Persistence of Functional Atrioventricular Accessory Pathways in Postseptated Embryonic Avian Hearts

Implications for Morphogenesis and Functional Maturation of the Cardiac Conduction System

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Background—During heart development, the ventricular activation sequence changes from a base-to-apex to an apex-to-base pattern. We investigated the possibility of impulse propagation through remnants of atrioventricular (AV) connections in quail hearts.

Methods and Results—In 86 hearts (group A, HH30–34, n=15; group B, HH35–44, n=65; group C, 5 to 6 months, n=6) electrodes were positioned at the left atrium, right ventricular base, left ventricular (LV) base, and LV apex. In group A, LV base activation preceded LV apex activation in the majority of cases (60%; 9 of 15), whereas hearts in group B primarily demonstrated an LV apex-to-base activation pattern (72%; 47 of 65). Interestingly, in group B, the right ventricular base (17%; 11 of 65) or LV base (8%; 5 of 65) exhibited premature activation in 25% (16 of 65) of cases, whereas in 26% (17 of 65), the right ventricular base or LV base was activated simultaneously with the LV apex. Morphological analysis confirmed functional data by showing persistent muscular AV connections in embryonic hearts. Interestingly, all myocardial AV connections stained positive for periostin, a nonmyocardial marker. Longitudinal analysis (HH35–44) demonstrated a decrease in both the number of hearts that exhibited premature base activation (P=0.015) and the number (P=0.004) and width (P=0.179) of accessory AV pathways with developmental stage in a similar time course. In the adult quail hearts, accessory myocardial AV pathways were functionally and morphologically absent.

Conclusion—Thus, impulse propagation through persistent accessory AV connections remains possible at near-hatching stages (HH44) of development, which may provide a substrate for AV reentrant arrhythmias in perinatal life. Periostin positivity and absence of AV pathways in the adult heart suggest that these connections eventually lose their myocardial phenotype, which implicates ongoing AV ring isolation perinatally and postnatally. (Circulation. 2007;115:17-26.)

Key Words: arrhythmia • conduction • electrophysiology • morphogenesis

Atrioventricular (AV) reentrant tachycardias involve the presence of an accessory myocardial AV pathway that bypasses the insulating annulus fibrosis; they are one of the most common arrhythmias in humans.1-3 In children, the first episode of this type of arrhythmia occurs before birth or in the first months of life in ~60% of cases and appears to resolve spontaneously in two thirds of cases before the age of 1 year.4,5 The natural course in fetuses or neonates is usually benign,5 but a radiofrequency catheter ablation procedure may be necessary to control the arrhythmia.6 Although a causal relationship between abnormal cardiogenesis and arrhythmogenesis has been hypothesized,7 the underlying mechanisms responsible for the development of these accessory pathways (APs) are still not completely understood.

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In the early tubular heart, the atrial myocardium is continuous with the ventricular myocardium, and the blood is driven in a caudal to cranial direction by a slow peristaltic contraction pattern that originates from the primitive pacemaker in the caudal sinus venosus region,8,9 with the endocardial cushions serving as primitive valves.10 Shortly there-
after, the emerging atrium and ventricle in the looped heart start to contract sequentially as a result of the development of alternating slow (sinoatrial region, AV junction, and outflow tract) and fast (atrial and ventricular regions) conducting regions, whereas propagation of the depolarization wave keeps following the direction of the bloodstream. Ultimately, however, ventricular activation shifts from this immature base-to-apex sequence to a mature apex-to-base pattern.

This transition in ventricular activation pattern reflects maturation of the His-Purkinje system (HPS) and coincides with completion of ventricular septation. Importantly, and almost simultaneously, the existing AV myocardial continuity, which is present on the entire circumference of the AV groove, has been found morphologically in postseptated embryonic and adult chick, mouse, and human hearts, the electrophysiological properties of which underlie the occurrence, and in many cases underlie early tachycardias. Nevertheless, the developmental mechanisms that underlie the occurrence, and in many cases underlie early and spontaneous disappearance of APs in fetuses and neonates, are incompletely understood.

We hypothesized that accessory AV connections that bypass the insulating annulus fibrosis are embryonic remnants of myocardium that retain their conducting properties in postnatal life. By analyzing the ventricular activation sequence in embryonic and adult quail hearts with extracellular electrode recordings and by correlating these electrophysiological data with morphology, we demonstrate that functional remnants of AV connections indeed remain present at late postseptational stages of embryonic heart development.

Methods

Experimental Preparations

Fertilized eggs of the Japanese quail (Coturnix coturnix japonica) were incubated at 37.5°C and 80% humidity. All animal experiments were in accordance with the institutional guidelines of the Leiden University Medical Center. After termination of incubation and staging according to Hamburger-Hamilton (HH) criteria, the embryonic hearts (HH30–34, n=15; HH35–44, n=65) were carefully isolated from the embryos after euthanization by decapitation.

The hearts were placed in a custom-built, fluid-heated, temperature-controlled tissue bath. Subsequently, the embryonic hearts were superfused (30±0.1°C) and the adult hearts were Langendorff perfused (65 mm Hg, 37±0.1°C with carboxygenated (95% O₂, 5% CO₂) Tyrode’s solution (in mmol/L): NaCl 130, KCl 4, KH₂PO₄ 1.2, MgSO₄ 0.6, NaHCO₃ 20, CaCl₂ 1.5, and glucose 10 (pH 7.35).

Electrophysiological Recording Protocol

Unipolar extracellular recordings were performed by consistently positioning 4 tungsten electrodes (tip: 1 to 2 μm; impedance 0.5 to 1.0 MΩ; WPI Inc, Berlin, Germany) on the left atrium, right ventricular base (RVB), left ventricular base (LVB), and left ventricular apex (LVA) (Figure 1). Electrograms were recorded with a high-gain, low-noise, direct-current biosignal amplifier system (Isomax; WPI Inc). The signals were band-pass filtered (300 Hz to 1 kHz) and notch filtered (50 Hz) before being digitized at a sampling rate of ≥1 kHz with a computerized recording system (Prucka Engineering Inc, Houston, Tex). Pacing was performed with a stimulator (EP-3, EP MedSystems Inc, West Berlin, NJ), which provided monophasic stimuli (strength 5 to 10 mA, width 1.0 ms). The embryonic hearts were stimulated at the high right atrium.

The hearts were in group A, hearts in group B with a stable spontaneous heart rate (HR) of at least 60 beats per minute (bpm; group B₁), and hearts in group C were allowed to beat spontaneously, whereas hearts with an HR of <60 bpm (group B₂) were stimulated at a fixed cycle length of 500 ms.

After baseline recordings in 15 hearts (HH38–41), 1 mL adenosine (0.3 mg/mL) was superfused on the heart to a final concentration of 0.03 mg/mL (0.11 μmol/L) in the tissue bath to analyze transitions in ventricular activation sequence after conduction through the AV node was slowed.

Definitions and immunohistochemistry are described in detail in the online-only Data Supplement.

Statistical Analysis

HR and AV interval were compared between groups with a 2-tailed Student t test for normally distributed values; otherwise, the Mann-Whitney U test was used (AV interval group B₁). The symmetry of the distribution was determined by measurement of the skewness value. For comparison of categorical variables (ventricular activation patterns, AP number, AP width), the χ² test was performed. Results are presented as mean±SD (range). A P value of <0.05 (2 tailed) was considered statistically significant. All analyses were performed with the Statistical Package for Social Studies version 11.0 (SPSS Inc, Chicago, Ill).

The online-only Data Supplement contains more information about methods used in this study. The authors had full access to and take responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

Results

Experimental Preparations

In 15 group A hearts (HH30–34), electrograms were recorded during stable heart rhythm of 143±30 bpm (AV interval range 65±19 bpm).
In 15 group B1 hearts (HH35–44), the HR (91 ± 36 bpm) and the AV interval (80 ± 15 ms) were not significantly different compared with group A (P = 0.414 and P = 0.415, respectively). During pacing of the right atrium (120 bpm) in the remaining 50 hearts in group B2 (HH35–44), the mean AV interval was 78 ± 28 ms (P = 0.758, compared with group B1). The 6 adult quail hearts showed an HR of 199 ± 52 bpm and an AV interval of 80 ± 7 ms. Table 1 summarizes the general (electrophysiological) characteristics of the quail hearts.

LV Activation Sequence: Base to Apex or Apex to Base?
Because initial studies mainly reported on LV activation patterns,12 we first analyzed the relationship between LVA and LVB electrograms. Hearts in group A primarily showed base-to-apex LV activation patterns (9 of 15, 60%), with LVB activation preceding LVA activation by 5 ± 4 ms (Table 2).

In contrast, hearts in group B mainly demonstrated apex-to-base LV activation patterns, with LVA activation preceding LVB activation by 4 ± 3 ms in 47 of 65 hearts (72%) (Table 2). In group B, no differences in LV activation patterns were observed between hearts that beat spontaneously and hearts driven by right atrium pacing (P = 0.843). Representative examples of electrode recordings in an embryonic heart from group A are shown in Figure 2A and 2B, and recordings in an embryonic heart from group B2 are shown in Figure 2C and 2D.

Global (LV and RV) Activation Patterns
Analysis of the more global ventricular activation patterns, which include RVB activation, revealed that quail hearts in group A demonstrated earliest ventricular activation at the LVB in 40% (n = 6) of cases, whereas the RVB was the site of earliest ventricular activation in 20% (n = 3) of cases (Table 2).

### Table 1. Developmental Stages of the Quail Hearts From Groups A, B, and C With Corresponding HRs and AV Intervals

<table>
<thead>
<tr>
<th>Developmental Stage, HH or months</th>
<th>SR/Paced</th>
<th>n</th>
<th>HR, bpm, mean ± SD (range)</th>
<th>AV Interval, ms, mean ± SD (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Group A</strong> (n = 15)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30 SR</td>
<td>5</td>
<td></td>
<td>140 ± 33 (100–184)</td>
<td>100 ± 15 (87–125)</td>
</tr>
<tr>
<td>31 SR</td>
<td>5</td>
<td></td>
<td>142 ± 41 (94–180)</td>
<td>93 ± 10 (82–107)</td>
</tr>
<tr>
<td>32 SR</td>
<td>1</td>
<td></td>
<td>175</td>
<td>115</td>
</tr>
<tr>
<td>33 SR</td>
<td>2</td>
<td></td>
<td>140 ± 11 (132–147)</td>
<td>137 ± 12 (129–146)</td>
</tr>
<tr>
<td>34 SR</td>
<td>2</td>
<td></td>
<td>141 ± 6 (137–145)</td>
<td>76 ± 2 (74–78)</td>
</tr>
<tr>
<td>Subtotal</td>
<td>15</td>
<td></td>
<td>143 ± 30 (94–184)</td>
<td>100 ± 20 (74–146)</td>
</tr>
<tr>
<td><strong>Group B1</strong> (n = 15)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>35 SR</td>
<td>1</td>
<td>76</td>
<td>76 ± 12 (63–92)</td>
<td>71 ± 9 (62–81)</td>
</tr>
<tr>
<td>36 SR</td>
<td>2</td>
<td></td>
<td>170 ± 5 (167–174)</td>
<td>91 ± 19 (78–105)</td>
</tr>
<tr>
<td>37 SR</td>
<td>2</td>
<td></td>
<td>76 ± 21 (61–90)</td>
<td>74 ± 20 (60–89)</td>
</tr>
<tr>
<td>38 SR</td>
<td>4</td>
<td></td>
<td>94 ± 17 (77–112)</td>
<td>72 ± 9 (61–78)</td>
</tr>
<tr>
<td>39 SR</td>
<td>3</td>
<td></td>
<td>77 ± 13 (63–90)</td>
<td>94 ± 18 (78–114)</td>
</tr>
<tr>
<td>Subtotal</td>
<td>15</td>
<td></td>
<td>91 ± 36 (41–174)</td>
<td>80 ± 15 (60–114)</td>
</tr>
<tr>
<td><strong>Group B2</strong> (n = 50)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>35 Paced</td>
<td>1</td>
<td>120</td>
<td>96 ± 40 (62–132)</td>
<td></td>
</tr>
<tr>
<td>36 Paced</td>
<td>4</td>
<td>120</td>
<td>88 ± 4 (85–91)</td>
<td></td>
</tr>
<tr>
<td>37 Paced</td>
<td>2</td>
<td>120</td>
<td>93 ± 46 (42–132)</td>
<td></td>
</tr>
<tr>
<td>38 Paced</td>
<td>3</td>
<td>120</td>
<td>71 ± 24 (47–140)</td>
<td></td>
</tr>
<tr>
<td>39 Paced</td>
<td>16</td>
<td>120</td>
<td>77 ± 28 (47–127)</td>
<td></td>
</tr>
<tr>
<td>40 Paced</td>
<td>5</td>
<td>120</td>
<td>77 ± 28 (47–127)</td>
<td></td>
</tr>
<tr>
<td>41 Paced</td>
<td>7</td>
<td>120</td>
<td>58 ± 10 (51–65)</td>
<td></td>
</tr>
<tr>
<td>42 Paced</td>
<td>4</td>
<td>120</td>
<td>78 ± 28 (41–140)</td>
<td></td>
</tr>
<tr>
<td>43 Paced</td>
<td>4</td>
<td>120</td>
<td>78 ± 28 (41–140)</td>
<td></td>
</tr>
<tr>
<td>44 Paced</td>
<td>2</td>
<td>120</td>
<td>78 ± 28 (41–140)</td>
<td></td>
</tr>
<tr>
<td>Subtotal</td>
<td>50</td>
<td>120</td>
<td>78 ± 28 (41–140)</td>
<td></td>
</tr>
<tr>
<td><strong>Group C</strong> (n = 6)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.5 months SR</td>
<td>6</td>
<td></td>
<td>199 ± 52 (134–251)</td>
<td>80 ± 7 (71–89)</td>
</tr>
</tbody>
</table>

SR indicates sinus rhythm.

*P = 0.758 (Student t test); †P = 0.414 (Student t test); ‡P = 0.415 (Student t test).
TABLE 2. LV Activation Sequences in Groups A, B, and C With Corresponding Locations of Earliest Ventricular Activation

<table>
<thead>
<tr>
<th>LV Activation Sequence</th>
<th>n (%)</th>
<th>LVB First</th>
<th>RVB First</th>
<th>LVA First</th>
<th>LVB or RVB or LVA</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Group A</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Base to apex</td>
<td>9 (60)</td>
<td>6</td>
<td>3</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Concurrent</td>
<td>5 (33)</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>5</td>
</tr>
<tr>
<td>Apex to base</td>
<td>1 (7)</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>1</td>
</tr>
<tr>
<td>Subtotal</td>
<td>15</td>
<td>6 (40)</td>
<td>3 (20)</td>
<td>...</td>
<td>6 (40)</td>
</tr>
<tr>
<td><strong>Group B</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Base to apex</td>
<td>7 (11)</td>
<td>5</td>
<td>2</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Concurrent</td>
<td>11 (17)</td>
<td>...</td>
<td>2</td>
<td>...</td>
<td>9</td>
</tr>
<tr>
<td>Apex to base</td>
<td>47 (72)</td>
<td>...</td>
<td>7</td>
<td>32</td>
<td>8</td>
</tr>
<tr>
<td>Subtotal</td>
<td>65</td>
<td>5 (8)</td>
<td>11 (17)</td>
<td>32 (49)</td>
<td>17 (26)</td>
</tr>
<tr>
<td><strong>Group B1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Base to apex</td>
<td>1 (7)</td>
<td>1</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Concurrent</td>
<td>3 (20)</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>3</td>
</tr>
<tr>
<td>Apex to base</td>
<td>11 (73)</td>
<td>...</td>
<td>...</td>
<td>9</td>
<td>2</td>
</tr>
<tr>
<td>Subtotal</td>
<td>15</td>
<td>1 (7)</td>
<td>...</td>
<td>9 (60)</td>
<td>5 (33)</td>
</tr>
<tr>
<td><strong>Group B2</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Base to apex</td>
<td>6 (12)</td>
<td>4</td>
<td>1</td>
<td>...</td>
<td>1</td>
</tr>
<tr>
<td>Concurrent</td>
<td>8 (16)</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>8</td>
</tr>
<tr>
<td>Apex to base</td>
<td>36 (72)</td>
<td>...</td>
<td>7</td>
<td>23</td>
<td>6</td>
</tr>
<tr>
<td>Subtotal</td>
<td>50</td>
<td>4 (8)</td>
<td>8 (16)</td>
<td>23 (46)</td>
<td>15 (30)</td>
</tr>
<tr>
<td><strong>Group C</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Base to apex</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td></td>
</tr>
<tr>
<td>Concurrent</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td></td>
</tr>
<tr>
<td>Apex to base</td>
<td>6 (100)</td>
<td>...</td>
<td>...</td>
<td>6 (100)</td>
<td>...</td>
</tr>
</tbody>
</table>

Values are n (%).

Interestingly, even at late stages of embryonic development (HH35–44) (group B), the LVA was the true site of earliest activation in only 32 of 65 (49%) hearts, whereas the RVB or LVB exhibited earliest ventricular activation in 11 (17%) and 5 (8%) cases, respectively. In the remaining hearts (17 of 65; 26%), concurrent activation of the LVA and RVB or LVB was observed (Table 2). Representative examples of electrogram recordings in embryonic hearts from group B, which displayed early RVB and early LV activation, are shown in Figure 3A/3B and 3C/3D, respectively.

In postseptated hearts (group B) with earliest ventricular activation of the LV B (n=5) or RVB (n=11), the AV intervals were 62±15 ms and 74±31 ms, respectively (P=0.540). Activation of the ventricular base occurred significantly faster in quails with a global base-to-apex pattern (69±26 ms) of ventricular activation than in quails with an apex-to-base pattern (83±22 ms) (P=0.005), which suggests that slow conduction through the AV node was indeed bypassed in these hearts.

Additional longitudinal analysis demonstrated early activation of the ventricular base in 93% (14 of 15) of preseptated HH30–34 hearts, whereas the ventricular base was prematurely activated in 60% (23 of 38) of postseptated HH35–39 and in only 37% (10 of 27) of postseptated HH40–44 hearts (P=0.015).

Ventricular Activation Patterns in the Adult Heart

In all adult quail hearts in group C (n=6; HR 199±52 bpm, AV interval 80±7 ms), the LVA was the location of earliest ventricular activation and was activated 5±4 ms before the LVB or RVB. Surface ECG recordings (n=4) did not reveal ventricular preexcitation: PR intervals were not shortened (69±2 ms, range 66 to 71 ms) and showed an isoelectric segment, and QRS complexes did not show a delta wave (31±2 ms, 29 to 33 ms). A representative example of extracellular and surface ECG recordings in an adult quail heart from group C is shown in Figure 4.

Effect of Adenosine on Ventricular Activation

Adenosine was administered in 15 (HH38–41) hearts from group B, which resulted in a rapid (1- to 2-minute) and marked increase in AV interval from 67±18 ms to 149±9 ms (P<0.001) and concurrent changes in ventricular activation pattern (P=0.022). For instance, in 44% (4 of 9) of hearts with an apex-to-base global ventricular activation pattern (9 of 15, 60%; AV interval 72±18 ms) at baseline, the ventricular activation pattern switched to base to apex (RVB, n=2; LVB, n=1; AV interval 149±12 ms), whereas in 11% (1 of 9) of the cases a concurrent ventricular activation pattern was observed (AV interval 140 ms). The ventricular activation sequence in hearts with a global base-to-apex pattern at baseline (5 of 15, 33%; AV interval 61±15 ms) remained unaltered, whereas the AV interval increased to 154±7 ms. In the remaining heart (AV interval 47 ms) with a concurrent ventricular activation pattern (LVB and LVA activation simultaneously) at baseline, adenosine increased the AV interval to 149 ms, and the RVB was shown to be the location of earliest ventricular activation. Interestingly, in hearts with base-first activation, conduction through the AP also decreased markedly, which indicates intrinsic AV nodal conduction properties.

Immunohistochemical Correlations With Electrophysiological Data

In all 16 sectioned postseptated embryonic quail hearts (HH35–44), an MLC2a-positive myocardial AV continuity was found at the right postero septal region. In all hearts, 1 or more mostly right-sided additional AV continuities could be identified until stage HH44. Left-sided continuities were frequently found in HH35–39 hearts (9 of 10; 90%), whereas only 1 of 6 (17%) of HH40–44 hearts showed a left-sided continuity (Table 3). All APs could be followed easily from section to section. Interestingly, all MLC2a-positive myocardial APs found in these embryonic postseptated hearts also stained positive for peristin, a nonmyocardial marker. In Figure 5A through 5H, representative examples of MLC2a and peristin staining in HH36 and HH39 embryonic hearts are given.

Longitudinal analysis showed that with increasing developmental stage, both the number (P=0.004) and width (P=0.179) of APs decreased. Whereas hearts at HH35–39 showed multiple broad APs in various locations, hearts at HH40–44 primarily harbored small AV continuities in the right postero septal and right midseptal regions, whereas the adult heart demonstrated complete fibrous annular isolation.
Morphological findings could not be directly correlated with electrophysiological data: Right- or left-sided APs were found both in embryonic hearts that displayed earliest ventricular activation at the RVB or LVB and in hearts with a concurrent or apex-to-base global ventricular activation pattern (Table 3). Morphologically, the APs showed no discriminating features, which could explain these different ventricular activation sequences.

Discussion

We analyzed ventricular activation patterns in embryonic and adult quail hearts with extracellular electrode recording techniques and correlated these activation patterns with the morphology of the insulating AV annulus. A key finding of this study is that although the LV activation pattern in septated hearts changed from an immature base-to-apex to a mature apex-to-base pattern, premature activation of the RVB and LVB remained present in 51% (33 of 65) of postseptated hearts up to stage HH44 (hatching at HH45–46). This premature ventricular base activation can morphologically be explained, as shown in this study, by persistent accessory myocardial continuities between atrium and ventricle.

Transition of the Ventricular Activation Sequence Versus Persistent Early Activation of the Ventricular Base

Whereas hearts at preseptational stages of development (group A) primarily exhibited an immature base-to-apex pattern of LV activation (9 of 15; 60%), hearts at postseptational stages of development (group B) demonstrated a mature apex-to-base LV activation pattern in the vast majority of cases (47 of 65; 72%). This transition from an immature base-to-apex to a mature apex-to-base LV activation pattern has been studied previously and is associated with maturation of the HPS.12–18 Optical mapping studies showed that this transition marks the emergence of mature “apex-first” epicardial breakthrough near the termini of the bundle branches and demonstrated that right and left bundle branch apical breakthrough sites appear at HH29 and HH35, respectively,13 which is consistent with the transition of the LV activation sequence that occurs at HH35 in the present study.

Unlike previous studies, we observed in postseptated hearts (HH35–44) that the ventricular base could still be “prematurely” activated in a significant number of cases (33 of 65; 51%). For instance, the RVB was activated before the LVA in 11 (17%) cases and the LVB before the LVA in 5 (8%) cases, whereas in another 17 (26%) hearts the ventricular base and LVA were activated simultaneously. This simultaneous activation can, given the position of our recording electrodes (Figure 1), most likely be explained by simultaneous conduction over 2 different pathways: the AV node/HPS on one hand and an AP on the other hand. Furthermore, in 44% (4 of 9) of hearts with an apex-to-base global ventricular activation pattern at baseline, the ventricular activation pattern switched to base to apex after administration of adenosine, which indicates conduction through an AP.

Thus, in contrast to previous studies, our data show that despite maturation of the HPS and transition of the LV activation sequence from base to apex to base, premature and direct activation of the ventricular base remained present in 51% of postseptated hearts at baseline.

Early Activation of the Ventricular Base in Postseptated Embryonic Hearts Can Be Explained by Persisting AV Continuities

In the present study, continuities between atrial and ventricular myocardium were found in the posterosetal region of the tricuspid annulus in all 16 postseptated quail hearts that were analyzed. In addition, in several hearts, 1 or more connections were found mostly at the right anteroseptal and
midseptal regions, whereas left-sided pathways were less frequently encountered (Table 3). The fact that left-sided APs were uncommonly found in late post-septated embryonic HH40–44 hearts (1 of 6; 17%) might reflect a developmental time difference in completion of left and right AV ring isolation, which agrees with a previous description that the left annulus fibrosis in the human adult heart is anatomically usually well formed and nearly always complete, in contrast to the poorly formed and at many sites deficient right annulus fibrosis. This is further supported by the demonstrated difference in AV interval between hearts with earliest ventricular activation at the RVB (74±31 ms) versus the LVB (62±15 ms) (P=0.540), and it may be speculated that different developmental mechanisms can be anticipated to cause the appearance of right- and left-sided APs.

Figure 3. A, Representative example of electrograms recorded in a postseptated HH39 quail heart (HRpacing 120 bpm, AV interval 96 ms) that demonstrate an apex-to-base LV activation pattern (LVA activation 1 ms earlier than LVB activation), with premature RVB activation. The RVB was activated 7 ms earlier than the LVA. B, Magnification. C, Electrograms recorded in a postseptated HH42 quail heart (HRpacing 120 bpm, AV interval 78 ms) that represent a base-to-apex LV activation pattern at this late developmental stage. The magnification (D) shows that the LVB was activated 6 ms before LVA activation. The RVB was also activated 1 ms before the LVA.

Normal Development of the Isolating AV Ring: Possible Fate of Persisting AV Connections and Periostin Expression

In the looped embryonic heart, the AV junction constitutes one of the slow conducting regions of the heart responsible for the sequential contraction pattern at this developmental stage. The subsequent separation of the atria and ventricles is thought to be caused by the fusion of the epicardially located AV sulcus with the endocardially situated AV cushions at the ventricular site of the junction. The processes that underlie atrial and ventricular myocardium dissociation are, however, still incompletely understood, and the tissues responsible for the formation of the annulus fibrosis yet remain largely unknown. Epicardium-derived cells that migrate through the developing AV-dissociated borderline have been followed in their differentiation and shown to become...
fibrils of the fibrous heart skeleton. During formation of the annulus fibrosis, the embryonic slow-conducting AV junctional myocardium becomes incorporated in the definitive atrium. With completion of this AV isolation, the junctional myocardium becomes incorporated in the definition of the annulus fibrosis, the embryonic slow-conducting AV node. At the ventricular insertion side of the AV junctional myocardium, we postulate that the myocardium of the APs found in the postseptated quail hearts consists of primitive remnants of the slow-conducting AV junctional myocardium in the looped heart.

This is in ample agreement with the relatively slow conduction through these pathways as found in our present study compared with the higher conduction velocity through the AV node/HPS and the decrease in conduction velocity through the AP after administration of adenosine.

Interestingly in the present study, anatomic AV myocardial continuities were found both in embryonic hearts exhibiting base-first activation and in hearts with a concurrent or apex-first activation pattern. On the basis of morphological data, we were unable to find any discriminating factors that can explain why some of the morphologically demonstrated APs in retrospect gave rise to premature ventricular activation and others did not. We propose that interembryonic variance in conduction properties of the AV connections on one hand and of the AV node/HPS on the other hand can be held responsible for this observation. Poor cellular coupling, a slow upstroke of the action potential, and perhaps “zig-zag conduction” or an unfavorable source–sink relationship at the ventricular insertion side may all contribute to the very slow conduction or even conduction block at the AP, which causes preferential activation via the AV node/HPS. In précis, the presence of an AP is required to give rise to ventricular preexcitation, but its mere presence does not assure the existence of a faster route for anterograde AV conduction.
mouse and chicken heart in the endocardial cushions that ultimately divide the primitive heart tube into a 4-chambered heart.\textsuperscript{39,40} Periostin is secreted during cushion mesenchym formation\textsuperscript{41} and has been suggested to induce myocardium to transform into mesenchym of a mixed phenotype, which can subsequently transdifferentiate into cells with a fibrous identity, whereas at late stages of development periostin may also serve to maintain the integrity of the fibrous tissues of the heart.\textsuperscript{41,42} At the boundary where myocardial cells directly face endocardial cushion tissue at the AV junction, periostin expression is enhanced and myocardial cells are replaced over time by dense fibrous periostin-positive tissue.\textsuperscript{43} Periostin is also abundantly present in epicardium and epicardium-derived cells.

On the basis of our observations that (1) the functionality, number, and width of persistent APs decreased with developmental stage, (2) the persistent APs all stained positive for periostin, and (3) APs were functionally and structurally absent in the adult quail heart, we assume that periostin expression in persistent myocardial APs perinatally results in inhibition of the myocardial phenotype by transdifferentiation of these myocytes into fibrous tissue. This implicates that these AV connections will disappear within the first weeks to months after birth.

This hypothetically ongoing process of postnatal isolation of the AV ring provides a good etiologic explanation for the clinical observation that AV reentrant tachycardias in human neonates spontaneously disappear before the age of 1 year in the majority of cases,\textsuperscript{4,5} which is further strengthened by the previously reported remarkable morphological transformations of the sinus node, AV node, and bundle of His, which similarly commences about 1 to 2 weeks after birth.\textsuperscript{44–46} Furthermore, local failure or a delay in this remodeling process until adolescence or adulthood may explain the occurrence of reentrant tachycardias later in life.\textsuperscript{46}

**Limitations of the Study**

The aim of this study was to investigate whether AV conduction remains possible via remnants of AV connections in postseptated hearts despite the well-known maturation of the HPS. Although we indeed showed that early activation of the ventricular base is present in a large number of postseptated hearts, which can be explained by the demonstrated persistent connections between atrial and ventricular myocardium, we did not demonstrate that the strands of tissue found by immunohistochemical staining were indeed the structures responsible for the recorded premature ventricular activation. For this, detailed mapping of impulse propagation via these connections and 1-to-1 correlation with morphology in all hearts will be necessary.

Furthermore, to meet the metabolic demands of the older embryonic hearts, we performed our experiments, similar to others,\textsuperscript{11} at subphysiological temperatures (30°C). Although this might have had an effect on our measurements (e.g., slower HRs or longer conduction times), the recorded AV intervals, time differences between apex and base activation, and the developmental stage at which the transition in LV activation sequence occurred were comparable to previous studies.\textsuperscript{12–16}
Conclusions
AV myocardial pathways that bypass the AV node remain present and functional in hearts at late postseptational stages of embryonic development and may provide a physiological substrate for AV reentrant tachycardias in perinatal and postnatal life. However, because (1) the number of embryonic hearts with premature ventricular base activation decreased significantly with developmental stage, (2) a decrease in both AP number and AP width was observed in a similar time course, (3) persistent APs are structurally and functionally absent in the adult heart, it is likely that these AV connections will disappear within the first weeks to months after birth. Further research should more precisely clarify the processes that cause the disappearance or persistence of these APs.

Disclosures
None.

References
CLINICAL PERSPECTIVE

The embryogenesis of the structures involved in atrioventricular (AV) conduction is not fully understood. Nonetheless, knowledge of the anatomic substrates that result in accessory pathway–mediated tachycardia has progressed from being of purely scientific interest to being integral to the management of patients. Within a short time, the primary heart tube transforms into a 4-chambered heart. Whereas sequential activation is initially caused by slow conduction over the circumferential AV continuity, the AV ring becomes isolated in later stages and conduction runs through the AV node/His-Purkinje system. As a result, ventricular activation changes from an immature base-to-apex pattern in preseptated hearts to a mature apex-to-base sequence in postseptated hearts. Abnormal development of the annulus fibrosis that results in accessory pathways may cause AV reentrant arrhythmias. Because these arrhythmias frequently occur in fetuses and neonates, we hypothesized that, during normal development, primitive AV connections that bypass the annulus fibrosis remain present even after development of the His-Purkinje system. We demonstrated that the annulus fibrosis in postseptated prenatal quail hearts is still far from complete, which resulted in functional AV myocardial pathways. We speculate that AV ring isolation continues postnatally, which implicates the disappearance of accessory AV connections within the first weeks after birth and thus provides an etiologic explanation for the clinical observation that AV reentrant tachycardias in human neonates spontaneously obliterate before the age of 1 year in the majority of cases. Local failure or a delay in this remodeling process of the isolating AV ring until adulthood may explain the occurrence of AV reentrant tachycardia later in life.
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