His Electrogram Alternans Reveal Dual Atrioventricular Nodal Pathway Conduction During Atrial Fibrillation
The Role of Slow-Pathway Modification
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**Background**—Traditional tools to study dual-pathway atrioventricular nodal (AVN) electrophysiology are not applicable in subjects with permanent atrial fibrillation (AF). The presence of fast-pathway (FP) and slow-pathway (SP) wavefronts and their possible modification remain uncertain in this condition. We demonstrated previously that His electrogram (HE) alternans can determine whether the FP or the SP reaches the His bundle on a beat-by-beat basis. We have now applied this novel index to monitor dual-pathway conduction and the effects of SP modification during AF.

**Methods and Results**—In 12 rabbit AVN preparations, HE alternans were confirmed during a standard A₁A₂ pacing protocol. During AF, in 9 of the 12 hearts, HE alternans indicated the presence of dual pathways. Successful SP modification guided by the HE alternans eliminated the SP, resulting in a predominantly FP conduction during AF in all hearts. This increased the average His-His interval (204±14 versus 276±51 ms, P<0.001). Morphological studies revealed that SP modification damaged only the posterior extension of the AVN.

**Conclusions**—We have demonstrated for the first time in rabbits that HE alternans permit “visualization” of dual-pathway electrophysiology and confirmed the presence of both FP and SP wavefronts during AF. This novel index has been used in a selective SP ablation that resulted in a significant slowing of the ventricular rate. HE alternans provide a new insight into the mechanisms of AVN conduction and could guide AVN modification for ventricular rate control in AF clinically. (Circulation. 2003;107:1059-1065.)

**Key Words:** fibrillation ■ atrioventricular node ■ His bundle ■ alternans ■ electrophysiology

Because slow-pathway (SP) targeted modification of the atrioventricular node (AVN) has been shown to reduce ventricular rate in patients with drug-refractory atrial fibrillation (AF), 1–4 dual-pathway electrophysiology is believed to be present in this condition. However, this remains a putative assumption, because the standard techniques to reveal dual wavefronts are not applicable during AF.

Recently, we demonstrated that a novel index, which we called His electrogram (HE) alternans, can be used to determine which wavefront, the SP or the fast pathway (FP), reaches the His bundle. 5 The term alternans implies that, depending on the atrial coupling interval, the HE amplitude exhibits 2 characteristic levels, low and high. Specifically, during beats with long coupling intervals (eg, sinus beats), the dominant FP wavefront first reaches the superior domain of the His bundle. The ensuing longitudinal activation of the superior His bundle fibers results in early and high-amplitude superior HEs (SHEs), whereas the transverse propagation into the inferior domain produces a later and lower-amplitude inferior HEs (IHEs). In contrast, during beats with short coupling intervals, the conduction blocks within the FP because of its longer effective refractory period (ERP). Now the dominant SP first reaches the inferior His domain, and the synchronized longitudinal activation of inferior fibers produces high-amplitude IHEs, whereas the transverse superior His bundle activation results in later and lower-amplitude SHEs. This phenomenon can be applied, by monitoring the His bundle electrogram alternans, to determine which wavefront is responsible for the propagation of the individual beats during generation of the standard AVN conduction curve. 5

We hypothesized that this novel index could be applied during AF as well. Accordingly, in the present study, we used HE alternans for monitoring AVN conduction during AF and attempted to answer the following questions: (1) Are dual pathways involved during AF? (2) Is it possible to selectively modify the SP conduction by using the HE alternans as a guide? (3) How does such modification affect ventricular rate during AF?

**Methods**
Rabbit AVN Preparations
The experiments were performed on 12 New Zealand White rabbit (Harlan, Indianapolis, Ind) atrial-AVN preparations that were instru-
mented as described previously. Briefly, after anesthesia with sodium pentobarbital (50 mg/kg), the heart was removed, placed in a glass chamber, and superfused with modified oxygenated Tyrode’s solution at 36°C, a pH of 7.3 to 7.4, and a flow rate of 35 mL/min. After trimming, the final preparation contained the triangle of Koch and the surrounding right atrial and ventricular tissues. (A photograph of an AVN preparation is shown in Figure 6)

**Electrical Stimulation and Recordings**

Bipolar leads (0.2 mm spacing) were custom-made from 125-µm Ag–AgCl Teflon-isolated wire and used to record atrial electrograms at the crista terminals and interatrial septum, as well as for atrial pacing. Roving bipolar electrodes were used to record SHEs and IHEs as reported previously. All electrodes were positioned with micromanipulators (WPI, M330). An 8-channel, programmable stimulator (AMPI, Master-8) was used for pacing. The recorded signals were amplified and filtered at 50 to 3000 Hz (Axon Instruments, CyberAmp 380), saved on tape (Vetter Digital, 4000A), and later digitized by AxoScope (Axon Instruments) at 200 µs per sample per channel.

**Pacing Protocol and Definition of Electrophysiological Terms**

All preparations were first paced at a basic cycle length (A1A1 interval) of 300 ms, and a standard AVN conduction curve was generated by interposing a premature A2 stimulus after every 20th basic beat A1. The prematurity coupling interval A1A2 was progressively shortened in 5-ms steps until the occurrence of AVN block. The resultant atrial-His conduction times A1A2 were plotted versus A1A2 prematurities. The AVN ERP was defined in a standard way.

Random, high-rate, atrial pacing was then used to simulate AF.

Coupling intervals in the range of 75 to 125 ms were generated by custom-written software. During AF, 2000 His-His (H-H) intervals were measured to determine the shortest, longest, and average values.

Low- and high-amplitude HEs were defined as 2 distinct signal levels recorded from the inferior and superior domains of the His bundle during different prematurities. Thus, the IHE was low during FP conduction at coupling intervals typically >170 ms. In contrast, the IHE was high during SP conduction at coupling intervals <170 ms. Because HE alternans were present in both the SHE and IHE recordings in a complimentary (opposite) manner, for simplicity of presentation, only IHE traces are illustrated in some figures.

The above observations were made in controls and repeated after SP modification.

**SP Modification**

To facilitate the precise location of the putative SP, we used miniature (<2-mm²) thermoelectric probes to cool the tissue in the inferior AVN approaches up to 15°C. The local block produced was fully reversible. Usually, clear modification effects were noted at the midpoint of the base of the triangle of Koch. The position was considered appropriate when the cooling resulted in a longer AVN ERP, no changes in the conduction time of the basic beats, and elimination of high IHEs during short coupling intervals or AF.

After determination of the best ablation position by cooling, a surgical cut was made (see Figure 6). Successful permanent block of the SP conduction was achieved in all 12 preparations. This was confirmed by repeating the protocol with generation of conduction curves and HE recordings during AF.

**Morphological Examination**

The AV conduction system was studied by serial sectioning perpendicular to the endocardial surface and oriented parallel to the AV conduction axis. As described previously, sections were cut at 7-µm steps, and every 10th section was retained. Alternate sections were stained by hematoxylin-eosin and Weigert–van Gieson stains. In this manner, it was possible to examine all major components of the conduction system, including the inferior approaches, the AVN, the superior approaches, and the penetrating bundle of His.

**Statistical Analysis**

All data are expressed as mean±SD where appropriate. Comparisons before and after SP modification were performed by paired Student’s t test. A value of P<0.05 was required for statistical significance.

**Results**

**Demonstration of Dual-Pathway Conduction During AF**

As previous reported, HE alternans were readily observed during the generation of the conduction curves. One example is presented in Figure 1 (left). For each prematurity A1A2, the conduction time A1A2 (gray data-points) is shown along with a pair of HEs. FP conduction (at long prematurities) resulted in a high SHE and a low IHE. In contrast, SP conduction (at short prematurities, arrows) produced an opposite phenomenon, with low SHE and high IHE. During AF (Figure 1, right), HE alternans were clearly evident. Note that a high SHE was always paired to a low IHE, and vice versa. This indicated that, in individual beats during AF, either the FP or the SP was dominating the AVN conduction. The described pattern was observed during AF in 9 of the 12 hearts.

Figure 2 shows another preparation, in which HE alternans were present while the conduction curve was being generated (Figure 2, left), confirming that both pathways were intact. However, during AF (Figure 2, right), only pairs of high IHEs and low SHEs were seen, indicating that only the SP conduction was present. This pattern was seen during AF in 3 hearts. There was no difference in average HH interval during AF in these 3 hearts compared with the other 9 hearts with mixed FP and SP conduction (204±14 versus 202±14 ms, P>0.05). However, as seen in Figures 1 and 2, the transition from FP to SP occurred at longer A1A2 in these 3 hearts (197±11 versus 161±18 ms, P<0.05). This might explain why there was a predominant SP in these 3 hearts during AF.

No preparation revealed solely FP conduction during AF (ie, only low IHEs).
Use of Localized Cooling to Guide the SP Modification

When the probe was properly placed, a graded elimination of SP conduction was achieved by progressive cooling. Figure 3 illustrates the observations in 1 heart during AF. Note that in controls at 36°C (A), the SP conduction was present in 60% of the beats (high IHEs). Cooling to 28°C (B) eliminated most of the high IHEs, so that 85% of all beats were conducted via the FP (low IHEs, *). At 20°C, the SP was completely blocked and the conduction used the FP exclusively (C). This progressive cooling was also associated with a graded slowing of heart rate (note that the total number of beats was reduced from 30 to 27 to 23 for the same time interval in Figure 3).

After exploration with the cooling probe, a surgical cut was placed to produce permanent SP modification, as shown below.

Effects of Permanent SP Modification on AVN Conduction Curve and HE Alternans

Figure 4 shows 1 example of the effect of permanent SP modification on AVN conduction curve (C) and HE alternans (A, B). Conduction curves are plotted as in Figure 1. IHEs corresponding to each prematurity A1A2 are shown before (A) and after (B) modification. A surgical cut eliminated HE alternans seen in A and resulted in only low IHEs (B). Accordingly, truncated left-hand, SP portion of control conduction curve was cut (C). See text for details.

The presence of only FP after the modification was confirmed by the only low IHE (Figure 4B), in contrast to the HE alternans observed before modification (Figure 4A). Note also that after the modification, the ERP of the FP shortened: the last low IHE was at A1A2=190 ms (B, *), versus A1A2=210 ms in control (A, *). (It should be noted, however, that the precise ERP of the FP before the ablation remains uncertain, because the FP wavefront might have been either blocked or just sufficiently delayed and replaced by the SP.)

The summarized data from all 12 hearts showed that SP modification guided by the HE alternans did not affect the basic AVN conduction time (68±7 ms in control versus 66±7 ms after modification, P>0.05) but significantly prolonged AVN ERP (from SP-determined ERP of 99±9 ms in control to FP-determined ERP of 147±21 ms after modification, P<0.001). Also, the ERP of the FP, determined by the IHE as illustrated in Figure 4 (*), was consistently shorter after the modification (147±21 versus 171±23 ms, P<0.001).

HE Alternans–Guided SP Modification During AF

The effects of permanent SP modification resembled those observed with extreme cooling (Figure 3). Figure 5A (left) is
Morphological Basis of Slow-Pathway Modification

Serial sections revealed that the cuts, which successfully blocked SP, inflicted damage within the inferior approaches (posterior extension) of the AVN. However, the major part of the AVN remained intact (Figure 6). This indicates that the inferior approaches are a major structure in which the formation of the SP wavefront takes place.

Discussion

Major Findings

The present study has demonstrated for the first time in rabbits that HE alternans provide a convenient way to monitor the dual-pathway electrophysiology and to guide AVN modification during AF. The occurrence of HE alternans during AF indicates that both FP and SP participate in AVN conduction. This study provides strong evidence suggesting that SP modification is the major underlying mechanism responsible for the therapeutic effect of this ablation procedure when used to control the ventricular rate during AF.

Dual-Pathway Conduction During AF

Dual-pathway electrophysiology was initially adopted to explain the AVN reentrant tachycardia (AVNRT). A model in which an FP wavefront originates in the superior AVN approaches and a SP wavefront originates in the inferior approaches has been generally accepted. This model has led to a successful clinical application of curative ablation of AVNRT. However, its applicability to the AVN conduction during AF remained putative.

It has been suggested that ablation of the SP or partial damage of the compact AVN might be the mechanism that explains the benefits of the treatment for ventricular rate control. This was indirectly supported by several clinical studies in patients with proven dual-pathway electrophysiology and AVNRT in which fast ventricular rate could be slowed after ablation.

In the absence of AVNRT, the dual-pathway conduction can be deduced only by a jump (>50 ms) in the AVN conduction curve for a small shortening of prematurity \( A_{AVN} \) (≤10 ms). This criterion is clearly not applicable for patients with chronic AF, in whom a standard electrophysiologic study cannot be completed. Thus, whether dual pathways or only the SP was involved in AVN conduction during AF has never been clarified.

HE Alternans: A Novel Index of Dual-Pathway Electrophysiology During AF

In a previous study, we demonstrated that the FP and the SP reach the bundle of His differently, producing characteristic HE alternans. Specifically, at long coupling intervals the FP reaches the superior domain of the His bundle first, resulting in a high SHE and a correspondingly low IHE. The roles become reversed at short coupling intervals (Figures 1, 2, and}

### Table: H-H Intervals (Shortest, Average, and Longest) and the Percentage of Beats With Low IHE (FP Conduction) and High IHE (SP Conduction) in 12 Preparations Before and After SP Modification During AF

<table>
<thead>
<tr>
<th></th>
<th>Shortest H-H, ms</th>
<th>Average H-H, ms</th>
<th>Longest H-H, ms</th>
<th>Low IHE (FP), %</th>
<th>High IHE (SP), %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>158±9</td>
<td>204±14</td>
<td>387±84</td>
<td>38.8±29.9</td>
<td>61.2±29.9</td>
</tr>
<tr>
<td>After SP modification</td>
<td>187±31</td>
<td>276±51</td>
<td>594±119</td>
<td>99.0±1.5</td>
<td>1.0±1.5</td>
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<td>( P )</td>
<td>(&lt;0.009)</td>
<td>(&lt;0.001)</td>
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5). By monitoring HE alternans, one can easily determine which wavefront reaches the bundle of His in a particular beat.

We have now applied the HE alternans to monitor, in effect to “visualize,” the AVN conduction on a beat-by-beat basis during AF (Figures 1 to 3 and 5). The data have demonstrated that both FP and SP are involved in AVN conduction during AF in most cases. In fact, up to 39±30% of the conducted beats during AF were with the FP signature (Table), producing a low IHE (Figure 1). In only 3 of the 12 cases did we see near-exclusive SP conduction during AF with high IHE (Figure 2). However, even in these 3 hearts, after SP modification, the dormant FP was revealed (Figure 5B).

HE Alternans–Guided Modification of AVN Inferior Approaches

Modification of the inferior AVN approaches was done by placement of surgical cuts close to but away from the compact AVN (Figure 6). This modification prolonged AVN ERP without affecting the basic conduction time, as previously reported. However, in cases with smooth conduction curves, it is difficult to determine whether SP was only partially damaged or totally blocked.

This uncertainty was resolved by the observation of the HE alternans. We noticed that in some cases, an initial prolongation of the AVN ERP after the first surgical cut could coexist with the presence of high IHE at the shortest A1A2 prematurities. This indicated that the SP was still not blocked. A subsequent more distal cut was needed to fully eliminate the HE alternans and establish an even longer ERP (determined now by the FP). In this regard, HE alternans were always a more sensitive criterion for SP modification compared with only monitoring the AVN ERP.

This study clearly demonstrated that SP modification resulted in a predominantly FP conduction during AF, evidenced by the observation of low IHE in the vast majority of the conducted beats (Figure 5). The average ventricular rate was significantly slowed after SP modification (Table). In addition, the shortest H-H intervals (thought to represent the functional refractory period of the AVN during AF) were significantly prolonged. The excellent correlation observed between the selective modification of the AVN input and the change in the HE alternans (Figures 3 through 5) validates the latter as a reliable index of dual-pathway electrophysiology during AF.

Substrate of SP and a Functional Model of Dual-Pathway Electrophysiology During AF

The morphological evidence indicates that SP modification inflicted damage within the inferior approaches to the AVN. However, the major part of the AVN remained intact (Figure 6). These results are consistent anatomically with the concept of an SP using the atrionodal input via the posterior nodal extensions.

Our findings could be explained by use of the following functional model. Figure 7A illustrates the beats with FP conduction during AF in an intact preparation. The FP wavefront activates the transitional cell region and reaches the superior domain of the His bundle, producing a high-SHE/low-IHE pair (see Figure 1). At that time, the SP, being formed in the inferior approaches, still traverses the posterior nodal extensions and the AVN itself.

Figure 7B illustrates the beats with SP conduction during AF in an intact preparation. In this case, the FP conduction is blocked, presumably within the “bottleneck” formed at the connection with the superior domain of the His bundle. It is therefore possible now for the SP to propagate all the way to the inferior domain of the His bundle, resulting in a high IHE.

According to this model, an FP pattern of HE in a given beat (ie, a low IHE) indicates that the fast wavefront was ahead of the slow wavefront for this beat (as in Figure 7A). Similarly, an SP pattern (ie, a high IHE) would indicate that the FP was blocked (as in Figure 7B).

Figure 7C illustrates the conduction during AF after SP ablation. Successful modification would shift the conduction pattern to the FP, resulting in a substantial reduction of the number of propagated impulses. In addition, for the majority of the conducted beats, only low IHEs would be recorded (Table; 99±1.5% of the beats).

Figure 7D explains why high IHEs could be seen sporadically during AF even after the SP modification (Table; 1±1.5% of the beats). In such cases, a block in the FP and
subsequent penetration of the AVN by intermediate wavefronts would produce a conduction pattern similar to the one illustrated in Figure 7B.

Our model supports the concept that the SP and the FP are not isolated cable-like structures. Not only does the morphological evidence argue against the presence of cable channels, but the functional electrophysiological observations also support the hypothesis that the atrionodal approaches are highly inhomogeneous conduction media. Although they may support 2, 3, or more functional wavefronts, especially during AF, no discrete boundaries between them have been delineated. The observations from the present study support similar conclusions. Thus, the removal of the SP produced a functional change in the remaining FP (Figure 4), an observation also made in patients and attributed to an electrotonic inhibition exerted by the SP. Furthermore, if the SP were an isolated cable, then any cut across the inferior approaches would eliminate its conduction. Instead, an “ideal” ablation placement was required, and even then, some high IHEs (ie, SP beats) were still present during AF.

Implications of the Reported Findings and Study Limitations

This study demonstrates that HE alternans are a useful novel tool to monitor dual-pathway electrophysiology on a beat-by-beat basis and can be used to guide AVN modification during AF.

This conclusion is based on the evidence obtained from rabbit hearts. However, HE alternans have also been demonstrated in vivo in dogs. Moreover, we have noticed HE changes resembling alternans in published clinical traces, although systematic clinical reports are not available and further evidence is needed to establish the presence and functional importance of HE alternans in humans.

References


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