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Human sinoatrial node structure: 3D microanatomy of sinoatrial conduction pathways

Thomas A. Csepe [a], Jichao Zhao [b], Brian J. Hansen [a], Ning Li [a], Lidiya V. Sul [a], Praise Lim [b], Yufeng Wang [b], Orlando P. Simonetti [c, d], Ahmet Kilic [d, e], Peter J. Mohler [a, d, f], Paul M.L. Janssen [a, d, f], Vadim V. Fedorov [a, d, *]

[a] Department of Physiology & Cell Biology, The Ohio State University Wexner Medical Center, Columbus, OH, USA
[b] Auckland Bioengineering Institute, The University of Auckland, Auckland, New Zealand
[c] Department of Biomedical Informatics, College of Medicine, The Ohio State University, Columbus, OH, USA
[d] Davis Heart & Lung Research Institute, The Ohio State University Wexner Medical Center, Columbus, OH, USA
[e] Department of Surgery, The Ohio State University Wexner Medical Center, Columbus, OH, USA
[f] Department of Internal Medicine, The Ohio State University Wexner Medical Center, Columbus, OH, USA

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ABSTRACT

Introduction: Despite a century of extensive study on the human sinoatrial node (SAN), the structure-to-function features of specialized SAN conduction pathways (SACP) are still unknown and debated. We report a new method for direct analysis of the SAN microstructure in optically-mapped human hearts with and without clinical history of SAN dysfunction.

Methods: Two explanted donor human hearts were coronary-perfused and optically-mapped. Structural analyses of histological sections parallel to epicardium (~13–21 μm intervals) were integrated with optical maps to create 3D computational reconstructions of the SAN complex. High-resolution fiber fields were obtained using 3D Eigen-analysis of the structure tensor, and used to analyze SACP microstructure with a fiber-tracking approach.

Results: Optical mapping revealed normal SAN activation of the atria through a lateral SACP proximal to the crista terminalis in Heart #1 but persistent SAN exit block in diseased Heart #2. 3D structural analysis displayed a functionally-observed SAN border composed of fibrosis, fat, and/or discontinuous fibers between SAN and atria, which was only crossed by several branching myofiber tracts in SACP regions. Computational 3D fiber-tracking revealed that myofiber tracts of SACPs created continuous connections between SAN #1 and atria, but in SAN #2, SACP region myofiber tracts were discontinuous due to fibrosis and fat.

Conclusions: We developed a new integrative functional, structural and computational approach that allowed for the resolution of the specialized 3D microstructure of human SACPs for the first time. Application of this integrated approach will shed new light on the role of the specialized SAN micro-anatomy in maintaining sinus rhythm.

1. Introduction

The sinoatrial node (SAN) is the primary pacemaker of the heart and responsible for initiating and regulating cardiac rhythm (Keith and Flack, 1907; Lewis et al., 1910; James, 1961; Boineau et al., 1988; Opthof, 1988; Boyett et al., 2000; Chandler et al., 2009; Fedorov et al., 2009; Fedorov et al., 2010a). SAN automaticity and conduction depends on the unique heterogeneous distribution of intracellular ion channels, Ca^{2+} handling proteins and autonomic receptors within the SAN (Monfredi et al., 2010; Dobrzynski et al.,

Abbreviations: BPM, beats per minute; CT, crista terminalis; Cx43, connexin43; Endo, endocardium; Epi, epicardium; IAS, interatrial septum; MRI, magnetic resonance imaging; OAP, optical action potential; RAA, right atrial appendages; RAFW, right atrial free wall; SACP, sinoatrial conduction pathway; SACT, sinoatrial conduction time; SAN, sinoatrial node; SND, sinus node dysfunction; SVC, superior vena cava.

* Corresponding author. Department of Physiology and Cell Biology, The Ohio State University Wexner Medical Center, 300 Hamilton Hall, 1645 Neil Avenue, Columbus OH, 43210-1218, USA.
E-mail addresses: fedorov.2@osu.edu, vadim.fedorov@osumc.edu (V.V. Fedorov).

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2013; Wu and Anderson, 2014) as well as the unique structure of the SAN complex (Fedorov et al., 2012). The specialized microanatomy allows the small SAN to pace the large atria efficiently by maintaining a balanced source-sink relationship (Joyner et al., 1986). Multiple factors affecting SAN structure could lead to sinus node dysfunction (SND) (Csepe et al., 2015), when the SAN inadequately paces the atria, which may result in a number of cardiac diseases such as heart failure, atrial fibrillation, malignant ventricular arrhythmias, and eventually cardiac arrest (Luu et al., 1989; Sumitomo et al., 2007; Faggioni et al., 2013; Hjortshoj et al., 2013; Alonso et al., 2014; Jensen et al., 2014). SND is the predominant prognosis for electric pacemaker implantation, which is currently the only available treatment (Jensen et al., 2014; Mangrum et al., 2000; Packer et al., 2009; Greenspon et al., 2012). Despite over a century of research on the SAN, limited knowledge of the relationship between the human SAN microarchitecture and SAN function remains a critical barrier to properly understanding SND mechanisms and developing new alternatives to implantable pacemaker therapy.

Since the discovery of the SAN by Keith and Flack in 1907, multiple studies have investigated the SAN structure and its role in the formation and regulation of sinus rhythm in human and different animal hearts (Keith and Flack, 1907; James, 1961; Opthof, 1988; Boyett et al., 2000; Fedorov et al., 2009; Fedorov et al., 2010a; Boineau et al., 1989; Beau et al., 1995; Sanchez-Quintana et al., 2005; Chandler et al., 2011). Located at the junction of the superior vena cava (SVC) and the right atrium, the human SAN structure consists of a compact mass of specialized cardiomyocytes enmeshed in a dense matrix of collagen, fibroblasts and fatty tissue (Csepe et al., 2015). In general, the macrostructural features of the SAN, such as SAN size, the relationship between increased collagen tissue percent with age (Lev, 1954; Alings et al., 1995), the defined SAN artery, and the banana-shaped 3D structure of the SAN, are generally accepted and agreed upon (James, 1961; Sanchez-Quintana et al., 2005; Chandler et al., 2011; Lev, 1954; Truex et al., 1967; Shiraishi et al., 1992) (Fig. 1). However, due to the complexities of this 3D structure, several microstructural features remain disputed and/or undefined.

Among the debates over human SAN microstructure are the contradictory hypotheses of how the SAN is electrically connected to the atria. One hypothesis is that the SAN is electrically insulated from the surrounding atria by a structural border of fibrosis, fat layers and myocyte discontinuity, and that functional and structural connection between the SAN and atria is limited to discrete SAN conduction pathways (SACPs) (James, 1961; Opthof, 1988; Fedorov et al., 2009; Fedorov et al., 2010a; Boineau et al., 1978; Bromberg et al., 1995; Schuessler and 2003). An alternative hypothesis is that SAN and atrial cells are extensively connected by diffuse inter-digitations of the SAN border with the atrial myocardium, and that no discrete pathways exist (Chandler et al., 2009; Sanchez-Quintana et al., 2005; Chandler et al., 2011; Anderson et al., 1998; Sanchez-Quintana et al., 2002). These discrepancies may be explained by methodological limitations of previous SAN studies, such as restricting analyses of SAN structure to 2D instead of utilizing a 3D computational model, insufficient spatial resolution of 3D structural studies, and/or conducting structural studies without functional mapping of SAN conduction.

The importance of the functional-structural SAN to atria connection lies in its fundamental role in the mechanism of atrial activation from SAN pacemaker activity (Fedorov et al., 2012; Joyner et al., 1986; Csepe et al., 2015) and the maintenance of normal sinus rhythm in the human heart. New methodologies need to be developed to resolve the discrepancy of the SAN-atrial connections. In the present study, we developed an integrated approach including high-resolution optical mapping, serial histological sectioning and computational 3D fiber tracking to provide for the first time a detailed description of the functionally-identified SAN structure and a 3D reconstruction of the specialized SACP microstructure in human hearts with and without SND.

2. Materials and methods

2.1. Optical mapping of coronary-perfused human atrial preparations

Explanted human hearts were obtained from Lifeline of Ohio in accordance with The Ohio State University Institutional Review Board. Patient-specific data can be found in Table 1. Explanted human hearts were cardioplegically-arrested and cooled to 4 °C in the operating room following cross-clamping of the aorta. Hearts were stored in cold cardioplegic solution (4 °C) during transport, dissection and cannulation. Human atrial preparations were isolated as previously described (Fedorov et al., 2010a, 2011), coronary-perfused and superfused with 36.5 ± 0.5 °C oxygenated Tyrode's solution under constantly maintained pH (7.35 ± 0.05) and pressure (55 ± 5 mm Hg) (Fedorov et al., 2010a, 2010b). Thus, stable heart rhythm, atrial conduction and repolarization were maintained in the entire preparation for 4–8 h (Fedorov et al., 2010a, 2011). The atrial preparations were immobilized with 10 μM blebbistatin and stained with voltage sensitive, near-infrared dye di-4-ANBDQBS (Fedorov et al., 2010a). All mapped atrial preparations excluded regions of poor coronary perfusion/ischemia.

Atrial preparations were mapped and optical action potentials (OAPs) were recorded using a high-resolution (optical field-of-view 3.3 × 3.3 cm^2, 330 μm resolution) CMOS camera (MiCAM Ultima-L, SciMedia Ltd, CA), which was focused on the epicardium (Epi) (Fig. 2). OAPs from the SAN and atria were analyzed using a custom Matlab computer program as previously described (Fedorov et al., 2009). As previously described (Fedorov et al., 2010a), OAP morphology and reconstruction of activation patterns allowed for the identification of the leading pacemaker, or area of earliest SAN depolarization, as well as areas of earliest atrial activation, or breakthrough sites where SAN activation exited the SAN and activated atrial myocardium through SACPs. To determine SAN activation during pacing, SAN action potentials were extracted from total optical signals (Lou et al., 2014). The preparations were instrumented with customized 2 bipolar pacing electrodes placed on the right atrial epicardial surface. Electrical activity was continuously recorded from a 2 mm bipolar sensing catheter (7Fr, 8 mm tip, Biosense Webster, CA) placed on the right atrial epicardial surface, and a far-field pseudo atrial ECG was recorded by two Ag–AgCl plaque electrodes (9-mm diameter).

2.2. Tissue dissection and staining

Two mapped heart preparations were chosen for detailed 3D reconstruction to represent SAN structure in non-SND (SAN #1) and SND (SAN #2) conditions, as SAN #2 had clinically-diagnosed SAN dysfunction as well as an implantable pacemaker and defibrillator. SAN activation maps were projected on the Epi surface of preparations to guide SAN histological dissection (Fig. 2). SAN pacemaker complex and surrounding atrial myocardium, including crista terminalis (CT), right atrial free wall (RAFW), SVC and interatrial septum (IAS), were formalin-fixed, paraffin-embedded and serial sectioned from Epi to endocardium (Endo) (Fig. 4). Histological sections were 5 μm thick, but to adjust for shrinking due to dehydration and compression of the tissue during processing, these sections more closely represented 7.5 μm thick tissue, and this size was used for subsequent analyses. Sections at average intervals of 21 μm and 13 μm for SAN #1 and SAN #2, respectively, were stained

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