Effects of complete heart block on myocardial function, morphology, and energy metabolism in the rat

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Introduction

Complete heart [atrioventricular (AV)] block (CHB) is a condition in which no conduction of electrical impulses occurs from atria to ventricles.1 CHB may lead to development of severe bradycardia and acute heart failure.2-5 The mainstay of the modern therapy of patients with CHB is implantation of a pacemaker in order to maintain normal (HR) and to control the symptoms caused by inadequate cardiac output (CO).6-7 CHB may be inherited but it is usually acquired and the most important causes include myocardial infarction, drug intoxication, surgery, rheumatic disease, infiltrative heart disease, myocarditis, and hypertensive heart disease. Acquired CHB is usually accompanied by signs and symptoms of reduced (CO). The prognosis of CHB has improved greatly after the invention of the pacemaker. During the pre-pacemaker era, CHB was associated with high mortality with sudden death, myocardial infarction, and progressive heart failure as the major causes for mortality. Animal models of CHB are a useful experimental tool for investigation of haemodynamic, electrophysiological, morphological, and metabolic consequences arising from bradycardia and volume-overload and from loss of AV synchrony. To date, there are several well characterized CHB models in both large-animals (pigs, dogs) and small-animals (rabbits, rats). The advantage of the small-animal models is that they are more cost-effective, easier to handle, and there are less societal and institutional impediments to their use for research purposes. CHB may be temporary or permanent. In the settings of permanent CHB, the heart must adapt to the chronic bradycardia, volume-overload, and increased wall-stress. This will initiate adaptive remodelling of the heart—post-CHB remodelling—to maintain normal haemodynamics.

The aim of this study was to evaluate the short-term and the long-term effects of CHB on cardiac function, morphology, and energy metabolism in the rat.

Methods

Animals

The experiments were conducted on male Sprague-Dawley rats weighing 220–240 g. All animals were fed with standard rat pellets and tap water ad libitum and housed in cages at 26 °C with 60% humidity and a 05.00–19.00 h light regimen. The study protocol was approved by the Ethics Committee for animal experiments at the University of Göteborg. The investigation conformed to the Guide for Care and Use of Laboratory Animals published by the U.S. Department of Health and Human Services.

Induction of CHB

Anesthesia was induced by incubating the animals with isoflurane. The rats were orally intubated and artificially ventilated (10 mL/kg, 70 strokes/min) with an admixture of isoflurane, oxygen and air using a rodent respirator (AstraZeneca, Mölndal, Sweden).

Normothermia was maintained with a heat blanket. Electrocardiogram was continuously monitored by means of oscilloscope. Induction of CHB was performed according to the operative procedure as previously described. Briefly, after midline sternotomy, the tip of the right atrial appendage was reflected laterally to provide access to the AV junction that is located in this area. This manoeuvre exposed the landmark for the epicardial approach to the AV node, a fat pad that is consistently found between the aortic root and the medial wall of the right atrium. This fat pad marks a point on the adventitial aspect of the aortic root corresponding to the commissure between the right and non-coronary leaflets of the aortic valve. A J-shaped Ø 0.3 mm needle was inserted 1 mm lateral and 1 mm posterior to the fat-pad parallel to the hearts long-axis for injection of 30 µL of 70% ethanol at a depth of 3–4 mm. This procedure was attempted in 20 animals, however, only one animal (1/20, i.e. ~5%) retained in permanent CHB after 3 h. We therefore developed a new approach by applying direct electrocautery to the same region using the commercially available diathermy equipment (Liare, model HFS 100, ALB SURGICALS, New Delhi, India) with L-shaped Ø 0.2 mm wire. This procedure was performed in 11 animals using the same anatomical approach as described earlier. The animals were observed for 15 min and further electrocautery was applied if CHB was transient. After establishment of stable AV block III on ECG, atropine was given to inhibit the vagal tonus and for final test of CHB permanence. The procedure as previously described. 8 Briefly, after midline sternotomy, the AV node, a fat pad that is consistently found between the aortic root and the medial wall of the right atrium. This fat pad marks a point on the adventitial aspect of the aortic root corresponding to the commissure between the right and non-coronary leaflets of the aortic valve. A J-shaped Ø 0.3 mm needle was inserted 1 mm lateral and 1 mm posterior to the fat-pad parallel to the hearts long-axis for injection of 30 µL of 70% ethanol at a depth of 3–4 mm. This procedure was attempted in 20 animals, however, only one animal (1/20, i.e. ~5%) retained in permanent CHB after 3 h. We therefore developed a new approach by applying direct electrocautery to the same region using the commercially available diathermy equipment (Liare, model HFS 100, ALB SURGICALS, New Delhi, India) with L-shaped Ø 0.2 mm wire. This procedure was performed in 11 animals using the same anatomical approach as described earlier. The animals were observed for 15 min and further electrocautery was applied if CHB was transient. After establishment of stable AV block III on ECG, atropine was given to inhibit the vagal tonus and for final test of CHB permanence. The lungs were hyperinflated and the thorax was closed. All animals received post-operative analgesia with buprenorphine (Temgesic, Reckitt & Colman, Hull, England; 0.05 mg/kg s.c.). Ten animals were used as the control group. Ten animals that did not retain AV block after the attempt with ethanol (with sinus rhythm) were defined as sham-operated controls.

Echocardiography

Transthoracic echocardiography was performed 1, 3, and 12 weeks after surgery according to the previously described protocol in 10 sham-operated and 6 CHB animals. The animals were anaesthetized lightly with isoflurane and were breathing spontaneously. The chest was shaved and the animals were placed on a heating pad in a shallow left lateral position. Electrocardiographic electrodes were placed on the paws and a standard lead (II) was recorded for heart rate measurements. The images were recorded on HDI 5000 (Philips Medical Systems) using 15 MHz linear transducer and analysed offline using an imaging analysis system (EchoPac, GE Vingmed Ultrasound, Horten, Norway) with digitally acquired data. Short axis two-dimensional views of the left ventricle at the papillary muscle level were used to obtain M-mode recordings. Anterior and posterior end-diastolic and end-systolic wall thickness and left ventricular (LV) internal dimensions in diastole and systole were measured using the leading edge method. Stroke volume (SV) and CO were measured non-invasively using standard echocardiography method on Doppler flow signal from the pulmonary artery according to the protocol previously described. All measurements were averaged at least on three consecutive cardiac cycles.

Intracardiac pressure measurement

Twelve weeks after induction of CHB, the animals were anesthetized and intubated. The chest was opened by removal of the anterior hemithorax allowing easy access to left and right ventricles for pressure measurements and for quick excision of the heart. A 21G needle connected to a pressure transducer was introduced into the left and right ventricles and the pressure curves were recorded for later offline measurements (Acknowledgement 8.1, Biopac, Goleta, CA, USA).

Quantification of creatine and energy-rich phosphometabolites

Myocardial content of creatine (Cr) and energy-rich metabolites, i.e. phosphocreatine (PCr), adenosine-three-phosphate (ATP), adenosine-di-phosphate (ADP), and adenosine-mono-phosphate (AMP), was measured at the end of the 12 weeks period. This analysis was performed in 6 CHB and 10 control animals. The apical half of the heart was freeze-clamped promptly after the end of the invasive pressure measurements for analysis of myocardial Cr, PCr, and total adenine nucleotides (TANs), i.e. the sum of ATP, ADP and AMP. The myocardial samples were extracted with 0.5 M pericloric acid. An aliquot of the homogenate was neutralized and the supernatant was used for measuring total Cr and TAN pool by means of HPLC (SMART system Pharmacia, Uppsala, Sweden) as previously described. The concentration of Cr, PCr, ATP, ADP, and AMP was calculated in nmol/mg protein.

Statistical analysis

Computer software (StatView 5.0.1) was used to perform standard statistical procedures. Unpaired and paired t-tests were used to detect significant differences between different treatments for interactions defined in advance. The value P < 0.05 was considered as statistically significant. All data are presented as mean ± SEM.

Results

Induction of CHB

Operative success with ethanol injection was only 5% (1/20), despite repeated injections and large amounts of ethanol (~1 mL). We therefore abandoned this method and developed an alternative approach by applying electrocautery to the same anatomical region. Our success rate was 54% (6/11). Two animals (18%) died due to the bleeding complications. The CHB animals recovered uneventfully and appeared healthy throughout the experimental period. There were no deaths during the follow-up.

Echocardiography

The data are summarized in the Table 1. The CHB animals had similar atrial rates but lower ventricular rates (P < 0.01) compared with the controls (Figures 1 and 2). Left ventricular dimension in diastole and LV mass were increased (both P < 0.05, Figure 2 and Table 1) compared with the controls. Relative LV wall thickness was decreased suggesting the presence of eccentric hypertrophy (P < 0.05). The LV hypertrophy developed simultaneously with LV dilatation and these findings were detectable already after 1 week and were sustained until the 12th week (P < 0.01). The SV increased by 2.5 times in the CHB group (P < 0.01) compensating for the lower HR (Figure 2) which was sufficient to maintain CO similar to the controls (Table 1). Cardiac output was preserved in the CHB animals, whereas myocardial contractility was decreased as reflected by FS at 3 and 12 weeks (P < 0.05).
effects of CHB in the rat

The data are summarized in the Table 2. There was no difference between the groups in regard to myocardial content of total Cr and PCR. Similarly, no difference was found in the myocardial TAN and PCR/ATP ratio.

Discussion

The most important results of this study could be summarized as follows. Induction of CHB in rats results in early, profound, and sustained cardiac remodelling with development of eccentric LV hypertrophy and preserved systolic function. These alterations were associated with normal myocardial Cr and TAN metabolism.

The model

Animal models of CHB can provide valuable insights into the haemodynamic, electrophysiological, morphological, and metabolic consequences of ventricular bradyarrhythmia 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 experimental CHB models, in both large-animals (dogs\textsuperscript{11}, pigs\textsuperscript{12}) and small-animals (rabbits\textsuperscript{13} and rats\textsuperscript{8}). Lee et al.\textsuperscript{8} have recently described CHB induction in rats by means of ethanol injection into the AV-node area. However, we were unable to reproduce their high success rate for induction of permanent CHB. It is true that most of the animals in our study developed CHB immediately upon the ethanol injection, but the CHB was transient, and only ~5% of the animals remained in CHB permanently. Therefore, we suggest direct electrocautery as the more reliable means of AV-node destruction. Studies in the large-animal models of CHB have yielded important insights into functional, structural, and electrophysiological remodelling processes that occur in response to acquired CHB. Nevertheless, compared with small-animals, these models are more expensive, more demanding, and there may be significant societal and institutional impediments to their routine use in biomedical research.

Echocardiography

Non-invasive and longitudinal evaluation with echocardiography was not previously attempted in the rat CHB model. This investigation has revealed important information about post-CHB LV remodelling and LV function. Already 1 week post-CHB, there was a marked increase in LV diameters and SV compensatory mechanisms to maintain normal CO. Similarly, LV mass increased through eccentric hypertrophy and was detectable at this time but without signs of disturbed LV function. During the next 3 weeks, the remodelling continued with increase in eccentric LV hypertrophy. Left ventricular contractile function decreased at 3 and 12 weeks, however, there were no signs of increased RV and LV filling pressures suggesting no presence of LV dysfunction. Decreased FS was not previously reported in the animal CHB models. It could be an indicator of functional decoupling of eccentric hypertrophied LV. However, the absence of increased filling pressures, both in LV and RV, speaks strongly against this hypothesis. Therefore, we speculate that this finding may be explained by the ‘Bodwich phenomenon’, i.e. force–frequency relationship (myocardial contractility decreases with increasing HR and vice versa in the case of positive force–frequency relationship).\textsuperscript{14,15} This hypothesis is supported by human data from elite athletes in whom a decreased FS has been demonstrated when compared to healthy controls.\textsuperscript{16,17}

### Table 1 Summary of basic characteristics and echocardiographic data

<table>
<thead>
<tr>
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<th>CHB, n = 6</th>
<th>Controls, n = 10</th>
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<tbody>
<tr>
<td></td>
<td>1 week</td>
<td>3 weeks</td>
</tr>
<tr>
<td>AR (bpm)</td>
<td>351 ± 12</td>
<td>353 ± 9</td>
</tr>
<tr>
<td>VR (bpm)</td>
<td>157 ± 6*</td>
<td>153 ± 15*</td>
</tr>
<tr>
<td>BW (g)</td>
<td>258 ± 12</td>
<td>337 ± 12**</td>
</tr>
<tr>
<td>IVS (mm)</td>
<td>1.8 ± 0.08</td>
<td>2.2 ± 0.1***</td>
</tr>
<tr>
<td>PWD (mm)</td>
<td>1.5 ± 0.07</td>
<td>1.9 ± 0.07***</td>
</tr>
<tr>
<td>RWT</td>
<td>0.32 ± 0.01*</td>
<td>0.35 ± 0.02*</td>
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<tr>
<td>LVdD (mm)</td>
<td>9.2 ± 0.3*</td>
<td>11.1 ± 0.4***</td>
</tr>
<tr>
<td>LVdS (mm)</td>
<td>4.5 ± 0.4</td>
<td>6.3 ± 0.5***</td>
</tr>
<tr>
<td>FS (%)</td>
<td>51 ± 3</td>
<td>44 ± 4***</td>
</tr>
<tr>
<td>LVm (g)</td>
<td>1.2 ± 0.1*</td>
<td>2.2 ± 0.2</td>
</tr>
<tr>
<td>SV (mL)</td>
<td>0.45 ± 0.04*</td>
<td>0.51 ± 0.04***</td>
</tr>
<tr>
<td>CO (mL/min)</td>
<td>71 ± 8</td>
<td>76 ± 5</td>
</tr>
<tr>
<td>CI (mL/min/kg)</td>
<td>27 ± 2</td>
<td>23 ± 2</td>
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AR, atrial rate (beats per minute); VR, ventricular rate (beats per minute); BW, body weight; IVS, septum; PWD, posterior wall thickness in diastole; RWT, relative wall thickness; LVdD, LV diameter in diastole; LVdS, LV diameter in systole; LVm, LV mass; FS, fractional shortening; CI, cardiac index.

*P < 0.05 vs. control.

**P < 0.05 (paired t-test within the group).

Invasive haemodynamic

There was no difference between the groups in LV and RV intraventricular pressures at 12 weeks after the CHB induction suggesting no development of congestive heart failure (CHF) (Figure 3).

Myocardial content of Cr and high energy phosphometabolites

The data are summarized in the Table 2. There was no difference between the groups in regard to myocardial content of total Cr and PCR. Similarly, no difference was found in the myocardial TAN and PCR/ATP ratio.

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Figure 1 Representative ECG tracing from an animal with CHB. There is dyssynchrony between P-waves (arrows) and QRS-complexes evidencing the presence of AV-block III. There is a widening of QRS complex suggesting an establishment of compensatory ventricular rhythm.

Figure 2 (A) Typical M-mode tracing from the animal with CHB and the control. There was a rapid and dramatic increase in LV dimensions early in the post-CHB phase which persisted until 12 weeks post-CHB. LVDd, LV diameter in diastole; LVDs, LV diameter in systole. (B) Typical tracing of a pulsed Doppler from the pulmonary artery in a CHB animal and a control. The SV was 2.5 times higher in the CHB animals. (C) Pulsed Doppler from the mitral annulus showing AV dissociation with no relation between E- and A-waves in a CHB animal. A, atrial filling wave; E, early diastolic filling wave.
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Intracardiac pressure measurements in systole (A) and diastole (B). There were no signs of increased filling pressures after 12 weeks, suggesting full functional compensation in the CHB animals despite the presence of pronounced LV dilatation and eccentric LV hypertrophy. LVp, LV pressure in systole; RVp, RV pressure in systole; LVpd, LV pressure in diastole; RVpd, RV pressure in diastole.

Myocardial content of creatine and high energy phosphometabolites at 12 weeks

<table>
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<tr>
<th></th>
<th>CHB (n = 6)</th>
<th>Control (n = 10)</th>
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<tbody>
<tr>
<td>PCr (nmol/mg)</td>
<td>9.0 ± 1.3</td>
<td>14.6 ± 3.0</td>
</tr>
<tr>
<td>Cr (nmol/mg)</td>
<td>57.9 ± 7.0</td>
<td>62.4 ± 4.6</td>
</tr>
<tr>
<td>ATP (nmol/mg)</td>
<td>12.5 ± 1.9</td>
<td>12.3 ± 1</td>
</tr>
<tr>
<td>ADP (nmol/mg)</td>
<td>12.6 ± 1.1</td>
<td>14.6 ± 1</td>
</tr>
<tr>
<td>AMP (nmol/mg)</td>
<td>8.0 ± 1.5</td>
<td>8.5 ± 1.0</td>
</tr>
<tr>
<td>TAN (nmol/mg)</td>
<td>32.9 ± 2.4</td>
<td>35.4 ± 2.4</td>
</tr>
<tr>
<td>PCr/ATP ratio</td>
<td>0.8 ± 0.1</td>
<td>1.0 ± 0.1</td>
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</table>

Table 2 Myocardial content of creatine and high energy phosphometabolites at 12 weeks

Myocardial energy metabolism

There were no biochemical signs of disturbed myocardial energy metabolism. Intracellular depletion of Cr and energy-rich phosphometabolites (PCr, ATP) is an important indicator of disturbed energy metabolism in the heart. Creatine and TAN depletion as well as disturbance in other parts of myocardial energy metabolism have been associated with different types of LV hypertrophy and CHF. In particular, Cr depletion appears to be an obligatory finding in the failing and hypertrophied heart and is evident of pathologic biochemical remodelling.18,19 Indeed, in the setting of chronic heart failure and advanced hypertrophy, Cr depletion is regarded as the major cause of decreased myocardial PCr content since ~70% of all Cr is phosphorylated in the form of PCr. However, after 12 weeks of chronic volume-overload due to total AV-block, we were unable to detect such a derangement. At this time point, the LV morphology was markedly altered by development of pronounced hypertrophy. Large body of evidence based on both human and animal experiments has shown that PCr/ATP ratio is decreased in the failing and hypertrophied heart.20–22 Indeed, in the setting of chronic heart failure and advanced hypertrophy, Cr depletion is regarded as the major cause of decreased myocardial PCr content (70% of all Cr is phosphorylated in the form of PCr). Therefore, if total Cr is unaltered, it is highly unlikely that PCr/ATP ratio—an indicator of myocardial energy reserve—is abnormal. Indeed, the results from the analysis of energy-rich phosphometabolites are directly congruent with the finding of an unaltered Cr content. On the other hand, our metabolic data differ from the previous reports in which other forms of volume-overload hypertrophy were reported to be associated with disturbed myocardial energy metabolism, i.e. decreased Cr and TAN levels.23,24 The reason for this discrepancy is not known. We speculate that the eccentric LV hypertrophy associated with chronic CHB is more adaptive and physiologic than hypertrophy associated with pure volume-overload. Recent studies have demonstrated that bradycardia solely may enhance angiogenesis in the heart mediated through increased myocardial synthesis and paracrine action of angiogenic factors.25–29 The normal capillary-to-myofibrillar ratio observed in large-animal CHB models support this speculation and provides anatomic base for supply–demand balance in terms of oxygen and substrate delivery.

Cardiac remodelling

Cardiac remodelling is a continuous process of alterations in genome expression, molecular, cellular, and interstitial changes that are manifested clinically as changes in size, shape, and function of the heart.30 This process is mainly viewed as a pathologic condition that occurs after myocardial damage, induced by ischaemia (infarction), pressure and volume overload (valvular diseases), inflammatory heart muscle disease (myocarditis), idiopathic dilated cardiomyopathy, and virtually after any type of myocardial damage. The process is influenced by haemodynamic load, neurohormonal activation, extent and location of myocardial damage, and probably many other factors which are currently the focus of clinical and experimental research.31–33 The cardiomyocyte is the major cardiac cell involved in the remodelling process.34 Other components that are involved include the interstitium, fibroblasts, collagen, and coronary vasculature.35–37 Circulating or locally generated neurohormones such as catecholamines, angiotensin-II, cytokines, and others are thought to play a major role in change of the myocardial phenotype by altering gene expression via activation of second messenger systems.38–40 Cardiac remodelling ultimately leads to development of progressive myocardial dysfunction during the time. It is generally accepted that early remodelling in response to pathologic stimuli (e.g. abnormal wall stresses) is an adaptive and useful response in the short term, but it is the continuation of the process that is regarded as maladaptive response. This small-animal model of post-CHB
remodelling offers possibility to study neurohormonal, metabolic, cellular, subcellular, and other processes involved in development of adaptive and more physiological LV remodelling in contrast to the pathologic remodelling. Insights into molecular mechanisms behind beneficial cardiac remodelling may be useful for the development of pharmacological and other interventions to attenuate and prevent progression of pathologic cardiac remodelling that leads to development of CHF. Our findings on LV remodelling and functional adaptation in this model are in agreement with those described in the canine model. One important limitation of this small-animal model of CHB at the present time is difficulty to implement different pacing strategies. This type of studies is more appropriate in large-animal models, although the future advances in pacemaker technology with development of smaller devices may allow pacing studies even in small-animal models.

In conclusion, we present the new approach to induce CHB in rats by means of direct electrocautery of AV node. Long-term CHB in rats leads to early, pronounced, and sustained cardiac remodelling with development of eccentric LV systolic mechanics in chronically failing rat heart. J Mol Cell Cardiol 2005;38:309–13.


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