Electrophysiological Mechanisms Underlying Rate-Dependent Changes of Refractoriness in Normal and Segmentally Depressed Canine Purkinje Fibers

The Characteristics of Post-Repolarization Refractoriness

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SUMMARY. Tissues from diseased hearts are known to exhibit post-repolarization refractoriness and rate-dependent changes of the refractory period that are often inconsistent with changes in action potential duration. To examine the electrophysiological mechanisms responsible for such rate-dependent changes of the refractory period, a narrow inexcitable zone was created by superfusing the central segments of Purkinje fibers with an 'ion-free' isotonic sucrose solution. The degree of conduction impairment could be finely regulated by varying the resistance of the extracellular shunt pathway. At intermediate or low levels of block, the refractory period remained unchanged or decreased, respectively, as the rate was increased. At relatively high levels of block, however, we observed marked increases of the refractory period in response to increases in the stimulation rate. The disparity of refractoriness between normally conducting fibers and fibers exhibiting discontinuous conduction characteristics and post-repolarization refractoriness increased dramatically as a function of increasing stimulation rate. With the aid of current clamp techniques, we demonstrate that the differential behavior is due to the interplay between rate-dependent changes (1) in the restitution of excitability at the site beyond the depressed zone secondary to changes in passive and active membrane properties and (2) in the intensity of local circuit current provided to that site by activity generated in the segment proximal to the zone of block. Our data suggest that rate-dependent changes of refractoriness in Purkinje tissue are principally governed by attendant changes in membrane resistance. (Circ Res 58: 257-268, 1986)

UNDER normal conditions, the refractory period of most cardiac tissues decreases as the heart rate is accelerated, and increases at slower frequencies (Hoffman and Cranefield, 1960; Moe et al., 1965). These changes are consistent with the rate-dependent changes in action potential duration (APD). Depressed tissues, however, often display rate-dependent changes of refractoriness inconsistent with changes in APD (Cranefield et al., 1971; Lazzara et al., 1975). Such anomalous behavior involving the specialized ventricular conduction system has been the subject of recent reports. Denes et al. (1975) observed no change or abbreviation of the effective refractory period (ERP) at slower heart rates in patients with intermittent bundle branch block. Other studies have shown 'unexpected' responses in the recovery of propagation in bundle branches with changes in heart rate (Nau et al., 1983) and examples of intraventricular aberrancy in patients with heart disease that are inconsistent with the Ashman phenomenon (Fisch et al., 1973).

It is assumed that, in at least some of the cases, the ERP outlasts the duration of the action potential (i.e., post-repolarization refractoriness) (Lazzara et al., 1975). The mechanisms responsible for the frequency-dependent changes of refractoriness in abnormally conducting tissue remains to be determined.

The present study employs a Purkinje fiber sucrose gap model to evaluate the rate dependence of ERP under conditions of normal and abnormal conduction and to assess the rate-dependent changes of active and passive membrane properties involved.

**Methods**

Unbranched free-running false tendons, dissected from the heart of anesthetized dogs, were mounted in a three-compartment tissue chamber. The central compartment was 1.5 mm wide. All three compartments were perfused with normal Tyrode's solution saturated with 95% O₂-5% CO₂ during a 1-hour equilibration period. The composition of the solution (in mM) was: NaCl, 137; KCl, 4-5; CaCl₂, 1.8; NaH₂PO₄, 0.9; NaHCO₃, 20; MgSO₄, 0.5; and dextrose, 5.5. The temperature was maintained at 37 ± 0.5°C.

After equilibration, the central segment was superfused with a purified 'ion-free' isotonic sucrose solution (300 mM) saturated with 100% O₂; CaCl₂ (0.1 mM) was added to the sucrose solution to prevent cellular uncoupling. The two outer compartments were perfused with normal Tyrode's solution. The preparations were stimulated with...
rectangular pulses (1–3 msec duration, twice threshold intensity) delivered through thin silver electrodes insulated except at their tips. The stimulated end of the preparation and the nonstimulated end beyond the gap will be referred to as proximal (P) and distal (D) segments, respectively. In some experiments, basic drive was simultaneously applied to both P and D segments.

Intracellular recordings were obtained differentially from one of the outer segments and referenced to ground from the other. Ag-AgCl electrodes placed in the two outer compartments were connected through a variable ohmic resistor (1 MOh), thus bridging the gap. Since the external shunt pathway provides the return route for most of the local circuit current, the degree of conduction block across the sucrose gap is a function of the impedance within this extracellular pathway.

The effective refractory period of the system and conduction curves were obtained by scanning the basic cycle with single stimuli (P2) delivered after each train of 15–25 basic beats. ERP in this model is defined as the shortest P1-P2 interval at which P2 generates a distal response. We evaluated refractoriness and conduction characteristics at several different levels of conduction impairment, created by adjusting the external shunt resistance (SR) to different values. In some experiments, we first evaluated refractoriness with all three compartments perfused with Tyrode’s solution. At each level of conduction impairment, the ERP and conduction characteristics were measured at four or more different basic cycle lengths (BCL) (range: 300–1500 msec). The preparation was allowed to beat at each new BCL for a period of 3–5 minutes before measurements were performed. Although, in most experiments, the rate of stimulation was altered from high to low rates (i.e., BCL of 300 to BCL of 1500 msec), in some, a random sequence of frequencies was used. After the complete frequency scan was performed, some BCL were studied again as an assessment of reproducibility.

Current Clamp Experiments

In four experiments, once the rate-dependent changes of ERP were evaluated as described above at two levels of shunt resistance (SR), the proximal segment was inactivated with Tyrode’s solution containing 25–30 mM KCl, and constant current pulses were applied across the sucrose gap to mimic proximal activity. Basic drive was delivered to the distal segment (test segment) through bipolar surface electrodes, and 200-msec-long constant current pulses were applied though the Ag-AgCl electrodes placed in the two outer compartments. Different levels of conduction impairment were simulated by changing the intensity of the current pulse. To make the values comparable to those of the previous runs, we initially adjusted the intensity of the current so that values of ERP at the fastest frequency (i.e., BCL = 300 msec) were similar to those obtained with normal activity in the proximal segment.

Current Threshold and Voltage Threshold

The current clamp technique was also used to assess changes in the current necessary to bring the test segment to threshold (Ith) as well as changes of threshold voltage, Vth (i.e., the membrane potential at which the cell develops a regenerative response). The apparent Vth was measured as the intersection of the two lines representing the final slope of the foot potential and the action potential upstroke (see Fig. 6, inset). Care was taken to evaluate Vth at a site within close proximity to the sucrose gap (0.5–1.0 mm from the gap) so that the measurements closely reflect the threshold voltage at the earliest site of activation. Curves relating Vth and Ith to the prematurity of the current pulse (coupling interval) were constructed at several different BCL.

Cable Analysis

A similar set-up was used to study the influence of frequency on passive cable properties. Hyperpolarizing constant current pulses were delivered after each train of basic drive. Differential recordings were obtained from two sites along the test segment. Care was taken to position the microelectrodes along the same longitudinal axis. The distance between the microelectrodes was measured with a micrometer.

The space constant (λ), input resistance (Rin) and membrane time constant (r_m) were calculated in long preparations (test segment 5.5–7.0 mm long; >3 λ). The λ was derived from a semilogarithmic plot of the steady state amplitude of the electronic potential (ΔV) vs distance (d) (Pressler, 1984). The Rin was defined as Vo/Io where Vo is the extrapolated value of the semilogarithmic plot at x = 0, and Io is the applied current. The r_m was defined as the time taken for the electronic potential to reach 84% of its final value (Fozzard, 1977). The intrinsic and extrinsic or specific electrical constants were determined from the values of λ, r_m, and Rin, using the following equations (Weidmann, 1952):

\[ r_1 = \frac{R_{in}}{\lambda}, \quad r_m = R_{in} \cdot \lambda \]

where \( r_1 \) is longitudinal resistance per unit length, \( r_m \) is membrane resistance times unit length, and \( R_{in} \) is membrane capacitance per unit length, and

\[ R_1 = \pi \cdot a^2 \cdot r_1 \]
\[ R_m = 2 \pi \cdot a \cdot r_m \cdot \phi \]
\[ C_m = \frac{r_m}{R_m} \]

where \( a \) is the radius of the fiber and \( \phi \) the folding factor for the internal and external membranes of the fiber (Schoenberg et al., 1975). The diameter of the Purkinje bundle was measured with a micrometer mounted in the stereomicroscope. The fiber diameter was estimated as 40% of the bundle diameter (Pressler, 1984).

In all experiments, the applied current was measured as the voltage drop across a 10 kΩ resistor placed in series with the negative output of the constant current unit (WPI PC-1). The amplified signals were displayed on a Tektronix oscilloscope and photographed with a Grass kymographic camera. APD was measured at 90% of repolarization in all cases.

Statistics

The results obtained at BCL of 300 and 1000 msec were expressed as mean ± SD. The significance of the difference between two means within the same experimental group were analyzed by a t-test for paired data.

Results

Rate-Dependent Changes in Effective Refractory Period

Isolated Purkinje fibers exhibiting normal conduction characteristics generally show a prolongation of APD and refractoriness following a deceleration of basic stimulation. These well-known characteristics are illustrated in Figure 1A, recorded from a Purkinje
FIGURE 1. Effect of rate of stimulation on the effective refractory period (ERP) under conditions of normal and impaired impulse conduction. Each panel shows transmembrane activity recorded from the P (top trace) and D (middle trace) segments of a Purkinje fiber mounted in a three-compartment chamber. The bottom trace shows the stimulus marker. The first beat in each panel is the last of a train of 20 beats elicited by stimulation of the P segment at a basic cycle length (BCL) of either 800 or 1000 msec. The two superimposed sweeps in each panel show the latest premature stimulus that failed to induce a proximal response that propagated across the gap and the earliest one that succeeded. Panel A: all three segments superfused with Tyrode's solution. Panel B: superfusion of the gap segment with sucrose solution. The SR was set at 10 KΩ. Panel C: sucrose gap; SR = 150 KΩ. The numbers denote the values of ERP. SR = shunt resistance. In this and in subsequent figures, the upstrokes were retouched. See text for further details.

fiber mounted in the three-compartment chamber but superfused with normal Tyrode's solution throughout. The two transmembrane records were obtained from the P and D segments in the two outer compartments. The first beat in each panel is the last of a train of 20 beats elicited by stimulation of the P segment at a BCL of either 800 or 1000 msec. Each panel shows two superimposed sweeps of the oscilloscope depicting the latest premature stimulus (applied to the P segment) that failed to induce a propagated response and the earliest that succeeded (the earliest induced proximal response was conducted). At a BCL of 800 msec, the ERP measured 277 msec (Fig. 1A, top) and the APD of P and D responses were 293 and 274 msec, respectively. After a change to a BCL of 1000 msec (Fig. 1A, bottom), a prolongation of ERP (275 to 300 msec) that paralleled the change in APD (P = 292 to 312 msec; D = 260 to 285 msec) was accompanied by a prolongation of ERP to 305 msec.

Panels B and C (Fig. 1) were recorded from the same preparation after the creation of a narrow inexcitable zone by superfusion of the central fiber segment with an ion-free isotonic sucrose solution. The degree of electronic interaction between the two excitable segments in the outer compartments was controlled by adjustments of the SR of the extracellular pathway. With a low resistance to the flow of local circuit current (SR = 10 KΩ; Fig. 1B), the behavior of the system was not very different from that shown in panel A. Deceleration of basic stimulation from a BCL of 800 to 1000 msec produced a prolongation of ERP (275 to 300 msec) that paralleled the change in APD (P = 292 to 312 msec; D = 260 to 285 msec). The lower values of APD reflect the presence of a central inactive segment. With a much greater impedance to flow of local circuit current (SR = 150 KΩ; Fig. 1C), the conduction characteristics and rate dependence of the refractory period were considerably altered. Impulse conduction across the inexcitable gap during basic stimulation was attended by a step delay of 37 msec, and only premature impulses occurring late in diastole succeeded in propagating (i.e., post-repolarization refractoriness). Each panel of Figure 1C is composed of two superimposed sweeps, in this case depicting the latest premature proximal response that failed to propagate and the earliest that succeeded. The change of stimulation rate (BCL = 800-1000 msec) produced a prolongation of APD (P = 302-320 msec; D = 261-291 msec) but an abbreviation of ERP (510-463 msec).

Figure 2 graphically illustrates the rate-dependent changes in ERP (top) and APD (bottom) observed in a similar experiment in which four different levels of conduction impairment were studied. Under normal conditions (normal Tyrode's solution in all three compartments; curve a), and with a low level of block (sucrose gap with SR = 10 KΩ; curve b), changes in ERP were consistent with changes in APD. As the degree of electronic interaction was reduced (i.e, the SR value increased), the absolute values of ERP progressively increased, post-repolarization refractoriness became apparent, and the rate dependence of ERP gradually reversed. At an intermediate level of block (curve c), the ERP remained relatively constant at all frequencies tested. At a still higher level of block (curve d), the curve assumed a negative slope with ERP decreasing as the BCL lengthened.

Because 1:1 conduction is required for the evalu-
Figure 2. Frequency-dependent changes in ERP (top) and APD (bottom) at different levels of conduction impairment. During normal conduction (curve a; Tyrode’s solution throughout) and at low level of block (curves b; sucrose gap; SR = 10 kΩ), changes in ERP parallel changes in APD. At higher levels of conduction impairment (curves c and d; sucrose gap; SR = 150 kΩ and ∞ resistance, respectively), ERP showed no change or a decrease at progressively slower frequencies.

Figure 3. Frequency dependence of ERP. Basic drive was applied to both ends of the preparation simultaneously in order to study the changes in ERP at higher frequencies under relatively high levels of block. Panel A: each panel shows the last beat of the train, the latest premature impulse (delivered only to the proximal side) that failed to propagate, and the earliest one that succeeded. Numbers denote ERP values. Under normal conditions (left), ERP was prolonged by 70 msec following a change of the BCL from 400 to 800 msec. Under abnormal conditions (sucrose gap; SR = 100 kΩ), the ERP was 1130 msec at a BCL of 400 msec and 355 msec at a BCL of 800 msec. Note the different time scales for the left and right panels. Panel B: complete frequency scan performed in the same experiment. Values of ERP are plotted as a function of the basic cycle length (BCL).
fractoriness, we examined the restitution and frequency-dependent characteristics of each element.

**The Source**

The action potential amplitude (APA) and the rate of rise of the action potential upstroke (dV/dt\text{max}) were measured as estimates of the amount of local circuit current generated by the source. Although these parameters accurately reflect the contribution of the source under conditions permitting normal conduction or at a low level of conduction impairment, they are poor measures of the effective source current at higher levels of conduction impairment where discontinuity of conduction gives rise to significant step delays. We therefore considered it appropriate to evaluate also changes of APD and action potential area (APArea).

In four experiments restitution curves of APA, (dV/dt)\text{max}, APD and APArea were performed at five different frequencies. Since delayed activity in the distal segment can importantly alter these parameters, the experiments were conducted under conditions of normal (Tyrode in the gap) or prompt (sucrose gap with low SR) proximal to distal conduction. Figure 4A shows the results of a representative experiment; each parameter is plotted as a function of the prematurity of the test beat (P1-P2 interval). During the initial 350 msec, the rate dependence of the restitution of each parameter was influenced by the concomitant changes in the APD of the preceding basic response such that the value of each parameter at a given interval was generally reduced as BCL increased. At longer intervals, APA showed a small but significant (see Table 1) reduction at the slower frequencies, dV/dt\text{max} remained unchanged, and APD and APArea were significantly increased with deceleration of basic stimulation. Thus, an action potential elicited at an interval of 800 msec

![Figure 4](http://circres.ahajournals.org/)}
Table 1

<table>
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<th>Experiment (msec)</th>
<th>APA (mV)</th>
<th>APD60 (msec)</th>
<th>dV/dtmax (V/sec)</th>
<th>AP area (V msec)</th>
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<td>1000</td>
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<td>340</td>
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Mean ± SD
300 107 ± 3.9 255 ± 16.8 492 ± 26 16.1 ± 5.7
Mean ± SD
1000 101 ± 4.7 344 ± 4.8 493 ± 22 20.9 ± 6.3

P <0.01 <0.05 NS <0.01

All values were obtained at the interval of 800 msec. BCL = basic cycle length; APA = action potential amplitude; APD60 = action potential duration at 90% repolarization. dV/dtmax = maximum upstroke velocity; AP area = action potential area; NS = statistically not significant.

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FIGURE 5. Rate-dependent changes of the ERP evaluated in the same experiment with normal electrical activity in the proximal segment (panel A, left) and with constant current pulses (panel A, right). Each panel is composed of two superimposed sweeps showing the latest proximal response (or current pulse) that failed to conduct (or activate) and the earliest that succeeded. The first beat is the last of a train of 20 beats elicited at a BCL of either 400 or 800 msec. The value of ERP obtained at a BCL of 300 msec with the protocol on the left was used to set the current intensity for the current clamp protocol on the right. Panel B shows the complete frequency scan performed in the same experiment. Closed circles = normal electrical activity at the proximal site; open circles = constant current pulses.

The data presented thus far clearly suggests that the time-dependent changes in source current may contribute to the disparate behavior observed in normal vs. segmentally depressed preparations. To evaluate the importance of the rate-dependent changes of the source component further, we compared the values of ERP obtained with impulse propagation across the gap with those determined in the same preparation after substituting the proximal electrical activity with constant current pulses applied across the gap.

Figure 5 illustrates a representative example. After the rate-dependent changes of ERP had been evaluated in the usual manner, the proximal segment of the preparation was inactivated by superfusion with Tyrode's solution containing 30 mM KCl. After each train of basic stimuli, a single constant current pulse, 200 msec long, was used to scan the cycle. The level of current employed (0.81 μA) was determined by adjusting the current intensity at a BCL of 300 msec to a level at which the value of ERP was similar to that obtained at the same frequency in the previous run. Each panel in Figure 5A shows two superimposed sweeps of the oscilloscope as in Figure 3 (same preparation). The pictures on the right illustrate the refractory periods recorded using the current clamp protocol. Each picture shows the latest application of the current pulse that failed to activate the fiber and the earliest that succeeded. The figure illustrates that a change in BCL from 400 msec to 800 msec produces similar changes in ERP with both protocols. The results of a complete frequency scan are graphed in panel B. Although some difference was observed between the two protocols, the rate-dependent changes in ERP remained a sensitive inverse function of the BCL. These data, together with those from three similar experiments, indicated that the degree to which time-dependent changes in the source current contribute to the frequency dependence of refractoriness in segmentally depressed fibers, in most cases, may be relatively minor.

The Sink

Using similar current clamp techniques, we next evaluated the influence of activation frequency on the excitability of the distal segment (the sink). Strength-interval curves were constructed by scan-
Figure 6. Restitution of Ith (top) and Vth following stimulation at four different BCL levels. Inset: each panel shows a superimposed picture with the subthreshold and suprathreshold current pulse delivered at an SI-S2 of 250 (left) or 350 msec. The upper trace is the transmembrane activity recorded from the test segment of the sucrose gap, and the bottom trace is the current monitor.

Passive Properties

In another series of six experiments, we examined the effects of stimulation frequency on the passive membrane properties of the sink (distal segment) to gain a better understanding of the marked frequency dependence of excitability. After each train of basic stimulation, the diastolic interval was scanned with single hyperpolarizing pulses. The voltage displacement was recorded at two or more sites along the test segment and the membrane electrical properties were calculated. Figure 7 plots these parameters (percentage of change from the values obtained at a BCL of 300 msec) as a function of the BCL. The results were all obtained at an S1-S2 interval of 800 msec. Somewhat to our surprise, we found little or no change in specific longitudinal resistance (Rl), but a marked increase in the specific membrane resistance (Rm), as the BCL was increased from 300–1000 msec (Fig. 7; Table 2). These changes were attended by a small but significant decrease in membrane potential measured at the same S1-S2 interval of 800 msec (Fig. 7; Table 2). Membrane capacitance (Cm) remained unchanged and the space constant (λ) increased from a value of 0.143 cm (± 0.022) at a BCL of 300 msec to 0.179 cm (± 0.04) at a BCL of 1000 msec (P < 0.01). We observed similar changes in cable properties at an S1-S2 of 400 msec.

Discussion

The Sucrose Gap Preparation as a Model for the Study of Refractoriness

Impaired conduction may be the result of homogeneous propagation of depressed responses (Wit et al., 1975) or the result of discontinuities of impulse propagation at one or more sites (Antzelevitch and Moe, 1981; Spach et al., 1982; Rozanski et al., 1984). The characteristics of impulse transmission across inexcitable segments of tissue created under simulated ischemic conditions are mimicked when an inexcitable gap is created by superfusion of the central fiber segment with an "ion-free" (isotonic sucrose) solution (Antzelevitch et al., 1980, Jalife and Moe, 1981). Post-repolarization refractoriness is a salient feature of both systems. However, the sucrose gap preparation offers an added advantage, in that electrical isolation of the two excitable segments in the outer compartments permits fine control of local circuit current flow through a variable resistance placed in series with the external shunt pathway. Moreover, the sucrose gap preparation provides for a clear definition between source and sink factors (discussed below). This feature of the system proved valuable in the evaluation of mechanisms.

Previous studies have shown the versatility of the sucrose gap preparation as a model of parasystole (Jalife and Moe, 1976, 1979; Antzelevitch et al., 1982, 1983), reflected reentry (Antzelevitch et al., 1980), tachycardia (Antzelevitch et al., 1983), brady-
Table 2

Cable Properties

<table>
<thead>
<tr>
<th>Experiment</th>
<th>BCL (msec)</th>
<th>λ (cm)</th>
<th>Rm (kΩ cm²)</th>
<th>Ri (kΩ cm)</th>
<th>Cm (μF/cm²)</th>
<th>Takeoff potential (mV)</th>
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<td>Mean ± SD</td>
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<td>0.143 ± 0.022</td>
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<td>1.55 ± 0.15</td>
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<td>Mean ± SD</td>
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<td>0.179 ± 0.040</td>
<td>8355 ± 2354</td>
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<td>&lt;0.01</td>
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All values were obtained at an S1-S2 interval of 800 msec. BCL = basic cycle length; λ = space constant; Rm = specific membrane resistance; Ri = specific longitudinal resistance; Cm = specific membrane capacitance; NS = statistically not significant.

cardia, and tachycardia-dependent block (Jalife et al., 1983). In the present study, by varying the external shunt resistance across the gap, we were able, in the same preparation, to evaluate refractoriness under conditions that approximate conduction in homogeneous systems and conditions that give rise to marked conduction delays.

The stability of the preparation permitted study of the ERP at several different frequencies and levels of conduction impairment. The same set-up and, in many experiments, the same preparation could be used to evaluate the rate-dependent changes in ERP and passive membrane properties under similar conditions.

Rate-Dependent Changes in ERP

Under normal conditions, rate-dependent changes in refractoriness parallel the attendant changes in action potential duration (APD). In the presence of a short inexdtable gap, when conduction characteristics approach the normal situation (sucrose gap with low shunt resistance), this relationship remains unaltered (Fig. 2). However, as higher levels of conduction impairment (greater shunt resistance values) are achieved, post-repolarization refractoriness develops, and the changes of ERP induced by changes in frequency depend progressively less on APD. At an intermediate level of conduction impairment, the ERP appears to become independent of the rate, and at high levels of block there is a clear inverse relationship between changes in ERP and APD in response to frequency alteration (Figs. 1–3, 5).

Why is Post-repolarization Refractoriness Longer at Faster Frequencies?

It is generally accepted that the determinants of refractoriness and conduction in heart tissue can be divided into source and sink factors, where the source is represented by cells already activated (or an extrinsic stimulus) and the sink is composed of tissue awaiting activation (Fozzard, 1979). The end of the refractory period, in this scheme, may be simply defined as the instant in time when the local circuit current generated by the source meets or exceeds the threshold current requirement of the sink. In the sucrose gap preparation, the source and sink can be readily distinguished as the proximal and distal excitatory segments, respectively. The interplay between the time and frequency dependence of the local circuit current provided by the source and the threshold current requirements of the sink is therefore the ultimate determinant of refractoriness.

Source

Several parameters of the action potential at the proximal site may be measured as estimates of the intensity of the source. These include the action potential amplitude, upstroke velocity, duration, and area. Clearly, it is difficult to generalize which of these parameters, or part thereof, participates in the process of conduction across the inexdtable gap. For example, in preparations manifesting prompt conduction, the upstroke (APA and dV/dtmax) and, possibly, a small part of phase 1 principally provide the current needed for excitation of the distal site. On the other hand, when conduction is delayed, a greater portion of the action potential is involved in providing the current necessary to maintain continuity of impulse propagation. In the latter case, APD and AParea become more important determinants of successful conduction, whereas dV/dtmax and peak amplitude (APA) lose significance (Fig. 4A). The results suggest that for a preparation with a
brief conduction time the level of local circuit current provided by the source is less at slower frequencies. However, under conditions of delayed conduction, the current intensity provided by the source is greater at slower frequencies (Fig. 4).

In other words, the contribution of the source is a function of the level of conduction impairment. When the ability to conduct an impulse is high, the refractory period will be brief. Thus, the frequency-dependent changes in the source that are of importance are those observed early in the cycle (P1-P2 < 350 msec Fig. 4A; P1-P2 = 300 msec (solid lines) in Fig. 4B). At these early intervals, all source parameters decrease, in some cases dramatically, as the BCL is prolonged. However, when conduction is impaired and post-repolarization refractoriness is evident, the frequency dependence of the source must be evaluated later in the cycle [P1-P2 > 350 msec in Figure 4A; P1-P2 = 800 msec (dashed line) in Figure 4B]. In this instance, there is an overall increase in the source contribution at longer BCL.

Previous reports on restitution of APD show similar results (Elharrar and Surawicz, 1983). It would thus appear that the frequency and time dependence of the source component, in part, contribute to the abbreviation of ERP at slower frequencies under conditions of impaired conduction. However, the persistence of frequency-dependent changes in ERP following substitution of the normal electrical activity of the proximal segment with time- and rate-independent constant current pulses of 200 msec duration (Fig 5B), indicates that changes other than those observed in the source component are involved.

Sink

Previous studies of excitability in cardiac tissues have generally been based on the construction of strength-interval curves using stimuli of 4- to 8-msec duration. Under conditions of delayed impulse propagation, where the effective source current may span the duration of an entire action potential, such brief stimuli are clearly inappropriate. To assess the excitability of the sink under conditions of impaired conduction, we therefore derived the strength-interval relationships using 200-msec current pulses. Under these conditions, the threshold current requirements decreased throughout diastole, as previously reported by Jalife and Moe (1981). Furthermore, our results demonstrate that the excitability of the sink improves markedly as BCL is prolonged (downward shift of strength-interval curves; Fig. 6A).

These results coupled with those of Figure 5 suggest that under conditions of post-repolarization refractoriness, changes in excitability may in large part account for the rate-dependent changes in ERP. The improvement of excitability observed at slower frequencies may, in turn, be due to several factors including: (1) decrease in takeoff potential, (2) increase in the threshold voltage (more negative values), and (3) changes in passive membrane properties as discussed below.

a) Transmembrane Potential

The progressive negative shifts of the take-off potential observed with increasing frequency (Fig. 7) may be attributable to an increase in maximum diastolic potential and overdrive suppression of phase 4 depolarization. In the steady state, both effects are, in large measure, believed to be due to an increase in electrogenic current resulting from stimulation of the Na-K pump (Vassalle, 1970). The decrease of take-off potential at slower frequencies results in improved excitability due to the closer proximity of the membrane potential to the threshold voltage (Fig. 7), as well as to secondary changes in the passive properties of the sink (increase in $R_m$, discussed below).

b) Voltage Threshold ($V_{th}$)

The smaller potential difference between membrane and threshold voltages at the slower frequencies was due not only to a deceleration-induced depolarization, but also to a negative shift of the threshold voltage (see Fig. 6). Similar results were reported by Jalife and Moe (1981) with 200-msec current pulses. The reason for the frequency-de-
Pendental changes in $V_{th}$ are not clear, but they may be related to the liminal length concept of Rushton (see Fozzard and Schoenberg, 1972). The liminal length may be defined as the length of fiber that must be raised above a given voltage threshold in order for a propagated action potential to be generated. At slower frequencies, we encounter longer space constants (due to increased $R_m$), and, therefore, a flatter voltage distribution along the length of the preparation. A liminal length may thus be achieved with a smaller stimulus, and the threshold voltage recorded near the current source (i.e., at a distal site near the sucrose gap border) should therefore be more negative (c.f. Fig. 7 of Fozzard and Schoenberg, 1972).

c) Passive Membrane Properties

Recent studies that focused on changes in passive properties accompanying frequency- and time-dependent changes in conduction velocity in dog papillary muscle preparations (Spach et al., 1982) and rabbit atrial trabeculae (Bredikis et al., 1981) have concluded that these changes are due principally to an alteration in cell-to-cell coupling (increased axial resistance). In light of these reports, we were surprised to find little to no variation in longitudinal (axial) resistance ($R_l$) in response to frequency changes in the dog Purkinje fiber preparations studied. Instead, we found marked changes in membrane resistance ($R_m$) at the different frequencies (Fig. 7). The higher $R_m$ at slower frequencies appears to account fully for the observed prolongation of the space constant. These changes are consistent with the rate-dependent changes in conduction velocity observed in normally conducting Purkinje fibers (Peon et al., 1978), and are probably responsible for the important changes in the excitability of the sink that affect conduction in fibers manifesting delayed impulse propagation.

These results concur with those recently reported by Pressler (1984) who found a near doubling of $R_m$ with no significant change in $R_l$ or $C_m$ in sheep cardiac Purkinje fibers studied under resting as compared with paced (2 Hz) conditions. In that study, as in the present one, the changes in $R_m$ appear to be due largely to the accompanying changes in membrane potential (take-off potential). The voltage dependence of $R_m$ is well known (Weidmann, 1952; Hall et al., 1963; Hellam and Studt, 1974). In changing BCL from 300 to 1000 msec, we observed a 56% increase in $R_m$ and a 5-mV depolarization (−85 mV to −80 mV), Hellam and Studt report a comparable change in $R_m$ (60% increase) over this range of voltage. This similarity, however, does not exclude other contributory factors such as extracellular cleft K⁺ accumulation (Kline et al., 1980).

The voltage dependence of $R_m$ is in large part due to inward rectification of the outward potassium currents (Hall et al., 1963). Because the steepness of this relationship is a function of the voltage range, it should be noted that, in diseased fibers, the rate-dependent changes in cable properties must, to some degree, depend on the level of depolarization produced by the disease state.

Although the qualitative changes presented in Table 2 and Figure 7 may be incontestable, we must hasten to point out some limitations of methodology and oversimplifications applied in the calculations of the cable constants. Chief among these is the calculation of $R_m$ based on the applied current without correction for the extracellular shunt current across the sucrose gap. Although we did not quantify the fraction of shunt current relative to the total current applied in this study, preliminary results from an on-going parallel study employing similar preparations provide some insight. Using the methodology of Kleber (1973), we measured an $r/\tau_c$ ratio of 0.45 ± 0.06 (four experiments) across the sucrose gap. Approximately 31% $r/(r + \tau_c)$ of the applied current is therefore shunted extracellularly across the gap (see Beeler and McGuigan, 1978). Consequently, the values of $R_m$, $r$, $\tau$, $R_m$, and $R_l$ must be considerably underestimated. Also affecting the calculated values for the specific electrical constants is our estimation of the fiber diameter as 40% of the diameter of the Purkinje bundle, based on the results of Pressler (1984). These sources of error may alter the absolute values of the cable constants in our study by as much as 50%, but are unlikely to alter the qualitative findings (Fig. 7).

Also deserving of comment is the fact that changes in the electrophysiological characteristics of the sink in this model may not apply for all disease states in which impulse conduction may be impaired. Rate-dependent changes of excitability, membrane potential, and passive membrane properties are likely to differ quantitatively, and perhaps qualitatively, in preparations depolarized to various levels following acute ischemia, stretch, or other insults.

The Inexcitable Gap

The results thus far discussed have implicated changes in the electrical properties of the source and, more importantly, the sink, in explaining the frequency dependence of conduction and refractoriness across an inexcitable gap created by superfusion of a central Purkinje fiber segment with an ion-free sucrose solution. The possible contribution of the inexcitable zone (gap) between source and sink deserved some comment.

Previous studies have demonstrated the passive function of the gap (Jalife and Moe, 1981; Antzellevitch and Moe, 1983a). The time-dependent changes of excitability, membrane potential, and passive membrane properties are likely to differ quantitatively, and perhaps qualitatively, in preparations depolarized to various levels following acute ischemia, stretch, or other insults.
Furthermore, the lack of frequency-dependent changes of \( R_t \) measured across the gap in the present study is consistent with the results of Pressler obtained through intracellular injection of current, and strongly suggests that local circuit current flow within the inexcitable gap is unaffected by the activity in the outer excitable segments. Moreover, in a preliminary study employing the methods described by Kleber (1973) to evaluate the \( r;e \) ratio within the sucrose gap, we found little to no change in response to frequency alterations (unpublished observation).

Finally, these effects of frequency on ERP are not limited to sucrose gap preparations, but occur in preparations in which narrow inexcitable zones are formed under ischemic (ischemic gap preparation) or pressure block conditions as well as in spontaneously depressed fibers (unpublished observations).

**Clinical Implications**

The results provide a mechanistic understanding for the anomalous changes in the refractoriness of the ventricular conduction system in response to changes in heart rate in patients with various degrees of conduction disturbance. Strikingly similar relationships were reported by Denes et al. (1975) in a study of bundle branch refractoriness in a group of patients with rate-dependent bundle branch block. In four of five patients in this group, the "expected" abbreviation of refractoriness with shortening of cycle length, observed in normal patients, did not occur. The similarity of these results to those observed in our preparations (Fig. 2; c.f. Fig. 4 of Denes et al., 1975) suggests that the mechanisms responsible for post-repolarization refractoriness and the altered rate dependence of the refractory periods in these patients may be similar to those operative in the sucrose gap model.

The study also highlights the dramatic development of disparity of refractoriness between normal and depressed tissues as the heart rate is accelerated (Fig. 3), setting the stage for the development of reentrant arrhythmias. Although the data are derived from Purkinje fiber preparations, it is tempting to speculate that similar mechanisms may be responsible for the anomalous heart rate dependence of refractoriness observed in depressed myocardial tissues (Cranefield et al., 1971; Lazzara et al., 1975) as well as the paradoxical increase in disparity of refractoriness and decrease in fibrillation threshold produced by the acceleration of the heart rate in ischemic hearts (Kent et al., 1973).

**Conclusions**

Rate-dependent changes in ERP in the sucrose gap model are related to rate-dependent changes of both current supply by the source and current requirements in the sink. Under normal conditions, both factors are greatly influenced by the APD; changes in ERP, therefore, parallel those of APD.

When an area of block is interposed between two excitable tissues, long conduction delays and post-repolarization refractoriness develop, and the effective current provided by the source and the excitability of the sink (late in diastole) become independent of APD. Under these conditions, the frequency-dependent changes in ERP may be opposite to those encountered under normal conditions. The abbreviation of the refractory period observed at slower frequencies may be explained by (1) an increase in the excitatory current provided by the source due to an increase in APD and AParea and (2) improvement of the excitability of the sink secondary to changes of passive electrical membrane properties.

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