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Impulse Initiation and Conduction in the Murine Atria: A Basis for Future Investigation of Sinus Node Dysfunction

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Abstract

The prevalence of atrial conduction defects and sinus node dysfunction increases with age. These age-related changes may play a critical role in establishing the substrate for the development of atrial fibrillation (AF), the most common sustained arrhythmia. Despite the association between atrial arrhythmias and age, little is known of the mechanisms that underlie changes in atrial electrophysiological function. Ongoing studies in our laboratory are focused on determining the mechanisms of atrial conduction defects associated with aging and disease. The purpose of this report is to present some initial studies of the murine sinus node and the approach we have taken to quantify conduction at the site of impulse initiation.

INTRODUCTION

Atrial fibrillation (AF), the major cause of stroke, is the most common cardiac arrhythmia currently affecting more than three million Americans. Treatment options for this disease are limited and often unsuccessful. It is well known that atrial conduction defects increase with age, which may explain the higher incidence of AF in the elderly population.

Sinoatrial (SA) node dysfunction is associated with numerous clinical symptoms ranging from dizziness and fatigue to rhythm disturbances and reentrant arrhythmias. A substantial deterioration of functional properties of the SA with age has been recognized [1–4]. Electrophysiological changes (e.g., decrease in heart rate and rate of slow diastolic depolarization, increase in maximum diastolic potential and action potential duration), together with structural alterations (e.g., decrease in the number of SA nodal cells, changes in cellular morphology, and increases in the collagen content) have been widely reported [4–8].

Studies have shown that fibrosis in the SA node increases with age [9;10]. This increase may contribute to the higher incidence of SA node dysfunction in the geriatric population. Cardiac myocytes are connected electrically and metabolically through gap junction channels. These structures have been shown to be essential components for normal cardiac impulse propagation [11]. Changes in electrical coupling between myocytes are also thought to contribute significantly to age-related degeneration of the SA node [3;10;12].

Many animal and human studies have described alterations in the abundance of sub-cellular localization of connexin proteins under a variety of pathological conditions [13–16]. This process is known as gap junction remodeling and is associated with discrete areas of conduction defects within the myocardium [17]. It has been hypothesized that gap junction remodeling disrupts wavefront propagation and thus contributes to an increased propensity for arrhythmogenesis in diseased myocardium [18].

In the mammalian heart, the specific functional role of the individual connexins in normal and abnormal propagation remains unknown [11]. The spatial distribution of connexins throughout the chambers of the heart is generally well preserved across different species [13;19;20]. Cx43

is expressed in both atrial and ventricular myocytes. In addition to Cx43, Cx40 is also expressed in the atrial myocardium. In contrast, Cx45 is expressed in the central region of the SA node.

It has been suggested that this sharp transition in connexin isotypes may play a critical role in pacemaker function by electrically isolating the SA node from the surrounding atrial muscle [10;12]. Conduction from the central pacemaking region to the atria is facilitated by strands of Cx40 and Cx43 positive cells that protrude into the central region [12]. Interestingly, the size of this Cx43 deficient region has recently been shown to progressively increase during aging [10].

Few experimental models are currently available to test specific pathophysiologic mechanisms associated with aging. Mice are the most used mammals for genetic manipulation to assess global physiological function. The recent development of new mouse models has provided an approach to investigate the role of specific proteins in complex processes of cardiac disease [21–24]. Mice also become senescent in substantially less time compared to larger mammals, making them an attractive species for high throughput aging studies. Similar to other mammals, the murine SA node and atria become progressively more fibrotic with age [12].

We hypothesize that with age, structural remodeling of the SA node and atria contributes to electrical remodeling. Furthermore, reduction in connexin protein levels contributes to age-related conduction changes in the SA node and atria. In this report we present an initial characterization of the SA node of one to three month old wild type mice. High-resolution optical mapping and microelectrode techniques were used to determine the location of the SA node and the electrophysiological characteristics at the site of impulse initiation. The approach and analysis techniques described here will be used in future studies of senescent wild-type and connexin deficient mice.

METHODS

Heart Isolation and Optical Mapping

One to three month old mice were heparinized and sacrificed with an overdose of CO₂; hearts were surgically removed via a thoracotomy. While the hearts were fully immersed in Tyrode's solution, the aorta was cannulated and Langendorff perfused at a constant pressure at 37 °C. The hearts were subsequently stained with a voltage-sensitive dye and high-resolution optical mapping studies were performed as previously described [21]. Recordings were made in the bin mode, which allows for an array of 64 × 64 pixels to be acquired at 947 frames/sec with 12-bit resolution in the absence of any pharmacological or mechanical motion-reduction techniques.

Spontaneous activity during sinus rhythm was recorded for 4 seconds. Maps of electrical activation were generated from signal-averaged movies as described previously [21;24;25]. To reveal the voltage dependent signal, the background fluorescence was subtracted from each frame. Ten to fifteen activation sequences were averaged to improve the signal-to-noise ratio of the fluorescent signal and to establish a more representative activation pattern minimizing beat-to-beat variability. A digital low pass filter (6th order Butterworth; cutoff of 200 Hz) was applied to each pixel.

Volume Conducted Electrocardiograms

A bipolar electrode mounted on a micromanipulator was used to record volume conducted electrocardiograms (ECG). Recordings were made using Ag-AgCl electrodes placed approximately 1 mm from the heart surface. ECGs were amplified, low pass filtered (cutoff of 300 Hz; 8 channel Axon Instruments CyberAmp), and digitally sampled (at 5 kHz; Digidata Digitizer and the Axon Instruments Axoscope software package). Continuous recordings were

obtained through the entire experiment and stored for offline analysis. Recordings were electronically tagged to indicate the time each optical recording was obtained.

Microelectrode Recordings

Glass microelectrodes were filled with 3M KCl and a final resistance of 10–30 M Ω was achieved. Recordings were made with a high impedance microelectrode amplifier as previously described [26]. The A/D system is capable of simultaneously acquiring up to 8 signals, allowing simultaneous recordings of synchronized microelectrode, ECG and camera output signals.

RESULTS AND DISCUSSION

SA node and atrial conduction defects become more prevalent with increasing age. Failure of conduction within the SA node may be an important contributing event that leads to electrical remodeling, setting the stage for AF. This work is aimed at establishing the basis for further investigation of age-related mechanisms underlying functional electrophysiological changes within the atria.

As a first step, we have begun to investigate electrical activation in the SA node and atria of adult mice. Figure 1 is an activation map showing the spread of electrical activity from the SA node to the surrounding atrial myocardium. This activity was recorded while the heart was beating in normal sinus rhythm at a rate of 240 beats per minute. The site of impulse initiation is shown in red, while regions that activate later are shown in blue. The SA node of the adult mouse is located along the medial and anterior aspect of the superior vena cava at the junction of the right atrium [27;28]. Spontaneous activation of this tissue results from the membrane potential slowly depolarizing during diastole until threshold for a new action potential is reached.

From these images we have quantified conduction of electrical impulses in the SA node region. The action potential characteristics of SA node cells have been divided into two categories. Central primary pacemaking cells have faster rates of slow diastolic depolarization and slower upstroke velocities. This region in the mouse measures $300 \times 150 \mu\text{m}$ [12]. Action potentials recorded from more peripheral secondary pacemaking cells have slower rates of diastolic depolarization and faster upstroke velocities. The peripheral region, including both primary and secondary fibers, measures $600 \times 300 \mu\text{m}$ [12]. Our initial measurement of conduction velocity from the peripheral region of the sinus node was $0.71 \pm 0.12 \text{ mm/msec}$ ($n=4$). To compare impulse conduction between the central and peripheral pacemaking regions, future mapping studies will be performed using higher magnification objectives.

Studies are also planned combining conventional microelectrode techniques with high-resolution imaging of electrical activity. Microelectrode impalements will be used to quantify resting membrane potential, action potential upstroke, duration and morphology in the SA node of control and aging mice.

The top trace in panel A of Fig. 2 shows a stable floating microelectrode recording from a region near the SA node. The bottom trace is the volume conducted ECG showing that the heart was beating spontaneously in normal sinus rhythm. The expanded traces in panel B show that the upstroke of the action potential occurred near the beginning of the p-wave in the volume conducted ECG confirming an atrial impalement near the SA node. The color activation map in panel C shows the pattern of electrical activation with the SA node in the center of the field of view. The black square indicates the approximate location of the microelectrode impalement. A Brightfield image of the preparation is shown on the left with the region that was optically mapped indicated by the black square. These data demonstrate our ability to simultaneously

map electrical activation, while precisely locating microelectrode impalements near the SA node.

Future studies in our laboratory have significant implications for the development of new therapeutic approaches for SA node dysfunction, atrial arrhythmias and structural heart disease. The data provided here represent our initial observations of impulse initiation in the SA node of 1 to 3 month old adult mice. Connexin deficient and senescent mice will be used in the future. The analysis of SA node conduction described will be used to quantify specific electrophysiological differences and changes in connexin expression levels associated with age. Ongoing experiments are designed to provide information for a broader understanding of the electrophysiological changes involved in aging.

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Fig. 1.

Optical map of impulse initiation in the SA node of a 3 month old control mouse. A. Representative atrial electrical activation map in normal sinus rhythm, with impulse of initiation originating in the SA node and spreading to the rest of the atria. B. Schematic of the SAN used for conduction velocity analysis, with dimensions based on the microelectrode studies of Verheick 2001. Orange—central SA node region, yellow—peripheral SA node region.

Fig. 2.

Simultaneous high resolution imaging and floating microelectrode recording. Panel A. Top trace—stable microelectrode recording; bottom trace—volume conducted ECG. Panel B. Expanded view showing that the upstroke of the action potential occurred at the beginning of the P-wave on the volume conducted ECG. Panel C. Left shows a Brightfield image of the preparation with the mapped region indicated with the black square. Right panel shows the pattern of electrical activation. White conduction vectors point away from the site of impulse initiation near the SA node region.