Functional anatomy of the murine sinus node: high-resolution optical mapping of ankyrin-B heterozygous mice

Alexey V. Glukhov,1 Vadim V. Fedorov,1 Mark E. Anderson,2 Peter J. Mohler,2 and Igor R. Efimov1
1Department of Biomedical Engineering, Washington University in St. Louis, St. Louis, Missouri; 2Department of Internal Medicine and Department of Molecular Physiology and Biophysics, Carver College of Medicine, University of Iowa, Iowa City, Iowa

Submitted 12 August 2009; accepted in final form 26 May 2010

Glukhov AV, Fedorov VV, Anderson ME, Mohler PJ, Efimov IR. Functional anatomy of the murine sinus node: high-resolution optical mapping of ankyrin-B heterozygous mice. Am J Physiol Heart Circ Physiol 299: H482–H491, 2010. First published June 4, 2010; doi:10.1152/ajpheart.00756.2009.—The mouse is widely used as a genetic platform to investigate the molecular mechanisms of sinoatrial node (SAN) pacemaking. Recently, it has been shown that isolated SAN cells from the ankyrin-B (AnkB)-deficient mice display severe pacemaking dysfunction similar to individuals harboring ankyrin 2 allele variants. However, these results have been limited to isolated SAN cells only and thus did not evaluate the functional anatomy of the widely distributed atrial pacemaker complex (e.g., the dynamic interaction of primary and subsidiary pacemakers). We studied pacemaker function in an intact mouse atrial preparation, which included the SAN, atrioventricular junction (AVJ), and both atria, excluding most of the septum. Optical mapping with a voltage-sensitive dye and CMOS camera ULTIMA-L was used to map spontaneous pacemaker activity with or without autonomic modulation in wild-type (WT) mice (n = 7) and in the AnkB heterozygous (AnkB+/−; n = 9) mouse model of human SAN disease. In WT mice, isoproterenol accelerated the SAN rate (for 10 μM: from 325 ± 19 to 510 ± 33 beat/min, P < 0.01) and shifted the leading pacemaker site superiorly by 0.77 ± 0.11 mm within the SAN. ACh decreased the SAN rate (from 333 ± 26 to 96 ± 22 beats/min, P < 0.01) and shifted the leading pacemaker either inferiorly within the SAN or abruptly toward the AVJ. After isoproterenol, AnkB+/− mice exhibited a larger beat-to-beat variability (SD of a cycle length: 13.4 vs. 3.6 vs. 2.5 ± 0.8 ms, P < 0.01 vs. WT mice), disorganized shift of the leading pacemaker (2.04 ± 0.37 mm, P < 0.05 vs. WT mice), and competing multiple pacemakers, resulting in beat-to-beat changes of the leading pacemaker location site between the SAN and AVJ regions. Notably, AnkB+/− mice also displayed a reduced sensitivity to ACh (rate slowing by 32 ± 12% vs. 67 ± 4%, P < 0.05, AnkB+/− vs. WT mice, respectively). In conclusion, AnkB dysfunction results in SAN abnormalities in an isolated mouse atria preparation. While AnkB dysfunction dramatically alters single SAN cell function, the mechanisms underlyng cardiac automaticity are clearly complex, and phenotypes may be partially compensated by the dynamic interaction of cells within the pacemaker complex. These new findings highlight the importance of the functional anatomy of the entire atrial distributed pacemaker complex, including the SAN and AVJ, and clearly demonstrate the role of AnkB in cardiac automaticity.

pacemaker; autonomic control; action potential; sinoatrial node

DESPITE THE CRITICAL IMPORTANCE of sinoatrial node (SAN) cardiac pacing, relatively little is known about SAN physiology or SAN cell biology compared with our more complete understanding of ventricular and atrial myocardium (4, 8, 9, 12, 14). To investigate the basic molecular mechanisms of SAN biology and disease, the mouse SAN is widely used as a genetic platform, allowing the modification of the expression and/or function of different genes that encode the corresponding ionic channels or Ca2+-handling proteins (20). However, many of these studies were performed using isolated SAN (21, 22, 28) or isolated cell (20, 23) preparations and therefore lack critical data regarding the interaction of the intact SAN with the surrounding atrial myocardium and atrioventricular (AV) junction (AVJ).

It has been known for over a century that pacemaker cells are widely distributed throughout the entire region located between the superior (SVC) and inferior vena cava (IVC) and between the crista terminalis and intra-atrial septum (8, 10, 18, 19). Canine and human studies (6, 7, 11, 15, 16, 31) in which potentials have been recorded from multiple electrodes simultaneously have revealed an extensive distributed system of atrial pacemakers (the atrial pacemaker complex), which includes but extends well beyond an anatomically defined SAN. The theory of the pacemaker complex postulates a frequent exchange of dominance among the multiple pacemakers coinciding with changes in heart rate and beat-to-beat cycle length (7, 29). Therefore, it is critical to investigate the mechanism of cardiac pacing with regard to the dynamic interaction of different regions of the distributed atrial pacemaker complex, including the SAN and secondary pacemakers.

Sick sinus syndrome is a common cardiac disease that affects 1 in 600 cardiac patients of >65 yr of age and results in the implantation of approximately half of all pacemakers used in the United States (1). Recently, human SAN disease has been linked to a dysfunction in cardiac ankyrin-B (AnkB) function (20). AnkB is essential for the normal membrane organization of SAN cell channels and transporters and is required for physiological cardiac pacing. It has been shown that AnkB haploinsufficient (AnkB+/−) mice display severe SAN dysfunction, similar to individuals harboring ankyrin 2 (ANK2) allele variants (20). However, this observation was studied in isolated SAN cells, and the significance of AnkB mutation in pacemaking function at the tissue level remains to be elucidated. In this study, we aimed to establish a model for SAN function in an isolated mouse atria preparation, which included the SAN as well as AVJ, using high-spatiotemporal resolution optical imaging.

MATERIALS AND METHODS

Mice. All procedures were carried out in compliance with the standards for the care and use of animal subjects as stated in the National Institutes of Health Guide of the Care and Use of Laboratory Animals.
Animals (NIH Pub. No. 85-23, Revised 1996). The Animal Care and Use Committees of Washington University and the University of Iowa approved the protocols. All animals used in this study received humane care in compliance with the Guide for the Care and Use of Laboratory Animals. Data were obtained from adult (age: 11–20 wk) wild-type (WT; \( n = H11005 \)) and AnkB heterozygous (AnkB \( H11001 / H11002; \( n = H11005 \)) mice (20). Four additional WT mice were studied as whole heart preparations.

Heart preparations. Isolated heart preparations were performed as previously described (17). Briefly, mice were anesthetized using a mixture of ketamine and xylazine with 100 units heparin. After a midsternal incision, the heart was removed and placed in oxygenated (95% O\(_2\)-5% CO\(_2\)), constant-temperature (37\(^\circ\)C), modified Tyrode solution of the following composition (in mM): 128.2 NaCl, 4.7 KCl, 1.19 NaH\(_2\)PO\(_4\), 1.05 MgCl\(_2\), 1.3 CaCl\(_2\), 20.0 NaHCO\(_3\), and 11.1 glucose (pH 7.35\(^\pm\)0.05). While bathed in the same solution, lung,

Fig. 1. Optical mapping of spontaneous pacemaker activity. A: photograph of the posterior surface of an isolated Langendorff-perfused mouse heart during a typical experiment. LA, left atrium; RA, right atrium; PVs, pulmonary veins; LV, left ventricle; RV, right ventricle. B: typical example of activation maps from both atria and ventricles during spontaneous sinus rhythm. Two color time scales for atrial and ventricular activation are shown with 34 ms of atrioventricular (AV) delay between the end of atrial activation and the beginning of ventricular activation. *Origin of activation. HR, heart rate [in beats/min (bpm)]; SAN, sinoatrial node. To better define the location of the primary pacemaker, isolated atria preparations were mapped (C). The preparation was oriented so that the RA was on the left side of the image. Anatomical features [the superior (SVC) and inferior vena cava (IVC) and crista terminalis] are marked by dashed lines. AVJ, AV juncture. D: activation map of the isolated mouse atria preparation during spontaneous rhythm. The average HR was not significantly changed after the isolation of the atria.

Fig. 2. Autonomic modulation of spontaneous atrial rhythm. A and C: dose-dependent effects of isoproterenol (Iso; A) and ACh (C) on the spontaneous rhythm (HR; in beats/min) of the isolated mouse atria preparation. B and D: relative changes (in percentages of control values) of atrial rhythm with Iso (B) and ACh (D). AnkB \(^{+/−}\), ankyrin-B (AnkB) heterozygous mice; WT, wild-type mice. *\( P < 0.05 \) compared with AnkB \(^{+/−}\) mice.
thymus, and fat tissue were dissected and removed. A short section of the aorta was attached to a custom-made 21-gauge cannula. After cannulation, the heart was superfused and retrogradely perfused with Tyrode solution passed through a 5-μm filter (Millipore) and warmed (37°C) using a water jacket and circulator (ThermoNESLAB EX7). Perfusion was performed using a peristaltic pump (Peri-Star, World Precision Instruments) with a constant aortic pressure of 60–80 mmHg, which was measured by a pressure amplifier (TBM4M, World Precision Instruments).

Two types of preparations were used: 1) intact whole heart (Fig. 1A) and 2) isolated atrial preparations (Fig. 1C). Four intact hearts were mapped to characterize the activation sequence from the intact SAN-driven mouse heart. The isolated heart was pinned at the apex to the Sylgard bottom of the chamber to prevent stream-induced movement. The right atrial (RA) and left atrial (LA) appendages were stretched and pinned to flatten them and allow optical measurements from the maximal surface area of the atria. A small silicon tube, fixed by silk to the nearby connective tissue, was inserted into the left ventricle through the pulmonary vein, LA, and tricuspid valve to prevent solution congestion and subsequent ischemia after the suppression of ventricular contractions. This also prevented acidification of the perfusion solution and the development of ischemia in the left ventricle.

For the isolated atrial preparation (Fig. 1C), the heart was cannulated as described above, the ventricles were dissected away, and the atria were stretched and then pinned to the bottom of a Sylgard-coated chamber and superfused with Tyrode solution at a constant rate of ~8 ml/min. Both the LA and RA as well as the AVJ were accessible in this preparation. A rim of ventricular tissue was preserved for pinning the preparation to prevent damage to the atria. The medial limb of the crista terminalis was cut to open the RA appendage, and the pacing electrode was placed on the edge of the RA appendage. The interatrial septal tissue was partially removed to reduce scattering of the optical signal from tissue that was not in focus. These procedures did not result in any irregularities of spontaneous rhythm.

The excitation-contraction uncoupler blebbistatin (10 μM, Tocris Bioscience) was used to eliminate motion artifacts from the optical signals (13). The application of blebbistatin induced a transient increase in cycle length. However, blebbistatin did not induce any shifts in leading pacemaker sites or changes in action potential duration, as has been associated with other contraction blockers (2, 26). This is consistent with what was observed in our previous studies using human tissue (11) and animal models (dog, rabbit, and rat) (13, 14, 17).

**Imaging system.** Hearts were stained with a voltage-sensitive dye [RH-237 (5 μl) in 1 mg/ml DMSO]. Coronary-perfused hearts were stained by perfusion with Tyrode solution including dye over a period of 5–7 min. Isolated atrial preparations were stained by direct application of the dye on the tissue. Staining with the voltage-sensitive dye RH-237 did not induce a significant change of SAN cycle length, as observed with di-4-ANNEPS (12).

Excitation light (530–40 nm) was generated by a 250-W xenon arc lamp with a constant-current, low-noise power supply (Oriel Instruments, Stratford, CT). The light was passed through a heat filter, a shutter, and an excitation filter (530–40 nm). A flexible light guide directed the bandpass-filtered light onto the preparation, and a shutter was used to ensure that the preparation was exposed to light only during image acquisition. The fluorescent light emitted from the preparation was long pass (>650 nm) filtered using an edge pass filter (Thorlabs) before reaching the camera. Emitted light was directed toward a MiCAM Ultima-L CMOS camera (SciMedia) with high spatial (100 × 100 pixels, 230 ± 20 μm/pixel) and temporal (1,000–3,000 frames/s) resolution. The acquired fluorescent signal was digitized, amplified, and visualized using custom software (SciMedia).

**Experimental protocol.** After isolation, motion suppression, and staining, preparations were equilibrated for 5–10 min before being imaged. Control maps of atrial activation during spontaneous rhythm were then made. To estimate pacemaker shift during autonomic stimulation, the location of the leading pacemaker site was plotted...
with orthogonal axes crossing at the IVC. The superior to inferior direction (from the SVC to the AVJ through the IVC) was along the ordinate. The lateral to mediate direction (from the RA to the LA through the septum) was along the abscissa (see Fig. 3A). To estimate SAN recovery time (SANRT), the atrial preparation was paced (S1S1 = 100 ms) through a pacing electrode located at the RA appendage for at least 1 min. SANRT was measured as the time interval between the last pacing beat and the first spontaneous beat originating from the SAN. Corrected SANRT was calculated as the difference between the SANRT and the resting cycle length measured before the SANRT pacing protocol. After control measurements, progressively increasing concentrations of isoproterenol (Iso) were tested. Iso was delivered through the perfusion solution and was applied for 10 min to reach steady state. The location of the leading pacemaker as well as SANRT were determined as described for control conditions. After Iso measurements, atrial preparations were washed out for 30 min before the delivery of ACh. After washout, additional staining as well as the additional injection of blebbistatin were performed as needed. ACh was applied using the same protocol as that described for Iso.

Data processing. A customized Matlab-based computer program was used to analyze the optical signals. Signals were filtered using the low-pass Butterworth algorithm at 200 Hz. The maximum upstroke derivative (dV/dt<sub>max</sub>) was calculated for each action potential using the normalized optical signal and its derivatives. Activation maps were constructed from activation times, which were determined from dV/dt<sub>max</sub>.

Statistical analysis. Values are expressed as means ± SE. Hypothesis testing was carried out using an unpaired Student t-test and χ²-analysis with Yates correction as appropriate. Values of P < 0.05 were considered statistically significant.

RESULTS

Distribution of primary pacemakers under control conditions. In the first series of experiments, we mapped the primary pacemaking activation site in intact WT mouse heart (n = 4). Figure 1B shows an example of the activation of an intact Langendorff-perfused heart during the simultaneous mapping of both atria and ventricles. During spontaneous sinus rhythm, the activation originated from a RA site near the SVC where the SAN is anatomically defined. After 11 ms of atrial activation and 24

---

![Figure 4. SAN dysfunction in AnkB<sup>+/−</sup> mice.](image)

**A**: SD of a cycle length (SD-CL; in ms) during spontaneous beating in the isolated atria of WT and AnkB<sup>+/−</sup> mice. **B**: coefficient of variation of a cycle length. **C**: beat-to-beat competition (labeled by red arrows) between the primary and secondary pacemakers in AnkB<sup>+/−</sup> mouse atria in response to sympathetic stimulation (30 nM Iso). Top, the red rectangle shows a fragment of recording where shift of a leading pacemaker appeared. This example is demonstrated in detail in the bottom. The two color contour maps represent the activation patterns during location of the leading pacemaker in both IVC (top) and SAN (bottom) regions. The two color scales show the corresponding activation time for each map. Optical recordings from different sites of preparation (marked by numbers) are shown beside the maps. The red and blue rectangles show the activation from the IVC and SAN, correspondingly. Abbreviations are the same as those in Fig. 1. *P < 0.05 compared with AnkB<sup>+/−</sup> mice.
ms of AV delay, the excitation originated in the center of the left ventricle and activated ventricles within 6 ms. The average AV delay was 40 ± 8 ms (n = 4).

To better define the location of the primary pacemaker, isolated atria preparations were mapped. The average heart rate did not change during the isolation procedure (334 ± 55 vs. 367 ± 18 beats/min, not significant). Figure 1C shows an image of a typical atrial preparation. We did not observe any significant difference in heart rhythm between WT and AnkB+/− mice under control conditions (Fig. 2A).

Under control conditions, the activation sequence of spontaneous excitation on the endocardial surface showed a similar pattern in all WT isolated atrial preparations studied (Fig. 1D). Activation originated in the anatomically defined SAN near the SVC with a wide wavefront that spread anisotropically throughout the RA and LA. Delayed activation in the septal margin of the SAN was observed in all mice, as has been previously shown in the mouse (26, 33) and in other mammals (12, 14, 27, 32). We observed a “typical” stable monofocal position of the leading pacemaker in all seven WT preparations under control conditions (blue circles in Fig. 3A). The area defined by the distribution of the leading pacemakers recorded in all preparations under control conditions (contoured by the blue dotted line in Fig. 3A) showed biological variability between different individuals (i.e., variability from preparation to preparation) and corresponded to the primary pacemaker area characterized previously in the mouse heart by histology, by immunolabeling of connexin43, connexin45, and HCN, and by microelectrodes (21, 33). In AnkB+/− mice, the area of leading pacemaker sites under control conditions was oblong in the inferior direction (see Fig. 5A). Control activation of an AnkB+/− mouse atria preparation is shown in Fig. 5C and did not significantly differ from WT mice (shown in Fig. 3C). The location of the leading pacemaker in all AnkB+/− mice was monofocal and stable under control conditions (see blue circles in Fig. 5).

**Sympathetic modulation.** In WT mice, Iso dose dependently accelerated the SAN rhythm (Fig. 2, A and B), shifting the leading pacemaker site superiorly (Fig. 3, A and B). In all WT mice, under each particular Iso concentration, we observed a stable monofocal origin of the leading pacemaker (red circles in Fig. 3) without any beat-to-beat pacemaker migration. The distance of the maximum pacemaker shift is shown in Fig. 3B for each preparation. In this case, we showed only the maximum anatomical displacement (red circles). The average shift was 0.77 ± 0.11 mm (n = 7). Figure 3D shows a representative example of atrial activation after Iso application. The earliest activation site (red circles) shifted superiorly during 1 μM Iso perfusion compared with control (blue circles in Fig. 3C).

We did not observe any significant differences in spontaneous atrial rhythm between WT and AnkB+/− mice either under control conditions or during Iso perfusion (Fig. 2, A and B). However, in contrast to WT mice, AnkB+/− mice displayed enhanced beat-to-beat rate variability in response to Iso (Fig. 4). Rate variability was calculated as the SD of cycle length. We also used the coefficient of variation of cycle length, i.e., the ratio of the SD to basic cycle length. AnkB+/− mice exhibited a significant increase in both the SD (Fig. 4A) and coefficient of variation of cycle length (Fig. 4B) compared with WT mice. Optical mapping revealed a beat-to-beat competition between several pacemakers, which resulted in heart rate irregularities. Figure 4C shows an example of the competition between the pacemaker located in the SAN area and the latent pacemaker located near the IVC during the perfusion of 30 nM Iso in AnkB+/− mouse atria. Two color contour maps show the acti-

---

**Fig. 5.** Effect of sympathetic stimulation on the maximum shift of the leading pacemaker in AnkB+/− mice. A: photograph of a typical preparation consisting of isolated adult mouse RA and LA. The orthogonal axes are the same as those in Fig. 3. The location of the leading pacemaker is shown by circles (blue circles for control conditions and red circles for 1 μM Iso perfusion). B: enlarged illustration of the pacemaker distribution. The distance of maximum pacemaker shift (shown by arrow) was calculated for each preparation (in mm). SEP, septum. C and D: representative examples of atrial activation under control conditions (C) and during 1 μM Iso perfusion (D). Abbreviations are the same as those in Fig. 1.
vation patterns for two successive spontaneous beats and the switching of leading pacemaker sites between the IVC and SAN area. Each beat represents a monofocal origin of the leading pacemaker migrating in a beat-to-beat manner. In this example, the pacemakers’ competition was observed with an 11:1 period (shown by red arrows) and was accompanied with pauses in the spontaneous rate (193-ms pause vs. 131-ms basic cycle length).

To calculate the shift of the leading pacemaker in the case of beat-to-beat pacemaker migration as observed in AnkB+/− mice under sympathetic stimulation, we used the maximum shift of a pacemaker. As a result, in AnkB+/− mice, 1 μM Iso induced in a more distributed shift of the leading pacemaker, without any predominant direction (Fig. 5, A and B). In five of nine AnkB+/− mice (55%), Iso shifted the leading pacemaker superiorly, as in WT mice. However, in the other mice, Iso shifted the leading pacemaker toward the AVJ (in 3 mice, 33%) or IVC region (in 1 mouse, 11%). As a result, during Iso perfusion, the distance of the maximum pacemaker shift significantly increased to 2.04 ± 0.37 mm (P = 0.012 vs. WT mice). Figure 5B shows pacemaker shift vectors and the corresponding magnitudes for all studied preparations. An example of atrial activation after the shift of the leading pacemaker to the IVC during 1 μM Iso is shown in Fig. 5D.

To estimate the SAN automaticity function, SANRT was measured in both WT and AnkB+/− mice. Two examples of SANRT measurements recorded under control conditions (Fig. 6C) and during 1 μM Iso perfusion (Fig. 6D) are shown for AnkB+/− mice. SANRT was defined as the interval from the last paced atrial activation to the first postpacing spontaneous beat. Figure 6C, top, shows how SANRT was calculated. There was a clear delay (SANRT = 290ms) before the emergence of the first spontaneous postpacing beat, which was reduced after the application of Iso (SANRT = 113 ms; Fig. 6D). Figure 6C, bottom, shows atrial activation maps recorded between the last pacing stimulus and the first postpacing spontaneous beat. It should be noted that during the Iso perfusion (Fig. 6D), the location of the first postpacing spontaneous beat was shifted superiorly and laterally compared with control conditions (Fig. 6C). However, no differences in either SANRT (Fig. 6A) or corrected SANRT (Fig. 6B) were observed between WT and AnkB+/− mice during Iso.

Parasympathetic modulation. As shown in Fig. 2, C and D, AnkB+/− mice exhibited a reduced sensitivity of pacemaking function when perfused with ACh: the maximum spontaneous atrial rhythm slowing at 10 μM ACh was 32 ± 12% versus 67 ± 4% in AnkB+/− and WT mice, respectively (P < 0.05). This difference was found for all ACh concentrations.
above 1 μM. Along with reduced heart rhythm slowing, AnkB+/− mice demonstrated a faster recovery of the SAN. As above for Iso, we measured the dose-dependent effects of parasympathetic stimulation on SANRT. Two examples of SANRT measurements recorded under control conditions (Fig. 7C) and during 1 μM ACh perfusion (Fig. 7D) are shown for one AnkB+/− mouse. In this example, SANRT was significantly prolonged during the perfusion of ACh (from 178 to 890 ms). Summarized data for SANRT and corrected SANRT changes during parasympathetic stimulation are shown in Fig. 7, A and B. Taken together, the observed data demonstrate a significantly diminished sensitivity of pacemaking function in AnkB+/− mice to parasympathetic stimulation compared with WT mice.

As shown in the activation maps in the Fig. 7D, bottom, ACh induced a shift in the location of the first postpacing spontaneous beat to a direction inferior to the SVC to the IVC. The same shift of the leading pacemaker location was observed in all mice during ACh perfusion. Figures 8 and 9 show the effect of parasympathetic stimulation on the location of the leading pacemaker site in both WT and AnkB+/− mice, respectively. In both WT and AnkB+/− mice, for all concentrations of ACh, we observed a stable monofocal origin of the leading pacemaker (red circles in Figs. 8 and 9) without any beat-to-beat pacemaker migration. The ACh-induced shift of the leading pacemaker was calculated during 1 μM ACh perfusion. In Figs. 8A and 9A, the region encompassing the leading pacemakers’ location in all preparations is contoured by a blue dotted line under control conditions and by a red dotted line during ACh perfusion. In both WT and AnkB+/− mice, parasympathetic stimulation resulted in a shift of the leading pacemaker inferiorly within the SAN or abruptly toward the AVJ (in 40% of WT mice and in 20% of AnkB+/− mice). No significant differences in the average distance of the maximum pacemaker shift were observed between WT and AnkB+/− mice [2.48 ± 0.89 (n = 7) and 1.68 ± 0.48 mm (n = 9) for WT and AnkB+/− mice, respectively, P = 0.42]. Figures 8 and 9 also show representative examples of the atrial activation before (C) and after (D) ACh application in both WT and AnkB+/− mice, respectively.

**DISCUSSION**

The atrial pacemaker complex in the mouse heart. The importance of understanding the underlying physiology of the SAN is clearly necessary for the development of new treat-
ments for SAN dysfunction in human disease (9). Common clinical phenotypes of SAN dysfunction or “sick sinus syndrome” include severe bradycardia, sinus arrest or block, alternating brady/tachyarrhythmias, and/or an inappropriate heart rate responses to autonomic stimulation (4).

Compared with ventricular or even atrial arrhythmias, the genetics of SAN dysfunction are less understood. Many genetic animal models have been developed to investigate the role of different proteins involved in pacemaker activity. However, the use of these models has been limited to a broad understanding of whole heart automaticity due to a lack of information regarding the status/location of the primary pacemaker site. These limitations have forced many studies (20, 23) to simply focus on describing SAN function at the single cell level. As such, these studies lack consideration of the role of other pacemaker cells widely distributed over the atria to form an extensive pacemaker complex. The concept of a pacemaker complex postulates that the leading pacemaker dynamically changes with interventions that change the heart rate by means of an anatomical shift of the leading pacemaker (6, 8, 11, 22, 32). The disorganized and widely distributed regions of the leading pacemaker site observed during sympathetic stimulation in AnkB/H11001/H11002 mice (see Fig. 5) compared with WT mice (Fig. 3) shows a dissociation within the pacemaker complex and can explain SAN dysfunction in individuals with ANK2 allele variants (20).

A similar, complex beat-to-beat pattern was observed in AnkB/H11001/H11002 mice during Iso perfusion (Fig. 4C). It should be noted that just dynamic competition between multiple pacemakers (i.e., electrotonically uncoupled pacemakers) rather than multifocal origin of the impulse results in differences in activation patterns as well as in the beat-to-beat rate variability observed in AnkB+/− mice versus WT mice. Taken together, these findings confirm the importance of the concept of the distributed atrial pacemaker complex in the regulation of the whole heart automaticity.

AnkB and SAN dysfunction. Ankyrins are adapter proteins that link critical membrane proteins (e.g., ion channels, transporters, receptors, and cell adhesion molecules) to the actin/spectrin-based cytoskeleton (5). Inherited loss-of-function variants in the AnkB gene (ANK2) cause AnkB syndrome ventricular arrhythmias (20, 25). A recent study (20) has also identified an important role for AnkB in SAN pacemaking. It has been shown that AnkB+/− mice display severe SAN dysfunction similar to individuals harboring ANK2 allele variants (20). Moreover, isolated SAN cells from AnkB+/− animals display abnormal membrane expression of Na+/Ca2+ exchanger (NCX)1, Na+-K+-ATPase, the inositol 1,4,5-trisphosphate receptor, and Cav1.3 (20). Consistent with these changes in cell surface expression, reduced NCX and Ca2+ current have been observed in AnkB-deficient SAN cells (20).

In the present study, we confirmed the presence of SAN abnormalities in AnkB+/− mice during sympathetic stimulation, as previously shown (20). In these mice, Iso resulted in a more disorganized shift of the leading pacemaker without any predominant direction (Fig. 5) compared with WT mice, where Iso resulted in a compact shift of the leading pacemaker toward the SVC (Fig. 3). A similar widely distributed area of the leading pacemaker location site was observed in AnkB+/− mice during control conditions as well (Fig. 5A, blue circles),
which can explain the increased t rate variability previously observed in intact mice (20). Moreover, we found that in contrast to WT mice, the pacemaker focus in AnkB\(^{-/-}\) mice was not stable and migrated in a complex beat-to-beat pattern. The average beat-to-beat spontaneous cycle length variability was significantly higher in AnkB\(^{-/-}\) mice compared with WT mice (see Fig. 4, A and B). The example shown in Fig. 4C illustrates the beat-to-beat competition between two pacemakers, revealing the abnormal electrical activity of the SAN. Such dynamic interactions between various pacemakers inside the distributed atrial pacemaker complex may mask the intrinsic abnormalities observed at the cellular level and partially compensate for them.

Hierarchy within the pacemaker complex is primarily based on a different ionic channel and Ca\(^{2+}\)-handling gene repertoire in the leading SAN pacemaker and secondary pacemakers (21, 24, 34). The different expression patterns of the genes throughout the pacemaker complex result in multiple pacemaker cell types with various intrinsic rates modulated by the nonuniform distribution of adrenergic and cholinergic receptors (3, 22, 30). The aberrant SAN electrical activity observed in AnkB\(^{-/-}\) mice is likely due to altered intracellular Ca\(^{2+}\) concentration transients associated with the aberrant membrane expression of Ca,1.3 and NCX1 and the corresponding reduction of both NCX current and L-type Ca\(^{2+}\) current recently demonstrated in AnkB-deficient SAN cells (20). Additionally, it can be referred to the functional heterogeneity of the SAN (22, 28) as well as its heterogeneous innervation (3, 12). It has been previously demonstrated in the rabbit SAN that after severing the superior from the inferior part of the SAN preparation, the ACh-induced deceleration was smaller in the inferior part than in the superior part (22). As shown in the present study, the area of the control distribution of leading pacemaker sites in AnkB\(^{-/-}\) mice was oblong in the inferior direction of the SAN region (see Fig. 4A), which would explain the lower sensitivity to ACh.

**Limitations.** According to Le Scouarnec et al. (20), AnkB\(^{-/-}\)-mice have SAN dysfunction with severe bradycardia and heart rate variability, which was confirmed in intact animals and isolated SAN cells. The present study confirmed heart rate variability but not bradycardia. The observed discrepancy could be related to the absence of basal sympathetic tone in the isolated atria preparation, which predominates in intact rodents. This might also account for the significant differences in basal heart rate between isolated atria in our study (~350–400 beats/min) versus that presented by Le Scouarnec et al. (20) (500–700 beats/min).
Moreover, heterogeneous sympathetic innervation of the intact heart could produce different spatial effects on the SAN and latent pacemaker cells compared with the homogeneous perfusion of ISO, as used in this study. Additionally, other signaling systems are lacking, including hormones and signaling peptides, which are known to modify neural and cardiac activity. It is supported by the fact that perfusion with ISO did not fully restore physiology of the intact animal. Clearly, additional work is required to fully understand the complex roles of this critical cardiac protein in vertebrate automaticity.

ACKNOWLEDGMENTS

The authors thank Dr. Deborah Janks for constructive comments and for reading the manuscript.

GRANTS

This work was supported by National Heart, Lung, and Blood Institute Grants HL-085369 (to I. R. Efimov), R01-HL-079031, R01-HL-62494, and R01-HL-70250 (to M. E. Anderson), and HL-084583 and HL-083422 (to P. J. Mohler) and by the Pew Scholars Trust (to P. J. Mohler).

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

REFERENCES


