A novel rabbit model of variably compensated complete heart block

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1Cardiovascular Research Programme, Hospital for Sick Children Research Institute; 2Pathology Division and 3Cardiology Division, Hospital for Sick Children; 4Department of Laboratory Medicine and Pathobiology, University of Toronto; and 5Department of Pediatrics, University of Toronto, Toronto, Ontario, Canada M5G 1X8

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Suto, Fumiaki, Sean A. Cahill, Gregory J. Wilson, Robert M. Hamilton, Ilana Greenwald, and Gil J. Gross. A novel rabbit model of variably compensated complete heart block. J Appl Physiol 92: 1199–1204, 2002.—Complete heart block (CHB) provides a useful substrate for study of bradycardia-dependent ventricular arrhythmias and cardiac function. Existing CHB animal models are limited by surgical recovery time and reliance on intrinsic escape rhythms. We describe a novel closed-chest rabbit model of CHB involving transcatheter radiofrequency (RF) atrioventricular (AV) node ablation and ventricular rate control with chronic transvenous pacing. Permanent CHB was achieved in 34 of 38 attempts overall. Procedural mortality due to cardiac tamponade (n = 2), airway complications (n = 2), and unknown causes (n = 5) occurred in nine animals. Survivors with CHB (n = 28) were maintained for ≥22 days, during which there were three late deaths related to infection (n = 1) or respiratory distress (n = 2). None of the survivors with CHB showed recovery of AV conduction or pacemaker capture loss during chronic ventricular pacing at about one-half normal sinus rates, and 25 animals surviving to death showed no overt signs of hemodynamic compromise such as lethargy, poor feeding, or respiratory distress. This approach provides a reproducible nonsurgical CHB model with adjustable ventricular rate control.

Atrial escape mechanisms for maintenance of heart rate and cardiac output. We have developed a closed-chest rabbit CHB model based on transcatheter radiofrequency (RF) AV node ablation and permanent transvenous ventricular pacemaker implantation. Our model is unique, because it minimizes surgical trauma and recovery time and enables chronic as well as acute ventricular rate control at reduced expense relative to large animal surgical CHB models.

METHODS

This investigation conformed with the Guide to the Care and Use of Experimental Animals published by the Canadian Council on Animal Care (2nd edition, 1993) and was approved by the Hospital for Sick Children Animal Care Committee.

Pacemaker insertion and AV node ablation. Healthy young adult male New Zealand White rabbits (3.5–4.0 kg) were orotracheally intubated and inhalationally anesthetized with a mixture of O2 (1 l/min), nitrous oxide (1 l/min), and halothane (1–1.5%) delivered in tidal volumes of 90–100 ml/breath at rates of 20–25 breaths/min. A six-lead surface electrocardiogram was recorded by direct acquisition to a 386-MHz personal computer running Axotape 1.1 software (Axon Instruments, Foster City, CA) via a custom-modified analog amplification system. Filters were set at 1 and 50 Hz. This system was also used to acquire and record intracardiac signals.

The neck was prepared and draped in standard sterile surgical fashion. A midline incision was made, and the right internal jugular vein was identified and dissected free of connecting tissue. The rabbit internal jugular vein receives two major tributaries: one was cannulated with a heparinized 5-F venous sheath (Daig, St. Jude Medical, St. Paul, MN), and the other was used for passage of a 7-F active fixation endocardial pacemaker lead (Medtronic, Minneapolis, MN) to the right ventricular apex (Fig. 1). A 5-F RF ablation catheter (Cordis Webster, Johnson & Johnson, Piscataway, NJ) was advanced through the sheath, fluoroscopically guided from the right internal jugular vein into the right heart, and manipulated in the midportion of the heart until atrial and ventricular electromograms were observed, ideally with an intervening His bundle potential (Fig. 2). RF energy from an electrocautery device (Vectroson V-10, Summit Hill Laboratories, Navesink, NJ) was applied at this site, typically for 30 s or, occasionally (n = 4), to a maximum of 60 s, until there was evidence of AV dissociation (Fig. 3). Successful AV node ablation was readily apparent by

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the presence of ongoing atrial rhythm with profound ventricular bradycardia or asystole; backup ventricular pacing was then instituted immediately.

The pacemaker lead was connected to an electronic pacemaker (model 5941 or 8084 modified to enable continuous high-rate pacing, Medtronic). Pacing threshold was determined, and the pacemaker was programmed in ventricular demand (VVI) or asynchronous (VOO) mode to deliver a ventricular rate approximating the animal’s resting sinus rate (typically 280–300/min) at twice threshold output to prevent acute hemodynamic decompensation after AV node ablation. Backup pacing could also be performed during the procedure with an external stimulator (model 5325, Medtronic) connected to the ablation catheter after it was repositioned in the right ventricle.

The permanent pacemaker was implanted in a dorsal pocket developed subcutaneously between the scapulae through a separate incision. The pacemaker lead was tunneled subcutaneously and connected to the generator, and the back and neck incisions were sutured closed. Permanent pacing was in VVI mode at a rate of 140/min, with generator output set at 5 V with a pulse width at least double the threshold value obtained during lead testing.

Cardiac performance. Cardiac performance before and after AV node ablation was assessed using M-mode echocardiography and hemodynamic measurements. Central venous and left carotid arterial pressures were measured directly before and after AV node ablation in a representative group of animals (n = 8). Central venous pressure was obtained by transducing the venous sheath. A second catheter was placed in the left carotid artery through a small incision in the vessel wall and used to obtain arterial pressure recordings and blood samples (~0.5 ml) for automated blood gas and hemoglobin measurements (model ABL510, Radiometer Medical, Copenhagen, Denmark). Central venous blood gases were obtained from the superior vena cava. An assumed oxygen consumption of 10 ml·kg⁻¹·min⁻¹ (7) was used to calculate cardiac output based on the Fick principle (6).

Echocardiographic short-axis parasternal views were obtained from a shaven window just to the right of the supine, spontaneously breathing animal’s sternum. Two-dimensional images and off-line M-mode measurements of ventricular chamber dimensions and wall thicknesses were recorded using an ATL HDI 5000 echocardiography machine with a 7-4 MHz broad-bandwidth transducer (Philips Medical Systems, Bothell, WA). These measurements were used to calculate left ventricular shortening fraction.

Pathological examination. Hearts were submitted for pathological assessment after 8 days of chronic ventricular pacing at 140/min in animals with CHB (n = 4). Rabbits were euthanized with intravenous pentobarbital (65 mg/kg). After rapid cardiectomy through a midline sternotomy, the aorta was cannulated and perfused retrograde with Ca²⁺-containing HEPES-buffered saline solution (in mM: 135 NaCl, 5.4 KCl, 1.0 CaCl₂, 1.0 MgCl₂, 10.0 HEPES, 10 d-glucose, pH 7.4,
with NaOH at 37°C) to wash out the blood. The heart was immersed in 10% buffered paraformaldehyde (pH 7.4). It was subsequently opened through a linear incision from the superior vena cava across the tricuspid valve to the apex of the right ventricle and another incision from the right ventricular apex through the right ventricular outflow tract across the pulmonic valve. This allowed visualization of the landmarks for obtaining a tissue block inclusive of the AV node and adjacent interatrial septum and interventricular septum. Because of the relatively small heart size, it was possible to include most of the interatrial and interventricular septa in a single block, which was routinely processed for histology, embedded in paraffin, sectioned at 5-μm intervals on a rotary microtome, and stained with elastic-trichrome to demonstrate myocardium in red and collagen in blue.

Data analysis. Mean ventricular-to-atrial ratios for successful vs. unsuccessful RF lesions were compared using the unpaired Student’s *t*-test. Changes in cardiac performance variables were assessed using one-way repeated-measures analysis of variance followed by pairwise comparison using Tukey’s test. *P* < 0.05 was considered statistically significant.

RESULTS

Feasibility. Permanent CHB was achieved in 34 of 38 attempts overall. All 4 failures occurred in the first 6 attempts, with 100% success in the latter 32 preparations in the series. Acute procedural mortality occurred in nine animals, including three in which CHB was not successfully induced. These nine deaths occurred in nine animals, including three in which CHB was not attainable with a ratio of 3.32 ± 2.61 (*P* < 0.001). Discrete His potentials were observed on 13 occasions, were associated with a ventricular-to-atrial ratio of 2.33 ± 1.62, and were 62% predictive of success in CHB induction. The combination of a discrete His potential and a ventricular-to-atrial ratio in the range 0.7–1.5 was 100% predictive of success in CHB induction (Fig. 4).

Hemodynamic findings. Before ablation, rabbits in sinus rhythm at 305 ± 33 beats/min had mean arterial and central venous pressures of 51.1 ± 7.1 and 5.3 ± 1.4 mmHg, respectively. After AV node ablation and with ventricular pacing at 140/min, the mean arterial and central venous pressures changed to 40.3 ± 6.1 (*P* = 0.013) and 7.1 ± 2.5 mmHg (*P* = 0.052), respectively. Interestingly, with an increase in the ventricular pacing rate to 280 beats/min, mean arterial pressure fully recovered to 50.8 ± 12.5 mmHg (*P* = 0.041 vs. pacing at 140/min, not significantly different from sinus rhythm), but central venous pressure remained elevated at 7.3 ± 2.1 mmHg. This likely reflects improvement in cardiac output without recovery of AV synchrony. Indeed, cardiac output declined acutely from 2.2 ± 0.8 l/min (*n* = 7) in sinus rhythm to 1.1 ± 0.2 l/min with ventricular pacing at 140 beats/min after AV node ablation (*P* = 0.008), partially recovering to 1.7 ± 0.4 l/min with an increase in pacing rate to 280 beats/min (Fig. 5).

Echocardiographic findings. Right parasternal short-axis M-mode measurements are provided in Table 1. None of these parameters changed significantly with AV node ablation and right ventricular pacing at 140 or 280 beats/min, as might be anticipated in the acute setting and in keeping with hemodynamic changes described above.

Pathological findings. The histopathology consistently showed a lesion in the interatrial septum, immediately superior to the AV node, consisting of an admixture of necrotic myocardium and early fibrosis.
with deposition of collagen around fibroblasts together with some macrophages among necrotic myocytes, consistent with thermal injury sustained 8 days before explantation. The AV node was partially or completely intact and demarcated from the adjacent interventricular septum by the central fibrous body. The connection between atrial myocardium and the AV node was interrupted by early fibrotic replacement of myocardium, providing histopathological corroboration of in vivo CHB (Fig. 6).

**DISCUSSION**

CHB in humans is most often an acquired condition. It typically complicates inferior wall myocardial infarction in older adults (1), surgical repair of congenital heart lesions in children (2), or alloimmune injury in the fetus (3). The intrinsic response to CHB, irrespective of its cause, is activation of a hierarchy of subsidiary pacemakers in the proximal His-Purkinje conduction system or in the ventricles themselves, usually resulting in a ventricular rate substantially slower than the sinus node or atrial rate. Inadequate or non-sustained escape mechanism activation can result in severe ventricular bradycardia or asystole, culminating in congestive heart failure, various arrhythmias, or sudden cardiac death. Consequently, CHB constitutes a prime clinical indication for permanent artificial pacemaker implantation.

CHB models can provide valuable insights into the hemodynamic and electrophysiological consequences of ventricular bradycardia, congestive heart failure, and loss of synchrony between atrial and ventricular activation. These models can also be used to assess the risks and benefits of various acute and chronic pacing strategies. Recognition of these advantages has led to development of several CHB models, most of them in larger animals such as dogs (11, 12) or pigs (4). Lee and colleagues (5) recently described CHB induction in rats on the basis of ethanol injection into the AV node area. Vos and colleagues (9–12) reported extensively on studies utilizing their surgical canine CHB model, which is based on an earlier description by Steiner and Kovalik (8). In all cases, dogs have been left in their intrinsic escape rhythm after CHB induction by injection of 37% ethanol directly into the AV junction via a right thoracotomy. These studies have yielded important insights into structural and electrophysiological remodeling processes that occur in response to acquired CHB. Nevertheless, dogs are relatively expensive to acquire and maintain, and there can be significant societal and institutional impediments to their routine use in biomedical research.

Responding primarily to cost issues inherent in the use of larger animals, Lee et al. (5) recently described a rat CHB model that essentially “miniaturizes” the basic canine model of Steiner and Kovalik (8). Approaching the heart through a midline sternotomy, they injected the AV junction with 70% ethanol under direct visualization of epicardial and vascular landmarks. Rat mortality, CHB induction success, and
complication rates were similar to those we experienced with our rabbit model. Postoperative recovery time was not specifically addressed in their report. In the rat model, there is obligatory reliance on intrinsic escape mechanisms, inasmuch as permanent pacing would undoubtedly pose a formidable technical challenge. The use of a transvascular approach to provide AV conduction injury and permanent ventricular pacing has inherent advantages in experimental models of bradycardia. Avoidance of thoracotomy and provision of ventricular rate support should ultimately enhance survival.

The electrophysiological predictors of success in CHB induction provide guidance for optimal catheter placement and refinement of our model. A low ventricular-to-atrial electrogram ratio suggests that the site of successful conduction system interruption is rather high on the AV septum in relation to the AV groove, as confirmed by histopathological evidence that successful RF lesions were actually located slightly proximal to the true AV node. A His bundle signal, if present, likely demonstrates an appropriately anteroseptal catheter position along the AV ring. The novel CHB model described here offers several potentially important advantages over preexisting models. Rabbits are relatively inexpensive and easy to procure and maintain compared with larger animals such as dogs. However, the techniques reported here should be readily transferable to larger species if rabbits are considered unsuitable in any specific investigational circumstances. Transcatheter RF AV node ablation allows for more rapid postprocedure recovery associated with less discomfort than does surgical thoracotomy; consequently, data gathering in the critical few days immediately after the procedure should be less difficult and more reliable. Postablation ventricular pacing prevents acute CHB-related death and congestive heart failure. Ventricular pacing also enables assessment of chronic rate-dependent electrical remodeling, repolarization changes attributable specifically to loss of AV conduction, and repolarization response to acute ventricular rate changes under various chronic conditions. Finally, our model allows for detailed investigation of morphological, hemodynamic, and electrophysiological effects of various pacing strategies in

<table>
<thead>
<tr>
<th>M-Mode Parameter</th>
<th>Sinus Rhythm (n = 6)</th>
<th>Pace 140/min (n = 7)</th>
<th>Pace 280/min (n = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IVS diastolic thickness, mm</td>
<td>0.28 ± 0.04</td>
<td>0.30 ± 0.06</td>
<td>0.30 ± 0.00</td>
</tr>
<tr>
<td>LV posterior wall thickness, mm</td>
<td>0.20 ± 0.09</td>
<td>0.19 ± 0.09</td>
<td>0.23 ± 0.15</td>
</tr>
<tr>
<td>LV end-diastolic dimension, mm</td>
<td>1.82 ± 0.08</td>
<td>1.81 ± 0.15</td>
<td>1.77 ± 0.21</td>
</tr>
<tr>
<td>LV systolic shortening fraction, %</td>
<td>26.50 ± 4.51</td>
<td>30.14 ± 7.29</td>
<td>27.00 ± 3.46</td>
</tr>
</tbody>
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Values are means ± SD. AV, atrioventricular; IVS, interventricular septum; LV, left ventricle; LVPW, LV posterior wall.

Fig. 6. Left: interatrial septum (top), atrioventricular node (AVN), central fibrous body (CFB), and superior portion of the interventricular septum (bottom). Trichrome staining shows muscle in red and collagen in blue; ×20. Right: inset magnified ×100. Thermal injury sustained 8 days earlier resulted in substantial necrosis to the atrial myocardium and subsequent replacement by early fibrosis (blue area) with numerous fibroblasts evident. Arrow, interruption in the bridge of atrial myocardium to the AVN, accounting for the CHB observed electrophysiologically.
CHB at levels ranging from the intact organism to subcellular gene expression.

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