Effects of the site and timing of atrioventricular nodal input on atrioventricular conduction in the isolated perfused rabbit heart

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ABSTRACT Programmed stimulation was used to study patterns of atrioventricular nodal propagation in an isolated rabbit heart preparation. Stimulation was done at the two major atrioventricular nodal input regions, the crista terminalis and interatrial septum, by use of various sequencing protocols. The influence of stimulation input interactions on atrioventricular nodal propagation was then evaluated with the use of simultaneous extracellular and intracellular recordings from the various regions of the atrioventricular node and His bundle. Activation of the atrioventricular node occurred predominantly via the stimulated input site, although perinodal conduction to the opposite input also occurred and could modify the atrioventricular conduction pattern. Engagement patterns of both AN and N fibers were dependent on the sequence of stimulation at the major input sites, and similar conduction times to the His bundle were often associated with different local activation times, depending on the site of premature stimulation. Conversely, if premature stimulation was timed to produce constant local activation times but the input site varied, then the time to His bundle activation could also vary. Atrioventricular nodal functional and effective refractory periods and conduction patterns were dependent on both the timing and pattern of engagement at the principal inputs to the atrioventricular node as well as the subsequent organization of activation of the various regions of the atrioventricular node. Furthermore, depending on the sequence of stimulation at the crista terminalis and interatrial septum, activation patterns were either organized or inhomogeneous in the AN and N regions of the atrioventricular node, consequently influencing the H-H interval, and in some instances resulting in conduction block to the His bundle. It is concluded that the relative timing of activation at the principal input regions of the atrioventricular node is critical to patterns of atrioventricular nodal propagation and subsequent conduction to the His bundle. Our results emphasize the complexity of atrioventricular nodal propagation during premature stimulation.


JANSE$^1$ demonstrated that normal antegrade atrioventricular nodal activation was dependent on the direction of the atrial wave front. Ferrier and Dresel$^2$ suggested that atrioventricular conduction was closely related to distal vs proximal sites of atrial stimulation. Later, Janse et al.$^3$ demonstrated the presence of dual inputs to the atrioventricular node: a posterior input via the crista terminalis entering the node between the coronary sinus ostium and internodal septum, and an anterior interatrial septal input entering the node as a broad wave front anterior to the ostium of the coronary sinus. These investigators stressed the fact that the activation sequence is a complex three-dimensional event. Furthermore, they demonstrated that the terminal portion of the atrioventricular node was activated in a rapid and synchronous manner. It would appear that the anterior input does not short-circuit the central part of the node and cannot excite the distal node directly. These authors felt that the anterior input curved in a posterior direction and merged with the posterior input before jointly activating the central nodal area. Zipes et al.$^4$ further demonstrated the summation of inputs in the rabbit atrioventricular node by dividing the atrium and floor of the coronary sinus, thereby establishing
two noncontiguous anatomically separate atrial inputs to the atrioventricular node. They also suggested that the rise time of action potential of nodal cells was voltage dependent, further underscoring the importance of summation in the atrioventricular node. Konishi and Matsuyma have also related changes in atrioventricular nodal input patterns to the success of atrioventricular nodal activation and propagation. More recent work by several authors has brought into sharp focus additional phenomena, including the concepts of inhomogeneous conduction, blind alleys and/or “dead-end” pathways, and collision of wave fronts, all of which may further influence observed patterns of conduction and refractory periods.

Recent studies in our laboratory have shown further that, as a result of these dual regions of atrioventricular nodal input, there exists the possibility of reentrant phenomena. For example, using programmed stimulation we induced unidirectional block in close proximity to one of the inputs, with subsequent reentry movement as the wave front from the other input retrogradely invaded the previously blocked area to produce a circus-movement tachycardia. It was also noted that the perinodal fibers of the atria surrounding the atrioventricular node could participate in the mechanisms of atrioventricular junctional reentry.

The present study was designed to further define the physiologic consequences of single and multiple activation inputs to the atrioventricular node in an isolated, perfused atrioventricular nodal preparation. In addition we attempted to determine the effects of the stimulation site and input interactions from the two major regions of atrial-atrioventricular nodal input, the crista terminalis and interatrial septum, on atrioventricular nodal refractory periods and conduction during programmed stimulation. This was done by the application of programmed stimulation at sites close to the crista terminalis and interatrial septal input regions to the atrioventricular node, singly and in combination, in conjunction with multiple simultaneous intracellular and extracellular recordings. These studies were done with the use of an isolated rabbit heart preparation, which is well suited for detailed electrophysiologic investigation of the atrioventricular node.

Methods

Preparations from the hearts of 24 young adult New Zealand white rabbits (weight 2 to 3 kg) were studied in a tissue bath and experiments were successfully completed in 13 preparations. Figures 1 to 9 illustrate representative electrophysiologic responses resulting from the various interventions.

The preparation. Each rabbit was anesthetized with sodium pentobarbital (30 mg/kg iv) and heparinized (1000 units iv). The chest was opened by a midsternal incision, and the heart was rapidly excised. After quickly removing apical tissue to ensure superfusion of the ventricular cavities, each heart was placed in a room temperature, modified Tyrode’s solution of the following composition (in units of mM/liter): NaCl 128.2, KCl 4.7, CaCl 1.3, MgCl 1.05, NaHCO3 20.1, NaHPO4 4.7, and glucose 11.1. The solution was constantly gassed with a mixture of 95% O2/5% CO2.

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 as a guide, the right heart was opened by an incision through the right ventricle and right atrium. After resection of the right ventricle, left ventricle, and left atrium, the preparation consisting of the right atrial appendage, sinus node, atrioventricular node, and proximal region of the His bundle 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 by means of a Haake circulating water bath with thermostat.

Electrical recordings. Surface electrogroms were obtained with Teflon-insulated silver bipolar electrodes placed at the crista terminalis, the interatrial septum, and the bundle of His. Tissue response was amplified with either a differential amplifier (Bloom Associates, Reading, PA) or a Hewlett-Packard bioelectric amplifier (model no. 8811A) before being displayed on a Tektronix storage oscilloscope (model no. D15).

Standard glass microelectrodes (1.0 mm outside diameter and 50 to 100 MΩ resistance) were filled with 3M KCl and K-PET were used to monitor cellular responses within the preparations. Tissue and cellular responses were recorded on a Grass Kymographic or a Polaroid camera after being displayed on the oscilloscopic screen.

Transmembrane potentials were recorded simultaneously from two or three microelectrodes in each preparation. One microelectrode was used as an intracellular reference and the others were moved to the various areas of the AN, N, and NH regions to map the pattern of activation through the atrioventricular node. Microelectrodes were mounted on micromanipulators (Sterling-Prior) for precise localization of impalements into the various regions of the atrioventricular node. Several coordinates of the impaled cells were noted to localize the area of recording. The terminology of Paes de Carvalho and Almeida, as applied to the zones of the atrioventricular node (e.g., AN, N, or NH), was used throughout the study. Transmembrane action potential contour and time of inscription were used to identify location and timing of activation of individual impaled fibers. The contour and timing of the action potentials were initially identified during spontaneous atrial rhythm and again after rapid random 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 typical of the so-called slow-response type in that they 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, a definite plateau, and were of higher amplitude. Notably, the characteristic configurations of the various cell types seen within the atrioventricular node during the spontaneous rhythm became distorted during premature atrial stimulation and reentry. This finding was especially true in the AN and N cells where nonuniform multicomponent action potentials were frequently observed.

The atrioventricular nodal functional refractory periods were determined with use of various combinations of basic drive at 400 msec (S1) and extrastimulus programs (S2). Refractory periods were measured from atrial electrograms recorded at the input sites and tissue responses at the site of the electrode in the His bundle. The A1−A2 interval was progressively decreased in
10 msec decrements until no response of the His bundle was observed. Refractory periods were determined during application of various patterns of 8 basic drive beats followed by premature stimulation from the interatrial septum and/or crista terminalis inputs to the atrioventricular node. The following protocols were used to identify the electrophysiologic responses.

(1) This protocol consisted of basic and premature stimulation from the same site, either crista terminalis or interatrial septum. Refractory curves were constructed by use of the timing of the basic and premature electrograms recorded nearest the site of stimulation of the H1-H2 intervals. For example, after eight basic drive stimuli (A1) in the region of the crista terminalis, premature stimuli (A2) were introduced at this site beginning with an A1-A2 interval of 300 msec and progressively decreasing by 10 msec until no response of the His bundle was observed. Similar stimulation patterns were used in the following protocols and any variations from this pattern are described.

(2) This protocol was one of basic stimulation from one site and premature stimulation from the opposite site (either crista terminalis or interatrial septum). Refractory curves were constructed by use of the timing of the electrograms of the basic and premature electrograms and the H1-H2 intervals, e.g., basic stimulation from the crista terminalis and premature stimulation from the interatrial septum.

(3) The final protocol was one of basic and premature stimulation from the same site with the addition of a second premature stimulus from the opposite site. The second premature stimulus, introduced close in timing to the first premature stimulus but at the opposite input site, was defined as a conditioning stimulus.

Other terminology is as follows: A1-A2, and A-A3, are intervals between the last basic and premature atrial electrograms recorded from the crista terminalis and interatrial septum, respectively and functional refractory period is the shortest H1-H2 interval achieved by successful premature stimulation from the designated stimulation sites.

The term “summation” is used to indicate a shortening of the H1-H2 interval in response to the change in the pattern of stimulation from the two principal input sites of the atrioventricular node (crista terminalis and interatrial septum) without change in the timing of the stimuli. Conduction delay and block indicate an increase in the H1-H2 interval in response to a change in the pattern of stimulation without a change in the timing of the stimuli.

**Stimulation.** All preparations were paced with bipolar silver stimulation electrodes insulated, except at the tip, with Teflon. Electrodes were placed at the crista terminalis and interatrial septum. The cycle length of the basic drive was 400 msec, except as noted. Rectangular pulses twice diastolic threshold and 1 msec in duration were delivered by a programmable digital stimulator (Bloom Associates).

**Results**

**Effects of active vs passive engagement at the crista terminalis and interatrial septum.** In figure 1, pairs of stimulating and recording electrodes were placed near the crista terminalis and interatrial septal input regions of the atrioventricular node. In figure 1, A, both basic drive and premature atrial stimulation were from the interatrial septum. The premature stimulus S2, introduced 150 msec after the last basic drive from the interatrial septum, produced a premature depolarization at fiber AN2 (located near the stimulating site in the atrial septum at fiber AN2 (located near the stimulating site in the interatrial septum.

**FIGURE 1.** Effect of activation site on atrioventricular conduction. A, Both the basic drive and premature stimulus were from the interatrial septum (IAS); B, stimuli were from the crista terminalis (CT). Although identical H1-H2 intervals (183 msec) were achieved in both A and B, local activation times after the premature stimulus were different (160 vs 152 msec). C, The local AN-AN activation time was the same as in B (152 msec), but the H1-H2 varied (183 vs 178 msec). Extracellular potentials of stimulation are designated by two small filled circles and extracellular recording sites by two small filled squares. The recording sites of the various microelectrodes are designated by stars. Action potentials were optimally amplified to measure activation time and identify the type of impaled atrioventricular nodal cell and hence voltage calibrations are not always included. With these methods the limit of resolution for activation times was +/− 1 msec. Only those findings that were consistently reproducible in individual animals are presented as representative. H = His electrogram; AN1 = action potential recorded at 1; AN2 = action potential recorded at 2; Sct = stimulus site at crista terminalis; Sias = stimulus site at interatrial septum.
the AN region) with an AN2 (drive)–AN2 (premature) interval of 160 msec and an H1-H2 interval of 183 msec. In figure 1, B, both the basic drive and premature stimuli were from the crista terminalis. To achieve the same 183 msec H1-H2 interval, the S1-S2 interval was adjusted to 150 msec with a local AN1 (drive)–AN1 (premature) interval of 152 msec. Adjustment of the S1-S2 interval at the interatrial septum to achieve a local AN2 (drive)–AN2 (premature) interval of 152 msec (identical to that in figure 1, B, when stimulation was from the crista terminalis) resulted in shortening of the H1-H2 interval to 178 msec (figure 1, C). As shown, for an identical H1-H2 interval of 183 msec to be obtained with the protocols illustrated in A and B, different local AN (drive)–AN (premature) intervals in the AN1 and AN2 regions were required. Conversely, similar local AN-AN intervals were associated with different H1-H2 intervals in B and C. In A and B the prematurity at the crista terminalis and interatrial septum were dissimilar (160 vs 152 msec), which resulted in similar H1-H2 intervals (183 msec). In contrast, when the prematurity at the crista terminalis and interatrial septum was of a similar degree (152 msec), the H1-H2 intervals differed (B and C).

As shown in figure 1, A and C, initial interatrial septal stimulation resulted in almost simultaneous depolarization of AN fibers 1 and 2. However, when the basic input and premature stimulus were from the crista terminalis there was a marked difference in timing between the two AN cells (figure 1, B). This suggests that depolarization of the AN region varied depending on the site of input of atrial stimulation. Moreover, as detailed in figure 1, B, after initial stimulation at the crista terminalis, AN2 was clearly activated before the inscription of the interatrial septal electrogram, suggesting that the crista terminalis input may have contributed to the AN2 depolarization. Similarly, stimulation from the interatrial septum (figure 1, A) caused depolarization of AN1 before the inscription of the crista terminalis electrogram. Hence, in this instance the crista terminalis input may have played only a passive role. It should be noted that to achieve a particular H1-H2 interval a variety of atrioventricular conduction patterns were observed in response to sequences of stimulation from the predominant inputs to the atrioventricular node. Earlier depolarization of fibers close to one input before evidence of depolarization of the electrograms at the same input suggested only a passive role of this particular input site.

**Effect of the site of stimulation on the sequence of activation of the atrioventricular node.** In figure 2, two curves of the atrioventricular functional refractory period in a representative preparation are illustrated in which A1-A2 is plotted against H1-H2. The solid line was generated when both stimulation and recording were from the crista terminalis and hence the A1-A2 intervals were measured from the local electrograms recorded at the crista terminalis region of input, and the dotted line was generated when stimulating and the recording sites were at the interatrial septum so that A1-A2 intervals for this curve were measured from the electrograms recorded at the interatrial septal site of input. The H1-H2 intervals were used to measure the output of the atrioventricular node and to determine the functional refractory period of the node. Clearly, the functional refractory period is dependent on the site of stimulation.

Two impalements were made in close proximity in the N region of the atrioventricular node (N1 and N2) (figure 3). The premature stimulus followed the basic drive by 170 msec. When the basic and premature stimuli were from the crista terminalis, N1 was depolarized slightly before N2 (figure 3, A) and when both the basic drive and premature stimuli were from the interatrial septum, the depolarization sequence of N1 and N2 was reversed (figure 3, B; arrows). In addition, the H1-H2 interval was different (138 vs 142 msec), although the S1-S2 was 170 msec in each instance. Although impaled N cells (1 and 2) were at closely adjacent sites, both the timing and configuration of the action potentials in the N region differed, depending on the site of input of atrial stimulation.

In figure 4, various depolarization sequences of the AN, N, and NH fibers are shown. In this experiment a more complex sequence of stimulation was used and the basic drive and premature stimulus were introduced from opposite sites. The sequence of basic depolarization of AN1, AN2, N3, NH4, and the His bundle is shown in figure 4, A. In this instance the premature stimulus was delivered near the interatrial septal region of input, which resulted in a depolarization sequence of AN2, N3, NH4, AN1, and then the His bundle. Notably the sequence and depolarization times between N3, NH4, and the His bundle were similar both for the basic drive and premature stimulus. However, the AN1-H1 interval of the premature beat was less than the drive (dashed lines indicate the activation of the His bundle). Hence, AN1 depolarization resulting from the premature beat did not appear to contribute to the activation of the His bundle. However, it is possible that the crista terminalis and AN1 were depolarized by perinodal spread from the interatrial septum to the region of the crista terminalis.

In figure 4, B, the basic drive stimulus was from the
interatrial septum and the premature stimulus was delivered at the crista terminalis. In this instance, the basic stimulus produced depolarization of AN, adjacent to the interatrial septal stimulation site and was followed by N1, AN1, and NH depolarizations and the His bundle electrogram, respectively. After premature stimulation from the crista terminalis, the order of atrioventricular nodal depolarization was AN1, N1, AN2, NH, and His bundle. There was an increase in the N1-H interval with the protocol illustrated in B (dashed lines indicate the His bundle electrogram) compared with that in A. The N1-H interval during the basic beats and N1-H interval during premature beats in A and B were quite different, suggesting different modes of engagement depending on the stimulation site.

In figure 5, A, a functional refractory curve is illustrated that was constructed in the usual manner when both basic and premature stimulation as well as measurements of A1-A2 intervals were from the interatrial septum. Considering only the A1-A2 interval of 170 msec (straight arrow) an H1-H2 of 205 msec was observed in this instance. The electrograms determining this curve of the functional refractory period are shown in figure 5, B. In figure 5, C, a similar pattern of stimulation at the interatrial septum is shown, but in addition, a conditioning stimulus was introduced at the crista terminalis 20 msec after premature stimulation of the interatrial septum. This conditioning stimulus moved the electrogram of the crista terminalis to the left in comparison with B. The H1-H2 interval shortened, suggesting a summation of the inputs from the crista terminalis and interatrial septum. This shortening of the H1-H2 interval is illustrated by the open triangles in figure 5, A. When the conditioning stimulus from the interatrial septum was omitted, as seen in figure 5, E, the H1-H2 interval increased as compared with that in C. In D the timing of the premature stimulus placed at the interatrial septal input was similar to that in B and C, but in addition, a conditioning stimulus was introduced at the crista terminalis 10 msec before the premature stimulation of the interatrial septum (A2). Consequently, another type of interaction with delayed conduction to the His bundle was seen and the H1-H2 interval increased from 170 to 215 msec (figure 5, A; open circles). Thus, the H1-H2 interval varied depending on the sequence and timing of stimulation at the input sites to the atrioventricular node. The electrograms used in determining the other points on this refractory curve are not shown.

In figure 6 additional examples illustrating various interactions resulting from the pattern of the stimula-

FIGURE 2. Curves of the atrioventricular nodal functional refractory period from the crista terminalis (ct) and interatrial septum (ias) in a typical preparation. The drive cycle length was 400 msec. Solid line = ct; dotted line = ias; dashed line = line of identity. The functional and effective refractory curves are dependent on the site of stimulation. The atrioventricular functional refractory period is increased when stimulation is from the ias vs ct. Inset. Schema of stimulation and recording sites. Abbreviations and notes as in figure 1.
FIGURE 3. Effect of the stimulation site on the sequence of atrioventricular nodal activation. The sequence of depolarization of fibers N1 and N2 were altered by the stimulation site of the premature beat. The H1-H2 interval was 138 msec in A and 142 msec in B. Abbreviations and notes as in figure 1.

Conduction patterns resulting from an interaction of stimulation from the interatrial septum and crista terminalis. Additional patterns of input interactions affecting atrioventricular nodal conduction after stimulation from the inputs at the crista terminalis and interatrial septal regions to the atrioventricular node are illustrated in figure 7 to 9.

In figure 7, the basic beats were introduced from the region of the crista terminalis. In figure 7, A, a premature stimulus introduced at the crista terminalis 155 msec after the basic drive engendered an H1-H2 interval of 223 msec. In B, both the basic drive and premature stimulus were similar to those in A except that a second premature conditioning stimulus was introduced at the interatrial septum 30 msec after the premature stimulus at the crista terminalis. The H1-H2 interval was shortened to 212 msec, suggesting summation. In C, stimuli were introduced simultaneously at the crista terminalis and interatrial septum 155 msec after the basic drive, and the H1-H2 interval was prolonged to 250 msec and an atrial echo beat was observed. In D, the interatrial septum was activated 5 msec earlier than the crista terminalis and block to the His bundle was observed. Hence, the possibility for interaction of the wave fronts entering the atrioventricular node from the crista terminalis and interatrial septum depended not only on the stimulation site, but also on the timing of activation at the two major input sites.

In figure 8 a series of microelectrode recordings from three N fibers (N1, N2, and N3) are displayed. The solid lines indicate the results of stimulation from only the crista terminalis, whereas the dotted and dashed lines indicate the results of stimulation from the interatrial septum.
FIGURE 4. Effect of input site, timing, and interaction of inputs on atrioventricular conduction. A. Basic drive was from the crista terminalis and the premature stimulus from the interatrial septum. The sequence of fiber depolarization was AN₂, N₃, NH₄, and AN₁. B. Basic and premature stimulus sites were reversed and the sequence of fiber depolarization was AN₁, N₃, AN₂, NH₄, and His bundle. In A the AN₁-H interval was shorter after the premature stimulus than after the basic drive so that fiber AN₁ appeared to play only a passive role. Abbreviations and notes as in figure 1.

FIGURE 5. Interaction of atrioventricular nodal inputs. A curve of the functional refractory period was produced (A) by introducing both the basic drive (S₁) and premature stimuli (S₂) from the interatrial septum, as shown in B. In C a similar pattern of stimulation was used, but in this instance the crista terminalis was stimulated 20 msec after the interatrial septum. The H₁-H₂ interval was shortened by this conditioning stimulus so that the atrioventricular refractory period was decreased to 190 msec (open triangles). When the crista terminalis was activated 10 msec earlier than the interatrial septum (D) the H₁-H₂ interval was lengthened to 215 msec (open circles in A). In E, the conditioning stimulus from the interatrial septum was omitted and the H₁-H₂ interval was increased compared with in C. Abbreviations and notes as in figure 1.
terminalis and interatrial septal stimulation was 5 msec. After stimulation from the interatrial septum or interatrial septum and crista terminalis the upstroke velocity of the action potential observed at cell N1 was more rapid than when stimulation was from the crista terminalis. This suggested that the main or predominant activation of cell N1 was from the interatrial septum.

The inscription of the action recorded from the N1 cell showed a slower upstroke velocity when stimulation was from the interatrial septum as compared with when stimulation was from the crista terminalis or crista terminalis and interatrial septum. Hence, the predominant influence of the activation appeared to be from the crista terminalis site of input in contrast to the N1 cell. The action potentials recorded at the N1 cell demonstrated three different configurations in response to the stimulation modes. Stimulation from the crista terminalis and interatrial septum produced the most rapid upstroke of the action potential from the N1 cell. Stimulation of only crista terminalis or interatrial septum caused a later inscription of the His bundle compared with stimulation of the crista terminalis and interatrial septum.

Further evidence demonstrating that the site and organization of the inputs from the crista terminalis and interatrial septum could produce both shorter and longer H-H intervals, as well as block to the His bundle, is shown in figure 9. In all instances there were 12 basic drive stimuli from the crista terminalis at a cycle length of 200 msec and in each case, after the eleventh drive stimulus, the interatrial septum was stimulated 20 msec later than the crista terminalis. In figure 9, B to F, the interatrial septum was also prematurely stimulated, with respect to the twelfth basic drive stimulus, from the crista terminalis. Since the tenth and eleventh sets of stimuli were common to all records, only comparison of the twelfth pair of stimuli will be discussed.

In figure 9, A, the resulting H1-H2 interval was 210 msec after the twelfth basic drive from the crista terminalis. In B, the twelfth basic drive at crista terminalis was modified by a premature stimulus introduced at the interatrial septum 5 msec earlier. Consequently, the H1-H2 interval was shortened by 25 msec to 185 msec. In C, the premature stimulus was preceded by a conditioning stimulus applied to the interatrial septum 15 msec earlier, which resulted in an H1-H2 interval of 188 msec. In D, introduction of the conditioning stimulus at the interatrial septum 40 msec earlier than that at the crista terminalis increased the H1-H2 interval to 212 msec (figure 9, D) and an even earlier conditioning stimulus at the interatrial septum (50 msec) produced block to the His bundle (figure 9, E). Introduction of the conditioning stimulus 60 msec before activation of the crista terminalis resulted in distortion of the N1 and N2 action potentials, and block to the His bundle was again observed (figure 9, F). Hence, small changes in the activation time of a second stimulation input to the atrioventricular node had considerable influence on the H1-H2 interval. The effects of stimulation from these inputs to the atrioventricular node alternatively caused shortening or lengthening of the H1-H2 interval, with distortion of the N fiber action potentials and finally block to the His bundle.

Discussion

Previous studies initially reported by Jansel' demonstrated differences in atrioventricular nodal activation
patterns resulting from interatrial septal vs crista terminalis stimulation. However, those studies did not compare the relative influence of stimulation at one input site vs the other on atrioventricular nodal conduction and, more importantly, the effects of input interaction resulting from various patterns of activation from the two input sites were not considered. In the present experiments, both the relative influence of the site of input of stimulation and the effects of stimulation from these sites were evaluated by programmed stimulation with the extrastimulus method and with the use of multiple simultaneous recordings of extracellular electrograms and those obtained with microelectrodes. Several important concepts emerged from the results of these studies.

Active vs passive engagement of the atrioventricular node. With multiple microelectrode recordings it was possible to demonstrate the relative influence of a particular wave front entering at one or the other input site on subsequent atrio-His conduction. At one end of the spectrum, engagement of the atrioventricular node occurred principally from one input site while the opposite input region played no role in the subsequent propagation of the impulse to the His bundle, and thus played only a passive role in the organization of atrioventricular conduction. Earlier studies by Janse and

**FIGURE 7.** Relative influence of the prematurity and site of stimulation on atrioventricular nodal conduction. The arrangement of the electrodes was similar to that in figure 5, but the basic drive was introduced at the crista terminalis region of input in this instance. B. A conditioning premature stimulus introduced at the interatrial septum 30 msec after premature activation at the crista terminalis shortened the H₁-H₂ from 223 to 212 msec. C. The crista terminalis and interatrial septum were prematurely stimulated, producing an increase in H₁-H₂. D. The interatrial septum was stimulated 5 msec before the crista terminalis and block to the His bundle resulted. See text for details. Abbreviations and notes as in figure 1.

**FIGURE 8.** Effect of premature stimulation at the crista terminalis, interatrial septum, and crista terminalis plus interatrial septum on the upstroke velocity of the action potentials. The results of the three types of stimulation are superimposed for each N₁, N₂, and N₃ fiber. The action potentials for N₁ and N₂ are offset 10 and 20 msec to the right and 25 and 50 mV downward, respectively. The most rapid upstroke of phase 0 of the action potential was observed with crista terminalis plus interatrial septum stimulation and was associated with the earliest His bundle electrogram. See text for further discussion. Abbreviations and notes as in figure 1.
others\(^2\,^3\) first demonstrated clearly the typical activation sequences of atrioventricular nodal conduction. However, in the present studies it was possible to show the relative influence of active vs passive activation patterns contributing to atrioventricular conduction. More specifically, as shown in figure 1, when both the basic drive and the premature stimulus were from the interatrial septum, depolarization of fibers AN\(_1\) and AN\(_2\) was entirely from the interatrial septum while the crista terminalis played only a passive role, as evidenced by inscription of the crista terminalis electrogram after both AN action potentials.

A second and related phenomenon demonstrated in the present studies was the effect of the site of input on local activation times. For example, a similar H\(_1\)-H\(_2\) interval could be produced in response to different local premature activation times (figure 1). On the other hand, if the local activation time between the basic and premature activation was held constant, as seen in panels B and C of figure 1, then the output or appearance of the electrogram at the His bundle varied. Consequently, the output or the H\(_1\)-H\(_2\) interval was predicated not only on the extrastimulus coupling interval, but also on the input side of the premature stimulus. It can be argued that the changes noted in the H\(_1\)-H\(_2\) intervals in this study were of small magnitude. However, they were reproducible and a consistent finding. Furthermore, these changes would generally elude clinical recognition if conventional methods and electrograms recorded from intracardiac catheter electrodes were used.

**Effect of the site of stimulation and conduction patterns resulting from various activation sequences.** Several variations and patterns of atrioventricular conduction could be identified by varying the site of stimulation as well as the sequence of stimulation from the two principal sites of input to the atrioventricular node. Although Mendez and Moe\(^17\) convincingly demonstrated that changes in configuration of the action potentials of atrioventricular nodal cells could be ascribed to the engagement patterns of the wave front, the present study further elucidates the complexity of this phenomenon.

In the present study the stimulation input site was shown to influence both the configuration of the atrioventricular refractory curve (figure 2) and the sequence of atrioventricular nodal depolarization (figure 3). In the latter example, changes in configuration and timing of the fibers N\(_1\) and N\(_2\) (figure 3) could be ascribed to differences in the depolarization of this particular fiber by the wave fronts resulting from stimulation of the crista terminalis vs the interatrial septum. Consequently, the H\(_1\)-H\(_2\) intervals as well as the difference in the time of depolarization between fibers N\(_1\) and N\(_2\), as seen in figure 3, were dependent on whether the premature stimulation was from the crista terminalis or the interatrial septum. These findings are consistent with those of Watanabe and Dreifus,\(^18\,^19\) who demonstrated that successful atrioventricular nodal propagation occurred when several apparently independent wave fronts were simultaneously engaged, whereas conduction delay and block resulted when inhomogeneous engagement of these wave fronts was observed. Additional activation patterns of atrioventricular nodal cells were also noted in several of the present experiments.

**FIGURE 9.** Effect of interaction from crista terminalis and interatrial septum inputs producing varying H\(_1\)-H\(_2\) intervals. In A through F there were 12 basic drive stimuli introduced from the crista terminalis plus a stimulus from the interatrial septum at a coupling interval of 20 msec after the eleventh drive stimulus. However, a conditioning stimulus was also introduced 5 to 60 msec before the twelfth basic drive. Electrodes representing N\(_1\) and N\(_2\) action potentials were impaled within 1 mm of each other and do not necessarily represent the sequence of activation. In B the H\(_1\)-H\(_2\) interval decreased from 210 to 185 msec and in C it decreased to 188 msec. However, in D, the H\(_1\)-H\(_2\) interval increased to 212 msec, and block to His bundle was observed in E and F. Note the delay and distortion of action potentials in F. Abbreviations and notes as in figure 1.
and representative examples are elucidated in figures 4 to 9 and detailed in the Results.

Hence, by use of programmed pacing and multiple simultaneous recordings it was possible to show that the stimulation input site, timing, and the effects of the input stimuli all play a role in determining the sequence and configuration of the action potentials of atrioventricular nodal cells and the conduction patterns to the His bundle.\(^3,5,13,15\) These results further support the conclusion of Zipes et al.,\(^4\) as well as recent work from our laboratory, indicating that specific stimulation programs between the crista terminalis and interatrial septal inputs could improve or delay atrioventricular nodal conduction.\(^13,15,21\)

Conversely, these studies further demonstrate that the effects of stimulation sequences at the major inputs to the atrioventricular node can also result in interference of wave fronts, producing inhomogeneous conduction and fragmentation, as noted previously,\(^1,3,18,19\) and block to the His bundle (figures 6 and 7). The distortions of the action potentials due to subthreshold depolarizations (figure 9) were similar to the inhomogeneous conduction patterns previously observed by Mendez and Moe,\(^17\) Watanabe and Dreifus,\(^18,19\) and Iinuma et al.\(^21\) and to the double-component action potentials demonstrated by Billette and Bonin.\(^9\) Finally, evidence for summation-enhancing atrioventricular conduction as well as block to the His bundle in the same preparation was observed in the present study (figure 9).

The present study also provides evidence for the role of the interatrial septal and crista terminalis regions of input in determining the properties of atrioventricular nodal conduction and refractoriness. The derived functional and effective refractory periods of the atrioventricular node appear to depend on both the site of stimulation relative to the principal inputs to the atrioventricular node as well as the site of atrial recording used to determine the timing and configuration of the action potentials of the atrioventricular node. Notably, the effect of stimulation from the two major atrioventricular nodal inputs appeared sufficient to result in discontinuous atrioventricular nodal refractory curves. Variations in the\(^1H_1-H_2\) interval were dependent on both the site and timing of atrioventricular nodal activation from the two principal input sites. Previous studies have shown that the input site (crista terminalis vs interatrial septum) influenced the manner of depolarization of the AN and N cells, while the NH region was activated by a more organized wave front from the upstream AN and N fibers.\(^13\) The use of multiple microelectrode impalements in recent studies from our laboratory\(^13,15,21\) support the observations of Zipes et al.,\(^4\) Konishi and Matsuyama and Janse et al.\(^5\) suggesting summation and cancellation of wave fronts. Furthermore, the present study supports the observation of Zipes et al.\(^4\) that, in the presence of summation of wave fronts, the inscription of the His bundle was earlier. In figure 8, stimulation of the crista terminalis and interatrial septum always produced the most rapid rise in the action potential upstroke in all three cells. However, the correlation between the upstroke velocity of the action potential and the conduction time to the His bundle was not always uniform since stimulation of the CT produced the slowest upstroke velocity in the N\(_1\) and the fastest in N\(_2\) and the upstroke velocity was intermediate in the N\(_1\) cell. Consequently, we suggest that different modes of stimulation could produce various conduction patterns. It is also possible that if many simultaneous impalements could be performed, an exact correlation of the upstroke velocity and conduction to the His bundle could be observed from a particular cell. Finally, it should be pointed out that in these studies no clear explanation can be offered for the observed phenomena. However, it seems that cancellation of wave fronts in the upper portion of the atrioventricular node by their collision would be associated with fragmentation and distortion of the action potentials, resulting in an increase in the\(^1H_1H_2\) intervals and finally block to the His bundle (figure 9). The alternative possibility, block in the downstream cells leading to lack of summation at the level of the N cells, seems less likely since earlier depolarization of N\(_1\) and N\(_2\) cells occurred in response to the premature stimulation at the interatrial septum (figure 9, A to E). Hence, cancellation of wave fronts in the upper part of the atrioventricular node by collision of wave fronts leads to delay and even block in the downstream cells.

The present observations should be considered in explaining the diverse and complex atrioventricular conduction patterns after stimulation from the two principal input sites. It is well established that alterations in atrioventricular nodal conduction patterns can be associated with reentry phenomena.\(^20\) Furthermore, we have recently observed reentry in an atrioventricular nodal-perinodal rabbit heart preparation, and have postulated that the presence of dual nodal input regions, rather than dual nodal pathways, may be sufficient to explain reentry.\(^15,21\) The present study provides further evidence for the complexity of atrioventricular nodal conduction based on the site and timing of inputs from the crista terminalis and interatrial septum, the two major atrial-nodal regions of input. With this experimental preparation it has been possible to elucidate
electrophysiologic phenomena that would not be as readily apparent or demonstrable in man, since electrode catheter recordings are limited in localizing the sites of input and the precise sequence of atrionodal depolarizations. However, these observations may help to explain some complex clinical electrophysiologic phenomena of atrioventricular nodal conduction.

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