Reflection After Delayed Excitation in a Computer Model of a Single Fiber

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Reflection (reflected reentry) is a case of reentry in a one-dimensional structure, divided into proximal and distal segments, in which tissue excited by a wave front propagating in a forward direction is reexcited by electrical activity coming backward from the original direction of propagation. Cases of reflection have been demonstrated in Purkinje fibers and in ventricular muscle preparations containing multiple fibers. Several mechanisms possibly responsible for reflected reentry have been proposed. However, the difficulty in the interpretation of the experimental results, as well as the limited number of different conditions in which reflection was obtained, has kept open the question about conditions and mechanisms for reflection. We have developed a computer model in which reflection occurs. The model involves a single fiber and uses the DiFrancesco-Noble equations for the Purkinje fiber to model the ionic currents. The results show that reflection is possible in a single fiber and that diastolic depolarization (automaticity) is not a requirement for reflection. Active membrane responses to a just-above-threshold stimulus were important for achieving the necessary time delay. Systematic simulations showed further that reflection occurred only when the right coupling conditions linked a short or long proximal fiber to a short distal segment.

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Reflection, or "reflected" reentry, is a special type of reentrant circuit in a one-dimensional structure, in which tissue excited by a wave front propagating in a forward direction is reexcited by electrical activity (reflected wave front) coming back from the direction of propagation.\(^1\)\(^-\)\(^7\) When propagation in both directions is over the same fibers (rather than over different fibers in a bundle), reflection is called true reflection.

Even though experiments on reflection started early in the century,\(^6\)\(^-\)\(^12\) the recent approach to the study of reflection was first initiated in the early seventies by Cranefield and colleagues\(^13\)\(^-\)\(^15\) and Wit and colleagues,\(^16\)\(^-\)\(^17\) who studied the propagation characteristics of Purkinje fibers with depressed excitability. In 1971, Cranefield, Hoffman, and collaborators\(^13\)\(^-\)\(^15\) showed that segments of Purkinje fibers, with normal conduction velocities of 2–4 m/sec, can conduct with apparent velocities of 0.01–0.1 m/sec when encased in high K\(^+\) agar. In 1972, Wit, Cranefield, and Hoffman\(^17\) demonstrated reentry in small loops (12–35 mm) of canine and bovine Purkinje fibers depressed by a solution containing a very high concentration of K\(^+\). Using unbranched bundles of Purkinje tissue with a central segment with depressed excitability, they observed\(^16\) a phenomenon similar to the one reported by Schmitt and Erlanger\(^10\) in which an impulse (stimulated) propagating in one direction of the bundle is followed by an impulse (reflected, nonstimulated) traveling in the opposite direction (return extrasystole). They proposed that the reflected response was caused by reentry at the level of the syncytial interconnections (microreentry), an explanation identical to the one used by Schmitt and Erlanger to interpret their observations. They also suggested the possibility of true reflection based on the observation that a fast action potential upstroke occurred in the depressed segment after the action potential in the tissue beyond the depressed segment.\(^13\)\(^,\)\(^16\)

In trying to demonstrate true reflection, a series of remarkable experiments were performed by Antzelevitch, Jalife, Moe, and associates in Purkinje,\(^18\)\(^-\)\(^21\) ventricular,\(^22\) and atrial\(^23\) muscle. They used a three-compartment tissue bath in which a zone of conduction block was created in the central segment either by perfusion with a solution containing a high concentration of potassium (central zone inexcitable) or by perfusing the central compartment with isotonic sucrose (electrical insulation of the extracellular space). The essential feature of both experimental conditions is that the central compartment can transmit only electrotonic potentials. The proposed mechanism for reflection was that the electrotonic potential transmitted through the inexcitable gap excites the distal tissue after a delay; the active response in the distal tissue causes an electrotonic potential that is transmitted over the same inexcitable gap and reexcites the proximal tissue. The delay across the inexcitable gap was long enough to allow the
proximal tissue to repolarize. More recently, reflection has been suggested as the mechanism underlying extrasystolic activity in ventricular tissue excised from a 1-day-old infarcted canine heart\(^ {24}\) and in a clinical case of incessant ventricular bigeminy in a patient with no evidence of organic heart disease.\(^ {25}\)

Despite the elegance of the experiments performed by Antzelevitch, Jalife, Moe, and associates,\(^ {18-23}\) interpretation of the experimental results is complicated by two factors.\(^ {2}\) First, it is well known that subthreshold potentials affect the automatic pacemaker of Purkinje fibers.\(^ {26,27}\) When they occur early in the cycle, the spontaneous discharge will be delayed, and when they occur late in the cycle, the spontaneous discharge is accelerated. Therefore, the reflected propagation might be instead a consequence of the automatic pacemaker of Purkinje fibers in the distal segment, modified by electrotonic interaction. This premise was supported by a computer modeling study of reflection\(^ {28}\) in which, for reflection to occur, it was necessary to incorporate pacemaking properties in the distal elements. On the other hand, the demonstration of reflection in strips of ventricular muscle (no diastolic depolarization) by Rozanski et al\(^ {22}\) and in thin strands of Purkinje fibers homogeneously bathed with a solution containing a high concentration of potassium\(^ {4}\) indicated that diastolic depolarization is not a requirement for reflection.

The second complicating factor is that inhomogeneities in the excitable gap or at the boundaries between the depressed segment with the normally excitable tissue may affect the interpretation of the experimental results. When asymmetric depression is present in the excitable gap, microcrenity of the type described by Schmitt and Erlanger\(^ {10}\) may occur.\(^ {2}\) When inhomogeneities exist at the boundaries, transmission between the proximal and distal excitable segments might not be purely electrotonic but a combination of electrotonic propagation and slow conduction.\(^ {2}\)

Another important question relates to the implications of these in vitro studies for the whole heart. The preparations in which reflection was demonstrated were isolated bundles of Purkinje fibers shorter than 10 mm, with the excitable zone (sucrose gap) in the middle. The fact that the size of the excitable segments of tissue were of the order of one or two (resting) space constants (2–4 mm) raises questions as to whether reflection is possible in long fibers. Furthermore, the use of single transmembrane recordings in the excitable segments makes it difficult to decide if the reflected responses are indeed propagated or just electrotonic responses.

To deal with some of the questions above, we developed a computer model in which reflection occurs under specific conditions. The model consists of a single fiber whose ionic currents are represented by the DiFrancesco-Noble equations.\(^ {29}\) The DiFrancesco-Noble model was chosen because it accurately reproduces the behavior of real Purkinje tissue during the refractory period and with respect to electrical stimulation.\(^ {30}\)

**Materials and Methods**

**Propagation and Membrane Models**

If propagation is planar, a fiber in a multifiber preparation can be modeled satisfactorily by a one-dimensional core-conductor model in which the extracellular impedance depends on the depth of the fiber considered.\(^ {31,32}\)

The model used was a continuous cable, described by the following equation:

\[
I_m = \frac{a}{2} \frac{\partial}{\partial x} \left( \frac{1}{R(x)} \frac{\partial V_m(x,t)}{\partial x} \right) - I_{ion} + C_m \frac{\partial V_m(x,t)}{\partial t} \tag{1}
\]

where \(I_m\) is the transmembrane current (\(\mu A/cm^2\)), \(a\) is the fiber radius (0.001 cm), \(R(x)\) is the intracellular resistance (0.250 k\(\Omega\)cm), \(V_m\) is the transmembrane potential (mV), \(I_{ion}\) is the DiFrancesco-Noble ionic current (\(\mu A/cm^2\)), and \(C_m\) is the specific membrane capacitance (1.2 \(\mu F/cm^2\)).

The cable equation was made discrete with a space step of 100 \(\mu m\) and a time step of 10 \(\mu sec\). The number of nodes depended on the simulation. The ends of the fiber were considered sealed (i.e., there was no intracellular current), so at both ends the boundary condition was \(\partial V_m/\partial x = 0\). The method to numerically solve Equation 1 has been described elsewhere.\(^ {30}\)

In this model, the intracellular and extracellular impedances are purely resistive, and the membrane impedance is a simple capacitance (as opposed to two capacitances\(^ {33,34}\)) in parallel with the ionic currents described by the DiFrancesco-Noble model of the membrane.\(^ {29}\) Despite the discontinuous nature of propagation in cardiac muscle\(^ {35-37}\) and because of the low resistance of the gap junctions,\(^ {38,39}\) we lumped the junctional resistance in with the intracellular axial resistance. The presence of capacitive effects in the gap junction\(^ {38,40}\) were neglected. The maximum conductance for the sodium current was set to 16.87 mS/cm\(^2\), one and a half times the standard conductance in the DiFrancesco-Noble model, so that the maximum rate of membrane depolarization fell into the normal range.

**Reflection Model**

To obtain reflection, incomplete isolation of fiber segments is needed. In the experimental studies,\(^ {18-22}\) this zone was created by perfusing the central segment of a three-compartment chamber with either isotonic sucrose (sucrose gap model) or a solution containing a very high concentration of potassium (ischemic model). The effect of the isotonic sucrose is the conversion of the extracellular space in the sucrose region into a nonconducting zone. Propagation between the excitable segments can be restored by connecting the extracellular spaces by an external resistance.

In our simulations, the sucrose gap was represented by a segment with high axial resistance (gap resistance). The proximal segment was the one where the external stimulus was applied (unless otherwise stated). The delay between the proximal and distal segments (P–D delay) was controlled in one of two ways: first, by adjusting the resistance of the segment simulating the sucrose gap; or second, by changing the coupling interval of a premature beat.

**Results**

**Mechanism of Reflection in a Single Fiber**

To study whether reflection was possible in a single fiber, we simulated a fiber that was near the size of experimental preparations. It had a length of 8 mm (80 nodes) and proximal and distal segments both 4 mm (40
FIGURE 1. Recordings showing transmembrane voltages calculated in a fiber with a length of 8 mm divided into proximal and distal segments, each 4 mm (40 nodes) long, for increasing values of the resistance between the proximal and distal segments: column A, 0.250 kOhm/cm; column B, 10.5 kOhm/cm; column C, 10.9438 kOhm/cm; column D, 10.9438 kOhm/cm; column E, 10.9439 kOhm/cm. Rows show transmembrane voltages at the beginning of the proximal segment (row 1), at the end of the proximal segment (row 2), at the beginning of the distal segment (row 3), and at the end of the distal segment (row 4). For each resistance, a stimulus was applied at time zero at the beginning of the proximal segment (row 1).

In all the simulations the diastolic depolarization current was set to zero so that there was no automaticity. The initial conditions for each node of the simulated fiber correspond to those occurring 600 msec after an action potential. In Figure 1, a progressive increase in the intracellular resistance between nodes 40 and 41 (to simulate the sucrose gap) is shown in five steps. Each column shows the V_m at the beginning of the proximal segment (node 1, row 1), at the end of the proximal segment (node 40, row 2), at the beginning of the distal segment (node 41, row 3), and at the end of the distal segment (node 80, row 4). Column A shows a control fiber in which the gap resistance is equal to the standard intracellular resistance and all action potentials (rows 1–4) are almost the same. As uncoupling increased between the proximal and distal segments, P–D delay increased (Figure 1, column B). In this case, the P–D delay was not enough to allow the proximal segment to recover its excitability, but the action potential in the distal segment caused an electrotonic prolongation of the action potential in the proximal segment.

Reflection occurred when P–D delay (≈300 msec) was enough to allow the proximal segment to recover its electrical excitability (Figure 1, column C). The top tracing shows that the reflected response caused propagation in the proximal segment. The P–D delay required for reflection in the model is similar to the P–D delay required for reflection in the experimental preparations.\(^{38–22}\) A still further decrease in coupling (with respect to that required for reflection) resulted in an action potential in the distal segment with no reflected response in the proximal segment (Figure 1, column D). This phenomenon might be the same as the "silent" period observed in studies of reflection in ventricular muscle.\(^{22}\) A further slight decrease in electrical coupling resulted in block, where no action potential was produced in the distal segment (Figure 1, column E). Note that in this case the electrotonic effect of the distal segment caused a shortening of the action potential in nodes of the proximal segment close to the gap.

Some conclusions can be extracted from the results in Figure 1 about the possibility of reflection. First, clearly it was possible to evoke a (true) reflected response in a single fiber (Figure 1, column C), since the model structure precluded microreentry. Second, diastolic depolarization was not a requirement for reflection, since the diastolic depolarizing current was inactivated during the simulations. Some conclusions can also be extracted about the mechanisms for reflection. First, the P–D delay had to be long enough to allow the tissue in the proximal segment to recover its excitability, or no reflection occurred (Figure 1, column B). Second, the distal segment had to be able to (re)excite the proximal segment. P–D delay alone was not enough to obtain reflection (Figure 1, column D). It is also interesting that for long delays between action potentials in the proximal and distal sites (Figure 1, columns C and D) the action potential in the distal site actually originated away from the gap, as seen from the fact the action potentials at nodes close to the gap (e.g., node 41) occurred later than at nodes far away from the gap (e.g., node 80). This phenomenon also was observed experimentally.\(^{22}\)

**Importance of the Geometry**

The previous section showed that reflection occurred between two nodes of a cable with proximal and distal segments of 4 mm. The fact that most of the experimental studies in which reflection was demonstrated were preparations whose proximal and distal segments were short (one to two resting space constants for Purkinje fibers, 2–4 mm) points to a possible role of segment length in the occurrence of reflection. Therefore, to study the importance of segment length on reflection, we tried to induce reflection in simulated fibers with proximal and distal segments of various lengths. The results are shown in Figure 2, where length combinations are grouped by regions.

There is one region of length combinations where reflection occurred (region II), and two regions where, despite the careful adjustment of the resistance between the proximal and distal segments, reflection never occurred (regions I and III). For configurations in region II, adjustment of the gap resistance caused enough P–D delay for the proximal tissue to recover its excitability and for the distal action potential to be able to (re)excite the proximal segment. Therefore reflection occurred, as described in the previous section for a cable with proximal and distal segments of 4 mm.

In region I, there was enough P–D delay for the proximal tissue to recover its excitability, but the distal action potential was not able to (re)excite the proximal
Reexcitation of the Distal than the Maximum Resistance

**Figure 2.** Graph showing the length of the proximal and distal segments and the occurrence of reflection. Reflection was obtained (by adjusting the resistance between the proximal and distal segments) only for preparations in the shaded region (region II). For preparations outside the shaded region (regions I and III), reflection was not obtained (despite adjustment of the resistance between the proximal and distal segments). The initial conditions for each node correspond to those occurring 600 msec after an action potential. Examples of resistances for particular length combinations identified on the figure are as follows: a, 27.38 kΩcm; b, 10.9438 kΩcm; c, 10.234791 kΩcm.

segment; therefore, reflection never occurred. In region III, there was not enough P–D delay for the proximal tissue to recover its excitability; therefore, reflection never occurred.

For distal segments between 3 and 6 mm, reflection was obtained for long lengths of the proximal segment, indicating that reflection is possible in a semi-infinite structure. On the other hand, reflection in an infinite structure (infinitely long proximal and distal segments) is not indicated, because reflection never occurred in long distal segments.

The resting membrane resistance of the DiFrancesco-Noble membrane was 20,000 Ωcm² (as calculated by the ratio of small increments of Vm over increments of ionic current), and the intracellular resistance used for proximal and distal segments was 250 Ωcm. Consequently, the (resting) space constant was 2 mm. Using this value for the space constant, the lengths of proximal and distal segments in Figures 2 (and other figures) can be expressed in terms of space constants, with greater generality.

**Reexcitation of the Proximal Segment by the Distal Segment**

Reflection did not always occur even with enough P–D delay, because the distal segment had to be able to reexcite the proximal segment. There were two cases in which there was enough delay but not reexcitation: lengths in region I (Figure 2) and lengths in region II (Figure 2) at certain gap resistances (Figure 1, column D).

Failure to reexcite in region I occurred when the gap resistance required to get enough P–D delay was larger than the maximum resistance that allowed propagation from the distal to the proximal segments, on stimulation of the distal segment. For any structure characterized by proximal and distal segments of fixed length, there was a maximum gap resistance for which there was conduction from the proximal to the distal segment on stimulation of the proximal segment; for larger resistances, there was block (such as Figure 1, column E). In region I, consider the structure whose proximal and distal segments were 6 and 2 mm, respectively. To get enough P–D delay, a gap resistance of 27.687 kΩcm was required. In comparison, the maximum gap resistance that allowed backward propagation was 9.907 kΩcm. Therefore, for this structure, reflection was not possible.

For structures in region II and gap resistances between the ones required for reflection and the ones that cause proximal–distal block, it was possible to obtain enough P–D delay for reflection but also distal–proximal block. The failure to reflect (after the success of the forward conduction) was caused by several factors. With proximal and distal segments both 4 mm and a gap resistance of 10.9438 kΩcm, there was proximal–distal conduction with a long enough delay for reflection but with distal–proximal block (Figure 1, column D). Using the same gap resistance and initial conditions, on stimulation of the distal segment, there was distal–proximal conduction, as expected. Therefore, a cause of block of the reflected response was the partial inactivation of the sodium channels for tissue close to the gap (presented below, Figures 4C and 4D). In contrast, when proximal and distal segments were 10 and 4 mm, respectively (gap resistance, 11.0718 kΩcm), on stimulation of the proximal segment, reflection was obtained, obviously including distal–proximal conduction. However, with the same gap resistance and initial conditions, on stimulation of the distal segment, there was distal–proximal block. In this case, the distal segment was able to stimulate the proximal segment when the proximal segment was close to threshold (the case of reflection, when Vm is near 60 mV) but not when it was fully repolarized (the case of external distal stimulation, when Vm is near –90 mV). Therefore, another cause for distal–proximal block, even with enough P–D delay for reflection, was that the proximal segment was too nearly recovered (too far away from threshold) to be stimulated by the distal segment. That is, there was a window of "supernormality" in phase 3 of the action potential in the proximal segment that allowed reflection.

**Mechanism of Delayed Excitation**

Reflection required a P–D delay long enough to allow the tissue in the proximal segment to recover its electrical excitability. Long delays occurred when the proximal segment stimulated the distal segment close to the threshold for excitation. Stimulation just above threshold caused, in short distal segments (shorter than 6 mm), delays of hundreds of milliseconds. In contrast, in long distal segments (larger than 6 mm), the delays were of tens of milliseconds. To understand the different mechanisms of delayed excitation, we studied two preparations with a proximal segment length of 6 mm and distal segment lengths of 4 and 6 mm, respectively.

**Short distal segment, just below threshold.** Figure 3 shows Vm, total ionic current, sodium activation parameter m, and sodium deactivation parameter h in a distal segment of length 4 mm at times 100, 200, 284, and 297
msec after stimulation in the proximal segment. Because the gap resistance was adjusted to the just-below-threshold stimulus level, no action potential was elicited in the distal segment; nonetheless, its response was not purely passive. During the first 100 msec there was a net negative (inward) total ionic current at nodes close to the gap (e.g., node 61) (Figure 3B). The inward current diminished with time. At 200 msec the total ionic current was positive at every node of the distal segment but less positive (more inward current) at nodes close to the gap. At 284 msec the situation was reversed, and the total ionic current was less positive (more inward current) at nodes far away from the gap. This contrast can be explained by the contribution of both the sodium activation and deactivation to the inward current. Since \( V_n \) was always more positive for nodes close to the gap (Figure 3A), the sodium activation parameter \( m \) was always more positive for nodes close to the gap (Figure 3C), and the sodium inactivation parameter \( h \) was always more positive for nodes away from the gap (Figure 3D). The product \( m'h \) determines at which nodes the inward current is larger (or at which nodes the total ionic current is smaller). Even though the inward (depolarizing) current was not strong enough to cause an action potential, its effect was to cause a redistribution of charge with a length constant larger than what would be expected from a passive response alone, resulting in an almost uniform \( V_n \) at 297 msec (Figure 3A). Note that \( V_n \) across the length of the distal segment neared the same value as time increased; this near equilibration was associated with long delays.

**Short distal segment, just above threshold.** With the gap resistance lowered slightly, the stimulus to the distal segment was just above threshold, and an action potential in the distal segment was elicited (Figure 4). A combination of passive and active responses close to the gap tended to cause a uniform \( V_n \) along the whole segment, as before. At 284 msec, \( V_n \) was approximately \(-60\) mV for every node in the segment (Figure 4A). At this time, even though the \( V_n \) was the same for every node, the state of activation of the sodium channels was not (Figure 4D). Therefore, the sodium current (inward) was larger for nodes far away from the gap. The sodium activation parameter \( m \) was approximately constant along the segment because of the short time constant of \( m (=0.150\) msec) (Figure 4C). On the other hand, the sodium inactivation parameter \( h \) was much smaller at nodes close to the gap than at nodes far away from the gap. This \( h \) gradient occurred because \( h \) decreases as \( V_n \) increases (gets less negative) and has a long time constant (=40 msec), and nodes closer to the gap were depolarized longer. Thereby, initiation of the excitation began away from the gap (at 297 msec) because \( m'h \) was higher there.

**Long distal segment.** With the longer distal segment and just-above-threshold stimulus, an action potential began in the distal segment (Figure 5) at the node closest to the gap (node 61). At 100 msec, for a long segment, \( V_n \) in nodes 60–70 varied between \(-53\) and \(-60\) mV (Figure 5A), whereas for a short segment, it varied between \(-60\) and \(-65\) mV (Figure 4A). There was a crucial difference in the behavior of the sodium activation \( m \) in these two ranges: for potentials more positive than \(-60\) mV, \( m \) has a higher value and changes more rapidly with \( V_n \) than for potentials more negative.

![Figure 3](http://circres.ahajournals.org/)

**Figure 3.** Graphs showing the just-below-threshold response of a short distal segment (4 mm) after stimulation at the beginning of the proximal segment (6 mm long). The different panels show transmembrane voltage (\( V_m \), panel A), total ionic current (\( I \), panel B), sodium activation (\( m \), panel C), and sodium deactivation (\( h \), panel D) at times 100, 200, 284, and 297 msec after the stimulus.
than −60 mV. For potentials below −60 mV, \( V_m \) can hover for a long time around a fixed value, but for potentials more positive than −60 mV, the \( V_m \) rises rapidly. Therefore, further stimulation for long segments caused an increase in sodium activation (Figure 5C) that activated nodes close to the gap rapidly. Conversely, the electrotonic interaction with the right portion of the distal segment, where \( V_m \) remained near baseline, meant that a smaller stimulus did not result in an action potential anywhere.

**Importance of the Frequency of Stimulation (Initial Conditions) on Reflection**

In the previous simulations, the initial conditions for each node of the simulated fiber corresponded to 600 msec after an action potential. To investigate the effect of the frequency of stimulation on reflection, we created a graph in Figure 6 similar to the one in Figure 2. In Figure 6, the initial conditions for each node of the simulated fiber correspond to 1,600 msec after an action potential (i.e., 1,000 msec after the previously used initial conditions). For the “late” initial conditions (1,600 msec), the gap resistances that cause the maximum P–D delay are greater than the corresponding gap resistances for the “early” initial conditions (600 msec) for every preparation. With the late initial conditions, some of the preparations formerly in region II (Figure 2, reflection) moved to region I (Figure 6, no reflection), and some preparations formerly in region III (Figure 2, no reflection) moved to region II (Figure 6, reflection).

**Recovery of Excitability During Diastole**

To understand the results in the previous section, the excitability of the fiber at 600 msec was compared with that at 1,600 msec after the onset of an action potential. The fiber was 10 mm long. The threshold for excitation was tested with the fiber not separated into segments. A test pulse of a duration of 100 msec was applied at one end of the fiber. A long stimulus was used, since stimulation across the gap leads to a long stimulation pulse. The current strength required for activation was 10% higher at 600 msec than at 1,600 msec. Slow recovery of excitability has previously been shown in ventricular myocytes and in a modified Beeler-Reuter model of the membrane (Delmar et al). The lower threshold explains why the gap resistances that caused maximum P–D delay (i.e., stimulation close to threshold) were greater for late initial conditions for every structure: higher resistance between the excitable segments meant less current between the segments. Furthermore, if higher gap resistances are required to cause the appropriate P–D delay for reflection, the distal segments (during phase 3 of the action potential in the proximal site) see a higher input impedance when trying to reexcite the proximal site. The higher resistance caused some of the preparations formerly in region II (Figure 2, reflection) to move to region I (Figure 6, no reflection).

**Reflection Using Premature Stimulation**

In the previous sections, reflection was obtained (when possible) by adjusting the gap resistance between the proximal and distal segments. To get enough P–D delay for reflection, the threshold gap resistance had to be adjusted in some cases with a precision of seven
FIGURE 5. Graphs showing just-above-threshold response of a long distal segment (6 mm) after stimulation at the beginning of the proximal segment (6 mm long). The different panels represent transmembrane voltage (Vm, panel A), total ionic current (I, panel B), sodium activation (m, panel C), and sodium deactivation (h, panel D) at times 50, 100, 146, and 149 msec after the stimulus.

FIGURE 6. Graph showing the length of the proximal and distal segments and the occurrence of reflection for initial conditions different from those in Figure 2. Reflection was obtained (by adjusting the resistance between the proximal and distal segments) only for preparations in the shaded region (region II). For preparations outside the shaded region (regions I and III), reflection was not obtained (despite adjustment of the resistance between the proximal and distal segments). The initial conditions for each node correspond to those occurring 1,600 msec after an action potential. Examples of resistances for particular length combinations identified on the figure are as follows: a, 38.995 kΩcm; b, 15.205 kΩcm; c, 11.0712 kΩcm.

significant figures. On the other hand, in real tissue,18–22 the P–D delay required for reflection was obtained by a combination of adjusting the gap resistance and premature stimulation. How does the gap resistance interact with premature stimulation? To investigate, the procedure we used was always the same: First, we chose a below-threshold gap resistance giving proximal–distal conduction. Second, we stimulated the proximal site with a basic stimulus, P1, at time zero and with a premature stimulus, P2 (these two stimuli caused two responses in the distal segment D1 and D2, respectively). Third, we adjusted the P1–P2 coupling to change the P2–D2 delay to get reflection, if possible.

In this first set of simulations, we used late initial conditions. For a preparation whose proximal and distal segments are both 4 mm long, a gap resistance of 15.205 kΩcm (threshold gap resistance) causes a P1–D1 delay of 367 msec, enough to obtain reflection. If instead a gap resistance of 15 kΩcm was used, a P1–D1 delay of 242 msec was obtained, which was not enough for reflection. However, the P2–D2 delay can be adjusted by changing the P1–P2 coupling interval. For a P1–P2 interval of 1,365 msec or greater, the P2–D2 delay was not enough for reflection to occur (Figure 7A). For a P1–P2 interval between 1,361 and 1,364 msec, enough P2–D2 delay (≈350 msec) can be obtained for reflection to occur (Figure 7B). For a P1–P2 interval of 1,360 msec or less, there is P2–D2 block (Figure 7C). Therefore, for a gap resistance of 15 kΩcm, there is a time window (for P1–P2 coupling) in the order of milliseconds for reflection to occur. These simulations agree with the experimental results.18–22
Using early initial conditions and integer kΩcm value, gap resistances (resulting from the truncation of the threshold gap resistance) showed a sharp contrast in the effectiveness of premature stimuli. The width of the P1–P2 coupling time window for reflection to occur was in the order of microseconds (instead of milliseconds). A possible explanation is given in the discussion.

**Gap Resistance Range for Reflection**

In the previous section we have shown that gap resistances close to the threshold gap resistance and premature stimulation can be combined to obtain reflection. For gap resistances further apart from threshold, the time window in which reflection can be obtained narrows, and the P1–P2 coupling that causes the maximum P2–D2 delay decreases. Therefore, the more favorable the gap resistance the less favorable must be the P1–P2 coupling, and vice versa.

What is the maximum decrease in the gap resistance (from the threshold gap resistance) such that, with a precision of 1 msec in the P1–P2 coupling interval, reflection is still possible? We evaluated a few examples, using a precision of 1 msec in the P1–P2 coupling interval. For a preparation whose proximal and distal segments are both 4 mm long (threshold gap resistance, 15.205 kΩcm), the gap resistance can be reduced to 12 kΩcm, and reflection still can be obtained by premature stimulation.

This much change gives a gap resistance range for reflection of approximately 20%. For a preparation whose proximal and distal segments are 10 and 5 mm long, respectively (threshold gap resistance, 12.0065 kΩcm), the gap resistance can be reduced to 11.5 kΩcm. This change is approximately 5%. In both cases, note how markedly the precision required for the coupling resistance diminishes when premature stimuli are allowed.

**Discussion**

**Relation of Gap Resistances and Coupling Intervals**

Consider a hypothetical “strength–interval” curve for the distal segment (Figure 8). Suppose the preparation responds according to the solid line in the figure. Further, suppose that stimulation just above threshold is essential for a delay long enough for reflection. Finally, suppose that the strength of the current (provided by the proximal segment) stimulating the distal segment is proportional to the reciprocal of the gap resistance. The P–D delay depends on the relation of the stimulation current to the threshold current. By using the late initial conditions and a structure in which reflection is possible (i.e., from Figure 6, region II), the gap resistance can be adjusted carefully to a threshold gap resistance (R₁, point A, Figure 8) to obtain enough P1–D1 delay for reflection. If the gap resistance is decreased (R₂, point B, Figure 8), the P1–D1 delay is
less than in the previous case because point B is further away from the threshold than point A, so reflection will not occur. The only way to get close to threshold (for a fixed \( R_{gap} \)) is by premature stimulation (point C); as the strength comes closer to threshold, the P2–D2 delay is increased until reflection occurs. If the \( R_{gap} \) is reduced still more (R2, point D), the P1–D1 delay is further decreased (D is still further away from the threshold than B). Premature stimulation brings the stimulus close to threshold (point E) at a shorter P1–P2 coupling time and a narrower range of times than before. (See text for further discussion.)

**P–D Delay, Hovering Voltage in the Distal Segment, and the Occurrence of Reflection**

One of the requirements for reflection to occur was enough P–D delay for the proximal segment to recover its excitability. The simulations using the late initial conditions (1,600 msec after the onset of the action potential) allowed us to further refine this requirement. For structures in region III at the early initial conditions (Figure 2), the maximum P–D delay obtained by adjusting the gap resistance was 150 msec. For structures in region III (distal too long) at the late initial conditions (Figure 6), P–D delays around 400 msec can be obtained, but still reflection did not occur. With the late initial conditions, less current was required for proximal–distal conduction (see previous section), resulting in stimulation closer to threshold and hence longer delays. For long delays, nodes of the distal segment close to the gap hover around a constant \( V_m \) for a long period of time. Reflection did not occur because the hovering voltage in nodes of the distal segment close to the gap was more positive than the refractory voltage in the proximal segment. Despite the long P–D delay, electrotonic coupling to the distal segment, together with the distal segment’s high hovering voltage, prevented the proximal segment from repolarizing and recovering its electrical excitability. For example, for a structure whose proximal and distal segments were both 7 mm, by using the late initial conditions and a gap resistance of 11.09885 kΩ cm, a P–D delay of 391 msec was obtained. Even so, the node of the proximal segment closest to the gap repolarized only to −51 mV. Reflection did not occur. Therefore, long P–D delays alone do not ensure recovery of excitability of the proximal segment: a hovering voltage in the distal segment low enough to be close to the threshold for excitation in the proximal segment also is required; otherwise, the proximal segment does not repolarize enough to be restimulated. For long delays, the hovering potential depends on the length of the distal segment. This requirement is satisfied by distal segments shorter than 6 mm, for both initial conditions (Figures 2 and 6).

**Relation to Experimental Findings**

The results obtained with experimental models of reflection \(^{18–22} \) (proximal and distal segments, <5 mm; basic pacing cycle length, between 1,000 and 2,000 msec; P–D delays caused by premature stimulation) have been reproduced accurately with the single-fiber model of reflection described above. As a consequence, even though we cannot rule out the possibility that reflection in multiple-fiber preparations is caused by syncytial reentry, the simulation results support the hypothesis that true reflection was the mechanism in the experimental models of reflection.

Because the model is constructed with an elevated axial resistivity in one segment of the cable, it is clear that reflection was achieved in the model by pure electrotonic transmission between the segments; i.e., there were no active responses in the gap. In experiments in which the proximal and distal segments were uncoupled by perfusing the central compartment (the gap) with a high concentration of extracellular potassium, \(^{22} \) the origin of the distal activation far away from the gap was explained by the possibility that potassium
leaking from the gap would render inexcitable tissue of the distal segment close to the gap. In the simulations presented here, for long P–D delays, the origin of activation in the distal segment was also far away from the gap. The mechanism, however, was partial inactivation of the sodium channels of nodes of the distal segment close to the gap (Figure 4). The mechanism of the simulation also could be what happened in the experiments.

An excellent earlier computer modeling study of reflection suggested that the long delay required for reflection to occur might require a combination of electrotonic transmission and modulation of the automatic pacemaker of Purkinje fibers. In the simulations presented here, reflection was obtained with the diastolic depolarization current of the Purkinje fiber model inhibited, showing that indeed diastolic depolarization is not a requirement for reflection. The different nature of the models that led to different conclusions makes difficult a more detailed comparison of the results. The demonstration of reflection in ventricular muscle, however, supports the idea that reflected activity can occur independent of a pacemaker mechanism.

Reflection and Segment Length

The simulations predict that reflection is possible in fibers with long or short proximal segments and short distal segments but not in fibers with long distal segments. This result is suggestive in extrapolating conclusions about reflection obtained in isolated (short) fibers to the whole heart. As far as we know, reflection has never been specifically documented in the literature in fibers with long segments whether proximal, distal, or bulk.

In all the simulations presented above, the diastolic depolarization current was set to zero to avoid automaticity. Setting this current to zero was desirable because it made possible a clear judgment as to whether true reflection occurred. Nonetheless, real Purkinje fibers include a diastolic depolarization current. In the smaller number of simulations that we have performed that include this current, longer P–D delays occur in fibers with long distal segments. It might be the case that the presence of a reduced diastolic depolarization current would allow reflection in fibers with long distal segments independent of automatic depolarization. A systematic evaluation of the interaction between electrotonic effects from the proximal segment and the diastolic depolarization of the distal segment will be complex, since there will be many degrees of interaction depending on segment length, current intensity, and degree of coupling.

Delayed Excitation

A crucial aspect of reflection is obtaining a long delay of excitation in the distal segment. One mechanism for obtaining long delays makes use of the long delays associated with series resistor–capacitor circuits, where a high (axial) resistance causes a long delay in charging (membrane) capacitance to a threshold voltage. This mechanism was the basis for only a part of the delay that occurred in the simulations here. The failure to reflect with late initial conditions (even with long P–D delay) and long distal segment suggested that resistor–capacitor delay alone was insufficient to produce reflection.

Another crucial aspect was stimulation of the distal segment to a degree that was just above threshold, initiating active changes in membrane conductances and thereby maintaining near-threshold V₄₅₉ for hundreds of milliseconds. A distal segment length of approximately two space constants allowed the entire segment to equilibrate in a near-threshold state. Longer fibers were not equilibrated, so some portion moved to excitation more quickly; shorter fibers could be equilibrated with long delays but were unable to reexcite the proximal segment. It appears to be the case that similarly long delays could occur by this mechanism during propagation down a fiber with an incompletely isolated interior segment as well as in the context of reflection.

Model Parameters

It is clear that the results of computer simulations are only as accurate as the parameters used to represent the fiber structure and membrane behavior. In “Materials and Methods,” a number of simplifications used to represent the fiber structure were enumerated. Among these are the representation of the actual structure of many interconnected cells by a single cylindrical structure and the representation of the complex grid of actual resistances and capacitances, including the actual structure of the narrow clefts and gap junctions, by those of the linear core-conductor model. It must be that at some level of microscopic detail these differences become highly significant, and we cannot rule out the possibility that a more accurate representation of the anatomic complexities of Purkinje fibers would have an effect on the results presented here. Even so, it has consistently been true that experimental–theoretical comparisons at a macroscopic level have shown close correspondence between Purkinje models using a cylindrical representation and experimental results for macroscopic propagation. Examples include such earlier reports as those of Spach et al as well as more recent comparisons involving premature stimulation. Since the electrotonic interactions of reflection occur at a macroscopic rather than microscopic scale, we think the cylindrical representation is a good starting point for a more detailed quantitative analysis.

The simulations reported here used a value for the maximum conductance of the sodium current 1.5 times the standard value in the DiFrancesco-Noble model for the Purkinje fiber. With the standard value, reflection still was obtained, but the reflected action potential had lower amplitude and slope than the action potentials reported in the experimental studies. With the revision, depolarization rates were closer to reported experimental depolarization rates. This raises some questions about the accuracy of the representation of the fast sodium current in the DiFrancesco-Noble model, which is based on experimental data collected by Colatsky. Even though Colatsky’s studies have provided more reliable information on the kinetics of the sodium currents in Purkinje fibers than previous studies, the major disadvantage of his data is that it was obtained in cooled fibers and the speed of the gate kinetics had to be adjusted to 37°C.

Conditions for Reflection

The results of the simulations show that reflection occurred in a single fiber when three conditions were
met: the delay between the proximal and distal segments was long enough for the proximal segment to recover its excitability, the hovering voltage in the distal segment was close to the threshold for excitation in the proximal segment, and the distal segment was able to reexcite the proximal segment. Diastolic depolarization was not a requirement for reflection, and microreentry was impossible. Just-above-threshold was not a proximal segment, and the distal was close to segment met: the delay.

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