Characterization of Refractoriness in the Sinus Node of the Rabbit

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SUMMARY Recovery cycles following premature atrial stimulation introduced during atrial pacing may fall into four categories: reset, incomplete interpolation, complete interpolation, and echo responses. It has been postulated that the transition from reset to incomplete interpolation may represent the point at which premature beats can no longer enter the sinus node and reset it and thus reflect the refractory period of the sinus node. The purpose of this study was to explore the electrophysiological basis underlying the transition from reset to incomplete interpolation and, to assess the spatial orientation of refractoriness in the sinus node. In 15 rabbit sinus node preparations, premature atrial stimuli were introduced at varying degrees of prematurity while intracellular potentials were recorded with a microelectrode in the sinus node. In 12 of 15 experiments, transition from reset to incomplete interpolation immediately followed a sudden reduction in action potential amplitude, rendering the action potential incapable of resetting the node. This point was interpreted as the effective refractory period of the sinus node. During the zone of incomplete interpolation, low voltage depolarizations were seen in the node, interfering with diastolic depolarization and delaying the recovery beat. These small depolarizations were absent during the zone of complete interpolation. In six experiments, the microelectrode was moved toward the crista terminalis in steps of 50 to 100 μm, and the stimulation sequence at each site was repeated. By examining relative action potential amplitude at various sites at the same premature interval, it was possible for us to construct curves showing the pattern of block of premature impulses. We found that progressively earlier premature beats are blocked at progressively greater distances from the node. Therefore, tissue between the crista terminalis and the sinus node provides a progressive gradation of refractoriness, rather than a discrete barrier. Circ Res 47: 742-756, 1980

TO measure refractoriness in most cardiac tissues, electrical activity proximal and distal to that tissue is recorded in response to premature stimulation. Depolarizations arising as the result of atrial stimulation are conducted retrogradely toward the sinus node which is engulfed by the approaching wavefront and is depolarized last (Sano et al., 1965). Electrical activity cannot be measured distally to the node, and therefore the conventional method of measurement of refractoriness cannot be applied to the sinus node.

Examination of the atrial return cycles following premature atrial stimulation may, however, provide insight into refractoriness of the sinus node. Premature atrial stimuli introduced late in the cardiac cycle appear to enter the sinus node and reset it, resulting in atrial return cycles that are slightly longer than the spontaneous cycle length. On the other hand, premature atrial stimuli introduced early in the cardiac cycle may be blocked en route to the sinus node, and interpolation may result. Kisch (1921) and Von Gonczy and Gyorgyi (1928) first observed interpolation of spontaneous atrial extrasystoles in animals and humans. These authors and others (Drury and Brown, 1926; Eccles and Hoff, 1934; Langendorf et al., 1962; Fleischmann, 1963; Friedberg and Schamroth, 1970; Goldreyer and Damato, 1971; Narula et al., 1972; Childers et al., 1973; Dhingra et al., 1975) have postulated that interpolation is due to encroachment of the premature impulse on the refractory period of the sinus node, resulting in block of the impulse. Bonke et al. (1971) and Klein et al. (1973) confirmed this hypothesis in isolated rabbit right atrial preparations. However, they did not demonstrate either the distribution of refractoriness in the sinus node or the site of block of premature impulses.

Assessment of sinus node refractoriness by analysis of atrial return cycles during spontaneous sinus rhythm may be distorted by variability in antegrade sinoatrial conduction time (Strauss and Geer, 1977). Consequently, accurate assessment of sinus node refractoriness can be accomplished only by eliciting premature responses during continuous atrial pacing. With this method, Childers et al. (1973) and Dhingra et al. (1975) studied refractoriness in the intact dog and human, respectively, but there have been no comparable in vitro studies.
Prystowsky et al. (1979) demonstrated that, as premature beats are introduced progressively earlier in diastole, the amplitude of the transmembrane potential recorded in the sinus node is reduced. In a preliminary series of experiments, they suggested that at a critical degree of prematurity the amplitude will be sufficiently reduced to result in failure of pacemaker reset.

This study seeks to characterize more clearly refractoriness of the sinus node during continuous atrial pacing. In particular, we shall attempt to examine the electrophysiological features underlying the transition from sinus node reset to interposition. This will involve examination of both the site at which early premature beats are blocked and the spatial orientation of refractoriness in the sinus node and adjacent tissue.

**Methods**

**Procedure**

Fifteen young rabbits of either sex weighing between 1.5 and 2.0 kg were anesthetized with intravenous pentobarbital (35-40 mg/kg). After being heparinized, the rabbits were killed by air embolization and the hearts were removed rapidly and placed in cool modified Tyrode's solution. The sinus node, crista terminalis, and surrounding right atrium (excluding the atrioventricular node) were dissected out and, with the endocardial surface up, were pinned to the wax-covered bottom of a Lucite chamber. The preparation was superfused with Tyrode's solution at approximately 10 ml/min and the temperature was held constant at 35.5 ± 0.5°C. The modified Tyrode's solution had the following composition: NaCl, 130 mM; KCl, 4.0 mM; NaH₂PO₄, 1.8 mM; CaCl₂, 2.7 mM; MgCl₂, 0.5 mM; dextrose, 5.5 mM; NaHCO₃, 18.0 mM in deionized, distilled water. The solution, gassed with a mixture of 95% O₂, 5% CO₂, achieved a pH of 7.2 to 7.3 in the bath.

A fine bipolar recording electrode, insulated except at the tip, was placed on the midportion of the crista terminalis to record a surface electrogram. A bipolar silver electrode was applied to the proximal end of the crista terminalis, and rectangular stimuli 2 msec in duration and 1.5 times threshold were introduced by a programmable pulse stimulator. Transmembrane action potentials were recorded using glass microelectrodes filled with 3 M KCl and having tip resistances between 20 and 35 MΩ.

After allowing 30-60 minutes for the preparation to equilibrate with the superfusate, the area of the sinus node was explored with the microelectrode until the sinus node pacemaker site was identified. Sinus node pacemaker cells were identified when an action potential was recorded that had the following characteristics: diastolic depolarization, gradual transition to the slow upstroke of the action potential, and the longest conduction time to the crista terminalis, exceeding 25 msec (see Fig. 1). The transmembrane potential, crista terminalis electrogram, and 50-msec time lines were recorded with a Siemens-Elema Mingograph 803 recorder at a paper speed of 200 mm/sec.

Spontaneous activity was recorded for a minimum of 30 seconds. The crista terminalis then was paced for 12 beats at a cycle length of 400 msec. This cycle length was chosen as the longest cycle length at which atrial capture consistently was achieved. The spontaneous cycle length for each preparation is shown in Table 1. After the 12th paced beat, a premature beat was introduced, and this was followed by a 2-second pause before beginning another train of paced beats. Premature atrial stimulation was commenced late in the cardiac cycle, and the coupling interval was shortened by decrements of 5–15 msec until the atrial effective refractory period was encountered.

In six hearts, the microelectrode then was moved from the sinus node to the crista terminalis in steps of 50 to 100 μm, and the stimulation sequence was repeated at each site. A single microelectrode was used for each experiment.

**Data Analysis**

For each pacing train, the crista terminalis (CT) and corresponding intracellular action potentials (AP) were defined as in Figure 1:

CT₁—the regularly paced CT depolarizations.
CT₂—the premature CT depolarization.
CT₃—the first CT depolarization following the premature beat.

AP₁, AP₂, AP₃—the corresponding intracellular action potentials.

The beginning of each action potential was defined as the intercept of tangents drawn to the

**Figure 1** Example of a tracing recorded from impalement in the sinus node. Upper tracing: Transmembrane action potential recorded by intracellular microelectrode. Lower tracing: Surface electrogram recorded from the crista terminalis (CT). Tangents are drawn to diastolic depolarization and the action potential upstroke, the intersection of these lines representing the take-off point of the action potential.
As sinus node cells undergo diastolic depolarization, premature responses elicited at different coupling distances will arise from different levels of membrane potential. Thus, the amplitude measured by the template method reflects the voltage displacement resulting from the premature beat, regardless of its timing in diastole. Furthermore, this method permits measurement of minimal displacements of membrane potential. The amplitude of AP2, measured by either the conventional or template methods, was expressed as a ratio to the immediately preceding regularly paced beat (AP1). This relative amplitude (AP2/AP1) therefore varied from zero to approximately 1.0.

Relative action potential amplitude was the only electrophysiological quantity that we examined in this study. This parameter provides only indirect assessment of membrane conductance, since neither voltage nor current is controlled. Furthermore, because electrical interactions between adjacent tissues within the electrically heterogeneous sinus node preparation may have independent effects on the action potential, relative amplitude does not specifically reflect membrane conductance. Nevertheless, these same disadvantages, which are inherent to any macroscopic preparation, apply to other widely measured quantities, such as the maximum rate of rise of the action potential. Results obtained in this study should be interpreted in light of these limitations.

The relative amplitude, measured by both methods, was plotted against prematurity at each site of implantation. Lines were drawn by hand through these points. Relative amplitude also was plotted against the distance from the sinus node at specific premature intervals. The points for this relationship were fit to a sigmoid function with the Hill equation, $Y = \frac{X^A}{B + X^A}$, where $A$ and $B$ are the variables to be optimized. Lines were drawn through these points, and goodness-of-fit was reported as the coefficient of determination, $r^2$, which was cal-

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**Table 1** Data from 15 Experiments

| Experiment | Spontaneous cycle length (msec) | Distance from sinus node to CT (μm) | Retrograde conduction time (CT,AP1) Mean ± sd (msec) | Transition from reset to incomplete interpolation (msec) | Transition from incomplete to complete interpolation (msec) | Atrial effective refractory period (msec) |
|------------|-------------------------------|-----------------------------------|------------------------------------------------------|--------------------------------------------------------|-----------------------------------------------------------|----------------------------------------|
| 1          | 460                           | 2200                              | 35 ± 2 (53)*                                        | 148                                                    | 131                                                       | 66                                     |
| 2          | 515                           | 1000                              | 12 ± 2 (45)                                         | 195                                                    | 151                                                       | 69                                     |
| 3          | 460                           | 1300                              | 43 ± 2 (43)                                         | 145                                                    | 125                                                       | 49                                     |
| 4          | 565                           | 1100                              | 41 ± 4 (52)                                         | 145                                                    | 114                                                       | 59                                     |
| 5          | 610                           | 1200                              | 47 ± 3 (59)                                         | 195                                                    | 145                                                       | 81                                     |
| 6          | 425                           | 1150                              | 24 ± 4 (32)                                         | 168                                                    | 150                                                       | 64                                     |
| 7          | 535                           | 1500                              | 53 ± 3 (36)                                         | 205                                                    | 130                                                       | 70                                     |
| 8          | 590                           | 1400                              | 52 ± 3 (30)                                         | 121                                                    | 104                                                       | 78                                     |
| 9          | 440                           | 1600                              | 41 ± 2 (22)                                         | 133                                                    | 110                                                       | 71                                     |
| 10         | 540                           | 1500                              | 40 ± 3 (27)                                         | 190                                                    | 97                                                        | 84                                     |
| 11         | 510                           | 1400                              | 48 ± 2 (24)                                         | 148                                                    | 105                                                       | 70                                     |
| 12         | 440                           | 1400                              | 60 ± 3 (24)                                         | 141                                                    | 109                                                       | 61                                     |
| 13         | 620                           | 1300                              | 36 ± 2 (20)                                         | 213                                                    | 153                                                       | 77                                     |
| 14         | 470                           | 2300                              | 62 ± 4 (30)                                         | 202                                                    | 146                                                       | 45                                     |
| 15         | 410                           | 2000                              | 17 ± 3 (31)                                         | 147                                                    | 112                                                       | 76                                     |

Mean ± sd

Retrograde conduction time from crista terminalis to sinus node (CT,AP1) was measured following the 12th regularly paced beat in each pacing sequence.

* Numbers in parentheses = number of intervals measured.

From these tracings, several intervals and amplitudes were measured, using a Sciences Accessory Corporation Grafpen connected to a DEC 11/03 computer:

1. Premature interval. The interval, CT1CT2, between the last regularly paced depolarization and the premature CT depolarization.
2. Recovery interval. The interval, CT2CT3, between the premature depolarization and first spontaneous recovery CT depolarization.
3. Encompassing interval. The interval, CT1CT3, which encompasses the premature CT depolarization.
4. Relative amplitude of the action potential. The amplitude of the premature action potential, AP2, was measured by two methods. With a conventional method (Strauss and Bigger, 1972), the amplitude was measured from the takeoff potential to the peak of the action potential (Fig. 2A). When premature beats are introduced early in the cardiac cycle, however, there is frequently a depolarization that cannot be measured by this conventional method. We therefore employed a template method that is similar to that described by Jongmsma and van Rijn (1972) and Lieberman et al. (1975) in their studies of the passive membrane properties of cultured heart cells. The template method is illustrated in Figure 2B. With this method the cardiac cycle containing the premature beat is superimposed on the preceding paced cardiac cycle which serves as a template. The premature beat causes a displacement of membrane potential, and the amplitude of this displacement can be measured as the vertical distance between the template and the test cycle. As sinus node cells undergo diastolic depolarization, premature responses elicited at different coupling intervals will arise from different levels of membrane potential. Thus, the amplitude measured by the template method reflects the voltage displacement resulting from the premature beat, regardless of its timing in diastole.
A. CONVENTIONAL METHOD

![Diagram of Conventional Method](image)

B. TEMPLATE METHOD

![Diagram of Template Method](image)

**Figure 2** Two methods of determining action potential amplitude. A. Conventional method: amplitude is measured from the takeoff point to the peak of the action potential. B. Template method: a tracing of the 11th regularly paced action potential (template—broken line) is superimposed on the last regularly paced action potential (solid line). The amplitude of the premature action potential (AP₂) is measured as the maximum vertical distance separating the template from AP₂. Both amplitudes of AP₂ are expressed as a ratio of the preceding beat (relative amplitude).

culated by the method of nonlinear least squares (Sedman and Wagner, 1976).

Recovery cycles following premature beats were defined as follows (Fig. 3):

1. Reset response. The time of the spontaneous recovery beat, CT₃, is set by the premature beat, CT₂, and the recovery interval, CT₂CT₃, is similar in duration to the spontaneous recovery cycle without a premature beat (Fig. 3, A and B).

2. Incomplete interpolation. CT₂ causes some delay in the appearance of CT₃, but CT₂CT₃ is shorter than a completely reset response (Fig. 3C).

3. Complete interpolation. The spontaneous recovery beat, CT₃, is set from CTᵢ and its timing is unaffected by CT₂. Hence the encompassing interval, CTᵢCT₃, approximates the spontaneous recovery cycle length without a premature beat (Fig. 3D).

**Results**

In all 15 preparations, the response following atrial premature stimulation could be separated into three zones: reset, incomplete interpolation, and complete interpolation (Fig. 3). Sinus node echo responses were seen infrequently in only five experiments, and cycles containing echo responses were not included in the analysis. Figure 4 shows an experiment in which both the recovery interval

![Figure 3](image)

**Figure 3** Examples of the three types of responses following premature beats (B-D) are compared to a spontaneous recovery cycle with no premature beat. A. Spontaneous recovery cycle without a premature beat. B. Reset response: the recovery cycle, CT₂CT₃, is nearly equal to the spontaneous recovery cycle, CTᵢCT₃ in panel A. The 15-msec lengthening is due largely to prolongation of retrograde conduction of the premature beat. C. Incomplete interpolation: CT₂ causes some delay in CT₃, and CT₂CT₃ is intermediate between complete interpolation and reset. D. Complete interpolation: CT₂ causes no delay in CT₃; CT₃ is set from CTᵢ and the interval CTᵢCT₃ (780 msec) is similar to the spontaneous recovery cycle in panel A (790 msec).
FIGURE 4  The encompassing interval (CT1CT3) and the recovery interval (CT2CT3) plotted as a function of prematurity (CT1CT2). The transition from reset to incomplete interpolation occurred at the sudden break in both curves. This represents the effective refractory period of the sinus node. The zone of complete interpolation can be recognized where CT1CT3 plateaued to a minimum value.

(T2CT3) and the encompassing interval (CT1CT3) were plotted as a function of the premature interval (CT1CT2). During the zone of reset, the recovery interval can be seen to remain relatively constant, the timing of CT3 being set by CT2. The transition from reset to incomplete interpolation results in sudden shortening of the recovery interval, demonstrated graphically by a break in the curve. The longest premature interval that is incompletely interpolated represents the effective refractory period of the sinus node.

The transition from incomplete to complete interpolation occurred when the encompassing interval (CT1CT3) reached a minimum. At this point, CT3 is set from CT1, and the CT1CT3 interval remains constant throughout the zone of complete interpolation.

Table 1 shows the spontaneous cycle length and the distance from sinus node to crista terminalis in all 15 experiments. Also tabulated are the retrograde conduction time, measured at a pacing cycle length of 400 msec, the point of transition from reset to incomplete interpolation (effective refractory period of the sinus node), the point of transition from incomplete to complete interpolation, and the atrial effective refractory period in all experiments.

Transition from Reset to Incomplete Interpolation

This transition was analyzed in conjunction with the relative amplitude of the premature action potential in the sinus node. The relative amplitude, measured by both conventional and template methods, was plotted as a function of prematurity, and lines were drawn through the points by hand as shown in Figure 5. The transition from reset to incomplete interpolation was accompanied by a fall in amplitude of the action potential according to one of two general patterns. In 10 cases, the transition was abrupt and was associated with a sudden fall in relative amplitude (Fig. 6A). In the other five cases, the transition was gradual and this was associated with a gradual fall in relative action potential amplitude (Fig. 6B). In 12 experiments, the transition from reset to incomplete interpolation occurred immediately after the rapid fall in relative amplitude of the action potential. In the other three, the transition occurred at premature intervals longer than those at which the fall in amplitude occurred, resulting in the appearance of larger depolarizations at this point of transition. This would suggest that, although the criteria for sinus node impairment were rigorously adhered to, the microelectrode was not, in fact, in the primary pacemaker site in these three experiments. Consequently, the potentials recorded at the transition to incomplete interpolation were larger than those that would have been recorded had the microelectrode been in the pacemaker site. In several experiments, the recovery interval, CT2CT3, was lengthened immediately after the transition, resulting in an accentuation of this break in the curve (Figs. 4 and 6A). This resulted when retrograde conduction of the premature beat was progressively prolonged (increase in CT2AP2) with earlier premature beats (Fig. 7).

At the point of transition, electrical depolarizations could be seen in the sinus node in all 15 preparations. In many cases these were displayed only as distortions of diastolic depolarization or of the repolarization phase of AP1, and their amplitudes could not be measured by the conventional method. In the three experiments mentioned above, these depolarizations were of greater amplitude. By analyzing relative amplitude of the action potential using the template method, we could ascribe amplitudes to the low amplitude depolarizations. By
FIGURE 6 Comparison of the relative amplitude of the action potential to zones of reset, incomplete interpolation, and complete interpolation. The sudden fall in relative amplitude occurred immediately before the transition from reset to incomplete interpolation (refractory period). A. Abrupt transition from reset to incomplete interpolation was associated with a sudden decrease in relative amplitude. B. Gradual transition was associated with a gradual fall in relative amplitude. In both cases the transition to complete interpolation was accompanied by the disappearance of low amplitude potentials from the node.
Retrograde conduction time (CT2AP2) can be seen to have become progressively prolonged with earlier premature beats (A to C) resulting in prolongation of the return cycle CT2CT3. In tracing D, the premature interval was short enough to have resulted in interpolation of CT2 and a marked decrease in CT2CT3.

Examining the relationship of relative amplitude measured by this method to prematurity (empty circles in Figures 5 and 6), we can see that there were depolarizations of small amplitude following the transition from reset to incomplete interpolation. This transition is associated with a change to a flatter shape of the curve of relative amplitude vs. prematurity. It is possible that this point, where the shape of the curve changes, represents the transition from a state where propagated action potentials penetrate the node to one where low voltage depolarizations enter the node as the result of either decremental conduction or passive spread of electrical activity. Therefore, the point of transition from reset to incomplete interpolation, which has been called the effective refractory period of the sinus node, represents a critical degree of prematurity at which the action potential amplitude is reduced sufficiently to prevent complete reset of the node. A subsequent section of this paper will explore the premise that, at this degree of prematurity, impulses in the crista terminalis which normally are of sufficient amplitude to enter and reset the node, encounter progressively more refractory tissue and therefore are blocked before they can enter and reset the node.

Incomplete vs. Complete Interpolation

The transition from incomplete to complete interpolation could be identified in all studies. This could be best appreciated when the encompassing interval was plotted against prematurity (Figs. 4 and 6), where complete interpolation could be identified as the point at which CT2CT3 plateaued to its shortest value.

In all experiments, there were several incompletely interpolated responses. The point of complete interpolation occurred at a mean premature interval of 125 ± 20 msec (mean ± sd), which was between the atrial effective refractory period (68 ± 11 msec) and the transition from reset to incomplete interpolation or sinus node effective refractory period (166 ± 30 msec). This is what one would expect since, at the point of complete interpolation, block probably occurs in tissue which is located between the atrium and the sinus node (see below).

Analysis of relative amplitude at the point of complete interpolation showed that, in all cases except one, the relative amplitude was zero. In the other experiment, impalement was again probably not in the pacemaker site. Even though the transition to upstroke was smooth and the timing was appropriate for a pacemaker impalement, the action potential was rather narrow and may have been recorded from the periphery of the node.

By the conventional method of measuring amplitude, usually there was no measurable amplitude at the point before transition from incomplete to complete interpolation. However, examination of tracings revealed a significant distortion of the diastolic portion of the action potential by low amplitude depolarizations. In 12 of 15 experiments, these small depolarizations were seen down to the point of complete interpolation. Figure 8 shows three tracings taken during incomplete interpolation and one tracing taken immediately after transition to complete interpolation. The low voltage depolarizations, which are easily appreciated in the early part of diastole, disappear during complete interpolation. Figure 9 shows two more examples in which the cycle immediately before complete interpolation (solid line) is superimposed on the first completely interpolated cycle (broken line).

These depolarizations, while incapable of resetting the sinus node, cause some delay in the timing of the spontaneous recovery beat (AP3 and its resulting CT3). The low amplitude depolarizations may occur at different times in the cardiac cycle. In Figures 8 and 9A, the depolarization falls in the early part of diastolic depolarization, whereas in Figure 9B the depolarization falls on the repolarization phase of the preceding action potential. These depolarizations appear to reduce the maximum diastolic potential and impose a phase delay in attaining maximum diastolic potential; this results in a delay in the appearance of the spontaneous recovery beat.

Another possible reason for the delay in appearance of the recovery beat during the zone of incomplete interpolation is a prolongation of antegrade conduction, AP3CT3, compared to the AP3CT3 incom-
A. FIGURE 8 Four tracings recorded within the sinus node. Transition from reset to incomplete interpolation (tracing A) is associated with low voltage potentials occurring in early diastole which persisted throughout the zone of incomplete interpolation (B and C). The transition to complete interpolation (D) is associated with the disappearance of these small depolarizations. There was considerable variation in AP3CT3 within each experiment. This took the form of shortening (occasionally up to 30 msec) or slight prolongation (usually less than 10 msec) compared to baseline antegrade conduction. Shortening was attributed to pacemaker shift, whereas prolongation may have been due to pacemaker shift or slowing of conduction. We compared AP3CT3 in the zones of complete and incomplete interpolation and found no significant difference between these intervals in 14 or 15 experiments. In one experiment, the mean value and range for AP3CT3 were longer during incomplete (38, 26–50 msec) than during complete interpolation (25, 21–30 msec). However, in this one experiment, the prolongation of AP3CT3 equalled only 17, 4–26% (mean, range) of the amount by which each response was incompletely interpolated.

By looking again at the graphs of relative amplitude of the action potential versus prematurity (Figs. 5 and 6), we can see that, using the template method, the transition from incomplete to complete interpolation coincides with the inability to record small depolarizations from the sinus node.

Retrograde Conduction

The retrograde conduction time from the crista terminalis to sinus node (CT1AP1) at the basic pacing cycle length of 400 msec is shown in Table 1. This conduction time remained constant throughout the pacing run in all 15 experiments. The retrograde conduction time of atrial premature beats (CT2AP2) was analyzed as a function of prematurity. Variable patterns were noted. In most preparations, the retrograde conduction time prolonged only slightly with increasing prematurity (Fig. 10, filled circles). However, in several preparations, CT2AP2 prolonged dramatically as premature intervals decreased (Fig. 10, empty circles). This prolongation of CT2AP2 began at long prema-

Figure 8: Four tracings recorded within the sinus node. Transition from reset to incomplete interpolation (tracing A) is associated with low voltage potentials occurring in early diastole which persisted throughout the zone of incomplete interpolation (B and C). The transition to complete interpolation (D) is associated with the disappearance of these small depolarizations.

Figure 9: Two examples of disappearance of low voltage potentials at the transition from incomplete (solid line) to complete interpolation (broken line). In A the small potential fell in the early part of diastolic depolarization, whereas, in B, it fell on the repolarization phase of the preceding action potential.

Figure 10: Retrograde conduction time of the premature beat (CT2AP2) as a function of prematurity in two experiments. The more common pattern (filled circles) showed a slight increase in retrograde conduction time at short premature intervals. Less commonly (unfilled circles), retrograde conduction prolonged markedly, beginning at longer premature intervals.
ture intervals and became most accentuated just before transition from reset to incomplete interpolation. In some cases this caused a significant prolongation of the recovery interval and, thus, in the plot of CT₂CT₃ vs. prematurity, CT₂CT₃ increased immediately before transition from reset to incomplete interpolation, accentuating the break in the curve (Figs. 4 and 6A).

In many cases there were qualitative aberrations in retrograde conduction that became more marked at earlier prematurity. These took the form of prolongation of the premature action potential (AP₂) or its fragmentation and slurring (Fig. 11). This fragmentation is displayed as a double hump of the action potential.

Retrograde conduction time also was plotted against distance from the crista terminalis to the impalement site for responses elicited at two different coupling intervals (400 and 195 msec), as shown in Figure 12. In both instances, the retrograde conduction time increased slightly until approximately half the distance between crista terminalis and sinus node, where it prolonged markedly. This prolongation near the node was more marked with beats that were more premature. This was seen in all six experiments. These data suggest a slowing of conduction as an impulse approaches the sinus node, more pronounced with earlier premature beats.

**Figure 11** Aberration of the premature action potential in the sinus node (AP₂). With progressive prematurity, AP₂ becomes broadened with a double hump appearance (tracings A to C). In panel D, the premature beat (CT₂) was completely interpolated and no corresponding depolarization was seen in the node.

**Figure 12** Retrograde conduction time (CT₂AP₂) as a function of distance from the crista terminalis at two coupling intervals (400 and 195 msec). A marked increase in retrograde conduction time occurred approximately midway from crista terminalis to sinus node and was more marked with the more premature beat.

**Relationship of Relative Action Potential Amplitude to Distance from the Sinus Node**

Analysis of relative amplitude of the action potential at various distances from the node should permit insight into the spatial orientation of refractoriness in the region of the sinus node. In six experiments, the microelectrode was moved from the sinus node to the crista terminalis in increments of 50-100 μm. At each site, premature stimulation of the crista terminalis was performed as previously described. The relative amplitude of the premature action potential was analyzed as a function of prematurity for each impalement site.

Figure 13 shows a series of curves representing 6 of 12 impalement sites in one experiment in which the distance from sinus node to crista terminalis was 1150 μm. As the microelectrode was moved farther from the node, the curve relating relative amplitude to prematurity moved to the left, and the range of prematurity over which the decrease in relative amplitude occurred became shorter. Since the sudden decrease in relative amplitude reflected refractoriness, there appears to be a gradual transition of refractoriness between the sinus node and crista terminalis. In the crista terminalis, the decrease in relative amplitude occurred immediately before the atrial effective refractory period. Refractoriness then progressively increased as the impalement site moved progressively closer to the sinus node. Similar results were obtained in the other five experiments.

A different analysis of the relationship between distance from the node and refractoriness was obtained by comparing relative amplitude of the action potential with distance from the node at the same degree of prematurity. Figures 14 and 15 show...
Relative amplitude of the premature action potential ($AP_2$) at six different distances from the sinus node (total distances from the node to crista terminalis = 1150 μm). Relative amplitude was measured by the conventional method. Lines were drawn by hand through each set of data points.

**Figure 14** Five tracings taken from different sites of impalement at $CT_1$ = 205 msec (transition from reset to incomplete interpolation). In the sinus node, only a low voltage potential resulted from the premature stimulus and was incapable of resetting the node. Movement of the electrode progressively farther from the node resulted in a progressive increase in the relative amplitude of the premature action potential. In the crista terminalis (1500 μm from the sinus node) the relative amplitude was 1.0.

The results taken from this form of analysis may be displayed graphically, as shown in Figure 16. Figure 16A shows the relative amplitude, measured by both the conventional and template methods, as a function of distance from the sinus node for a single premature interval of 205 msec. When such an analysis was performed for different premature intervals, a series of lines was obtained, as shown in Figure 16B. Each pair of lines represents a specific premature interval. As can be seen, the onset of block, as determined by the rapid decline in relative amplitude, moves progressively farther from the node with earlier premature beats. In the experiment shown in Figure 16B, at the point of transition from reset to incomplete interpolation (205 msec), block begins between 500 and 700 μm from the node.
Using the same format as Figure 14, six tracings are shown with a premature interval of 130 msec (transition from incomplete to complete interpolation). At this interval, the relative amplitude of the premature action potential began to decrease farther from the node than with the premature interval displayed in Figure 14, and only low voltage depolarizations remained at 500 μm. By 400 μm, these were totally blocked and were not seen in the sinus node itself.

while, during complete interpolation (130 msec), block begins between 1000 and 1200 μm from the node. Similar analysis was performed in the other five experiments, and the distances at which block begins in all six complete experiments are shown in Table 2. Experiments 2 and 5 are two of the cases in which the fall in relative amplitude did not coincide with the transition from reset to incomplete interpolation, indicating that the first impalement might not have been in the primary pacemaker site. This probably explains why the apparent site of block is closer to the sinus node. The other four experiments show general agreement, and all six confirm the concept that, with greater degrees of prematurity, block occurs farther from the node. During incomplete interpolation (lines at 205 and 155 msec in Figure 16B), low voltage depolarizations are present in the node. At 155 msec, these depolarizations can be appreciated only by examining the curve measured by the template method.

The tissue between the sinus node and crista terminalis appears to represent a transition of re-
fractoriness with a progressive increase in refractoriness as the sinus node is approached. As a result, progressively earlier beats are blocked farther from the pacemaker site, offering greater protection to the sinus node from disturbance by premature beats.

Discussion

Interpolation of an atrial premature beat has been suggested to arise from encroachment of that premature beat on the refractory period of the sinus node. At the effective refractory period, there should exist some electrophysiological phenomenon accounting for the sudden inability of the premature impulse to reset the sinus node. We chose to examine the amplitude of the action potential as one factor affecting the ability of an impulse to cause cellular excitation and to relate changes in amplitude to the changes in recovery response following premature beats. In 12 of 15 experiments, the transition from reset to incomplete interpolation occurred immediately after the rapid fall in relative amplitude of the action potential in the sinus node. Therefore, the transition from reset to incomplete interpolation may represent a critical point at which the amplitude of the depolarization of the pacemaker cell is no longer sufficient to result in sinus node reset.

Whereas the effective refractory period of the sinus node could be definitely identified in most preparations, occasionally the transition between zones was less sharp, and it was more difficult to assign a precise value to the refractory period (Fig. 6B). Therefore, although this methodology appears to be able to measure the refractory period in most cases, it may be unable to do so in some circumstances. Recording and stimulating at a site near the sinus node would allow this method to be used in the absence of intracellular recording. It may be possible to extend this technique to measure sinus node refractoriness by intracardiac electrograms in situ situations, including human subjects.

This study has provided an explanation for the electrophysiological basis underlying incomplete interpolation. The low voltage depolarizations, seen in the node following transition from reset to incomplete interpolation, persist throughout the zone of incomplete interpolation. They result in a delay in the spontaneous sinus node recovery beat by perturbing the terminal part of phase 3 and/or the early part of phase 4 of the transmembrane action potential.

The introduction of these low amplitude depolarizations into the sinus node is comparable to previous experiments performed in Purkinje fibers (Weidmann, 1951; Jalife and Moe, 1976). Weidmann demonstrated that small cathodal electrotonic depolarizations introduced early in diastole resulted in delay of the next spontaneous beat, the duration of the delay being related to the magnitude of the electrotonic depolarization. Jalife and Moe demonstrated that in addition to amplitude of the electrotonic depolarization, duration and timing of the depolarization also were important. Depolarizations of greater duration produced greater delay. The timing of the electrotonic depolarization was found to produce a biphasic response, with late depolarizations accelerating and early depolarizations delaying the following spontaneous beat. Sano et al. (1978) found similar effects in the rabbit sinus node when they introduced electrotonic depolarizations which varied in timing, amplitude, and duration. In the present study, the experimental design did not permit a manipulation of these variables. However, the low voltage depolarizations seen in early diastole in our preparations may reflect a situation that might arise physiologically in response to premature atrial beats. Indeed, the timing and magnitude of these depolarizations are comparable to those introduced by Sano, and the delay of the spontaneous recovery beats in our study is of a nature similar to that noted by the previous three studies.

Results obtained from experiments using electrically homogeneous preparations may be extrapolated cautiously in an attempt to explain phenomena noted in larger preparations, such as those used in this study. Electrical events seen in these large preparations may not reflect membrane properties and may be complicated by the effects of surrounding tissue. Nevertheless, while recognizing these limitations, it may be of value to examine the ionic mechanism of sinus node automaticity to help explain the delay in automaticity induced by low voltage depolarizations. Noma and Irisawa (1976) demonstrated that, in an electrically homogeneous preparation of rabbit sinus node, automaticity results from a voltage-dependent decay of an outward potassium current in the face of a background inward sodium current. The rate of decay of the potassium current is voltage-dependent, with more rapid deactivation occurring at more negative mem-

| Experiment | CT,CT2 (msec) | Site of block (μm) | CT,CT2 (msec) | Site of block (μm) |
|------------|---------------|--------------------|---------------|--------------------|
| 2          | 195           | 300-400            | 151           | 500-700            |
| 3          | 145           | 400-600            | 125           | 600-800            |
| 4          | 145           | 600-800            | 114           | 800-1000           |
| 5          | 162           | 200-400            | 145           | 300-500            |
| 6          | 168           | 700-900            | 150           | 1100-1300          |
| 7          | 205           | 500-700            | 130           | 1000-1200          |

Table 2: Site of Block of Premature Impulses in Six Experiments
brane potentials. Thus, if the maximum diastolic potential is reduced by the low voltage depolarizations described in this study, then the rate of deactivation of the outward potassium current and the rate of diastolic depolarization will be reduced. This mechanism may play a role in the modification of automaticity seen with incompletely interpolated beats.

An alternative explanation for the delay in the appearance of the atrial recovery beat seen in incomplete interpolation is that the premature beat alters the properties of the cardiac tissue, causing a prolongation of antegrade conduction (Langendorf et al., 1962; Fleischmann, 1963; Goldreyer and Damato, 1971; Childers et al., 1973). This prolongation occurred in only one preparation and accounted for a small percentage of the amount by which the response was incompletely interpolated. Therefore, this mechanism appears not to be a significant factor in incomplete interpolation in our experiments.

The site of block of premature impulses may be identified by the zone of marked reduction in relative action potential amplitude. In Figure 16, this decrease in relative amplitude occurs over a distance of 300–400 μm, rather than at a discrete point, making it impossible to localize exactly the site of block. Furthermore, the zone of block varies with the degree of prematurity, more premature beats being blocked farther from the sinus node. The farther block occurs from the node, the more protection is afforded to the pacemaker site. In the reset zone, block is insufficient to prevent propagated action potentials from entering and resetting the node. During incomplete interpolation, only small potentials enter the node which, although unable to reset the node, delay the spontaneous return beat. During complete interpolation, beats are blocked at a sufficient distance from the node to prevent even low amplitude potentials from entering the node.

Therefore, the sinus node and surrounding atrial tissue is best thought of as a unit with a gradual transition of electrophysiological properties between the primary pacemaker and atrial tissues. The term "refractory period of the sinus node," in fact, should be considered as a measure of refractoriness in the sinus node unit. By sinus node unit, we imply the primary pacemaker site, the remainder of the sinus node, and the perinodal fibers that lie between the sinus node and the crista terminalis (Strauss and Bigger, 1972). At the effective refractory period (transition from reset to incomplete interpolation), we are in fact measuring refractoriness outside the pacemaker site, for it is properties of these tissues that prevent action potentials from entering the node. Similarly, at the transition from incomplete to complete interpolation, it is the refractory period of tissue 500 to 1000 μm from the node that determines whether the impulse reaches the node. Thus, the degree of prematurity at which transition from incomplete to complete interpolation occurs is, as one would expect, between the effective refractory periods of the atrial tissue and the sinus node.

Marked prolongation of retrograde conduction occurred in many experiments immediately before and after the effective refractory period. Late in the zone of incomplete interpolation it is conceivable that delay in arrival of the impulse at the sinus node might be sufficient to allow the sinus node to recover from its refractory period and again be excitable. Thus a secondary zone of reset might appear. This was not seen in our study.

Examination of retrograde conduction provides suggestive evidence that, besides a generalized increase in refractoriness as the sinus node is approached, there are also localized inequalities of refractoriness in adjacent tissues. Retrograde conduction time increases dramatically as impalement moves closer to the sinus node (Fig. 12). This suggests that there is considerable slowing of conduction in this region, confirming previous reports (Sano and Yamagishi, 1965; Bonke et al., 1969). It is important to note that earlier premature beats undergo much greater slowing of conduction (Figs. 10 and 12) as shown by other authors (Bonke et al., 1969; Klein et al., 1973). Furthermore, progressively earlier premature action potentials may demonstrate morphological aberration (Fig. 11). The widening and fragmentation of the action potentials were seen in over one-half of these experiments. In the atrioventricular (AV) node, it has been suggested that this fragmentation of action potentials (double humps or notches) is due to excitation of a neighboring tissue with passive spread of potential into the area of impalement (Hoffman et al., 1959; Alanis and Benitez, 1964; Mendez and Moe, 1966). It is probable that the fragmentation seen here represents a similar phenomenon in the sinus node. An alternative explanation is that the impalement site may be activated by wavefronts from two different directions, separated in time by differential conduction velocities or by different lengths of the pathways of conduction. In synthetically grown strands of cardiac muscle, Lieberman et al. (1973, 1974) demonstrated that notchting and fragmentation of action potentials could be explained on the basis of cable properties rather than interaction of colliding wavefronts. Although experiments have not been done in isolated sinus node preparations, it seems unlikely that the variability in the fragmentation of the action potential seen within a fraction of one cardiac cycle could be explained on this basis. It is possible that, in our experimental design, fragmentation represents differential conduction velocity in different tissues, particularly prominent with early premature beats. Therefore, whereas the impaled pacemaker site is not excitable due to continued refractoriness, a premature beat may excite adjacent tissue and result in the passive spread of electrotonic impulses into the pacemaking
region. This suggests that, as well as a generally progressive gradation of refractoriness from the atrium to sinus node tissue, there are also localized inhomogeneities in refractoriness within the sinus node unit. These inhomogeneities would provide the appropriate substrate for the initiation of reentry circuits (Han et al., 1968; Allessie and Bonke, 1979).

**Clinical Implications**

Routine evaluation of sinus node function usually entails measurement of sinoatrial conduction and sinus node automaticity. Another important property, that of refractoriness, usually is not assessed. In this study, an animal model has been presented in which one can, in the majority of cases, measure refractoriness in the sinus node unit. By observing the return atrial cycle following right atrial stimulation, the effective refractory period can be identified as the point of transition from reset to interpolated responses. Intracellular recording is not necessary for this identification, and it is envisioned that this measurement could be carried out easily using intracardiac catheter electrodes in intact animals or humans.

In the technique described in this study, we employed continuous atrial pacing prior to the introduction of the premature beat. Atrial pacing was chosen rather than allowing the preparation to beat spontaneously in order to standardize the experiments and to avoid further variability due to differences in antegrade conduction. When an atrial premature beat is introduced following a regular sinus beat, the coupling interval recorded in the sinus node itself depends on the antegrade conduction time of the last spontaneous beat, as well as the retrograde conduction time of the atrial premature beat. The antegrade conduction time may be extremely variable and thus may contribute to a false approximation of the effective refractory period of the sinus node (Strauss and Geer, 1977). During atrial pacing, both the basic impulse and the premature impulse are propagated in the same direction, which is analogous to the method of determination of the AV nodal refractory period. Consequently, the premature interval between atrial depolarizations more closely reflects the coupling interval of action potentials in the sinus node itself. During the sinus rhythm, the coupling interval in the sinus node exceeds the atrial coupling interval by the sum of antegrade and retrograde conduction. When the premature beat is introduced during sinus rhythm, the atrial coupling interval at which incomplete interpolation occurs will underestimate sinus node refractoriness; indeed it is possible that the effective refractory period might not be identified during sinus rhythm if the sum of antegrade conduction of the last spontaneous beat, the atrial premature coupling interval, and the retrograde conduction of the premature beat exceeds the sinus node refractory period. Thus, the technique of premature beats introduced during continuous atrial pacing theoretically should be more accurate than when introduced during sinus rhythm. Attention should be directed toward ensuring sinus node capture during atrial pacing (Grant et al., 1979).

In support of the view that the introduction of premature beats during atrial pacing is preferable to their introduction during sinus rhythm, Dhingra et al. (1975) employed both methods to define the transition from reset to interpolation in patients with normal sinus node function. They found that this transition was defined more frequently when premature beats were introduced during atrial pacing than during sinus rhythm. The usefulness of the measurement of refractoriness in identifying patients with sinus node dysfunction awaits further clinical investigations in which the normal range of values must be defined fully and the ability of this method to separate patients with normal from those with abnormal sinus function must be elucidated. It is possible that measurement of refractoriness, in conjunction with measurement of conduction and automaticity, may increase the sensitivity and selectivity of clinical investigations.

**Acknowledgments**

The authors are deeply indebted to Dr. Andrew G. Wallace for his support and constructive criticisms of the manuscript, to Amalia Hutchison for her valuable technical assistance and data analysis, to Renate Wend for her preparation of the manuscript, and to Laura Philpot for her aid in data analysis.

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Circ Res. 1980;47:742-756
doi: 10.1161/01.RES.47.5.742

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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