Cardiac electrophysiology and the susceptibility to sustained ventricular tachycardia in intact, conscious mice

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Lujan HL, DiCarlo SE. Cardiac electrophysiology and the susceptibility to sustained ventricular tachycardia in intact, conscious mice. Am J Physiol Heart Circ Physiol 306: H1213–H1221, 2014. First published February 21, 2014; doi:10.1152/ajpheart.00780.2013.—Cardiac electrophysiological dysfunction is a major cause of death in humans. Accordingly, electrophysiological testing is routinely performed in intact, conscious humans to evaluate arrhythmias and disorders of cardiac conduction. However, to date, in vivo electrophysiological studies in mice are limited to anesthetized open-chest or closed-chest preparations. However, cardiac electrophysiology in anesthetized mice or mice with surgical trauma may not adequately represent what occurs in conscious mice. Accordingly, an intact, conscious murine model of cardiac electrophysiology has the potential to be of major importance for advancing the concepts and methods that drive cardiovascular therapies. Therefore, we describe, for the first time, the use of an intact, conscious, murine model of cardiac electrophysiology. The conscious mouse model permits measurements of atrioventricular interval, sinus cycle length, sinus node recovery time (SNRT), SNRT corrected for spontaneous sinus cycle, Wenckebach cycle length, the ventricular effective refractory period (VERP), and the electrical stimulation threshold to induce sustained ventricular tachyarrhythmias in an intact, complex model free of the confounding influences of anesthetics and surgical trauma. This is an important consideration because anesthesia and surgical trauma markedly reduced cardiac output and heart rate as well as altered cardiac electrophysiology parameters. Most importantly, anesthesia and surgical trauma significantly increased the VERP and virtually eliminated the ability to induce sustained ventricular tachyarrhythmias. Accordingly, the methodology allows for the accurate documentation of cardiac electrophysiology in complex, conscious mice and may be adopted for advancing the concepts and ideas that drive cardiovascular research.

refractory period; sinus node recovery time; atrioventricular interval; ECG

CARDIAC ELECTROPHYSIOLOGICAL studies are routinely performed in intact, conscious humans to evaluate arrhythmias and disorders of cardiac conduction. However, to date, in vivo electrophysiological studies in mice are limited to anesthetized open-chest or closed-chest preparations (5, 27). However, cardiac electrophysiology in anesthetized mice or mice with surgical trauma may not adequately represent what occurs under physiological and functionally relevant conditions.

Similarly, it is very difficult to induce ventricular arrhythmias in wild-type mice with programmed electrical stimulation (1, 5, 7, 14, 19, 32, 42). For example, Maguire and colleagues (32) could not induce ventricular tachycardia with programmed electrical stimulation in any of the 25, 9- to 15-wk-old C57 mice, and induced nonsustained ventricular tachycardia in only 1 of 6, 50-wk-old C57 mice. In fact, of the 41 C57 mice of all ages studied, only 1 mouse had ventricular tachycardia and it was nonsustained. This difficulty may be due, in part, to the reliance on murine models that do not mimic the clinical or physiological situation. Specifically, the confounding influences of anesthetics, surgical trauma, or the use of crystalloid perfused hearts that are devoid of many of the vital components of blood may account for this effect. In this context, the limited use of intact, conscious mice is of major concern. In fact, whenever possible, studies of cardiovascular physiology and pathophysiology should be conducted in conscious, complex models to avoid the complications associated with the use of anesthesia and surgical trauma (29–31).

The limited number of murine models that mimic the clinical and physiological situation presents a significant weakness because mice have become increasingly important as models for human cardiovascular diseases, and cardiac electrophysiology in anesthetized mice or mice with surgical trauma may not adequately represent what occurs in conscious mice (5, 6, 27). Specifically, anesthesia and the inflammation associated with surgical trauma may markedly alter cardiac electrophysiology (5, 9, 22, 28, 34, 38). Accordingly, there is a need for a chronically instrumented, intact, conscious murine model for cardiac electrophysiological testing.

Therefore, we describe, for the first time, the use of an intact, conscious, mouse model for cardiac electrophysiology testing. The conscious mouse model permits measurements of atrioventricular interval, sinus cycle length, sinus node recovery time (SNRT), SNRT corrected for spontaneous sinus cycle, Wenckebach cycle length, the ventricular effective refractory period (VERP), and the electrical stimulation threshold to induce sustained ventricular tachyarrhythmias in an intact, complex model free of the confounding influences of anesthetics and surgical trauma. This is an important consideration because anesthesia and surgical trauma markedly reduced cardiac output and heart rate as well as altered cardiac electrophysiology parameters. Most importantly, anesthesia and surgical trauma significantly increased the VERP and virtually eliminated the ability to induce sustained ventricular tachyarrhythmias.

METHODS

Experimental Subjects

All surgical and experimental procedures involving animals were reviewed and approved by the Institutional Animal Care and Use...
Committee and conformed to the American Physiological Society Guiding Principles in the Care and Use of Animals. Studies determining cardiac electrophysiology parameters and the susceptibility to sustained ventricular arrhythmias were conducted in 24 male C57BL/6J mice (15 wk of age), a strain commonly used in transgenic studies (5).

Initially, fifteen mice were chronically instrumented. No mice died during the initial surgical instrumentation; however, two mice died during the recovery period, both within 48 h of the initial surgery. These animals had lethal pulmonary edema. None of the mice had instrumentation failure; thus data were obtained in 13 chronically instrumented, intact, conscious mice. Nine additional male C57BL/6 mice (15 wk of age weighing 31.4 ± 1.2 g) were studied to determine effects of acute anesthesia and surgical trauma on cardiac electrophysiology parameters. Complete data were obtained from all 9 anesthetized, open-chest mice.

**Surgical Procedures**

*Instrumentation.* All surgical procedures were performed using aseptic surgical measures (29–31). Thirteen adult, male C57BL/6 mice (29.3 ± 0.6 g before instrumentation and 31.2 ± 1.1 g after completion of the studies) were studied to determine cardiac electrophysiology parameters and the susceptibility to sustained ventricular tachyarrhythmias in intact conscious mice. Mice were anesthetized with sodium pentobarbital (60 mg/kg ip) and supplemental doses (10–20 mg/kg ip) were administered if the mice regained the blink reflex or responded during the surgical procedures.

The hearts were approached via a left thoracotomy through the second intercostal space. Teflon-coated stainless steel wire electrodes (0.003 in., part no. 316 SS 7/44T, Medwire, Mount Vernon, NY) were sutured 1–2 mm apart with 8.0 silk on the surface of the left ventricle and atrial appendage as previously described in rats (39, 40), and a 1.6-mm silicone cuff-type Doppler ultrasonic flow probe (Iowa Doppler Products, Iowa City, IA) was positioned around the ascending aorta as previously described in mice (29). The stimulating and flow probe wires were tunneled subcutaneously and exteriorized at the back of the neck. Subsequently, ECG electrodes (DataSciences International, Small Gauge Lead Coupler Kit: 276–0065-001) were sutured with 6.0 silk subcutaneously in a modified lead II configuration, tunneled subcutaneously, and exteriorized at the back of the neck as previously described in mice (29, 30). At least 10 days were allowed for recovery. During the recovery periods, the mice were handled, weighed, and acclimatized to the laboratory and investigators.

**Experimental Procedures**

Conscious, unrestrained mice were studied in their home cages (standard mouse polycarbonate cage, 17 cm width × 27 cm length × 12 cm height) during the light cycle for all experiments. Cardiac output and the ECG were recorded by taping the leads to single-stranded stainless-steel wires from a miniature fluid and electric swivel (Alice King Chatham Medical Arts, Hawthorne, CA). The atrial or ventricular pacing leads were connected and secured with tape to teflon-coated stainless steel wire electrodes (0.003 in., part no. 316 SS 7/44T, Medwire, Mount Vernon, NY). The ECG signals were initially amplified (1,000 times) with a Grass P5 11 differential preamplifier and high-impedance probe (HIP 511GA, Grass Instruments, Quincy MA). The low- and high-pass filters were set at 0.3 Hz and 10 kHz. Cardiac output was recorded by connecting the pulsed Doppler flow probe wires to a multichannel ultrasonic flow-dimension system with 20-MHz high-velocity modules (Baylor College of Medicine). The Doppler flow-dimension system measures blood flow velocity in kilohertz of Doppler shift, which is directly proportional to absolute blood flow as determined with an electromagnetic system (23). The temperature within the cage was monitored and maintained near the thermoneutral zone for mice of approximately 29–31°C (41) by use of a circulating water pad under the cage and a Presto HeatDish Plus Parabolic Heater. Mice were allowed to adapt to the laboratory environment for ~2 h to ensure stable hemodynamic conditions.

After the stabilization period, beat-by-beat, steady-state heart rate, cardiac output, and ECG parameters were recorded over 10–15 s. Subsequently, the atrioventricular interval (AV interval), sinus cycle length (SCL), sinus node recovery time (SNRT), SNRT corrected for spontaneous sinus cycle length (cSNRT), and Wenckeback Cycle Length (WCL) were determined as previously described in anesthetized mice (5). Briefly, the PowerLab stimulator delivered current via the leads attached to the atrial appendage. The current was recorded via an amp meter (Radio Shack, 22–805) in series with the atrial appendage stimulating electrode. Atrial pacing thresholds were determined and stimulation was performed for 1.0-ms pulse widths at the capture current (0.002 μA).

The AV interval, SCL, SNRT, and cSNRT were determined during atrial pacing at a frequency of 8.4, 10, and 11.6 Hz for ~30-s durations (5). The AV interval was measured as the time from the last paced stimulus to the onset of the QRS complex (Fig. 1, AV interval). The SNRT was measured as the time from the last paced stimulus to the onset of the P wave (Fig. 1, SNRT). To control for differences in sinus rate, SNRT was normalized to resting heart rate by subtracting the SCL from SNRT (cSNRT = SNRT − SCL). SCL was determined from at least 60 consecutive cycles before the pacing period. A period of at least 60 s was allowed to elapse between each successive pacing.

The WCL was determined during incremental increases in atrial pacing frequency performed for 1.0-ms pulse widths at twice the capture current. The WCL was defined as the minimum cycle length...
that fails to conduct through the AV node as indicated by missed ventricular contractions. Missed ventricular contractions were detected by both the ECG and cardiac output waveform (Fig. 2A). The WCL is an index of AV nodal conduction where increases in the WCL represent depressions in AV nodal conduction and decreases in WCL represent enhancements in AV nodal conduction.

On an alternate day (>48 h later), the ventricular refractory period and the electrical stimulation threshold to induce sustained ventricular tachyarrhythmias were determined as previously described in conscious rats (39, 40).

Briefly, the Grass SD9 stimulator delivered current via the leads attached to the left ventricle. The current was recorded via an ammeter (Radio Shack, 22–805) in series with the ventricular stimulating electrode. Ventricular pacing thresholds were determined and stimulation was performed for 1.0-ms pulse widths at twice the capture current. The ECG was monitored and sent through a window discriminator. The window discriminator was fitted with a switch that allowed every 10th R-wave to trigger the Grass SD9 stimulator, sending one pulse through the ventricular stimulating electrodes. The delay on the stimulator allows the R wave-stimulus interval to be progressively shortened. The ventricular refractory period represents the shortest R wave-stimulus interval capable of generating a cardiac response. In Fig. 3A, the stimulus was within the refractory period and did not depolarize the heart. In contrast, in Fig. 3B, the stimulus was outside the refractory period and depolarized the heart. Accordingly, the next sinus complex entered the ventricle during the ventricular refractory period and resulted in a compensatory pause until the next sinus complex entered the ventricle.

The electrical stimulation threshold to induce sustained ventricular tachyarrhythmias was determined as previously described in conscious rats (39, 40). Briefly, a PowerLab stimulator delivered trains of pulses through the ventricular stimulating electrodes at a frequency of 50 Hz and duration of 10 ms. The current delivered was recorded via an ammeter (Radio Shack, 22–805) in series with the ventricular stimulating electrode. The intensity of the trains was increased in 2-μA increments every second. The electrical stimulation threshold to induce sustained ventricular

![Fig. 2. Analog recording of cardiac output, the electrocardiogram (ECG), and the electrical stimulus voltage sent through the atrial electrodes (Stimulus), during the protocol to measure the Wenckebach cycle length (WCL) in an intact conscious mouse. WCL was defined as the minimum cycle length (maximum frequency) of atrial pacing that produced atrioventricular (AV) node Wenckebach phenomenon. The frequency of atrial stimulation was increased in 0.1-Hz increments until the stimulation failed to conduct through the AV node. During a period of atrial stimulation at 14.4 Hz or 69.44 ms (A), the stimulus was successfully conducted to the ventricle as indicated by the cardiac output and ECG tracings. However, during a period of atrial pacing at 14.5 Hz or 68.97 ms (B), the AV node was unable to conduct all of the pulses. In this animal, the WCL was 69.44 ms.](http://ajpheart.physiology.org.org)
period (23.25 ms) and did not depolarize the heart. In contrast, in sinus rhythm appears upon termination of the stimulation without plexes with concomitant absence of cardiac output (Fig. 4). Normal sustained ventricular tachycardia (39, 40). Ventricular tachyarrhythmias was determined as the minimum current causing refractory period represents the shortest R wave-stimulus interval capable of to trigger a Grass SD9 stimulator. The delay on the stimulator allows the window discriminator was fitted with a switch that allowed every 10th R-wave windowing to the physiological range. Adequacy of ventilation was determined at the end of the experiment in a subset of animals via analysis of blood gases (Opti CCA T/S Blood Gas Analyzer, Global Medical Instrumentation, Ramsey, MN). The surgical instrumentation and experimental protocols were conducted as described above for the conscious animals. The level of anesthesia was adjusted for each animal to completely inhibit corneal reflexes and the withdrawal response to firm toe pinch.

**Preparation of Heart Sections**

Following the completion of the chronic studies in five mice, the hearts were excised under deep anesthesia and processed as recently described (30). The heart was quickly rinsed in 10 mM Tris, 0.9% NaCl, 0.05% thimerosal in 10 mM phosphate buffer, pH 7.4 (TPBS), then immersion fixed in formaldehyde/zinc fixative for 60 min, washed in TPBS (3 × 10 min), then cryoprotected overnight in 30% sucrose (prepared in half-strength TPBS). The left and right atrial appendages were removed, and the appendages and ventricles were embedded in OCT compound. The ventricles were sliced transversely from the apex to the base (short axis) at 10-μm thickness with the use of a cryostat. An interval of 300 μm was maintained between the ventricular sections. Similarly, the atrial appendage was sliced distal to proximal (short axis) at 10-μm thickness. No interval was maintained between the atrial sections. All sections were thaw mounted on Superfrost Plus slides (30) and stained with Masson trichrome for quantitative analysis of tissue injury from the implanted leads.

**Data Analysis**

All physiological recordings were sampled at 4 kHz, and the data were expressed as means ± SE. The final values for AV interval, SNRT, and cSNRT are the maximum value obtained during atrial pacing at frequencies of 8.4, 10, and 11.6 Hz. These frequencies were chosen for two reasons. First, these frequencies are within the physiological range (500, 600, and 700 beats/min). Second, these frequencies are within the range of previous studies with anesthetized animals [6.67 (400 beats/min), 8.3 (500 beats/min), and 10 Hz (600 beats/ min)] (5).

The maximum value obtained during atrial pacing at frequencies of 8.4, 10, and 11.6 Hz was chosen because of the pioneering work of Berul and colleagues (5). These investigators reported the maximum value from all three pacing drives in the calculations of SNRT because this approach is analogous to the methods used in human studies (33).

Cardiac output was evaluated using an ultrasonic range-gated pulsed Doppler flowmeter (21). Blood flow (Q) (in ml/min) was calculated by Q = Doppler shift frequency (in kHz) (AV interval, SNRT, and cSNRT are the maximum value obtained during atrial pacing at frequencies of 8.4, 10, and 11.6 Hz) by 10.220.33.6 on June 25, 2017 http://ajpheart.physiology.org/ Downloaded from

**RESULTS**

The protocol to measure AV interval and SNRT in an intact conscious mouse is shown in Fig. 1. Specifically, Fig. 1 is an original recording of cardiac output, the electrocardiogram (EGC), and the electrical stimulus voltage sent through the atrial appendage electrodes (Stimulus). The AV interval was measured as the time from the last paced stimulus to the onset of the QRS complex (AV). The SNRT was measured as the time from the last paced stimulus to the onset of the P wave (SNRT).

Nine additional adult, male C57BL/6 mice (31.4 ± 1.2 g) were studied to determine effects of acute anesthesia and surgical trauma on cardiac electrophysiology parameters. The mice were anesthetized with a mixture of pentobarbital and ketamine (0.033 mg/g each, ip) (5). Animals were intubated orally and artificially ventilated with a small-animal ventilator (SAR-1000; CWE, Ardmore, PA). The mice were placed on a feedback-based temperature control system (model no. 40–90-8; FHC, Bowdoin, ME) for monitoring and maintaining body temperature within the physiological range. Adequacy of ventricle. In this example the effective refractory period was 23.5 ms.

tachyarrhythmias was determined as the minimum current causing sustained ventricular tachycardia (39, 40). Ventricular tachycardia was identified on the electrocardiogram as rapid, wide QRS complexes with concomitant absence of cardiac output (Fig. 4). Normal sinus rhythm appears upon termination of the stimulation without the use of defibrillation shocks.

**Effects of Anesthesia and Surgical Trauma**

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Figure 2 presents the protocol to measure the WCL in an intact conscious mouse. Specifically, Fig. 2 is an original recording of cardiac output, the ECG, and the electrical stimulus voltage sent through the atrial appendage electrodes (Stimulus). The frequency of atrial stimulation was increased in 0.1-Hz increments until the stimulation failed to conduct through the AV node. During a period of atrial stimulation at 14.4 Hz or 69.44 ms (Fig. 2A), the stimulus was successfully conducted to the ventricle as indicated by the cardiac output and ECG tracings. However, during a period of atrial pacing at 14.5 Hz or 68.97 ms (Fig. 2B), the AV node was unable to conduct all of the pulses. In this animal, the WCL was 69.44 ms.

The ventricular refractory period was measured in intact conscious mice using the protocol shown in Fig. 3. Specifically, cardiac output and the ECG were recorded. The ECG was sent through a window discriminator. The window discriminator was fitted with a switch that allowed every 10th R-wave to trigger a Grass SD9 stimulator. The delay on the stimulator allows the R-wave-stimulus interval to be progressively shortened. The ventricular refractory period represents the shortest R wave-stimulus interval capable of generating a cardiac response. In Fig. 3A, the stimulus was within the refractory period (23.25 ms) and did not depolarize the heart. In contrast, in Fig. 3B, the stimulus was outside the refractory period and depolarized the heart. Accordingly, the next sinus complex entered the ventricle during the ventricular refractory period and resulted in a compensatory pause until the next sinus complex entered the ventricle. In this example the effective refractory period was 23.5 ms.

The protocol to determine the electrical stimulation threshold to induce sustained ventricular tachycardia in intact conscious mice is shown in Fig. 4. Trains of pulses (frequency 50 Hz and duration of 10 ms), started at the dotted line and recorded by the ECG electrodes, were delivered through the ventricular stimulating electrodes. The current delivered was recorded via an Amp meter in series with the stimulating electrodes. The intensity of the trains was increased every 1 s in ~2-μA increments. Ventricular arrhythmia was identified by both the electrocardiogram as rapid, wide QRS complexes and a decrease in cardiac output. In this animal, the threshold current required to induce ventricular tachyarrhythmias was 104 μA. Normal sinus rhythm reappears upon termination of the stimulation without the use of defibrillation shocks (data not shown).
Resting cardiac output and heart rate, for the 13 intact, conscious mice, and the 9 open-chest, anesthetized mice are presented in Fig. 5. Anesthesia and surgical trauma were associated with a significantly lower cardiac output and heart rate.

Anesthesia and surgical trauma were also associated with significantly altered cardiac electrophysiology parameters. The cardiac electrophysiology parameters (AV interval, SCL, SNRT, cSNRT, and WCL) for the 13 intact, conscious mice, and the 9 open-chest, anesthetized mice are presented in Fig. 6. Anesthesia and surgical trauma significantly increased AV interval, SCL, SNRT, and WCL.

Anesthesia and surgical trauma also significantly increased the ventricular effective refractory period and virtually eliminated the ability to induce sustained ventricular arrhythmias (Fig. 7). Specifically, anesthesia and surgical trauma were associated with a more than doubling of the VERP (Fig. 7A) and virtually an inability to induce sustained ventricular tachycardia (Fig. 7B). This is consistent with the pioneering work of Maguire and colleagues (32). Specifically, these pioneers documented that the inducibility of ventricular tachycardia is markedly different between the strain and age of mice and is most powerfully correlated with the ventricular effective refractory period.

Placement of the ventricular stimulating electrodes produced minor tissue injury as documented by the photomicrographs of ventricular sections from one chronically instrumented mouse heart (Fig. 8). The 10-μm sections were taken from the apex through the base (short axis) of the left ventricle at 300-μm intervals and processed with Masson trichrome stain. The collagen (i.e., blue stain) documents minor tissue injury due to placement of the ventricular stimulating electrodes since no blue is shown on hearts without ventricular stimulating wires (29, 30).

![Fig. 5](image)

![Fig. 6](image)

![Fig. 7](image)
Similarly, placement of atrial appendage stimulating electrodes produced minor tissue injury as documented by the photomicrographs of sections from the right (not instrumented) and left (instrumented) atrial appendages from one chronically instrumented mouse heart (Fig. 9). The 10-μm sections were taken from the apex through the base (short axis) of the appendages at 10-μm intervals and processed with Masson trichrome stain. The collagen (i.e., blue stain) documents minor tissue injury due to the placement of the atrial appendage stimulating electrodes.

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Fig. 8. Photomicrographs of ventricular sections from one chronically instrumented mouse heart. The 10-μm sections were taken from the apex through the base (short axis) of the left ventricle at 300-μm intervals and processed with Masson trichrome stain to determine the extent of epicardial surface damage. The collagen (i.e., blue stain) documents minor tissue injury due to placement of the ventricular stimulating electrodes.

Fig. 9. Photomicrographs of sections from the left atrial appendage showing electrode placement (A, B, and C) and from the right atrial appendage with no electrode placement (D, E, and F). The 10-μm sections were taken from the apex through the base (short axis) of the appendages at 10-μm intervals and processed with Masson trichrome stain to determine the extent of atrial appendage surface damage. The collagen (i.e., blue stain) documents minor tissue injury due to the placement of the atrial appendage stimulating electrodes. A comparison of the left and right atrial appendages (electrode implantation vs. no implantation, respectively) provides an approximation of the extent of the atrial appendage surface damage.
injury due to the placement of the atrial stimulating electrodes. A comparison of the right (not instrumented) and left (instrumented) atrial appendage provides an approximation of the extent of the injury.

DISCUSSION

This study documents the determination of cardiac electrophysiology parameters, and the susceptibility to sustained ventricular tachyarrhythmias in intact, conscious mice (Figs. 1–4). The procedures and protocols are straightforward and measure parameters similar to those routinely recorded in conscious humans to evaluate arrhythmias and disorders of cardiac conduction. The procedures conducted in conscious C57BL/6J mice, a strain commonly used in transgenic studies, can be utilized in genetically modified models to enhance our understanding of single gene defects and their electrophysiological phenotypes in intact, conscious animals (10, 11, 15, 18, 43).

Furthermore, the cardiac electrophysiology protocols can be initiated in the conscious state after the resolution of the inflammation that occurs during the initial surgical preparation. This is an important consideration because anesthesia and surgical trauma significantly altered resting cardiac output and heart rate (Fig. 5) as well as altered the cardiac electrophysiology parameters (Figs. 6 and 7). Most notable is the more than doubling of the VERP and a dramatic decrease in the ability to induce sustained ventricular tachycardia (Fig. 7). This is consistent with the pioneering work of Maguire and colleagues (32) who documented that the inducibility of ventricular tachycardia is powerfully correlated with the ventricular effective refractory period.

Until relatively recently (42), it was believed that ventricular tachyarrhythmias were impossible in the adult mouse heart with a ventricular surface area less than 100 mm² (12, 17, 35, 44). However, Vaidya and colleagues (42) demonstrated, for the first time, that the normal Langendorff perfused mouse heart is capable of sustained ventricular arrhythmias. The authors documented that the induction of ventricular arrhythmias in the mouse heart was difficult, variable, and dependent on the electrode position, stimulus strength, and pacing frequency. Similarly, Maguire and colleagues (32) could induce ventricular tachycardia with programmed electrical stimulation in only 1 of 41 C57 mice of all ages. Furthermore the ventricular tachycardia was nonsustained and of very short duration. This difficulty and variability may be due, in part, to the reliance on models that may not mimic the clinical or physiological situation. Specifically, isolated hearts are devoid of humoral influences and neuronal regulation, and most often use a hemoglobin-free perfusate which requires an unphysiologically high arterial oxygen tension (16) and coronary perfusion rate (3). Furthermore, because the perfusate is devoid of many of the vital components of blood, the crystalloid perfused hearts exhibit significantly more edema than blood-perfused hearts (13, 37), and increased edema alters myocardial function and contributes to ultrastructural damage (2). Similarly, anesthesia and surgical trauma alter cardiac electrophysiology (Figs. 6 and 7). In sharp contrast, in the intact, conscious mouse, the induction of sustained ventricular tachycardia is consistent, and the current strength can be measured for comparison of arrhythmia susceptibility with interventions (39, 40). Accordingly, this report provides a resource for investigators using wild-type mice or available spontaneous or engineered mouse mutants, for the study of cardiac electrophysiology parameters and arrhythmia susceptibility.

Limitations

It is important to note that mice may not reflect human cardiovascular physiology as closely as larger mammals because electrophysiology properties are species dependent as a result of differences in heart size, body mass, oxygen consumption, and heart rate (4). Accordingly, extrapolation of mouse data to the human physiological condition may be limited; and the murine model may best be used to establish “proof of concepts” that will need to be confirmed in other models that more closely approximate human physiology (30). However, mice are and will continue to be an important model for human cardiovascular research and will be essential for progress in understanding cardiovascular function in health and disease.

Furthermore, the mouse has significant advantages over other experimental models for the investigation of electrophysiological properties. Specifically, the mouse is readily available, inexpensive, has a high throughput, and the ability to create genetically modified models.

Another potential limitation is the tissue damage imposed by implantation of the stimulating wires. Histology of atrial and ventricular tissue documents mild tissue injury. It is possible that the tissue injury explains, in part, differences between conscious and anesthetized data.

Perspectives

Cardiac electrophysiological testing, in humans, occurs in the absence of anesthetic agents and surgical trauma, and the hearts are perfused with blood. Furthermore, in this conscious, complex setting, regulated systems, feedback controls, and redundant control mechanisms dominate. Accordingly, investigations of cardiac electrophysiology must be conducted in conscious, complex models with multiple systems and regulatory strategies to fully appreciate the physiological context (8, 26). Currently, in mice, these investigations are mainly performed under anesthesia; and thus the facilitation of translational aspect of the findings are limited because anesthesia alters these regulatory mechanisms (20). This paper addresses these concerns by describing the application of existing technology, surgical techniques and experimental protocols for the study of cardiac electrophysiology and arrhythmia susceptibility in chronically instrumented, intact, conscious mice. Investigators may be encouraged to adopt these existing procedures to their investigations of cardiac electrophysiology since highly reliable data can be obtained in mice under physiological conditions.

It is important to acknowledge that the data gathered from experiments performed at many levels, from molecular to human, have been and will be critical for understanding cardiac electrophysiology (30). Accordingly, a wide range of investigations, rather than a single model of cardiac electrophysiology, is required (25). In this context, the conscious mouse provides an additional tool for understanding cardiac electrophysiology (36).

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CONSCIOUS MURINE MODEL OF CARDIAC ELECTROPHYSIOLOGY

H1221

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AUTHOR CONTRIBUTIONS
Author contributions: H.L.L. and S.E.D. performed experiments; H.L.L. and S.E.D. analyzed data; H.L.L. and S.E.D. interpreted results of experiments; H.L.L. and S.E.D. prepared figures; H.L.L. and S.E.D. edited and revised manuscript; H.L.L. and S.E.D. approved final version of manuscript; S.E.D. drafted manuscript.

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