High-Resolution, Three-dimensional Fluorescent Imaging Reveals Multilayer Conduction Pattern in the Atrioventricular Node

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Background—The atrioventricular node (AVN) is the only normal electrical link between the upper and lower chambers of the heart. The AVN modulates transmission of impulses, thus coordinating the contraction of the atria and ventricles.

Methods and Results—Structural and functional complexity, combined with the absence of adequate experimental techniques, has complicated attempts to directly evaluate the three-dimensional electrical activity of the AVN. Thus, despite a century of research by conventional electrophysiologic and histologic methods, even the existence of conduction through AVN is still debated.

Conclusions—Using a novel combination of microelectrode recordings and high resolution fluorescent imaging with voltage-sensitive dyes, we have for the first time clearly demonstrated three-dimensional conduction through the AVN. (Circulation. 1998;98:54-57.)

Key Words: atrioventricular node • conduction • mapping • imaging

Since the first morphologic description by Tawara,1 the atrioventricular node (AVN) has been one of the most studied, yet least understood, structures of the heart. The three-dimensional pattern of activation of the AVN has been mostly speculative because of the exceptional architectural and functional complexity of this rather small region2 and the lack of appropriate experimental techniques. The glass microelectrode has been the only reliable tool to visualize electrical responses of AVN cells from different layers. However, microelectrodes cannot provide a map of activation. This is because at a single position, cells located closer to the surface may be activated substantially earlier than deeper cells. Moreover, the depth of microelectrode impedance is either unknown or very difficult to verify. Finally, simultaneous use of more than two microelectrodes has proved to be prohibitively difficult.2

The application of voltage-sensitive dyes and optical imaging techniques to studies in cardiac electrophysiology is becoming increasingly common. However, this new technology is being used mostly to map electrical responses of thin layers of epicardial cells3 or isolated cells and cellular cultures4 that are considered morphologically and functionally “flat” two-dimensional fields. Because the AVN in the rabbit heart is located immediately below the endocardial surface5 and, judging by microelectrode experience and histologic sections,6 the deepest cellular layers are less than 500 µm away, we suggested earlier that multicomponent optical recordings from the AVN area observed in our previous experiments may carry signatures of electrical activity from several layers at the same time.6 We hypothesized that three-dimensional mapping of the AVN might be feasible if high-resolution fluorescent imaging and microelectrode recordings were combined.

Methods

The isolated rabbit AVN preparation as well as the experimental techniques have been previously described in detail,6 thus will be outlined only briefly. Rabbits (n = 5) of either sex weighing 2 to 3 kg were anesthetized, and after a midsternal incision, the heart was removed and placed onto a Langendorff apparatus, where it was retrogradely perfused with oxygenated (95% O2, 5% CO2) room-temperature, modified Tyrode’s solution. The heart was stained by a bolus injection with 350 µL of 2 mmol/L solution of di-4-ANEPPS in DMSO. After staining, the heart was removed from the Langendorff apparatus and the right atrial AVN preparation was dissected.6 The final preparation contained right atrial tissues with the triangle of Koch and the AVN. This preparation was pinned down on a thin silicon disk and superfused at 37.0 ± 0.1°C, with a flow rate of 30 mL/min; 12.5 mmol/L BDM was added to suppress contraction-induced distortion of optical signals. As recently shown, this concentration of BDM has no adverse effect on rabbit AVN conduction.7

The preparations were paced at a cycle length of 300 ms, at twice diastolic threshold voltage, by a bipolar electrode located in the sinus node area. Bipolar electrograms were continuously recorded from low crista terminalis and the bundle of His. Optical recordings of electrical activity were performed simultaneously from 256 sites with a state-of-the-art photodiode array imaging system.6,8

Results

Similar to previously described records6 from the AVN area, we found that optical signals recorded from the distal AVN region, close to the bundle of His, consistently exhibited multiple components. Thus in the time interval between an...
atrial input activation and the subsequent activation of the bundle of His, two distinct optical action potentials could be identified. We hypothesized that the first of these components represented depolarization of cells located in the superficial envelope of transitional cells, whereas the second component represented the delayed depolarization of cells in the compact nodal region.

To test this hypothesis, we impaled a glass microelectrode to record AVN cellular action potentials (AP). The narrow depth of field and large numerical aperture of the optical lens and the transparency of the glass microelectrode minimized optical interference. Deeper impalement provided an AP typical for a distal nodal cell (Figure 1, bottom left panel). The inscription of this AP preceded or coincided with the bundle of His electrogram activation and was absent when the latter was missing because of conduction block (in this example, a 3:2 Wenckebach cycle was observed and the second beat was blocked in the AVN). It is evident that the AP of this deeper cell always coincided with the second component of the optical signal. AVN block was always associated with the absence of both the cellular action potential and the second component in the optical signal. Finally, dissociation between the two optical components clearly reflected the overall AVN delay. That is, a marked dissociation in the first beat (blue strip) was due to the long delay between the atrial and His activations. In contrast, the shorter conduction delay in the third beat (yellow strip), which followed the blocked one, resulted in less separation between the components in the optical signal.

When the microelectrode was slightly withdrawn vertically, a more superficial transitional cell was impaled (Figure 1, bottom right panel). Although it cannot be proven that this cell had exactly the same x-y coordinates as the previously impaled cell, it is safe to estimate the horizontal dislocation to <100 μm. This cell had a faster upstroke and depolarized earlier after the atrial activation in each beat. In addition, it was activated even when AVN block was present. Thus this was a typical AVN transitional cell. Note that its AP coincided with the first of the two components in the optical signal. Moreover, both the cellular AP and the first of the optical components in each beat were related to the atrial activation and significantly preceded the bundle of His activation.

The above observations were reproduced in all five hearts and confirmed that the optical signal contained information from at least two wave fronts: one representing the activation of the superficial envelope of transitional cells and a second one representing the ensuing depolarization of the deeper compact nodal layers (Figure 1). The optical recordings were also able to discriminate the occurrence and location of conduction block. Note, for example, that in the left bottom panel of Figure 1, the optical signal in the second (blocked) atrial beat did not exhibit an appreciable second hump, suggesting that the block of the wave front occurred at a substantial distance before reaching the source of the optical signal. In contrast, in the right bottom panel of Figure 1, a small but distinct second hump in the optical signal in the second (blocked) atrial beat is evident, suggesting that a fading wave front stopped closer to the source of the optical signal.

The primary advantage of optical imaging can be appreciated when mapping of the spread of activation is attempted. Microelectrodes cannot accomplish such a task. In fact, successful high-resolution intranodal mapping of AVN activation has not been previously reported. Figure 2 illustrates the individual optical signals obtained in one preparation during propagation of a single atrial beat. Note that the characteristic dissociation of the optical signals into two components becomes progressively more pronounced when moving from the input (left) to the output (right) of the AVN.
The ratio of the amplitudes of the two components is not constant. This can be explained by the variable thickness of the layer of transitional cells on the endocardium, which causes different relative contributions to the total optical signal of the transitional and the deeper nodal cells. Thus a relatively larger amplitude second component should be expected at more distal (right) coordinates where the late-depolarized deeper cells are located. On the other hand, a larger-amplitude first component should be expected at the proximal (left) coordinates, where the earliest-depolarized transitional cells are abundant. At certain intermediate coordinates, the two components are of comparable amplitude.

We constructed activation maps of conduction through the AVN by using either only the first or only the second of the two optical signal components. Pairs of such activation maps are shown in Figure 3. First components in the distal node were significantly (see Figure 2, rows 12 to 14, columns 12 to 16) smaller relative to second components. However, it was of comparable amplitude with first components recorded from the proximal node. It is evident from Figure 3 that two distinct wave fronts were present during the propagation of both the basic (left panels) and the premature (right panels) beats. The first wave front (upper maps) ran over the envelope of transitional cells and brought the excitation from the pacing site to the AVN region. This wave front encircled the opening of the coronary sinus (shown in black) and activated the viewing area within 30 ms during the basic beat and within 55 ms during the premature beat. The second wave

**Figure 2.** Two hundred fifty-six simultaneous optical traces were obtained from a 6×6 mm area that includes the atrioventricular node (AVN). Each trace was recorded from a 320×320 μm spot. Different colors are used to distinguish signals from the posterior approaches to AVN (blue), the anterior approaches (green), and the compact nodal area (red). Signals in black are from the area of the opening of the coronary sinus and were ignored during the analysis. This scan was performed during a premature atrial beat with a coupling interval of 140 ms.

**Figure 3.** Activation maps during basic (left) and premature (right) beats. The activation times at each individual location were determined by −(dF/dt)_{max} (F=fluorescence). The isochronal lines were drawn with a 5-ms resolution. Spread of activation is shown as white-to-red gradient. The left pair of maps illustrates the conduction of a basic drive beat at a cycle length of 300 ms; the right pair of maps was obtained during the propagation of a premature beat with a coupling interval of 180 ms. See text for details.
front (the bottom maps) was only traceable in a portion of the viewing field that corresponds to the location of the compact node and the more distal nodal region. Conduction in this deeper region of nodal fibers was initiated after and probably by the superficial wave front. This conduction was substantially slower. This slowing of conduction was especially pronounced during premature beats. In Figure 3, it took 100 ms to transverse the deeper cellular layers; the entire surface was activated within 55 ms. In addition, the area of slow conduction, defined by the presence of secondary components in the optical recordings, was consistently larger during propagation of premature atrial beats. The phenomena described above were consistently observed in all five preparations.

Discussion
The novel combination of microelectrodes and high-resolution optical imaging with voltage-sensitive dyes can now be successfully added to the arsenal of tools used to study the electrophysiology of the AVN. This novel integration of techniques has helped to directly demonstrate the previously deduced pattern of multilayer AVN conduction and to provide the first detailed three-dimensional mapping of transmission of basic and premature beats through the AVN. This has been achieved by reconstructing the three-dimensional pattern from two-dimensional optical mapping assisted by the functional information obtained with microelectrodes and macroelectrodes.

This creates an exciting possibility to gain a deeper understanding of the many still controversial properties of AVN conduction. Among those are the filtering role of the AVN during high-rate irregular rhythms such as atrial fibrillation and the fundamental issue of whether or not the AVN truly conducts or is an electrotonically modulated oscillator. Furthermore, application of the optical imaging technique may help to visualize dual pathway structures (if they exist) and to correlate specific bipolar potentials that were clinically described with the AVN cellular responses. Finally, the combination of microelectrode recordings with optical techniques offers a powerful new approach for studying electrical conduction in other complex biologic nonhomogeneous structures with a multilayer architecture.

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