Rate-dependent Changes in Excitability of Depressed Cardiac Purkinje Fibers as a Mechanism of Intermittent Bundle Branch Block

Jose Jalife, M.D., Charles Antzelevitch, Ph.D., Vito Lamanna, M.D., Ph.D., and Gordon K. Moe, M.D., Ph.D.

SUMMARY When the heart rate is accelerated, rate-dependent intraventricular block may occur. This block has been attributed to abnormal action potential prolongation in a diseased conducting pathway. Less often, intraventricular block develops during slowing of the heart rate and has been explained in terms of phase 4 depolarization in potentially automatic cells within the diseased fascicle. We tested these hypotheses in isolated bundles of Purkinje fibers placed in a three-chambered tissue bath. In one group of experiments, conditions of localized injury and depressed excitability were mimicked by superfusing the central segment with sucrose solution. Action potentials were initiated in the proximal segment while the slope of phase 4 of cells in the distal end was controlled by intracellular ramps of current of either polarity. In these preparations, phase 4 depolarization facilitated rather than retarded propagation across the depressed segment, even at takeoff potentials as low as −45 mV. In a second group, depressed excitability was induced by exposing the three fiber segments to Tyrode’s solution that contained high concentrations of KCl and CaCl₂ or isoproterenol (0.1 μg/ml). Under these conditions, Purkinje fibers did not undergo phase 4 depolarization and did not generate abnormally prolonged action potentials. These preparations showed a biphasic time dependence of conduction during premature stimulation or in response to changes in the basic cycle length. Conduction impairment and block were manifest at either side of an optimal interval or cycle length. Our results suggest that phase 4 depolarization and abnormally prolonged action potentials are not necessary conditions for intermittent block. Both tachycardia and bradycardia-dependent intraventricular conduction abnormalities may be associated with time-dependent variations in the excitability of depolarized conducting fibers as well as in the amplitude of the slow responses generated by these fibers. These alterations can be explained in terms of regulation of slow inward current by the intracellular calcium concentration.

THE PHENOMENON known as “intermittent (heart rate-dependent or transient) bundle branch block” was first described by Lewis in 1913 and demonstrated experimentally by Drury and Mackenzie in 1934. In most cases, heart rate–dependent bundle branch block occurs when a critical heart rate is exceeded, and has been attributed to a prolongation of recovery in the diseased bundle branch. Less common cases have been reported in which bundle branch block develops after long diastolic intervals or during slowing of the heart rate.

Several hypotheses have been proposed to explain these phenomena, including concealed conduction, hypoxia, vagal effects and supernormality of the affected bundle. Recently, Singer et al. demonstrated that depolarization of depressed Purkinje fibers can lead to propagation impairment and can produce slow conduction or even blockade. Their experiments suggested that conduction abnormalities associated with prolongation of the cycle length could be the result of the development of phase 4 depolarization in potentially automatic cells. Based in part on Singer’s hypothesis, Rosenbaum et al. proposed a model in which entrance block and phase 4 depolarization (phase 4 block) in the diseased fascicle play a major role in the development of bradycardia-dependent bundle branch block. On the other hand, premature excitation during abnormally prolonged action potential duration in the tissues surrounding the depressed zone would result in tachycardia-dependent bundle branch block.

This is an appealing model that is supported by some clinical and experimental evidence. Yet, recent electrophysiologic experiments have demonstrated that phase 4 depolarization can accelerate, rather than retard, conduction in linear bundles or across areas of depressed excitability. Other experiments have shown that block of premature impulses across a depressed zone need not be associated with abnormally prolonged action potentials. However, all of these experiments were done using normally polarized Purkinje fibers and may not be representative of what actually occurs in the clinical situation, in which injured tissues may be involved.

Recently, we provided preliminary evidence indicating that an area of depressed excitability in an isolated false tendon may lead to rate-dependent conduction alternations. We demonstrated that slow diastolic depolarization in localized areas of the bundle can facilitate or impair conduction, depending on the position of the pacemaking cells with respect to the site
of block. In addition, experiments from several laboratories have demonstrated rate-dependent alterations of excitability in partially depressed tissues that do not undergo phase 4 depolarization. These alterations have been related to changes in the amplitude of the slow-response action potential, as a function of the basic cycle length.

In the present study, we used the sucrose gap preparation of cardiac Purkinje fibers to determine the input-output relations as well as the frequency dependence of conduction and excitability in normal and depressed tissues.

Methods

Purkinje fibers were excised from anesthetized (sodium pentobarbital, 35 mg/kg i.v.) sheep and dogs or were obtained from calf hearts supplied at slaughter. Linear, unbranched preparations were placed in a three-chambered tissue bath and superfused with oxygenated (95% oxygen, 5% CO₂) Tyrode’s solution. Unless otherwise indicated, the composition of the solution was (mM): Na⁺ 155, K⁺ 4.0, Cl⁻ 142, HCO₃⁻ 24, H₂PO₄ 0.9, Mg²⁺ 1.0, Ca²⁺ 1.8, and dextrose 5.6. The temperature was maintained at 37 ± 0.5°C and the pH was 7.4 ± 0.5.

The preparations were allowed to equilibrate for 1 hour while driven at various basic cycle lengths (BCLs) with biphasic pulses (1–2 msec) delivered by a stimulator (P6i, Frederick Haer) through one of two pairs of thin bipolar electrodes on the surface of the lateral segments. Transmembrane potentials were recorded from these segments as previously described. The first derivative of the transmembrane potential (dV/dt) was obtained by an electronic differentiator that was linear between 100 and 1000 V/sec. The amplified signals were displayed on an oscilloscope (Tektronix, 565) and photographed with a Grass (Model C4) kymographic camera.

Determination of Conduction–Membrane Potential Relation

In the first group of experiments in Purkinje fibers we studied, the relationship between membrane potential, excitability and conduction of impulses initiated in the proximal segment across the sucrose gap, while the slope of phase 4 depolarization in the distal element was controlled by the application of long ramps or steps of intracellular current. In a second group, depressed excitability was induced by exposing the three fiber segments to a modified Tyrode’s solution containing high concentration of KCl and CaCl₂, or isoproterenol (0.1 μg/ml).

In the current-injection experiments, the proximal and distal segments of the fiber were superfused with Tyrode’s solution that contained 4 mM KCl, while the central segment was superfused with isotonic sucrose solution. A variable resistor (0–100 kΩ), connected to two Ag–AgCl electrodes, was used to bridge the gap (2 mm long) and to modulate the degree of conduction impairment between proximal and distal segments. When the conditions of high-degree block were achieved, the proximal segment was driven by applying trains of 10–20 basic stimuli (BCLs 500–1000 msec) separated by 2–4-second pauses. The last stimulus in the train was used to trigger another digitally controlled P6i unit that delivered a slowly rising ramp of current through an intracellular microelectrode, located at an intermediate point between the rubber membrane and recording electrodes, but within less than 100 μm from the intracellular recording electrode in the distal segment. The intracellular current ramp, of variable slope and polarity, was programmed to start at the maximum diastolic potential of the last basic response in the train and to terminate at the moment of the first stimulus after the pause. The intracellular stimulation technique has been described in detail.

Determination of Rate-dependent Conduction Characteristics

Input-output characteristics were studied by premature stimulation techniques during sucrose superfusion of the central segment. The conducting properties of the preparation were studied at various cycle lengths and shunt resistance values, and time-dependent changes of excitability were assessed by scanning the diastolic interval of the distal (test) segment with relatively long (100–200 msec) depolarizing current pulses, applied across the gap. Current was measured as the voltage drop across a 1000-Ω resistor in series with the negative side of the circuit.

The distal segment was superfused with Tyrode’s solution containing KCl at concentrations of 4–25 mM and CaCl₂ concentrations of 1.8–20 mM. No attempt was made to correct osmolarity. In some experiments, isoproterenol, 0.1 μg/ml, was added to the Tyrode’s instead of the high calcium. Lidocaine (20 μg/ml) was used in some preparations to abolish fast-channel activation.

Results

Can Phase 4 Depolarization Cause Entrance Block?

The influence of diastolic depolarization on excitability and conduction was studied in a group of eight experiments. Figure 1 shows the results when all three segments of a long Purkinje fiber (1.2 cm) placed in the three compartment bath were superfused with 6 mM KCl Tyrode’s solution. In all panels, the sweep started with the last of 10 basic responses initiated in the proximal segment (P) at a BCL of 500 msec. The last response was followed by a 3-second pause, after which a new train of stimuli was started. Panel A shows the control; PD conduction time, measured at 50% of peak amplitude, was 3 msec. At a control takeoff potential of −82 mV, the maximum upstroke velocity (dV/dt max) of the impaled cell in the distal element (D) was 320 V/sec. In panel B, a slowly rising hyperpolarizing ramp was applied through an intracellular microelectrode located very close to the recording site in D. Upon termination of the ramp, an action potential initiated in the proximal segment activated D at a time when its membrane potential had been increased to −88.5 mV. Consequently, the amplitude
and dV/dt max of the distal response increased to 110 mV and 340 V/sec, respectively, but the PD conduction time remained constant at 3 msec. In panel C, a ramp of the opposite polarity depolarized the membrane of the distal segment to −65 mV and decreased amplitude (76 mV) and dV/dt max (120 V/sec). Conduction velocity, however, was not appreciably affected; the PD conduction time remained at about 2 msec (not shown). In panel D, the amplitude of the depolarizing ramp was increased. At its peak, this ramp brought the membrane potential to −52 mV and must have completely inactivated the fast inward current, as demonstrated by the greatly diminished amplitude and slow upstroke velocity of the response. However, even at this level of depolarization, the effects on conduction velocity were relatively minor; the PD conduction time, measured at half the amplitude of the slowly rising response in D, was less than 5 msec. The results shown in figure 1 are typical of five experiments, and demonstrate that phase 4 depolarization itself cannot be responsible for blockade of impulses into conducting fibers undergoing progressive loss of membrane potential during long diastolic intervals.

**Can Phase 4 Depolarization Facilitate Conduction?**

The results from an experiment in which we studied the influence of diastolic depolarization on conduction across a depressed segment during sucrose superfusion of the central chamber are shown in figure 2. The two outer segments were superfused with 4 mM KCl Tyrode's solution. Three superimposed oscilloscopic sweeps are shown. The top traces were recorded from the proximal segment and the middle traces from the distal segment with its dV/dt displayed in the bottom traces. In all sweeps, the initial action potential is the last of a series of 10 evoked in P by bipolar stimulation at a BCL of 500 msec. A 50-kΩ shunt resistance was used to bridge the gap. At this rate, complete PD block occurred, and only subthreshold depolarizations were apparent in the distal segment. Under control conditions, a postmature action potential initiated in P after a diastolic interval of 1850 msec activated the distal fiber after 141 msec (discharge A). This long delay occurred despite the normal configuration of the distal action potential and despite its large amplitude (89 mV) and dV/dt max (475 V/sec). In the next sweep, application of a depolarizing ramp to the distal segment increased its slope of phase 4 depolarization and facilitated its approach to threshold. When, at the end of the ramp, the proximal segment was stimulated, propagation was again successful, but the PD conduction time was reduced to less than 59 msec. This occurred even though the amplitude and dV/dt of phase 0 were significantly decreased in the distal segment, as a result of a reduction of the takeoff potential. In contrast, when the slope of phase 4 was inverted by the application of a hyperpolarizing ramp, complete block was induced, and only the electrotonic image of the proximal action potential was apparent in the distal trace.

This experiment indicates that activation across an area of complete inexcitability by electrotonic currents can be greatly facilitated by the development of phase 4 depolarization in fibers distal to the zone of block. Facilitation could also be demonstrated during the application of ramps that depolarized the membrane of cells in the distal segment to levels at which the fast sodium inward current was completely inactivated. This is illustrated in figure 3, obtained from an experiment in which sucrose superfusion during 1 hour had produced complete PD block when no shunt bridged the gap. In panel A, the last two action potentials of a train of 10, initiated in the proximal segment (top trace) at a BCL of 1000 msec, were blocked and induced only subthreshold depolarization in the distal segment (bottom trace). After a 3100-msec pause, another response generated in P failed to propagate across the gap (panel A). The records in panel B were taken several seconds later. Eight hundred milliseconds after the last response in the train (second action potential in panel B), a hyperpolarizing step was applied in the distal segment, and was maintained for more than 5 seconds. During the step, the membrane potential of the distal cell (bottom trace in panel B) changed from the control value of −82 mV to −94.5 mV. Again, the third proximal response did not propagate through the gap, but it induced a subthreshold depolarization smaller in amplitude than the control. In
the conditions of high-degree block, the development of phase 4 depolarization in cells within or distal to the area of block can greatly facilitate propagation, even when diastolic depolarization in the recipient cells displaces the takeoff potential to $-60 \text{ mV}$ or less.

**Is Phase 3 Block a Result of Abnormal Action Potential Prolongation?**

The input-output relationships of cardiac tissues across an area of depressed conductivity can be studied in the sucrose gap. The degree of conduction impairment can be controlled with an ohmic resistor connected to the outer chambers, and the conducting properties of the system can be assessed by applying progressively earlier stimuli to the proximal segment.

The experiment illustrated in figure 4 was taken from a calf Purkinje fiber–sucrose gap preparation in which a 20-kΩ resistance was used to shunt the gap. Under these conditions, action potentials (P) initiated in the proximal segment (top in panels A–C) at a BCL of 2000 msec activated the distal segment (bottom in panel C, the resting membrane potential had been returned to the control value. After the train, a long (7 seconds) intracellular ramp of the opposite polarity gradually depolarized the cell in the distal segment. When an action potential was again induced after a delay interval of 3100 msec (i.e., when the ramp had depolarized D by about 20 mV), a greatly enhanced electrotonic depolarization brought the membrane to a threshold potential of $-49 \text{ mV}$ and gave rise to an action potential that had all the characteristics of a slow response. From this experiment, it is clear that under

**Is Phase 3 Block a Result of Abnormal Action Potential Prolongation?**

The input-output relationships of cardiac tissues across an area of depressed conductivity can be studied in the sucrose gap. The degree of conduction impairment can be controlled with an ohmic resistor connected to the outer chambers, and the conducting properties of the system can be assessed by applying progressively earlier stimuli to the proximal segment.

The experiment illustrated in figure 4 was taken from a calf Purkinje fiber–sucrose gap preparation in which a 20-kΩ resistance was used to shunt the gap. Under these conditions, action potentials (P) initiated in the proximal segment (top in panels A–C) at a BCL of 2000 msec activated the distal segment (bottom in panel C, the resting membrane potential had been returned to the control value. After the train, a long (7 seconds) intracellular ramp of the opposite polarity gradually depolarized the cell in the distal segment. When an action potential was again induced after a delay interval of 3100 msec (i.e., when the ramp had depolarized D by about 20 mV), a greatly enhanced electrotonic depolarization brought the membrane to a threshold potential of $-49 \text{ mV}$ and gave rise to an action potential that had all the characteristics of a slow response. From this experiment, it is clear that under

---

**Figure 2.** Phase 4 depolarization facilitates while hyperpolarization prevents activation of distal (D) segment by proximal (P) impulse across the sucrose gap in a normally polarized dog Purkinje fiber (4 mM KCl Tyrode's in panel D). Three superimposed traces are shown. At a basic cycle length of 500 msec, all proximal impulses were blocked. In the control, a 1850-msec diastolic interval permitted recovery; distal activation was successful at a long P-D delay (A, middle trace); dV/dt was maximal (trace A; bottom). A depolarizing ramp (duration 1.8 sec; peak current 0.1 μA) applied during diastole through a microelectrode in D brought the membrane closer to threshold; PD conduction was greatly accelerated (B, middle trace), but dV/dt was much less than control (trace B, bottom). When the polarity of the ramp was inverted (middle trace in C), complete block was manifest (peak current 0.18 μA). Shunt resistance is 50 kΩ. Spikes have been retouched.

**Figure 3.** Phase 4 depolarization facilitates propagation even at very low levels of membrane potential of a dog Purkinje fiber in sucrose gap. Top traces show the proximal (P) and bottom traces the distal (D) cells. Same impalement in all panels. The basic cycle length is 1000 msec; shunt resistance = $\infty$. A is the control; B, hyperpolarizing step (7-second) increased degree of block. In C, a depolarizing ramp increased the amplitude of the electrotonus and permitted distal activation at takeoff potential of $-49 \text{ mV}$. Proximal spikes retouched.
panels A–C) after a constant delay of 55 msec. Premature stimuli (P₂), applied after every tenth basic beat, scanning the basic cycle at progressively earlier intervals, gradually increased the duration of the electrotonic "foot" that preceded the distal action potential, and delayed the D discharge time (A–B). Premature stimulation at P₁P₂ intervals of 650 and 530 msec produced prolongation of the P₁D₂ interval to 80 and 180 msec in A and B, respectively. Premature P₂ stimuli at intervals earlier than 530 msec were blocked at the gap, and they induced only subthreshold depolarizations in the distal segment (fig. 4C). Panel D shows the complete scan in the same experiment. The P₁D₂ interval is plotted in the ordinate scale as a function of the P₁P₂ interval. The time of activation of the distal segment increased progressively with the prematurity of P₂. The effective refractory period of the system, defined as the longest premature interval at which stimulation of the proximal segment fails to generate a distal response, can greatly outlast repolarization from a previous action potential. These data suggest that tachycardia-dependent conduction abnormalities in partially depolarized tissues need not be associated with abnormally prolonged action potentials.

Alternative Explanations for Intermittent Bundle Branch Block?

It is very unlikely that injury of a fascicle by ischemia or stretch can lead to identical conditions as superfusion of a fiber segment with ion-free sucrose solution. It is indeed more reasonable to assume that myocardial injury involving one of the bundle branches should lead to partial depolarization of groups of cells within the affected bundle, creating the conditions necessary for intermittent bundle branch block.

The recordings shown in figure 5 were obtained from a preparation exposed in its whole length to a solution containing 20 mM KCl to mimic the environmental conditions that might prevail in depressed cardiac tissues. In this experiment, three microelectrode impalements were maintained at approximately equidistant sites in an unbranched canine false tendon. The top trace was recorded from a cell that was within less than 1 mm from the stimulating bipolar electrode. The middle trace is from an intermediate recording site 5 mm distal to the stimulating electrode. The bottom trace corresponds to an impalement in the far end of the preparation about 1 cm from the bipolar electrode. Stimulation at a relatively slow rate (BCL of 1500 msec) generated the initial two slow responses recorded with the most proximal electrode. These responses were blocked somewhere before they reached the intermediate electrode, producing only subthreshold electrotonic depolarizations, which were completely absent in the most distal recording site (bottom trace). However, as the interval between stimuli was diminished for the third and fourth responses, the amplitude of the depolarization in the middle portion of the fiber increased progressively, and subthreshold depolarizations were manifest in the bottom trace, suggesting that premature stimulation facilitated conduction and shifted the block to a more distant site.

Thus, rate-dependent conduction disturbances can be demonstrated in partially depressed conducting bundles that do not undergo phase 4 depolarization. These disturbances can become manifest either when the heart rate is accelerated or as a consequence of deceleration. One way to better understand the mechanism of these biphasic phenomena is to study the input-
output relations of Purkinje fiber preparations under the conditions of high KCl-induced depolarization. Figure 6 shows the results from one of these experiments. Two cells located at the extremes of the fiber were impaled. In all panels, several superimposed traces are shown. The upper traces were recorded from a site very close to the bipolar stimulating electrodes. The lower traces were taken from a site 12 mm distal to the bipolar electrode. The preparation was driven with trains of 10 stimuli at a BCL of 500 msec and test stimuli were applied after every tenth basic beat to scan the long diastolic interval separating the trains. At the basic stimulation frequency, all slow responses were blocked before they reached the distal cell, as shown by the brief duration of the proximal action potentials and the subthreshold depolarizations in the distal trace during the initial responses of all panels. However, when test stimuli were applied at progressively later intervals, progressively greater subthreshold depolarizations were induced in the distal cell until, at stimulus intervals of 180 msec (arrow, panel A) the distal depolarization reached threshold and a propagated response ensued. The proximal-to-distal (peak-to-peak) conduction time for this response was about 100 msec.

All slow responses initiated at intervals between 200 and 480 msec were conducted to the distal site. However, at test intervals of 385 msec or longer (panel C), PD conduction time increased progressively until at the stimulus interval of 680 msec (arrow, panel C) complete block reappeared. Only a subthreshold depolarization was again apparent in the distal impalement. The amplitude of this depolarization decreased progressively at the later intervals and no distal responses were obtained thereafter.

**Are Diastolic Changes in Excitability Responsible for Rate-dependent Block?**

The biphasic time dependence of the amplitude of the distal activity in response to premature stimulation can also be demonstrated during the application of relatively long depolarization current pulses across the sucrose gap. A comparison of the time-dependent changes in the effects produced by these pulses in normal vs depolarized fibers is illustrated in figure 7.

In the experiment shown in figure 7A, the test compartment was perfused with Tyrode's solution containing 4 mM KCl, whereas the opposite chamber was perfused with 25 mM KCl Tyrode's during isotonic sucrose perfusion of the central chamber. Each of the nine superimposed traces started with the last of a series of 10 basic beats initiated in the test segment by bipolar stimulation (BCL of 1000 msec). The diastolic interval was scanned with 300-msec (0.7 μA) depolarizing pulses applied after every tenth basic beat. Pulse application at progressively later intervals with constant current amplitude produced, as expected, progressively greater depolarizations. A similar scan was done in the same fiber during superfusion of the test segment with 20 mM KCl and 20 mM CaCl₂ Tyrode's solution produced quite different results (fig. 7B). In the absence of phase 4 depolarization, square-current pulses (duration 300 msec, amplitude 1.60 μA) produced depolarizations that increased progressively in amplitude and then decreased as they were applied later in diastole.

The data presented in figures 6 and 7B demonstrate that in the absence of any apparent changes in resting membrane potential during diastole, depressed fibers show a biphasic time course in their response to depolarizations scanning the diastolic interval; and conduction impairment phenomena analogous to intermittent bundle branch block can be demonstrated at fast and slow rates, in the absence of abnormally prolonged action potentials as well as in the absence of phase 4 depolarization. Figure 8 further illustrates this point in a similar preparation in which the test segment had been depolarized by continuous superfusion with 25 mM KCl Tyrode's solution that contained 10 mM CaCl₂ and 0.1 μg/ml isoproterenol. Depolarizing current pulses, 200 msec in duration and constant amplitude of 2.4 μA, were used to drive the test segment at various cycle lengths. In panel A, the initial five pulses, applied at a cycle length of 300 msec, induced only subthreshold depolarizations of the test segment. However, when the cycle length was abruptly changed to 600 msec, all depolarizations reached threshold and induced active responses. The amplitude of these re-

---

**Figure 6.** Rate-dependent facilitation and conduction of slow responses. Dog false tendon exposed to a solution containing 20 mM KCl and 9 mM CaCl₂ and stimulated at a basic cycle length of 2000 msec. Premature stimuli were applied every tenth beat with an increasing delay to 'scan' the diastolic interval. The superimposed records in all three panels were recorded within 3 minutes. The upper record in each of them corresponds to a site close to the bipolar stimulating electrode; the lower record, to a site 12 mm apart. Stimulus artifacts and upstrokes have been retouched.
sponses increased progressively during the first four beats, and then declined asymptotically toward a new steady-state amplitude of 63.5 mV. When this train was stopped and a single pulse of the same amplitude and duration was applied after an interval of 3600 msec, the membrane potential again could not reach threshold and no active response was generated. Acceleration (panel B) or deceleration (panel C) from the optimal stimulus cycle length of 600 msec to 300 and 3600 msec, respectively, was accompanied by sudden changes in the amplitude of the electrotonic depolarization that became subthreshold at both sides of the optimal value.

These frequency-related phenomena are extremely reproducible and very much dependent on the concentration of potassium in the solution bathing the test compartment. In fact, the time dependency of the changes in the amplitude of the electrotonic events can be reverted from "normal" (fig. 7A) to biphasic (fig. 7B) by changing the potassium concentration from 15 to 20 mM. Figure 9 shows a series of traces at KCl 15 mM. The Tyrode's solution also contained 10 mM CaCl₂ and 1 µg/ml isoproterenol to enhance slow-channel activation as well as 20 µg/ml lidocaine to ensure that the fast sodium channels were not activated. Several superimposed sweeps are shown. In all panels, the initial action potential is the last of a series of 10 in response to 300-msec (3.1 µA) depolarizing current pulses (BCL of 1000 msec). In panel A, test depolarizing current pulses of fixed amplitude (2.4 µA) and duration (300 msec) were used to scan the diastolic interval after every tenth basic pulse. The test pulses induced progressively greater depolarizations of the cell membrane. When the current strength was increased in small steps (panels B, C, D and E), the electrotonic depolarizations reached threshold only at the later intervals. However, the amplitude of the active responses decreased progressively even though their takeoff potentials occurred at more negative values as the pulse was applied later during diastole (panels C, D and E).

Figure 10 shows the results obtained in the same preparation when the K⁺ concentration was raised to 25 mM. Under these conditions, the biphasic nature of the amplitude of the electrotonic potential as a function of time became clearly apparent. In panel A, when the current amplitude was relatively low the electrotonic depolarizations gradually increased, and then decreased as they were induced at progressively later intervals. In panels B, C and D, by increasing the amplitude of the current in very small steps, we predictably increased the number of active responses generated at the intermediate intervals. In panel E, when the current amplitude was further increased, all depolarizations started at intervals longer than 240 msec reached threshold. However, their amplitude decreased gradually at progressively later intervals.

**Discussion**

**Phase 4 Depolarization Can Facilitate Propagation**

In 1967, Singer et al. showed that when isolated canine Purkinje fibers were subjected to stretch, hypoxia or other interventions, there was often a decrease in their maximum diastolic potential. If the membrane potential was reduced sufficiently, action potential amplitude and upstroke velocity also decreased. Since dV/dt of phase 0 can be an important determinant of conduction velocity in excitable tissues, Singer et al. suggested that phase 4 depolarization, which decreases takeoff potential (and thus amplitude and dV/dt), can lead to propagation impairment and can be responsible for the ("entry and exit") block of impulses into and out of clusters of potentially automatic cells. Thus, under one set of circumstances, phase 4 depolarization would represent an effective barrier that would prevent impulses from propagating into a de-
pressed zone. Under other circumstances, phase 4 depolarization in cells proximal to or within the depressed zone would lead to a lower action potential amplitude and dV/dt, hence offering a relatively weak input to cells located more distally.

We have focused our attention on the former hypothesis and have examined it in preparations in which the slope of phase 4 depolarization of the recipient units, and thus their takeoff potentials, were controlled by the application of current ramps during diastole. Our experiments clearly indicate that entrance block at slow rates of stimulation need not be explained in terms of phase 4 depolarization, even when diastolic depolarization shifts the takeoff potential to levels at which the sodium channels are completely inactivated (fig. 1D). Propagation can still be sustained through the slow inward current and, under these conditions, time to excitation of the depolarized fiber remains practically unchanged despite a decrease in the upstroke velocity.

When an impulse initiated in the proximal segment is blocked at the junction with the inexcitable element in the sucrose gap, a subthreshold electrotonic potential may be recorded in the segment distal to block. The local circuit current flow between proximal and distal tissues may be able to bring the distal element to its threshold potential, but only after a delay imposed by the resistance-capacitance (RC) properties of the system and by the length of inexcitable tissue through which local circuit currents have to travel. Obviously, if the length of the inexcitable gap is too long or if excitability of the post-gap segment is totally depressed, conduction could be lost. However, if this segment is normal or is partially depolarized but capable of generating slow-response action potentials, the success of propagation would depend primarily on the amplitude of the electrotonic depolarization and on the threshold potential for the regenerative current. In fibers undergoing phase 4 depolarization, there is a progressive increase in membrane resistance and space constant, as well as a gradual approach to the threshold potential. Thus, one may assume that if slow diastolic depolarization develops in the tissue distal to the zone of block, conduction would be accelerated.

This thesis has been amply confirmed by our experiments (figs. 2 and 3), and is of particular importance in cases of diastolic depolarization in partially depressed fibers in which fast sodium channels are completely inactivated (fig. 3). Again, under these conditions, phase 4 depolarization can greatly facilitate electrotonic transmission and relieve the blockade. Accordingly, it is very unlikely that bradycardia-dependent conduction impairment in a specialized bundle can result from entrance block secondary to phase 4 depolarization in excitable cells within or distal to a depressed zone in the bundle.

Our experiments do not completely rule out the involvement of phase 4 depolarization in the generation of frequency-dependent conduction disturbances. Indeed, in a recent study, we confirmed Singer's idea that bradycardia-dependent conduction abnormalities can sometimes result from exit block associated with diastolic depolarization in cells proximal to an area of depressed excitability. However, although phase 4 depolarization may contribute to a decrease in amplitude of proximal responses and may therefore facilitate the development of block, its presence is not absolutely necessary. Frequency-dependent changes in the magnitude of the slow inward current can alter the amplitude of action potentials in normally polarized Purkinje fibers proximal to a zone of block, and can lead to changes in the efficacy of electrotonic inputs to activate tissue distal to the block. These changes are independent of the maximal upstroke velocity, and they have been shown to occur in the complete absence of slow diastolic depolarization. Thus, some exam-
Mechanism of Tachycardia-dependent Block

Rate-dependent conduction disturbances in specialized pathways are most often observed when the heart rate is accelerated or during premature atrial or ventricular complexes. Their mechanism also is not well understood. These disturbances have been attributed to incomplete recovery of excitability generated by an abnormal prolongation of action potential duration in damaged and partially depressed fibers within the diseased fascicle. Although, theoretically, abnormal action potential prolongation could indeed explain the mechanism of tachycardia-dependent bundle branch block, the hypothesis is untenable in light of the well-known effects of cell damage and membrane depolarization, which can lead to marked action potential shortening rather than prolongation. In addition, as demonstrated in the experiments of Downar et al., in which transmembrane potentials were recorded in situ from ischemic tissues or in which isolated cardiac cells were superfused with "ischemic" blood, membrane damage and depolarization can lead to postdepolarization refractoriness despite a significant abbreviation of the action potential. Furthermore, our experiments (see fig. 4) indicate that frequency-dependent conduction abnormalities associated with rapid heart rates need not be related to abnormally prolonged action potentials. In fact, it appears likely that localized depression in a bundle branch can set the stage for conditions similar to those that prevail in other nonhomogeneous systems, such as the sucrose gap preparation and the atrioventricular node, and can generate functional block between normal and partially depressed tissues. If so, the development of tachycardia-dependent bundle branch block should be predicted by input-output characteristics comparable to those described here. Indeed, similar input-output relations, postdepolarization refractoriness and tachycardia-dependent block have been found in Purkinje fibers in which the central segment was rendered excitable by superfusion with an "ischemic solution".

Rate-dependent Block, a Unified Theory

Our experiments provide an alternative explanation for intermittent conduction disturbances associated
with changes in the heart rate. We have demonstrated that conduction delay and block can occur in a rate-dependent manner when Purkinje fibers are exposed to Tyrode’s solution containing high concentrations of KCl and CaCl₂. Under these conditions, conducting fibers do not develop slow diastolic depolarization and do not generate abnormally prolonged action potentials, except in cases in which very long delays in activation of distal elements electrotonically postpone repolarization of proximal sites (fig. 5). In these preparations, scanning the diastolic interval with progressively earlier premature beats yields a biphasic time-dependence of conduction along the bundle, with conduction impairment and block at either side of an optimal interval. Our results also suggest that these changes are associated not with slow diastolic depolarization, but with time-dependent variations in the excitability of the depolarized conducting fibers as well as in the amplitude of slow-response action potentials generated by these fibers. Thus, both bradycardia- and trachycardia-dependent block in the His bundle or one of its branches may have an alteration in the excitability of depressed fibers as a common cause. In fact, several features of the characteristic behavior in clinical cases of intermittent bundle branch block can be readily explained by the phenomena demonstrated in this study. These include not only the time-dependent changes in atrioventricular and His-Purkinje conduction during diastolic scanning, but also most of the conduction alterations demonstrated electrocardiographically during progressive slowing of the heart rate.⁶-⁸ These phenomena are readily reproducible in the isolated preparation (figs. 8–10) and they can be attributed to frequency-dependent changes in excitability and amplitude of slow response action potentials.

One additional behavioral characteristic demonstrated in this study has important clinical implications. In their perturbation experiments in patients with intermittent bundle branch block, Rosenbaum et al.⁹ demonstrated that in most cases, two frequency ranges, one fast, one slow, of conduction impairment are separated by and appear to “compress” an intermediate range of frequencies at which conduction is normal. They termed this phenomenon the “bellows-like effect” because the dimensions of each of the individual ranges varied greatly from patient to patient. According to Rosenbaum et al., the significance of the bellows-like effect becomes even more apparent when the time course of development and disappearance of intermittent bundle branch block is studied in patients at different stages of the underlying disease. When severe conduction impairment is present, the intermediate frequency range of successful propagation is absent or extremely narrow, but during recovery it widens in either direction, at the expense of the two ranges of block. These phenomena can be readily mimicked in the sucrose gap preparation. Of course, it is very unlikely that injury of a conducting bundle by ischemia, inflammatory diseases or stretch can generate conditions identical to those present in the sucrose gap. Yet, experiments in which the test segment is superfused with high-KCl Tyrode’s solution can provide important clues for the mechanism of these changes.

As demonstrated in figures 9 and 10, successful activation of a depressed segment across an area of block in a conducting bundle is much more probable at intermediate ranges of frequencies, and depends not only on the amplitude of the electrotonic potential across the block but also on the extracellular potassium concentration. Accordingly, membrane depolarization induced by cell damage in patients who develop intermittent bundle branch block might tend to unmask the biphasic nature and frequency dependence of slow response action potentials in localized regions of the diseased fascicle. The bellows-like effect demonstrated by Rosenbaum et al.⁹ may also be associated with progressive restoration of membrane potential and gradual recovery of normal excitability in these fibers.

**Ionic Basis of Intermittent Block**

The mechanism responsible for these time- and frequency-dependent changes in slow response amplitude is not well understood. Obviously, diastolic changes in membrane resistance are not responsible, for in the absence of electrotonic input the resting membrane potential is stable throughout the diastolic interval. In addition, unpublished data from our laboratory have shown that when experiments similar to those shown in figures 7B, 9 and 10 are performed using hyperpolarizing pulses of constant amplitude and duration, no time-dependent variations in the amplitude of the electrotonus are demonstrable. The data in this study are more consistent with a time- and frequency-dependent change in the excitability of depressed fibers associated with changes in calcium conductance. This idea is supported by the voltage clamp experiments of Hiraoka and Sano,⁹³ who have shown that the slow inward current can increase with premature excitation with a certain range of intervals. Further support is provided by the results of Weingart et al.⁹⁴ in Purkinje fibers treated with strophanthidin. Digitalis compounds promote calcium uptake by cardiac tissues, and Langer and Serena⁹⁵ showed that during the administration of strophanthidin, the repriming of slow inward current and twitch contraction follows a biphasic time course very similar to that found in our slow-response experiments (fig. 9). Biphasic time-dependent variations in slow response amplitude have also been shown in mammalian ventricular muscle.²²

The concentration of intracellular calcium modulates gating processes of transmembrane currents in various tissues.³⁶ In Purkinje fibers, an increase in the intracellular calcium concentration increases inward currents.³⁷ Moreover, experiments in frog atrium³⁸ indicate that the membrane conductance to slow-channel current can be enhanced by a previous depolarization in a voltage- and time-dependent manner. Noble and Shimoni³⁹ explained these phenomena in terms of activation of the slow inward current by the intracellular calcium concentration. Following this line of argument and based on our experiments, we propose that in
depressed Purkinje fibers, the amplitude of slow-response action potentials is maximal at intermediate frequencies of stimulation. At very low frequencies, the diminished intracellular free calcium concentration would lead to a reduction in the amplitude of the slow inward current responsible for excitation. However, a slow recovery from inactivation of this current would prevent excitation at very rapid frequencies; but proof of this hypothesis requires further experimental work.

Acknowledgment
The authors thank Judy Hefferton and Diana Warburton for skilled technical assistance and LaVerne Tinker for typing the manuscript.

References
21. Cukierman S, Paes de Carvalho A: Frequency-dependent excitability on "membrane" slow responses of rabbit left atrial trabeculae in the presence of Ba²+ and high K+. J Gen Physiol 79: 1017, 1982
37. Isenberg A: Cardiac Purkinje fibers. The slow inward current component under the influence of modified Ca²⁺. Pflugers Arch 371: 61, 1977
Rate-dependent changes in excitability of depressed cardiac Purkinje fibers as a mechanism of intermittent bundle branch block.
J Jalife, C Antzelevitch, V Lamanna and G K Moe

Circulation. 1983;67:912-922
doi: 10.1161/01.CIR.67.4.912

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1983 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/67/4/912