Reentrant Pathway During Ventricular Echoes Is Confined to the Atrioventricular Node
High-Resolution Mapping and Dissection of the Triangle of Koch in Isolated, Perfused Canine Hearts

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Background—During ventricular echoes, reentrant excitation is supposed to involve 2 functionally distinct pathways in the atrioventricular (AV) nodal area. The exact pathway of reentrant excitation is unknown. The objectives of this study were to analyze electrical activity in the AV nodal area after ventricular stimulation and during ventricular echoes and to assess the role of perinodal atrial tissue in AV nodal reentry.

Methods and Results—In 16 isolated, blood-perfused canine hearts, multiterminal electrodes were used to map electrical activity in Koch’s triangle after ventricular stimulation and during ventricular echoes. The subendocardial cell layers were chemically destroyed in 3 hearts. Incisions in the posterior approach to the compact node were made in 6 hearts. The apex of the triangle of Koch was surgically dissociated from the perinodal atrial tissue in 5 hearts. Retrograde atrial activation occurred via 2 distinct endocardial exit sites. Ventricular echoes could be induced in all hearts irrespective of the atrial activation pattern. Simultaneous retrograde activation of both exit sites often preceded reciprocation. Ventricular echoes were demonstrable after chemical destruction of the endocardium and after surgical dissociation of the perinodal atrial tissue from the AV node.

Conclusions—Our data show that the reentrant pathway during ventricular echoes is confined to the AV node. The tissue that connects the node to the endocardial exit sites has to be excluded from the reentrant circuit responsible for single echoes. (Circulation. 1999;100:1346-1353.)

Key Words: atrioventricular node reentry mapping electrophysiology

During ventricular echoes, reentrant excitation is supposed to involve 2 functionally distinct pathways in the AV nodal area, an anterior “fast” pathway that has a long refractory period and a posterior “slow” pathway with a shorter refractory period. This widely accepted concept of dual AV nodal physiology provides a theoretical basis for AV nodal reentry manifesting as atrial or ventricular echoes, as well as for sustained AV nodal reentrant tachycardia (AVNRT). Although AV nodal reentry has been subjected to clinical and experimental investigation since it was described by Mines in 1913, controversy continues regarding the precise pathway of reentrant excitation and the participation of perinodal atrial tissue in the circuit. Literature provides evidence suggesting that the reentrant pathway involves atrial tissue well outside the specialized area. In 1981, Sung et al demonstrated earliest atrial activation in the anterior AV nodal area close to the His bundle during retrograde fast-pathway conduction, whereas earliest activation was found near the coronary sinus (CS) orifice during retrograde slow-pathway conduction. Jackman et al suggested that these exit sites represent the atrial insertions of the slow and fast pathways. Observations made by other investigators support an intranodal location of the reentrant circuit. Detailed reviews on this issue have recently been published by Janse et al and McGuire et al.

The aims of the present study were to analyze electrical activity in the triangle of Koch and the perinodal atrial tissue after ventricular stimulation and during ventricular echoes and to assess the role of perinodal atrial tissue in AV nodal reentry.

Methods

This study conformed to the guiding principles of the American Physiological Society. Studies were performed in isolated, blood-perfused hearts obtained from 27 mature mongrel dogs (weight 18 to 30 kg) of either sex.
Preparation of Hearts
The methods of preparation and perfusion of isolated hearts have been described elsewhere. Briefly, after deep anesthesia, the heart was excised and the aortic root was cannulated to permit Langendorff perfusion. Interference from sinus rhythm during pacing was avoided by resection of the sinus node.

Bipolar hook electrodes (interelectrode distance 1 mm) were placed in the right atrium and right ventricle. Stimulation was achieved with 2-ms-long pulses at twice diastolic threshold. Recording electrodes were placed in the high right atrium and over the His bundle. Diacetyl monoxime (10 to 15 mmol/L) was added to the perfusate to dampen contraction.

Experimental Protocol
In 16 hearts, multiterminal electrodes were used to map electrical activity in the AV nodal area. The left atrial septum was mapped with a single roving electrode in 4 hearts. In 3 of the 16 hearts, the endocardial cell layers were destroyed by phenol application. In a second group of experiments, incisions were made in the posterior approach to the compact node in 6 hearts. In 5 hearts, the compact AV node was surgically dissociated from the surrounding atrial tissue. After the study, the hearts were preserved for histological analysis.

Stimulation Protocol
Programmed atrial and ventricular extrastimulation and incremental pacing were performed. Up to 2 programmed extrastimuli after every eighth beat of a paced rhythm were introduced to induce AV nodal echoes. Whereas antegrade AV nodal conduction was stable throughout the experiment, ventriculoatrial (VA) conduction sometimes deteriorated during the course of the experiment. In those cases, isoproterenol was added to the perfusate and titrated upward (0.05 to 0.5 μg/min) until stable VA conduction was achieved.

Mapping Plaques
Two multiterminal electrodes were used. One contained 84 terminals (silver wire, diameter 200 μm) arranged in a 12×7 matrix at interelectrode distances of 2.5 mm. Ten additional terminals were positioned at distances of 2.5 mm along a silicone tube (5 mm diameter), which was inserted into the proximal CS. The indifferent pole was placed either at the aortic root or, to reach a high level of common-mode rejection, in the middle of the matrix 1 mm from the endocardial surface.

The second electrode contained 96 terminals arranged in a 12×8 matrix at interelectrode distances of 1 mm. Each terminal consisted of a recording and a reference electrode (silver wire, diameter 100 μm); the reference electrodes were cut 1.5 mm shorter. In this way, 96 quasi-unipolar recordings were obtained. Remote signals were attenuated, and unipolar characteristics were preserved.

Signal Processing
A customized data-acquisition system allowed simultaneous recording of 96 channels at a sample frequency of 1 kHz/channel. Signals were amplified 256-fold and band-pass-filtered with lower and upper cutoff frequencies of 0.1 and 500 Hz, respectively. The registrations were stored on a hard disk of an IBM-compatible computer system. The signals from the bipolar atrial and His bundle electrodes were amplified with a gain of 500 to 1000, band-pass filtered between 0.1 and 500 Hz, and stored on an 8-channel digital audiotape recorder (DTR 1801, Biologic).

Analysis of Electrical Activation
The dV/dt was calculated for each electrogram by use of a computer algorithm. The point of maximum negative dV/dt was selected as the time of local activation. In case of doubt, recordings from contiguous sites were taken into consideration to determine the activation times. Isochronal maps were constructed manually by connecting points with the same time of local activation.

Phenol Application
Small (5×5 mm) pieces of filter paper were soaked with phenol (75%) and applied on the endocardium in the triangle of Koch, the perinodal tissue, and the floor of the proximal CS for 2 minutes. In this concentration, phenol causes necrosis to a depth of ~300 μm. After application of each piece of filter paper, the effects on conduction parameters and ventricular echoes were determined.

Dissection of Triangle of Koch
Before dissection, unipolar and bipolar recordings from a single roving bipolar electrode (electrode distance 0.5 mm) were used to localize the most proximal part of the His bundle and therewith the approximate position of the compact AV node, which in dogs has a length of 1 to 1.5 mm. The site with the largest H-V interval in the bipolar and unipolar recordings and an initially negative His deflection in the unipolar recording was considered the compact node–penetrating bundle interface.

Incisions (1.5 to 3 mm deep) were made in the posterior approach to the compact node in 6 hearts. The first incision was made at the base of the triangle of Koch, extending from the tricuspid valve annulus (TVA) to the posterior edge of the CS orifice and into the floor of the CS. Consecutive incisions were made, each ~2 mm more anterior than the last. After each incision, the effects on conduction and reentry were determined. In 4 hearts, the most anterior incision was made close to the presumed location of the posterior aspect of the compact AV node. In the other 2 hearts, additional incisions were made until ventricular echoes could no longer be induced.

In 5 hearts, the roof of the CS and the upper part of the interatrial septum were cut away. The interatrial septum was carefully trimmed down to the muscular AV septum. The interatrial groove lying posterior to the aorta was trimmed down to the right atrial trigone (central fibrous body). The anterior walls of the right and left atria were cut away down to the parietal right and left AV junctions, respectively. An incision was made at the presumed position of the posterior aspect of the compact AV node, extending from the mitral valve annulus to the TVA. These dissections circumscribe the posterior part of the muscular AV septum corresponding to the apex of the triangle of Koch (Figure 1). Electrodes were positioned inside the truncated area and on the right and left atrial walls. Switching between stimulation of the remaining atrial tissue, the truncated area, and ventricular stimulation, the incisions were carefully extended or deepened until complete electrical dissociation between the truncated area and the remaining atrial tissue was achieved in both antegrade and retrograde directions.

Histology
In the hearts that were used for activation mapping, the position of the mapping electrode was marked. The endocardial exits were identified and marked with fine needles. Serial sections (5 μm) were
cut perpendicular to the TVA and stained with hematoxylin-eosin and elastic van Gieson and were examined with light microscopy.

In the 6 hearts in which the posterior approach to the AV node was incised, serial sections were cut parallel to the TVA. In the 5 hearts in which the triangle of Koch was dissected, serial sections were again cut perpendicular to the TVA.

For the histological definition of the compact AV node and the transitional cells, we applied the criteria of Anderson et al.13 that the compact node is a well-recognized half-oval of small cells closely adherent to the central fibrous body. Transitional cells are usually palely staining and are frequently separated into small fascicles by connective tissue septa.13

Results

Atrial Activation Sequence During Ventricular Stimulation

The 16 hearts in which activation mapping was performed revealed 2 distinct atrial exit sites during ventricular stimulation. One was in the anterior area toward the apex of the triangle of Koch, close to the site where His signals were recorded. The other exit site was located posteriorly between the TVA and the orifice of the CS. Activation in the CS ran from the proximal electrode (orifice) to the distal electrode. Earliest activation of the left atrium occurred later (12±4 ms [mean±SD]) than earliest activation of the right AV junction.

In 5 of the 16 hearts, earliest atrial activation was found at the anterior site during pacing at long cycle lengths (≥600 ms). As illustrated in Figure 2, during incremental pacing (steps of 20 ms), the sequence of atrial activation changed gradually until earliest atrial activation was recorded at the posterior site. Although the change in the activation pattern suggests a shift from retrograde fast- (Figure 2A) to retrograde slow-pathway conduction, earliest atrial activation was delayed by only 4 ms (Figure 2C).

In the other 10 hearts, the retrograde impulse activated the atrium using both exit sites concurrently over a wide range of paced cycle lengths. The activation maps revealed 2 early sites with intrinsic negative deflections, indicative of areas where activation arises, separated by recording sites with later local activation times. An example is shown in Figure 3. The site of earliest atrial activation was recorded in the posterior area. The activation spread posteriorly, toward the orifice of the CS, and anteriorly. A second exit site, activated 12 ms after the posterior exit site, was in the anterior area near the apex of the triangle of Koch.

In 6 of these 10 hearts, the posterior exit site was activated slightly before the anterior exit site during pacing at long cycle lengths (≥600 ms). During incremental ventricular pacing or after closely coupled ventricular extrastimuli, activation of the posterior exit site occurred increasingly later than activation of the posterior exit site. In some cases, the posterior exit site could completely mask the anterior site. In 2 of the 10 hearts, the anterior exit site was activated before the posterior site even at shorter cycle lengths. In the 2 remaining hearts, activation of the exit sites occurred at random, independently of the paced cycle length.

Dual-Pathway Physiology

Single ventricular echoes were consistently induced in all hearts. Atrial echoes occurred only sporadically and could not be reproducibly induced to allow mapping. Sustained
VA conduction delay compared with Figure 3 is 25 ms. Again, the activation pattern and signal morphology match those in Figure 3. The tracings in the lower right of Figure 5 show His bundle electrograms of the ventricular echo and baseline atrial stimulation.

Mapping was also performed with the high-resolution, quasi-unipolar electrode. Although this electrode was specifically designed to unveil low-frequency signals, no distinct potentials were discerned that could be attributed to activation of the compact node or the transitional cell zone.

Histology of Exit Sites

The sites of endocardial breakthrough were found to be in atrial myocardium, well away from the compact AV node and the transitional cell zone. The myocardium between the exit sites and the AV node was not specialized in terms of histological characteristics, nor were histologically discrete or insulated tracts identified.

Phenol Application

Ventricular echoes were still inducible after application of phenol. Retrograde and antegrade AV nodal conduction parameters did not differ before and after phenol application. Light microscopy of histological sections revealed a mean zone of necrosis to a depth of 475 μm (range 350 to 600 μm).

Incisions in Posterior Approach to Compact AV Node

In 4 of the 6 hearts, the most anterior incision was made at the presumed location of the posterior aspect of the compact node. Antegrade and retrograde conduction parameters were only slightly affected: the A-H interval increased by 5±3.8 ms (mean±SD) and the V-A interval by 11±6.5 ms (mean±SD).

Ventricular echoes were still inducible after the most anterior incision. Figure 6A shows electrograms of ventricular echoes before and after dissection. Note that the third and fourth incisions were made anteriorly relative to the location of the posterior exit site. Figure 6B shows a serial section from the same heart. The distance between the most anterior incision and the compact node is ≈2 mm. In 1 of the 2 hearts in which additional incisions were made, histology could not be interpreted because the area of interest was destroyed by the incisions. In the other heart, the incision that abolished echo responses passed through the compact node.
Dissection of Triangle of Koch

Electrophysiology

Ventricular echoes occurred after dissection in all 5 hearts. Figure 7 shows recordings from the right and left atria and the His bundle during programmed ventricular extrastimulation before and after dissection. The ventricular echo in Figure 7A was induced by an extrastimulus with a coupling interval (S1-S2) of 330 ms during a paced cycle length of 600 ms. Figure 7B shows a ventricular echo after dissection (S1-S2 540 ms, basic cycle length [BCL] 800 ms). Note that the recordings from the right and left atria show no electrical activity, which demonstrates that the atrial tissue outside the truncated area was electrically dissociated.

Antegrade functioning of the AV node was preserved in the 5 hearts after dissection (Table). Interestingly, after dissection, 1 heart revealed discontinuous AV conduction, with an A-H jump of 170 ms (Figures 8, 9A, and 9B). The antegrade effective refractory period of the fast pathway lengthened from 220 to 260 ms, thereby exposing the slow pathway, which was concealed before dissection. As shown in Figure 9C, an atrial extrastimulus with a coupling interval of 240 ms induced an atrial echo, which then reciprocated to the ventricles. Recordings from the left and right atria demonstrate that the truncated area was electrically isolated from the perinodal atrial tissue.

Histology

Histology is illustrated in Figure 10 on the basis of the heart discussed in Figures 7 through 9. Progressing from posterior to anterior, the sections at the site of the incision between the TVA and the mitral valve annulus show remnants of atrial tissue at the mitral and tricuspid valve annuli. The atrial surface of the muscular AV septum is depleted of atrial and specialized tissue (Figure 10A). Sections immediately anterior to the incision pass through the posterior part of the compact node. A zone of transitional cells extends to the TVA and to the mitral valve annulus (Figure 10B). Approaching the middle of the compact node, almost all the tissue at the atrial surface of the muscular AV septum is histologically specialized (compact and transitional cells), with only a narrow rim of overlying atrial myocardium (Figure 10C). At the anterior exit site, transitional cells still connect the distal compact node to the atrial myocardium (Figure 10D). More anteriorly, the contact between compact node and atrial myocardium is lost, which indicates the junction between compact node and penetrating bundle (Figure 10E).14

Discussion

New Observations

In this study, high-resolution mapping of atrial activation after ventricular stimulation shows 2 AV nodal exit sites. These sites are situated in atrial myocardium, well away from the AV node. The exit sites and the atrial tissue that connect the node to these sites have to be excluded from the reentrant
pathway during ventricular echoes for the following reasons: (1) ventricular echoes occurred irrespective of the atrial activation pattern; (2) synchronous retrograde activation of both exit sites often preceded ventricular echoes; and (3) ventricular echoes occurred after chemical destruction of the endocardial and subendocardial tissue, as well as after surgical dissociation of the perinodal atrial tissue, including both exit sites, from the AV node.

**Dual Atrial Exit Sites Versus Dual Pathways**

Activation of the right atrium after ventricular stimulation occurred via 2 distinct endocardial exit sites. During ventricular pacing with short cycle lengths or after closely coupled ventricular extrastimuli, the preferential route of atrial activation was via the posterior exit site, which suggests functional differences between the 2 distinct areas.

The exit sites observed in the present study correspond to the sites of earliest atrial activation described both in animal studies and in clinical settings. The studies of Sung et al3 and of McGuire et al 15, 16 suggest that during VA conduction, strands of atrial cells that connect the AV node with the endocardial exit sites are the substrate of the fast and slow pathways. The results of the present study, however, show clearly that these hypothetical pathways could be completely disconnected from the compact node without abolishment of the ventricular echoes.

**Canine Dual AV Nodal Physiology**

Although dual AV nodal pathways manifested as ventricular echoes, none of the hearts showed discontinuous AV nodal

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**Antegrade AV Nodal Conduction Parameters in 5 Hearts Before and After Dissection**

<table>
<thead>
<tr>
<th>Heart</th>
<th>Before Dissection</th>
<th>After Dissection</th>
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<td>ERP&lt;sub&gt;ad&lt;/sub&gt;</td>
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<tr>
<td>5</td>
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ERP indicates effective refractory period; FRP, functional refractory period; WCL, Wenckebach cycle length; and AVN, AV node.

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**Figure 8.** Antegrade AV nodal conduction (A<sub>2</sub>-H<sub>2</sub>) in response to progressively premature atrial extrastimuli (A<sub>1</sub>-A<sub>2</sub>) after every eighth beat of a BCL of 600 ms during baseline and after dissection of triangle of Koch. Extrastimuli with coupling intervals of 240 and 230 ms (*) were followed by atrial echoes.

**Figure 9.** A and B, Dual AV nodal pathways after dissection of triangle of Koch manifested by a sudden prolongation of AH interval (145 to 315 ms) after 10-ms decrease in coupling interval (A<sub>1</sub>-A<sub>2</sub>) at BCL of 600 ms. C, Further decrease of A<sub>1</sub>-A<sub>2</sub> interval induced an atrial echo (A<sub>e</sub>), which reciprocated to the ventricles. Recordings from right (RA) and left (LA) atria demonstrate electrical dissociation of truncated area. Abbreviations as in Figure 7.
function curves during baseline electrophysiological study. Because discontinuous AV nodal conduction can be observed in the majority of patients undergoing electrophysiological study, this might indicate a substantial difference between human and canine AV nodal physiology. A possible explanation for this apparent discrepancy is provided by the observation made in the heart discussed in Figures 8 and 9: dual pathways are present, but if the differences in effective refractory periods between the fast and slow pathways are not sufficiently distinct, slow-pathway conduction will be concealed.

A study by Moe et al. emphasized the significance of vagal-sympathetic interactions for AV nodal functioning. Differences in antegrade AV nodal electrophysiology compared with clinical findings might therefore also be due to denervation of the isolated hearts.

The occurrence of ventricular echoes with smooth AV nodal function curves is a common finding in experimental and clinical settings, and patients presenting with AVNRT do not necessarily demonstrate discontinuous AV conduction. Thus, conduction delay due to decremental conduction properties, rather than differences in conduction velocities, is essential for AV nodal reentry to occur.

Where is the Site of AV Nodal Reentry?

One of the objectives of the present study was to assess the role of perinodal atrial tissue in AV nodal reentry. This intent is complicated by the fact that the anatomic definition of the AV nodal area is still subject to controversy. Transitional cells, separated from each other by connective tissue septa, surround the more closely packed midnodal cells. This zone of midnodal cells is called the compact node. Some investigators only consider this compact part of the specialized area. Anatomic and electrophysiological studies of the AV junction, however, suggest that the AV node comprises all the different cell groups that determine its functional properties.

In the present study, progressive restriction of the reentrant circuit excluded the perinodal atrial tissue. The anatomic and functional potentials for a dual-transmission system within the AV node certainly exist. In their original study, Mendez and Moe suggested that "...the upper region of the node was functionally and spatially split into two effective pathways." Techniques such as extensive mapping with (multiple) microelectrodes and high-resolution extracellular mapping after resection of the endocardium and the subendocardial atrial tissue should be helpful in the attempt to locate the exact site of reentry within the AV node.

Study Limitations

It is generally thought that atrial or ventricular echoes represent a "single-beat expression" of AVNRT. In contrast to the findings of the present study, the recent results of surgical and catheter ablation techniques in humans with AVNRT suggest that damage to the AV node is not a prerequisite for cure. There are several possible explanations for this discrepancy: (1) The persistence of single echoes after selective slow-pathway ablation in patients with AVNRT is a common finding. If successful ablation for AVNRT could only be achieved by complete destruction of
the circuit, this should equate with eradication of the echo beat. Radiofrequency or surgical lesions, even if placed well away from the compact AV node, certainly modify the complex architecture of the AV junction. This might disturb the delicate balance that seems to be necessary to sustain circus movement, while the circuit is still intact and allows single echoes. (2) The success of radiofrequency ablation in abolishing AVNRT at sites well away from the compact AV node can be attributed to remote effects on the transitional cells or the compact node itself. It has been shown that sequences of 60-second 25-W radiofrequency pulses increase myocardial temperature to >50°C at distances as great as 10 mm.26 (3) Recent concepts suggest intranodal microreenery as the basis for single AV nodal echoes, whereas some forms of AVNRT are ascribed to macroreentry with the participation of perinodal atrial tissue.27 (4) The mechanism of the ventricular echoes induced in dog heart is different from the mechanism of AVNRT in humans.

Despite the fact that dual AV nodal physiology seems to be a normal property of the dog heart, sustained AVNRT could not be induced. Although it has been shown that the conduction system in dog and in humans is basically similar,11 extrapolation of the results of the present study to the pathophysiology of human hearts with AVNRT should be considered with care. The question whether single echoes and sustained AV nodal reentry use the same substrate remains to be elucidated. Comprehensive understanding of the arrhythmogenesis in the AV junction is hampered by the lack of an accurate model of AVNRT in an experimental setting.

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References

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