Localization of Na+ Channel Isoforms at the Atrioventricular Junction and Atrioventricular Node in the Rat

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Background—The electrical activity of the atrioventricular node (AVN) is functionally heterogeneous, but how this relates to distinct cell types and the 3-dimensional structure of the AVN is unknown. To address this, we have studied the expression of Na,1.5 and other Na+ channel isoforms in the AVN.

Methods and Results—The rat AVN was identified by Masson’s trichrome staining together with immunolabeling of marker proteins: connexin40, connexin43, desmplakin, atrial natriuretic peptide, and hyperpolarization-activated and cyclic nucleotide–gated channel 4. Na+ channel expression was investigated with immunohistochemistry with isoform-specific Na+ channel antibodies. Na,1.1 was distributed in a similar manner to Na,1.5. Na,1.2 was not detected. Na,1.3 labeling was present in nerve fibers and cell bodies (but not myocytes) and was abundant in the penetrating atrioventricular (AV) bundle and the common bundle but was much less abundant in other regions. Na,1.5 labeling was abundant in the atrial and ventricular myocardium and the left bundle branch. Na,1.5 labeling was absent in the open node, penetrating AV bundle, AV ring bundle, and common bundle but present at a reduced level in the inferior nodal extension and transitional zone. Na,1.6 was not detected.

Conclusions—Our findings provide molecular evidence of multiple electrophysiological cell types at the AV junction. Impaired AV conduction as a result of mutations in or loss of Na,1.5 must be the result of impaired conduction in the AVN inputs (inferior nodal extension and transitional zone) or output (bundle branches) rather than the AVN itself (open node and penetrating AV bundle). (Circulation. 2006;114:1360-1371.)

Key Words: atrioventricular node □ conduction □ sodium channels □ connexins □ immunohistochemistry

As the only normal conduction pathway between the atria and ventricles, the atrioventricular node (AVN) functions to delay and regulate action potential conduction between the atria and ventricles.1 Since the first anatomic description by Tawara in 1906,2 the AVN has been one of the most investigated but least understood regions of the heart.3 For example, although there are abundant histological and electrophysiological data concerning AVN conduction,1 it has been difficult to correlate electrophysiological recordings with distinct cell types and the 3-dimensional structure of the AVN.1 In part, this is a consequence of the exceptional functional and architectural complexity of this small region.1 The cardiac voltage-gated Na+ channel, Na,1.5, is known to play a major role in the generation and conduction of the action potential.3 Recently, various naturally occurring mutations in Na,1.5 have been associated with familial cases of block4-6 or slowing7,8 of atrioventricular (AV) conduction. In addition, heterozygous mutant mice lacking 1 copy of the Na,1.5 gene have impaired AV conduction (as well as other cardiac conduction defects).2 Recently, various neuronal Na+ channel isoforms have also been shown to be expressed in the heart (sinoatrial node and ventricle) and to be functionally important.10,11 The distribution of neuronal Na+ channel isoforms in the AVN is unknown. The aim of the present study was to investigate the distribution of cardiac and neuronal Na+ channel isoforms in and around the AVN. The study has revealed a complex distribution of different cell types (based on Na+ channel expression) at the AV junction.

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Methods

Adult male Wistar rats weighing 250 to 300 g (University of Leeds, Leeds, UK) were killed according to the United Kingdom Animals (Scientific Procedures) Act, 1986. The heart was removed, and a
A region that included the triangle of Koch was dissected; a typical preparation is shown in Figure 1A. Preparations, still pinned to a silicon rubber base, were frozen in 10% gelatin (porcine type A; Sigma, Poole, United Kingdom) or Jung tissue-freezing medium (Leica Instruments GmbH, Wetzlar, Germany) at $-50^\circ$C in isopentane cooled by liquid N$_2$ and stored at $-80^\circ$C until use. The frozen preparations were secured on the chuck of a cryostat (CM 1850; Leica Microsystems GmbH, Bensheim, Germany) with Jung tissue-freezing medium and serially sectioned (at $-15^\circ$C) at 10-µm thickness perpendicular to the AV junction. Sets of sections were collected at $\approx$500-µm intervals from the coronary sinus to the common bundle. Sections were mounted on Superfrost Plus slides (BDH, Poole, United Kingdom) and stored at $-80^\circ$C until use. Some sections were stained with Masson’s trichrome as described previously. Others sections were immunolabeled (as described previously) for 5 Na$^+$ channel isoforms (Na$_1.1$, Na$_1.2$, Na$_1.3$, Na$_1.5$, and Na$_1.6$) and 5 marker proteins: connexin40 (Cx40), connexin43 (Cx43), desmoplakin I/II (DP), atrial natriuretic peptide (ANP), and...
hyperpolarization-activated and cyclic nucleotide–gated channel 4 (HCN4). Data for Na\textsubscript{1.1}, Na\textsubscript{1.3}, Na\textsubscript{1.5}, Cx43, DP, ANP, and HCN4 from 1 preparation are shown in Figures 1 through 8, but similar data were obtained from 2 other preparations (except Na\textsubscript{1.1} and Na\textsubscript{1.3} were studied in 2 preparations only, and DP was studied in 1 preparation only). In addition, at the level of the penetrating AV bundle only, similar data for Na\textsubscript{1.1}, Na\textsubscript{1.3}, and Cx43 were obtained from 3 other preparations, and similar data for Na\textsubscript{1.5} were obtained from 1 other preparation. Cx40 was studied in 2 preparations. Nav1.2 and Nav1.6 were studied in 3 preparations (level of the penetrating AV bundle only). In 1 case, sections (treated as above) were cut from a whole-heart preparation (excluding the apex of the ventricles) in the sagittal plane. See the online-only Data Supplement for further details of the methods used.

The authors had full access to the data and take full responsibility for their integrity. All authors have read and agree to the manuscript as written.

**Results**

At the rat AV junction, immunohistochemistry was used to investigate the expression of Na\textsubscript{1.1}, Na\textsubscript{1.2}, Na\textsubscript{1.3}, Na\textsubscript{1.5}, and Na\textsubscript{1.6}; the skeletal isoform, Na\textsubscript{1.4}, was not investigated. Na\textsubscript{1.2} and Na\textsubscript{1.6} were not detected (in nodal tissue or in atrial and ventricular muscle) and will not be considered further. In addition, to help distinguish different cell types, Cx40, Cx43, DP, ANP, and HCN4 were immunolabeled as markers: Cx40, a gap junction protein, is expressed in the AV conduction system\textsuperscript{14}; Cx43, another gap junction protein, is the principal connexin in the working myocardium (but is sparse or absent in the AVN)\textsuperscript{14}; DP, a constituent of desmosomes, is expressed in all myocytes\textsuperscript{15}; ANP, a body-fluid control hormone, is expressed by atrial muscle only\textsuperscript{16}; and HCN4, the principal isoform responsible for the pacemaker current, $I_f$, is expressed in the AVN.\textsuperscript{13} From preparations that included the triangle of Koch (with borders defined by the coronary sinus, tendon of Todaro, and tricuspid valve annulus), sets of 10-μm sections (cut perpendicular to the AV junction) were collected at ~500-μm intervals. The position of the 14 sets of sections in 1 preparation is shown in Figure 1A.

Data for Cx43, DP, ANP, HCN4, Na\textsubscript{1.1}, Na\textsubscript{1.3}, and Na\textsubscript{1.5} from the preparation shown in Figure 1A are shown in Figures 1 through 8.

**Figure 2.** Expression of Cx43, DP, ANP, and HCN4 in inferior nodal extension. A, Masson’s trichrome–stained section. B, Adjacent section labeled for Cx43 (green) and DP (red). C, Adjacent section labeled for Cx43 (green) and ANP (red). D, Adjacent section labeled for HCN4 (green) and Cx43 (red). Scale bars = 100 μm. AM indicates atrial myocardium; VM, ventricular myocardium.
Inferior Nodal Extension

Nomenclature used for the AV junction follows the recommendations of Cosio et al. Figures 2 through 4 show labeling at the level of the inferior nodal extension (Figure 1A, level 4). Figure 2A shows a Masson’s trichrome–stained section (connective tissue is stained blue, and myocytes are stained red). The inferior nodal extension is outlined in Figure 2A (and all other panels in Figures 2 and 4). The inferior nodal extension was in close proximity to the atrial muscle but separated from the ventricular muscle by connective tissue and the tricuspid valve (Figure 2A). The myocytes in the inferior nodal extension were generally stained a lighter red than the surrounding atrial and ventricular muscle (Figure 2A). Figure 2B shows that DP (red signal) was expressed in all myocytes (at cell margin), including those of the inferior nodal extension; as expected, it was not expressed in connective tissue. Cx40 was not expressed in the inferior nodal extension (data not shown). Cx43 was expressed in the atrial and ventricular muscle (at intercalated discs) but not in the inferior nodal extension (eg, Figure 2B, green signal). Figure 2C shows that ANP (red signal) was expressed in the atrial muscle (present in intracellular vesicles) but not in the inferior nodal extension or ventricular muscle. Figure 2D shows that HCN4 (green signal) was expressed in the inferior nodal extension (at the cell membrane; there was also intracellular labeling) but not in the atrial or ventricular muscle (dull green signal in the atrial and ventricular muscle in Figure 2D is background only). The unique pattern of expression in the inferior nodal extension (DP-positive/Cx43-negative/ANP-negative/HCN4-positive) allowed it to be distinguished from the nearby atrial muscle (DP-positive/Cx43-positive/ANP-positive/HCN4-negative). Figure 3 shows high-magnification images of Na,1.1 labeling (green signal) in different regions. Na,1.1 labeling was present in the atrial and ventricular muscle in the outer cell membrane; there was also a nonspecific labeling of nuclei (Figures 3A and 3G). Na,1.1 labeling was also present in the inferior nodal extension (Figure 3D). Figure 4 shows immunolabeling of Na,1.3 and Na,1.5 (green signal) at the inferior nodal extension. There was labeling of Na,1.3 (bright green labeling, some highlighted by arrows, in Figure 4A) but not in myocytes (dull green labeling in Figure 4A is background only). Na,1.3 colocalized with a neuronal cell marker, neurofilament 160 (Data Supplement Figure I), and this shows it to be present in nerve fibers/cell bodies. There was little or no innervation (as detected by Na,1.3 labeling) in the atrial and ventricular muscle, but there was innervation (relatively weak) in the inferior nodal extension (Figure 4A). There was dense labeling of Na,1.5 in the atrial and ventricular muscle (Figure 4B). There was labeling of Na,1.5, although weaker than in atrial and ventricular muscle, in the inferior nodal extension (Figure 4B). This was confirmed by semiquantitative assessment of Na,1.5 labeling (Data Supplement Figure II). As expected, there was no labeling of Na,1.5 in connective tissue (Figure 4B). Control experiments confirmed that the Na,1.5 antibody was specific (Data Supplement Figure III).

Penetrating AV Bundle

Continuous with the inferior nodal extension is the superior enclosed part of the AVN, described by Tawara as the penetrating AV bundle. Figures 5 and 6 show labeling at the level of the penetrating AV bundle (Figure 1A, level 9). At this level, the tissue architecture was more complex than at the inferior nodal extension. The penetrating AV bundle consisted of an ovoid group of myocytes, which were separated from the surrounding atrial and ventricular muscle by connective tissue (Figure 5A). The myocytes in the penetrating AV bundle were stained a lighter red than the surrounding atrial and ventricular muscle (Figure 5A). Figure 2B shows that DP (red signal) was expressed in all myocytes (at cell margin), including those of the inferior nodal extension; as expected, it was not expressed in connective tissue. Cx40 was not expressed in the inferior nodal extension (data not shown). Cx43 was expressed in the atrial
below). Once again, DP was expressed in all myocytes, including those in the penetrating AV bundle and the transitional zone (Figure 5B). Cx40 was expressed in the penetrating AV bundle, but only in the lower half (Data Supplement Figure IV). Cx43 was not detectable in the penetrating AV bundle or transitional zone (Figure 5B). ANP was not expressed (Figure 5C), whereas HCN4 was expressed (Figure 5D), in the penetrating AV bundle and transitional zone. There was no Nav1.1 labeling (apart from nonspecific labeling of nuclei) in the penetrating AV bundle (Figure 3E). Once again, Nav1.3 labeling was only observed in nerve fibers and cell bodies (Figure 6A). Innervation (as judged by Nav1.3 labeling) was abundant in the penetrating AV bundle (more abundant than in inferior nodal extension; Figure 6A). There was much less abundant innervation in the transitional zone (similar to that of the inferior nodal extension; Figure 6A). Figure 6B shows that there was no labeling of Na1.5 in the penetrating AV bundle. In the transitional zone, there was labeling of Na1.5, it was weaker than that in the atrial and ventricular muscle (as in the case of the inferior nodal extension). This was confirmed by semiquantitative assessment of Na1.5 labeling (Data Supplement Figure II).

**Common Bundle**

Figures 7 and 8 show labeling at the level of the common bundle (Figure 1A, level 11). At this level, the tissue architecture was even more complex. The common bundle consisted of a triangle of pale red–staining myocytes, which were separated from surrounding atrial and ventricular myocytes by connective tissue (Figure 7A). At this level, the zone of transitional myocytes was again present (Figure 7A), but it was smaller than at the level of the penetrating AV bundle (Figure 5A). In addition, there was another region of nodal-like myocytes (Figure 7A), and this we refer to as the termination of the tricuspid valve AV ring bundle (it is not, however, the termination of the tract of nodal-like myocytes; see below). This region of nodal-like myocytes lies above, but separate from, the transitional zone (Figure 7A). Once again, DP was expressed in all myocytes, including those in the common bundle, transitional zone, and termination of the AV ring bundle (Figure 7B). Cx40 was expressed throughout the common bundle (Data Supplement Figure V). Weak punctuate labeling of Cx43 was present in the common bundle, but Cx43 was not detectable in the transitional zone and termination of the AV ring bundle (Figure 7C). ANP was expressed in the atrial muscle but not elsewhere (Figure 7C), whereas HCN4 was expressed in the common bundle, transitional zone, and termination of the AV ring bundle (Figure 7D). There was no Na1.1 labeling (apart from nonspecific labeling of nuclei) in the common bundle (Figure 3F) or the termination of the AV ring bundle.
(Figure 3C), whereas there was labeling in the transitional zone (Figure 3B). Labeling of Na,1.3 (once again in nerve fibers and cell bodies) was abundant in the common bundle and was present but much less abundant in the transitional zone, and there was little or no labeling in the termination of the AV ring bundle (Figure 8A). Na,1.5 labeling was present but less abundant (than in atrial and ventricular muscle) in the transitional zone and absent from the common bundle and termination of the AV ring bundle (Figure 8B); this was confirmed by semiquantitative assessment of Na,1.5 labeling (Data Supplement Figure II).

### Left Bundle Branch

In the left bundle branch, as in the common bundle, Cx40 and HCN4, but not Cx43, were expressed (Data Supplement Figure V). Whereas Na,1.5 was not expressed in the common bundle, it was expressed in the left bundle branch, 1.5 mm from the common bundle (Data Supplement Figures II and V). DP and Na,1.1 were expressed, whereas Na,1.3 was not expressed in the left bundle branch (data not shown). Strikingly, ANP was expressed in the left bundle branch (data not shown). The right bundle branch was not investigated.

### Around the Tricuspid Annulus

Nodal/nodal-like myocytes are not confined to the triangle of Koch and continue as the AV ring bundle around the tricuspid valve.18 The expression pattern in the AV ring bundle on the opposite side of the tricuspid valve to the AVN was the same as that of the transitional zone but different from that of the common bundle: DP-positive (data not shown), Cx40-negative (data not shown), Cx43-negative (Data Supplement Figure VI), ANP-negative (data not shown), HCN4-positive (Data Supplement Figure VI), Na,1.1-positive (Data Supplement Figure VII), Na,1.3-positive (data not shown), and Na,1.5-positive (Data Supplement Figure VII).

### Summary

The Table summarizes the expression pattern in the different tissues. On the basis of the expression pattern, 3 types of nodal/nodal-like myocytes were identified: ring bundle myocytes were present in the inferior nodal extension, the transitional zone, and the AV ring bundle on the opposite side of the tricuspid valve to the AVN; nodal myocytes were expressed in the open node (nodal tissue immediately prox-
imal to the penetrating AV bundle), the penetrating AV bundle, and the termination of the AV ring bundle; and His bundle myocytes only differed from nodal myocytes in their expression of Cx40 and were located in the penetrating AV bundle (lower part), common bundle, and bundle branches.

For all 14 levels investigated from the preparation shown in Figure 1A, Masson’s trichrome–stained sections are shown in Figure VIII of the Data Supplement, and the distribution of the different tissues and the 3 types of nodal/nodal-like myocytes is summarized in Figure 1C. The distribution of the 3 types of nodal/nodal-like myocytes (from Figure 1C) was mapped onto the original preparation (Figure 1A). Figure 1A shows the following: ring bundle myocytes (light blue) in the inferior nodal extension continued as the transitional zone adjacent to the open node; nodal myocytes (green) in the open node projected into the penetrating AV bundle; nodal myocytes (green) were also present in the termination of the AV ring bundle; and His bundle myocytes (purple) starting in the lower part of the penetrating AV bundle continued in the common bundle. Figure 1B shows a schematic of the whole of the tricuspid valve AV ring bundle. Figure 1B shows that the penetrating AV bundle, transitional zone, and inferior nodal extension are just 1 part of the AV ring bundle that projects around the tricuspid valve. Whereas much of the AV ring bundle is made up of ring bundle myocytes, the termination of the tricuspid valve AV ring bundle (above the AVN) comprises nodal myocytes. Figure 1B shows that the termination of the tricuspid valve AV ring bundle is not the termination of the tract of nodal/nodal-like myocytes; it continues as the mitral valve ring bundle.

Previously, we have shown (using optical recordings of action potentials) that in the rabbit, pacemaker activity at the AV junction originates in the inferior nodal extension. In similar experiments, we observed that in the rat, pacemaker activity at the AV junction also originates in the region of the inferior nodal extension. From the leading pacemaker site, the action potential propagated at a slow velocity (4 to 12 cm/s) along a discrete pathway (the slow pathway) before exiting into the atrial muscle (data not shown). The approximate position of the slow pathway as defined by these experiments corresponds to the inferior nodal extension.

**Discussion**

The present study shows for the first time the distribution of different Na$^+$ channel isoforms at the AV junction. Na$_{1.1}$ and Na$_{1.5}$ were distributed in a similar manner, Na$_{1.3}$ was only present in neuronal tissue, and Na$_{1.2}$ and Na$_{1.6}$ were not detected. The study has revealed a complex organization of multiple myocyte types (including 3 types of nodal/nodal-like myocytes) at the AV junction that must be the substrate of the complex electrophysiology of the AVN, including slow conduction, dual-pathway electrophysiology, and AVN reentry.

**Relation to Previous Studies**

Petrecca et al$^{20}$ previously reported evidence of a lower level of expression of Na$_{1.1}$ in the mid-nodal cells of the rabbit AVN and the results of the present study from the rat AVN are consistent with this. But Petrecca et al$^{20}$ used an antibody raised against a conserved region of the Na$_{1.1}$ subfamily (the antibody, therefore, could not discriminate between different Na$^+$ channel isoforms); there have been no previous studies

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**Figure 6.** Expression of Na$_{1.3}$ and Na$_{1.5}$ in penetrating AV bundle. A, Na$_{1.3}$ (green) and Cx43 (red) double-labeled section. B, Na$_{1.5}$ (green)-labeled section. Sections are adjacent to those in Figure 5. Scale bars=200 μm. AM indicates atrial myocardium; VM, ventricular myocardium.
using antibodies for specific Na$^+$ channel isoforms. In the present study, the labeling of HCN4 in the rat AVN is similar to that in the rabbit AVN reported by Dobrzynski et al. Ko et al reported that Cx43 is expressed in the inferior nodal extension in the rabbit, whereas in the present study of the rat and in a study by Dobrzynski et al of the rabbit, the inferior nodal extension was Cx43-negative. This discrepancy is likely to be the result of confused nomenclature only; the “inferior nodal extension” in the study of Ko et al probably corresponds to the tract of Cx40-expressing (in the rat; Data Supplement Figure IV) or Cx43-expressing (in the rabbit; unpublished observations) His bundle myocytes. Above the “inferior nodal extension” in the study by Ko et al, there is a tract of Cx43-negative myocytes (termed “transitional cells” by Ko et al), and this corresponds to the inferior nodal extension in the present study (Figure 1) and the study by Dobrzynski et al.

Electrophysiologically, 3 types of myocytes have been identified at the AV junction (atrinodal [AN], N [nodal], and nodo-His [NH] myocytes), and it is tempting to speculate that they may be related to the 3 types of nodal/nodal-like myocytes identified in the present study. In the rabbit, whereas atrial myocytes have an upstroke velocity of >100 V/s, the upstroke velocity of AN (ANCO and ANL), N, and NH myocytes is 48, 18, and 17 V/s, respectively. In comparison, in the present study, the intensity of Na$^+$ labeling in atrial myocytes was 79±5% (of labeling in ventricular myocytes), whereas the intensity of Na$^+$ labeling in ring bundle myocytes, nodal myocytes, and His bundle myocytes was 34±5/39±4% (inferior nodal extension/transitional zone), 18±6/14±3% (open node/termination of AV ring bundle), and 24±7% (common bundle), respectively (Data Supplement Figure II). At the level of the penetrating AV bundle and common bundle, there was a second group of nodal-like myocytes, and these were referred to as transitional myocytes (although they were indistinguishable from the nodal-like myocytes of the inferior nodal extension). These nodal-like myocytes (ring bundle myocytes) are transitional in that although atrial myocytes show abundant expression of Na$^+$, and nodal myocytes in the penetrating AV bundle show no expression, they show an intermediate...
level of expression (Data Supplement Figure II). In the rabbit, transitional myocytes (with a transitional histological appearance and presumed to be AN myocytes) have also been identified.18,22

Understanding the Electrophysiology of the AV Junction

The AVN has dual inputs (slow and fast pathways) from the atrial myocardium, and this is the substrate for AVN reentry.23 The slow pathway proceeds parallel to the tricuspid valve annulus from beneath the coronary sinus to the penetrating AV bundle near the apex of the triangle of Koch, whereas the fast pathway enters the penetrating AV bundle superiorly from the direction of the atrial septum. The results from the present study help to understand this dual-pathway electrophysiology and other aspects of the electrophysiology of the AV junction.24,25

In the atrial (and ventricular) myocardium, the upstroke velocity (100 to 160 V/s) and amplitude (90 to 110 mV) of the action potential are high,22,26 and this is because the voltage-dependent Na\(^{+}\) current, $I_{Na}$, is responsible for it. In accordance with this, in the present study, we observed

Summary of Expression of Marker Proteins and Na\(^{+}\) Channel Isoforms in and Around the AVN

<table>
<thead>
<tr>
<th>Color Code (Figure 1C)</th>
<th>DP</th>
<th>Cx40</th>
<th>Cx43</th>
<th>ANP</th>
<th>HCN4</th>
<th>Na(<em>{1.1}$/Na(</em>{1.5})</th>
<th>Na(<em>{1.2}$/Na(</em>{1.6})</th>
<th>Na(_{1.3})*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atrium</td>
<td>Pink</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
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<tr>
<td>Ventricle</td>
<td>Red</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
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<tr>
<td>Ring bundle myocytes: inferior nodal extension and transitional zone</td>
<td>Light blue</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Nodal myocytes: open node, penetrating AV bundle (upper part) and AV ring bundle</td>
<td>Green</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>His bundle myocytes: penetrating AV bundle (lower part) and common bundle</td>
<td>Purple</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Left bundle branch</td>
<td>Not shown</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
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<tr>
<td>Connective tissue</td>
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<td>−</td>
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+ Indicates expression; −, no expression.

*Labeling present in nerve fibers and nerve cell bodies.
labeling of both Na\textsubscript{1.5} and Na\textsubscript{1.1} in the atrial myocardium (eg, Figures 3 and 6 and Figure II in the Data Supplement). The high conduction velocity in atrial myocardium (eg, 70 cm/s\textsuperscript{3}) can be explained by the high upstroke velocity of the action potential (as a result of the expression of Na\textsubscript{1.5} and Na\textsubscript{1.1}) and the abundant expression of the medium conductance gap junction protein, Cx43 (for example, in Figure 5) in the atrial myocardium.

In the slow pathway, the upstroke velocity and amplitude of the action potential have been reported to be intermediate between those in the atrial myocardium and the penetrating AV bundle (41 V/s and 70 mV, respectively\textsuperscript{26}). Consistent with this, in the present study, we observed labeling of both Na\textsubscript{1.5} and Na\textsubscript{1.1} in the inferior nodal extension, but the labeling of Na\textsubscript{1.5} was less abundant than in the atrial myocardium (Figures 3D and 4B and Figure II in the Data Supplement). The low conduction velocity of the slow pathway/inferior nodal extension (4 to 12 cm/s in the present study; 7 to 8 cm/s in the study by Nikolski et al\textsuperscript{19}) could be the result of the reduced expression of Na\textsubscript{1.5}. It could also be the result of the lack of expression of the medium and high conductance gap junction proteins, Cx43 and Cx40, in the inferior nodal extension (Cx45 is expressed instead\textsuperscript{13}).

The location of the transitional zone means that it could be the fast pathway into the penetrating AV bundle from the direction of the atrial septum. In the fast pathway (and AN cells), the upstroke velocity (40 to 60 V/s) and amplitude (70 to 80 mV) of the action potential are again intermediate between those in the atrial myocardium and the penetrating AV bundle.\textsuperscript{22,26} Consistent with this, we observed labeling of both Na\textsubscript{1.5} and Na\textsubscript{1.1} in the transitional zone, but the labeling of Na\textsubscript{1.5} was less abundant than in the atrial muscle (Figures 3B and 6B and Figure II in the Data Supplement). Conduction time via the fast pathway is shorter than that via the slow pathway. If the transitional zone does constitute the fast pathway, the difference in conduction time between the slow and fast pathways (ie, between inferior nodal extension and transitional zone) is unlikely to be the result of differences in the expression of Na\textsuperscript{+} channels or connexins (because expression in the 2 regions was similar; Table). Instead, the shorter conduction time via the fast pathway is perhaps the result of the shortness (≈1 mm) of the tract of Cx43-negative myocytes with reduced Na\textsubscript{1.5} expression in the transitional zone that the action potential has to propagate across to reach the open node (the length of the slow pathway/inferior nodal extension is much greater; Figure 1A).

In the penetrating AV bundle (and N cells), in contrast to atrial muscle, the upstroke velocity (18 V/s) and amplitude (50 to 80 mV) of the action potential are low,\textsuperscript{26} and this is because the L-type Ca\textsuperscript{2+} current, I_{Ca,L}, rather than I_{Na}, is responsible for it. In cells likely to be from the penetrating AV bundle, there is no I_{Na}.\textsuperscript{20} Consistent with this, we observed no labeling of either Na\textsubscript{1.5} or Na\textsubscript{1.1} in the penetrating AV bundle (Figures 3E and 6B and Figure II in the Data Supplement). The absence of Na\textsubscript{1.5} or Na\textsubscript{1.1} expression continued from the open node, through the penetrating AV bundle and common bundle to the proximal left (and presumably right) bundle branch. Consistent with this, in the NH cells in this region, the upstroke velocity (20 to 30 V/s) and amplitude (60 to 80 mV) of the action potential are still low.\textsuperscript{26} Slow conduction through the AVN is responsible for the delay between activation of the atria and ventricles in the cardiac cycle, and the absence of Na\textsubscript{1.5} and Na\textsubscript{1.1} from the open node to the proximal left and right bundle branches will at least contribute to the slow conduction. However, the expression pattern of connexins is also expected to contribute: From the open node to the proximal left bundle branch, the medium conductance gap junction protein, Cx43, was not expressed or was poorly expressed (eg, Figure 6); however, from the penetrating AV bundle to the left bundle branch, the high-conductance gap junction protein, Cx40, was expressed (eg, Data Supplement Figure IV).

In the rabbit and dog, the upstroke velocity and amplitude of the action potential and conduction velocity are high in the common bundle,\textsuperscript{22,26} and this is inconsistent with the lack of expression of Na\textsubscript{1.5} and Na\textsubscript{1.1} in the common bundle in the rat (Figures 3F and 8B and Figure II in the Data Supplement). It is possible that this reflects a species difference: Because a rat is 10 to 100 times smaller than a rabbit or dog, and yet the PR interval is approximately the same,\textsuperscript{27} it follows that Na\textsubscript{1.5} may be absent from a greater proportion of the AV conduction pathway. In the common bundle, Mazgalev et al\textsuperscript{24} have described distinct electrophysiologies in the mid and lower nodal myocytes. Does this correspond to the upper Cx40-negative and lower Cx40-positive nodal myocytes in the penetrating AV bundle in the rat, as shown in Figure IV of the Data Supplement?

The pattern of Na\textsubscript{1.3} labeling was different from the pattern of labeling of myocytes by other antibodies, but it was similar to that of neuronal tissue at the AV junction in the studies by Petrecca et al\textsuperscript{28} and Anderson.\textsuperscript{18} Furthermore, in the present study, Na\textsubscript{1.3} labeling was colocalized with labeling of the neuronal cell marker, neurofilament 160 (Data Supplement Figure I), and therefore, it is likely that Na\textsubscript{1.3} is expressed by neuronal tissue. Petrecca et al\textsuperscript{28} and Anderson\textsuperscript{18} showed that neuronal innervation is abundant in the nodal tissues at the AV junction (compared with the working myocardium). In the present study, Na\textsubscript{1.3} labeling was minimal in atrial and ventricular muscle, present (but not abundant) in the specialized pathways into the AVN (inferior nodal extension and transitional zone), and abundant in the open node and penetrating AV bundle. Therefore, innervation may be controlling AV conduction at the point at which conduction will be the most severely constrained by the absence of Na\textsubscript{1.5}, Na\textsubscript{1.1}, Cx40 (in part, at least), and Cx43 (to a large degree, at least). Interestingly, in humans, innervation is more abundant in the transitional zone than in the compact node and penetrating bundle.\textsuperscript{29}

**Why Do Mutations in or Knockout of Na\textsubscript{1.5} (SCN5A) Result in AV Conduction Defects?**

The role of Na\textsubscript{1.5} in AV conduction has recently been highlighted by the discovery of inherited AV conduction
disturbances (block or slowing of AV conduction) linked to mutations in SCN5A and its conduction. Na⁺ channels play a major role in conduction, and this is the first study to map in detail the distribution of Na⁺ channels throughout the AV junction. The expression of Na⁺ channels was nonuniform at the AV junction and allowed the identification of various types of nodal/nodal-like myocytes.

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Disclosures
None.

References
CLINICAL PERSPECTIVE

As the only conduction pathway between the atria and ventricles, the atrioventricular node (AVN) functions to delay and regulate action potential conduction between the atria and ventricles. The electrical activity of the AVN is heterogeneous, but how this relates to distinct cell types and the 3-dimensional structure of the AVN is unknown. Sodium channels play a major role in the generation and conduction of the action potential, and recently, various naturally occurring mutations in the cardiac sodium channel Na\(_1\).5 have been associated with familial cases of block or slowing of atrioventricular conduction. We have studied the expression of Na\(_1\).5 and other sodium channel isoforms in the AVN using immunohistochemistry. Na\(_1\).1 and Na\(_1\).5 were abundant in the atrial and ventricular myocardium and the left bundle branch; present at a reduced level in the inferior nodal extension, transitional zone, and atrioventricular ring bundle; and absent in the open node, penetrating bundle, and common bundle. Na\(_1\).3 was present in neuronal tissue (but not myocytes) and was abundant in the penetrating bundle and the common bundle but was much less abundant in other regions. Our findings provide molecular evidence of multiple electrophysiological cell types at the atrioventricular junction and help us to understand the delay in conduction through the AVN as well as dysfunction of the AVN (eg, as a result of mutations in Na\(_1\).5).
Localization of Na\textsuperscript{+} Channel Isoforms at the Atrioventricular Junction and Atrioventricular Node in the Rat
Shin Yoo, Halina Dobrzynski, Vadim V. Fedorov, Shang-Zhong Xu, Tomoko T. Yamanushi, Sandra A. Jones, Mitsuru Yamamoto, Vladmir P. Nikolski, Igor R. Efimov and Mark R. Boyett

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