Development of a model of complete heart block in rats

RAN D ALL J. L EE, 1 RICHARD E. SIEVERS, 1 G. JO SEPH GALLING HOU S E, 1 AND PHILIP C. URSELL 2

1 Department of Medicine and Cardiovascular Research Institute, and 2 Department of Pathology,
University of California, San Francisco, California 94143-1354

Lee, Randall J., Richard E. Sievers, G. Joseph Gallighouse, and Philip C. Ursell. Development of a model of complete heart block in rats. J. Appl. Physiol. 85(2):758-763, 1998.—Atrioventricular (AV) block is a useful substrate for the study of cardiac physiology. The objective of this investigation was to develop a straightforward and reproducible model of permanent AV block in rats. Working through a sternotomy, we used an epicardial fat pad between the aortic root and the right atrial wall of the rat as a landmark for the site for injection of 70% ethanol (5–10 µl) into the myocardium 3 mm below the epicardial surface. Stable, complete heart block was produced in 23 of 28 rats (82%) with a success rate of 100% in the last 16 rats of the series. Saline injection produced no heart block in 15 rats. A separate group of 14 animals was allowed to recover. Chronic heart block was achieved in all ethanol-injected animals for up to 7 days before death. The survival rate in the recovered rats was 90% in the ethanol-injected group and 100% in the saline-injected control group. Acute hemodynamic changes following the production of heart block consisted of an increase in central venous pressure, a decrease in systolic blood pressure, a decrease in left ventricular pressure, and a decrease in change in pressure over time. Chronic hemodynamic changes demonstrated a return to baseline of the central venous pressure, a persistent decrease in systolic blood pressure, and a decrease in left ventricular pressure. After the rats were killed and the hearts were dissected, discrete areas of myocardial damage were identified histologically in the atrial septum near the AV conduction axis tissue in the ethanol-injected hearts. Complete heart block was associated only with lesions extending into the specialized muscle of the AV node or His bundle. Focal mild hemorrhage, inflammation, and damaged myocardial fibers were observed in the acute stage, whereas healing lesions were characterized by granulation tissue and fibrosis replacing conduction tissue. The simple technique described provides a reproducible model for permanent, complete heart block and the study of cardiac function.

atrioventricular node; atrioventricular block; His bundle; conduction tissue; specialized cardiac muscle
In the present age, space and cost considerations often necessitate use of smaller, less expensive animal models (3, 21, 28), and, for these reasons, the rat is a popular species. To our knowledge, rats have been used only infrequently as models of heart block. A preliminary study of catheter-induced electrical injury resulting in heart block in rats was reported many years ago (1). This report, however, lacked a detailed description of the technique, histological verification of the site, and extent of injury and success rates. The objective of the present study was to devise a convenient and reproducible model of complete heart block in the rat, a species that is still relatively inexpensive. Although the thorax must be opened for the epicardial approach, morbidity and mortality can be minimized, and the success rate is very high. This technique can be not only used in physiological investigations for production of heart block but also adapted to deliver biologically active substances into the AV conduction axis.

MATERIALS AND METHODS

Surgical technique. The study protocol was approved by the Committee for Animal Research of the University of California at San Francisco. The investigation conforms to the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH Publication No. 85–23, revised 1985).

Albino Sprague-Dawley rats (210–225 g) were anesthetized by pentobarbital sodium (40–50 mg/kg ip), positioned supine on an animal surgery table, and held in a stable position by paw restraints. The animals were kept on a delta-phase isothermal pad. This pad maintained the animals at 37°C for the duration of the surgery. After intubation, the animals were ventilated with a Harvard respirator (model 683, Harvard Apparatus, South Natick, MA). Electrograms were displayed on a physiological recorder and recorded on paper, together with surface leads I and II.

A 30-gauge needle connected to a microliter syringe (Hamilton, Reno, NV) was used to inject the solutions into the myocardium. To facilitate the direction of the needle toward the nodal tissue, the needle had been prepared by making a 90° bend in the shaft 3 mm from the tip. Thus the needle could only be inserted into the myocardium up to a maximum of 3 mm from the epicardial surface. After midline sternotony and pericardiotomy, the tip of the right atrial appendage was reflected laterally to provide access to the AV junction in this area. This maneuver exposed the landmark for the epicardial approach to the AV node, a fat pad consistently located 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 noncoronary leaflets of the aortic valve (Fig. 1).

The tip of the needle penetrated the epicardial surface at a point 1 mm posterior and 1 mm lateral to the fat pad. Directed toward the apex of the heart (i.e., in the long axis of the heart), the needle was inserted up to its bend. The angled portion of the needle was maintained parallel to the ascending aorta at all times.

When the insertion of the needle resulted in momentary, complete AV block (as determined by electromechanical dissociation of the heart and electrocardiogram [ECG]), 5–10 μl of 70% ethanol were injected. Twenty-eight rat hearts were injected with ethanol. Hearts were reinfused with ethanol if the heart block resolved within 10 min. Permanent heart block was defined as stable, complete heart block lasting at least 60 min. Many of these animals survived for several days to 1 wk for histological analysis of the AV conduction axis. A control group of 15 rats underwent an identical procedure except that saline was used instead of ethanol. In six of the saline-injected rats, the needle was inserted repeatedly up to five times to test whether the mechanical trauma alone could induce heart block.

Once the technique of AV nodal injection was developed and validated by the production of permanent AV block in the first group, a second group of 14 animals (10 ethanol injected, 4 saline control) was used to test the feasibility of creating a chronic model for complete heart block. The same technique was employed to inject this second group of rats. The chest was closed with 5–0 Prolene (Davis and Geck). The layers of skin were closed in a whipstitch fashion. A mediastinal tube was placed to evacuate air. After sternal closure, the mediastinal tube was aspirated by syringe before tube extraction. The endotracheal tube was frequently suctioned during the recovery of the animals, and atropine (1 mg/kg sc) was administered perioperatively to reduce pulmonary secretions. The animals were allowed to awaken from anesthesia, and the endotracheal tube was removed. The animals were kept on the isothermal pad during recovery for maintenance of body core temperature. After recovery, the rats had free access to standard rat chow and water. Before death, the electrogram of each animal was checked to confirm complete heart block.

Hemodynamic recordings. Additional animals were used to determine the hemodynamic effects of complete heart block. The rats were divided into three groups: control (baseline animals, n = 7), acute (1 h after the production of heart block, n = 6), and chronic (5–7 days after the production of heart block, n = 4). The rats were anesthetized by pentobarbital sodium (40–50 mg/kg ip), positioned supine on an animal surgery table, and held in a stable position by paw restraints. The right internal jugular vein was cannulated with a 22-gauge angiocath needle and connected to a pressure

---

**Fig. 1. Epicardial fat pad marks approach to atrioventricular (AV) node.** A: right atrial appendage (Raa) is reflected laterally with forceps to expose epicardial fat pad (arrowhead) between aortic root (Ao) and medial wall of right atrium. Bent portion of needle, positioned parallel to aorta, will penetrate epicardial surface at a point 1 mm lateral and 1 mm posterior to fat pad. B: fat pad (fp) is identified adjacent to noncoronary (nc) and right coronary (rc) sinuses of aortic valve. a, Atrium; v, ventricle. Magnification ×25; stained with Masson’s trichrome.
transducer to record central venous pressure. The right carotid artery was cannulated with a 22-gauge angiocath needle and connected to a pressure transducer to record systemic blood pressure. The maximum level of left ventricular pressure was obtained by direct left ventricular puncture with a 19-gauge needle connected to a pressure transducer. Heart rate was calculated from the ECG. Hemodynamic monitoring was performed over a 2-min period for each group, to ensure stable hemodynamic recordings. Measurements were the average of three separate cardiac cycles. Hemodynamic measurements were obtained from animals with a narrow QRS complex. The transducer measurements were recorded with the analog-to-digital MacLab chart recorder. ANOVA was used to determine whether the means of control, acute, and chronic conditions were significantly different.

Histology. All rats were killed 1 wk after surgery by an overdose of pentobarbital sodium. After thoracotomy, the hearts were rapidly excised and rinsed in cold saline. The apical half of the ventricles of each heart was cut away and discarded. The remainder was then immersed in 4% buffered paraformaldehyde (pH 7.4) for 1 or 2 days. Under a dissecting microscope, excess tissue at the base of each heart was trimmed. To orient the specimen during embedding, a frontal cross section of the base of the heart was made in a plane roughly parallel to the right ventricular outflow tract; sectioning commenced at this flat surface. The specimen was dehydrated in graded alcohols and xylene before paraffin embedding. Serial 10-µm sections were cut and laid out on a tray. Every 20th section was stained with hematoxylin and eosin for orientation, and, from those containing specialized muscle, every 20th section (staggered by 10 from the hematoxylin and eosin sections) was stained with Masson’s trichrome. The result was a composite view of the AV conduction axis of the rat heart cut in the classical transverse plane of section (10).

RESULTS

Surgical results. Representative surface ECGs from before and 1 h after 70% ethanol or saline injections into the AV node area are shown in Fig. 2. Injection of ethanol into the AV node created heart block, whereas saline produced no noticeable difference in the surface electrogram, compared with the baseline state.

Momentary block of AV conduction and lack of blood on aspiration of the syringe all but confirmed the position of the needle in the rat AV node or bundle, similar to what has been reported in dogs (4). Subsequent injection of ethanol produced stable heart block. Saline-injected rats also showed AV block, but normal sinus rhythm returned within 10–15 s. Moreover, up to five injections of 5–10 µl of saline produced no change in the P-R interval or permanent AV dissociation in each of six rats.

Stable, complete AV dissociation was produced in 23 of 28 rats after ethanol injection. In five rats only transient heart block was created, which occurred early in the series. Perforation of the left ventricle probably occurred in seven rats. Heralded by the appearance of bright red blood, this complication required mild pressure with a cotton swab to terminate the bleeding. Laceration of the lung occurred in two rats, resulting in a collapsed lung. All of these complications happened during the early developmental phase of this project. Complete heart block was achieved in each of the last 16 rats of the series without any complications. In every case, heart block was attained after only the first (11 animals) or second injection (4 animals).

In a second group of 10 ethanol-injected rats, chronic heart block was obtained by allowing the rats to recover postoperatively. The survival rate at 7 days was 90% (9 of 10 animals), compared with 100% (4 of 4) for saline-injected animals. In the period spent in developing the technique, the majority of postoperative deaths could be attributed to respiratory failure due to increased secretions plugging the endotracheal tube. Survival was markedly improved by frequent suctioning of the endotracheal tube and administration of atropine (1 mg/kg sc) perioperatively to reduce pulmonary secretions.

Hemodynamic response. Acute and chronic hemodynamic changes after the production of heart block are presented in Table 1. With onset of complete heart block, there was an increase in central venous pressure (P = 0.015) and a nonsignificant decrease in systolic blood pressure (P = 0.032) but a significant decrease in left ventricular pressure (P < 0.001). Later, the central venous pressure returned to baseline, but there was a persistent decrease in systolic blood pressure and a significant decrease in left ventricular pressure (P < 0.001).
Table 1. Hemodynamic effects of complete heart block

<table>
<thead>
<tr>
<th></th>
<th>HR, beats/min</th>
<th>CVP, mmHg</th>
<th>SBP, mmHg</th>
<th>DBP, mmHg</th>
<th>LVP, mmHg</th>
<th>LVEDP, mmHg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control rats (n = 7)</td>
<td>417 ± 0.8</td>
<td>1.7 ± 0.7</td>
<td>133 ± 7.1</td>
<td>101 ± 12.3</td>
<td>135 ± 4.8</td>
<td>1.8 ± 0.8</td>
</tr>
<tr>
<td>Acute complete heart block (n = 6)</td>
<td>178 ± 11.5</td>
<td>4.8 ± 0.7</td>
<td>89 ± 12.9</td>
<td>49 ± 7.2</td>
<td>90 ± 4.8</td>
<td>3.8 ± 0.2</td>
</tr>
<tr>
<td>Chronic complete heart block (n = 4)</td>
<td>185 ± 29.6</td>
<td>1.8 ± 1.2</td>
<td>119 ± 18.0</td>
<td>77 ± 17.5</td>
<td>94 ± 6.5</td>
<td>4.0 ± 1.7</td>
</tr>
<tr>
<td>P values</td>
<td>P &lt; 0.001</td>
<td>P = 0.015</td>
<td>P = 0.032</td>
<td>P = 0.017</td>
<td>P &lt; 0.001</td>
<td>P = 0.369</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of rats. HR, heart rate; CVP, central venous pressure; SBP, systolic blood pressure; DBP, diastolic blood pressure; LVP, left ventricular pressure; LVEDP, left ventricular end-diastolic pressure. P values determined by ANOVA.

Normal anatomy of the AV conduction axis in the rat heart. Three noninjected rat hearts were dissected and serially sectioned to investigate the normal anatomy of the AV conduction axis. In sections made in the transverse plane of section, the specialized cardiac muscle could be clearly identified by its histological features (Fig. 3). The approximate dimensions of the AV node were 0.7 $\times$ 0.6 $\times$ 0.1 mm. The compact portion of the node abutted the central fibrous body on its right side. Because of offsetting of the tricuspid valve, however, the AV node was physically much closer to the mitral valve, which attached to the fibrous skeleton at this point (Fig. 3). The specialized muscle of the compact node was continuous with that of the AV bundle in more anterior sections. The bundle was a distinctive aggregate of specialized muscle completely surrounded by collagen (Fig. 3). The branching bundle was a triangular structure at the crest of the ventricular septum.

Localization of lesions in the ethanol- and saline-injected hearts. The histopathology of the AV node area was assessed in 12 ethanol- and 5 saline-injected hearts. Ethanol-induced myocardial damage appeared as necrotic muscle in various phases of inflammation and repair, depending on time elapsed since injury (Fig. 4). Coagulative necrosis of muscle, mild hemorrhage, and polymorphous inflammation were observed in the acute stage ($<$ 4 days, n = 2), whereas the chronic stage (4 days to 1 wk, n = 10) was characterized by granulation tissue replacing specialized muscle. Among the ethanol-injected hearts, seven were from animals with permanent, complete heart block. The extent of the damage in these seven hearts was variable. Five hearts had focal lesions involving essentially only the site of the specialized muscle. The ECG of these five animals revealed a narrower QRS complex than that of the other two animals, which had more widespread granulation tissue involving the atrial septum, crest of the ventricular septum, and the AV bundle. Thus, in the hearts from the animals with permanent, complete AV dissociation and a wide QRS complex, there was necrosis of, or reparative tissue replacing, the AV node and AV bundle. Five of the ethanol-injected rats submitted for histological examination demonstrated only short-term heart block ($<$ 30 min). Histological examination...
of the hearts from these rats demonstrated perinodal injury involving working muscle in the interatrial septum or the ventricular septum, sparing the specialized muscle comprising the AV node and bundle.

In contrast, the saline-injected rat hearts showed little pathology. The hearts from these animals had scant hemorrhage denoting the area of injection, the architecture of the conduction axis was preserved in every heart, and the specialized muscle was histologically normal.

**DISCUSSION**

This investigation demonstrates a straightforward and reproducible method of injecting the AV conduction axis in rats. The accuracy of the injection site was validated by the production of AV block. A new finding from this study is that an epicardial fat pad, corresponding to the position of the commissure between the right and noncoronary leaflets of the aortic valve, can be used as a landmark to guide placement of the needle used to inject the AV node area. Starting at a point 1 mm posterior and 1 mm lateral to the fat pad and in a line parallel to the ascending aorta (i.e., directed toward the apex of the heart), the needle tip can easily be made to penetrate the AV node 3 mm below the epicardial surface.

In designing our method, we adapted a surgical approach used in large animals (25). This simple procedure does not require special equipment. Because the injection is through the medial wall of the right atrium, there is minimal effect on morbidity or mortality due to the needle puncture. Furthermore, the open-chest model facilitates electrophysiological experiments such as those employing programmed electrical stimulation. Finally, by allowing animals to recover, we have shown that this method can be useful in chronic preparations. In our series of rats, onset of complete heart block was associated with an increase in central venous pressure, which returned to normal over time, and a more permanent reduction in systolic blood pressure and left ventricular pressure.

Previous studies of the rat AV conduction axis have focused on the histology and ultrastructure of the specialized muscle and have not correlated the gross anatomy with the microscopic anatomy and electrophysiology (2, 12–14, 17). In the present study, we documented histologically the extent of damage to the specialized muscle and correlated these findings with the electrophysiological data. Our structural data from normal hearts corroborate those from other studies defining the normal microscopic anatomy of the conduction axis (2, 10, 11, 26). Each heart in which permanent, complete heart block had been achieved contained a lesion obliterating either the AV node or AV bundle. In many hearts, the limited extent of the lesion underscored the accuracy of the method of localization. A few hearts showed a larger lesion, which extended into the specialized muscle. In any case, the interruption of the specialized muscle was the structural basis for nonconduction of the electrical impulse through the AV conduction axis.

This simple surgical method in rats provides an inexpensive model with which to conduct sophisticated experiments in cardiac physiology and molecular biology. The recent advances in the field of cardiovascular gene transfer have inspired visions of repair and even regeneration of damaged myocardium, possibly as an
adjunct to conventional medical therapy or alternative to cardiac transplantation (15, 23). Thus far, studies of gene therapy directed at myocardial cells have focused on the restoration of contractility, and little attention has been paid to the potential of gene therapy in modulating cardiac conduction. The ability to reliably perform AV nodal injections facilitates the study of the effects of drugs and biological substances on cardiac conduction, as measured by changes in the P-R interval of the surface electrocardiogram. In addition, the open-chest model allows programmed electrical stimulation of the atrium to be performed during pharmacological, autonomic blockade to determine the AV node block cycle length and the AV node effective refractory period (6), thus increasing the sensitivity of detection of a change in electrical conduction through the AV conduction axis.

In conclusion, we describe a reliable technique for producing complete heart block in rats. The ability to successfully inject substances into the AV conduction axis provides an easy and precise physiological means of assessing cardiac conduction.

We thank Margaret Mayes for assistance in the preparation of the histology specimens.

This study was supported in part by the National Institutes of Health (NIH) Program of Excellence in Molecular Biology (Grant HL-43821) and the University of California at San Francisco Gene Therapy Core Center (NIH Grant DK-47766).

Address for reprint requests: R. J. Lee, MU East Tower, Box 1354, Univ. of California, San Francisco, 500 Parnassus Ave., San Francisco, CA 94143-1354.

Received 25 July 1997; accepted in final form 13 April 1998.

REFERENCES


