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Optical Mapping of the Isolated Coronary-Perfused Human Sinus Node

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Objectives We sought to confirm our hypothesis that the human sinoatrial node (SAN) is functionally insulated from the surrounding atrial myocardium except for several exit pathways that electrically bridge the nodal tissue and atrial myocardium.

Background The site of origin and pattern of excitation within the human SAN has not been directly mapped.

Methods The SAN was optically mapped in coronary-perfused preparations from nonfailing human hearts (n = 4, age 54 ± 15 years) using the dye Di-4-ANBDQBS and blebbistatin. The SAN 3-dimensional structure was reconstructed using histology.

Results Optical recordings from the SAN had diastolic depolarization and multiple upstroke components, which corresponded to the separate excitations of the SAN and atrial layers. Excitation originated in the middle of the SAN (66 ± 17 beats/min), and then spread slowly (1 to 18 cm/s) and anisotropically. After a 82 ± 17 ms conduction delay within the SAN, the atrial myocardium was excited via superior, middle, and/or inferior sinoatrial conduction pathways. Atrial excitation was initiated 9.4 ± 4.2 mm from the leading pacemaker site. The oval 14.3 ± 1.5 mm × 6.7 ± 1.6 mm × 1.0 ± 0.2 mm SAN structure was functionally insulated from the atrium by connective tissue, fat, and coronary arteries, except for these pathways.

Conclusions These data demonstrated for the first time, to our knowledge, the location of the leading SAN pacemaker site, the pattern of excitation within the human SAN, and the conduction pathways into the right atrium. The existence of these pathways explains why, even during normal sinus rhythm, atrial breakthroughs could arise from a region parallel to the crista terminalis that is significantly larger (26.1 ± 7.9 mm) than the area of the anatomically defined SAN. (J Am Coll Cardiol 2010;56:1386–94) © 2010 by the American College of Cardiology Foundation

Despite numerous detailed studies of the origin of excitation in the sinoatrial node (SAN) of many animal species, only excitation of the atrial myocardium and indirect measurements of SAN function have been recorded in humans (1–3). The major obstacle is the inability of epicardial and endocardial electrode mapping to determine the excitation origin and slow propagation of action potentials within the 3-dimensional (3D) structure of the SAN before it activates the adjacent atrial myocardium (4–6). Numerous epicardial (7,8) and endocardial (9) mapping studies demonstrated anatomically widespread sites of early atrial activation, which sometimes fire simultaneously. Such multifocal activation started simultaneously during normal sinus rhythm (SR) in humans in 2 to 5 foci located >1 cm apart. The atrial breakthroughs were reported to arise at the epicardial and/or endocardial region along the crista terminalis (CT), over a region 7.5 cm (8,9) in length. That region is significantly larger than the length of the anatomical SAN, which is only 10 to 18 mm (10,11). Thus, the origin of excitation and pattern of transmural conduction within the human SAN remain unknown.

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Several hypotheses were proposed to explain the relationship between anatomical structure and function of the SAN (4). The Boineau-Schuessler SAN model (4,12) hypothesized discrete sinoatrial exit pathways (SEPs) to explain the multi-

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focal atrial surface activation and shifting site of earliest activation. Boineau and Schuessler hypothesized that depolarization originates and slowly spreads within the SAN and then is transmitted to the atria via several specialized conduction SEPs. We recently presented functional and structural evidence in support of the Boineau-Schuessler hypothesis for the canine SAN, which is anatomically and functionally similar to the human SAN (5,13). In that study, we developed a new analytical approach that was utilized to resolve the intramural activation pattern of the canine SAN from high-resolution optical action potential recordings (6).

The present study shows for the first time, to our knowledge, optically mapped, coronary-perfused intact human atria, including the SAN during normal SR. Histology was used to investigate the relationship between function and the 3D anatomic structure of the human SAN.

Methods

In vitro preparation of the human SAN. Optical mapping studies were conducted in 4 isolated coronary-perfused preparations of the human SAN, similar to our previous study of the human atrioventricular junction (14). Explanted donor hearts were obtained from Mid-America Transplant Services. Hearts were rejected for transplantation due to age and/or cardiac arrhythmias and were donated for research in accordance with a protocol approved by the Washington University School of Medicine Institutional Review Board. Hearts were cardioplegically arrested in the standard fashion and then were removed with intact superior vena cava (SVC) and inferior vena cava (IVC), as shown in Online Figure 1. Hearts were stored in cold cardioplegic solution at 4°C during transport, dissection, and cannulation, with a total cold ischemic time of 60 to 80 min. The cardioplegic solution contained (in millimoles per liter): 110 NaCl, 1.2 $CaCl_2$, 16 KCl, 16 $MgCl_2$, 10 $NaHCO_3$. Online Table 1 presents patient demographics.

The proximal right and left coronary arteries were separately cannulated with a custom-made polyethylene cannulae (inside diameter 0.86 mm; outside diameter 1.27 mm). The ventricles were removed, and all ventricular branches of the left and right coronary arteries were ligated. The entire atrial preparation was positioned in a temperature-controlled glass chamber with the right atrial (RA) posterior epicardium facing the optical apparatus. The preparation was instrumented with 2 bipolar pacing and recording electrodes placed on the RA free-wall epicardium and the intra-atrial septum (IAS) (Online Fig. 1). The human atrial preparations were superfused (80 ml/min) and coronary perfused using 2 pumps (Peri-Star 291, WPI, Sarasota, Florida) under a constant pressure of 55 ± 5 mm Hg with oxygenated (95%/5%, O_2/CO_2) modified Tyrode's solution containing (in millimoles per liter): 128.2 NaCl, 1.3 $CaCl_2$, 4.7 KCl, 1.05 $MgCl_2$, 1.19 NaH_2PO_4, 25 $NaHCO_3$, and 11 glucose. Temperature and pH were continuously maintained at 36 ± 0.5°C and 7.35 ± 0.05, respectively.

Experimental protocol. The preparations were equilibrated during SR in the tissue chamber for 60 to 90 min before the measurements were taken. Blebbistatin (10 to 20 μmol/l) was perfused to suppress motion artifacts in the optical signals (15). The preparations were stained with 10 to 40 μmol/l di-4-ANBDQBS, an infrared voltage-sensitive dye, via coronary perfusion (16). The SAN preparations were restained with the dye during the experiment as needed. No measurements were performed until 5 min after completing the restaining procedure. Stability of the preparation was periodically verified by measuring SR cycle length (CL).

Abbreviations and Acronyms

- **3D** = 3-dimensional
- **CL** = cycle length
- **CT** = crista terminalis
- **CV** = conduction velocity
- **IAS** = intra-atrial septum
- **IVC** = inferior vena cava
- **OFV** = optical field of view
- **RA** = right atrial
- **SACT** = sinoatrial conduction time
- **SAN** = sinoatrial node
- **SEP** = sinoatrial exit pathway
- **SR** = sinus rhythm
- **SVC** = superior vena cava

Fluorescent signals were recorded from the epicardial optical field of view (OFV) ranging in size from 30 × 30 to 40 × 40 mm^2 with a spatial resolution of 300 to 400 μm/pixel at a rate of 1,000 frames/s using a 100 × 100 Ultima-L CMOS camera (SciMedia, Tokyo, Japan). The OFV included portions of the SVC, inferior SAN, CT, and IAS regions. Programmed atrial stimulation was used to measure atrial conduction properties at different CLs (CL = 500 to 1,000 ms).

Optical mapping data analysis and interpretation. A custom Matlab computer program was used to analyze the optical action potentials (OAP) in the SAN and the atria as previously described (6). Optical recordings contained fluorescent signals from the myocardium up to a depth of 1 to 3 mm (16–19). The methodological details of the SAN optical mapping are summarized in a review (20).

Sinoatrial conduction time (SACT) was the time between the earliest excitation in the SAN and the earliest atrial activation (breakthrough) determined by the second optical upstroke, which represented atrial excitation (Fig. 1). Activation times and corresponding conduction velocities (CV) were defined in the SAN layer using 50% of the SAN OAP amplitude (AP50%) (Fig. 1B) (6,21,22). The atrial layer activation times and pattern were defined by the $-dV/dt_{max}$ of the atrial OAP component (Fig. 1B). The conduction block zone for atrial activation was defined as an area with CV <20 cm/s (Fig. 1C).

The slope of the slow diastolic depolarization was determined by measuring the slope of a linear fit of diastolic depolarization and normalizing its amplitude to the OAP amplitude.

Histology. Histology was performed as previously described (21). After optical mapping experiments, human SAN preparations (n = 4) were perfused with 3.7% formaldehyde, frozen in isopentane, cryosectioned perpendicular

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