EXPERIMENTAL STUDIES

Role of the Perinodal Region in Atrioventricular Nodal Reentry: Evidence in an Isolated Rabbit Heart Preparation

HIROYUKI IINUMA, MD, LEONARD S. DREIFUS, MD, FACC, TODOR MAZGALEV, MD, ROHN PRICE, ERIC L. MICHELSON, MD, FACC

Philadelphia, Pennsylvania

To evaluate the role of perinodal tissue in the genesis of atrioventricular (AV) nodal reentry, echo beats were induced by programmed stimulation in nine superfused rabbit atrial-atrioventricular nodal preparations. In these preparations, extracellular bipolar electrograms were recorded simultaneously from the crista terminalis, interatrial septum and His bundle, along with microelectrode action potentials from cells in the AV node and perinodal tissues. Three patterns of AV reentry were observed: 1) In four preparations, with the introduction of basic and premature stimuli at the crista terminalis input region to the AV node, block occurred within the AV node, anterograde conduction continued along the perinodal fibers and entered the AV node by way of the interatrial septal input region. Reentry occurred via retrograde nodal conduction. 2) In two other preparations, with the introduction of basic and premature stimuli at the interatrial septal input region, conduction blocked in retrograde manner within the AV node, and retrograde conduction occurred via the perinodal tissues. It subsequently entered the node by way of the crista terminalis input region, and continued in an anterograde direction through the AV node, with reentry via the interatrial septal region and perinodal fibers. 3) In three preparations, basic and premature stimuli at the crista terminalis region blocked in the perinodal tissue near the crista terminalis, but conducted slowly in an anterograde fashion through the AV node. To complete the reentry circuit, the stimuli exited at the interatrial septal region and conducted in retrograde fashion via the perinodal tissues to reenter the node at the crista terminalis. Moreover, in four other preparations, surgical interruption of the perinodal tissue prevented reinitiation of reentrant phenomena. These studies demonstrate the critical role of the perinodal tissues as a necessary link in the AV nodal reentrant mechanism in this preparation.

Atrioventricular (AV) nodal reentrant rhythms are a common cause of supraventricular tachycardia in human beings. An abundance of information is now available demonstrating the phenomenon of dual AV nodal pathways in patients with paroxysmal supraventricular tachycardia (1-6), although it has been further recognized that reentrant arrhythmias may also incorporate either manifest or concealed bypass tracts as part of the circuit (7). Early evidence for a dual AV transmission system was suggested by Mendez and Moe (8). Janse et al. (9) presented experimental evidence that reentrant circuits could occur within the AV node, whereas Watanabe and Dreifus (10) demonstrated evidence of concealed, but never manifest reentry within the node. However, precise localization of the reentrant pathways responsible for AV nodal reentry utilizing dual pathways has not been clearly defined. Recently, Mazgalev et al. (11) used microelectrode mapping techniques in isolated rabbit heart preparations to further characterize AV nodal reentry. In this preparation, two major input regions to the node, the crista terminalis and the interatrial septum, are readily identified. Using the technique of programmed stimulation, Mazgalev et al. (11) demonstrated that unidirectional block of premature stimuli near either the crista terminalis or interatrial septal input regions to the AV node could predispose to reentrant phenomena, and suggested the possible importance of dual AV nodal input regions and the perinodal tissue in this mechanism. The present study was designed to further elucidate this hypothesis using multiple simultaneous mi-
Microelectrode and extracellular recordings and programmed pacing methods in isolated rabbit heart preparations.

Methods

We studied in vitro preparations from 13 young adult New Zealand white rabbit hearts (weight 2.5 to 3.5 kg). Figure 1 illustrates the anatomic landmarks utilized in these experiments. Figures 2 to 4 represent the electrophysiologic responses observed consistently in each of the nine preparations in which sequential activation was determined by multiple microelectrode impalements. In addition, the effects of an incision in the perinodal tissues on the initiation of atrioventricular (AV) nodal reentry was studied in four other preparations in which only extracellular electrograms were recorded to monitor the reentrant movement.

Experimental preparation. Each rabbit was anesthetized with sodium pentobarbital (30 mg/kg body weight intravenously) and heparinized (1,000 units, intravenously). The chest was opened by a midsternal incision and the heart was rapidly excised. After quick removal of apical tissue to ensure more thorough superfusion of the ventricular cavities, the heart was placed in a room temperature modified Tyrode's solution of the following composition (concentrations are in units of mmol/liter): sodium chloride, 128.2; potassium chloride, 4.7; calcium chloride, 1.3; magnesium chloride, 1.05; monosodium bicarbonate, 20.1; monosodium phosphate, 4.7 and glucose, 11.1. The solution was constantly gassed with a mixture of 95% oxygen and 5% carbon dioxide.

After the tissue was fixed to a paraffin block, a probe was passed through the apical end of the right ventricle, through the tricuspid valve, into the right atrium and out through the superior vena cava. With the probe used as a guide, the right side of the heart was opened by an incision through the right ventricle and right atrial anterior margin to the interatrial septum to expose the endocardial surface. After resection of the right ventricle, left ventricle and left atrium, the preparation consisting of the right atrial appendage, sinus node and AV node was fixed to the bottom of a tissue bath (volume 45 ml) and superfused with a constantly oxygenated solution at a rate of 10 ml/min. A temperature of 37 ± 1°C was maintained with a Haake circulating water bath and a thermostat.

Electrical recordings. Surface electrograms were obtained from Teflon-insulated, silver bipolar electrodes placed at the crista terminalis, the interatrial septum and the bundle of His. The recording electrodes were always placed most proximal at each input region (Fig. 1 to 4). In addition, bipolar stimulating electrodes were also placed at the crista terminalis and interatrial septum. Tissue response was amplified using either a differential amplifier (Bloom Associates) or a Hewlett-Packard bioelectric amplifier (model 8811A) before being displayed on a Tektronix memory oscilloscope (model DI5).

Standard glass microelectrodes filled with 3.0 M potassium chloride were used to monitor cellular responses within the preparations. Tissue and cellular responses were recorded on a Polaroid camera after being displayed on the oscilloscopic screen.

Transmembrane action potentials were recorded with two or three microelectrodes simultaneously in each preparation. These microelectrodes were sequentially moved to the various areas of the AV node, conventionally designated the AN, N and NH regions to map the course of impulse transmission through the AV node. Activation sequences were mapped in this manner for both stimulated and reproducibly inducible reentrant beats in each preparation. Programmed pacing was used to induce arrhythmias as described later. Activation sequences were determined using the stimulus artifact for time reference. Microelectrodes were mounted on micromanipulators (Stoelting-Prior) for precise impalements into the various regions of the AV node. Several coordinates drawn from the superior-anterior and right-left directions on the AV node were used to localize the area of recording in the impaled cells. The terminology of Paes de Carvalho (12) as applied to the zones of the AV node was used throughout the study, but the location of the
N region was more closely identified using the descriptive criteria of Janse et al. (13).

Transmembrane action potential contour and time of inscription were used to identify location (for example AN, N or NH) and timing of activation of individual impaled fibers. The contour and timing of the action potentials were initially identified during spontaneous atrial rhythm as well as after premature stimulation from the right atrial appendage. Recordings from AN fibers displayed a rapid phase 0 with a distinctive narrow apex. Recordings from N fibers were

Figure 2. Intranodal block with anterograde perinodal conduction and retrograde atrioventricular nodal reentry. This and subsequent figures are arranged in the following manner and exceptions are noted accordingly: CT = stimulus artifacts and electrograms recorded from the area of the crista terminalis (CT), 1–8 = action potentials recorded from various fibers within the atrioventricular node and perinodal fibers with similar numerical designations in the schematic (B) with stars (*) designating the locations of microelectrode recordings; His = electrograms recorded from the region of the His bundle (His); IAS = stimulus artifacts and pacing electrograms recorded from the interatrial septum (IAS). Action potential amplitude units are in millivolts. Time units are in ms. Columns to the right of Figures 2A, 3A, 3C and 4A with headings SJ, Sz and (Ae) indicate the activation times measured from the respective stimulus artifact to the inscription of the local action potential or electrogram for each of the recording sites. In Figures 2B, 3B, 3D and 4B, the upper left schematic = basic stimulus (S1), lower left = premature stimulus (S2) and right = reentry beat (Ae). Vertical bars = 50 millivolts; A1 = atrial electrogram in response to S1; A2 = atrial electrogram in response to S2; Ae = atrial echo electrogram; dashed lines = activation sequence of premature stimulus; dotted lines = activation sequence of reentrant beat; S1 = stimulus artifact of last basic drive; S2 = stimulus artifact of premature stimulus; solid lines = activation sequence of basic drive.
typical of the slow response type and showed a slowly rising phase 0 with absence of phase 1 and were of low amplitude. The NH cells were characterized by a rapid phase 0 and definite plateau and were taller in amplitude. Notably, the usual characteristic configurations of the various cell types seen within the AV node during the spontaneous rhythm became distorted during premature atrial stimulation and reentry. This finding was especially true in the AN and N cells as nonuniform multicompontent action potentials were frequently observed. Perinodal fibers were identified by their location lateral and inferior to the border of the coronary sinus and atrial wall and by their more rapid phase 0 and more distinct phase 1 (Fig. 1).

Each preparation was paced at a basic cycle length of 350 to 450 ms with a rectangular pulse ($S_1$) (1 ms in duration and 2 times diastolic threshold) applied through bipolar electrodes at the crista terminalis or interatrial septum using a programmable digital stimulator (Bloom Associates). Premature stimuli ($S_2$) were applied after every eight basic drive beats with a decreasing $S_1S_2$ interval until reentrant beats or tachycardia was induced. From these recordings, we studied the activation sequence within the perinodal region and

**Figure 3.** A. Anterograde intranodal block with perinodal conduction and retrograde atrioventricular nodal reentry after stimulation from the crista terminalis. B. Schematic diagram. C. Intranodal block with retrograde perinodal conduction and anterograde atrioventricular nodal reentry after stimulation from the interatrial septum in the same preparation. D. Schematic diagram.

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AV nodal tissues during basic, premature and reentrant beats, and attempted to identify the activation sequence of each beat. The actual sites of microelectrode impalement are identified by stars in Figures 2 to 4. In each instance, three schematic diagrams are displayed to identify the sequential activation of the basic, premature and echo action potentials and electrograms. Solid lines indicate the activation sequence of a basic beat ($S_1$). Dashed lines indicate the activation sequence of a premature beat ($S_2$). Dotted lines indicate the activation sequence of an echo beat. The terms "input area" and "region" are used in these studies to identify the earliest site of activation as recorded by microelectrodes in the area of the crista terminalis or interatrial septum. It should be noted that conduction into and out of the AV node was by way of these two major input regions.

Results

The sequence of activation within atrioventricular (AV) nodal and perinodal tissues in representative preparations from which optimal records were obtained is diagrammed in Figures 2 to 4, which are arranged similarly. The activation sequences are shown as recorded for the last basic drive beat ($S_1$), the premature beat ($S_2$) and the resultant reentrant beats. Only the activation sequence of the first reentrant beat is shown even when sustained reentrant tachycardia was established. From the analysis of these records, three patterns of reentry were demonstrated, two involving intranodal conduction block and one dependent on perinodal conduction block.

Intranodal conduction block with reentry: crista terminalis stimulation. Examples of this pattern of reentry are shown in Figures 2 and 3. The last beat of the basic drive ($S_1$) from the crista terminalis is shown in Figure 2B (schematic) by solid lines. After introduction of the premature beat ($S_2$) at the crista terminalis, conduction (Fig. 2B, dashed line) decreased near location 3 (N region), which...
responded with a local response (Fig. 2A, straight arrow) and anterograde block. However, simultaneously, anterograde excitation propagated successfully via the perinodal fibers with activation of points 8 through 6, located in the perinodal region, followed by activation at the interatrial septal region and activation of cell 5. Activation by way of the interatrial septal input region may have also been responsible for the S2 responses of cell 4 (NH) and His bundle (H2) activation. In addition, further retrograde propagation also produced the third and taller action potential located at point 3 (Fig. 2A, curved arrow), which was followed by activation and reexcitation of cells at points 2 and 1 and the crista terminalis (Fig. 2A, Ae) and subsequent activation in an anterograde fashion by way of perinodal fibers and interatrial septum (Fig. 2B). The initial graded response at point 5 (Fig. 2A, dotted arrow) could be ascribed to an abortive reentrant excitation from cells in the vicinity of point 3. The delayed inscription of action potentials in response to S2 at points 6, 7 and 8 in comparison with that from the S1 stimulus may have resulted from divergent or branched pathways from the principal excitation wave front. In subsequent figures, representation of the basic beat (solid line), premature beat (dashed line) and reentry-sequential depolarizations (dotted line) are utilized unless otherwise indicated.

Figure 3 shows another preparation in which basic and premature stimulation were applied at the crista terminalis, resulting in intranodal block and anterograde conduction via perinodal fibers with activation of the node by way of the interatrial septum. Subsequent retrograde AV nodal conduction exiting by way of the crista terminalis completed the reentrant loop. In Figure 3A, the action potential at point 2 after the extrastimulus S2 (arrow) was very small compared with the response to the last basic stimulus and conduction to point 3 did not occur. Hence, the His bundle response to S2 (H2) may have resulted from excitation through perinodal tissues and the AN region close to the interatrial septal input (points 6 through 3). The later appearance of the action potential at point 6 in response to S1 resulted from the organization of the wave front in this instance. S2 produced a delay in depolarization of fibers 5 and 6 in comparison with the interatrial septum as A2 and fiber 6 were simultaneously inscribed, and depolarization of fiber 5 followed fiber 6 in this instance. The third action potential recorded at point 2 followed the second depolarization at point 3, suggesting that a reentrant beat had propagated to the crista terminalis in a retrograde fashion through AV nodal tissues (for example, point 1) and then conducted via perinodal tissue (points 6 through 4) and the interatrial septum to point 3 in the node (Fig. 3A and B). This circus movement was terminated by the local nonpropagated response at point 3. This pattern of reentry was observed in four of the nine preparations with inducible reentry.

Intranodal block with reentry: interatrial septal stimulation. In Figure 3C and D, the preparation was the same as in Figure 3A, but the stimulation site was moved to the interatrial septum with the microelectrodes impaled in cells close, but not identical to those in Figure 3A. In this case, the AN cell (point 3) close to the stimulation electrode at the interatrial septum responded with a local response to S2 (arrow), suggesting that intranodal conduction block occurred close to this cell, while fibers 6,5,4 and the crista terminalis (A3) were depolarized, followed by anterograde depolarization of nodal fibers 1 and 2. Subsequent His bundle depolarization (H2) was delayed and apparently occurred by means of anterograde intranodal conduction. Excitation of AN cells near the crista terminalis produced the third action potential at point 3, which in turn induced the third sequence of depolarizations of the perinodal tissues (points 4 through 6), the interatrial septum (Ae), crista terminalis (Ae) and points 1 and 2 (Fig. 3D). This pattern of reentry with intranodal block and reentry via retrograde perinodal conduction was observed in two of nine preparations. It should also be noted that in Figure 3A and C, the H1H2 interval was quite different (230 versus 278 ms) in response to crista terminalis and interatrial septal stimulation, although the A1A2 coupling interval was 140 ms in both instances.

Perinodal conduction block with reentry: crista terminalis stimulation. An example of reentry resulting from perinodal block is shown in Figure 4, which is organized similarly to Figures 2 and 3. In Figure 4, both the basic (S1) and the premature extrastimulus (S2) were introduced at the crista terminalis region. However, a response to S2 was observed only at the crista terminalis recording site (Ae) and microelectrode sites 1 and 2. There was failure of direct propagation via the perinodal fibers to sites 5 and 4 and the interatrial septum. In response to S2, activation of the AN region (point 2, asterisk) was followed by delayed activation at site 3 (dotted line, arrow), with subsequent activation of the interatrial septum (Ae), points 4 and 5, the crista terminalis (Ae) and subsequently fibers 1 and 2. Because the activation time from point 3 to the His bundle was unusually short for this sequence compared with the basic drive (3-H), the H2 response most likely resulted from anterograde conduction from the crista terminalis input region. Hence, excitation of the N region (not recorded) presumably depolarized previously unexcited AN cells close to the septal (IAS) input (point 3), and was followed by activation of sites 4, 5, 1 and 2 in that sequence. That is, the second action potentials recorded at points 3, 4 and 5 were most likely not responses to S2, based on the short AH interval and altered activation sequence, but rather were part of a reentrant pathway that was accompanied by the excitation of the crista terminalis and points 1 and 2 (Fig. 4B, dotted line). In this instance, the reentry was terminated as anterograde conduction ceased after the cells at point 2 were depolarized. This type of reentry was observed in three of the nine preparations demonstrating reentry, and was only seen with crista terminalis stimulation.
Interruption of perinodal conduction. In four other preparations, an additional intervention was done to further define the role of the perinodal region in AV nodal reentry. In each case, reentry was reproducibly present. In each preparation, a fine sharp scalpel was used to interrupt the perinodal pathway between the crista terminalis and interatrial septal regions. In each instance after surgical incision of the perinodal tissue (Fig. 5, dotted line), anterograde conduction continued to the His bundle along the usual AV nodal pathway, although the crista terminalis-His interval during sinus rhythm increased from 70 to 95 ms and the S2H2 interval (S1S2 = 140 ms) increased from 222 to 251 ms, but reentrant tachycardia could not be reinitiated by the extrastimulus method (Fig. 5).

Figure 4. Perinodal block with anterograde atrioventricular nodal conduction and perinodal reentry after premature stimulation at the crista terminalis. A, The basic (S1) and the premature stimulus were introduced at the crista terminalis. After S2, conduction occurred in an anterograde direction through the atrioventricular node and exited by way of the interatrial septum. Reentry through the crista terminalis was by way of the perinodal fibers. B, Schematic diagram.
Critical role of perinodal tissue in AV nodal reentry. Earlier studies by Watanabe and Dreifus (10) demonstrated concealed reentry within small portions of isolated rabbit atrioventricular (AV) nodal tissue, but manifest reentry confined exclusively to the AV node was never observed. Hence, it became apparent that the perinodal tissue could play a critical role and offer an "extranodal" pathway and a necessary link for a manifest reentrant mechanism. In the present study, evidence is presented from an isolated rabbit heart preparation indicating the critical role of the perinodal tissue in various patterns of AV nodal reentry. In this preparation, reentry confined to the AV node was never observed. Three patterns of reentry were observed: 1) after intranodal block when basic and premature stimuli were introduced at the crista terminalis with reentry by means of retrograde nodal conduction; 2) after intranodal block when basic and premature stimuli were introduced at the interatrial septum with reentry by means of retrograde perinodal conduction; and 3) anterograde perinodal block associated with delayed anterograde AV nodal conduction exiting at the interatrial septum after stimulation at the crista terminalis, and reentry by way of retrograde perinodal conduction with the impulse reentering the AV node at the crista terminalis to complete the reentrant loop.

This study also emphasizes the critical role of the two predominant input regions to the node, the crista terminalis and interatrial septum in permitting reentrant phenomena (11). In this isolated rabbit heart preparation, the existence of two major atrial input regions to the AV node was critical to reentrant excitation and provided conduits for reciprocating rhythms by way of the node and perinodal regions (11). Moreover, the site of premature stimulation (crista terminalis versus interatrial septum) also influenced the pattern of reentry. Finally, surgically interrupting the perinodal tissues without penetrating or damaging the node was consistently sufficient in abolishing reentry in preparations previously susceptible to induction of reentrant beats. These phenomena are illustrated in the four figures chosen to represent the 13 experimental preparations.

Furthermore, it can be inferred that the concept of dual AV nodal pathways may in some instances incorporate a perinodal link. Although not as dramatic as the extranodal pathways associated with the Wolff-Parkinson-White syndrome or concealed bypass tracts, perinodal fibers may exhibit differences from intranodal fibers with respect to both conduction and refractory periods sufficient to cause block in one pathway while conduction could occur in the other pathway, providing the necessary requirements for a reentrant movement. In another recent series of experiments (14) using the same preparation, discontinuous curves typical of dual AV nodal pathways could be constructed when the perinodal region was one of these pathways.

Clinical implications. Potential limitations of these studies should be considered in the interpretation of the results and their application to understanding AV nodal reentry in human beings. These findings were observed in the isolated AV node of rabbits and may not be analogous to reentrant AV tachycardia in human subjects. In addition, although multiple, simultaneous and sequential electrograms and microelectrode recordings were utilized in these studies, more sophisticated mapping with brush and three-dimensional mi-
Microelectrode techniques may be required to provide a more detailed confirmation of presumed reentrant mechanisms. Despite these limitations, several clinical implications are suggested. Atrioventricular reentry has been generally accepted as a mechanism of supraventricular tachycardia, although the precise location of dual nodal pathways has never been demonstrated. Notably, both our previous (11) and present studies could not identify manifest reentry occurring within the confines of the AV nodal tissues themselves, but always required an extranodal link to complete the reentrant circuit. Clinically, of course, it is not possible to localize sites of block and potential reentry with the precision afforded by this in vitro preparation. In addition, it is not always possible to distinguish retrograde nodal conduction from conduction by way of a paraseptal bypass tract or other perinodal tissues (15). However, it has been appreciated clinically that the site of stimulation can be critical to the initiation of reentrant arrhythmias incorporating the AV node (13). Finally, the effects of prototypical drugs, such as verapamil, propranolol and digitalis, in this preparation will be of considerable interest. Such studies should shed further light on the relevance of these in vitro findings to the understanding of the mechanisms of supraventricular tachycardia incorporating the AV node and possibly perinodal fibers in reentrant circuits.

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References


