Atrioventricular conduction with and without AV nodal delay: two pathways to the bundle of His in the rabbit heart

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Hucker WJ, Sharma V, Nikolski VP, Efimov IR. Atrioventricular conduction with and without AV nodal delay: two pathways to the bundle of His in the rabbit heart. Am J Physiol Heart Circ Physiol 293: H1122–H1130, 2007. First published May 11, 2007; doi:10.1152/ajpheart.00115.2007.—The electrophysiological properties of atrioventricular (AV) nodal dual pathways have traditionally been investigated with premature stimuli delivered with right atrial pacing. However, little is known about the functional characteristics of AV nodal inputs outside of this context. Superfused rabbit triangle of Koch preparations (n = 8) and Langendorff-perfused hearts (n = 10) were paced throughout the triangle of Koch and mapped electrically and optically for activation pattern, electrogram and optical action potential morphologies, stimulation thresholds, and stimulus-His (S-H) intervals. Optical mapping and changes in His electrophysiology were used to confirm the activation pathway. Pacing stimuli ≥2 mm above the tricuspid valve caused fast-pathway activation of the AV node and His with a threshold of 2.4 ± 1.6 mA. An area directly below the coronary sinus had high thresholds (8.6 ± 1.4 mA) that also resulted in fast-pathway excitation (P < 0.001). S-H intervals (81 ± 19 ms) for fast-pathway activation remained constant throughout the triangle of Koch, reflecting the AV delay. Stimuli applied <2 mm from the tricuspid valve resulted in slow pathway (SP) excitation or direct His excitation (4.4 ± 2.2 mA threshold; P < 0.001 compared with fast pathway). For SP/His pacing, S-H intervals showed a strong dependence on the distance from the His electrode and were significantly lower than S-H intervals for fast-pathway activation. SP/His pacing also displayed characteristic changes in His electrophysiology. In conclusion, optical maps and S-H intervals for SP/His activation suggest that AV conduction via SP bypasses the compact AV node via the lower nodal bundle, which may be utilized to achieve long-term ventricular resynchronization.

resynchronization therapy; atrioventricular node; dual-pathway electrophysiology; optical mapping; slow pathway

IN NORMAL PHYSIOLOGY, THE atrioventricular node (AVN) serves as the gateway to the His-Purkinje system and is responsible for the delay between atrial and ventricular excitation. The AVN is located near the apex of the triangle of Koch (24), and it has at least two inputs, each with unique electrophysiological properties (22). The fast pathway (FP) input to the AVN, near the triangle apex, has a relatively short conduction delay and long refractory period, whereas the slow pathway (SP) input, in the isthmus between the tricuspid annulus and the coronary sinus ostium in both rabbits and humans (14, 16, 20), possesses a relatively long conduction delay and relatively short refractory period (16). The distinct functional characteristics of these pathways are critically important in clinical AVN reentrant tachycardia (AVNRT).

Because of the clinical importance of dual-pathway electrophysiology in AVNRT, studies investigating AVN inputs, and the SP in particular, have done so in the context of differential conduction between the FP and SPs, through the use of premature stimuli (S2 beats) applied in the high right atrium or on the crista terminalis (CrT) (20, 29). SP activation in such a manner engages the normal atrial myocardium, transitional cells (TCs), and the SP itself, with the end result of slower conduction to the His than through the FP. Without a premature S2 stimulus, the SP has very little involvement in atrioventricular (AV) conduction, normally acting as a dead end pathway (1). Therefore, there have been few studies that have investigated SP physiology without premature stimuli, although it has been established that the SP possesses unique pacemaking properties (8).

It has been commonly accepted that each AVN input funnels into a common pathway to the His bundle (21). However, the recent demonstration of “His electrogram alternans” by Zhang et al. (29) suggests that there may in fact be two separate pathways to the His bundle. This study revealed characteristic changes in His electrograms, which were caused by whether the His bundle was excited by the SP or the FP, arguing that the influence of the activating pathway spreads farther than the AVN to the His itself.

Because of the clinical sequelae associated with right ventricular pacing (18, 26), direct His bundle pacing has emerged as a cardiac resynchronization therapy (6, 7, 25, 27), and therefore the clinical relevance of His activation has increased. As its name implies, direct His bundle pacing stimulates the His bundle with a pacing catheter placed on the His bundle, at the apex of the triangle of Koch. However, to our knowledge, no studies have investigated pacing stimuli applied within the triangle of Koch, directly stimulating the SP and FP. We hypothesized that pacing stimuli applied in this region may reveal whether two pathways to the His bundle exist. In this study, we mapped conduction, electrogram morphologies, and pacing thresholds for pacing stimuli applied throughout the triangle of Koch. Our data suggest that the SP can be paced in a relatively large area of the right atrium to produce His activation in the rabbit, while avoiding the AV delay. However, the direct clinical relevance of this study will have to be proven in humans in the future.

METHODS

New Zealand White rabbits (n = 18, 2.5–3 mo old, 2–3 kg) were anesthetized with 100 mg/kg pentobarbital sodium and 1,000 IU heparin intravenously, after which a midsternal thoracotomy was performed and the AV node was isolated. The atrial appendages were isolated and ligated, and the atrioventricular groove was opened to the AV node. Heparin was administered intravenously, after which a midsternal thoracotomy was performed and the AV node was isolated. The atrial appendages were isolated and ligated, and the atrioventricular groove was opened to the AV node.
performed and the heart removed. All experiments were approved by the Animal Studies Committee of Washington University. The heart was Langendorff perfused with oxygenated (95% O₂-5% CO₂) Tyrode at 37°C and received 50 μl of 5 μM Di-4-ANEPPS (Molecular Probes, Eugene, OR) over 5 min. We conducted a study in superfused isolated AV junctions (n = 8) and in Langendorff-perfused hearts (n = 10) with the AV junction exposed via right atriotomy (a more complete description of the Langendorff-perfused preparation is provided in the online data supplement).² For superfused experiments, the AV junction was dissected in cold Tyrode (0°C), and the sinoatrial node was removed. The preparation was superfused at 30 ml/min with Tyrode containing 15 mM of the excitation-contraction uncoupler 2,3-butanedione monoxime (Sigma, St. Louis, MO) to prevent motion artifacts (4). A 16 × 16 photodiode array was used with an optical mapping system that has been described previously (9). Optical signals were sampled at 1.5 kHz, averaged, and low-pass filtered at 120 Hz. Optical activation maps displayed the optical signal derivative, which corresponds to wave fronts of excitation.

Electrogram recordings. Electrodes were placed on the interatrial septum (IAS) and CrT, and a quadrupole electrode on the His bundle recorded both the superior and inferior His electrogram (SHE and IHE, respectively) to monitor fast-His and slow-His excitation. Changes in His excitation and His electrogram morphology can occur from changes in the AVN excitation pathway, previously referred to as His electrogram “alternans” (28, 29) because of the specific pacing protocol and alternating conduction pathways. If the AVN and His are excited by the SP (i.e., slow-His excitation), the IHE has a larger amplitude than when the AVN is activated by the FP. Conversely, when the FP excites the AVN and His (i.e., fast-His excitation), the SHE has a larger amplitude than with slow-His excitation. The location of each electrode is shown in Fig. 1A.

A fourth roaming electrode was used to record electrograms throughout the triangle of Koch. A schematic of the triangle of Koch is shown in Fig. 1A. The reference lead was located 3 mm from the electrode tip. The Teflon-coated 0.13-mm Pt/Ir wire tip had 0.07 mm stripped to mimic a clinical hemispheric tip. This electrode was mounted on a force transducer (FORT25; World Precision Instruments, Sarasota, FL) to control contact force, ensuring that contact force was minimal and consistent between locations. The electrode was moved in 1-mm increments by a motorized micromanipulator throughout the triangle of Koch (grid in Fig. 1A). Electrograms were recorded at 1.5 kHz (National Instruments, Austin, TX), and each electrode location was digitally photographed.

Stimulation protocol. The preparation was paced from two electrodes: the IAS electrode and the roaming electrode (Fig. 1A). IAS pacing was constant at 2× threshold (~2 mA), with 2-ms pulses and 300-ms cycle length (Fig. 1B). IAS pacing simulated sinus pacing and was used to mask the AV junctional rhythm, which originates in the SP (8), as well as to maintain tissue excitability in a consistent state as the roaming electrode was moved from location to location. Stimulation thresholds were determined with a ramp of unipolar pulses (0.5-ms pulses, 300-ms cycle length, amplitude ranging from 0.33 to 10 mA and increasing by 0.33 mA with each pulse; Fig. 1B). Each ramp pulse was delivered 45–60 ms before an IAS pacing pulse (Fig. 1B). The pacing ramp was delivered from the roaming electrode for anywhere from 14–24 locations throughout the triangle of Koch (grid in Fig. 1A), allowing quick determination of threshold for each location. Pacing threshold was defined as the amplitude of the ramp pulse that caused a shift in the activation pattern from IAS pacing to stimulation beginning from the roaming electrode.

Statistics. Statistical correlations were determined with the Pearson correlation coefficient R using a two-tailed Student’s t-test to determine whether the null hypothesis of R = 0 was true, indicating that the two variables were not correlated (13). Group comparisons were performed with a two-sample Student’s t-test. Data are presented as means ± SD.

RESULTS

Identifying the SP. The schematic in Fig. 1A indicates the approximate location of the inputs to the SP and FP. Although the anatomic substrate of the SP is often thought to be the inferior nodal extension (INE) (16, 20), the anatomic substrate of the FP is less well defined and consists of TCs that overlay the compact AVN. During IAS pacing, the AVN is activated by the FP, and the SP acts as a dead-end pathway. Yellow electrograms all have a sharp signal immediately after the pacing artifact, which signifies fast conduction through the atrial myocardium. Yellow OAPs have atrial action potential morphology. Blue electrograms contain both a fast signal directly after the pacing artifact (similar in timing to that seen in yellow traces) and another sharp spike ~80 ms later, which reflects His excitation. Blue OAPs have two humps that are the summation of atrial and His excitation. The first hump corresponds to atrial acti-

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² Supplemental material for this article is available online at the American Journal of Physiology: Heart and Circulatory Physiology website.
vation, whereas the second hump has the distinct plateau phase of His activation. Brown and purple electrograms contain several components. The first component matches in time the signals seen in the yellow electrograms and is followed by complex low-amplitude biphasic recordings that are consistent with slow conduction during the interval between atrial and His excitation. Brown electrograms are located near the anatomic location of the AVN, and brown OAPs have a nodal morphology. Purple electrograms and OAPs are located along the SP. There is overlap in the characteristics of the brown and purple electrograms and OAPs. Traces shown in black were difficult to classify because they share characteristics of two types of activation. White traces were recorded from dead ventricular tissue and therefore contain only noise.

In Fig. 2C, the sharpest spikes in the electrograms correspond to the dF/dtmax of the fluorescent optical signals. In the nodal and SP traces, the slow conduction characteristics of the electrograms correlate well with the OAPs from the same location. One exception is the spike seen in the nodal electrogram, which occurs during the plateau of the OAP. On the basis of the timing of this spike relative to the bipolar IHE, this spike most likely reflects excitation of the nodal-His (NH) region of the node or the lower nodal bundle (LNB), where excitation accelerates into the His bundle (2). Supplemental Fig. 1 shows the electrograms and OAPs from a second preparation to show that the signal morphologies were consistent between preparations.

Pacing stimuli applied within the triangle of Koch produced different activation patterns, depending on where the stimuli were delivered. Figure 3A provides a schematic of the location of the conduction system in this preparation for clarity, similar to the schematic shown in Fig. 1A. In this schematic, the approximate location of TCs has been marked with the cross-hatched area. Figure 3B illustrates the activation pattern caused by IAS pacing. Activation originated from the IAS electrode and spread rapidly across atrial tissue and TCs that lie above the conduction system in the triangle of Koch, which activated the FP of the AVN. Because the activation map displays dF/dt, there is no conduction visible from ~50 to 70 ms when there is exclusively slow AVN conduction (which has a low amplitude dF/dt). After this period, His excitation occurred, which actually began in the LNB region (shown in orange in Fig. 3B). The corresponding SHE is also displayed, showing that the interval between IAS pacing and His excitation was ~70 ms.

At many pacing locations, pacing from the roaming electrode caused an atrial/FP activation pattern similar to that seen with IAS pacing. Figure 3C illustrates FP activation of the AVN with a pacing stimulus applied close to the SP on the lower CrT. From this location, a fast wave of excitation originated from the location of the roaming electrode (square pulse on Fig. 3C, bottom left), spread across the atrial tissue and TCs that lie above the conduction system in the triangle of Koch, and then produced His excitation after a period where no conduction was visible. In the SHE trace shown in Fig. 3C, the His electrogram did not change morphology from IAS pacing, maintaining a fast-His morphology.

When the roaming electrode was placed within ~2 mm of the tricuspid valve within the triangle of Koch, it excited the SP (Fig. 3D). SP activation appeared as a slow, narrow activation pattern that moved toward the apex of the triangle of Koch.
After it reached the AVN region, excitation continued toward the His bundle without pause (which can be seen in the timing of the SHE trace in Fig. 3D with His excitation occurring 43 ms after stimulation) and also spread retrogradely across the IAS. In the SHE trace shown in Fig. 3D, the His electrogram morphology changed to a slow-His morphology with a smaller amplitude during SP pacing (compare SHE morphology in Fig. 3B and D) and returned to its original morphology after the ramp ended. Notice that the interval between stimulation and His excitation (the S-H interval) is paradoxically longer for fast-His excitation than for slow-His excitation. This phenomenon is explored further in Fig. 7. Slow-His electrograms were observed in six of eight superfused experiments.

Similar optical activation patterns for SP and atrial/FP activation were observed in the AV junctions of Langendorff-perfused hearts. The whole heart preparation is shown in Fig. 4A. Activation maps of SP pacing are shown in Fig. 4, B and C, with the corresponding IHE trace shown in Fig. 4C, bottom. Similar to Fig. 3D, SP pacing in the whole heart produced a slow narrow activation pattern followed without pause with His excitation (Fig. 4B). In the intact heart, His activation was followed by a robust ventricular optical signal that could be seen through the overlying atrial tissue (Fig. 4C). Atrial activation preceded ventricular activation by ~10–15 ms (light blue activation in Fig. 4C).

IAS pacing (Fig. 4, D–F) produced a fast wave of excitation that spread across the atrial tissue and TCs (Fig. 4D). After atrial activation, excitation spread from the AVN region in two directions: the wave front of His excitation spread toward the His electrode and a wave front of decremental conduction spread down the SP and died out (Fig. 4E). Ventricular activation followed His excitation (Fig. 4F). A small amplitude fast-His potential is seen in the IHE with IAS pacing (i.e., the IHE deflection is larger with SP pacing than with IAS pacing).

Pacing within the triangle of Koch. Figures 2–4 illustrate that the SP location can be identified with electrograms and OAP morphology, and SP vs. FP activation can be differentiated with activation patterns and His electrogram morphology in both the superfused and whole heart preparations. Using both activation patterns and His morphology, we identified which activation patterns occurred for multiple pacing locations throughout the triangle of Koch. Figure 5 displays a summary of where the different activation patterns occurred and the pacing thresholds in the superfused experiments. Supplemental Fig. 21 shows a representative example of pacing stimuli and activation patterns recorded from many pacing locations throughout the triangle of Koch. Generally, pacing stimuli applied within ~2 mm of the tricuspid valve excited the SP (similar to Figs. 3D and 4B) or the His directly when pacing near the apex of the triangle of Koch. Direct His stimulation was defined as fast conduction directly after stimulation, which had a S-H interval of <10 ms and occurred in a small area near the His electrode. On average, the pacing threshold for SP/His pacing was 4.4 ± 2.2 mA. Pacing stimuli applied further away from the tricuspid valve generally excited atrial tissue, producing FP excitation of the AVN with an average stimulation threshold of 2.4 ± 1.6 mA (P < 0.001 compared with SP/His pacing thresholds). Additionally, there was one area between the coronary sinus and the tricuspid valve that had very high pacing thresholds (8.6 ± 1.4 mA; P < 0.001 compared with atrial/FP thresholds). Pacing this region excited atrial tissue and the FP of the AVN. OAPs from this region were quite noisy and very dissimilar from the large-amplitude atrial OAPs recorded from other areas of the triangle of Koch.

Fig. 3. Schematic of the conduction system (A), similar to Fig. 1A, is shown with the approximate location of TCs shown as cross-hatched area. Activation patterns during IAS pacing (B), atrial activation from the roaming electrode (C), and SP excitation (D) are shown, with corresponding SHE traces shown at bottom. Pacing locations for A–C are indicated with square pulses.
High pacing thresholds and very low OAP amplitudes suggest that few excitable cells are located in this region. There were several instances where different activation patterns occurred at different thresholds, typically with atrial activation followed by SP or His excitation at higher stimulus intensities. Very similar pacing thresholds for each activation type were observed in the Langendorff-perfused hearts, with the SP/His threshold statistically the same as the right ventricular pacing threshold.

It is important to note that, once SP activation of the His bundle has occurred, the SP would excite the His bundle throughout the duration of the stimulation ramp in a 1:1 fashion. However, this was not the case for all pacing locations that produced FP excitation. As shown in Fig. 6, pacing near the superior border of the AVN often disrupted AV conduction by causing 2:1 AV block or prolonged AV conduction. In the example shown in Fig. 6, IAS pacing conducted 1:1 to the His before the stimulation ramp crossed threshold, seen in both the electrograms and the OAP recorded above the His bundle. Once the stimulation ramp crossed threshold, it excited atrial
tissue (seen in the shift in both the OAP and the CrT trace), but excitation did not propagate to the His. In the next beat, excitation propagates to the His (seen in the OAP and the IHE), and conduction continues in this 2:1 fashion. Therefore, AV conduction was disrupted by pacing in this location. Pacing stimuli delivered to this area of the triangle of Koch caused 2:1 AV block or prolonged AV conduction in five of eight experiments. Once AV conduction was disrupted, it did not fully recover in any of the five experiments, suggesting that the effect of pacing in this area was not neurologically mediated.

The S-H interval was measured for each pacing location. The S-H interval usually stabilized three to five pulses after threshold was reached, and these stable values were measured. S-H intervals at high-stimulus intensities, where a greater amount of tissue was presumably depolarized by far-field stimulation, were discarded because the S-H intervals at the end of the ramp were generally different from the stable S-H intervals. Rarely, S-H intervals did not stabilize and linearly decreased throughout the ramp, in which case the range of S-H intervals was noted. Also, we discarded from the analyses any S-H interval that was recorded after AV conduction was disrupted, as shown in Fig. 6. Data from one representative experiment are shown in supplemental Fig. 3A.1

S-H intervals were divided into two groups, those associated with atrial/FP activation and those associated with SP/His activation. When plotted against the horizontal distance from the His electrode, atrial S-H intervals showed no distance dependence (P = not significant; Fig. 7A) but instead remained at a constant level of 81 ± 19 ms. This constant interval between an atrial stimulus and His excitation is the AV delay. Surprisingly, S-H intervals associated with SP and His excitation exhibited a strong correlation with distance from the His electrode (P < 0.001; Fig. 7B). If SP excitation experienced the AV delay, one would expect a nonlinear jump from direct His excitation at small distances from the His electrode to values at or above the AV delay of 81 ± 19 ms. Moreover, the S-H intervals during SP pacing remained below the AV delay measured from atrial/FP activation (81 ± 19-ms delay for FP excitation and 53 ± 25-ms delay for SP excitation ≥4 mm from the His electrode; P < 0.001). S-H intervals for SP/His excitation from each superfused experiment are shown in supplemental Fig. 3B.1

Figure 7C shows an example of S-H intervals measured during SP pacing and IAS pacing with the last ramp stimulus, which excited the SP, and the first IAS pacing pulse after the ramp. The S-H interval was 31 ms for the last ramp stimulus and increased to 64 ms when the His bundle was excited by IAS pacing. Paradoxically, the S-H interval for the SP was shorter than the S-H interval for the FP. The shifts from slow-His to fast-His potentials are seen in both the IHE and SHE traces. Similar S-H interval trends for both atrial and SP excitation were observed in Langendorff-perfused hearts, with SP S-H intervals shorter than FP S-H intervals on average (see supplemental Fig. 4).1

DISCUSSION

Our results support previous findings that several layers of conduction exist in the triangle of Koch (10). Now, we further demonstrate that these layers can be differentially engaged with varying stimulus strengths and pacing locations. We found that stimulation thresholds for atrial excitation are significantly lower than for SP/His activation, with the exception of one area beneath the coronary sinus. Using the activation pattern documented by optical mapping, as well as slow-His
electrograms, we have verified SP excitation and found that not only do S-H intervals decrease linearly as the stimulus location approaches the His bundle for SP excitation, whereas S-H intervals associated with atrial/FP activation remain nearly constant, but also that paradoxically S-H intervals for the SP are shorter than those recorded for FP activation.

Because of the different modalities used to study the AV junction, there is a “lack of common terminology” for its components (15). For instance, many previous studies, including studies from our group, have referred to the AVN extension located in the isthmus between the coronary sinus and the tricuspid valve as the “posterior nodal extension” (8, 16). However, a naming task force has indicated that the INE more accurately describes its location when the heart is anatomically oriented (5). Previous studies have described layers of atrial cells and TCs overlying the components of the conduction system in the triangle of Koch, such as the INE, AVN, and His bundle (1). Functional studies have provided evidence that the INE is the anatomic substrate of the SP (16, 20); however, there is debate regarding whether the INE itself, the TCs overlying the INE, or a combination is the true substrate of the SP (19). Our results cannot distinguish which cell layer or structure produced SP excitation; however, our optical mapping results confirm that this pathway can be reliably excited by pacing stimuli delivered within 2 mm of the tricuspid valve within the triangle of Koch.

Plotting S-H intervals of atrial excitation against the distance from the His electrode revealed no correlation (Fig. 7A). The S-H interval of atrial activation is composed of two intervals: conduction time in the atrial layer and FP (S-AVN interval) and AVN conduction to the His bundle (the AV delay). Because conduction in the atrial layer is fast (~35 cm/s) (23), small changes in the distance between the stimulating electrode and the His electrode change the S-AVN interval minimally. Therefore, the main determinant of the S-H interval is the AV delay, which remains essentially constant.

On the other hand, plotting S-H intervals of SP excitation vs. distance from the His electrode showed a strong correlation (Fig. 7B). A greater dependence on distance would be expected for SP activation because, as the name implies, the SP conducts slowly (~7 cm/s) (23). Therefore, one would expect the S-AVN interval to decrease slightly as the distance between the stimulus and the AVN decreased. However, once conduction time in the SP is minimal, the S-H interval should be determined by the AV delay, which is 81 ± 19 ms according to the atrial activation data (Fig. 7A). As the His bundle is approached further, the S-H interval should then jump to a very small value when direct His activation occurs. Instead, the raw data (shown in supplemental Fig. 3) and the pooled data in Fig. 7B show S-H intervals almost entirely <81 ms and a linear decrease of the S-H interval as the His electrode is approached. Activation patterns for SP pacing in Figs. 3D and 4B show SP excitation proceeding linearly to the His bundle without pause. These data suggest that there is no dependence of the SP/His S-H interval on the AV delay, implying that SP excitation avoids the AV delay and excites the His bundle directly.

How can the SP conduct to the His more quickly than the FP? Many studies have investigated the rate-dependent properties of the SP vs. the FP in the context of premature stimuli, most commonly with pacing stimuli applied in the high right atrium or to the CrT (20, 29). On the basis of these studies, the SP was given its name because excitation took longer to reach the His bundle than through the FP. However, we found that SP excitation reached the His bundle faster than FP excitation. Despite this apparent paradox, our results are fully consistent with the large body of evidence concerning the SP for two
reasons. First, SP excitation in previous studies traveled the full length of the SP, which our data indicate would have an S-H interval similar to FP excitation (Fig. 7B, right). In the context of a premature beat, this activation would take even longer. In the absence of pacing stimuli applied directly to the SP, SP conduction to the His only occurs when a premature stimulus encounters a refractory FP (Fig. 8A). Because conduction traveling the full length of the SP takes the same amount of time as the AV delay (or longer with premature stimuli), it would be impossible to recognize that SP conduction avoided the AV delay. Only through pacing the SP incrementally along its length, as we did in this study, would it become apparent that SP excitation does not experience the same AV delay as FP excitation. Second, in this study, we engaged the SP directly, potentially avoiding any conduction delay that may occur at the interface of the atrial tissue with the SP (Fig. 8A). This interface may be responsible for an additional delay in conduction, which would slow SP conduction even further (23).

In this study, we paced at 300 ms within the triangle of Koch, close to the AVN itself. Therefore, one interpretation of our data is that because of the His bundle proximity, pacing stimuli excited the His bundle directly when stimuli were delivered within 2 mm of the tricuspid annulus and the SP was not involved. Direct stimulation of the His bundle would certainly result in short S-H intervals. However, this possibility is unlikely for two reasons. First, SP activation was confirmed by being visualized with optical mapping. Second, pacing stimuli applied much closer to the His bundle itself (such as on Fig. 5, right, and the right side of the grid shown in supplemental Figs. 2 and 3) produced atrial activation with an AV delay of ~80 ms or longer throughout the entire pacing ramp. Therefore, it seems that the tissue area initially captured by the pacing stimulus was rather small. Also, the S-H intervals that were analyzed were measured three to five beats after the pacing ramp reached threshold (i.e., ≤2 × the pacing threshold), which also limits the extent of tissue that was excited at that point in the pacing ramp.

There is a growing consensus in the literature that the His, LNB, and the INE form a continuous structure distinct from the compact AVN tissue on a morphological, molecular, and functional basis (1, 17, 20). We interpret our data to suggest that, instead of a final common pathway as classically suggested by Mendez and Moe (21), there are instead two pathways to the His bundle: one through the compact node and the other through the LNB. Medkour et al. (20) and Khalife et al. (16) put forth similar arguments suggesting that the INE connects to the His bundle through the LNB and that the FP passes through the compact node. The concept of two pathways to the His is supported by changes in His electrogram morphologies (i.e., slow-His and fast-His electrograms). The distinct His electrogram signatures of FP or SP activation indicate that the activating pathway influences how the His bundle, or which part of the His bundle, is depolarized (29).

Based on this dual-pathway concept, we suggest that SP excitation begins in either the inferior TCs or the INE itself and travels via the INE to the His bundle through the LNB with a gradient of conduction velocities, as shown in the schematic in Fig. 8B. As excitation approaches the His, the level of connexin 43 increases (17) and conduction velocity increases until the His bundle is reached. FP activation from the atrial tissue surrounding the AVN funnels into the compact node via the TCs that overlie the AVN. The AVN delay is due to the green compact nodal tissue and its connecting TCs (3), after which excitation passes to the His.

Interestingly, we identified a small region below the coronary sinus where stimulation thresholds were significantly higher than the surrounding myocardium. This area may serve as a localized area of block, contributing to anisotropy in the atrial myocardium and may play a role in atrial flutter and fibrillation, similar to the block zone in the intercaval region that can maintain typical atrial flutter (11).

Clinical implications and limitations. The existence of an SP “bypass tract” in the right atrium expands the area that potentially could be paced to achieve His bundle excitation with direct His bundle pacing procedures, alleviating the difficulty of pacing the small His region located close to aorta (7). Locating the SP pacing location could be guided by the slow conduction characteristics in SP electrograms, shown in Fig. 2 and shown clinically (12), which are used to guide SP ablations during AVNRT procedures.

SP pacing may very well have lower pacing thresholds than those required for His bundle pacing (27) because of the fibrous tissue surrounding the His bundle. Our experiments in the rabbit revealed that SP pacing and direct His pacing had pacing thresholds that were not statistically different. However, in the rabbit, the endocardial side of the His bundle is only covered by a small amount of connective tissue, whereas, in the human, the His bundle is encased in the fibrous tissue of the central fibrous body. Therefore, it is possible that SP pacing in the human will have a lower threshold than His bundle pacing. Additionally, pacing thresholds for right ventricular epicardial pacing and SP/His pacing were not statistically different in the whole heart experiments (supplemental Table 1), suggesting that clinical SP pacing thresholds may be close to pacing thresholds for right ventricular pacing. However, this study was conducted in normal rabbit AV junction preparations.
where no attempt was made to disrupt AV conduction. Whether His capture from the SP will be possible in a diseased AV junction remains to be shown.

Conduction curves are the gold standard used to investigate AVN dual-pathway electrophysiology. Because of the number of pacing locations investigated in this study, it was not possible to construct conduction curves for each. Although conduction curves are very useful clinical tools, optical mapping in our preparations confirmed the distinct activation patterns of FP vs. SP activation (Figs. 3 and 4), and our optical mapping of SP excitation corresponds very well to SP optical mapping performed during standard S1-S2 protocols (23). Additionally, slow-His potentials provided another line of evidence for FP vs. SP participation in conduction.

GRANTS

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REFERENCES