Electrical Excitability of Atrioventricular Nodal Cells

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ABSTRACT

The electrical threshold of A-V nodal cells of isolated rabbit heart preparations was estimated by techniques which permitted stimulation and recording through the same micropipette. Cells within the node were significantly less excitable than adjacent atrial and ventricular tissues; thresholds exceeded $5 \times 10^{-6}$ amp in some instances. Recovery of excitability was delayed well beyond repolarization; in cells of the midnodal region, "diastolic" threshold was not attained until 0.2 to 0.5 seconds after restoration of resting membrane potential. A cumulative effect of frequency upon excitability (fatigue) was demonstrated. At high driving rates, the lag in recovery was further delayed, and the late diastolic threshold was increased. Responses to single-cell stimulation indicate that summation may be an important feature of propagation within the node.

ADDITIONAL KEY WORDS

fatigue of A-V transmission atrioventricular conduction summation in A-V node

In muscle cells and in the specialized conducting tissues of atria and ventricles, electrical excitability is fully restored at the moment of complete repolarization (1). In the His-Purkinje system, and in the specialized conducting tissue of Bachmann's interatrial band, a brief period of supernormal excitability is demonstrable just prior to the restoration of the resting membrane potential (2, 3). In the A-V node, on the other hand, a number of observations suggest that the tissue remains relatively refractory for a significant period of time after the termination of the action potential (1). For example, the functional refractory period of the A-V transmission system, defined as the minimal attainable interval between two ventricular responses, both propagated from the atrium, usually exceeds the action potential duration of any intranodal cell. Furthermore, the conduction time of a premature response from atrium to ventricle may still be prolonged long after the expiration of the A-V functional refractory period (4).

Frequency-dependent changes in A-V conductivity also support the hypothesis that excitability lags behind repolarization. The P-R interval increases with the driving frequency (except, of course, for adrenergically mediated rate changes), although the functional refractory period of the transmission system diminishes. Furthermore, the effect of frequency on conductivity is cumulative. When the atrial driving frequency is abruptly increased, several or many beats may occur before the conduction time stabilizes at a higher value. This observation suggests that the lag in the recovery of conductivity in nodal cells is subjected to a further delay by a kind of fatigue (5, 6).

We may assume that conduction velocity within the node, as in any excitable tissue, is a function of the "margin of safety," and that the latter is determined by the stimulating efficacy of the action potential and by the excitability of the tissue. Delay in the...
A-V propagation of premature responses could therefore represent incomplete recovery either of excitability, or of the amplitude or upstroke velocity of the action potential, or both. The present study was undertaken to determine whether the excitability of nodal cells could be directly assessed by intracellular stimuli, whether the recovery of excitability is in fact delayed beyond full repolarization, and whether the frequency-dependent depression of nodal conductivity can be related to cumulative effects upon excitability.

**Methods**

### IN-VITRO STUDIES

Hearts were rapidly removed from rabbits stunned by a blow on the head. Trimmed preparations of the excised tissue, with ample exposure of the A-V node (7), were pinned in a paraffin bed and perfused with oxygenated Tyrode's solution at a temperature between 37° and 38°C. The calcium concentration of the perfusion medium was reduced to 1.8 mM/liter, to reduce the contractility of the preparation (8). The sinus node was excised, and the preparation was driven with bipolar silver electrodes on the crista terminalis or on the bundle of His. The driving pulses were of 2-msec duration, provided by a Tektronix pulse generator, triggered at approximate intervals by counting from a 100-kc crystal oscillator (9), and passed to the preparation through an isolation transformer. Microelectrodes were drawn from Pyrex capillary glass and filled with either 3M KCl or 2M K citrate. Microelectrode resistance values ranged from 4 to 20 megohms. Action potentials were recorded through a negative capacitance amplifier. Extracellular electrograms were recorded from bipolar, silver electrodes on the crista terminalis close to the ostium of the coronary sinus, and in some experiments from the His bundle. The recordings were displayed on a Tektronix 505 oscilloscope and photographed with a Grass camera.

The terminology used to define intranodal recording sites is that applied to the subdivisions of the A-V node by Paes de Carvalho and de Almeida (10). We have used the term AN for cells which are anatomically and temporally close to atrial tissue, in an area in which dissociation commonly occurs (11). Cells referred to as N were classified not only by their temporal and spatial location and the configuration of their action potentials, but also on the basis of function. Cells which fired after the first 20% but before the final 20% of the total transnodal conduction time were classified as N; block of premature atrial responses could be readily demonstrated within this region, but not below it. Cells which fired during the final 20% of the normograde conduction interval, in which action potentials began to approach the configuration of His

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**Figure 1**

Schematic diagram of circuits used for intracellular stimulation and recording. ME = microelectrode; P = potentiometer; C = calibrator; A1 = amplifier for recording transmembrane action potential; A2 = current monitor.
bundle fibers, and in which transmission appeared to be obligatory were classified as NH.

The technique used for stimulating and recording through the same microelectrode is shown in Figure 1. The current source at the upper left was triggered at the desired interval to provide a positive pulse which, applied within the cell, was a depolarizing stimulus. The millisecond relay closed that portion of the circuit only for the duration of the pulse. The amplification circuitry (A₁), protected by the diode shunt to ground during the passage of stimulating pulses, recovered quickly enough to record the presence or absence of an active response (see Figure 3, A and B). In later experiments, the millisecond relay and diode were replaced by a field-effect transistor electronic switch which, when open, offered nearly infinite impedance in parallel with the microelectrode, and thus less attenuation of recorded action potentials than when the lower impedance diode was utilized. In Figure 1, the potentiometer (P) permitted bucking to zero potential. The calibrator (C) provided 100 mv calibration signals. The pulse current strength monitor (A₂) measured the potential drop across the 100-kilohm resistor. The 30-kilohm resistor served as a load resistor for the current source when the diode was in the conducting state and thus offered negligible resistance.

External stimulation of selected areas was accomplished by passing cathodal pulses through a monopolar silver-silver chloride electrode with a tip diameter of 100 μ. Responses were monitored by recording an atrial electrogram from the crista terminalis, an electrogram or transmembrane action potential from the His bundle, and a transmembrane action potential immediately adjacent to the tip of the stimulating electrode, usually within 100 μ.

Strength-duration curves for both intracellular and external stimulation, constructed with pulse durations from 0.5 to 40 msec, were essentially flat beyond 4 msec. Accordingly, strength-interval curves were obtained with pulses of 4, 5, or 8 msec.

In the studies of frequency-dependent changes, the preparations were atropinized to prevent possible cholinergic effects of driving stimuli (12). Atropine sulfate was injected into the bath to achieve a concentration of 7 μg/ml, after which perfusion was stopped for 5 minutes. Test doses of atropine were found in the specialized conducting tissue of the ventricle, and significantly higher values were found in myocardial cells of atrium and ventricle. Much higher thresholds were encountered as impediments were made progressively closer to the midsodal region, and cells at the temporal midpoint (in the "N" region) were

Results

**EXCITABILITY OF SINGLE CELLS**

In preliminary studies, the electrical thresholds of excitability of single units in atrium, His bundle, peripheral Purkinje fibers, and ventricular myocardium were determined to serve as a basis of comparison for excitability measurements in intranodal cells. In all such experiments, the lowest thresholds were found in the specialized conducting tissue of the ventricle, and significantly higher values were found in myocardial cells of atrium and ventricle. Much higher thresholds were encountered as impediments were made progressively closer to the midsodal region, and cells at the temporal midpoint (in the "N" region) were

![Representative threshold values determined by intracellular stimulation.](image-url)
often not excited by the maximum current pulses available through the stimulating network (3 to 5 μamp).

Representative thresholds taken from a number of experiments are shown in Figure 2. These are plotted, not as statistically significant means, but merely to give some idea of the order of magnitude of the differences observed. The open bars represent thresholds determined with intracellular current pulses delivered late in diastole, at a time when the strength-interval curve was nearly flat. The shaded bars indicate thresholds determined at the termination of the previous action potential, i.e., at the moment of full repolarization.

In atrial and ventricular myocardium, the excitability was fully restored as soon as the resting membrane potential had been reached, and no further change was apparent as the test pulse was delivered later in diastole. In peripheral Purkinje fibers, and occasionally in cells of the His bundle, the cells were "supernormally" excitable just preceding full repolarization. Intranodal cells, on the other hand, were never fully excitable immediately after repolarization, but recovered slowly, sometimes for several hundred milliseconds, before the late diastolic threshold was reached. This lag in the recovery of excitability appeared to be greater in magnitude and duration as the N region of the node was approached.

A detailed comparison of the threshold requirements of representative cells of the His bundle and the lower transitional NH region from one preparation, and a plot of the time course of recovery, are shown in Figures 3 to 5. In Figure 3, the initial response in each panel is the last of a series of seven evoked by rhythmic stimulation of the atrium. In A and B, subthreshold and just suprathreshold pulses were applied 230 msec after the last basic response of the cell, and about 60 msec after full repolarization. The diastolic threshold in this assay was 3 × 10^-7 amp. In C and D, the test pulse was shifted to 135 msec after the last driven response, at a time when the cell was nearly but not
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When intracellular stimuli were applied to cells within the A-V node, just proximal to the His bundle, the electrical excitability was found to be lower, and recovery of the late diastolic threshold was delayed beyond full restoration of the resting membrane potential. Figure 4, B was $7 \times 10^{-7}$ amp, more than from a low nodal (NH) cell from the same preparation as that of Figure 3. The diastolic threshold was assessed by an intracellular test pulse delivered 320 msec after the last driven response of the cell; the threshold pulse of Figure 4, B was $7 \times 10^{-7}$ amp, more than double the threshold of the His cell. In C and D the test pulse was moved 125 msec earlier. Although this position was 30 msec after full repolarization, the threshold had increased to $9 \times 10^{-7}$ amp. In all such determinations, repeated estimates of late diastolic threshold were made to be certain that changes in the impalement of the cell under study were not the cause of the observed threshold alterations.

Strength-interval curves obtained in the cells represented in Figures 3 and 4 are compared in Figure 5. Following the last driven pulse at the basic frequency, the threshold was repeatedly assessed by test pulses applied to the His cell, on the left, and the NH cell, on the right. The strength-interval curves are drawn below the corresponding action potentials, drawn from an enlarged projection of the actual responses. The diagrams indicate that the excitability of the His cell, fully restored before complete repolarization, remained constant between 250 and 350 msec after the last driving stimulus, although the threshold of the NH cell had not quite reached a constant value at 400 msec, or about 150 msec after full repolarization. The figure also indicates that the delayed recovery of excitability is not quantitatively related to the slight hyperpolarization in the early phase of diastole. Complete curves like that displayed in Figure 5 were not regularly obtained, but in many experiments, a lag between repolarization...
and recovery of full excitability was demonstrated by showing that a current pulse which was suprathreshold in late diastole failed to excite the impaled cell when the interval between driving and test pulses was diminished. Observations of this type were considered valid only when the late pulse was again shown to be effective.

Estimates of the excitability of NH cells were relatively easy to obtain, but cells in the AN and N regions of the node were difficult to hold long enough for precise measurements. Threshold currents were considerably higher than in other cardiac tissues, so much so that in some of the earlier experiments, mechanical displacement of the microelectrode regularly accompanied the injection of current pulses approaching the presumed threshold.

When pulses of relatively high magnitude are applied, there is room for doubt whether an observed response is in fact an action potential initiated by the action of the stimulus upon the impaled cell per se or the result of activity induced by the recruitment of a number of neighboring elements. This doubt cannot be completely overcome, but tests of latency, applied in a manner illustrated in Figure 6, provide circumstantial evidence. The preparation was driven from the atrium. In Figure 6, A, a test pulse delivered to the AN cell 530 msec after its full repolarization was ineffective; in B, a pulse of $6.5 \times 10^{-7}$ amp was just effective. An action potential, stemming from the

Response of a single cell in the AN region to threshold intracellular stimulus. Top trace, atrial electrogram; second and third traces, transmembrane action potentials recorded from AN cell and His bundle, respectively. Bottom trace, current monitor (peak of current pulses indicated by arrows). Calibrations in A, 50 mv, 100 msec, $1 \times 10^{-7}$ amp, respectively. Time calibration represents 20 msec in C, D, and E. In A, subthreshold stimulus defines amplitude and shape of shock artifact. In B, pulse amplitude of $6.5 \times 10^{-7}$ amp. Time relations of responses propagated from atrium (C), from His bundle (D) and from stimulated cell (E) shown in bottom row at higher sweep speed.
shock artifact, yielded a response which was propagated to the atrium and to the His bundle.

If the test response shown in Figure 6, B, originated in cells adjacent to the impaled cell or, by intracellular current flow, in cells at some distance from it, then the latency of the response propagated in both directions from the impaled cell should be less than the corresponding AN-H1 and AN-A intervals for responses propagated through the node from atrium and from His bundle, respectively. A comparison of these propagation intervals is shown at higher sweep speed in C, D, and E. C indicates the conduction intervals for antegrade conduction, D the corresponding values for retrograde conduction of a response initiated in the bundle of His below the recording microelectrode. In E, the intervals resulting from application of the intracellular stimulus are shown on the same time scale. Although it is difficult to pinpoint the moment at which the active response in the AN cell begins, the conduction times to the atrium and to the His bundle are clearly longer than those for the same distances in C and D. The results imply that a response initiated in a single nodal cell, or in a restricted cluster of cells, propagates appreciably more slowly than a broad wave front initiated by activation from the atrium or from the His bundle. In other words, spatial summation appears to be a significant feature of intranodal conduction.

Intracellular stimulation of the N cells in the middle of the A-V node of the rabbit was exceedingly difficult. The impalements were hard to maintain for sufficient periods of time, and in many experiments current pulses of $2 \times 10^{-8}$ amp and higher failed to elicit responses. However, it was possible in some experiments to stimulate cells clearly identifiable by their temporal and electrical characteristics as N cells, as distinguished from AN or NH cells. When N cells could be stimulated, the thresholds were higher than those of NH or AN cells in the same preparations.

The response of an N cell to injection of a threshold current pulse is shown in Figure 7. In A, a large shock artifact accompanies injection of a subthreshold current pulse at a test interval of 400 msec. In B the current was increased to $3 \times 10^{-8}$ amp, and a response of the impaled cell appears. Propagation times to the atrium and the His bundle were 125 and 80 msec, considerably longer than the corresponding intervals for propagation of the basic response at the left.

Cells low in the N region were sometimes

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

**Figure B**

Responses of an unusually excitable cell low in the N region. Arrangement of tracings as in Figure 6; N cell action potential in second trace from top. In A, threshold pulse is $9.8 \times 10^{-8}$ amp. Time relations of responses propagated from atrium, His bundle, and from the stimulated N cell shown at high sweep speed in B, C, and D. Current calibration in A, $1 \times 10^{-8}$ amp; time calibration in D, 100 msec for A, 20 for B, C, and D. Voltage calibration, 50 mv. Basic cycle length, 650 msec. In B conduction time from N cell to His bundle, 12 msec; in C, N cell to atrium, 30 msec. In D conduction intervals, measured from beginning of stimulus were 65 and 19.5 msec to atrium and His bundle, respectively, measured from end of stimulus artifact, corresponding intervals were 59 and 13.5 msec.
sufficiently excitable to permit more detailed study of their characteristics. Responses of one such cell are shown in Figure 8. The preparation was driven from the atrium with a cycle length of 650 msec. After seven basic beats, a threshold test pulse of $6.8 \times 10^{-7}$ amp was introduced at an interval of 440 msec after full repolarization. The induced N cell response propagated back to the atrium and down to the His bundle (A). Segments B, C, and D of Figure 8 indicate, at higher sweep speed, the conduction times for responses initiated in the atrium (B), His bundle (C) and by intracellular stimulation of the impaled N cell (D). The conduction times from the N cell to the atrium and to the His bundle in D were longer than the corresponding intervals recorded in B and C, illustrating again that summation is a significant feature of intranodal propagation.

Recovery of excitability in N cells was delayed well beyond full repolarization. Pertinent test data, obtained in the same cell as Figure 8 but at a basic cycle length of 400, are shown in Figure 9. Diastolic threshold, at a test interval of 425 msec after the salt driven response in A, was $8 \times 10^{-7}$ amp (as compared with $6.8 \times 10^{-7}$ at the test interval of 550 msec in Fig. 8). At barely threshold strength, the response shown in Figure 9, A failed to propagate to the atrium, and its conduction time to the His bundle was appreciably longer than in Figure 8 even though the S2 test interval was longer than the basic cycle length of responses propagated from the atrium. Repetition of the same pulse in Figure 9, B, at

**Figure 9**

Failure of propagation from stimulated N cell. Same cells and arrangement of tracings as Figure 8; preparation driven from atrium. In A, threshold pulse initiates response which is propagated to His bundle but not to atrium; in B, propagation failure in both directions (note action potential durations in A and B). In C, same pulse fails to excite when moved earlier in cycle. Interval between driving and test stimuli in A and B exceeds basic cycle length.
Response of N cell to premature atrial stimulation. Same cells as in Figures 8 and 9. Calibrations in A, 50 ms, 50 mV.

FIGURE 10

Response of N cell to premature atrial stimulation. Same cells as in Figures 8 and 9. Calibrations in A, 50 ms, 50 mV.

The response interval in the N cell was 118 ms. In Figure 9, B, on the other hand, direct excitation of the N cell failed to propagate in either direction when the response interval in the N cell was 425 ms. In Figure 10, B, the A1A2 interval was reduced to 98 ms. The impaled N cell fired, but propagation to the His bundle failed. The N cell responses in Figure 10 developed soon after full repolarization, at a time when the maximum available current from the stimulator would have failed to excite the cell. The stimulating efficacy of the propagating wave front must therefore have been considerably in excess of the current pulses applied to the single cell in Figure 9.

EXCITABILITY AS MEASURED BY EXTERNAL STIMULATION

Because of the difficulty of holding good impalements of midnodal cells long enough to conduct complete studies of the time course of recovery, attempts were made to assess the excitability in this region by means of cathodal stimuli applied externally through a chlorided silver wire of 100 μm diameter. At the outset, this technique did not appear to be feasible, as it has been shown that cells of the N region are commonly overlain by cells characteristic of the AN region. However, results compatible with discrete excitation of N cells were obtained in the experiments illustrated in Figures 11 and 12. In these experiments, a transmembrane action potential was recorded from an N cell as close as possible to the stimulating electrode. This record was used to mark the success or failure of the stimulating pulse, and to establish the timing of the stimulus with respect to the local action potential in this area. The temporal location of the impaled N cell in Figure 11 is indicated by the somatic action of Figure 9 and 10. The impaled cells in Figure 10 were the same as those represented in Figures 8 and 9, and the basic cycle length of atrial stimulation (400 ms) was the same as in Figure 9. A premature atrial response delivered at an A1A2 interval of 105 ms traversed the node, yielding a response interval in the His cell of 134 ms, and in the impaled N cell of 118 ms. In the trial illustrated in Figure 9, B, on the other hand, direct excitation of the N cell failed to propagate in either direction when the response interval in the N cell was 425 ms. In Figure 10, B, the A1A2 interval was reduced to 98 ms. The impaled N cell fired, but propagation to the His bundle failed. The N cell responses in Figure 10 developed soon after full repolarization, at a time when the maximum available current from the stimulator would have failed to excite the cell. The stimulating efficacy of the propagating wave front must therefore have been considerably in excess of the current pulses applied to the single cell in Figure 9.
the responses in A, in which the preparation was driven by an atrial stimulus, and in B, in which the stimulus was applied to the His bundle distal to the impaled unit. In C, a late diastolic stimulus of subthreshold strength was applied to the N region. The shock artifact was large in the bipolar atrial record, but reasonably small in the transmembrane recordings. In D, the stimulus strength was just suprathreshold (3 X 10^-4 amp). An active response is apparent in the adjacent N cell, and propagation back to the atrium occurred with a conduction interval of 78 msec, approximately the same as the retrograde conduction time shown in B. Propagation to the His bundle did not occur.

When the stimulus amplitude was slightly increased, propagation to the His bundle also occurred, with an interval of 26 msec, comparable to that observed in A. Because the conduction intervals were not significantly different from those for responses propagated from the atrium or from the His bundle, it
is apparent that: (1) a wave front of substantial breadth was generated by the stimulus (i.e., excitation was more or less simultaneously achieved in a cluster of cells), and (2) the stimulus did not "spread" to cells in the AN or NH regions, in which case the antegrade and retrograde conduction intervals should have been appreciably less than those recorded.

In experiments of this type, a substantial increment in the stimulus amplitude often resulted in an abrupt abbreviation of antegrade or retrograde conduction times or both, clearly the result of recruitment of fibers much closer to the His bundle and atrium. This result on occasion precluded the construction of a strength-interval recovery curve, for as the stimulus strength was increased to assess early diastolic excitability, more excitable cells remote from the stimulated site were fired. A complete curve in an experiment in which this artifact did not occur is illustrated in Figure 12. The late diastolic threshold, at 1100 msec, was estimated at $1.4 \times 10^{-8}$ amp. As the test pulse interval was diminished, threshold values increased progressively to a value of 0.78 at 105 msec. To check for possible polarization of the stimulating electrode, the threshold at 655 msec was reassessed. Although the effective stimulus amplitude was now increased over the initial value (open circle), the broken line drawn at this level intersects the strength-interval curve at about 450 msec, indicating that excitability recovery lagged at least 300 msec beyond full repolarization at the stimulated site.

**FREQUENCY-DEPENDENCE OF A-V CONDUCTIVITY**

In the absence of changes induced by autonomic nerve stimulation or other agencies which alter the specific conductivity of A-V
Cumulative prolongation of internodal conduction time. Isolated rabbit heart preparation. Atrial electrogram above, transmembrane potential of an NH cell below. In A, basic cycle length changed from 610 to 210 msec; in B, from 210 to 610 msec. Time and voltage calibrations in A, 20 msec, 50 mv.

FIGURE 14

Cumulative prolongation of internodal conduction time. Isolated rabbit heart preparation. Atrial electrogram above, transmembrane potential of an NH cell below. In A, basic cycle length changed from 610 to 210 msec; in B, from 210 to 610 msec. Time and voltage calibrations in A, 20 msec, 50 mv.

nodal cells, acceleration of the atrial driving frequency beyond a certain limit is accompanied by an increase in the A-V conduction time. That the frequency-dependent depression of conductivity is cumulative is apparent in Figure 13. The data from which this figure was drawn were obtained from an exposed dog heart, driven at basic cycle lengths between 250 and 810 msec by rhythmic stimuli applied to the right atrium. At each of the basic cycle lengths represented by the squares in the figure, the A-V conduction time was measured after a stable state was reached. Some of the points in the curve were obtained as the driving frequency was increased; others were obtained during stepwise deceleration. The conduction time increased progressively as the basic cycle length diminished in the range below 510 msec. The cumulative nature of the change is illustrated by the companion curves. The open circles indicate the A-V conduction times of test atrial responses initiated at various times after each sixteenth driving stimulus, at a basic cycle length of 646 msec. The conduction time was constant down to an A1A2 interval of 420 msec, and then rose progressively at shorter intervals. The separation of the basic curve (squares) and the test curve (circles) is a measure of the cumulative effect of frequency. The filled circles represent A-V conduction times for test responses initiated at various intervals after the last of 16 driving stimuli at a basic cycle length of 260 msec. Full recovery of conductivity was not yet achieved when the A1A2 interval was increased to 800 msec. Longer test intervals could not be assessed because spontaneous pacemaker activity intervened. The two test curves, which describe a kind of hysteresis loop, indicate that full recovery of conductivity occurred about 400 msec after a basic response when the frequency was slow, but was grossly delayed when the driving frequency approached the maximum level at which 1:1 transmission was possible.

The test conductivity curves of Figure 13 are clearly not the result of concomitant changes in the over-all refractory period of the system. The arrows at the lower left of the figure indicate the functional refractory period at the basic cycle lengths of 646 msec (open circle) and 260 msec (filled circle), respectively.

Fatigue of A-V conductivity at high driving rates was also readily demonstrable in the isolated rabbit preparations. The time required to reach a stable state was significantly greater than in the dog heart in situ, perhaps because of less efficient perfusion. The superimposed sweeps in Figure 14, A, illustrate the progressive change in the conduction time from atrium to a cell in the NH region following a shift from a basic cycle of 610 to 210 msec. The first upstroke of the NH cell is the last of a long series of responses at the
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A preparation was driven by stimuli applied to the His bundle at a basic cycle length of 700 msec in A, and 200 msec in B, C, and D. In parts A, B, and C, the S2S2 test interval was constant at 500 msec. The threshold at this interval at the slow frequency was $4 \times 10^{-7}$ amp (A). Between A and B the driving frequency was increased. The record in B indicates that the previously successful stimulus was now subthreshold, although the S2S2 test interval was unchanged. The new threshold was found to be $5 \times 10^{-7}$ amp (C), an increase of 20%. In D, at the same basic cycle length as C, the same current pulse was 610-msec cycle length; the A-NH conduction interval was stable at 80 msec. The progressively later upstrokes were inscribed at each eighth cycle following the abrupt acceleration of the driving frequency. Equilibrium was reached, at a conduction interval of 156 msec, after about 200 beats (nearly 40 seconds). In B, the much quicker reverse adaptation is illustrated. The latest action potential is the last of a series at a cycle length of 210 msec; progressively shorter conduction times were recorded when the cycle length was abruptly increased to 610, and equilibrium was eventually reached at the original A-NH interval of 80 msec.

Cumulative depression of conductivity could represent a shift to the right of the strength-interval curve, or to an elevation of the diastolic threshold, or to frequency-dependent alterations in the action potential, alone or in combination. Studies of single-cell excitability as a function of driving frequency were undertaken to test whether depression of conductivity is associated with changes in the diastolic threshold.

In the preparation represented by Figure 15, intracellular stimuli were applied to a cell in the NH region of the rabbit node. The preparation was driven by stimuli applied to the His bundle at a basic cycle length of 700 msec in A, and 200 msec in B, C, and D. In parts A, B, and C, the S2S2 test interval was constant at 500 msec. The threshold at this interval at the slow frequency was $4 \times 10^{-7}$ amp (A). Between A and B the driving frequency was increased. The record in B indicates that the previously successful stimulus was now subthreshold, although the S2S2 test interval was unchanged. The new threshold was found to be $5 \times 10^{-7}$ amp (C), an increase of 20%. In D, at the same basic cycle length as C, the same current pulse was...
effective 100 msec earlier. Within the resolution of the stimulating circuit, the threshold was "flat" during this portion of the cycle.

Diastolic threshold estimations were made at several intermediate basic frequencies in the same NH cell as that in Figure 15. Threshold, measured at a constant test interval of 500 msec, was constant as the basic cycle was reduced to 500 msec, and increased progressively as the basic cycle was reduced to 200 msec.

Complete strength-interval curves were not obtained at two different driving rates in the same cell, but the observations recorded in Figure 16 indicate that the lag in recovery of excitability is shifted to the right at higher frequencies. The action potential is that of an NH cell, and the preparation was driven from the His bundle at a basic cycle of 700 msec in A and B, and 250 msec in C. In this series of measurements, two successive identical current pulses were passed through the microelectrode. In A, the threshold pulse of $8 \times 10^{-7}$ amp was effective at a test interval of 590 msec, but not at 250 msec. In B, the first of the pair of intracellular pulses was delayed to 260 msec, but the current strength was unchanged. Both pulses were now successful. The recovery of excitability at this frequency (basic cycle, 700 msec) was thus complete at approximately 80 msec after full repolarization. At the more rapid driving rate (basic cycle 250 msec, Fig. 16, C) the late diastolic threshold, determined at the same test interval as in A, was increased to $1.1 \times 10^{-6}$ amp, an increment of 37.5%. Furthermore, the recovery of excitability was delayed. The first test pulse shown in part C ($S_1S_2 = 330$ msec) was the latest position at which the test pulse of $1.1 \times 10^{-6}$ amp was unsuccessful. In other words, the lag of recovery was prolonged an additional 100 msec at the higher driving rate.

**Discussion**

Indirect studies of the A-V transmission system, based on input-output relationships, suggest that the margin of safety for propagation is low. Although direct measurements of the excitability of nodal cells have not previously been reported, it has been conjectured that nodal cells are poorly excitable, and that the high threshold, coupled with a slowly rising action potential, accounts for the slow conduction (1). It has also been conjectured, without direct evidence, that the excitability of cells at the atrio-nodal junction is delayed beyond full restoration of the resting membrane potential. It was suggested that in this respect the junctional cells behave similarly to atrial and ventricular cells which have been depressed under the influence of hypothermia, or of certain drugs and metabolic inhibitors (1).

The studies reported here demonstrate that the electrical excitability of cells within the A-V node is indeed low; that the recovery of excitability lags beyond full repolarization; and that the frequency-dependent depression of conductivity (fatigue) is associated with a parallel reduction of excitability.

**EXCITABILITY OF NODAL CELLS**

Although the electrical threshold of nodal cells, including the "transitional" AN and NH zones, is significantly higher than in cells of the adjacent atrial and ventricular tissues, individual cells which were responsive to the intracellular injection of brief current pulses were found in all strata of the node. Threshold requirements rise very sharply as the center of the node is approached from either the atrial or ventricular margin; in the temporal midpoint of the node, pulses of as much as $5 \text{ amp}$ sometimes failed to cause responses. If we assume that excitability is one of the determinants of conduction velocity, then the relatively poor conductivity of nodal tissue can be ascribed in part to its low excitability.

The recorded values for the threshold of cells in the midnodal region must be interpreted with caution; the injection of stimuli through an intracellular pipette is hardly a physiological mode of excitation. The results may reflect a difference in the connectivity of the cells and in the internal resistance of the connections, rather than a fundamental difference in the properties of the excitable membranes.
membrane. If we can assume low resistance connections between cells in a 3-dimensional syncytium (13), then a depolarizing current pulse introduced into one element may be countered by the flow of repolarizing current from numerous neighboring elements. In accord with Noble's analysis of excitation in a branching system, the critical area of membrane which must be depolarized will depend not only on the characteristics of the membrane itself, but also on the geometric relationship with adjacent cells (14). In the A-V node it is possible that a propagated response to an intracellular stimulus can occur only when a pulse delivered through a point source is sufficiently strong to depolarize not only the impaled cell but its neighbors as well.

In spite of these reservations, the observed changes in excitability of single cells as a function of time and of frequency must be physiologically significant.

LAG IN RECOVERY OF EXCITABILITY

Unlike cells of the atrium or ventricle, which normally reach the diastolic level of excitability at or before full repolarization, cells within the node recover much more slowly. The lag in recovery, already marked in the transitional NH region (Fig. 5), extended several hundred milliseconds into phase 4 in cells of the N region. During part of this time, but not all of it, cells of the node are often slightly hyperpolarized (Figs. 5 and 12).

The functional or effective refractory period of the A-V transmission system can be defined as the shortest attainable interval between two ventricular responses both propagated from the atrium (4). This measurement includes the longest refractory period of any element in the conducting series plus the increment in conduction time of the second of the pair of responses. Although under certain circumstances the limiting refractory period is subnodal (for example, in the His bundle or its peripheral branches (15, 16), the barrier to propagation of premature atrial responses can usually be assigned to the A-V node itself. Because the effective refractory period determined in this way often greatly exceeds the action potential duration of any intranodal cell, it is obvious that much of the response interval represents the delayed transmission of the test response in tissue not yet fully recovered; the demonstrated lag in the recovery of excitability must account, at least in part, for the delay.

FREQUENCY-DEPENDENT REDUCTION IN EXCITABILITY

The cumulative depression of conductivity at higher driving frequencies, also noted in clinical records by Jedlicka (17), is related to parallel changes in excitability. The strength-interval curve appears to be shifted upward and to the right. The results suggest two recovery processes with widely different time courses. The first, which we may identify with the relatively refractory period or the "lag" in recovery of excitability, is shifted to the right when the driving frequency is increased (Fig. 16). Although complete strength-interval curves were not obtained at the higher frequencies, it is clear that the slope of threshold recovery must change, for although the functional refractory period of the tissue diminishes as the cycle is reduced, the end of the relatively refractory period is displaced to the right. In other words, the duration of the relatively refractory phase must be prolonged as the frequency is increased.

The second phase of excitability recovery, which we may ascribe to a frequency-dependent "fatigue," has a much more protracted time course. Dissipation of fatigue was so slow, in fact, that the "diastolic" excitability appeared to be constant for several hundred milliseconds after the last of a series of driven responses at a short basic cycle (Fig. 15). Clearly, the threshold must decline eventually, but the rate of change was too slow to detect within the resolution of the stimulating circuit. It is perhaps pertinent that recovery from the cumulative prolongation of A-V conduction time in the dog heart (Fig. 13) also passed through a plateau between 600 and 600 msec. A similar step in the recovery of conductivity was observed in other experiments as well.
The physico-chemical mechanism of the observed time-dependent and frequency-dependent changes in excitability is not apparent, and it would be premature to speculate about it, inasmuch as there is doubt whether the generation of the action potential in the node has the same ionic basis as in other cardiac cells (18).

**THE WEAK LINK IN A-V TRANSMISSION**

It is often stated that the weakest link in the A-V conduction chain lies in the atrio-nodal junction (1, 19). The results of the present study do not support this conclusion, unless it be argued that the atrio-nodal junction includes the temporal center of the node. The lowest excitability and the greatest lag in recovery were observed in the midnodal region. Accordingly, it is this area, rather than the atrial margin, which should be expected to determine the success or failure of propagation of premature beats, and to define the minimal output interval between sequential responses. In our experience (13), as in that of Paes de Carvalho and de Almeida (10), this is true; the latest atrial premature beat which fails to reach the His bundle dies at the margin of N and NH rather than in the atrio-nodal transitional zone (Fig. 10). Indirect evidence also supports this concept; a late but blocked atrial premature beat can be facilitated by very slight pre-excitation of the His bundle, just in advance of the prior basic response (15). Earlier premature responses can, of course, be extinguished at higher levels within the node (13, 16).

**SUMMATION IN A-V NODAL PROPAGATION**

Several of the observations made in this study indicate the importance of summation as a feature of nodal transmission. Whenever a single cell was stimulated through a micro-electrode, no matter how late in the recovery cycle, the propagation time to the atrium and to the His bundle exceeded the corresponding conduction intervals for responses initiated in the His bundle or the atrium. In a number of cases, successful excitation of the impaled cell occurred without propagation to the atrium or the His bundle (Fig. 9), although an earlier response initiated in the atrium successfully traversed the node (Fig. 10). In view of previous observations of the characteristics of intranodal propagation of premature responses (13), and of the nature of excitation in a branching system with low resistance connections (14), these results are not surprising. The action potential of a single unit in a field of fully polarized cells may not provide enough energy to accomplish the depolarization of its immediate neighbors; a more or less uniform wave front, involving a large number of units in parallel, will provide a much more effective stimulus.

**References**

ELECTRICAL EXCITABILITY OF A-V MODAL CELLS

Electrical Excitability of Atrioventricular Nodal Cells
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