Development of the Right Ventricular Inflow Tract and Moderator Band

A Possible Morphological and Functional Explanation for Mahaim Tachycardia


Abstract—Atriofascicular accessory bundles with AV-node like conduction properties can sustain atrioventricular (AV) re-entrant tachycardia (Mahaim tachycardia). During early embryogenesis, the AV canal is situated above the primitive left ventricle (LV), and a right AV connection has not been achieved yet. We studied the formation of the right ventricular (RV) inflow tract in relation to the developing cardiac conduction system and hypothesized a morphological explanation for functional atriofascicular bypass tracts. Analysis of lacZ-expression during sequential stages of cardiogenesis was performed in CCS-lacZ transgenic mice (E9.5 to 15.5). Embryos were stained for β-galactosidase activity and the myocardial marker HHF35. At early stages CCS-lacZ expression was observed in a ring surrounding the AV canal, which connected at the inner curvature to the primary fold. The first sign of formation of the (CCS-lacZ negative) RV inlet component was a groove in the CCS-lacZ positive tissue of the primary fold. Outgrowth of the RV inlet tract resulted in division of the primary fold in a septal part, the trabecula septomarginalis and a lateral part, the moderator band, which extended laterally up to the right AV ring. Electrophysiological measurements in embryonic hearts (E15.5) in which the right atrium (RA) and RV were isolated from the left atrium (LA) and LV supported the functionality of this AV-connection via the moderator band, by demonstrating sequential atrial and ventricular activation in both RA/RV and LA/LV preparations. In conclusion, our observations may provide a possible morphological and functional explanation for atriofascicular accessory pathways via the moderator band, underlying Mahaim tachycardia. (Circ Res. 2005;96:776-783.)

Key Words: cardiac conduction system ■ cardiac development ■ electrophysiology ■ arrhythmia ■ Mahaim tachycardia

Atrioventricular (AV) reentrant tachycardias are based on the presence of accessory myocardial bundles connecting atrial and ventricular tissue, thus bypassing the insulating function of the AV groove. An unique arrhythmia based on reentry is the so-called Mahaim tachycardia. In Mahaim tachycardia, the accessory AV pathway is formed by atriofascicular accessory fibers, which can sustain exclusively antidromic AV reentrant tachycardias.1 During Mahaim tachycardia, antegrade conduction occurs over an accessory pathway with AV node–like conduction properties. The proximal insertion of these fibers is localized to the lateral, anterolateral, or posterolateral part of the tricuspid annulus; distally the fibers connect to the right ventricular free wall or the right bundle branch.1,2 The origin of Mahaim fibers and their functional characteristics (such as AV node–like conduction properties and, as recently reported, spontaneous automaticity3) have not yet been explained.

The precursors of the cardiac conduction system have been described as junctional rings of specialized myocardium based on histological criteria.4 It has been hypothesized that right-sided AV accessory bundles originate from embryological remnants of the primitive ring of specialized tissue surrounding the orifice of the tricuspid valve during the early stages of the development of the conduction system.5 This hypothesis was supported by observations in human embryos using the HNK1 marker, which was found in the right, but not in the left, AV ring.6 However, these findings cannot explain the occurrence of Mahaim reentrant tachycardias.

Several transgenic mouse models have been used to study the developing cardiac conduction system.7,8 Results of these studies showed that the area stained by the markers used to delineate the embryonic cardiac conduction system is more extensive than the mature cardiac conduction system. In
2001, Rentschler et al demonstrated the ability of the CCS-lacZ transgenic mouse model to delineate the embryonic and mature cardiac conduction system. In this model not only the atrial, but also the ventricular components of the cardiac conduction system, showed β-galactoside staining. Interestingly, during further study, we found that anatomic regions derived from the developing specialized conduction system correlated with areas prone to arrhythmias later in life.10

During early embryogenesis, the AV canal is situated entirely above the primitive left ventricle, and a right AV connection has not been achieved yet. We hypothesized that the outcome of the process of outgrowth of the right ventricular inflow tract and the remodeling of the primary fold tissue may provide a developmental basis for the occurrence of functional Mahaim fibers. The development of the right ventricular inflow tract in relation to the developing cardiac conduction system in the CCS-lacZ strain of transgenic embryos was therefore examined. Our analysis of this murine model suggests that Mahaim tachycardias may be explained from a developmental morphological origin.

Materials and Methods

Morphological Analysis

A transgenic strain of CCS-lacZ mice was used to investigate the formation of the right ventricular inflow tract in relation to the developing cardiac conduction system. The generation of a stable transgenic line of CCS-lacZ reporter mice and staining for β-galactosidase activity has been described.9 Embryos were provided by the Leon H. Charney Division of Cardiology of the New York University School of Medicine (New York, USA), where the mice were maintained according to institutional and NIH guidelines. Twenty-three murine embryos ranging from gestational age E9.5 to E15.5 days post coitum (d.p.c.) were studied (the morning of the vaginal plug was designated as 0.5 d.p.c.). Embryos were fixed at 10.5. At this early unseptated stage, there is myocardial continuity at the AV canal (AVC). AV canal is still situated above the primitive left ventricle. CCS-lacZ-positive myocardium lining the AV canal is continuous with the primary fold (PF) at the inner curvature of the heart. HHF35 staining, Bar=100 µm. LA indicates primitive left atrium; LV, primitive left ventricle; RA, primitive right atrium; RV, primitive right ventricle.

Electrophysiological Measurements

A total of 8 embryos with a gestational age of 15.5 d.p.c. were used for electrophysiological measurements. Two wild-type Swiss pregnant mice were euthanized by cervical dislocation; the uterus was removed and placed in a heated 0.9% NaCl solution (37°C). Hearts were dissected from the embryos, and a cut was made to isolate the right atrium (RA) to right ventricular (RV) continuity over the lateral right atrioventricular junction. The RV section included the apical part of the ventricle with the insertion of the crossing moderator band. This specimen did not contain any parts of the atrioventricular nodebundle tissue, ensuring that possible conduction could only take place by way of a myocardial connection over the right atrioventricular junction. The left specimen contained the complete left atrium (LA) and left ventricle (LV) but also the atrial septum and the main part of the ventricular septum as well as both great arteries. The atrioventricular node, bundle of His, and right and left bundle branches were also part of this specimen. Both preparations were simultaneously placed in the same tissue bath containing heated (±30 to 32°C) oxygenated (95% O2 and 5% CO2) Tyrode’s solution containing Na+ 150, K+ 5.6, Ca2+ 1.5, Mg2+ 0.6, H2PO4− 1.2, Cl− 132.4, SO4− 0.6, HCO3− 25, glucose 5, and pyruvate 5 mmol/L. A fixed reference electrode was placed in the tissue bath and simultaneous electrogram recordings from the right and left atrial and ventricular surfaces were performed by metal unipolar microelec-
trodes (TM33BKT 1 to 2 MΩ, World Precision Instruments, Inc). Electrodes were positioned at the free wall of each cardiac chamber assuring no contact with the AV ring. Signals were fed into an amplifier (ISO-DAM8A, World Precision Instruments, Inc), filtered (typically between 1 and 500Hz), and written on paper with a speed of 25 to 100 mm/s (Graphitec MARK 12 DMS 1000, Western Graphitec). If no spontaneous activity was present, preparations were stimulated in the atrium and/or ventricle using a monophasic stimulus, with a width of 2 ms and a strength of 2 times the diastolic threshold. The experiments were performed under a microscope allowing visual confirmation between electrical and mechanical activity (contraction) of atria and ventricles.

Results

Morphological Data

E9.5 and E10.5

In the earliest stages studied, cardiac septation has not yet commenced. The atrial myocardium is continuous with the myocardium of the ventricles via the atrioventricular canal. At these early unseptated stages, the AV canal is still situated above the primitive left ventricle, and the outflow tract is situated above the primitive right ventricle. A ring of CCS-lacZ positive myocardium surrounding the entire AV canal is present. In the inner curvature of the heart, this ring of tissue connects to another lacZ-positive ring, the primary fold, as is
demonstrated in Figure 1. The primary fold demarcates the border between the ventricular inlet segment of the heart, the primitive left ventricle, and the ventricular outlet segment of the heart, the primitive right ventricle.

**E11.5 and E12.5**

At stage E11.5, atrial and ventricular septation have been initiated, but not completed. The ventricles connect through the primary interventricular foramen. As in the previous stages, the myocardium surrounding the AV canal stains positive for CCS-lacZ and there is continuity of the atrial myocardium with the ventricular myocardium via the AV canal. The AV canal is still situated mainly above the primitive left ventricle and connects at the inner curvature to the CCS-lacZ–positive tissue of the primary fold. However, at stage 11.5, a myocardial groove toward the right ventricle is formed. This groove, which precedes the formation of the tricuspid valve, is embedded in the CCS-lacZ positive tissue of the primary fold, as demonstrated in Figure 2a (arrow). At stage E12.5, the groove has widened, leading into the primitive right ventricle, as demonstrated in Figure 2b (arrow). Figure 2c shows a dorsal view of a 3-D reconstruction of the same E12.5 embryo. Figure 2d demonstrates the level of section of Figure 2b on a ventral view of the 3-D reconstruction, in which the CCS-lacZ–negative structures have been rendered transparent, demonstrating the myocardial groove in the CCS-lacZ–positive myocardium.

**E13.5 and E14.5**

Septation has progressed, and at stage E14.5, ventricular septation has been completed. At these stages, atrioventricular valves can be distinguished. The CCS-lacZ–positive atrioventricular ring bundle (AVRB) remains visible in these stages and surrounds both the tricuspid and mitral valves (Figure 3a). On the right side, CCS-lacZ–positive tissue of the AV ring becomes continuous with lacZ staining in the right ventricle (Figure 3b). The atrioventricular sulcus tissue has not fused with the atrioventricular cushion tissue yet, and therefore, no fibrous tissue is present in the primitive AV canal. Consequently, the atrial myocardium is still continuous with the ventricular myocardium at this stage.

The right ventricular inflow tract has further expanded at these stages, as is demonstrated by the outgrowth of myocardium in the right ventricular dorsal wall. This has resulted in a shift of the right side of the AV canal, to become positioned above the primitive right ventricle. The newly formed myocardium of the right ventricular inflow tract, which is wedged in between the tissue of the primary fold, does not express CCS-lacZ (Figure 3c and 3e, arrows). This outgrowth of the right ventricular inflow tract results in a division of the primary fold tissue into two branches. Thus, the primary fold is now divided into a septal part, the trabecula septomarginalis, and a lateral part, the moderator band, that transverses the lumen of the right ventricle, as is demonstrated in Figures 3d, 3f, and 4 for stage E14.5 and E13.5, respectively. The trabecula septomarginalis, which contains the right bundle branch, represents the border between the inlet septum and the trabeculated septum of the muscular ventricular septum. The trabecula septomarginalis is connected to the moderator band, which runs via the apex of the right ventricle up to the right ventricular lateral wall and, more cranially, to the right side of the AVRB (Figures 3b, 3d, 3f, and 4, arrows).
At stage E15.5, CCS-lacZ positive myocardium, although less marked than in previous stages, can still be distinguished at the AV junction. Strands of fibrous tissue are now observed at the AV junction, but myocardial continuity between atria and ventricles is still present, as demonstrated by the presence of CCS-lacZ-positive myocardium at the AV junction (data not shown).

As in the previous stages, the right ventricular moderator band can be distinguished as a lacZ-positive structure connecting via the apex of the right ventricle to the right part of the AV junction. The right ventricular inflow tract is still recognizable as lacZ-negative myocardium in the right ventricular dorsal wall. Thus at this stage, a lacZ-positive connection between atrial and ventricular tissue, other than the AV node connection, is still present.

Electrophysiological Measurements
As mentioned, by cutting the heart, we aimed to include the AV node in the LA/LV preparation and the apical part of the ventricular septum (containing the insertion of the moderator band) in the RA/RV preparation. In the first 5 preparations, only an electrical connection between the LA/LV was present. By including increasingly more of the apical septum in the RA/RV preparation, the preparation from the 6th embryo showed only an electrical connection between the RA and RV chambers and not between the LA and LV. However, in preparations from the 7th and 8th embryos, a sequential electrical and mechanical activation of atria and ventricles was observed in both RA/RV and LA/LV preparations. As can be seen from Figure 5 (embryo 8) slow but spontaneous and sequential activity was present in the RA and RV (Figure 5a). Although no spontaneous activity was present in the LA/LV preparation, pacing the LV clearly resulted in retrograde 2:1 conduction (1:1 LV capture was visually confirmed by contraction of the LV). Figure 5b and 5c show the whole mount (b) and the section (c) of the same embryo 8 as in Figure 5a. The specimen was cut as described in the Materials and Methods section, demonstrating that the isolated RA and RV were only myocardially connected at the lateral AV junction. Study of sections of both the LA/LV specimen and the RA/RV specimen showed that fibrous tissue was starting...
to dissociate the atria from the ventricles (not shown). In the RA/RV specimen depicted, the myocardial connection was only clearly discernable over a distance of 9 sections (45 μm). This implicates that conduction of the electrical impulse could only have traveled over this connection, along the free wall of the RA to the RV.

**Discussion**

It is well established that AV-accessory pathways can be composed of abnormal fibers, which bypass the insulating AV groove. Furthermore, histological studies have demonstrated that atrioventricular connections can be composed of specialized myocardium. Electrophysiological studies demonstrated that atriofascicular fibers as present in patients with Mahaim tachycardias display properties very similar to that of the AV node and the presence of AV node–like cells in Mahaim pathways has been reported. Figure 6a demonstrates a schematic drawing of the cardiac conduction system with a Mahaim fiber. Figure 6b and 6c show surface ECG and intracardiac recordings in a patient with Mahaim tachycardia. The potential presence of AV node–like cells in these pathways is supported by the occurrence of spontaneous automaticity in Mahaim fibers, as reported recently. However, the mechanism whereby nodal cells, possibly underlying the frequency-dependent propagation of these tracts, may be present at this site, remains unclear.

In the present study, we examined the developing cardiac conduction system in the CCS-lacZ mouse, to provide a possible explanation for the occurrence of functional atriofascicular bypass tracts causing Mahaim AV reentrant tachycardias. Mahaim-fibers, mostly atriofascicular fibers, connect proximally to the lateral right atrium or right AV junction and insert distally in the right bundle branch or right ventricular free wall. As mentioned, the conduction properties of these fibers have been attributed to AV node–like specific conduction tissue in the proximal part of these fibers, whereas the distal part inserts in the right bundle branch. Thus far, no developmental model of the cardiac conduction system has been able to demonstrate the presence of a connection of the right bundle branch with the right AV ring.
The explanation of the presence of a lacZ-positive moderator band as a primary fold–derived structure requires understanding of formation of the right ventricular inflow tract. In early embryological stages, the primary fold delineates the border between the primitive left and right ventricle. During outgrowth of the right ventricle, the primary fold follows a process of division and growth (see summary). Newly formed, CCS-lacZ–negative myocardium will form the dorsal inflow portion of the right ventricle, laterally demarcated by the moderator band. Medially, the inflow septum and the crista supraventricularis of the muscular ventricular septum are derived from the primary fold, with the trabecula septomarginalis (which contains the right bundle branch) as a border structure between the inlet septum and the trabeculated part of the muscular septum. In the present study, the CCS-lacZ–positive right ventricular moderator band could be traced from the trabecula septomarginalis via the apex of the right ventricle all the way up to the lateral wall of the right ventricle. This band connected with the right atrioventricular ring, thus providing a direct connection with the right ventricular apex, supplying a potential pathway for reentry, as seen in Mahaim tachycardia. These data are supported by observations in the MinK-lacZ knockout murine embryo. The formation of the right ventricular moderator band in relation to the development of the right ventricular inflow tract and the cardiac conduction system is summarized schematically in Figure 7.

To electrically insulate the atria from the ventricles, continuity of the annulus fibrosus is important. In the stages we examined, the development of the fibrous skeleton of the atrioventricular junction had not been completed. However, a mature electrical activation pattern, with a delay between the atrial and ventricular signal on the ECG, is already present around the time that ventricular septation is completed. This suggests that there is already a functional insulation between atria and ventricles, which was confirmed in 6 of 8 embryos showing AV conduction in only one of the RA/RV or LA/LV preparations. However, in the other 2 embryos, conduction from the atrium to the ventricle and/or vice versa could be demonstrated in both RA/RV and LA/LV preparations, indicating that next to conduction over the AV node, a second functional AV connection was present. Although we did not perform high-resolution mapping to show that conduction indeed occurred over the lateral AV ring and the moderator band to the right bundle branch, the data presented in Figure 5 indicate that, at this developmental stage, a functional accessory connection running from the right atrial free wall to the right ventricle was present. Interestingly, the location of this accessory connection is similar to the most common location of a so called Mahaim fiber in patients. The existence of accessory AV bundles with conductive capacities bypassing the insulating annulus may produce reentrant tachycardias, as is the case in the WPW syndrome. It has been suggested that remnants of embryonic atrial conduction tissue may serve as AV- bypass tracts. Using the epitope HNK1, the presence of a right, but not a left, AV ring was demonstrated in human embryos. In the CCS-lacZ reporter mouse, both the right and the left AV ring demonstrate β-galactosidase staining. It has been reported that AV junctional cells surrounding both the tricuspid and mitral annuli resemble nodal cells in their cellular electrophysiology. The exact mechanism of why Mahaim tracts may become or remain functional is not clear. Several mechanisms can be hypothesized to be responsible for the occurrence of functional accessory pathways, such as failure of regression of embryonic conduction tissue, failure of apoptosis, or re-expression of the embryonic phenotype. Indeed, several studies have reported the occurrence of specialized tissue in patients.

**Figure 6.** a, Mahaim fibers (MF) consist of atriofascicular fibers, which can possess AV node–like conduction properties. Proximal insertion of these fibers is localized to the lateral, anterolateral, or posterolateral part of the tricuspid annulus; distal insertion is to the right ventricular free wall or the right bundle branch (RBB). Arrows indicate the direction of conduction over the Mahaim fiber. b, Surface ECG during Mahaim tachycardia, showing an antidromic tachycardia. c, Intracardiac recordings during rapid atrial pacing. Arrows point at the Mahaim-potential. a indicates atrial signal; m, Mahaim potential; v, ventricular signal; AVN, atrioventricular node; LBB, left bundle branch bundle; SN, sinus node.
bypass tracts and demonstrated the presence of myocardial cells running through the fibrous insulating AV tissue. The frequent occurrence of Mahaim tachycardia and WPW syndrome in patients with Ebstein anomaly, may relate to the persistence of embryological functional bypass tracts during an abnormal development of the right AV junction, or renewed differentiation of cardiomyocytes at these sites, as described in the WPW model of Patel et al. Apoptosis, which under normal circumstances occurs postnatally in the right ventricle, may play a crucial role in peri/postnatal morphogenesis. Apoptosis can also involve the human cardiac conduction system and failure of apoptotic morphogenesis may result in the persistence of embryonic conduction tissue. The role of genetic reexpression (a well-known mechanism in the genesis of hypertrophic cardiomyopathy), or activation of the embryonic gene program in the persistence of expression of these functional bypass tracts warrants further investigation.

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