Determinants of Postrepolarization Refractoriness in Depressed Mammalian Ventricular Muscle

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SUMMARY. Functional determinants of postrepolarization refractoriness were studied with microelectrodes in isolated cat and dog ventricular muscle preparations mounted in a three-chambered bath. Frequency-dependent conduction delay and block were readily manifested when the central segment (1 mm) was superfused with high potassium (20–30 mM) Tyrode's solution. Conduction disorders were attributed to postrepolarization refractoriness involving slow recovery in the amplitude of elicited subthreshold depolarizations in depressed fibers distal to the central blocked zone. Investigations of subthreshold phenomena in homogeneously depressed tissues indicated that a relatively large local response participated in the voltage displacement induced by subthreshold depolarizing currents. The local response was blocked by tetrodotoxin (10 μg/ml) or verapamil (2 μg/ml) when resting membrane potential was near −70 or −50 mV, respectively. At either level of reduced membrane potential, gradual recovery in diastolic excitability correlated closely with time-dependent recovery of the local response, the rate of which was also proportional to the current intensity applied. Thus, postrepolarization refractoriness in depressed ventricular muscle fibers is a function of the time for recovery of active subthreshold properties (the local response), as well as intensity of excitatory current input. These factors may play a role in the development of delayed conduction and reentry that occur at faster heart rates under ischemic conditions. (Circ Res 55: 486–496, 1984)

CONDUCTION in normally polarized ventricular muscle is a relatively rapid process where excitability is restored upon complete repolarization of membrane potential (Spear and Moore, 1974). During ischemia, excitability of cardiac fibers decreases to levels where conduction through affected tissue becomes markedly delayed and nonhomogeneous (El-Sherif et al., 1977; Spear et al., 1983). Under these conditions, the incidence of conduction disorders and arrhythmias is increased at faster heart rates (Hope et al., 1974; El-Sherif et al., 1977). Rate-dependent aberrations in depressed tissues are believed to involve a prolonged refractory period that outlasts the duration of the action potential, i.e., postrepolarization refractoriness (Lazzara et al., 1975). The relationship of this property to arrhythmogenesis is that conduction delay and reentry is likely to occur at longer than expected basic cycle lengths, thus expanding the period of ventricular vulnerability well into diastole (Singer et al., 1981). It is generally thought that postrepolarization refractoriness in depressed myocardium is due to prolonged recovery from inactivation of regenerative inward currents (Gettes and Reuter, 1974; Kohlhardt et al., 1975; Shimoni, 1981). However, this mechanism may not completely account for relatively long refractory periods that may exceed 500–1000 msec (Senges et al., 1979; Jalife and Moe, 1981; Antzel-evitch and Moe, 1983). Thus, while postrepolarization refractoriness probably imposes conditions favoring conduction disturbances and arrhythmias in situ, a detailed understanding of its determinants is lacking.

The present investigation studied the mechanism of postrepolarization refractoriness and related frequency-dependent block in isolated depressed ventricular muscle. When conduction was impaired, frequency-dependent aberrations were related to slow recovery of excitability in depressed fibers beyond a site of block. Changes in diastolic excitability were due to slow recovery of nonregenerative subthreshold currents (the local response) which were shown to be an important determinant of postrepolarization refractoriness in depressed tissues.

Methods

Frequency-Dependence of Impaired Conduction

Papillary muscles and ventricular trabeculae, approximately 1 mm in diameter and 5–10 mm long, were isolated from hearts excised from anesthetized (sodium pentobarbital, 30 mg/kg) cats and dogs. Left ventricular epicardial strips (approximately 1 × 1 × 10 mm) were also obtained from some hearts by cuts made parallel to the fiber orientation with a double razor blade. Tissues were threaded through preformed holes in the latex membranes of a three-chambered bath in which the central chamber, i.e., gap, was 1 mm wide. Preparations were allowed to equilibrate for 1 hour while superfused at 37°C with oxygen-
ated (95% O<sub>2</sub>, 5% CO<sub>2</sub>) Tyrode’s solution. Unless otherwise stated, the composition of Tyrode’s solution was (in mm): NaCl, 137; KCl, 4; NaH<sub>2</sub>PO<sub>4</sub>, 0.9; NaHCO<sub>3</sub>, 20; CaCl<sub>2</sub>, 1.8; MgSO<sub>4</sub>, 0.5; and dextrose, 5.5.

A total of 31 tissues were isolated for study. In a first series of seven experiments, the effects of altering basic cycle length (BCL) on impaired impulse conduction were studied using a model of segmental block described previously (Rozanski et al., 1984). Conduction impairment was established by superfusing the central segment with Tyrode’s solution containing a K<sup>+</sup> concentration ([K<sup>+</sup>]) of 20–30 mm while the outer segments were exposed to 4 mm [K<sup>+</sup>]-Tyrode’s. This induced a narrow zone of block where the degree of conduction impairment could be modulated by changing the resistance of a shunt pathway connecting the outer chambers. Preparations were driven at BCL ranging from 3000 to 200 msec with rectangular stimuli (1–2 msec in duration, 2.5 times diastolic threshold intensity; Frederick Haer, P6i) delivered through silver bipolar electrodes placed at either end of the preparation. Transmembrane recordings were obtained from fibers 0.5–1 mm from the latex membranes at points proximal (P) and distal (D) to the central blocked zone. Amplified signals were displayed on an oscilloscope (Tektronix, 565) and photographed on 35-mm film (Grass, C4R). Conduction between proximal and distal sites was measured as the time interval between the midpoints of action potential upstrokes.

Under these conditions of conduction impairment, proximal activity generates an electrotonically mediated subthreshold depolarization (SD) in fibers distal to the blocked zone. A regenerative action potential develops when the SD reaches threshold voltage (V<sub>t</sub>), an event manifest as a “foot” preceding distal activation (Antzelievitch and Moe, 1981; Jalife and Moe, 1981). In the context of the present study, the SD is considered to be the “stimulus” for distal (all-or-none) excitation and, therefore, either does, or does not, induce a regenerative upstroke. Accordingly, conduction failure is defined as the inability of the SD to generate an action potential beyond the blocked zone.

**Determination of SD Characteristics**

Fourteen additional preparations were studied in the three-chambered bath in which the central segment was superfused with an isotonic sucrose solution containing 0.1 mm CaCl<sub>2</sub> and equilibrated with 100% O<sub>2</sub>. Transmembrane potentials were recorded differentially from the test (distal) segment which was limited to a length of approximately 1 mm. The test segment was driven at a BCL of 1000 msec with constant current pulses (5 or 10 msec duration, 2.5 times diastolic threshold intensity) applied through Ag-AgCl electrodes in the outer chambers. The remaining outer (proximal) segment was superfused with 25 mm [K<sup>+</sup>]-Tyrode’s to suppress regenerative responses and facilitate current passage. Test current pulses (100 msec) of either polarity were applied, through the same Ag-AgCl electrodes, at progressively shorter intervals after every 10th basic current pulse. Current was measured as the voltage drop across a 1000 ohm resistor in series with the negative side of the circuit.

Characteristics of SD generated by current pulses were studied under control conditions and at levels of reduced resting membrane potential (RMP) associated with depressed fast channel (approximately −70 mV) and slow channel (approximately −50 mV) mediated action potentials. Reduction in RMP to the desired level was achieved by elevating the superfusate [K<sup>+</sup>]. We also investigated time-dependent changes in SD amplitude by scanning diastole with current pulses of constant magnitude. In these experiments, a train of 10 basic current pulses (BCL = 1000 msec) was followed by a 2-second diastolic pause during which identical test pulses were applied at progressively earlier intervals. The current applied during a control diastolic scan was chosen to be just subthreshold in late diastole.

Since mixing of solutions through the extracellular space at the Tyrode’s-sucrose boundary must occur to some extent (Kleber, 1973), it is possible this factor might introduce artifacts in the voltage response of test fibers to subthreshold currents. Therefore, 10 other experiments were conducted, using a suction electrode to apply current to small ventricular muscle segments (approximately 2 × 2 mm) homogeneously superfused in a single chamber. The suction electrode, filled with normal or modified Tyrode’s solution, was made of polyethylene tubing (inside diameter, 0.86 mm) and coupled to a constant current source through an Ag-AgCl electrode. Current was measured in the same manner as described above.

All data values are expressed as mean ± SEM. Analysis of variance was performed for simultaneous comparison of data from the three levels of RMP studied. When a significant difference among groups was indicated by the initial analysis, individual paired comparisons were made using a Bonferroni modified t-test (Wallenstein et al., 1980). Differences were considered significant at P < 0.05. Drugs used in these experiments were: tetrodotoxin (TTX; Sigma), verapamil (Isoptin; Knoll), lidocaine hydrochloride (Astra), and acetylstrophanthidin (Lilly).

**Results**

**Frequency-Dependent Conduction Delay and Block**

The effects of altering BCL on impaired impulse conduction were studied in seven preparations. Figure 1 typifies the response observed in all seven experiments to an abbreviation in BCL. In this example, the gap was perfused with 30 mm [K<sup>+</sup>]-Tyrode’s which partially depolarized fibers at the proximal and distal gap boundaries to −78 and −76 mV, respectively. At BCL = 1500 msec (panel A), conduction was 1:1 and the SD generated by electrotonic current from proximal tissue activated the distal segment with constant delay (P – D = 28 msec). At BCL of 700 msec (panel B), steady state proximal action potential amplitude was reduced by 4 mV and stable 2:1 block developed (P – D = 38 msec). This pattern evolved from an alternation in SD amplitude with no beat-to-beat change in the input (action potential amplitude) from the proximal segment. Thus, as illustrated in this figure, the SD represents a critical link for the continuation of propagation from the proximal to distal segment.

Figure 2 shows representative traces of a complete BCL scan from the same experiment as shown in Figure 1. Panels A and B demonstrate the change in conduction from a 1:1 to a 2:1 pattern in response to shortening BCL from 1000 to 800 msec. This BCL...
FIGURE 1. Frequency-dependent conduction block. Transmembrane recordings from fibers proximal (P) and distal (D) to a zone of block (gap [K+] = 30 mM) are shown. Panel A: BCL = 1500 msec. Panel B: two superimposed traces are shown at BCL = 700 msec. For purpose of clarity, only the proximal action potential corresponding to a successfully conducted beat is shown. Potentials traced from original records. Cat papillary muscle. Shunt resistance = 0 Ω.

change was attended by a 3-mV decrease in the amplitude of the proximal action potential, but during the 2:1 rhythm, there was no beat-to-beat change in this parameter. When BCL was 600 msec (panel C), only every third SD excited the distal segment (P − D = 47 msec), resulting in a repetitive 3:1 conduction pattern. Following a conducted impulse (first distal action potential), successive proximal action potentials were elicited progressively larger SD (dashed line) that eventually induced a distal action potential. The progressive growth in SD amplitude was even more apparent at higher degrees of block, achieved by stimulating at still shorter BCL, as shown in panel D. At this BCL (500 msec), a 5:1 pattern evolved (P − D = 48 msec) where successive blocked beats were associated with progressively larger SD. As in panels B and C, the gradual increase in SD amplitude could not be accounted for by a concomitant change in proximal action potential amplitude. Further BCL shortening.

FIGURE 2. Steady state conduction patterns as a function of BCL. Note the progressive increase in peak SD amplitude at high degree of block (panels C and D: dashed lines) even though proximal action potential amplitude (input) at steady state BCL is constant. Distal upstrokes retouched. Same experiment as Figure 1.

FIGURE 3. Postrepolarization refractoriness. Panel A: premature proximal action potentials were elicited at progressively shorter intervals after every tenth basic beat (BCL = 2000 msec). Only premature action potentials at coupling intervals of 950 msec or less are shown. Cat papillary muscle; gap [K+] = 25 mM; shunt resistance = 10 kΩ. Panel B: Dynamic changes in peak SD amplitude could be mimicked with constant current pulses applied through Ag-AgCl electrodes in the outer tissue chambers. Cat papillary muscle; gap [K+] = 30 mM. Panel C: marked time-dependent changes were also observed in epicardial strips. Cat epicardial strip; gap [K+] = 30 mM. Time calibration bar is 500 msec for panels A and C and 450 msec for panel B.
(less than 300 msec) resulted in complete block, with little or no change in SD amplitude (not shown).

**Postrepolarization Refractoriness**

Figures 1 and 2 suggest that the time for recovery of distal excitability for impulses transmitted across the gap may last several hundred milliseconds, indicating the effective refractory period of the system greatly outlasts the action potential duration of recipient fibers. This phenomenon, termed postrepolarization refractoriness (Lazzara et al., 1975), was evaluated in four of seven segmental block preparations, of which Figure 3A is an example. In this experiment, premature proximal responses (P2) were induced at progressively shorter intervals after every 10th basic beat (P1; BCL = 2000 msec). The effective refractory period, defined as the longest P1-P2 where P2 fails to excite the distal segment, was between 800 and 875 msec, far longer than the duration of the basic distal action potential (179 msec, measured at 50% of repolarization). Premature proximal action potentials that blocked at intervals of 800 msec or less were associated with progressively smaller SD in partially depolarized distal fibers (RMP = -74 mV). To test whether this change in SD amplitude was due to a concomitant decrease in depolarizing current reaching the distal segment, constant current pulses were applied across a high [K+] induced-zone of block, through Ag-AgCl electrodes located in the outer chambers. An example of such an experiment is given in panel B in which only potentials from the distal segment are shown. Using the same pacing protocol as in panel A, identical current pulses (100 msec) applied at intervals of 640 msec or greater elicited SD of sufficient amplitude to excite the distal segment (RMP = -72 mV). However, the same current pulse applied at intervals progressively earlier than 640 msec did not activate the distal segment, and elicited progressively smaller SD. Panel C shows another current pulse experiment conducted in an epicardial strip to determine whether diastolic change in SD amplitude might be due to the presence of latent pacemaker elements (i.e., Purkinje fibers) in the distal segment. As shown in panel C and found in three additional experiments, diastolic change in SD amplitude occurred in the absence of Purkinje fibers, and thus probably represents an inherent property of depressed ventricular muscle.

The time-dependent increase in amplitude of SD elicited by propagating action potentials or by constant current pulses suggests that an important determinant of refractory period is the time for recovery of SD amplitude relative to threshold voltage. However, the underlying basis for this recovery is difficult to assess quantitatively in view of the heterogeneous excitability present under segmental block conditions (Antzelevitch and Moe, 1981). It is possible that activation of inward nonregenerative current contributes to the morphology and peak amplitude of the SD. Furthermore, time-dependent changes in SD amplitude could be explained on the basis of a concomitant decrease in the threshold voltage, or to a change in passive properties. To investigate these possibilities in more detail, voltage- and time-dependent properties of the SD were studied using the sucrose gap and suction electrode techniques where homogeneous excitability in recipient fibers could be achieved.

**SD Characteristics as a Function of RMP**

The voltage response of ventricular muscle fibers to subthreshold currents was studied at RMP levels near -90, -70, and -50 mV, in a total of 24 preparations—14 with the sucrose gap and 10 with the suction electrode technique (see Methods). The morphology of the SD induced by square current pulses was a function of the RMP from which it developed, regardless of the procedure used to pass current. Furthermore, at a given RMP level, no quantitative differences (other than current magnitude) were observed between the two techniques, and thus data from both series of experiments were pooled. Figure 4 illustrates the characteristic SD recorded at each level of RMP studied in both sucrose gap (panel A) and suction electrode (panel B) experiments. In the presence of 4 mM [K+] Tyrode’s solution, ventricular muscle fibers had a RMP near -90 mV (-89.5 ± 1.6 mV; n = 18), and the SD elicited by a subthreshold current pulse was more or less typical of a passive (RC) change in potential (panels A1 and B1). In 16 preparations superfused with Tyrode’s containing 10–15 mM [K+] (12.1 ± 0.5 mM), RMP decreased in each case to approximately -70 mV (-69.2 ± 1.3 mV) and excitability was reduced, as evidenced by an increased current requirement for excitation and a more positive threshold voltage (Vth), compared with control (see Table 1). At this RMP level, the induced SD was no longer monophasic, but rather, consisted of an early transient component (panels A2 and B2). Finally, a different SD was recorded in all of 11 experiments in which RMP was reduced to near -50 mV (-54.5 ± 1.3 mV).
TABLE 1
Resting Membrane Potential (RMP) and Threshold Voltage (Vth) of Fibers with Depressed Fast and Slow Channel Action Potentials

<table>
<thead>
<tr>
<th>[K+] (mM)</th>
<th>RMP (mV)</th>
<th>Vth (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>-89.5 ± 1.6 (18)</td>
<td>-69.2 ± 1.3* (16)</td>
</tr>
<tr>
<td>10-15</td>
<td>-69.2 ± 1.3* (16)</td>
<td>-54.7 ± 1.5* (10)</td>
</tr>
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All data values were determined at mid-diastole (BCL = 1000 msec) in preparations studied with the sucrose gap and suction electrode. Threshold voltage (Vth) was estimated from the take-off potential measured at the inflection point where the SD merged into phase 0 of the action potential. Numbers in parentheses represent number of preparations. Simultaneous comparisons were made using an analysis of variance followed by a modified *t*-test when a significant difference among groups was indicated.

* Significantly different from control.
† Significantly different from depressed fast response.

result of inward-going rectification (McDonald and Trautwein, 1978). However, in depressed fibers, a relatively large but nevertheless nonregenerative inward-current component, hereafter referred to as the local response, also participated in the voltage displacement induced by subthreshold depolarizing currents. This is indicated by the experiments shown in Figure 5.

Figure 5A illustrates the characteristic SD recorded at RMP near -70 mV, consisting of an early transient component that was absent during hyperpolarization. Superfusion of 10 µg/ml TTX eliminated the transient component (panel B), indicating that the SD generated from RMP near -70 mV is composed, in part, of a local response mediated by the fast channel. Blockade of the local response was achieved by adding either TTX (n = 3) or lidocaine (5 µg/ml; n = 3) to the superfusate. Figure 5C gives an example of the SD typically recorded at RMP near -50 mV. At this membrane potential level, the late component appeared only with depolarizing pulses. When the same fiber was exposed to 2 µg/ml verapamil (panel D), the late component was abolished. This type of response was observed in all seven experiments testing verapamil, indicating that at RMP near -50 mV, the SD is composed of a slowly developing local response mediated by the slow channel.

Time-Dependent Changes in SD Amplitude and Excitability

Change in SD amplitude as a function of time was assessed by applying identical current pulses at progressively earlier intervals after the last action potential in a train of 10 basic beats. Qualitatively similar results were obtained in both sucrose gap and suction electrode experiments. Figure 6 illustrates a typical diastolic scan recorded at each of the three levels of RMP. Each panel shows nine superimposed traces of phases 3 and 4 of the last action potential in the train and the SD generated by identical depolarizing current pulses. Also shown are threshold current (Ith) and voltage (Vth) values.

![Figure 5](http://circres.ahajournals.org/) Subthreshold voltage responses elicited at reduced RMP. Current pulses that just failed to produce a regenerative upstroke were applied to preparations mounted in the sucrose gap. Panel A: voltage responses (bottom trace) generated by current pulses (top trace) of either polarity in a fiber at RMP of -70 mV (10 mM [K+]). Panel B: the same pulses applied after superfusing 10 µg/ml TTX. Panel C: the voltage response induced by a depolarizing pulse in a fiber with RMP of -57 mV (20 mM [K+] 10 µg/ml TTX) exhibited a slowly developing component that was blocked by verapamil (2 µg/ml; panel D).
FIGURE 6. SD amplitude and excitability. Each panel shows nine superimposed traces of phases 3 and 4 of the last action potential in a train of 10 basic beats (BCL = 1000 msec) and the SDs generated at various intervals by identical test current pulses. Also shown are threshold current (I_{th}) and voltage (V_{th}) values determined at the same intervals. Panel A: dog papillary muscle; 4 mm [K⁺]; 1.95 µA test current pulse. Panel B: dog papillary muscle; 14 mm [K⁺]; 3.15 µA test current pulse. Panel C: dog trabecula; 24 mm [K⁺]; 3.3 µA test current pulse. All preparations were studied in the sucrose gap.

determined at the same time intervals. Under control conditions (panel A), there was little or no diastolic change in peak SD amplitude. This was found in 14 of 16 control experiments. The two preparations which did show a change in SD amplitude were depressed (RMP = −72 and −78 mV) despite being exposed to normal Tyrode's solution. Measured in four preparations, excitability (represented by the inverse of I_{th}) recovered soon after complete repolarization and remained relatively constant throughout the 2000 msec test interval (panel A, triangles).

In fibers depressed to RMP near −70 mV (panel B), peak SD amplitude exhibited a time-dependent increase that correlated closely to a gradual decrease in I_{th}. This change in excitability, however, was not due to a change in V_{th} (circles). In general, peak SD amplitude increased with a curvilinear or linear time course with a concomitant increase in excitability (n = 4). Linear regression analysis of I_{th} vs. peak SD amplitude of the experiment shown in panel B yielded a correlation coefficient of −0.878. Diastolic changes in SD amplitude were also observed at RMP of −50 mV as shown in panel C. In all 11 preparations studied, marked but variable time dependence of SD amplitude was observed. In some preparations, a biphasic change was recorded, but others exhibited an N-shaped or curvilinear time course. Because of this variability, a quantitative comparison of the changes at RMP levels of −50 and −70 mV could not be made. However, as measured in three experiments and shown in panel C, the time course of change in SD amplitude corresponded closely to an increase in excitability with no change in V_{th}.

Linear regression analysis of I_{th} vs. peak SD amplitude of the experiment shown in panel C yielded a correlation coefficient of −0.931.

The morphology of the SD in depressed fibers suggests that excitability during a depolarizing current step is greatest at the peak of the SD (Bailey et al., 1973) where the local response occurs. Furthermore, the apparently slow kinetics of the local response at −50 mV predicts that all-or-none excitation would take longer to achieve from this membrane potential than at −70 mV or control (Fig. 6). This was found to be the case when the latency of excitation was compared for all three conditions. Latency is defined as the time interval from the beginning of a just threshold depolarizing pulse to the moment V_{th} is attained. The mean latency for slow channel-mediated action potentials (75.0 ± 1.9 msec; n = 10) was significantly greater than that for depressed fast response (22.6 ± 2.3 msec; n = 10) and control (26.4 ± 1.9 msec; n = 10) action potentials. The latencies under the latter two conditions were not significantly different (analysis of variance; modified t-test).

Time-Dependence of the Local Response

In Figure 7A, test current pulses of either polarity were applied to a muscle preparation with RMP of −70 mV. The pacing protocol and application of test pulses was the same as in Figure 6. The peak amplitude of responses elicited by depolarizing pulses (closed circles above zero) increased progressively while hyperpolarizing pulses (closed circles below zero) delivered at the same intervals generated relatively constant voltage responses. The open circles show the voltage responses of the same fiber after superfusing 10 µg/ml TTX. Action potentials during the train (slow responses) were maintained by applying higher intensity basic current pulses. The progressive growth in SD amplitude to a constant depolarizing input was blocked by TTX, indicating that the magnitude of the local response increased as a function of time following a depressed action potential. Furthermore, the constant voltage response to hyperpolarizing pulses indicates no change in passive properties. Results similar to those shown in panel A were also obtained when lidocaine (5 µg/ml; n = 3) was used instead of TTX. Figure 7B shows the voltage response (closed circles) to current pulses in a fiber with RMP of −57 mV. A biphasic
growth in peak SD amplitude occurred with depolarizing pulses with no time-dependent change in the amplitude of hyperpolarizations. Verapamil (2 \( \mu \)g/ml) superfusing the same fiber (open circles) greatly attenuated the basic action potentials (not shown) and all but completely eliminated the growth in SD amplitude. Results similar to those shown in panel B were obtained in five additional experiments. Thus, at RMPs associated with slow channel-mediated action potentials, the local response also changes in magnitude as a function of time.

The role of the local response in excitability was evident after administering channel-blocking agents which concurrently attenuated the local response and reduced excitability. On the other hand, non-toxic concentrations of acetylstrophanthinid increased the magnitude of the local response and, consequently, excitability, as well. The effect of acetylstrophanthinid on SD amplitude is shown in Figure 8. In this series of four sucrose gap experiments, of which Figure 8 is an example, the test segment was pretreated for 30 minutes with normal Tyrode's containing 0.1 \( \mu \)g/ml acetylstrophanthinid. After pretreatment, RMP was reduced with high [K\(^+\)]-Tyrode's plus acetylstrophanthinid, and test current pulses were applied before and after washout of the drug. In panel A, identical current pulses of either polarity were applied to a preparation with RMP of \(-72\) mV. In the presence of acetylstrophanthinid, peak SD amplitude increased markedly with time (open circles above zero) and, at later intervals, successfully reached threshold voltage (V\(_{th}\)) to elicit action potentials. No time-dependent change in the voltage response to hyperpolarizing pulses was evident (open circles below zero). After washing out acetylstrophanthinid for 15 minutes, RMP was unchanged and identical current pulses induced SDs of smaller amplitude and with a time-dependent change of lesser slope (closed circles). Responses to hyperpolarizing pulses were the same as those in the presence of the drug. Similar although less dramatic effects were also noted at \(-50\) mV. In the example shown in panel B (RMP = \(-55\) mV), 0.1
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μg/ml acetylstrophanthidin augmented peak SD amplitude at all intervals compared with that in the absence of the drug. As in the experiment of panel A, RMP and the responses to hyperpolarizing pulses remained constant. The inset of each panel shows two superimposed traces of the SD generated by the same current pulse and at the same coupling interval (1000 msec) during control (C) and in the presence of acetylstrophanthidin (AS). Results similar to those in Figure 8 were obtained in three of four experiments, with one preparation showing little or no effect of acetylstrophanthidin at either level of reduced RMP.

Effect of Changes in Current Input

Recovery of peak SD amplitude, indicative of a recovery in excitability, was readily apparent with constant current pulses of low intensity that failed to excite depressed test fibers (Fig. 6). However, in all depressed preparations, the rate of recovery of SD amplitude was also a function of input current intensity. The experiment of Figure 9 illustrates this point. In this example, RMP of test segment fibers was reduced to −72 mV, and consecutive diastolic scans were performed, each with a depolarizing current pulse of slightly greater intensity. The same impalement was maintained throughout this procedure. The peak amplitude of the voltage response (ordinate) is plotted as a function of the coupling interval (abscissa) after the last action potential in a train of 10. The family of amplitude-interval curves shown in this figure demonstrates a greater rate of change in peak response amplitude with increased current intensity. This is signified by a shift in the curve upward and to the left. A family of curves qualitatively similar to that shown in this figure was also observed in fibers with RMP near −50 mV, although the range of current intensities required to elicit these changes was higher (not shown).

Discussion

Postrepolarization Refractoriness

Frequency-dependent conduction similar to that demonstrated in the present study is a common characteristic of experimentally depressed cardiac tissues (Cranefield et al., 1971; Downar et al., 1977; Antzelevitch and Moe, 1981; Jalife and Moe, 1981), as well as diseased human ventricle (Singer et al., 1981; Gilmour et al., 1983). In many cases, periodicities in conduction have been shown to correlate with changes in the prepotential (SD) recorded in fibers within or just beyond a region of low excitability (Paes de Carvalho and de Almeida, 1960; Cranefield et al., 1971; Antzelevitch and Moe, 1981; Massara and Paes de Carvalho, 1982) demonstrated that the change in the magnitude of subthreshold events in depressed atrial myocardium reflects a concomitant change in excitability that, in turn, determines the frequency dependence of conduction. They also found the shape of the action potential invading a slow response region to be an important determinant for continued propagation. Thus, when the safety factor for conduction is reduced to a relatively low level, conduction aberrations may emerge, due to a gradual change in diastolic current requirements of depressed tissue (sink), as well as the magnitude of current supplied (source) by the invading wavefront (Antzelevitch et al., 1983).

The inability of depressed fibers to respond to propagating impulses of greater frequency or prematurity has been attributed to postrepolarization refractoriness (Lazzara et al., 1975), a property shared by fibers displaying depressed fast and slow channel-mediated action potentials (Merideth et al., 1968; Cranefield et al., 1972; Arita et al., 1983). The currently held view is that it is determined by a lack of availability of regenerative inward current secondary to slow recovery from inactivation (Gettes and Reuter, 1974; Kohlhardt et al., 1975). It is apparent from our experiments that other factors may play a role. For example, in Figure 2D, the distal segment was refractory to propagated impulses (BCL = 500 msec) over an interval of approximately 2500 msec. However, the distal segment was not refractory to bipolar stimuli applied directly to it at much shorter intervals. The time-dependent increase in SD amplitude with constant current input (Fig. 3) indicates that postrepolarization refractoriness is related, in part, to a slow recovery of diastolic excitability. Measurements of threshold current (Ith) with time in fibers with depressed fast and slow response action potentials support this hypothesis (Fig. 6).

The Local Response

The experiments of Figure 5 demonstrate that all-or-none excitation of depressed ventricular fibers is
preceded by a subthreshold potential change composed of a relatively large active component superimposed on a passive electrotonic depolarization. This active component or local response (Hodgkin, 1938) represents a nonregenerative activation of inward current that, in addition to anomalous rectification (McDonald and Trautwein, 1978), contributes to the larger voltage response of subthreshold depolarizing currents compared with hyperpolarizing pulses. It is clear from Figure 5 that the ionic conductance mediating the local response in depressed fibers depends on the RMP from which it develops. This figure also demonstrates that, when excitability is reduced, the local response represents a large fraction of the total SD amplitude. In these examples, the fast channel (panel A) and slow channel (panel C) mediated local responses represented 31% and 42%, respectively, of the total subthreshold voltage displacement.

The basis of the local response induced by a subthreshold depolarizing current may be explained in terms of space- and voltage-related phenomena. The former hypothesis involves the concept of liminal length (Rushton, 1937; Fozzard and Schoenberg, 1972), which predicts that if too small an area of membrane is raised above threshold, the activation of inward currents would be suppressed by adjacent passive membrane, and all-or-none depolarization would not develop. Moreover, as a result of the shallow slope of the current-voltage relationship near RMP for both I_Na and I_L (Dudel and Rudel, 1970; New and Trautwein, 1972), a relatively small inward current is activated by a small voltage step. If this inward current is insufficient to overcome background outward current, all-or-none depolarization would not develop, and only a transient active response would occur. In the present investigation, the large local response in depressed fibers compared with control (Fig. 4) is likely the result of two factors: (1) an increased K+ conductance secondary to an elevation in extracellular [K+], and (2) a shift in steady state threshold voltage to less negative membrane potentials (Table 1). Increased K+ conductance would necessitate a larger opposing local response to reach a less negative V_th. This predicts that elevation of extracellular [K+] increases liminal length and consequently threshold current requirements for excitation.

The SD generated from normal RMP by identical current pulses showed little or no time-dependent change in amplitude (Fig. 6A). Excitability recovered soon after complete repolarization of membrane potential and remained relatively constant throughout diastole, as found in other studies (Spear and Moore, 1974). At each level of reduced RMP, marked concurrent changes in peak SD amplitude and excitability were clearly evident (Fig. 6, B and C). Similar changes in diastolic excitability have been reported in Purkinje (Jalife et al., 1983) and atrial (Cukierman and Paes de Carvalho, 1982) fibers exhibiting slow channel-mediated action potentials. Our experiments further demonstrate that a marked diastolic change in excitability is also characteristic of fibers in which the fast channels are partially inactivated. This predicts that conduction aberrations can occur in partially depolarized fibers, examples of which are shown in Figures 1 and 2.

The observed changes in diastolic excitability cannot be attributed to a concomitant shift in threshold voltage to more negative potentials. As demonstrated in Figure 6 (panels B and C), measured V_th was constant at the same time elicited SD was increasing progressively. Moreover, the constant peak voltage response to hyperpolarizing pulses at each RMP level (Fig. 7) indicates no time-dependent change in passive (RC) properties. Instead, gradual increase in excitability can be explained by a time-dependent recovery of the local response generated by a given input current. This is indicated by the effects of TTX and verapamil shown in Figure 7.

**Effects of Acetylstrophanthidin and Current Input Intensity**

The underlying mechanism of local response recovery is yet uncertain, as is whether the changes at -70 and -50 mV are governed by similar or different processes. However, the experiments testing the effects of acetylstrophanthidin (Fig. 8) suggest the recovery of the local response is related to Na-K pump activity or to changes in intracellular [Ca++]

If, during diastole, electrogenic pump activity gradually declines (Falk and Cohen, 1984), a time-dependent increase in outward current would result. Thus, at later intervals, a generated local response would encounter less of an opposing outward current and would be greater in magnitude. Since pump inhibition also leads to an increase in intracellular free [Ca++] (Langer, 1968), it is possible that local response recovery for a given cell is related to a diastolic change in the concentration of this ion. It has been shown that intracellular Ca++ modifies ionic channel conductance (Isenberg, 1977; Colquhoun et al., 1981). For example, an oscillatory increase in intracellular [Ca++] regulates the transient inward current (carried by Na+) in Purkinje fibers exposed to toxic concentrations of cardiac glycosides or to high extracellular [Ca++] (Kass et al., 1978). Intracellular Ca++ has also been implicated in the regulation of slow channel conductance (Isenberg, 1977; Shimoni, 1981). Therefore, diastolic changes in intracellular [Ca++] in ventricular muscle fibers may regulate the conductance through the ionic channels mediating the local response.

Change in diastolic excitability of depressed fibers was not only a function of time, but, also, of current input intensity (Fig. 9). This was the case at either level of reduced RMP tested. The mechanism(s) of this property probably relates to the space and voltage factors involved in the generation of the local response, as discussed earlier. Nevertheless, Figure
9 predicts that, in addition to a time-dependent change in diastolic excitability (current sink), postpolarization refractoriness should also depend on the intensity of current input (current source) such that the smaller the input, the greater the refractory period. This may in part explain the inverse relationship of BCL and refractory period illustrated in Figure 2 and observed in other studies of conduction in low excitability tissues (Merideth et al., 1968; Lazzara et al., 1975; Gilmore et al., 1983). It would be predicted that at faster steady state frequencies, the current-generating property of depressed tissue (represented by V_{max} action potential amplitude, and duration) is reduced. This decrease in current input, in addition to the temporal inhibitory interactions of subthreshold depolarizations (Antzelevitch and Moe, 1983), would tend to lengthen refractory period.

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