Interrelationships Between Automaticity and Conduction in Purkinje Fibers

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ABSTRACT
Microelectrode studies of transmembrane potentials of canine Purkinje fibers show that phase-4 depolarization causes voltage-dependent changes in conduction and responsiveness similar to those occurring during repolarization at comparable levels of potential. Abnormalities ranged from simple slowing of conduction to decrement, unidirectional and bidirectional block, and unexcitability. Reentrant excitation also developed. Significant conduction disturbances usually appeared at —75 to —70 mv; decrement and advanced block at —65 to —60 mv, or below. Because the threshold potential of normal Purkinje cells is approximately —70 mv, depolarization to lower levels implies shifts in this variable toward 0. Determinations of threshold potential confirm such shifts. It may be further inferred that significant abnormalities would most likely occur in fibers in which threshold potential is shifted toward 0 or membrane responsiveness impaired. Alterations in conduction due to phase-4 depolarization provide a reasonable explanation for various peculiarities of cardiac rhythm, including occurrence of conduction disturbances and reentrant rhythms at low heart rates, exit and entry block about parasystolic foci, instability of peripheral Purkinje pacemakers, and supernormal conduction. Circumstances that enhance phase-4 depolarization are common in diseased hearts, indicating that this mechanism may be a significant factor in human arrhythmias.

ADDITIONAL KEY WORDS phase-4 depolarization transmembrane potentials arrhythmia latent pacemakers Purkinje fibers diastolic membrane potential pacemaker threshold potential conduction disturbances cardiac electrophysiology canine heart

Conduction in excitable tissues is determined by many variables. Among these, the amplitude of the action potential and the maximum rate of change of membrane potential during its upstroke (dV/dt, phase 0) are of singular importance (1). In Purkinje fibers, these two variables bear a predictable relationship to the level of membrane potential at the time of excitation: below a critical value, the lower the membrane potential at the time of excitation, the lower the amplitude and dV/dt of the resultant action potential (2). Therefore, sufficient reduction in membrane potential before excitation should result in altered conduction (1). In the heart, conduction disturbances due to reduced levels of membrane potential usually are the result of propagation in incompletely repolarized fibers, as in premature systoles initiated during phase 3 of repolarization (3, 4). However, the cyclic decrease in diastolic potential resulting from slow diastolic (phase 4) depolarization of automatic cells can be expected to result in conduction disturbances comparable to those at similar levels of membrane potential during repolarization (Fig. 1).

The occurrence of conduction disturbances...
due to phase-4 depolarization would be important in relation to any explanation of the mechanisms responsible for various abnormalities of cardiac rate and rhythm. It would provide a reasonable mechanism for a number of peculiarities of conduction that are otherwise difficult to explain: e.g., conduction disturbances associated with low heart rates and long diastolic intervals; the exit and entry block thought to occur around parasystolic foci, and the instability of certain idioventricular pacemakers. It also would afford one explanation for reentry and for supernormal conduction.

Our studies were undertaken to test the possibility that phase-4 depolarization of Purkinje fibers can result in significant conduction abnormalities, to characterize the nature of such disturbances, and to identify conditions under which they might occur.

Methods

Mongrel dogs were anesthetized with sodium pentobarbital, 30 mg/kg, administered intravenously. The heart was removed rapidly and free-running strands of Purkinje fibers (false tendons) together with small segments of attached ventricular muscle were dissected from the endocardial surface of the ventricles and pinned to a
wax block in a Lucite chamber. The tissue was continuously perfused with modified Tyrode's solution containing (in millimoles per liter): NaCl, 137; NaHCO₃, 12; dextrose, 11; KCl, 2.7; NaH₂PO₄, 3.8; MgCl₂, 1.0 and CaCl₂, 2.7. The stock Tyrode's solution was equilibrated with a mixture of 95% O₂ and 5% CO₂; the same gas mixture was introduced directly into the tissue bath. The tissues were kept at 37°C unless otherwise specified.

Transmembrane potentials were recorded through microelectrodes filled with 3M KCl and with a d-c resistance of 20 megohms. The electrodes were mounted rigidly in micromanipulators and positioned in the tissue under microscopic control. The bath was connected to a ground by means of a large electrode filled with 3M KCl. In both the microelectrodes and the bath electrode, a silver wire coated with AgCl made contact with KCl. The first stage of the recording system consisted of conventional cathode followers capable of neutralization of input capacitance (Bioelectric Inst.). The signals were then led through d-c amplifiers (Tektronix, types 3A74 and 3A72), after which they were displayed on a dual-beam oscilloscope (Tektronix, type 565). For voltage calibration, a 100-mv signal was introduced between the bath and ground. In some instances, bipolar surface electrograms were recorded through fine silver wire electrodes, Teflon coated except for the tips. Signals were photographed with a Grass oscilloscope camera on film or paper. Records were obtained at low sweep velocities to show the full range of changes in transmembrane potentials during the complete cycle and at high sweep velocities to show details of action potential upstrokes and of the sequence and time-course of activation.

The preparations were stimulated at a regular rate through fine Teflon-coated silver wire electrodes inserted into the ventricular muscle. Rectangular pulses were delivered by Tektronix, type 161 pulse generators. Frequency of stimulation is indicated in terms of interval between stimuli, or cycle length, in milliseconds. The pulses were led through radiofrequency oscillators isolated from ground. In some experiments depolarizing and hyperpolarizing current pulses were introduced intracellularly through a glass microelectrode. A resistance of 100 megohms was placed in series with the current source. In these experiments transmembrane potentials were recorded differentially through adjacent intracellular and extracellular microelectrodes. Current was measured by recording the voltage drop across a 1-megohm resistor in series with the stimulating circuit.

The change of membrane potential during phase 0 of the action potential (dV/dt) was electronically differentiated with respect to time by means of the R-C circuit of an operational amplifier (Tektronix, type 0). The magnitude of the maximum rate of change of membrane potential is indicated by the amplitude of the differentiated spike. Records of dV/dt were calibrated by introducing sawtooth signals of known amplitude (100 mv) and duration (0.2 to 1 msec) between the tissue bath and ground, and differentiating the signal recorded through the microelectrode and cathode follower. These calibrat-

![Figure 2](http://circres.ahajournals.org/)

**Panel A**, schematic representation of typical preparation of isolated strand of canine Purkinje fibers and attached ventricular muscle (hatched area). Abbreviations: DR₁, both stimulating electrodes implanted at one end of the preparation. DR₂, one stimulating electrode implanted at each end of the preparation. P and D refer to microelectrodes at sites near to ("proximal") and distant from ("distal") the site of stimulation. E, electrodes for recording surface activity. Panels B and C show typical records of transmembrane potentials recorded at low (B) and high (C) sweep velocities. Lines showing time marks indicate 0 potential. dV/dt of phase 0 of the distal action potential is indicated by the amplitude of the differentiated spike of high sweep velocity records (C, bottom trace). Interval between action potential upstrokes indicates interelectrode conduction time.
ing signals were provided by the oscilloscope sweep circuit. This procedure was used to eliminate errors caused by slight variations in neutralization of input capacity during the experiment. The amplitudes of the differentiated spikes were linearly proportional to the rate of change of voltage in the range of 0 to 500 v/sec, with a slight fall-off from linearity in the range of 500 to 1,000 v/sec.

In most experiments, transmembrane potentials were simultaneously recorded at sites near to ("proximal") and distant from ("distal") the sites of stimulation (Fig. 2) under control conditions, at intervals during the development of phase-4 and generalized diastolic depolarization and during attempts to reverse the ensuing changes. Development of phase-4 depolarization was facilitated by decreasing the frequency of stimulation alone or in conjunction with one of the following: increased stretch, cessation of the final equilibration of the Tyrode solution in the tissue bath with O,

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and CO,

2

reduction in the concentration of Ca

2+

, K

+ or both, of the Tyrode solution, or exposure of the tissue to a toxic concentration of ouabain. Reversal of induced changes was accomplished by techniques known to suppress phase-4 depolarization, e.g., increased frequency of stimulation, reduction in temperature of the perfusate from 37°C to 30-34°C, perfusion with potassium-rich Tyrode’s solution, and by techniques that increase maximum levels of diastolic potential, such as addition of epinephrine (final concentration 10⁻⁶ g/liter) and introduction of hyperpolarizing current pulses). Specific experimental techniques are indicated in the text or figure legend in each instance. Time and voltage calibrations are indicated for each figure.

Measurements of dV/dt for studies of membrane responsiveness were made on action potentials initiated at representative levels of membrane potential by means of the following: a) stimuli applied through surface electrodes at selected intervals during phase 3 and phase 4; b) hyperpolarizing and depolarizing current pulses, 40 to 100 msec in duration, introduced intracellularly through a microelectrode just before the anticipated arrival of the next propagated action potential. Timing of the stimulus was such that the propagated excitation wave arrived late during the current pulse, by which time membrane potential had achieved its final imposed value, i.e., membrane capacitance was almost fully charged. Determinations were made under control conditions, at selected intervals during development of phase-4 depolarization and generalized diastolic depolarization, and during reversal of such changes.

Threshold potential also was measured in some experiments. For these determinations, depolarizing current pulses, 40 to 100 msec in duration, were introduced intracellularly at the point of maximum diastolic potential and at the end of phase 4, just before the expected arrival of the next propagated action potential. Threshold potential was considered to be the lowest level to

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\text{We have used the term membrane responsiveness to describe the relationship between the level of transmembrane potential at the time of stimulation and the maximum rate of change of potential during the response elicited by the stimulus.}
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which membrane potential could be reduced in this manner without occurrence of excitation.

Results

EFFECTS OF DIASTOLIC DEPOLARIZATION

In each instance, a sustained low rate of stimulation, sometimes alone and usually in conjunction with one of the experimental procedures listed, resulted in the development of spontaneous phase-4 depolarization. The extent of the depolarization usually could be regulated by varying the frequency of stimulation—the longer the diastolic interval, the

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**FIGURE 4**

Decrement due to phase-4 depolarization. Records in A were obtained at a low sweep velocity; in B through I at a fast sweep. A, control conditions. Note some phase-4 depolarization even at fast rate of stimulation (CL [cycle length] 630 msec). B through I. Effects of progressive increase of phase-4 and development of generalized diastolic depolarization during period of sustained stimulation at a low rate (CL 2000 msec). Note progressive reduction in amplitude and upstroke velocity of action potentials and development of slowly rising prepotentials (E through I) at both recording sites. Greater than expected dv/dt of the distal action potential (D through I) suggests that in large measure the response reflects retrograde activation of the distal recording site. Purkinje spike (arrow) of bipolar electrogram undergoes similar reduction in amplitude. Action potential upstrokes, which are superimposed in B, become increasingly separated due to prolongation of interelectrode conduction time. Latency, between stimulus (S) and proximal response, also increases markedly. Interval between Purkinje and muscle spikes of the electrogram does not increase because in this experiment the Purkinje component of the electrogram was recorded immediately adjacent to the muscle. Since phase-4 depolarization does not develop in ordinary muscle, conduction in muscle was not impaired. Increases in amplitude of stimulus artifact (S) denote associated depression of excitability.
FIGURE 5

Development of unidirectional and bidirectional block and electrical unexcitability due to unchecked progression of phase-4 depolarization. One stimulating wire was inserted into each end of the preparation (see Fig. 2, DR). Panels in vertical row I show records obtained during stimulation from one end. Records in II were obtained during stimulation from opposite end. The dV/dt of phase 0 of the distal action potential is indicated by the amplitude of the differentiated spike. Except in 1A, dV/dt is so low that no spike is discerned. Time lines are 10 and 100 msec apart. Comparisons of end diastolic potentials in A-E, row I, show the changes in voltage-time course of phase-4 depolarization that result in development of generalized diastolic depolarization.

The development of phase-4 depolarization was accompanied by marked changes in action potentials and by altered conduction. The magnitude of these changes could be related to the decrease in diastolic membrane potential. Appreciable slowing of conduction usually appeared first when the action potential upstroke was initiated at a membrane potential between −75 and −70 mv. Advanced conduction disturbances, including decremental conduction, unidirectional and bidirectional block, and alterations in the sequence of activation occurred only with further reduction in membrane potential to −65 mv or less. The most marked abnormalities occurred in conjunction with generalized diastolic depolarization. If membrane potential was not restored toward normal, complete block and unexcitability ensued. Depolarization to less than −70 mv was uncommon in fibers maintained under normal conditions. Advanced conduction disturbances were therefore correspondingly uncommon in these preparations. The changes resulting from the development of phase-4 depolarization are illustrated in Figures 3 through 5.

The most common sequence of events is shown in Figure 3. In this experiment, on a preparation mounted under excessive tension, increasing the cycle length of the drive from 500 msec (120/min) in A to 1,000 msec (60/min) in C resulted in the development of phases 3 and 4 eventually changed, and membrane potential no longer returned to the control maximum diastolic level after each action potential. The changes in voltage-time course and loss of maximum diastolic potential also tended to become progressively more marked with time, with the result that membrane potential eventually was reduced throughout phase 4. This condition will be designated as generalized diastolic depolarization. Significant reduction of maximum diastolic membrane potential and development of generalized diastolic depolarization occurred only rarely in fibers maintained under normal conditions; i.e., those in which phase-4 depolarization developed in response to rate slowing alone.
of both phase-4 and generalized diastolic depolarization. Membrane potential at the initiation of the action potential upstroke was reduced to \(-68\) mV at the proximal recording site and \(-57\) mV at the distal one. The amplitude and dv/dt of both action potentials, but particularly of the distal one, were reduced and interelectrode conduction time prolonged. Further progression of generalized diastolic depolarization was associated with still greater deterioration of the action potentials and additional slowing of conduction (Fig. 3D, E). In E the upstroke of the action potential recorded from the distal site preceded the proximal upstroke; therefore, the sequence of activation of the two recording sites, which were approximately \(1.5\) cm apart, was reversed. The most reasonable explanation for this reversal is that the development of local block between the stimulating electrode and proximal recording site resulted in prior activation of the distal site. Excitation then spread retrograde to the proximal site. The alternate possibility that the distal response in E was a spontaneous event is virtually ruled out by the finding that addition of epinephrine resulted in gradual restoration of the normal sequence of activation of the two recording sites (see Fig. 10).

Figure 4 illustrates the development of graded responses and decremental conduction due to phase-4 depolarization caused by a sustained low rate of stimulation and reduction in concentrations of \(\text{Ca}^{2+}\) and \(\text{K}^{+}\) in the Tyrode solution to 1.35 and 2.0 mM, respectively. The extent to which conduction was slowed is remarkable considering the small interelectrode distance (less than \(2\) cm). The progressive changes in the rate of rise and configuration of transmembrane action potentials and slowing of conduction in this figure also emphasize the self-perpetuating nature of the decrement. The slowly rising, low-amplitude action potentials resulting from excitation of partially depolarized fibers caused a more gradual depolarization of adjacent cells than would normal action potentials. Responses resulting from excitation of these adjacent cells showed a further decrease in rate of rise and amplitude. This in turn further reduced their effectiveness as stimuli for unexcited tissue. In this sense decrement could be considered a regenerative process. The bottom trace in each panel shows the corresponding changes in configuration of a simultaneously recorded bipolar surface electrogram. There is progressive diminution in the amplitude of the spike resulting from Purkinje fiber activation. The altered configuration of the electrogram serves to emphasize the possibility that phase-4 depolarization of automatic cells might cause aberration of the QRS complex of the electrocardiogram.

In the experiment shown in Figure 5, stimulating electrodes were inserted into each of the two ends of the preparation so that the site of origin of activity, and thus the direction of propagation, could be reversed. Transmembrane potentials recorded during stimulation at one end are shown in vertical row i and during stimulation at the opposite end in vertical row n. When the records in iA were obtained, membrane potential at the two recording sites had been reduced to \(-78\) mV and \(-60\) mV respectively. This was the result of phase-4 and generalized diastolic depolarization that had developed in response to a sustained low rate of stimulation and increased stretch. Despite the reduction in amplitude and dv/dt of action potentials initiated at these levels of membrane potential, conduction was still fairly well maintained (note superimposition of the upstrokes). Records in iB-E show that, in this experiment, unchecked progression of diastolic depolarization eventually culminated in failure of propagation and in local unexcitability (D-E). By this time, membrane potential had been reduced to \(-69\) mV and \(-49\) mV at the two recording sites. Immediately thereafter, the site of stimulation was changed to the opposite end of the preparation, and propagated activity reappeared (nA). This reappearance of propagated activity at the same recording site on reversal of the direction of stimulation suggests that block due to phase-4 and generalized depolarization may be unidirectional. The unidirectional character of the changes is most reasonably ex-
Row i: Records of transmembrane potentials to show development of reentrant excitation in fiber undergoing phase-4 depolarization. P and D designate driven action potentials recorded at proximal and distal sites. P' and D' designate reentrant activity at the same sites. Time lines are 100 msec apart. A, reentrant activity occurs at both recording sites. Note that interelectrode conduction time is markedly prolonged for both driven (62 msec) and reentrant (133 msec) beats and that sequence of activation of the two recording sites is reversed during the reentrant beats. B, Reentrant excitation wave propagates to distal site (action potential D') somewhat earlier than in A but decrements before reaching the proximal site so that only a local response or electrotonic potential (P_r) is recorded there. C, no evidence of reentrant activity at either site. Line showing time marks does not indicate 0 potential.

Row ii: Schematic representations of possible mechanisms for reentrant activity seen in row i. Diagrams show two parallel Purkinje fibers, X and Y. Conduction is generally depressed in both fibers due to effects of phase-4 depolarization. Dark area in fiber X represents a region of more advanced, but variable, block. Solid lines with arrows indicate orthograde spread of activity. Dashed lines with arrows indicate retrograde spread. Undulating lines with arrows indicate very slow conduction through region of block. Arrows indicate direction of spread. P and D refer to proximal and distal recording sites. In nA and B, block in the dark area is unidirectional. Depending on conditions of responsiveness and conduction in the region of block, retrograde spread of activity may result in reexcitation of both recording sites (nA) or just of the distal site (nB). Bidirectional block in the hatched area would abolish or prevent reentry (nC modified after Schmitt and Erlanger [27], p. 340).

explained in terms of differences in membrane potential at the two ends of the preparation. Eventually, continued progression of diastolic depolarization (nB-E) culminated in failure.
Abnormalities due to phase-4 depolarization reversed by an increase in frequency of stimulation. Time marks on records made at high sweep speed are 10 msec apart. A, control conditions, preparation stimulated at cycle length of 700 msec. B, development of phase-4 depolarization in response to reduced rate of stimulation (cycle length 1350 msec) and hypoxia. Note reduced amplitude and dV/dt of action potentials as compared to controls and the prolonged interelectrode conduction time. C, resumption of faster rate of stimulation suppressed phase-4 depolarization and reversed the abnormalities. Comparison of records obtained immediately (C, top) and 5 min (C, bottom) after the increase in rate shows that restoration to control conditions was not immediate.

of propagation in this direction as well (nD, E). At this stage, block was bidirectional. The occurrence of reentrant beats in conjunction with development of phase-4 depolarization due to increased stretch in the preparation is shown in Figure 6.

REVERSAL OF ABNORMALITIES DUE TO PHASE-4 DEPOLARIZATION

Restoration of diastolic membrane potential to normal resulted in reversal of the abnormalities described. This could usually be accomplished by use of techniques known to suppress phase-4 depolarization. The simplest method was to gradually increase the frequency of stimulation. In most instances an appropriate reduction in cycle length resulted in restoration of normal levels of maximum diastolic potential, normalization of the action potentials, and reversal of conduction disturbances (Fig. 7). In some preparations, usually those exhibiting the most marked degrees of generalized diastolic depolarization, increasing the rate of stimulation either had no significant effect or caused further deterioration of transmembrane potentials. If maximum diastolic potential of such fibers could be sufficiently increased by some other means, e.g., addition of epinephrine, recovery subsequently ensued. Reduction in the temperature of the perfusate from 37° C to 30-34° C or infu-
Abnormalities due to phase-4 depolarization reversed by increase in potassium concentration of the perfusate. Same preparation as in Figure 7. Cycle length of stimulation and potassium concentrations indicated under the corresponding records. A, control conditions. B, development of phase-4 depolarization. Amplitude and dV/dt of action potentials as well as maximum diastolic potential are reduced at both recording sites and interelectrode conduction time is prolonged. C, reversal of abnormalities on doubling potassium concentration of the perfusate. Interelectrode conduction time is restored to control values. Amplitude and dV/dt of both potentials are still somewhat diminished due to persistence of slight phase-4 depolarization and reduction in maximum diastolic potential (11 and 13 mv less than in A at proximal and distal sites, respectively) resulting from the increase in potassium concentration.

The low membrane potential caused by generalized diastolic depolarization and the resulting changes in action potential could be restored to or toward normal by epinephrine (Figs. 9 and 10). Epinephrine, in addition to its effects on automaticity, restores maximum diastolic potential of partially depolarized fibers toward normal values (9-12). For certain of the more deteriorated preparations, the increase in maximum diastolic potential was not accompanied by an increase in automaticity. Usually, however, epinephrine also enhanced automaticity of these fibers. This was shown by increased phase-4 depolarization and appearance of spontaneous beats (Fig. 9). Comparison of driven and spon-
Effects of l-epinephrine on transmembrane potentials from partially depolarized (membrane potential at excitation — 53 mv) Purkinje fiber. Phase-4 and generalized diastolic depolarization resulted from sustained low rate of stimulation (cycle length 2000 msec) and hypoxia. A, partially depolarized fiber. B through C, addition of l-epinephrine (E), final concentration 10^{-4} g/liter resulted in increase in maximum diastolic potential and in amplitude and dV/dt of action potentials. Also note occurrence of spontaneous beats due to increased phase-4 depolarization. Combination of driven and spontaneous beats cause bigeminy. The first action potential in each pair is driven; the second, spontaneous. Subsequent increase in frequency of stimulation to cycle length 630 msec resulted in further improvement due to suppression of phase-4 depolarization (low and high sweep velocity records in H through I). Amplitude of differentiated spike indicates dV/dt of driven action potentials.

FIGURE 9

Effects of l-epinephrine on transmembrane potentials from partially depolarized (membrane potential at excitation — 53 mv) Purkinje fiber. Phase-4 and generalized diastolic depolarization resulted from sustained low rate of stimulation (cycle length 2000 msec) and hypoxia. A, partially depolarized fiber. B through C, addition of l-epinephrine (E), final concentration 10^{-4} g/liter resulted in increase in maximum diastolic potential and in amplitude and dV/dt of action potentials. Also note occurrence of spontaneous beats due to increased phase-4 depolarization. Combination of driven and spontaneous beats cause bigeminy. The first action potential in each pair is driven; the second, spontaneous. Subsequent increase in frequency of stimulation to cycle length 630 msec resulted in further improvement due to suppression of phase-4 depolarization (low and high sweep velocity records in H through I). Amplitude of differentiated spike indicates dV/dt of driven action potentials.

Native action potentials (Fig 9B-G) reveals a greater reduction in amplitude and dV/dt of the latter because of greater reduction of end-diastolic membrane potential due to phase-4 depolarization. Under these circumstances, greater improvement than that caused by epinephrine alone could usually be achieved by increasing the frequency of stimulation and thus suppressing the epinephrine-induced phase-4 depolarization (Fig. 9H-I). Figure 10 shows improvement in conduction in a deteriorated preparation resulting from restoration of membrane potential toward more normal levels by epinephrine. It will be noted that in this preparation (also used for the records shown in Fig. 3) improvement in conduction was associated with normalization of the sequence of activation of the two recording sites. That the improvement induced by epinephrine was due to the increase in membrane potential during phase 4 and not to any change in the relationship between membrane potential and membrane responsiveness is indicated by the curves in Fig. 11 and will be discussed later. The response to epinephrine began within 30 to 60 sec after administration and reached a maximum within approximately 2 to 10 min. Following recov-
Conduction disturbances resulting from development of generalized diastolic depolarization reversed by l-epinephrine. Preparation is the same as in Figure 3. A, record of transmembrane potentials from partially depolarized fiber is the same as that in Figure 3E. dV/dt is so low that a differentiated spike was not recorded. Compared to control conditions (Fig. 3A), conduction is much depressed and the sequence of activation of the two recording sites is reversed. B through F. Addition of l-epinephrine (E), final concentration 10^{-6} g/liter, results in increase in levels of diastolic membrane potential and amplitude and dV/dt of the action potentials and in improvement in conduction. The sequence of activation of the two recording sites returns to normal between B and D.

VOLTAGE DEPENDENCE OF THE OBSERVED PHENOMENA

Although the abnormalities developing in conjunction with phase-4 depolarization were clearly related to the degree of depolarization, in some instances the abnormalities were so marked as to suggest that membrane responsiveness had been reduced to a greater extent than would be predicted from the level of membrane potential.

To test this possibility, the relationship between maximum dV/dt and level of membrane potential was determined for the same fibers under control conditions, during development of progressive phase-4 and generalized diastolic depolarization, and during recovery. In the experiments illustrated in Figures 13 and 14, measurements of dV/dt under control conditions were made on action potentials initiated at representative levels of membrane potential by stimulating the preparation at selected intervals during repolarization and during phase 4. For the fiber undergoing phase-4 and generalized diastolic depolarization and for the fiber "recovering" from this process, measurements of dV/dt were made on action potentials initiated at the end of phase 4. In the experiment shown in Figure 11 on the other hand, measurements of dV/dt in the partially depolarized fiber were made on action potentials initiated at similar levels of membrane potential by use of hyperpolarizing and depolarizing current pulses introduced through an intracellular microelectrode. Comparison of the curves in these figures shows that, irrespective of technique used to alter...
Relationship between $dV/dt$ of action potentials from a canine Purkinje fiber and level of membrane potential at which they were initiated. Curve connecting filled circles shows relationship for the normal fiber under control conditions. Determinations were made on action potentials initiated at selected levels of membrane potential by stimulating the preparation at intervals during and after repolarization. Open circles show measurements made on the same fiber after the development of generalized diastolic depolarization in response to a sustained low rate of stimulation (cycle length 2000 msec) and anoxia. Measurements were made on action potentials initiated at selected levels of membrane potential by means of depolarizing and hyperpolarizing current pulses introduced through intracellular microelectrodes. Open triangles show determinations made during recovery of the same fiber following administration of l-epinephrine. Comparison of the curves shows that with respect to $dV/dt$, action potentials initiated in the partially depolarized fiber and during epinephrine-induced recovery were similar to those obtained from the same fiber under control conditions at comparable levels of membrane potential.

membrane potential, maximum values of $dV/dt$ of action potentials initiated in the partially depolarized fiber were quite similar to those obtained for the same fiber under control conditions at comparable levels of membrane potential. The comparability of $dV/dt$ of action potentials initiated, at similar levels of membrane potential, in normal and partially depolarized fibers is also demonstrated by the records of transmembrane potentials in Figure 12. These findings preclude impairment of membrane responsiveness, i.e., a change in
Comparability of amplitude and dV/dt of action potentials initiated at similar levels of membrane potential in the same fiber under control conditions and following development of diastolic depolarization. Maximum dV/dt indicated by amplitude of differentiated spike on bottom trace. A, normally functioning fiber stimulated at a rate of 120/min (cycle length [CL] 500 msec). B, after development of generalized diastolic depolarization in response to a sustained low rate of stimulation 30/min (CL 2000 msec), and hypoxia. C, after artificially increasing end-diastolic membrane potential to the control value by means of a hyperpolarizing current pulse introduced intracellularly through glass microelectrode. The transmembrane potential at the time of excitation was —90 mv in A, —58 mv in B, and —90 mv in C. Amplitude and dV/dt of a propagated action potential initiated during passage of the hyperpolarizing pulse (C) are similar to those of the action potential initiated under control conditions (A). The true outline of the early repolarization phase of the action potential in C, which is distorted by the break portion of the stimulus artifact, is indicated by dash.

SHIFTS IN THRESHOLD POTENTIAL

The threshold potential of Purkinje cells in normal sheep (13) and dogs (unpublished observations, D. H. Singer, R. Lazzara, and B. F. Hoffman) is approximately —70 mv. In view of this, the finding that phase-4 depolarization sometimes decreased membrane potential to less than this value without the occurrence of spontaneous firing (Figs. 3-5, 7-10, 12, 15) implies that there must have been a shift in threshold potential toward zero potential in these fibers.

Further evidence in support of such a shift is provided by the records of transmembrane potentials obtained from spontaneously beating automatic cells during periods of observation ranging from ½ to 2 hr (Fig. 15). Development of phase-4 depolarization was facilitated by cessation of the final equilibration of the Tyrode solution in the tissue bath with O2 and CO2. The sequence of transmembrane potentials in this figure suggests the occurrence of a gradual but progressive shift in threshold potential (defined as the level of membrane potential at the onset of rapid depolarization) toward zero potential. Shifts of threshold potential to levels as low as —50 mv were noted. These changes were accompanied by reduction in maximum diastolic potential and progressive decrease in the amplitude and maximum dV/dt of the action potential. The shift in threshold potential seemed to precede significant lowering of maximum diastolic potential and the development of generalized diastolic depolarization. It is important to note that similar shifts in threshold potential were not encountered in “normal” fibers; i.e., those in which only a decrease in frequency of stimulation was used to induce...
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Relationship between level of membrane potential and maximum upstroke velocity (dV/dt) of action potentials recorded under control conditions (closed circles) and during development of generalized diastolic depolarization (open circles). Diastolic depolarization was caused by a stretch and a low rate of stimulation (30/min).

Phase-4 depolarization. Conversely, if abnormalities that had resulted from progression of phase-4 and generalized depolarization were reversed by exposure to epinephrine, there seemed to be an associated restoration of threshold potential toward the normal level. A limited number of direct measurements of threshold potential made on the same Purkinje fiber under control conditions and after the development of marked phase-4 and generalized diastolic depolarization confirmed the occurrence of a shift in threshold potential toward zero.

Discussion

PHASE-4 DEPOLARIZATION AS A CAUSE OF CONDUCTION DISTURBANCES

As could be predicted from the studies of Weidmann (2), the cyclic reduction in diastolic membrane potential resulting from spontaneous phase-4 depolarization in automatic (latent pacemaker) cells caused reduction in amplitude and dV/dt of action potentials initiated in these cells and alterations in conduction and excitability comparable to those that occur if a response is initiated during repolarization at corresponding levels of membrane potential (3, 4). The abnormalities were voltage dependent and could be reversed by measures which suppressed phase-4 depolarization and restored diastolic potential to normal levels.

Although the relationship between level of membrane potential and conduction velocity...
was not exactly the same for each fiber, conduction usually was well maintained until diastolic potential was reduced to —75 to —70 mV. Significant abnormalities usually appeared first at levels of membrane potential of —70 mV or less. Further progression of depolarization resulted in increasingly marked depression of conduction and excitability and culminated in decrement, unidirectional and bidirectional block and unexcitability. Decrease and advanced block usually did not appear with any consistency until membrane potential had been reduced to the vicinity of —65 to —60 mV or below. The most marked abnormalities occurred in fibers in which progression of phase-4 depolarization was associated with a change in voltage-time course of such a nature that generalized diastolic depolarization ensued.

The mechanisms responsible for development of generalized diastolic depolarization are not yet defined. However, it is known that phase-4 depolarization in normally functioning automatic cells is accompanied by an increase in membrane polarization resistance (8, 14). This, in turn, is thought to reflect decreased conductance to potassium during phase 4 (15-17). Therefore, observations that development of generalized diastolic depolarization also is accompanied by a progressive increase in membrane resistance (unpublished observations, D. H. Singer, R. Lazzara, and B. F. Hoffman) may reasonably be interpreted to suggest that the process is related to a further decrease in sodium conductance during phase 4. These observations further suggest that development of generalized diastolic depolarization is not due to an increase in potassium conductance. A decrease in membrane resistance would be expected under such circumstances. The rapidity with which generalized diastolic depolarization sometimes developed and its prompt reversal in some fibers by decreasing the driven cycle length also suggests that loss of intracellular potassium ion was not the primary cause.

The indicated relationships between membrane potential and conduction are of interest from at least two standpoints. First, maintenance of relatively unimpaired conduction at membrane potentials greater than —70 to —75 mV is puzzling in light of the considerable decrease in amplitude and dv/dt of action potentials generated at these levels. Similar findings for incompletely repolarized fibers (4) have been attributed to a decrease in current required for excitation (13) because membrane potential is closer to the threshold potential and to an increase in the space constant of the fibers which results from an increased membrane resistance (8, 14) during phase 3. Both of these factors enhance the
effectiveness of electrotonic current spread and would tend to compensate for deterioration of the action potentials. In this manner, conduction may be preserved at lower levels of membrane potential than would otherwise be possible. Since reduced current requirements for excitation (13) and increased membrane resistance (8, 14) also occur during phase-4 depolarization, this explanation may be applicable to our experiments as well.

Second, these relationships permit certain deductions regarding the circumstances in which phase-4 depolarization is likely to result in advanced conduction disturbances. Since the threshold potential of normal Purkinje fibers in vitro is approximately −70 mv, if diastolic potential falls with reasonable rapidity to a value less than this, spontaneous firing will occur. At this level of membrane potential, the fiber is still capable of generating an effective action potential, and conduction is only moderately impaired. Therefore, it may be inferred that phase-4 depolarization is unlikely to result in advanced conduction disturbances in Purkinje fibers that are functioning normally in the sense that threshold potential and membrane responsiveness are normal. The absence of significant conduction disturbances in fibers in which phase-4 depolarization developed solely in response to low rates of stimulation is best explained in these terms.

In addition, it may be inferred that major conduction disturbances could occur in fibers in which threshold potential is shifted toward 0 or in which membrane responsiveness is impaired or both. Such shifts in threshold potential would enhance the effects of phase 4 depolarization by permitting a reduction in diastolic potential to levels at which significant depression of conduction would be likely to occur; i.e., to less than −70 mv. Impairment of membrane responsiveness, which results in the generation of action potentials of reduced amplitude and dV/dt at all levels of membrane potential, could sufficiently enhance the depressive effects of phase-4 depolarization to lead to significant conduction disturbances even at levels of membrane potential greater than −70 mv; i.e., in fibers with a normal threshold potential. Finally, severe conduction disturbances are most likely to occur as a result of simultaneous alterations in both variables in the directions indicated.

Under the conditions of our experiments, the maximum rates of depolarization (dV/dt of phase 0) of action potentials initiated at similar levels of membrane potential in the same fibers were comparable under control conditions and after development of phase-4 depolarization. Insofar as dV/dt of phase 0 is a valid index of the ability of a fiber to generate an inward sodium current on excitation (1, 2, 18), these findings preclude altered responsiveness as a causal factor in the abnormalities resulting from phase-4 depolarization. On the other hand, the fact that advanced conduction disturbances did not occur until diastolic potential had been reduced to less than −70 mv implies that their development may have been related to a shift in threshold potential toward 0 in the involved fibers. Successive shifts in this variable to lower and lower levels could be responsible for the progressive deterioration of transmembrane potentials and depression of conduction and excitability seen in the course of these studies. A limited number of measurements of threshold potential showed that the changes in activity noted in these experiments were accompanied by shifts in threshold potential towards zero. In addition, the observation that the alterations in threshold potential preceded either significant reduction in maximum diastolic potential or the onset of generalized diastolic depolarization are in accord with the hypothesis that the relationship is a causal one.

According to the ionic hypothesis, threshold potential is thought to be that level of membrane potential at which there is sufficient activation of “sodium carrier” to initiate a regenerative response. The value of the threshold potential is determined by a number of time and voltage-dependent variables (19). It seems likely that in our experiments the slow rate of phase-4 depolarization resulted in gradual inactivation of the sodium-carrier system and thus in a shift in threshold poten-
tial towards zero. This concept is based on the observation that slowly rising depolarizing current pulses elicit a smaller negative current carried by Na\(^+\) than do abruptly rising pulses of comparable magnitude (20) and that small, sustained depolarizing current pulses may depress membrane responsiveness to subsequent stimulation (21). Gradual inactivation of sodium carrier due to slow depolarization would necessitate reduction of diastolic potential to progressively lower levels before sufficient carrier could be made available to permit excitation. Threshold potential would, therefore, shift toward 0. Conversely, suppression of slow diastolic depolarization and restoration of normal levels of diastolic membrane potential would tend to return threshold potential toward normal. Evidence that suggests that the hyperpolarizing effects of epinephrine (9, 10) and other catecholamines (22) are accompanied by a shift in threshold potential away from 0 is in accord with this.

However, since development of phase-4 depolarization in otherwise normal fibers only rarely resulted in reduction in diastolic potential to less than −70 mv, inactivation of sodium carrier due to the low rate of depolarization could not be the sole factor underlying alterations in threshold potential observed in our experiments. It may be that the additional procedures (e.g., hypoxia, increased stretch on the fibers, exposure to ouabain, alterations in K\(^+\) and/or Ca\(^{2+}\) concentrations of the perfusate) necessary to induce development of progressive phase-4 depolarization also resulted in a reduction in total available sodium carrier.

CONDUCTION DISTURBANCES DUE TO PHASE-4 DEPOLARIZATION AS A POSSIBLE CAUSE OF ARRHYTHMIA

The observation that phase-4 depolarization of automatic cells can result in significant alterations in conduction and excitability has important implications in relation to the understanding of both the mechanisms underlying a variety of disturbances of cardiac rate and rhythm, and the mode of action of antiarrhythmic agents. Conduction disturbances resulting from a reduction in diastolic potential due to phase-4 depolarization could be widespread or confined to a small area. The specific manifestations would be determined by the location of the involved fibers and the extent to which they had become depolarized by the time propagated activity caused their excitation.

In the intact heart, widespread involvement of the Purkinje system could result in a diffuse and nonspecific pattern of intraventricular block; involvement of the bundle branches might cause bundle branch block and involvement of the His bundle a condition simulating atrioventricular block. Sufficient reduction of diastolic potential in both bundle branches or the His bundle thus might be one cause of advanced or complete “atrioventricular” block and Stokes-Adams attacks. The spontaneous variations in degree of block so commonly seen in patients could be readily explained in terms of changes in the number of involved cells and extent of phase-4 depolarization due to alterations in such variables as cardiac rate, degree of stretch on the specialized fibers, and local concentration of catecholamine or potassium.

Paradoxically, enhanced phase-4 depolarization in the His-Purkinje system could result not only in slowing of conduction or block but also in “supernormal” (23, 24) conduction. Since automatic cells begin to depolarize spontaneously immediately on completion of repolarization, membrane potential is maximum early in the cycle and declines progressively thereafter. Action potentials initiated in such cells during the latter part of repolarization and immediately after completion of depolarization may be of greater amplitude and dV/dt, and may, therefore, propagate more rapidly than those initiated late in the cycle. Thus, under conditions in which enhancement of phase-4 depolarization occurs in the His bundle and causes impaired atrioventricular transmission, atrioventricular conduction of premature systoles might be more normal than that of the basic beats (25). Enhancement of phase-4 depolarization in the bundle branches or more peripheral portions...
of the His-Purkinje system could, in similar fashion, result in supernormality of intraventricular conduction. Under normal conditions, maximum diastolic potential is attained immediately after the end of phase 3. In deteriorated fibers, after the onset of generalized diastolic depolarization, the entire voltage-time course of diastolic potential is altered and the maximum value may be reached quite late in the diastolic interval. For this reason it would not be reasonable to assume that a period of supernormality of conduction would always bear a fixed temporal relationship to the preceding action potential.

On the other hand, if enhancement of phase-4 depolarization was confined to small clusters of automatic cells, it could result in development of localized areas of block, which might be unidirectional or bidirectional depending on level of membrane potential. A parasystolic focus is generally thought of as a localized collection of automatic cells that undergo varying degrees of phase-4 depolarization. It is, therefore, tempting to think that localized conduction disturbances resulting from reduced levels of diastolic potential of the cells in the focus are responsible for many of the peculiarities of parasystole (23, 26). Such conditions provide the most reasonable explanation for the exit and entry block thought to occur about such foci. Changes in degree and direction of block, in conjunction with spontaneous changes in extent of phase-4 depolarization, could explain both alterations in parasystolic rate and the frequency with which parasystolic beats propagate to the remainder of the heart. Increases in rate and extent of phase-4 depolarization would reduce amplitude and dv/dt of action potentials initiated in cells of the focus and thus reduce the likelihood of successful propagation. Sufficient depolarization could prevent propagation of the parasystolic impulse altogether. Conversely, increases in levels of diastolic potential due to reduction in extent of phase-4 depolarization would decrease the degree of block and so facilitate reappearance of the parasystolic beats. Small variations in levels of diastolic potential due to spontaneous variations in extent of phase-4 depolarization could also readily explain the intermittent character of some parasystolic rhythms. Similarly, spontaneous changes in levels of diastolic potential in conjunction with variations in extent of phase-4 depolarization of a peripheral Purkinje pacemaker could also be an important factor in the intermittence and instability of such pacemakers. Such changes could result in asystole when these foci serve as the primary pacemaker of the heart, as in complete atrioventricular block.

The irregularity and intermittence of many peripheral Purkinje fiber or “low idioventricular” pacemakers is in itself quite puzzling, particularly when contrasted with the stability of foci in more proximal portions of the specialized conducting system, including the His bundle. If, however, as suggested by our experiments, very low rates of phase-4 depolarization predispose to instability of the threshold potential, then the phenomenon may be related to intrinsic differences in rate of phase-4 depolarization of the several fiber types. Since the peripheral Purkinje fibers exhibit the lowest rate of phase-4 depolarization, their erratic behavior may simply reflect fluctuations in spontaneous firing rate and degree of depolarization which result from alterations in threshold potential caused by changes in environmental conditions. Instability of the threshold potential with successive shifts toward 0 in conjunction with changing environmental conditions could also be a factor in the progressive depression of pacemaker activity that so often characterizes the dying heart (23).

Since both localized slowing of conduction and unidirectional block are thought to underlie the occurrence of reentrant excitation (23, 26, 27), it seems reasonable to suppose that phase-4 depolarization is a significant factor in the development of reentrant rhythms (Fig. 6) ranging from single extrasystoles to runs of ectopic tachycardia and fibrillatory arrhythmia. Under these circumstances, variations in diastolic potential, and consequently in the degree and direction of block resulting from variations in the rate and extent of
phase-4 depolarization would provide an explanation for alterations in the characteristics of the reentrant beats as well as for changes in their frequency.

Development of conduction abnormalities is often a rate-dependent phenomenon. Most often such abnormalities develop in conjunction with increases in rate and shortening of the cycle length, as in the case of the aberration of the QRS complex resulting from early atrial premature systoles or a rapid supraventricular tachycardia (23, 26). Such conduction disturbances are readily explained in terms of impulse spread through incompletely repolarized fibers (i.e., fibers still in phase 3). Similar conduction disturbances have also been reported in conjunction with a reduction in heart rate and prolongation of cycle length, for example, the QRS aberration of late supraventricular escape beats (28-30) and the bundle branch block that may appear during carotid-sinus massage (31). These abnormalities cannot be due to spread through incompletely repolarized fibers because the long diastolic interval should permit adequate time for full recovery prior to advent of the next impulse. Long diastoles, however, also facilitate phase-4 depolarization of automatic cells. Sufficient reduction of diastolic membrane potential due to this process could, therefore, cause such conduction disturbances. The tendency for ectopic rhythms to develop during periods of low heart rate and the frequent occurrence of such rhythms in instances of advanced or complete heart block with a slow idioventricular pacemaker (23) similarly could be explained in terms of the development of reentrant excitation in conjunction with enhancement of phase-4 depolarization during the long diastolic intervals.

These data also indicate that suppression of phase-4 depolarization and restoration of maximum diastolic potential toward normal should prevent development of related conduction disturbances and reentrant rhythms and should reverse such abnormalities once they have been established. In suppressing arrhythmia, the beneficial effects of potassium or of increasing heart rate by pacing may be due, at least in part, to prevention of conduction disturbances due to phase-4 depolarization. In light of recent evidence that low concentrations of procainamide also may reverse conduction abnormalities of this type (32), it is not unreasonable to suppose that this action may be a significant factor in its antiarrhythmic activity.

Agents which increase maximum levels of diastolic potential should also improve or abolish conduction abnormalities due to phase-4 depolarization, irrespective of their effects on this variable. A great part of the effectiveness of catecholamines in improving conduction in depressed hearts (33) and in suppressing certain types of ventricular tachycardias (34-36), despite their propensity for enhancing automaticity, is almost certainly due to the hyperpolarizing actions of these agents on partially depolarized fibers (see Figs. 9 and 10). This explanation seems even more likely in light of evidence that the hyperpolarizing actions of catecholamines need not be accompanied by enhancement of phase-4 depolarization (11, 12). In some instances, epinephrine-induced hyperpolarization of abnormal cells has been accompanied by inhibition of this process (11, 12). The effectiveness of catecholamines in enhancing responsiveness of refractory ventricular fibrillation to countershock (37) may also be explained on this basis, the increase in maximum diastolic potential resulting in generalized improvement of conduction and a decrease in fragmentation of the excitation wave.

**APPLICABILITY OF FINDINGS TO THE IN SITU HEART**

Automatic cells are so widely distributed throughout the specialized tissues, particularly the His-Purkinje system, that it seems reasonable to believe that phased depolarization is an important factor in the development of conduction disturbances and reentrant excitation in the in situ heart.

On the basis of our experiments, it may be postulated that this mechanism is likely to be operative in circumstances known to enhance automaticity and increase the extent of depolarization of the involved cells: (a) low
heart rates with resultant long diastoles; (b) increased stretch of specialized fibers as might result from severe cardiac dilation; (c) exposure to toxic concentrations of ouabain; (d) reduction in serum potassium and ionized calcium concentrations; (e) ischemia and hypoxia. Factors that depress membrane responsiveness to any considerable extent, such as exposure to moderate or high concentrations of standard antiarrhythmic agents (13, 38), would also increase the likelihood of the occurrence of significant abnormalities due to this cause. Conversely, factors that suppress phase-4 depolarization or increase maximum levels of diastolic potential, or both, should have the opposite effects.

Since circumstances that facilitate development of phase-4 depolarization are common in the human heart and particularly the diseased human heart, it is reasonable to suppose also that this mechanism may be operative in producing conduction disturbances and arrhythmias in man.

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Interrelationships Between Automaticity and Conduction in Purkinje Fibers
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