Atrioventricular Ring Reentry in Embryonic Mouse Hearts

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Background—During development, AV conduction switches from base-to-apex to apex-to-base conduction after emergence of the conduction system. We hypothesize that after this transition, the bulk of the AV ring, although no longer required for AV conduction, remains transiently able to conduct, providing a potential arrhythmia substrate. We studied AV conduction during this transition and its sensitivity to autonomic modulation.

Methods and Results—Simultaneous voltage and Ca\(^{2+}\) mapping with RH-237 and Rhod-2 was performed with 2 CCD cameras in embryonic mouse hearts \(n=43\). Additionally, isolated calcium mapping was performed in 309 hearts with fluo-3AM. Propagation patterns in voltage and Ca\(^{2+}\) mapping coincided. Arrhythmias were uncommon under basal conditions, with AV block in 14 (4%) and junctional rhythms in 4 (1%). Arrhythmias increased after stimulation with isoproterenol—junctional rhythm in 9 (3%) and ventricular rhythms in 22 (6%)—although AV block decreased (3 hearts, 1%). Adding carbachol after isoproterenol caused dissociated antegrade and retrograde AV ring conduction in 30 (8.6%) of E10.5 and E11.5 hearts, occurring preferentially in the right and left sides of the ring, respectively. In 2 cases, reentry occurred circumferentially around the AV ring, perpendicular to normal propagation. Reentry persisted for multiple beats, lasting from 3 to 22 minutes. No episodes of AV ring reentry occurred in E9.5 hearts.

Conclusions—AV ring reentry can occur by spatial dissociation of antegrade and retrograde conduction during combined adrenergic and muscarinic receptor stimulation. Critical maturation (>E9.5) seems to be required to sustain reentry.

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Key Words: atrioventricular node ■ conduction ■ tachycardia

Atrioventricular conduction undergoes major remodeling during development. The early heart tube exhibits a peristaltic, continuous propagation from the primitive atria to the primitive ventricles. After looping, the atria and ventricles become distinguishable, and AV conduction occurs through the AV ring (AVR), with a continuous propagation sequence from the atria via the AVR to the base of the ventricles, followed by the apex, and finally the outflow tract. Later on, with the emergence of the conduction system, the electrical impulse is delivered from the primitive AV node to the apex, which then propagates to the rest of the ventricular tissue.1–3 At that point, AV conduction through the bulk of the AVR is no longer necessary for ventricular activation and yet remains (at least transiently) able to conduct. We hypothesize that this redundancy may provide a substrate for reentry because different electrophysiological properties of AV conduction via the nascent AV node and remaining conduction via the AVR are to be expected.

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AV node conduction is heavily regulated by autonomic tone. We have recently shown that maturation of sympathetic receptors precedes that of the parasympathetic receptors.4 Here, we show that during simultaneous sympathetic and parasympathetic receptor stimulation, reentry in the embryonic AVR could be induced. Spatial dissociation of antegrade and retrograde conduction led to sustained reentry in most cases required participation of atrial tissue. In 1 case, the entire reentrant circuit appeared to be confined to the AVR. These findings support the hypothesis that the AVR, without participation of a mature AV node, can sustain reentry over an extremely small spatial scale. Speculations in relation to the adult form of AV nodal reentrant tachycardia (AVNRT) also are proposed.

Methods

Dissection of Embryonic Mouse Heart

Pregnant mice with different embryonic age groups were first sedated by inhalation of isoflurane and then killed by cervical dislocation.5 The uterus was dissected, and whole embryos were exposed. Embryonic mouse hearts were then isolated with a dissecting microscope. During dissection, the embryonic mice were bathed in modified oxygenated Tyrode’s solution containing (mmol/L) 136

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Optical Mapping

Isolated E9.5 to E11.5 embryonic mouse hearts (n=43: E9.5 hearts, n=14; E10.5 hearts, n=13: and E11.5 hearts, n=16) were incubated with 5 μmol/L Rhod-2-acetoxyethyl ester dissolved in dimethyl sulfoxide and pluronic F-127 for 40 minutes, followed by a 5-minute incubation with 5 μmol/L RH-237. The embryonic mouse hearts were then washed in Tyrode’s solution before transfer to an experimental chamber (T=37°C) on a modified inverted microscope. Green light (520±20 nm) from a high-intensity light-emitting diode (Luxeon, Calgary, Canada) was delivered via the microscope lens into the hearts. Fluorescence was collected through the lens and split with a 630-nm dichroic mirror. Passed light (>630 nm) was filtered through a 710-nm long-pass filter (voltage signal); reflected light (<630 nm) was filtered through a 585±20-nm filter (Ca²⁺ signal). All filters were obtained from Chroma (Rockingham, VT). Figure 1 shows a schematic of the setup. Ca²⁺ and voltage signals were simultaneously recorded with 2 electron-multiplier CCD cameras operating at 200 to 500 frames per second (Photometrics Cascade 128+, Tucson, Ariz) with a spatial resolution of 128×128 pixels. Spontaneous activations were mapped before and after the addition of Tyrode’s solution containing the β-adrenergic agonist isoproterenol (ISO; 1 μmol/L) and/or the muscarinic receptor agonist carbachol (CCh; 10 μmol/L). Diacetylmyoinosylxime was used as an electromagnetic uncoupler to reduce motion artifact in 22 specimens. Experiments were conducted at 37°C. For technical simplicity, taking advantage of the higher signal-to-noise ratios of Ca²⁺ indicators and the uniform correspondence of voltage and Ca²⁺ propagation maps, additional experiments (n=309) were conducted using isolated Ca²⁺ mapping with fluo-3AM with either a single Photometrics Cascade camera as above or with another CCD camera (model LCL 811K, Watc America, Las Vegas, Nev) using an ATi video capture card (30 frames per second). A few experiments were conducted at room temperature to visualize activations with the slower frame rate.

Data were then analyzed with custom software. After spatiotemporal filtering, the onset of action potentials or Ca²⁺ transients was detected by finding frames with a fluorescence that crossed the median fluorescence value going up; the offset was assigned to frames whose fluorescence crossed the median going down. Action potential duration (APD) and Ca²⁺ transient duration were measured as the time delay between onset and offset. Propagation wave front was assigned to points at the onset of action potentials or Ca²⁺ transients and wave back to points at the offset. AV Ca²⁺ transient conduction time was measured by marking the time difference between the activation of the atria and ventricle. All fluorophores were purchased from Molecular Probes (Eugene, Ore). ISO, CCh, and other chemicals were from Sigma Chemical Co (St Louis, Mo). The Student t test was used to compare means, and a value of P<0.05 was considered statistically significant.

Results

Simultaneous Voltage and Ca²⁺ Recordings

Activation sequence and propagation patterns were equivalent between voltage and Ca²⁺ maps in all 43 specimens subjected to dual mapping. Figures 2 and 3 show examples. The mean ventricular APD was 166±34 ms. The mean ventricular Ca²⁺ transient duration in the ventricle measured at 50% repolarization was 375±58 ms (P<0.001). Atrial APDs measured 145±41 ms; atrial Ca²⁺ transient duration measured 183±25 ms (P<0.05). In E9.5 hearts, AV conduction proceeded via the AVR in a base-to-apex pattern. In E10.5 and E11.5 hearts, however, the earliest ventricular activation occurred at the apex, consistent with a maturing conduction system, as suggested previously (Figure 3 for E9.5 and E10.5, Figure 2 for E11.5).1,2,6,7 The close agreement between activation sequences in voltage and Ca²⁺ maps validates the use of Ca²⁺ transients to map activation sequence.

Autonomic Modulation of Voltage and Ca²⁺

In response to 1 μmol/L ISO, heart rate increased from 122±24 to 140±26 bpm (n=99), and AV conduction time shortened from 234±6 to 205±5 ms (n=63), indicating the presence of functional β-adrenergic receptors throughout the atria, AV, and ventricles. ADP prolongation accompanied the increases in heart rate induced by ISO, from 166±34 to 240±29 ms (P<0.03). However, the Ca²⁺ transient duration did not change significantly after ISO (375±58 versus 358±62 ms; P>0.05). ADP decreased and Ca²⁺ transient duration increased again after the addition of CCh, but the differences did not reach statistical significance compared with baseline or ISO values. In another group of embryonic hearts exposed to ISO, 10 μmol/L CCh reversed the changes in AV conduction time and heart rate induced by ISO in E10.5 (basal: 282±11 ms, n=13; ISO: 212±9 ms, n=12; P<0.001; ISO+CCh: 280±14 ms, n=12, P<0.001) and E11.5 hearts (basal: 295±15 ms, n=13; ISO: 218±18 ms, n=13, P<0.01; ISO+CCh: 286±18 ms, n=13, P<0.01) but not in E9.5 hearts (basal: 285±14 ms, n=9; ISO: 222±14
ms, n=9, P<0.01; ISO+CCh: 229±12 ms, n=9, P=NS). These findings suggest that muscarinic receptors do not become functional in embryonic mouse hearts until E10.5.

Arrhythmias in the Embryonic Heart
The Table summarizes arrhythmias of various types noted in the course of these studies. Under basal conditions, the most common arrhythmias were second- and third-degree AV block (14 of 347, 4%). After β-adrenergic stimulation with 1 μmol/L ISO, AV block improved or resolved, and AV junctional (9 of 347, 2.6%) and ventricular ectopic (22 of 347, 6.3%) beats were observed. In the presence of ISO, ventricular ectopic beats conducted retrogradely to the atria in 12 embryonic mouse hearts, which also sometimes occurred in the presence of both ISO and CCh. The respective incidences of these arrhythmias when hearts were exposed to ISO and CCh are listed in the Table.

More interestingly, in 30 of 347 (8.6%) of the hearts studied, spatial dissociation of antegrade and retrograde conduction through the AVR, analogous to dual AV nodal conduction, was observed. Figure 4 shows an example of an E10.5 heart with dual voltage and Ca2+ mapping in which the optical field was positioned to focus predominantly on the AVR, with some portion of atrial and ventricular tissue also visible. Antegrade conduction occurred exclusively via the AVR-L (right side of the image), reached the ventricle, and turned retrogradely through the right side (left side of the image). After 2 such cycles, the third atrial activation failed to conduct antegrade, and tachycardia failed to sustain (blocked atrial activation in Figure 4C). After this, a new atrial activation (starting in the right portion of the atrium and traveling to the left) propagated antegrade through the AVR-L, retrogradely activating the atria.

Tachycardia was nonsustained in 19 of 30 episodes. Figure 4 shows an example of an E10.5 heart with dual voltage and Ca2+ mapping in which the optical field was positioned to focus predominantly on the AVR, with some portion of atrial and ventricular tissue also visible. Antegrade conduction occurred exclusively via the AVR-L (right side of the image), reached the ventricle, and turned retrogradely through the right side (left side of the image). After 2 such cycles, the third atrial activation failed to conduct antegrade, and tachycardia failed to sustain (blocked atrial activation in Figure 4C). After this, a new atrial activation (starting in the right portion of the atrium and traveling to the left) propagated antegrade through the AVR-L, retrogradely activating the atria.

In 11 cases, sustained reentry was obtained. Figure 5 (and movies 1 and 2 online) illustrates an example of sustained AV reentry in which antegrade conduction proceeded via the AVR-R and retrograde conduction via the AVR-L. After the atria were activated, the Ca2+ transient conducted slowly to the ventricle through the AVR-R. As the ventricles were being activated, the Ca2+ transient conducted retrogradely through the AVR-L to the atria, with a ventricle-atrial conduction time shorter than antegrade AV conduction. The space-time plots shown in Figure 5B (corresponding to the arrows in Figure 5A) indicate that conduction was continuous in both the antegrade and retrograde directions. This reentrant pattern repeated itself for multiple beats, lasting 12 minutes. Sustained dual-pathway conduction lasted from 3 to 22 minutes. The occurrence of dual pathways in the AVR was observed in 10.2% (21 of 205) of E10.5 hearts and 12.8% (9 of 70) of E11.5 hearts after perfusion of ISO and CCh but did not occur in E9.5 hearts (n=72).

Of interest, autonomic receptor stimulation occasionally induced reentry localized purely to the AVR without any
apparent participation of either the atria or ventricles. In 2 examples (1 nonsustained), reentry was circumferential around the AVR, with propagation perpendicular to antegrade or retrograde conduction and bystander activation of the atria and ventricles. Figure 6 shows an example in which propagation proceeded from left to right in the forward-facing side of the AVR and then turned and propagated right to left in the back of the AVR. This is best appreciated in the online movie 3. In this case, ventricular activation occurred every other time that the activation reached the leftmost portion of the AVR, possibly the connection point of the AVR with the nascent ventricular conduction system.

Discussion

To the best of our knowledge, this is the first demonstration that embryonic hearts can exhibit an arrhythmia that relies on dual AVR physiology, resembling AVNRT in the adult heart. The most significant finding was the spatial dissociation of antegrade and retrograde conduction pathways in the AVR in E10.5 and E11.5 embryonic hearts during combined exposure to ISO and CCh. Because AVNRT is a common form of supraventricular tachycardia in children and adults, it is intriguing that an early correlate of this arrhythmia can occur in the embryonic heart before the AV node has matured. At this stage, the AVR is still a circumferential band of slowly conducting tissue separating atria and ventricles. Despite the functional but still immature anatomy of the AV conduction system, dual-pathway AVR conduction could be unmasked by ISO and CCh perfusion, with spatial dissociation of antegrade and retrograde conduction to either side of the AVR.
Notably, the observed episodes of dual-pathway AVR conduction induced by combined β-adrenergic and muscarinic receptor stimulation occurred in E10.5 and E11.5 hearts, with no episodes in E9.5 hearts. We recently showed that ISO increased heart rate and Ca$^{2+}$ transient amplitude and shortened AV conduction time in E9.5 to E11.5 hearts, but CCh reversed the effects of ISO only in E10.5 to E11.5 hearts. Thus, unlike β-adrenergic receptors, muscarinic receptors do not appear to become functional until E10.5. These findings suggest that dual-

![Figure 5: AVR reentry in a fluo-3AM–loaded E11.5 embryonic mouse heart after administration of ISO (1 μmol/L) and CCh (10 μmol/L) (T=23°C). A, Pseudocolored snapshots of calcium fluorescence at the various times indicated during dual AVR pathway conduction. Red indicates high calcium; yellow and green, intermediate calcium; and black-blue, low calcium. Right panel in the second row shows an isochronal map of propagation during dual AVR pathway conduction, with AV conduction on the AVR-R and ventricle-atrial conduction on the AVR-L. Colored squares indicate the areas from which the calcium transient tracings shown in C were obtained. B, Line activation maps of calcium fluorescence showing continuous antegrade propagation from atria to AVR-R to ventricles along the white arrow in the isochronal map in A and continuous retrograde conduction from ventricles to AVR-R to atria along the black arrow. C, Calcium transient traces from the atria (A), AVR-R, and AVR-L of the AVR and ventricle at the sites indicated in A during dual AVR conduction. Note that the peak of the calcium transient in the AVR-L is after that of ventricle, indicating the retrograde conduction of calcium transient from ventricle to AVR-L. See movies 1 (baseline propagation) and 2 online. RA indicates right atrium; LA, left atrium; RV, right ventricle; LV, left ventricle; and ΔF/F, relative calcium fluorescence ratio.

![Figure 6: “Sideways” AVR reentry. An E11.5 heart after ISO and CCh showing transverse propagation around the AVR where its circumference serves as the reentrant pathway. The specimen had been stained with fluo-3AM at 37°C. A, Consecutive snapshots (times in bottom left) showing propagation from left to right of the AVR from 0 to 180 ms, accompanied by ventricular activation (120 ms). From 240 to 480 ms, propagation proceeds from right to left at a location slightly higher than before, most likely in the posterior AV ring (dimmer signal). From 540 to 630 ms, left-to-right propagation occurs again across the front side of the AV, this time without ventricular activation. From 630 to 780 ms, propagation is from right to left along the backside of the AVR. B, Raw Ca fluorescence snapshot of the heart. Circled numbers 1 through 5 indicate locations of tracings shown below. At most locations, the Ca transients show double humps, reflecting left-to-right propagation along the front side of the AVR, superimposed on right-to-left propagation along the backside of the AVR for each reentrant cycle. This pattern can be seen more clearly in movie 3 online. Abbreviations as in Figure 5, plus OFT indicates outflow tract.>}
pathway AVR conduction may require the presence of functional muscarinic receptors. One possibility is that the expression of muscarinic receptors in the AVR is still heterogeneous at E10.5 to E11.5, so CCh only reverses the ISO-enhanced conduction in certain regions, creating a heterogeneous substrate facilitating dissociation of AVR conduction pathways. In E9.5 hearts, the lack of muscarinic receptors may preclude combined autonomic receptor stimulation from creating a sufficiently heterogeneous substrate for dual-pathway AVR. Alternatively, the AVR at E9.5 may be too small to support dual pathways. Finally, it is possible that AVR reentry also depends in some way on a functional AV node and ventricular conduction system, which is not yet present in E9.5 hearts, as indicated by the activation sequence (Figure 2).

Correlations between our findings and the pathophysiology of adult AVNRT remain speculative, and we can only hypothesize how the specific activation patterns in embryos might relate to those of AVNRT. The embryogenesis of nodal and perinodal tissues that form the substrates of AVNRT is unclear. After looping of the heart tube, the primitive atria and ventricles appear as distinct protuberances, whereas the AVR area remains smoothly tubular. This AVR area carries the electrical impulse from atria to ventricles, proceeding sequentially in a peristaltic fashion. The primitive AV node forms in the AV junction between the 2 ventricles as a localized bundle identifiable in rabbits by neurofilament 160 (NF-160) staining,3 which is contiguous with the ventricular conduction system.8 The development of the conduction system is correlated with an apex-to-base activation of the ventricles.1-3 However, before the AV node is formed, conduction between the atria and the ventricles occurs through the AVR. This implies that a transition between AVR conduction and AV node conduction must take place by which AV propagation via the AVR is replaced by propagation through the AV node. Once that occurs, conduction through the AVR becomes superfluous. To electrically disconnect the AVR from the ventricles, propagation through the AVR has to be uncoupled from the ventricles and funneled to the AV node for it to become the sole AV connection. During this transition, progressive uncoupling of the AVR and the ventricles occurs. The AVR, however, remains connected to the nascent AV node.

Despite the high success rate of curative radiofrequency ablation, the anatomic substrate that sustains AVNRT remains unclear. Since the initial demonstration of functional dual AV nodal conduction by Moe et al10 and Mendez and Moe,10 the specific anatomic locations of the conduction pathways have been the subject of debate. Moreover, their embryologic origin remains unexplored. Optical and multielectrode mapping data in a rabbit model have suggested that perinodal atrial tissue participates in the reentrant circuit.11-13 We hypothesize that the AVR could form these inputs to the AV node, which in turn could serve in the adult as part of the perinodal reentrant circuit during AVNRT.

Finally, we cannot distinguish whether these dual pathways were functionally induced or anatomically based. The observation that in sinus rhythm, conduction through both sides of the AVR appeared uniform may favor a functional mechanism. More detailed studies are needed to determine the basis for the dual AVR pathways in these embryonic hearts.

In this study, we found that arrhythmias are relatively uncommon in embryonic mouse heart under basal conditions but can be elicited by β-adrenergic and muscarinic receptor stimulation. An important caveat, however, is that we studied the embryonic hearts in vitro rather than in vivo. Whether similar arrhythmias occur in vivo embryonic hearts under physiological conditions remains to be determined.

Conclusion

The present study is, to the best of our knowledge, the first to demonstrate that dual-pathway AVR conduction can occur in embryonic hearts before the AV node has developed into a discrete anatomically defined structure.

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Disclosures

None.

References


**CLINICAL PERSPECTIVE**

The embryogenesis of the structures involved in AV conduction is unclear. It is known that at an initial heart tube stage, propagation proceeds sequentially in a peristaltic fashion from the primitive atria to the ventricles, which are activated from base to apex. With maturation of the conduction system, however, the ventricular activation sequence reverses because Purkinje fibers deliver propagation to the apex first, which then proceeds toward the base. We reasoned that after maturation of the conduction system, the so-called peristaltic activation mechanism from atria to ventricles becomes redundant and can provide a substrate for reentry. We mapped propagation in embryonic mouse hearts at different stages of development during which this transition occurs. By using dual autonomic modulation, both sympathetic and parasympathetic, we show that reentry in the AV ring can occur, during which spatial dissociation of antegrade and retrograde conduction pathways is present, similar to what happens in the clinically common AV nodal reentry. We speculate that the primitive AV ring may evolve to participate in the perinodal tissue that contributes to this common adult arrhythmia.
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