His Electrogram Alternans Reveal Dual-Wavefront Inputs Into and Longitudinal Dissociation Within the Bundle of His

Youhua Zhang, MD, PhD; Saroja Bharati, MD; Kent A. Mowrey, MS; Shaowei Zhuang, MD; Patrick J. Tchou, MD; Todor N. Mazgalev, PhD

Background—His electrogram (HE) amplitude and morphology changes were observed in our previous studies during transition from “fast” to “slow” atrioventricular nodal (AVN) conduction. This phenomenon and its significance for the dual-AVN electrophysiology are not well recognized and have not been studied.

Methods and Results—Experiments were performed on 17 healthy rabbit atrial-AVN preparations during standard programmed electrical pacing. HEs were mapped along the His bundle with roving surface electrodes, along with recording of cellular action potentials (APs). HEs recorded from the superior margin of the His bundle were of greater amplitude during basic beats and decreased substantially, by 42±19% (P<0.01), when premature A1A2 shortened to 178±20 ms. In contrast, the HEs from the inferior margin increased dramatically, 2.9±1.7 times (P<0.01), during short A1A2, and remained high until AVN block occurred. In addition, during long A1A2, the superior HEs consistently preceded the inferior by 1.9±0.7 ms. In contrast, at short A1A2, the superior HEs occurred 2.7±0.8 ms after the inferior. Cellular AP recordings demonstrated clearly the presence of and the transition between early (fast) and late (slow) excitation wavefronts that accompanied HE alternans.

Conclusions—The morphological-electrophysiological evidence from the AV junction suggests that fast and slow wavefronts reach the His bundle differently, producing functional longitudinal dissociation into 2 domains. The characteristic HE alternans recorded from these domains are a new sensitive tool to determine the presence of distinctly different wavefronts and their participation in the conduction during reentrant or other arrhythmias. These findings provide further understanding of the mechanisms of dual-AVN electrophysiology. (Circulation. 2001;104:832-838.)

Key Words: electrocardiography ■ atrioventricular node ■ His bundle ■ electrophysiology

Although early understanding of dual-atrioventricular-node (AVN) electrophysiology and AVN reentrant tachycardia (AVNRT) comprised a longitudinal dissociation into α- and β-pathways confined to the compact AVN and followed by a common final pathway,1 recent studies established that the atrial approaches to the AVN remote from the compact node are also involved in AVNRT. Moreover, longitudinal dissociation in the His bundle has been proposed as the underlying substrate of some arrhythmias.2-4 We have previously observed that His bundle electrogram (HE) changes (an increase in HE amplitude) occur during very short premature coupling intervals.5,6 We have also searched recent literature and found HE alternans in traces published by others.7,8 This phenomenon and its significance are not recognized, however, and have not been critically evaluated. Accordingly, the present study was designed to systematically evaluate the HE alternans and their possible role in the mechanism of the dual-pathway AVN electrophysiology and arrhythmias originating from the AV junction.

Methods

Rabbit AVN Preparations

The experiments were performed on 17 New Zealand White rabbit atrial-AVN preparations, instrumented as previously described.9 Briefly, after sodium pentobarbital (50 mg/kg) anesthesia, the heart was removed and placed in an oxygenated Tyrode’s solution (in mmol/L: NaCl 128.5, KCl 4.7, CaCl2 1.3, MgCl2 1.05, NaHCO3 25, NaH2PO4 1.19, and glucose 11.1; pH 7.3 to 7.4 at 35.5°C; saturated with 95% O2/5% CO2; flow rate 35 mL/min). After trimming, the final preparation contained the triangle of Koch and the surrounding right atrial tissues (see Figure 2).

Electrical Stimulation and Recordings

Bipolar leads, custom-made from 125-μm Ag-AgCl Teflon-isolated wire with 0.2-mm spacing, recorded atrial electrograms at the crista terminalis (CrT) and interatrial septum (IAS). Roving bipolar electrodes, used to obtain HEs, were initially placed far superior (or inferior) to the penetrating His bundle and were gradually moved down (or up) until clear His signals were first seen. These HEs are referred to as superior or inferior HEs, respectively. The 2 domains were also explored longitudinally at 0.5-mm steps over an ~4-mm distance starting at the apex of Koch’s triangle (Figure 2).
platinum-iridium leads of similar design were used for atrial pacing (2 ms, twice the diastolic threshold). All electrodes were positioned with micromanipulators (WPI, M330) and connected to optically isolated stimulator units (WPI, A360) and an 8-channel programmable stimulator (AMPI, Master-8) or to high-resistance, differential-input probes and then to a 16-channel programmable amplifier at 50 to 3000 Hz (Axon Instruments, CyberAmp 380). Signals were monitored on a storage oscilloscope (Tektronix 2216) and digitally saved on tape (Vetter Digital, 4000A) for offline computer analysis (AxoScope, Axon Instruments).

All preparations were paced at a basic cycle length (interval A1A1) of 300 ms, typically from the CrT. The mean basic conduction time A1H1 (from CrT to His) was 77±12 ms. The AVN conduction curve was generated by periodic interruption of the basic A1A1 drive with progressively shorter premature stimuli A2 until the occurrence of AVN block, and the A2H2 conduction times were plotted versus A1A2 prematurities.

For quantitative evaluation of HEs alternans, we determined relative changes in amplitude for each recording site so that only the effects of the A1A2 prematurity (and not those of electrode-tissue contact or amplification) were included. Thus, the terms “high” and “low” refer to HE amplitude at shorter prematurities versus basic rate. For bipolar recordings, the wire tips were oriented parallel to the edge of the tricuspid valve. In several cases, monopolar signals were recorded and juxtaposed with the bipolar HEs. Tape-recorded data were digitized by AxoScope (200 μs per sample per channel). All data are expressed as mean±SD where appropriate. The paired t test was used to compare HEs during slow- and fast-pathway conduction. Significance was assumed at a value of P<0.05.

We used standard glass microelectrodes to record action potentials (APs) from single AVN cells. Anatomic location, AP morphology and amplitude, and dAP/dt (~10 V/s), as well as cycle-length dependency, were used to identify signals originating from the compact region.

**Modifications of AV Nodal Approaches**

To correlate the HE changes with the putative presence of fast and slow wavefronts, the anterior or posterior inputs were cooled (range 35°C to 15°C) with miniature thermoelectric probes.9 The small size of the probes (2 mm2) permitted localized barriers for propagation to be produced that selectively affected each of the wavefronts. The cooling effects were fully reversible.

In 4 preparations, we applied surgical cuts to isolate the anterior and posterior AVN inputs while preserving the compact node, as previously reported.10 In these cases, pacing was applied at either the posterior or anterior inputs separately or in combination. HEs were recorded from the inferior His domain along with cellular AP from nearby His fibers.

**Morphological Examination**

The AV conduction system was studied by serial sectioning. As described previously, sections were cut at the 6-μm level. Each 10th section was retained.11 Alternate sections were stained by hematoxylin-eosin and Weigert–van Gieson stains. In this manner, a total of 776 sections were examined.

**Results**

**Morphological Evidence and the Working Model**

The AVN was situated adjacent to the tricuspid valve with its superior and inferior approaches on the right AV septal junction. Serial sections revealed that most of the fibers from the inferior aspect became the compact part of the AV node adjacent to the central fibrous body (CFB). These fibers, after piercing into the CFB, formed the major, inferior portion of the penetrating bundle. At that point, the node still retained the superior approaches (Figure 1). The superior AV nodal fibers were fewer and last to penetrate the CFB in the
formation of the bundle. The penetrating AV bundle was quite short, and this formed the branching bundle that gave off the posterior radiation of the left bundle branch fibers on top of the ventricular septum. The branching bundle became the bifurcating bundle and gave off the anteriormost fibers of the left bundle branch and the right bundle branch fibers.

On the basis of morphological architecture, we developed the following working hypothesis (Figure 2). The penetrating His bundle forms 2 asymmetrical functional domains at the junction between the atrial approaches and the penetrating axis. The inferior domain (brown) contains the majority of fibers and is directly linked to the major component of the AVN (red), whereas the superior domain (blue) is smaller and connected to the septum via transitional fibers (violet).

Within the penetrating His bundle, the 2 domains communicate across the fiber length (small green arrows).

The fast wavefront uses the “bottleneck” formed by selective transitional fibers from the anterior septal input into the superior His domain (Figure 2C, large green arrow). Therefore, at long prematurities, the superior HEs (Figure 2C, red and yellow electrodes) would be generated by the fast wavefront first, and their relative amplitudes would be high. The inferior domain would be activated across the fiber orientation (small green arrows), thus yielding later and low-amplitude inferior HEs (black electrodes).

With prematurity shortening, the fast wavefront “dies” within the transitional cells, whereas the propagating slow wavefront reaches the inferior domain (Figure 2D, large green arrow). The simultaneous activation of a large number of inferior fibers plus the propagation along their axis result in a high-amplitude inferior HE. In contrast, the superior domain will now be invaded by this slow wavefront transversely (small green arrows), resulting in later and low-amplitude superior HEs.

Several lines of evidence will be presented in support of this working hypothesis.

**Cycle Length- and Position-Dependent Changes in HE Amplitude**

HE mapping revealed unique, characteristic, cycle length—and position-dependent alternans in all preparations. A typical example of such signal morphology changes during programmed stimulation is illustrated in Figure 3. The top panel shows superior HEs plotted against the A1A2 prematurities. The middle panel shows simultaneously recorded inferior HEs. The bottom panel is the conduction curve. The superior HEs were of greater amplitude during longer prematurities and diminished when A1A2 shortened (in this case, to 160 to 150 ms). The average reduction of superior HEs was 42±19% (P<0.01, range 17% to 72%, n=17). In contrast, the inferior HEs were of smaller amplitude during long prematurities but increased dramatically with shortening of A1A2 (here at A1A2=170 to 160 ms) and remained high until AVN block. The average increase of inferior HEs was 194±168%, P<0.01, range 30% to 690%, n=17.

The above-described behavior was observed when HEs were recorded up to 4 to 5 mm from the apex of the triangle of Koch along the penetrating His bundle. The average prematurity at which the alternans occurred was A1A2=178±20 ms, and the inferior HE increases usually preceded the superior HE decreases. The HE alternans were more pronounced and easier to see in the inferior domain.

Although larger relative changes in the HEs were observed when the bipole were carefully oriented along the tricuspid valve ridge (Figure 2), similar alternans were recorded by monopolar electrode configuration (not shown).

**Timing of the Superior and Inferior HEs**

Shortening of A1A2 affected not only the relative amplitudes of the HEs but also the timing between the superior and inferior signals, as illustrated in Figure 4 from one experiment. At long coupling intervals, the superior HE always preceded the inferior one (panel A, average 1.9±0.7 ms). At A1A2=170 ms, the 2 HEs were almost simultaneous (panel D), and further shortening of A1A2 was associated with a progressively earlier inferior HE in relation to the superior HE (panels E and F, average 2.7±0.8 ms). The small changes in the relative timing are consistent with conduction across the small transverse dimensions of the penetrating bundle.
They were reproducible and were observed in each preparation at various His recording sites.

HE Alternans and Their Relation to Nodal and His Cellular Activities

Figure 5 shows that during basic beats or at long prematurities, conduction via the fast wavefront promptly activated the impaled N cell and produced a low-amplitude inferior HE (panel A). Premature shortening attenuated this wavefront and, beginning from A₁A₂=190 ms, the AP gradually dissociated into early and late components. The former component gradually declined to an electrotonic hump (panels B through F, arrows), whereas the latter grew and was present until AVN block occurred (curved arrows). Panels G and H detail the response during beat A₂ for 16 progressively shorter A₁A₂ prematurities from 300 to 118 ms (illustrated with different colors). For clarity, the cellular traces are shifted downward with each prematurity, whereas the His electrograms are superimposed on the same trace. The separation of the AP upstroke into early decremental and progressively late components with shortening of A₁A₂ is shown in G (arrows), and the time derivatives dAP/dt in H demonstrate the increasing delay between the arrival of the fast and slow wavefronts at this impaled fiber (arrows). Note that for all A₁A₂=190 ms (red traces) and shorter, the penetrating His bundle has been reached by the slow wavefront first (high-amplitude HE), even though (judging by the inscription of AP, panels G and H) the fast wavefront may still have contributed to this fiber’s depolarization for A₁A₂ as short as 150 ms (green traces).

Figure 6A illustrates nodal-His (NH) APs recorded close to the inferior His electrode during long-lasting, irregular, atrial rhythm. The atrial spikes, a, can be seen on the superior HE trace (⊥ indicates blocked beats). Note that the amplitude alternans in the superior versus inferior HE were always reversed, ie, high superior–low inferior and vice versa. On the basis of previous analysis, we concluded that alternative slow and fast wavefronts reached the His bundle during individual beats of the tachycardia. For example, the slow wavefront produced the high-amplitude inferior and low-amplitude superior HEs (pairs marked with *). This can also be concluded from the longer conduction delays in these beats, compared with the beats with high superior–low inferior HE (pairs marked with ⊥), conducted via the fast wavefront. No correlation was determined, however, between the dramatic HE alternans and the cellular NH AP parameters (amplitude, dV/dt, or diastolic interval). Conversely, the cellular activity in the compact node (Figure 6B, N-AP) was highly inhomogeneous, containing low-amplitude and multihumped APs corresponding to the blocked atrial beats (⊥). Again, the pattern of His engagement could not be deduced from the individual cellular N-APs but were easily determined from the HE alternans (* and ⊥ pairs).
Effects of Modifications of AVN Approaches on HE Alternans

Figure 7 (A through C) illustrates cooling of the anterior input. In control, both the basic A1 and premature A2 beats were conducted via the fast wavefront, and low-amplitude inferior HEs were observed as expected (panel A). At 30°C (panel B), the basic beats still traversed the cooled region and conducted via the fast wavefront (notice conduction delay prolongation from 72 to 79 ms). The premature beat, however, was conducted via the slow wavefront (high-amplitude inferior HE, curved arrow). Further cooling to 24°C completely blocked the fast wavefront. High-amplitude inferior HEs were inscribed in both the basic and premature beats (panel C, curved arrows), and there were reduced superior HEs.

Figure 7 (D through G) illustrates cooling of the posterior input in a different heart. In control, the transition from fast to slow wavefront propagation occurred at A1A2=180 ms, when the high-amplitude inferior HE appeared (panel D, curved arrow). Cooling of the posterior input to 33°C slightly delayed this slow wavefront, so that the fast wavefront prevailed again (panel E, low-amplitude inferior HE, *). At even shorter prematurity, however, A1A2=130 ms, the fast wavefront was fully eliminated (panel F, high inferior HE, curved arrow), and now posterior input cooling (panel G) did not change the His activation pattern but only prolonged the premature beat conduction time. Note that posterior cooling did not affect the conduction of the basic beats A1. The simultaneous AP recordings (not shown) confirmed this conclusion by demonstrating that only the later AP components (like those marked with curved arrows in Figure 5) were selectively delayed (or blocked) during posterior cooling.

Observations from 1 of the 4 preparations with separated atrial inputs into the AVN are illustrated in Figure 8. This model permitted independent pacing from either the CrT, the IAS, or both input sites. The HEs and the cellular APs were recorded from the inferior His domain (D, inset), and only the responses to the premature beat A2 are shown. In panel A, shortening of IAS premature A1A2 from 185 to 155 ms illustrates the transition between fast and slow wavefront conduction. The former was evident until A1A2=175 ms (low-amplitude HE and pronounced electrotonic foot development in the AP upstroke, arrow). At A1A2=155 ms, the fast wavefront has been eliminated (blocked), and the high-amplitude HE (red trace, *) indicated that the His bundle was engaged via a slow wavefront. Pacing from the CrT input (panel B) resulted only in high-amplitude HEs (red traces, *). Simultaneous pacing (panel C) resulted in a His engagement pattern similar to the one seen in panel A. The shorter conduction delays in this case, however, suggested a summation of the 2 wavefronts.

The conduction curves (shown only for A1A2 in the range 185 to 155 ms) along with the HEs indicated which wavefront was dominant, depending on the prematurity and the pacing site. All 8 data points in panel D marked as slow wavefront (black symbols) were associated with high-amplitude inferior HEs (red traces in A, B, and C). The fast wavefront (black symbols) was present only when the IAS input was paced at prematurities A1A2≥175 ms and produced low-amplitude HEs (black traces in A and C).

Discussion

Major Findings

The present study provides direct evidence that a substantial portion of the penetrating His bundle is normally involved in dual-pathway electrophysiology. The fast and slow wavefronts reach the penetrating AV bundle via 2 different inputs, and the bundle itself is longitudinally dissociated into superior and inferior domains, respectively. This duality is in accordance with the morphological findings12–14 that the penetrating bundle initially contains a large number of fibers coming directly from the inferior aspect (the compact node), whereas a relatively smaller number of fibers from the superior aspect join the bundle later.

Our results have revealed that the early (fast) wavefront longitudinally activates the superior portion of the His bundle, whereas the late (slow) wavefront engages the inferior domain. During fast wavefront propagation, the His bundle...
activation is in a superior-to-inferior direction. During slow wavefront propagation, in contrast, it is just the opposite.

**Dual Inputs Into the His Bundle**

The concept of electrophysiologically dual pathways encompassing the AVN approaches and the compact node itself is well accepted. Although arguments still continue about their number and whether they constitute functional or anatomic substrates, the dual pathways are commonly considered to be atrionodal entities. The penetrating bundle is usually considered a cable-like structure surrounded by the CFB and distanced from the supraventricular reentrant loop by an interposed common final pathway.

Our results provide evidence that the transition from node to bundle may be more complex and that instead of a homogeneous common final pathway, there are functionally distinct domains in the His bundle. The detailed morphological evidence previously described suggests that the His bundle is not simply a compact AVN extension but instead has a rather heterogeneous connection with the atrial site of the AV conduction axis. As demonstrated in the present study (Figure 1), the major components of the deeply located inferior compact node fibers, after penetration into the CFB, progressively form the inferior domain. Superiorly, however, the penetrating His bundle is in contact with transitional cells that form part of the anterior input. The present results indicate that the activation of the His bundle takes place differentially via the superior and inferior domains.

The superior His domain was activated earliest during basic rhythm (Figure 4), but progressive shortening of premature A1A2 resulted in reduction of the recorded HE (Figure 3). This behavior is in agreement with the established functional properties and anatomic location of the fast wavefront. Excessive decrement and/or block of the fast wavefront at short A1A2 permitted the slow wavefront to traverse the compact region and invade the inferior His domain. The latter was activated earliest at short prematurities (Figure 4) and was associated with a sharp increase of the inferior HE (Figure 3).

**Longitudinal Dissociation Within the His Bundle**

The proposed dual inputs into the penetrating bundle are conceptually related to the property of functional longitudinal dissociation of conduction within the bundle, which is by no means new. Histological studies demonstrated longitudinal separation of Purkinje strands by fine collagen septa and the presence of specialized intercellular junctions within each strand in the His bundle. Furthermore, functional transverse interconnections exist in the His bundle, and transverse conduction velocity is always less than the longitudinal propagation.

Clinical observations have suggested a longitudinal dissociation within the His bundle. Scherlag et al observed fractionated His bundle potentials in dogs. Later investigators rather freely used this concept to propose the existence of longitudinally separate fast- and slow-conducting His fibers in AVNRT or other arrhythmias.
HE Alternans and Dual-Pathway Physiology

Although the present results are in agreement with the previously proposed concept of longitudinal distal His bundle dissociation, they extend it further by demonstrating that the dual-pathway electrophysiology encompasses a much larger substrate, including the atrial approaches and the AVN, as well as the proximal nodal-bundle junction and at least a part of the penetrating bundle. In particular, we demonstrated that different His bundle domains were selectively activated by either the fast or the slow wavefronts.

The morphological evidence (Figure 1) suggests that the inferior domain contains the majority of fibers. Such asymmetrical anatomic architecture defines the inferior domain as a potential source of the strong driving force. When reached by the slow wavefront at short prematurities, the simultaneous activation of the vast number of inferior fibers produces the characteristic augmented inferior HE (Figures 3, 5, and 7).

In contrast, the superior HE is generated by a smaller number of fibers. They are simultaneously and longitudinally activated by the early (fast) wavefront at long prematurities. The decrement of the fast wavefront with premature shortening reflects on the declining HEs (Figure 3), and when it is blocked and replaced by the slow wavefront, the superior domain is transversely invaded. The transverse propagation is slower and less synchronous, therefore producing a weaker driving force responsible for the low superior HEs at short prematurities.

Proper orientation of the recording bipolar electrodes is important in accentuating the effects of the differential activation of the superior and inferior His domains. These effects, however, could be observed even with monopolar electrodes, suggesting that the profound differences in anatomic content of the domains and the properties of longitudinal versus transverse conduction are the major mechanisms underlying the HE alternans.

Implications of the Reported Findings and Study Limitations

These results expand our basic understanding of AV conduction by revealing that dual-pathway electrophysiology does not end with the AVN proper but rather also extends into the junctional region between the node and the penetrating His bundle. The AVNRT may therefore use the duality of the penetrating His bundle as well. The possibility to record His alternans may serve as a new tool in determining whether the fast or the slow wavefronts underlie the propagation of particular atrial beats. Thus, HE alternans during fast atrial rates (Figure 6), Wenckebach periodicity, or atrial fibrillation suggests an alternating participation of both wavefronts. Distinguishing the pattern of electrical excitation of the His bundle may provide valuable guidance during modifications in the triangle of Koch designed to alter the properties of the AV transmission.

Although the dual inputs into and the functional longitudinal dissociation within the His bundle were revealed by HE alternans in these rabbit hearts, there are interspecies differences. Although anatomically, there are some similarities in the formation of the penetrating AV bundle in the CFB in rabbits and humans, further electrophysiological evidence is needed to establish the presence and the functional importance of His alternans in humans.

Acknowledgments

This study was supported in part by grants from the American Heart Association, Ohio Valley Affiliate (9807701) and from the NIH-NHLBI (RO1-HL-60833-01A1).

References


His Electrogram Alternans Reveal Dual-Wavefront Inputs Into and Longitudinal Dissociation Within the Bundle of His
Youhua Zhang, Saroja Bharati, Kent A. Mowrey, Shaowei Zhuang, Patrick J. Tchou and Todor N. Mazgalev

Circulation. 2001;104:832-838
doi: 10.1161/hc3301.092804

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2001 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/104/7/832

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation is online at:
http://circ.ahajournals.org//subscriptions/