Electrophysiological Properties of the Rabbit Sinoatrial Perinodal Fibers

By Harold C. Strauss and J. Thomas Bigger, Jr.

ABSTRACT

In the rabbit, the sinus node is surrounded by a group of cells which we have termed perinodal fibers. Since sinus node impulses preferentially proceed to the crista terminalis and then spread to the rest of the atrium, we selected the zone of perinodal fibers lying between the sinus node and the crista terminalis for study. Values of transmembrane potentials and the maximum rate of rise (Vmax) of phase 0 in these perinodal fibers were intermediate between those in sinus node and crista terminalis fibers. Values of phase-0 amplitude and Vmax obtained from perinodal fibers near the crista terminalis were greater than those values from perinodal fibers near the sinus node. Membrane responsiveness was determined using premature stimulation or potassium-induced depolarization. In perinodal fibers, recovery of phase-0 Vmax of premature responses to peak value showed a strong time dependence outlasting full repolarization and extending throughout diastole. The increasing values of Vmax during diastole occurred despite spontaneous diastolic depolarization. When time was minimized as a variable, using potassium-induced depolarization, Vmax was also shown to be dependent on membrane activation voltage. In addition, recovery of phase-0 amplitude of premature responses to peak value was shown to be somewhat time dependent. The resistance of phase-0 amplitude and Vmax to the depolarizing effects of increased external potassium concentration was greater in perinodal fibers than it was in ordinary atrial fibers. We were able to show that premature responses capable of exciting the crista terminalis blocked in the zone of perinodal fibers.

KEY WORDS potassium-induced depolarization electrotonic interaction transmembrane potential sinoatrial junction voltage dependence membrane responsiveness premature stimulation time dependence sinoatrial entrance block

Recent interest in human sinus node function has led to the study of sinus

automaticity (1-6) and sinoatrial conduction (5, 7, 8) in patients. Since sinus node activation is not reflected in the electrocardiogram and occurs much earlier than the onset of the P wave (9), interpretation of its behavior is made inferentially by analysis of atrial cycles. Analysis of sinus node automaticity and sinoatrial conduction is facilitated by postulating the existence of an electrophysiological sinoatrial junction. According to this concept, impulses arising in the node traverse this

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a junctional zone before reaching the crista terminalis. In the normal heart, the zone introduces a delay between the time of impulse formation in the node and the initial depolarization of the crista terminalis. In the abnormal heart, the zone may delay or completely block the transmission of impulses (10). Hence the sinoatrial junction could play a critical role in modulating sinus node activity.

Anatomic evidence suggesting the importance of such a zone has come in part from postmortem studies on patients with atrial arrhythmias due to myocardial infarction. These studies (11) revealed that the sites of damage occurred “at the junction of the node with the right atrium and sinus intercavum.” In addition, in histologic studies on the sinus node, James et al. (12) found three types of cells—P cells (pacemaker cells), ordinary atrial cells, and a group of transitional cells located between P cells and ordinary atrial cells. These transitional cells may constitute the junction that is under investigation in this study.

Whether such a zone actually exists has remained a controversial subject. Microelectrode studies have been conducted on the sinus node and contiguous areas (13-17), but definite electrophysiological evidence for the existence of a sinoatrial junction has not been previously shown. For example, Sano and Yamagishi (15) examined propagation of excitation from the sinus node to the atrium in rabbit right atrial preparations. They concluded that there did not seem to be any bridgelike structure or special conduction pathways between the sinus node and the crista terminalis. Because the available electrocardiographic and histopathologic evidence mentioned above argues so strongly for a functional sinoatrial junction, we chose to carefully reexamine the electrophysiological properties of the sinoatrial border in an isolated rabbit right atrial preparation. Our results lead us to believe that we have demonstrated the existence of a distinct junctional zone between the sinus node and the crista terminalis which consists of fibers with characteristic electrical properties; we have termed these cells perinodal fibers.

**Methods**

Rabbits (1.5-2.5 kg) were killed by a blow on the head, and their hearts were excised quickly and dissected in cool modified Tyrode's solution. Preparations were pinned to the wax-lined bottom of a 30-ml Lucite tissue chamber and studied in a manner similar to that described by Paes de Carvalho et al. (13). The atrioventricular (AV) node was excised from the preparations. Modified Tyrode's solution had a millimolar composition of NaCl 137.0, KCl 3.0, NaH2PO4 1.8, CaCl2 2.7, MgCl2 0.5, dextrose 5.5, and NaHCO3 12.0 in deionized, twice-distilled water. Tyrode's solution in the reservoir bottles and in the tissue bath was equilibrated with 95% O2-5% CO2. Modified Tyrode's solution continuously perfused the tissue bath at a constant temperature (35.5±0.5°C) and flow rate (10 ml/min).

Transmembrane potentials were recorded through glass microelectrodes filled with 3M KCl with resistances of 18-35 Mohms. Microelectrodes were connected by silver-silver chloride wires to amplifiers with high input impedance and input capacity neutralization (Bioelectric Instrument Co., NF-1). The output from the amplifier was displayed on a dual-beam cathode-ray oscilloscope (Tektronix, RM-565). The rate of rise (V) of phase 0 of the transmembrane potential was obtained by electronic differentiation and displayed on the oscilloscope (18, 19), and 50- or 100-msec time marks, provided by a time-mark generator (Tektronix, 184), were also displayed on the oscilloscope trace. The oscilloscope images were viewed directly and photographed (Grass, C-4 camera). These photographic records were analyzed by projecting them onto a calibration grid.

The preparations were either allowed to beat spontaneously or electrically stimulated by rectangular pulses from an arrangement of waveform and pulse generators (Tektronix). The preparation was stimulated from a site high on the crista terminalis unless otherwise specified. The duration and amplitude of the basic driving and the test stimuli and the interstimulus interval could be varied independently. Stimulating pulses were isolated from ground by stimulus isolators (Bioelectric, ISA) and delivered to the preparations through closely placed pairs of insulated silver electrodes. The driving stimulus was 2 msec in duration and twice threshold; the test stimulus was 2-4 msec in duration and three to four times threshold. Membrane responsiveness (18, 19) was determined during maintained impalement of a single fiber by stimulating at a constant cycle.
length with the driving stimulus and placing the test stimulus at desired interstimulus intervals in every eighth cycle. In all instances, the onset of depolarization of both basic and premature responses was used as the reference point for determining the coupling interval between responses.

**DEFINITIONS**

The terms used in the text are defined in the following manner. (1) *Membrane activation voltage* is the transmembrane voltage at which a distinct increase in the rate of depolarization occurs, i.e., at which a response is initiated. This term is used because diastolic depolarization occurs in sinus node and perinodal fibers and, thus, no true resting potential exists in these fibers. When the transition between phases 4 and 0 becomes so gradual that no distinct point of increase in the rate of depolarization occurs (sinus node), membrane activation voltage is equated with the voltage defined by the intersection of lines drawn tangentially to phase 4 and phase 0 of a normal response (20). (2) *Action potential amplitude* is the difference between the membrane activation voltage and the most positive membrane voltage attained. (3) *Maximum diastolic voltage* is the most negative transmembrane voltage attained during the diastolic period. In sinus node and perinodal fibers, it occurs at the end of phase 3 of the transmembrane potential. (4) *Action potential duration* is measured from the onset of phase 0 (phase of rapid depolarization) to the point at which repolarization is 90% complete. (5) *Membrane responsiveness* is the relationship between the maximum rate of rise (Vmax) of phase 0 and the membrane activation voltage. Premature stimulation was used to elicit responses at different values of membrane activation voltage and, thereby, to determine the membrane responsiveness of a fiber. Where the time constant for

**FIGURE 1**

A representation of the endocardial surface of the rabbit right atrial preparation. The zone of perinodal fibers (densely stippled area) lies between the sinus node (stippled area) and the crista terminalis. In this experiment, an imaginary line (black line in diagram) was drawn perpendicular to the crista terminalis from the sinus node to a point in the atrial appendage. Transmembrane potentials were recorded from cells along this line in the sinus node, the zone of perinodal fibers, the crista terminalis, and the atrial appendage. The traced transmembrane potentials were aligned to correspond to the temporal relationships between action potentials from these four cell types during sinus rhythm. In sinus rhythm, depolarization of the sinus node fiber (top trace) preceded depolarization in the perinodal fiber (second trace) by 35 msec. Conduction time to the crista terminalis fiber (third trace) was shorter, 15 msec. Conduction time to the atrial fiber (fourth trace) was 8 msec. Transmembrane potentials recorded from the sinoatrial node were characterized by spontaneous phase-4 depolarization and smooth transmission to phase 0. In perinodal fibers, phase-4 depolarization was less rapid and action potential duration shorter than that seen in sinus node fibers. There was no diastolic depolarization in either the crista terminalis or the atrial fibers.
reactivation (21) is sufficiently small relative to the rate of change of transmembrane voltage during repolarization, e.g., in Purkinje fibers, the membrane responsiveness relationship is similar whether it is determined by premature stimulation or by voltage-clamp techniques. Similarly, potassium has been shown to be equally effective in reducing membrane activation voltage without shifting the membrane responsiveness relationship (21).

**DATA ANALYSIS**

Results are expressed in the text as means ± se. The t-test for paired samples was used to determine the significance of data obtained from the same cells prior to and after an intervention (22).

In the text, the values of membrane activation voltage and maximum diastolic voltage are expressed as absolute numbers, i.e., a membrane activation voltage of —65 mv is expressed as 65 mv.

**Results**

**ACTION POTENTIAL OF SINUS NODE**

In all 36 preparations, we found a discrete zone whose dimensions are roughly depicted in Figure 1 (densely stippled area). Invariably, cells in this zone between the sinus node and the sinoatrial ring bundle on the crista terminalis exhibited the following transmembrane potential characteristics (Figs. 1, 3): (1) an amplitude and an upstroke velocity of phase 0 greater than those in sinus node cells, (2) little or no phase 1 (early rapid repolarization), (3) a phase 2 (plateau) longer than that seen in ordinary atrial fibers, which merged gradually with the rapid phase of repolarization (phase 3), (4) termination of phase 3 with a definite hyperpolarization, and (5) phase-4 (diastolic) depolarization slower than that in sinus node fibers and insufficient to cause self-excitation at sinus rates as low as 60/min. We have termed these cells perinodal fibers. In ten experiments, cells with similar transmembrane potential characteristics were also found on the septal, caudal, and cephalic sides of the sinoatrial node in caval tissue. We did not find cells with these transmembrane potential characteristics in the right atrial appendage.

In 18 different preparations, the crista terminalis was stimulated at a constant cycle length of 500 msec. Table 1 gives the range of

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<th>Characteristics of Perinodal Fibers</th>
<th>Transmembrane Potentials</th>
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<td>Rhythm</td>
<td>CL (msec)</td>
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<td></td>
<td>500</td>
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<td>Near crista terminalis (12)</td>
<td>D</td>
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<td>Entire PNP zone (41)</td>
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<td>Near sino node (5)</td>
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<tr>
<th>Duration (s)</th>
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The values of action potential characteristics are means ± se. Values were determined during stimulation of the crista terminalis at a constant cycle length of 500 msec (120/min) (D) or during spontaneous activity (S). CL = cycle length, MAV = membrane activation voltage, MDV = maximum diastolic voltage, V_{max} = maximum rate of rise, and PNP = perinodal fiber. Number of experiments performed is given in parentheses.
values of membrane activation voltage, maximum diastolic voltage, phase-0 amplitude and $V_{\text{max}}$, and action potential duration from 41 different perinodal fibers. There was great variation among the measurements from individual cells. To determine if the variability was related to the location of the cell in the zone of perinodal fibers, four experiments were performed in the following manner. The preparation was stimulated from an atrial site near the crista terminalis. Recordings were obtained from one crista terminalis cell (fixed reference point) midway down the length of the crista terminalis. A second microelectrode was used to record from 10–20 sites along the imaginary perpendicular line shown in Figure 1. We used the conduction time between the crista terminalis and the perinodal fiber as a measure of the temporal separation between points of impalement in the zone of perinodal fibers and the reference point in the crista terminalis.

Membrane activation voltage, maximum diastolic voltage, and phase-0 amplitude and $V_{\text{max}}$ all decreased as a function of the conduction time from the reference site (crista terminalis) to the perinodal fiber sampling sites (Fig. 2), i.e., each of the values decreased as the conduction time between the crista terminalis and the perinodal fiber increased. Phase-0 $V_{\text{max}}$ showed the greatest decrease as the sampling microelectrode was moved away from the crista terminalis. Although the transmembrane potential characteristics showed a gradual transition across the zone of perinodal fibers (Table 1, Fig. 2), the characteristics of the potentials at each border were quite different (Table 1).

In sinus rhythm, impulses arising in the sinus node are conducted through the zone of perinodal fibers to the crista terminalis and then to the rest of the atrium. When the preparation was paced from a driving site on the crista terminalis, impulses proceeded from this site through the zone of perinodal fibers to the sinus node, i.e., the sequence of activation of the perinodal fibers was reversed. To determine the influence of reversing the sequence of activation on perinodal fiber transmembrane potentials, cells were impaled and action potentials were recorded during periods of spontaneous beating and during
regular stimulation (Table 1). When the rhythm was changed from spontaneous to driven, small but significant increases in membrane activation voltage and phase-0 amplitude occurred only in perinodal cells near the crista terminalis (Table 1), and only those fibers near the sinus node showed a significant decrease in V\text{max} (average 63%); in two experiments the change was negligible when the sequence of activation was reversed.

**Determinants of Phase-0 V\text{max in Perinodal Fibers}**

**Premature Responses (Voltage and Time Dependence).**—To evaluate membrane responsiveness in perinodal fibers near the crista terminalis, responses to premature stimulation were observed. Membrane activation voltage, phase-0 V\text{max}, and the coupling interval of premature responses were determined (Fig. 3). Premature action potentials initiated at similar values of membrane activation voltage but at different coupling intervals in the

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

Voltage and time dependence of V\text{max} of premature responses in perinodal fibers. Recordings were obtained from a single perinodal fiber throughout this experiment. The crista terminalis was stimulated every 410 msec. In A, the perinodal fiber action potential and its first time derivative (V) are shown. B-I show premature responses at increasing coupling intervals. In the bottom traces, the initial deflection is the V of phase 0 of the driven response. The earliest response is seen in B. As the coupling interval increased, responses were initiated at higher values of membrane activation voltage (B-C) until phase 3 was terminated. Once phase 3 was terminated, later stimuli initiated responses at lower values of membrane activation voltage (C-I). In E, a premature response was initiated at the same value of membrane activation voltage as was the regular driven action potential. Note that the value of V\text{max} of this premature response was less than that of the regular driven response. Even when action potentials were evoked during the period of hyperpolarization terminating phase 3 (F and C), V\text{max} was still not at peak value. Peak values of V\text{max} of phase 0 were reached only when premature responses were initiated late in diastole (I).
diastolic period (Fig. 3E) had different values of \( V_{\text{max}} \) (Fig. 4). The relationship between \( V_{\text{max}} \) of premature responses and membrane activation voltage suggested that membrane activation voltage was not the only determinate of \( V_{\text{max}} \).

To ascertain the role of time in this membrane responsiveness relationship, \( V_{\text{max}} \) was plotted against coupling interval (Figs. 4, 5). Although recovery toward peak value of the \( V_{\text{max}} \) of premature responses was initially rapid, the perinodal fibers showed continuing recovery throughout the diastolic interval (Fig. 5). The increasing value of \( V_{\text{max}} \) of the premature responses during the diastolic interval occurred despite the spontaneous depolarization and the decreasing value of membrane activation voltage during diastole. In five different experiments (cycle length 500 msec), \( V_{\text{max}} \) of premature responses was 50%, 75%, and 87.5% of peak value at coupling intervals of 134.0 ± 12.6, 171.8 ± 17.4, and 230.8 ± 23.0 msec, respectively. In these five experiments, the perinodal fiber action potential duration averaged 107.8 ± 6.5 msec; therefore, at a time when average repolarization was 90% complete, average phase-0 \( V_{\text{max}} \) was less than 50% of its peak value (Fig. 5), showing that the dependence of \( V_{\text{max}} \) of premature responses on time in perinodal fibers is greater than it is in atrial and crista terminalis fibers (see below).

In crista terminalis fibers, \( V_{\text{max}} \) of premature responses was voltage dependent as in perinodal fibers. However, the large time dependency seen in perinodal fibers was absent from the crista terminalis fibers (Fig. 6). In both fiber types, recovery of \( V_{\text{max}} \) of premature responses seemed to be primarily affected by membrane activation voltage, but in the perinodal fibers an additional time effect on the recovery process was noted (Figs. 4, 6).

![Membrane responsiveness curves of an ordinary atrial fiber (A) and a perinodal fiber (B and C). In the ordinary atrial fiber (A), the distribution of points shows the voltage dependence of \( V_{\text{max}} \). In this experiment, points at the bottom of the curve were not obtained because of the distance between the stimulating and recording electrodes. Data from the perinodal fiber (B and C) were obtained from the experiment illustrated in Figure 3. In B, although \( V_{\text{max}} \) is voltage dependent, different values of \( V_{\text{max}} \) were obtained from action potentials initiated at identical values of membrane activation voltage (also see Fig. 3E). For example, a vertical line up from the abscissa at a value of 74 mV would intersect the curve at two points. Plotting the recovery of \( V_{\text{max}} \) of premature responses as a function of the coupling interval (C) clearly demonstrates the time-dependent recovery of \( V_{\text{max}} \) to peak value.](http://circres.ahajournals.org/)

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The relationship between recovery to peak value of $V_{\text{max}}$ of premature responses (top) and the voltage time course of the perinodal fiber transmembrane potential (bottom). The fiber was stimulated every 500 msec, and every eighth cycle was interrupted by premature stimuli at various times during diastole. Note that, although initial recovery of $V_{\text{max}}$ was rapid, further significant recovery occurred throughout diastole. Peak values of $V_{\text{max}}$ were attained at the end of the diastolic interval (peak $V_{\text{max}} = 70 \text{ v/sec}$). Vertical dashed lines dropped from the curve at 50%, 75%, and 87.5% of peak values of $V_{\text{max}}$ are shown intercepting the voltage time course of the transmembrane potential below to indicate the respective coupling intervals. For example, $V_{\text{max}}$ was 50% of the peak value when the coupling interval was 157 msec. Note that at 50% of peak $V_{\text{max}}$ the vertical dashed line intercepts the transmembrane potential near the end of repolarization at a value of transmembrane voltage that is nearly identical to that seen at the end of diastole. Thus, action potentials initiated at identical levels of transmembrane voltage but at different coupling intervals have different values of $V_{\text{max}}$ (Fig. 4).

Membrane responsiveness in a crista terminalis fiber. In A, $V_{\text{max}}$ is plotted as a function of the coupling interval and in C as a function of the membrane activation voltage. As in Figure 5, the transmembrane potential has been traced (B) to demonstrate the relationship between the coupling interval of the premature response and the voltage time course of the crista terminalis action potential. Initial recovery of $V_{\text{max}}$ was more rapid in the crista terminalis fiber than it was in the perinodal fibers, and, when phase 3 had reached maximum diastolic transmembrane voltage at 200 msec, $V_{\text{max}}$ was 96% of its peak value. The membrane responsiveness curve (C) demonstrates that $V_{\text{max}}$ is less time dependent in this fiber type than it is in the perinodal fiber. Similar results were obtained in all of four other experiments. In five experiments on crista terminalis fibers, $V_{\text{max}}$ was at 50% of peak value when it was initiated at a membrane activation voltage of $77.6 \pm 1.2 \text{ mv}$.  

Potassium-Induced Depolarization (Voltage Dependence).—To gain further information about the voltage dependence of phase-0 $V_{\text{max}}$ in the perinodal fibers, time was minimized as a variable by using potassium to depolarize the cells in four preparations (Figs. 7, 8). Changes in "steady-state" values of membrane activation voltage were measured at the end of 500-msec cardiac cycles. As the potassium concentration of the perfusate was changed from 3 mm to 20 mm, steady-state values of membrane activation voltage and phase-0 amplitude and $V_{\text{max}}$ were continuously monitored in atrial and perinodal fibers.
The effect of elevated potassium concentration ([K\(_o\)]) on a perinodal fiber and an ordinary atrial fiber. Action potentials recorded from the perinodal fiber (PNF) were longer in duration than those of the atrial fiber (At). The atrial fiber recording site was 8 mm lateral to the crista terminals (near end of black line in Fig. 1). The rate of rise of the perinodal fiber action potential is recorded on the bottom trace and that of the atrial fiber is shown as an inserted spike in the second trace. The crista terminals of the preparation was stimulated every 500 msec. Under control conditions (A), membrane activation voltage = 75 mv, amplitude = 90 mv, Vmax = 32 v/sec in the perinodal fiber and membrane activation voltage = 86 mv, amplitude = 116 mv, Vmax = 240 v/sec in the atrial fiber. The tissue was then exposed to an isosmotic perfusate containing 20 m/m potassium. B–E were obtained during the 15-minute period required to attain steady-state conditions (F). Although membrane activation voltage was 11 mv higher in the atrial fiber than in the perinodal fiber at [K\(_o\)] of 3 mM, membrane activation voltage was approximately the same (55 mv) in the two fiber types at [K\(_o\)] of 20 mM. At this membrane activation voltage (55 mv, [K\(_o\)] of 20 mM), phase-0 amplitude was much greater in the perinodal fiber (68 mv) than it was in the atrial fiber (12 mv). Similar results were obtained in four different experiments.

until equilibration was complete. In both atrial and perinodal fibers, at potassium concentrations between 3 mM and 20 mM, Vmax was strongly dependent on steady-state values of membrane activation voltage (Fig. 8). Membrane activation voltage and Vmax at an external potassium concentration of 3 mM were less in perinodal fibers than in atrial fibers. In the perinodal fibers at an external potassium concentration of 20 mM, responses with mean values of phase-0 Vmax of 7.9 ± 3.0 v/sec were elicited at a membrane activation voltage of 56.6 ± 1.8 mv. It is of note that premature stimuli were incapable of evoking responses during repolarization at this same value of membrane activation voltage (56.6 mv) when the external potassium concentration was 3 mM.

In atrial fibers, as potassium concentration increased, Vmax fell to values of less than 2 v/sec at a mean membrane activation voltage of 61.4 ± 1.4 mv. When the external potassium concentration was 20 mM, the mean membrane activation voltage was 55.3 ± 1.5 mv. In perinodal fibers, the membrane responsiveness determined by potassium-induced depolarization and that determined by premature stimulation were different (Figs. 4, 8). On the other hand, in atrial fibers, membrane responsiveness determined by potassium-induced depolarization and that determined by premature stimulation were very similar (Figs. 4, 8).

DETERMINANTS OF PHASE-0 AMPLITUDE IN PERINODAL FIBERS

Premature Responses (Voltage and Time Dependence).—To evaluate the determinants
The relationship between $V_{\text{max}}$ and membrane activation voltage during potassium-induced depolarization for an atrial fiber and a perinodal fiber. Membrane activation voltage and $V_{\text{max}}$ were measured continuously during the increase in external potassium concentration ([K]$_o$) from 3 to 20 mM (Fig. 7). In the atrial fiber, peak $V_{\text{max}}$ ([K]$_o$ = 3 mM) was 240 v/sec, $V_{\text{max}}$ was at 50% of its peak value when membrane activation voltage was 73 mV, and $V_{\text{max}}$ was less than 2 v/sec when membrane activation voltage was 61 mV. In the perinodal fiber, $V_{\text{max}}$ was 32 v/sec at a membrane activation voltage of 75 mV ([K]$_o$ = 3 mM). As [K]$_o$ increased, $V_{\text{max}}$ was 50% of its peak value at a membrane activation voltage of 64 mV and 5 v/sec when membrane activation voltage was 55 mV ([K]$_o$ = 20 mM). Using potassium-induced depolarization, reduction in membrane activation voltage was brought about independent of changes in transmembrane voltage during repolarization and diastole. Thus, we were able to demonstrate that $V_{\text{max}}$ of both fiber types was voltage dependent.

of phase-0 amplitude in perinodal fibers, premature responses were elicited and their membrane activation voltage, coupling interval, and phase-0 amplitude were determined (Fig. 9). In ten different preparations, perinodal fiber phase-0 amplitude was clearly demonstrated to be strongly dependent on the membrane activation voltage. Similar results were obtained in crista terminalis and atrial fibers.

**Potassium-Induced Depolarization (Voltage Dependence).**—To gain further information about the voltage dependence of phase-0 amplitude in perinodal fibers, time was minimized as a variable by using potassium to depolarize the cells in four preparations as previously described (Figs. 7, 10). Changes in steady-state values of membrane activation voltage and phase-0 amplitude were measured at the end of 500-msec cardiac cycles. In perinodal fibers at potassium concentrations between 3 and 20 mM, phase-0 amplitude was strongly dependent on steady-state values of membrane activation voltage (Fig. 10). A similar voltage dependence of phase-0 amplitude was demonstrated using premature stimulation (Fig. 9). However, in perinodal fibers, the relationships between phase-0 amplitude and membrane activation voltage determined by potassium-induced depolarization and those determined by premature stimulation differed. For any given reduction in membrane activation voltage, the percent decrease in phase-0 amplitude was less when potassium was used to depolarize the membrane. For example, at a reduced value of membrane activation voltage—65 mV—phase-0 amplitude was 81 mV during potassium-induced
The relationship of phase-0 amplitude to the coupling interval of the premature response (A) and to the membrane activation voltage (MAV) (C) in the perinodal fiber whose action potential is shown in B. In A, phase-0 amplitude of the premature response rose rapidly from 0 to 80 mV over a very brief period of 27 msec near the end of phase 3. Thereafter, the amplitude of phase 0 reached a maximum value of 93 mV 175 msec after the basic action potential upstroke when the premature response was elicited at maximum diastolic voltage. Then, diastolic depolarization began to occur and, as the coupling interval of the premature responses increased, membrane activation voltage and phase-0 amplitude decreased. In C, phase-0 amplitude was plotted as a function of membrane activation voltage, and an S-shaped relationship was obtained. Thus, recovery of peak values of phase-0 amplitude for premature beats in perinodal fibers was less time dependent than was recovery of peak values of Vmax.

Discussion

In our studies we found a discrete junctional zone lying between the sinus node and the crista terminalis. This junctional zone was solely composed of fibers with characteristic electrophysiological properties which differentiated them from sinus node and crista terminalis fibers. We named these fibers sinoatrial perinodal fibers. Further, we found fibers with similarly shaped transmembrane potentials located on the septal, caudal, and cephalic sides of the sinus node. Thus, perinodal fibers are strategically situated around the sinus node. Studies on activation of the atrium during sinus rhythm in isolated rabbit hearts (13, 15) have shown that conduction through the septal, caudal, and cephalic sides of the sinus node is sufficiently slow to minimize the role of these areas in normal atrial activation. In rabbit hearts, sinus node impulses preferentially proceed to the crista terminalis and then spread to activate the rest of the atrium (15). In so doing, the wave front of emerging sinus node impulses must pass through the zone of perinodal fibers lying between the sinus node and the crista terminalis. For this reason, we chose to study...
The relationship between phase-0 amplitude and membrane activation voltage in both atrial (left) and perinodal fibers (right) during potassium-induced depolarization. Phase-0 amplitude and membrane activation voltage were continuously monitored in both fiber types as external potassium concentration ([K]) was changed from 3 to 20 mM. In the atrial fiber, control phase-0 amplitude was 116 mV at an activation voltage of 86 mV ([K]o = 3 mM). During potassium-induced depolarization, the atrial fiber phase-0 amplitude decreased to 50% of control at a transmembrane voltage of 64 mV and to 7 mV at an activation voltage of 55 mV (steady state at [K]o = 20 mM). Despite the demonstrated voltage dependence of phase-0 amplitude in both fiber types and depolarization to identical values of membrane activation voltage ([K]o = 20 mM), the decrease in phase-0 amplitude was clearly less in the perinodal fiber than in the atrial fiber. In both fiber types, phase-0 amplitude was a function of membrane activation voltage under conditions that minimized the role of time-dependent recovery to peak values of phase-0 amplitude.

the properties of the junctional zone of perinodal fibers on the crista terminalis side of the sinus node.

Recordings of transmembrane potentials were obtained from perinodal fibers throughout this junctional zone between the sinus node and the crista terminalis. We found that the shape of the transmembrane potentials was somewhat intermediate between those recorded from sinus node and crista terminalis fibers. In addition, mean values of membrane activation voltage, maximum diastolic voltage, phase-0 amplitude and Vmax, and action potential duration (Table 1) of perinodal fibers were intermediate between those values from sinus node and crista terminalis fibers. However, despite the presence of diastolic depolarization, under the conditions of this study, which included rates as low as 80/min, these perinodal fibers did not discharge spontaneously and were, therefore, different from sinus node fibers. Other electrophysiological properties mentioned below differentiated them from crista terminalis fibers.

To determine how the junctional zone of perinodal fibers might modulate sinus node impulses proceeding to the crista terminalis, we recorded transmembrane potentials from different perinodal fibers lying along an imaginary line that was perpendicular to the crista terminalis. We felt that such a sampling of fibers along this line would be roughly representative of the sequence of fibers that sinus node impulses would depolarize on their way to the crista terminalis (15). As the microelectrode moved from the sinus node side to the crista terminalis side of the zone of perinodal fibers, there was a progressive
Block of premature atrial depolarizations in the zone of perinodal fibers. Transmembrane potentials were recorded from a perinodal fiber (PNF) and a fiber in the crista terminalis (CT) separated by a distance of 5 mm. The basic rhythm was spontaneous, but following every eighth spontaneous cycle a premature response was elicited by stimulating the crista terminalis (vertical arrows point to test stimuli). During spontaneous rhythm, the conduction time between the perinodal and crista terminalis fiber recording sites was 10 msec. In A, the test stimulus was introduced and the coupling interval of the premature crista terminalis response was 67 msec. Block occurred between the two recording sites. In B, the coupling interval was increased to 72 msec, and the impulse conducted to the perinodal fiber recording site with great delay—42 msec. In C, the coupling interval was increased to 102 msec, and the conduction time decreased to 10 msec—the minimum value seen in diastole. Clearly, the zone of perinodal fibers can serve as a site of block for premature atrial depolarizations and prevent their entry into the sinus node. In B and C, premature responses in the perinodal fiber clearly show two components during depolarization (transition indicated by the angled arrows).

increase in the values of phase-0 amplitude and Vmax. This implied that the safety factor (9) and the rate of transmission of impulses increased as impulses moved through the zone of perinodal fibers from the sinus node to the crista terminalis. Furthermore, the opposite occurred as the impulses moved from the crista terminalis to the sinus node. These results are supported by previously reported isochronal maps documenting the sequence of activation of emerging sinus node impulses (15) and by measurements of conduction times in rabbit atrium (13). A comparison of this junctional zone of perinodal fibers to the AV junctional zone of fibers suggests a partial, but useful, analogy. Impulses propagating from the atrium to the ventricle undergo an initial deceleration followed by an acceleration as they pass through the AV junction (9). On the other hand, automatic impulses leaving the sinus node propagate with increasing velocity through the zone of perinodal fibers to the crista terminalis.

Since the zone of perinodal fibers is located between the sinus node and the crista terminalis, one would expect electrotonic interactions (9) to occur between these three fiber types in accordance with the “space constant.” Changing the rhythm of the preparation from spontaneous to driven (Table 1) reversed the sequence of depolarization in the zone of perinodal fibers and permitted a limited evaluation of electrotonic influences on the perinodal fiber transmembrane potential.
Electrotonic influences were more marked on the sinus node side than on the crista terminalis side of the zone of perinodal fibers.

To further determine the physiological role of the zone of perinodal fibers, we examined the effect of premature responses on phase-0 V_max and amplitude of transmembrane potentials recorded from perinodal fibers. Such an evaluation permits some extrapolation of these findings to the in vivo situation where circumstance often calls on these cells to deal with premature beats. Premature stimulation has been used to determine the membrane responsiveness of various cardiac fibers (18, 19). Ordinarily, where the time constant for reactivation (21) is sufficiently small relative to the rate of change of voltage during repolarization, the membrane responsiveness relationship is similar to that found in Purkinje fibers (18, 19, 21). However, in perinodal fibers, recovery of V_max of premature beats to peak value was less strongly dependent on the instantaneous membrane activation voltage than it was in the atrial fibers. When V_max was plotted as a function of the coupling interval, it became apparent that recovery of V_max of premature beats to peak value was also time dependent. This time dependence was so pronounced that, when repolarization was 90% complete, V_max had still not reached 50% of peak value. In addition, V_max of premature responses continued to increase in diastole despite the decrease in membrane activation voltage due to spontaneous diastolic depolarization. These findings contrasted with those reported for Purkinje fibers (21) in which, during diastolic depolarization, V_max of premature responses decreased.

Membrane responsiveness was determined for crista terminalis fibers to provide further evidence of the differences between perinodal fibers and crista terminalis fibers. The distribution of the data points in the membrane responsiveness relationships indicated that the degree of time dependence in the recovery of V_max of premature responses to peak value was much less marked in crista terminalis fibers than in perinodal fibers. The full recovery time (9) of crista terminalis fibers should, therefore, be shorter than that of perinodal fibers.

To determine the degree of voltage dependence of V_max in perinodal fibers, time had to be minimized as a variable. Although the voltage-clamp technique has been traditionally used to this end, the architecture of this zone of fibers precluded the successful application of this technique to the study of these fibers. For this reason, we attempted to alter membrane activation voltage in a steady-state fashion by using potassium to depolarize the membrane while the preparation was stimulated at a constant cycle length. Use of this procedure depended on the reasonable assumption that in atrial and perinodal fibers the relationship between V_max and steady-state values of membrane activation voltage was not significantly altered when membrane activation voltage was altered by changing external potassium concentration (21). Throughout the period of potassium-induced depolarization of the membranes, voltage dependence of V_max was observed in both perinodal fibers and atrial fibers.

In both perinodal fibers and atrial fibers, membrane responsiveness was determined by premature stimulation and by potassium-induced depolarization. In atrial fibers, the membrane responsiveness relationships determined by these two techniques were virtually identical. This similarity in the responsiveness relationships would be predicted for fibers that have a short time constant for reactivation (21). In addition, these results would seem to validate the use of potassium-induced depolarization for the determination of membrane responsiveness. In perinodal fibers, the membrane responsiveness relationships determined by the two techniques were different. This difference in the responsiveness relationships shown by the comparison of the two techniques clearly demonstrated that the time dependence of the recovery of V_max of premature responses to peak value was greater in perinodal fibers than in atrial fibers. Full recovery time also persisted much longer than action potential repolarization in frog atrial
fibers (24). Haas et al. (24) postulated that this might be explained by assuming that the time constant for reactivation was much longer than that for inactivation (21, 23).

In rabbit atrial and nodal cells as well as in other cardiac fibers of other animal species, it is hypothesized that the action potentials are composed of two distinct components—a rapid component and a slow component—of depolarization (25-28). The slow depolarizations have been recorded in the presence of increased external potassium concentration and catecholamines in the perfusate (25, 26, 28). In our experiments on potassium-induced depolarization of the membrane, (external potassium concentration of 20 mM), action potential amplitude was much greater in perinodal fibers than it was in atrial fibers. Regardless of the mechanism underlying this difference, the perinodal fiber action potential upstroke showed a greater resistance to elevated potassium compared with ordinary atrial fibers (Fig. 7). These observations combined with similar observations from experiments on specialized atrial fibers (29) and sinus node fibers (30) could explain the findings of Vassalle and Hoffman (31) who documented the occurrence of electrocardiographic sinoatrial block with sinoventricular conduction in the presence of hyperkalemia.

Because of its strategic location, the zone of perinodal fibers serves as the site of normal conduction delay for impulses entering and leaving the sinus node. Thus, premature atrial responses initiated late in diastole should collide with the emerging sinus node impulse and not gain entrance into the sinus node. The pause following these beats should be fully compensatory (17). Premature atrial responses initiated in the middle of diastole should gain access to the sinus node after traversing the zone of perinodal fibers. The duration of the return cycle would be determined by the time required for the atrial premature response to traverse the zone of perinodal fibers to the sinus node, the return sinus cycle, and the conduction time back to the atrium. Since the refractory period in perinodal fibers is longer than that in atrial fibers, one would predict that premature atrial depolarizations would cross the zone of perinodal fibers with increasing delay as they were initiated earlier in diastole. Han et al. (32) and Bonke et al. (17), using the isolated rabbit atrial preparation, showed that as the coupling interval of a premature atrial depolarization was shortened the time required for the impulse to conduct to the sinus node increased. We found that the zone of perinodal fibers could serve as the site of conduction delay and block for premature atrial depolarizations, delaying or preventing their access to the sinus node (Fig. 11). Premature atrial depolarizations occurring so early in the diastolic period that they block in the zone of perinodal fibers would correspond to interpolated atrial premature depolarizations and would explain the findings of Han et al. (32) and Bonke et al. (33), who also found that early premature atrial depolarizations failed to discharge the sinus node. Similar results have been obtained in patient studies by different investigators (5-8) who observed early atrial premature depolarizations that were interpolated and concluded that these early atrial premature depolarizations did not gain access to the sinus node.

Impulses generated within the sinus node are unable to propagate to the atrium when conduction in the zone of perinodal fibers becomes depressed due to injury (16) or drugs, e.g., digitalis (18). Thus, block between the sinus node and the crista terminalis has been demonstrated in rabbit hearts. In addition, potassium can produce block between the crista terminalis and the atrial appendage in the rabbit (34). In humans, focal pathologic changes demonstrated to occur at the sinoatrial junction (11) could lead to sinoatrial block between the sinus node and the crista terminalis. In addition, digitalis excess is thought to be responsible for 22% of the cases of sinoatrial block (10). Thus, it would seem that, clinically, block between the sinus node and the crista terminalis might be the mechanism for sinoatrial block in the sick sinus syndrome (35) or in digitalis excess but that block between the crista terminalis and the atrial appendage might only be of
ELECTROPHYSIOLOGY OF THE PERINODAL FIBERS

importance under conditions such as hyperkalemia.

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