action potential, indicating a lag in the recovery of excitability.

Complete curves like those displayed in Figure 6 for 200-msec pulses were obtained regularly, and a lag in the recovery of excitability was demonstrated in all experiments, particularly at long current pulse durations. When the duration of the current pulses was decreased to 100 and 75 msec (filled triangles and unfilled squares, respectively), and the same approach was used to measure Vth and Ith, the relationships were shifted to the left even though current requirements were significantly increased at all intervals longer than 1500 msec (lower panel). Finally, as a result of the increased current amplit-

### Table 1 Influence of Shunt Resistance and Cycle Length on Conduction across the Sucrose Gap

<table>
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<th>Date</th>
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<td>10†</td>
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BCL = basic cycle length; APD = action potential duration measured at 90% repolarization; P = proximal; D = distal; P-D = P-D conduction time during stimulation; ERP = effective refractory period; FRP = functional refractory period; SR = shunt resistance across the gap.

* Preparation beating spontaneously.
† Partially depolarized fiber.
‡ Spontaneous cycle length decreased after sucrose (540 msec).
§ APD decreased after sucrose (P = 500 msec; D = 460 msec).
Figure 6 Threshold potential and strength-interval curves obtained in a calf Purkinje fiber mounted in the sucrose gap. Data were obtained by scanning the diastolic interval with relatively long depolarizing current pulses applied across the gap every 10th basic beat. Top: two superimposed tracings from original transmembrane recordings illustrate the key for plotting threshold potential (Vth) expressed in mV, as a function of the position of the test pulse. Bottom: superimposed tracings of current records indicate the key for the corresponding threshold currents (Ith) expressed in μA. Symbols in both panels represent results obtained with depolarizing pulses with durations of 200 msec (filled circles); 100 msec (unfilled squares); and 75 msec (filled triangles). Vth and Ith were determined as the difference between the peak subthreshold depolarization induced by a square pulse, and the "take-off potential of an active response initiated at the break of a slightly stronger pulse.

Amplitude of the Vth-interval curve (top panel) flattened and deviated to more negative potentials. An increase of the current amplitude applied across the sucrose gap is, in many ways, analogous to a decrease in the shunt resistance in the electrotonic propagation experiments (cf. Figs. 1 and 2). In the case of Figure 2, a low shunt resistance permitted a rapid membrane depolarization of the distal segment towards threshold at all P2 intervals, with relatively supernormal excitation during late repolarization. Similarly, when 75-msec current pulses were used for the scan (Fig. 6, black triangles), both Vth and Ith remained constant throughout most of phase 4, and a supernormal period of excitability clearly was apparent as a decrease in the amount of current necessary to bring the membrane potential to threshold during the later part of the repolarization phase.

The results presented so far strongly suggest that the characteristics of the input and, more importantly, the passive membrane properties of the cells in the distal compartment, determine the changes in the excitability and conduction across the blocked region. However, the possibility exists that these changes might result from artifacts created by ionic alterations in the boundaries of the sucrose compartments or from alterations of the membranes of cells located in the sucrose chamber, and thus they may not truly represent accommodation or threshold shifts occurring at the site of the impaled cells. These possibilities were studied in three preparations in which current pulses were applied through a microelectrode located very close (50 μm or less) to the recording electrode in the test compartment.

Figure 7 shows a comparison of the threshold requirements of two different cells from the same dog Purkinje fiber preparation. Current was applied across the gap (A1-3) or through a closely located microelectrode (B1-3). In all panels, Vth and Ith were determined at two different intervals after 10 basic driven responses (BCL = 2000 msec), and were measured according to the format illustrated in Figure 6. Several superimposed traces are shown in each panel of Figure 7 to illustrate the threshold values obtained with pulse durations of 10 msec...
FIGURE 7 Changes in threshold potential (top traces) and threshold current (bottom traces) during diastole, in response to depolarizing current pulses applied across the gap \( (A_1 - A_3) \), or through an intracellular microelectrode \( (B_1 - B_3) \) located within less than 50 \( \mu \)m of the recording site in the test compartment. Broken lines were drawn to stress the comparable inclinations of the "threshold voltage-interval" curve, regardless of the experimental technique. Spikes were retouched. See text for further details.

At each test interval, current amplitude was increased very gradually until threshold was reached; the broken lines were drawn by connecting the peaks of the largest subthreshold depolarization at each duration, and the slopes of those lines indicate the changes in excitability during diastole. As this figure shows, the results are almost identical regardless of the technique used. The briefest pulses \( (A_1, B_1) \) required relatively larger current amplitudes (bottom traces), but they rapidly depolarized the membranes of these two cells and threshold was reached at relatively large membrane potentials (—68 mV in \( A_1 \); —76 mV in \( B_1 \)). In panels \( A_2 \) and \( B_2 \), in which duration was 50 msec, the current necessary to bring the membrane to threshold decreased significantly, but in both panels threshold potential was less negative, and a slight inclination of the threshold lines was manifest. When the duration of the pulses was increased to 300 msec \( (A_3, B_3) \), these changes were grossly apparent. Threshold current decreased at both intervals (note change of calibration in \( B_3 \)), and threshold potentials shifted to significantly less negative levels. In addition, the slope of the threshold line was clearly manifest in both panels. Furthermore, current threshold was also greater at the earlier intervals both when pulses were applied across the gap \( (A_3) \), and through an intracellular microelectrode \( (B_3) \). Finally, these two panels also show that, as a result of the slowly rising depolarizations and of the shift of the threshold potential to less negative levels, the amplitude of the action potentials fired by the earlier pulses was significantly lower than the amplitude of responses initiated by later pulses (also apparent in Fig. 6), suggesting the occurrence of a partial voltage-dependent inactivation of the inward current responsible for the action potential upstroke. In fact, as indicated by the encircled points in both panels of Figure 6, complete inactivation was the rule when the relatively long current pulses were initiated very early during diastole.

Clearly, under conditions of impaired conductivity, or when these conditions are mimicked by the application of long current pulses of low amplitude, the amount of current necessary to reach threshold and the threshold potential itself are critically dependent on the passive membrane properties of the cells beyond the blocked area, and on the time-dependent changes of these properties during diastole.

Symmetry of the Preparation

We have demonstrated in previous publications (Jalife and Moe, 1976, 1979) that the conduction characteristics of the tissue are in part dependent on the symmetry of the preparation. In the experiments described in Figures 1-5, the fiber was placed asymmetrically across the three chambers. The proximal fiber segment was significantly longer than the distal segment, permitting successful activation in the P-D direction with unidirectional block in the opposite direction. This is demonstrated in Figure 8, recorded from a preparation driven at a BCL of 2000 msec and with a shunt of 35 K\( \Omega \). The first response is the last of a basic series of 10 initiated in the proximal segment (upper trace). The P-D conduction time was 80 msec. A test stimulus was applied to the distal segment at an interval of 1100 msec; i.e., at a time when almost full recovery in the P-D direction was expected (see Fig. 5B, filled circles). Activation of the P segment...
was not successful; only a small subthreshold depolarization was apparent. Figure 8 also shows that the duration of the action potential initiated in D by the test stimulus was extremely brief. This abbreviation probably resulted from repolarizing current provided by the resting areas beyond the block (Mendez et al. 1969; Sasyniuk and Mendez, 1971).

The Conditions for Reflections

When the conditions of external impedance and symmetry are appropriate, and when the impulse initiated in the proximal segment is delayed sufficiently across the gap, electrotonic transmission in the reverse direction can re-excite the proximal end of the fiber (Antzelevitch et al., 1980). The example of this phenomenon shown in Figure 9 illustrates the critical dependence on the P₁-P₂ interval. The top and bottom traces are recordings from proximal and distal segments, respectively, in a symmetrical preparation (total length ~8 mm). P was driven at a BCL of 2000 msec and, with a shunt resistance of 20 KΩ, P₁-D₁ conduction time was 70 msec. Test stimuli were delivered in all panels at progressively briefer P₁-P₂ intervals. In panel A, a stimulus applied to P₂ at 900 msec was transmitted to D with a P₂-D₂ conduction time of 164 msec. This discharge occurred during the refractory period of P₂, but its electrotonic effects were apparent as a "hump" during early repolarization and as a significant increase in the duration of the P₂ action potential. In B, the P₁-P₂ interval was abbreviated to 860 msec, resulting in further delay of the response in the distal segment (P₂-D₂ = 340 msec). This response occurred late in the repolarization phase of P₂, and it was reflected to the proximal segment with a D₂-P₁ conduction time of 200 msec. The electrotonic manifestation of the reflected P₁ discharge was now apparent in the distal segment as a hump during the repolarization phase of D₂ and a lengthening of its action potential duration. In C, a premature stimulus at 850 msec was blocked in the gap and the distal segment did not reach threshold. Electrotonic effects were manifest in both segments as subthreshold depolarization of the distal end (bottom trace), and as a shortening in the duration of the P₂ action potential (upper trace). At the point in the cycle at which success or failure of excitation was critically poised, very slight variations in the position of the test stimulus resulted in relatively large variations in the delay between proximal and distal elements. When the time of activation of the distal element exceeded the refractory period of the proximal segment (Fig. 9B), the impulse was reflected as a closely coupled premature beat.

Frequency Dependence of Coupled Reflection

The patterns of manifest ectopic activity in the reflection model are very much dependent on the basic frequency (Antzelevitch et al., 1980). We have studied changes in these patterns as a function of heart rate over wide frequency ranges, and we have found that, although these changes are qualitatively similar to those of the parasystole model (Jalife and Moe, 1979), there are important quantitative differences between the two models.

In the example shown in Figure 10, the fiber was positioned symmetrically across the three chambers.
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(totallength ~ 10 mm). The top traces in this figure are transmembrane potentials from the distal segment and the lower traces are from the proximal end of the fiber. In this experiment, the shunt resistance was maintained at 10 KΩ and P was driven at cycle lengths ranging from 850 to 1200 msec in steps of 50 msec. At each of the four illustrated cycle lengths, responses were recorded until a stable pattern of coupled reflections was established. In panel A, the proximal segment was driven at BCL = 850 msec. Coupled reflection occurred on a 3:1 basis. Each reflected response was followed by a compensatory pause, and the manifest pattern of "ectopic" activity was a trigeminal rhythm in the proximal segment (panel A, lower trace). The P-D and D-Pref conduction intervals were approximately constant at about 360 and 240 msec, respectively. This pattern of trigeminy persisted in spite of step-wise changes in BCL to 900, 950, and 1000 msec (not shown). However, the conduction intervals decreased gradually at each BCL until, at 1050 msec (panel B), a pattern of alternating bigeminy and trigeminy was established. P-D intervals were short whenever the preceding P action potential failed to activate the distal segment, and they increased progressively during the bigeminal couplets. This cyclic pattern remained stable until the BCL was increased to 1100 msec (panel C). At this cycle length, a bigeminal rhythm with compensatory pauses was established. Proximal activity brought the distal segment to its cycle length. The abscissa and ordinate represent the driven cycle length of the proximal segment, expressed in seconds. No reflected responses occurred at the longer cycle lengths. Proximal action potentials activated D in a 1:1 manner (DCL:PCL = 1:0), with increasing conduction intervals as the driven cycle was abbreviated. At cycle lengths shorter than 1400 msec, block across the gap occurred at various DCL:PCL levels, including 1.5, 2.0, 3.0, and 4.0. Manifest reflected responses were observed at various patterns including bigeminy (50% PVC's), trigeminy (33.3% PVC's), and quadrigeminy (25% PVC's).

**Discussion**

**Impulse Conduction in Homogeneous and Nonhomogeneous Tissues**

When an impulse propagates along a homogeneous cable of excitable tissue that is nowhere insulated from its saline environment, the wavefront may be considered, at least macroscopically, to move at a uniform velocity. A plot of the distance traversed (x) against the time elapsed (t) will be a straight line with a slope that defines the velocity (Moe et al., 1956; Rosenblueth, 1958a). At the microscopic level, of course, the straight line may be, like the approximations defining a "smooth" curve in the integral calculus, a series of small steps. If we consider an orderly linear sequence of excitable cells, each 100 µm in length, arranged end to end, and connected by low resistance pathways, concepts derived from the local circuit theory of propagation (Hodgkin, 1937; Hodgkin and Huxley, 1952) suggest that cell A in the series reaches threshold and fires cell B after a small but discrete interval, and that the action potential in cell B in turn supplies depolarizing current and initiates a local action potential in cell C (see also Fozzard, 1979). In other words, conduction at this microscopic level is probably "step-wise" rather than smooth and
uninterrupted, but with technically feasible recording methods it appears to be continuous. These concepts do not, of course, demand that depolarization within a single cell be everywhere simultaneous; we are only emphasizing that in "normally" excitable cells, as in a strand of Purkinje fibers, the propagation may be step-wise when time and distance are resolved to sufficiently small units.

The sucrose gap technique is a convenient method for "amplifying" the resolution of time and distance, to enable us to distinguish between a continuous function and a step function; in other words, to deal with milliseconds and millimeters rather than with microseconds and micrometers. In such a nonhomogeneous system, two active elements are connected by a passive segment that permits an axial flow of current, and the step-wise nature of propagation becomes readily apparent. When an impulse initiated in the proximal segment is blocked at the junction with the passive element in the gap, a slowly rising electrotonic potential is recorded beyond the blocked region. The axial current flow between the tissue proximal and distal to the region of block may be sufficient to bring the distal element to threshold, but only after a delay imposed by the amplitude and rate of rise of the electrotonic depolarization (Fig. 6-7). These variables are, of course, determined by the length of the passive element, by the geometry of the preparation, and, more importantly, by the RC properties of the system.

Additional factors are important, including the threshold potential; the partial inactivation (accommodation) of the ionic channels (sodium or calcium, depending on the membrane potential) during the slow rise of the electrotonic potential (Hodgkin and Huxley 1952); the presence of inward-going rectification (Noble and Tsien, 1968); and the upstroke velocity of the action potential (dv/dt of "phase 0"). These factors will also determine the magnitude of the delay of the active response in the distal element; however, all these determinants will depend ultimately on the passive electrical properties of the tissues involved.

The Functional Refractory Period of the System

In a homogeneous system (homogeneous with respect to all properties of the excitable tissue, including excitatibility, conductivity, and refractoriness), in which the velocity of an impulse in fully recovered tissue is defined as a straight line (i.e., dx/dt is constant), we can define the end of the absolute refractory period by another straight line exactly parallel to the first (dx/dt of "phase 0"). These factors will also determine the magnitude of the delay of the active response in the distal element; however, all these determinants will depend ultimately on the passive electrical properties of the tissues involved.

![Figure 12A: diagram to illustrate propagation of impulses in a homogeneous system during the relative refractory period. B: theoretical input-output relationships of premature responses in a homogeneous system, as affected by recording distance. FRP: functional refractory period; ARP: absolute refractory period; RRP: relative refractory period; ERP: effective refractory period. Adapted from Rosenblueth (1958a). See text for further explanation.](image)

If dx/dt increases monotonically, an impulse at ts initiated at any time between ti and ts will encounter tissue less refractory and it will travel initially faster, but it could not reach any point x < x2 earlier than the impulse initiated at the earlier time. The behavior of this system can be defined by the input-output relationship that would be recorded at distances x1 and x2 (Fig. 12B). The FRP of the system is thus seen to be a function, not only of the duration of the "true" refractory period (ti), but also of the duration of the relative refractory period and of the distance traversed. At any distance greater than x2, the FRP will be constant with a duration, D1-D2, that is equal to the total refractory period of the system.

The major point of Figure 12 is that, in a homogeneous system, the relationship between input and output (Fig. 12B) must terminate on the left in a plateau or in a curve of diminished slope that ends abruptly at some point lower than that plateau. If at any point the slope changes sign (i.e., if any point to the left of ts falls above the plateau of x2), the system cannot be homogeneous in the sense we have chosen.
As we have already indicated in the description of Figure 2, except for the longer P-D conduction times and for the presence of a supernormal phase of conduction, the behavior of the Purkinje fiber-sucrose gap preparation, at relatively low SR values (see also Fig. 5), is not very different from that of the homogeneous system we have just described. This is not surprising; the sucrose gap serves only to increase the distance through which the local circuit currents have to travel to induce a response in the next active element. The introduction of a shunt pathway decreases the time constant and increases the amplitude of the passive response, and thus it "decreases" the degree of inhomogeneity of the system. The presence of the supernormal phase of conduction introduces only a minor complexity. Whether the supernormality is within the phase of relative refractoriness (Fig. 2) or at the end, the input-output relationship must still terminate abruptly during the descending branch (Fig. 5A, SR = 10 KΩ) or in a plateau (Fig. 5B, SR = 20 KΩ).

These concepts, except for the addition of a supernormal phase, were carefully considered by Rosenblueth (1958b) in his commonly overlooked consideration of the mechanism of Wenckebach cycles in AV transmission. He concluded that the accepted concept, progressive delay and "fatigue" of the transmission process until block and a "rest" period reset the cycle, was incompatible with the behavior to be expected in a homogeneous system. The cyclic process could be explained more readily in a system in which a finite interruption of transmission occurred at some junctional site. Rosenblueth's "synaptoid" hypothesis was elegantly confirmed by Paes de Carvalho and de Almeida (1960): a transmembrane potential record of a cell in the AV node with a constant input cycle length demonstrated progressively increasing electrotonic delay in the impaired cell until excitation failure occurred, a pattern very similar to the inset in Figure 1.

Because propagation across the sucrose gap is electrotonic, and because this preparation is not a homogeneous system, it is a useful model in which to study conduction block processes, their mechanisms, and their frequency-dependence. When the shunt resistance was set at a relatively high value, i.e., when a high degree of conduction impairment existed from P to D, the FRP of the system outlasted the repolarization phase (Fig. 5). Under these circumstances, the FRP did not depend on the true refractory period but on the passive membrane properties of the tissues involved. Progressive changes in these properties during diastole were responsible for the success or failure of activation of the distal segment. These time-dependent changes during diastole can explain periodic patterns of conduction in cardiac Purkinje fibers (Wenemark and Bandura, 1974; Jalife and Moe, 1979, Fig. 12; Antzelevitch et al., 1980, Fig. 5; this paper, Fig. 10B).

In the AV node (Merideth et al., 1968; Ferrier and Dresel, 1973, 1974), as in the sucrose gap preparation under high SR conditions, or during the application of long current pulses of low amplitude, the duration of the FRP outlasts the action potential duration, threshold current can be greatly increased early in diastole, and excitability gradually recovers at later intervals (Merideth et al., 1968). In both systems, the input-output plot may be smooth until the input interval encroaches on the zone of the FRP of the recipient units, and in both systems the output intervals can increase gradually as the input intervals are abbreviated below the FRP. Although it does not necessarily follow that there is a single and identical mechanism involved, the behavioral similarities become even more striking when the input-output curves at progressively higher SR values (Fig. 5) are compared with the curves obtained by Zipes and Fischer (1974) when studying the time course of the effects of verapamil, D600, manganese, and lanthanum on AV conduction. These agents block the channels of the inward current responsible for the upstroke of the AV nodal action potential (Zipes and Mendez, 1973; Zipes and Fischer, 1974), and by doing so, they directly depress AV nodal conduction and deviate the input-output relationship to the right (cf. Zipes and Fischer, 1974, Figs. 3–6).

In considering the nature of these changes we should bear in mind, however, that similarity of behavior of the depressed AV node on the one hand, and of the Purkinje fiber-sucrose gap preparation at progressively higher SR values on the other, does not prove unity of cause. Nevertheless, the existence of a gradual increase in the excitability of AV nodal cells during diastole leads us to speculate whether these progressive changes in threshold may not reflect a time-dependent change in the passive electrical properties of the nodal cells.

Geometry of the System and Conduction Block

Unidirectional block is the rule when the preparation is positioned asymmetrically across the sucrose gap (Jalife and Moe, 1976, 1979). If, for example, the proximal segment is longer than its counterpart, action potentials will propagate in the P-D direction, but they may be blocked in the opposite direction. When activation across the gap was not successful, the local circuit currents from the depolarized cells to the cells beyond the block hastened the repolarization of the active cells (Mendez and Moe, 1966; Mendez et al., 1969), while they, of course, caused a partial depolarization of the cells beyond the block (Fig. 8). The acceleration of the repolarization phase can be remarkable (more than 50% in Fig. 8). Sasyunik and Mendez (1971) recorded refractory periods of about 40 msec in cells proximal to a site of block in Purkinje-muscle preparations. These cells would otherwise have remained refractory for 200 msec or more.
Another manifestation of block in the sucrose gap preparation was the presence of an electrotonic "foot" in the action potentials of the cells beyond the block. Electrotonic effects were also manifest as "humps" interrupting the repolarization phase and as changes in the duration of the action potential of the recipient fibers (Figs. 9 and 10).

The critical dependence on the geometry was further confirmed when, in some experiments, an attempt was made to readjust the preparation to a more symmetrical position. After this was done, propagation became more symmetrical in both directions. An explanation for this characteristic behavior can be found in the concept of liminal length (Rushton, 1937) which relates the amount of tissue that must be depolarized beyond threshold, to the geometry of the system. Based on this concept, it can be suggested that successful activation of the segment distal to the block depends on the amount of tissue in the proximal segment that exerts a depolarizing influence. This further suggests that premature reentrant activity cannot occur in the atria or ventricles unless a critical mass of tissue exists beyond an area of depressed conductivity.

The Conditions for Reflection

Although we found some variability from one experiment to another (Table 1), the degree of retardation of a driven response across the gap in a given preparation can be controlled accurately by means of the shunt pathway bridging the gap. This delay can be made to exceed 200 to 400 msec for a 2-mm length of depressed tissue when the SR is set at a relatively high value. If the geometry of the preparation is appropriate, and if recovery of excitability has already occurred in the driven end of the fiber, the impulse will be reflected to the proximal segment as a closely coupled premature beat (Fig. 9).

Reflection rarely has been considered in the relevant clinical literature, but it is much more easily demonstrated experimentally than circus movement reentry (Antzelevitch et al., 1980), and it has been demonstrated in theoretical computations of excitable cables with changing geometry (Goldstein and Rall, 1974). In reflection, as in circus movement reentry, an area of impaired conductivity is a prerequisite. The area of block can be provided experimentally by the sucrose gap or by perfusing the central chamber with a solution containing a high KCl concentration instead of sucrose (Antzelevitch et al., 1980). No circuitous loop is required for such a reentry, but in many respects it does behave like a circuit. One may no longer speak properly of conduction, either fast or slow, but the electrotonic transit time imposed by the passive properties of the area of block is, in most respects, equivalent to a conduction interval. As in a true circuit, the functional refractory period of the system is the ultimate determinant of the minimal coupling interval (Fig. 9); but also as in a true circuit, the conduction interval and its variations with frequency may be more important than the FRP in determining the overall coupling intervals.

Coupled Reflection vs. Parasystole

Although no sharply defined boundaries exist between the two mechanisms, the different sequences of patterns of manifest ectopic activity that accompany changes in the basic heart rate should provide important clues for the distinction between reentrant and parasystolic mechanisms. In both sucrose gap models, the patterns of ectopic activity that result from the interaction between two active segments across an area of impaired conductivity depend on the magnitude of the electrotonic influence. In the parasystole model, electrotonic depolarizations of small-to-moderate amplitude can either delay or accelerate the next pacemaker discharge, depending on their timing. As a result of this biphasic influence, a number of mathematically obligatory patterns emerge when the basic cycle length is changed over wide frequency ranges.

Pattern shifts also accompany changes of the BCL in the reflection model. These shifts are obligatory consequences of the time- and frequency-dependent alterations in the passive membrane properties of the tissue. Although these changes are, indeed, qualitatively similar to those obtained in the parasystole model, subtle quantitative differences exist that result from the larger electrotonic influence, and from the absence of a delaying effect of the electrotonic depolarizations in the reflection model. The wide zones of complex patterns of ectopic activity that separate the zones of bigeminy, trigeminy, and quadrigeminy in the parasystole model (Moe et al., 1977) were either very narrow (Fig. 10B) or completely absent (Fig. 11) in the reflection model. Furthermore, due to the fact that no spontaneous activity was involved in the reflection model, and because the FRP of the system and the apparent conduction intervals are both functions of the heart rate, manifest coupled reflections disappeared completely at the slower frequencies (Fig. 11).

Fixed coupling is considered to be an attribute of reentrant premature beats, and, within limits, it probably is (Antzelevitch et al., 1980). As a corollary, fixed coupling is thought to be incompatible with a parasystolic mechanism; beyond certain limits, it is (Moe et al., 1977). The limits are defined by the frequency domains within which the constant coupling intervals occur; under certain conditions, depending on the heart rate, fixed coupling fails to be a satisfactory diagnostic clue to the mechanism of ventricular extrasystole. However, if the patterns of extrasystolic activity can be defined as a function of the heart rate, a much more reliable diagnostic criterion can be derived.
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