Proximal Atrioventricular Bundle, Atrioventricular Node, and Distal Atrioventricular Bundle Are Distinct Anatomic Structures With Unique Histological Characteristics and Innervation

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Background—Direct 3D analysis (ie, stereotaxic analysis of 3 planes) has shown that the atrioventricular (AV) node (AVN) is continuous with only specialized myocardium of the proximal AV bundle (PAVB) and distal AV bundle (DAVB) or His bundle. The purpose of the present study was to determine whether the PAVB, AVN, and DAVB possess histological features distinct from each other and from the ordinary myocardium.

Methods and Results—A protocol that preserves the cytoplasmic and interstitial integrity of the tissue and permits serial sections of the AV junction region to be made in 3 orthogonal planes showed that the PAVB, AVN, and DAVB are characterized by myocardium aggregated into fascicles containing ~8 myofibers. Myofibers within the fascicles are coiled or spiraled about each other; and spiraling is most compact in the PAVB. Collagen encases individual fascicles and segregates primary fascicles into secondary fascicles. Fascicles, and not myofibers, are in parallel array in the PAVB, interwoven in the AVN, and parallel in the DAVB. Narrow junctions of parallel fascicles separate the AVN from the PAVB and DAVB. Myocytes, which are largest in DAVB, possess clear perinuclear regions; thin finger-like end processes, which are most numerous in the AVN; uniform, delicate cross-striations; and intercalated disks, which are broader in the PAVB and form short stacks in the AVN. Sheaves of nerve terminals, including boutons, are as found in skeletal muscle.

Conclusions—The PAVB, AVN, and DAVB have distinct histological features. Collagen septation of primary and secondary fascicles presents natural barriers within the tissues and to surrounding myocardium and structures. These findings confirm that the AV junction region contains a specialized conduction system that is anatomically isolated from ordinary myocardium. (Circulation. 2000;101:1049-1059.)

Key Words: atrium ■ atrioventricular node ■ conduction ■ collagen ■ myocytes ■ nervous system

The atrioventricular (AV) junction region and the organization of its ordinary and specialized myocardial tissues have assumed great importance as the focus of radiofrequency catheter ablation therapy of common arrhythmias. The proximal AV bundle (PAVB; atrial bundle), AV node (AVN; Knoten), and distal AV bundle (DAVB; AV or His bundle) were described almost a century ago as an AV bridge of specialized myocardium connecting the atria to the ventricles in many species, including sheep, cat, dog, and humans in dissected hearts and in transverse plane serial sections.1 Nevertheless, the existence of this anatomic bridge was only recently confirmed (Figure 1, top).2 The tissues have also been demonstrated to form a functional bridge with discrete lesions at (1) the DAVB that elicited idioventricular rhythms3 and (2) at both the PAVB-AVN and AVN-DAVB junctions that evoked hierarchical automaticity of the AVN with respect to the DAVB, whereas nearby atrial myocardium showed the sinus rhythm.4 Moreover, although the tissues exhibit distinguishing anatomic and electrophysiological features,1–6 the identity of different components of the AV bridge myocardium remains controversial.7,8

This present report represents a continuation of our correlated anatomic and electrophysiological studies.9–11 The purpose of the present study was to determine whether histological criteria can be used to distinguish the PAVB, AVN, and DAVB from each other and from the surrounding ordinary myocardium.

Methods

Eleven adult mongrel dogs weighing 9 to 15 kg were anesthetized with sodium pentobarbital (30 mg/kg IV). As previously described,1,2,9–11 prefixation and postfixation 5% sucrose buffer rinses and Karnovsky’s fixative were used to limit osmotic damage,12 and the hearts were flattened naturally through spreading on a paper towel before fixing. The AV junction region yielded 1 to 3 blocks,
depending on the orientation used for sectioning and heart size. Paraffin-embedded tissues blocks were serially sectioned at a thickness of 10 to 15 μm in the parallel (n = 5), perpendicular (n = 4), or transverse (n = 2) plane (Figure 1, bottom). All sections were mounted on glass microscope slides and stained with Goldner’s trichrome, Masson’s trichrome, Verhoff’s elastica, or hematoxylin-phloxine-safran and studied in photomicrographs taken at intervals of 0.5 mm for transverse and perpendicular sections and of 0.25 mm for parallel sections. At selected sites, every section was studied. Goldner’s trichrome was most useful; myocardium stained brick red; nuclei, black; nerves and ganglia, magenta; red blood cells, orange; and connective tissue, blue to green.

Data Analysis

The size of structures were determined through direct measurement with the use of an ocular micrometer and from photomicrographs, with photographs of the 1-cm or 0.1-mm rulers enlarged to the same magnification.

Results

Each plane of section provides histological information that is not apparent in the other planes (Table). This is exemplified in Figure 2, which shows the macroscopic histology of the PAVB, AVN, and DAVB in the 3 orthogonal planes. We noted that anatomic landmarks and tissues have a regular relation with each other that does not vary in different hearts or with the size of the heart; this is exemplified by a small sinus appearing regularly in the anteroinferior lip of the coronary sinus ostium, as shown here in the perpendicular and transverse sections (Figures 2B and 2C1).

Histology of the PAVB

The PAVB is composed of small parallel myocardial fascicles (not myofibers as first described in dissected hearts) along its full length. Parallel fascicles are displayed in long axis in parallel (Figures 3A and 3D) and perpendicular...
Figure 2. Direct 3D analysis of AV junction region in selected serial orthogonal sections. A and B, Vertical lines indicate section level in C. B, C1, Coronary sinus ostium (CSO) and black dot identify a regularly appearing sinus in lateral, inferoanterior lip of coronary sinus ostium. A, Parallel plane: the PAVB, AVN, and DAVB span AV junction region as a myocardial continuum confluent with right bundle-branch (RBB); medial atrionodal bundle (MAB) and lateral atrionodal bundle (LAB) are located on either side of coronary sinus (CS); and superior atrionodal bundle (SAB) is superior to AVN. Dashed lines indicate Koch’s triangle. B, Perpendicular plane: collagen encasement imparts a gray tone to PAVB, AVN, and DAVB in contrast to dark staining of medial atrial wall (MAW) and ventricular septum (VS) myocardium. White arrowheads delineate MAW annular, subepicardial circumferential lamina. Thin sleeve of central fibrous body connective tissue separates AVN from MAW. C, Transverse plane. C1, MAB and LAB converge. C2, Collagen septa separate vertical stack of PAVB fascicles atop VS. C3, Convergence of SAB and PAVB. C4, SAB is superior to AVN, which is surrounded by halo of central fibrous body connective tissue. Perpendicular lamina myofibers cascade over central fibrous body and descend into medial leaflet. C5, DAVB within central fibrous body and astride VS. Note specialized tissues are outside MAW. C1, CSFo and star indicate CS fossa; LA, left atrium; n, noncoronary posterior aortic valve sinus wall; and TT, tendon of Todaro. Calibration bar in A, B, and C1. (Goldner’s trichrome.)
Figure 3. A, Orthogonal parallel plane PAVB is a loose aggregate of linearly arrayed myocardial fascicles intermixed with fat vacuoles that condenses at PAVB-AVN junction (Junct) and is separated from MAW by a large blood vessel (white arrows). B, From box in A, myocytes with uniform cross-striations (black arrowheads) and unusual broad intercalated disks (open arrows) that display a central density (inset). C, Sheaves of nerve terminals (curved black arrows) impinge along lengths of myofibers. Myocytes contain evenly distributed cross-striations (black arrowheads). D, From box in A, parallel fine fascicles (Fasc) composed of strands of twisted myofibers (black and white arrowheads), many showing fine finger-like processes (inset). Sheaves of nerve endings (curved black arrows) contact fascicles and capillaries (top right). Collagen is interspersed between fascicles. Inset, Myocyte end processes are >30 μm long.

Figure 4. A, Orthogonal perpendicular plane PAVB is an orderly array of fine-caliber parallel myocardial fascicles separated by collagen lamina with large blood vessels within its substance and flanking its borders (white arrows). B, From box in A, primary fascicles. C, From B, myocyte with a clear perinuclear zone (thin black arrow) and intercalated disks (open arrows). Capillary in cross section (short curved black arrow). D, From box in A, numerous small fascicles with spiraling myofibers (white arrowheads) separated by collagen (CT). Large blood vessel borders PAVB luminal aspect (open arrows across bottom). (Goldner’s trichrome.)
(Figures 4A and 4D) and in cross section in transverse (Figures 5A and 5D) sections.

Surprisingly, light microscope high-magnification views revealed that what appear to be small single myofibers are in reality several myofibers twisted into single strands (Figures 3D and 4D). These are seen in parallel sections (Figure 3D) but are most distinct in perpendicular sections (Figure 4D). Neither individual myofibers nor their disposition within the fascicle is apparent in transverse sections (Figure 5D).

Connective tissue stains show that individual primary fascicles are encased by collagen. Collagen septa segregates several primary fascicles into larger secondary fascicles. Collagen encasement of individual fascicles can be seen in parallel sections at high magnification (Figure 3D) and in perpendicular sections at low magnifications (Figure 4A). Figure 4A shows the collagen investment prevents casual contact among fascicles within the PAVB and isolates the PAVB fascicles from the myocardium of the medial atrial wall and ventricular septum. Transverse sections clearly show secondary fascicles separated by collagen septa at low magnification (Figures 5A and 5D). In contrast, elastic fibers are restricted to the walls of larger blood vessels and the endocardium.

Goldner’s trichrome–stained parallel sections show that unusual sheaves of nerve endings extending along lengths of myofibers (Figure 3C) terminate perpendicular to the long axis of the myofiber as fascicles of boutons, tendrils, and varicosities (Figure 3D).

PAVB myocytes exhibit 4 unique features: a clear perinuclear zone (Figure 4C), delicate evenly dispersed cross-striations (Figures 3B to 3D), thin cytoplasmic finger-like, end processes (Figure 3D, inset), and wide intercalated disks containing a central density (Figure 3B, inset). The numbers of intercalated disks may be underestimated, however, because they may be more difficult to detect in the tightly twisting and spiraling myofibers.

The PAVB is also characterized by numerous ganglia nestled among its fascicles (Figures 5A, 5B, and 5D), large blood vessels within its substance (Figures 4A and 5A) and flanking its borders (Figures 3A, 4A, and 4D), and fat vacuoles that bestow a loose appearance on the PAVB in parallel sections (Figure 3A) and are prominent at its ventricular septal apposition (Figures 5A and 5B). Fat vacuoles are also a prominent feature of the ordinary atrial myocardium, at this same level (Figures 5A and 5B).
Histology of the AVN

Several major distinctions exist between the AVN and PAVB. The AVN is composed of a meshwork of interwoven myocardial fascicles, not myofibers as previously described.\(^1,3,13-16\) The interwoven AVN meshwork immediately follows the PAVB-AVN junction (Figure 3A), at which point the AVN is also encased within the central fibrous body (Figures 2B and 2C\(^4\)). The result of this massive whorl of fascicles can be appreciated in perpendicular sections, where primary fascicles displayed in cross-section are located next to fascicles in the long axis (see Figure 7D). Although the AVN exhibits parallel fascicles along its periphery (Figures 6A and 7A), thin end processes interlace these fascicles to deeper ones. In this way, the peripheral myofibers become part and parcel of the entire meshwork. Intercalated disks, displayed in short stacks, join myocyte thin processes (Figure 6B). No large blood vessels or ganglia and few to no fat vacuoles were found.

The AVN exhibits many anatomic characteristics of the PAVB. Myofibers are aggregated into fascicles. Collagen encasement of fascicles, which is discernible in parallel sections at high magnifications (Figure 6D), is most apparent in perpendicular sections (Figures 7A, 7B, and 7D). Transverse sections show segregation of primary fascicles (Figure 8C) into secondary fascicles by collagen septa (Figure 8D). Twisted and spiraling myofibers within the fascicles are apparent in both parallel and perpendicular sections (Figures 6D and 7D). AVN myocytes display a clear cytoplasmic perinuclear clear region (Figure 6C) and delicate uniform cross-striations along their full extent, including their fine finger-like processes (Figures 6C, 6D, and 7C). Fascicles of nerves pass over thin myocyte processes (Figure 6B) and terminate on AVN myocytes (Figure 6D, inset). The variety of fascicles of nerve terminals is especially apparent in the AVN as varicosities, tendrils, and boutons (Figure 6D).

Histology of the DAVB

The DAVB is also composed of myocardial fascicles, not single myofibers as previously described.\(^1,3,13-16\) and differs from the AVN and PAVB in several major aspects. It is a narrow bundle; its fascicles are the largest (Figures 9 to 11, compare Figures 3D, 6D, and 9D) and are disposed in clusters in transverse sections (Figure 11). DAVB myocytes display intercalated disks that are longer (Figure 10C) and thin end processes are less numerous (Figure 10D). The fascicles contain tendrils of white nerve fibers that interlace the...
myofibers (Figures 11C and 11D), but packets or fascicles of nerve endings are not apparent (Figure 9D).

The DAVB exhibits many histological features in common with the PAVB and AVN, such as the presence of primary fascicles (Figure 9D) with collagen encasement (Figures 9A and 10A) and secondary fascicle segregation by collagen septa (Figures 9D, 10D, 11A, 11B, and 11D). Twisted and spiraling intrafascicular myofibers are apparent in both parallel and perpendicular sections (Figures 9B, 9D, and 10D). Myocytes display a clear perinuclear zone (Figure 10B) and delicate uniform cross-striations along their full extent (Figures 9B to 9D). As in the case of the AVN, no large blood vessels or ganglia and few to no fat vacuoles are found.

Histology of the PAVB-AVN and AVN-DAVB Junctions
The PAVB and DAVB junctions with the AVN are narrow, which bestows on the AVN its bulbous shape (Figure 2A). Both junctions are composed of fascicles in parallel array; abrupt ordering of the myofibers occurs at the PAVB-AVN junction (Figure 3A) and at the AVN-DAVB junction (Figure 6A). The PAVB-AVN junction is most pronounced, in that the rather broad PAVB abruptly narrows (Figure 2A) to a region of <0.25 mm in length and width (Figure 3A). In contrast, the DAVB remains fairly uniform in size after the abrupt change in fascicle ordering, as seen in both parallel (Figure 2A) and perpendicular (Figure 2B) sections.

Histology of the Ordinary Myocardium of the AV Junction Region
Ordinary atrial and ventricular septal myocardium overlay and underlay the specialized AV bridge tissues and are arrayed in the well known layers or sheets of myocytes with little to no collagen separating individual myofibers or tissues. Only ordinary atrial myocardium contacts the endocardium. Myocytes exhibit little to no branching and are joined together in an end-to-end and side-by-side fashion at myocyte step processes by prominent intercalated disks. No intercalated disk connections were found between the ordinary atrial or ventricular septal myocytes and the AV bridge myocytes. No direct appositions were found, as the latter was segregated by collagen and connective tissue.

Discussion
Morphological features of the PAVB and histology of the AV bridge tissues are demonstrated here for the first time. The PAVB is a relatively extensive structure. It spans the area between the coronary sinus ostium and the posterior half of the medial leaflet and is as long as the AVN and DAVB combined (Figure 2A). The PAVB has been overlooked in conventional studies because the PAVB region is completely (Hudson,3 Figure 2–11; and Smith et al,17 Figure 2) or partially (Lev et al,13 Figure 1) excluded, as seen in schematics of AV junction region tissue blocks. When the PAVB
region is schematized, none of its features appear in the published histological sections (Becker and Anderson, Figure 1; and Inoue and Becker, Figure 1).

Fascicular compartmentation of the AV bridge tissues into primary fascicles are similar only to Purkinje fibers, which in fact consist of several myofibers tightly packed into small fascicles (Sommer and Jennings, Figure 30) and has been directly demonstrated through the diffusion of iontophoresed Lucifer yellow. Secondary fascicles are unique to the AV bridge tissues and provide an anatomic basis for multiple inputs to and through the AVN.

Connective tissue investment of AV bridge fascicles is the collagen type and not elastic fibers, as commonly believed. Collagen septa were shown here to prevent the direct contact of fascicles within the same tissue and to prevent even casual contact of the AV bridge tissues with both atrial and ventricular septal myocardium. This latter factor is an important consideration because the PAVB, which is subjacent to medial atrial wall epicardium, is adjacent to the ventricular septum along its full extent (Figures 2C2 and 2C3).

Spiraling, twisted intrafascicular myofibers are unique to the AV bridge tissues among the organ systems of the body. Myofibers spiraling is most compact in the PAVB and less compact in the DAVB. Thus, the small caliber and compact spiraling myofibers could provide an anatomic basis for different conduction velocities within the tissues. Relative fiber spiraling under basal conditions is PAVB > AVN > DAVB, and relative fiber size is PAVB < AVN < DAVB.

Sheaves of nerve terminals including tendrils (sympathetic), varicosities (parasympathetic), and boutons (somatic) impinge on AV bridge myofibers and small blood vessels (Figures 3D and 6D). This is contrary to the current belief that nerves within the AVN are restricted to single nerve fibers and that specialized canine AV junction tissues are devoid of parasympathetic fibers.

Clear perinuclear regions were found in the AV bridge myocytes. These represent glycogen storage and are a hallmark of the ventricular conduction system, which uses anaerobic glycogen metabolism, as opposed to the aerobic metabolism preferred by working myocardium, and permits delineation of this system during surgery through the Lugol’s reaction (Sommer and Jennings, Figure 29).

Myocyte finger-like end processes (Figures 3D, inset, and 7C) explain the knobby protrusions from the body of the

Figure 8. A, Orthogonal transverse plane AVN displays a dense oval knot of myocardium, encased within central fibrous body and overlaid by perpendicular (curved arrows) and circumferential (straight arrows) lamina, and superior atrionodal bundle (broad arrow). B, From a region in A, primary fascicles. C, From box in B, primary fascicles separated by collagen. D, From A, secondary fascicles separated by collagen septa. (Goldner’s trichrome.)
**Figure 9.**
A, Orthogonal parallel plane DAVB displays large parallel myocardial fascicles separated by collagen lamina. B, From region in A, fascicle with twisting myofibers (arrowheads) contain myocytes with uniform cross-striations and clear cytoplasmic perinuclear zones (arrows). C, Intercalated disks (open arrow) are narrow. D, From A, large parallel fascicles with twisting myofibers, thin myofiber branches (arrowheads), intercalated disks (open arrows), and capillaries (small curved arrows). (Goldner’s trichrome.)

**Figure 10.**
A, Orthogonal perpendicular plane DAVB displays large parallel myocardial fascicles separated by collagen. B, Three primary fascicles in cross section surrounded by collagen. C, From B, intercalated disk (open arrows) joining myocytes at myocyte soma. D, Large parallel secondary fascicles with primary fascicles separated by collagen and thin myocyte projections containing cross-striations (arrowheads). (Goldner’s trichrome.)
myocytes displayed in 3-D electron micrograph reconstructions of AVN myocytes by Thaemert. The latter study was limited to 11-μm lengths. We show the processes are multiples of 10 μm in length (Figures 3D, inset, and 7C).

Points of Controversy
A number of investigators have examined the AV junction histologically. Although the same plane of section was used, major discrepancies are still apparent in schematics of the AV junction region and in different studies by the same workers. The real AV junction region tissues were revealed by (1) preservation of the hearts with a protocol that maintains the ultrastructural integrity of paraffin-embedded tissues (Racker, Figures 1-8 to 1-11), (2) natural flattening of the hearts so that structures could be viewed in orthogonal planes at right angles to each other in 360 degrees, and (3) systematic study of the histological details of the entire AV junction region in 3 orthogonal planes in photomicrographs. A comparison of our data with that generated with conventional protocols reveals that the latter resulted in osmotic damage with a loss of cytoplasmic (eg, Hudson, Figures 2-12C and 2-13C; James, Figures 3B and 3D; and Becker and Anderson, Figure 10) and interstitial (eg, James, Figures 2B, 6, 7, and 9; James, Figures 6C and 6D, the rightward component of the compact AVN (Becker and Anderson, Figure 8A; Inoue and Becker, Figures 2B, 3, and 5 to 7), the overlay and transitional myofibers (James, Figures 2A and 3B; Becker and Anderson, Figure 2A), with the posterior nodal extension and compact AVN

**Figure 11.** A, Orthogonal transverse plane DAVB displays clusters of primary DAVB myocardial fascicles grouped into secondary fascicles. B, Secondary fascicles separated by collagen septa. C, Myocyte profiles in cross section. D, Secondary fascicles in cross section surrounded by capillaries, small blood vessels (arrow), white nerve tendrils (open curved arrows), dense varicosities of various shapes, and collagen. (Goldner’s trichrome.)
(Medkour et al, 24 Figure 2) and the AV nodal ring input being analogous to part or all of the circumferential lamina encircling the tricuspid valve (McGuire et al, 25 Figures 11 and 12) and all of the ordinary atrial myocardium of the AV junction region (Sanchez-Quintana et al, 26 Figure 2). Moreover, the PAVB is aligned with the medial or upper leg of Koch’s triangle (Figure 2A). Importantly, the circumferential lamina runs parallel to the annulus, aligned with the lower leg of Koch’s triangle; these myofibers are referred to as the posterior nodal extension (Medkour et al, 24 Figure 2) and the AV nodal ring myofibers (McGuire et al, 25 Figures 11 and 12). Because the ordinary myocardium is physically isolated from the AVN and PAVB by connective tissue and collagen ensheathment, this connective tissue isolation prevents even casual contact of the 2 classes of myocardium.

What Are the AV Junction Region Transitional Cells? Transitional cells are envisioned as connecting ordinary atrial myocardium to the AVN as listed above (eg, Ho et al22) and are in fact ordinary atrial myocardium that appear to be contiguous with the AV bridge tissues when not carefully viewed in 3 planes.

What Is the AVN or the Compact AVN? What has been termed by many investigators the AVN (Hudson, 3 Figure 2-12; Lev et al, 13 Figure 4; Truex and Smythe, 15 Figure 3; James, 23 Figure 8) or the compact AVN (Becker and Anderson, 16 Figures 2 and 4; Inoue and Becker, 19 Figure 2A; Ho et al, 22 Figure 2) is in fact restricted to the PAVB-AVN junction, which is composed of fine-caliber parallel fascicles. Because the PAVB-AVN junction is outside of the central fibrous body and not even protected by Todaro’s tendon (Figure 2A), as is the PAVB (Figures 2C2 and 2C3) and because of its minute size (Figure 3A), it is more sensitive to heat, and discrete lesions are more likely to cause complete AVN block. On the basis of the present results, the area anterior to the PAVB-AVN junction is an interwoven mass of myocardium (Figures 2A and 3A), is coincident with the appearance of the central fibrous body (sleeve of connective tissue separating AVN from the medial atrial wall (Figure 2B), and should be defined as the AVN.

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