Subdiaphragmatic murine electrophysiological studies: sequential determination of ventricular refractoriness and arrhythmia induction

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Gutstein, David E., Stephan B. Danik, Jedd B. Sereisky, Gregory E. Morley, and Glenn I. Fishman. Subdiaphragmatic murine electrophysiological studies: sequential determination of ventricular refractoriness and arrhythmia induction. Am J Physiol Heart Circ Physiol 285: H1091–H1096, 2003.—Programmed electrical stimulation (PES) is a crucial aspect of the evaluation of the risk of arrhythmias in cardiac patients and provides a powerful tool for understanding the mechanisms of arrhythmia in experimental models. Whereas PES in the mouse is well characterized, the procedures allowing for follow-up studies in the same animal have not been developed. In this report, we describe a novel subdiaphragmatic approach that allows for repeat electrophysiological studies in the mouse. Under inhaled anesthesia, PES was performed in 36 wild-type mice via a stimulating electrode introduced through an epigastric incision and placed directly into the diaphragmatic surface of the heart. The procedure was repeated 7 days later. Ventricular effective refractory periods (VERP) did not change significantly between the initial and follow-up trials. Chronic treatment with amiodarone, however, was associated with a 70% prolongation in VERP from initial to follow-up studies (P < 0.001). In addition, PES of a genetically modified strain with sudden cardiac death, the connexin43 conditional knockout mouse consistently induced lethal polymorphic ventricular tachycardia. Thus sequential PES in mice is feasible with the use of a subdiaphragmatic approach, yields reproducible VERP values, and can be used to follow pharmacologically induced changes in VERP and identify mice at risk of lethal ventricular arrhythmias.

MATERIALS AND METHODS

Study preparation. Thirty-six nine-month-old male wild-type 129/Sv mice were used for this study. All studies were performed in accordance with the regulations of the Institutional Animal Care and Use Committees of the New York University School of Medicine and the Veterans Administration New York Harbor Healthcare Medical Center (New York, NY). In preparation for electrophysiological study, hair was removed from the epigastric region with a depilatory (Nair, Church & Dwight; Princeton, NJ). The mice were anesthetized with isoflurane (1.5 vol%; Baxter; Deerfield, IL) and immobilized on a heating pad set to 37°C. ECG recordings. Electrocardiographic signals were amplified with a Honeywell ECG amplifier (Honeywell; Morristown, NJ), converted from analog to digital with an ACQ-16 Acquisition Interface and recorded with Ponemah Physiology Platform software (Gould; Valley View, OH). Electrocardiographic intervals were calculated from limb leads recorded at the beginning of each study. QRS amplitude was determined from lead II.

Electrophysiological study. After the electrocardiogram was recorded, a 1-cm midline incision was made in the epigastric region. A custom-designed cardiac stimulating electrode with a 200-μm monopolar platinum tip (model UE-GM1, Haer; Bowdoinham, ME) mounted on a micromanipulator (Fine Science Tools; N. Vancouver, BC, Canada) was inserted through the diaphragm directly into contact with the apical surface of the heart (Fig. 1). Electrode contact was ensured by monitoring of the electrocardiographic activity recorded from the stimulating electrode.

PES was performed with a Programmable Stimulator (model 2352, Medtronic; Minneapolis, MN). Output was set for programmed electrical stimulation (PES) to evaluate the propensity of genetically altered mice to develop complex ventricular arrhythmias. However, these protocols for PES in the mouse have been described for use only as isolated studies and are not amenable to longitudinal examination. We have developed a procedure for PES in mice that allows for repeat studies, making it possible to follow progressive changes in electrophysiological parameters resulting from genetic, pharmacological, or surgical manipulation in individual animals. Moreover, this novel procedure reliably identifies mice at risk for lethal ventricular arrhythmias.

SUDDEN CARDIAC DEATH STRIKES an estimated 400,000 Americans each year and is a major cause of mortality throughout the world (21). Because of the difficulties associated with identifying the majority of patients at risk, the expense of implantable cardiac defibrillators and the lack of ideal pharmacological therapies, identification of genes involved in sudden arrhythmic death, and the development of new preventive strategies are of paramount importance (7, 11, 16). To identify such target genes, several groups (5, 12, 14, 15) have used in vivo electrophysiological testing with programmed electrical stimulation (PES) to evaluate the propensity of genetically altered mice to develop complex ventricular arrhythmias. However, these protocols for PES in the mouse have been described for use only as isolated studies and are not amenable to longitudinal examination. We have developed a procedure for PES in mice that allows for repeat studies, making it possible to follow progressive changes in electrophysiological parameters resulting from genetic, pharmacological, or surgical manipulation in individual animals. Moreover, this novel procedure reliably identifies mice at risk for lethal ventricular arrhythmias.

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at twice the stimulating threshold in all animals. Pulse width was 1.0 ms for all studies. PES consisted of pacing with a train of eight beats, followed by a single extrastimulus for the determination of ventricular effective refractory period (VERP). This protocol was repeated at pacing cycle lengths of 120, 100, and 80 ms. Double extrastimuli were added at each cycle length to test for inducible arrhythmias. Induced non-sustained ventricular tachycardia was defined as a ventricular arrhythmia consisting of at least three beats and lasting up to 30 s. Sustained ventricular tachycardia was defined as that lasting >30 s (19).

After pacing at the first site, the stimulating electrode was withdrawn by using the micromanipulator along one axis, repositioned 1 mm laterally, and reinserted through the diaphragm, and the pacing protocol was repeated. VERP was calculated as the average from the two sites and was determined for each cycle length. After the initial pacing study, the electrode was removed and the peritoneum and skin were sutured closed. The animals were observed for 7 days, when the electrophysiological study was repeated. After the repeat study, the animals were euthanized.

To test whether the subdiaphragmatic PES technique could detect time-dependent changes in electrophysiological parameters, we performed electrophysiological studies before and after treatment with amiodarone (Wyeth-Ayerst; Philadelphia, PA) in a cohort of male wild-type 129/Sv mice. For this substudy, 12 mice were loaded with 100 mg/kg of amiodarone, followed by 25 mg·kg⁻¹·day⁻¹ amiodarone diluted in sterile water with 5% dextrose (D5W) and injected IP for 2 wk. Nine matched controls were injected with an equal volume of D5W daily. Baseline electrocardiograms and electrophysiological studies as described above were performed before and immediately after courses of treatment.

To test the efficacy of this protocol in the induction of lethal ventricular arrhythmias, we performed PES via the subdiaphragmatic approach in three male connexin43 cardiac-specific conditional knockout mice (Cx43 CKO; α-MHC-Cre: Cx43flo/flox) and three male Cx43fl/o littermate controls (9).

Statistics. Data are expressed as means ± SE. All values from trials 1 and 2 were compared with a two-tailed paired t-test (except for induced arrhythmias, which were compared with a χ² test) using Microsoft Excel software. P < 0.05 was considered statistically significant.

RESULTS

Subdiaphragmatic approach for electrophysiological study is well tolerated. In a pilot experiment, three wild-type mice survived for several weeks after electrophysiological testing from the subdiaphragmatic approach without any obvious impairment. The procedure was well tolerated, with no gross disruption of the diaphragm or respiratory distress during or immediately after the procedure. All animals awoke within minutes of cessation of anesthesia and were ambulating normally shortly thereafter. On the basis of the pilot study, we tested whether measurable changes in electrophysiological parameters occurred within 1 wk of the initial study.

We studied 36 mice; all tolerated the initial PES protocol (trial 1) and underwent follow-up testing (trial 2). There was no difference in the weight of the animals from trial 1 to trial 2. Mean weight before trial 1 was 29.8 ± 0.5 g versus 30.3 ± 0.4 g before trial 2 (P = not significant). There was no gross damage to the heart or visible pericardial blood on postmortem examination. To determine whether microscopic damage resulted from subdiaphragmatic PES, hearts from a subset of six animals were removed on death after follow-up electrophysiological testing, fixed in 10% formalin, and embedded in paraffin for histological evaluation. No evidence of fibrosis or intramyocardial hemorrhage was seen on hematoxylin-phloxine-saffron (HPS)-stained histological sections from these hearts (Fig. 2).
Electrocardiographic intervals remain stable between initial and follow-up studies. To evaluate the stability of measurements afforded by repeat electrophysiological study with this approach, we measured basic electrocardiographic indexes before each study from six-lead electrocardiograms (Fig. 3). Electrocardiographic indexes, including P wave duration, PR interval, QRS duration, R-R interval, and QRS amplitude from each mouse in the two separate trials, were compared pairwise. Given the difficulty identifying the murine QT interval (20), a pairwise comparison was possible in only 9 of 36 mice studied. As shown in Table 1, there were no significant differences in any of the measured parameters between the initial and follow-up trial. Because of the lack of correlation between the end of the T wave and complete ventricular repolarization in the mouse (6), however, we regard the VERP as a more reliable measure of ventricular repolarization than the QT interval.

Electrophysiological indexes are reproducible on follow-up testing. To establish the reproducibility of electrophysiological parameters from initial to follow-up testing, we compared the stimulating thresholds and VERP values obtained from initial and repeat trials in the 129/Sv mice. Stimulating threshold was calculated as the mean threshold obtained from two separate pacing sites per trial. PES consisted of a train of eight paced beats, followed by single extrastimuli, from which VERP was derived (Fig. 4). Electrophysiological indexes, including both stimulating thresholds and VERP at pacing cycle lengths of 120, 100, and 80 ms, were unchanged from trial 1 to trial 2 (Table 2).

Self-terminating ventricular arrhythmias are induced in a minority of wild-type mice. Because PES can be a powerful trigger for the induction of sustained arrhythmias, we monitored our study population carefully for ventricular ectopy induced by the pacing protocol. The electrophysiological testing protocol included both single and double extrastimuli for all pacing rates at two different sites in all animals. As expected, no sustained or nonsustained supraventricular tachycardias were induced. Ventricular tachycardia was induced in 19 of 72 trials (26.4%; 72 trials included 36 initial and 36 follow-up trials). The propensity toward induced arrhythmias was not significantly different in the initial trials compared with the follow-up trials. Induced arrhythmias were seen in 9 of the 36 initial trials (25.0%) and in 10 of the 36 follow-up trials (27.8%; P = 0.79). Ventricular tachycardia was reproducible in both trials in only 5 of the 14 mice in which an arrhythmia was induced. Of the induced ventricular arrhythmias, 18 were nonsustained and one was sustained. The nonsustained runs of ventricular tachycardia averaged 19.5 ± 2.9 beats (range 3–42 beats), with a mean cycle length of 48.2 ± 1.6 ms, corresponding to a mean duration of <1 s per run of nonsustained ventricular tachycardia. In one mouse, a 60-s run of self-terminating ventricular tachycardia with an average cycle length of 48.0 ms was induced during trial 1 but no ventricular tachycardia was induced on follow-up testing.

Table 1. Electrocardiographic data in wild-type mice from two sequential trials

<table>
<thead>
<tr>
<th></th>
<th>Trial 1</th>
<th>Trial 2</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>P wave duration, ms</td>
<td>23.0 ± 0.6</td>
<td>22.8 ± 0.6</td>
<td>0.73</td>
</tr>
<tr>
<td>PR interval, ms</td>
<td>38.8 ± 0.8</td>
<td>39.2 ± 0.4</td>
<td>0.54</td>
</tr>
<tr>
<td>QRS duration, ms</td>
<td>11.2 ± 0.2</td>
<td>11.4 ± 0.2</td>
<td>0.31</td>
</tr>
<tr>
<td>R-R interval, ms</td>
<td>133.2 ± 3.9</td>
<td>131.7 ± 2.5</td>
<td>0.68</td>
</tr>
<tr>
<td>QRS amplitude, μV</td>
<td>101.6 ± 7.2</td>
<td>88.7 ± 5.9</td>
<td>0.06</td>
</tr>
<tr>
<td>QT interval, ms</td>
<td>80.2 ± 3.0</td>
<td>78.7 ± 5.3</td>
<td>0.66</td>
</tr>
</tbody>
</table>

Values are group means ± SE. Paired statistical analyses of individual mice were used to determine significance between trial 1 and trial 2; n = 35 for all measurements except QT interval, which was detectable in both pretrial electrocardiograms in only 9 mice. There were no significant differences in any of the above comparisons.
Table 2. Electrophysiological data in wild-type mice from two sequential trials

<table>
<thead>
<tr>
<th></th>
<th>Trial 1</th>
<th>Trial 2</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stimulating threshold, µA</td>
<td>208 ± 6</td>
<td>210 ± 4</td>
<td>0.75</td>
</tr>
<tr>
<td>VERP, ms</td>
<td>40.2 ± 1.3</td>
<td>39.8 ± 1.0</td>
<td>0.79</td>
</tr>
<tr>
<td>VERP100, ms</td>
<td>41.8 ± 1.2</td>
<td>40.0 ± 0.9</td>
<td>0.17</td>
</tr>
<tr>
<td>VERP90, ms</td>
<td>42.7 ± 1.2</td>
<td>42.2 ± 1.0</td>
<td>0.68</td>
</tr>
</tbody>
</table>

Values are group means ± SE. VERP, ventricular effective refractory period (80, 100, and 120 ms). Paired statistical analyses of individual mice were used to determine significance between trial 1 and trial 2; n = 36 for all measurements. There were no significant differences in the above comparisons.

Subdiaphragmatic PES is useful for following pharmacologically induced changes in ventricular repolarization. To investigate whether the subdiaphragmatic PES technique could be used longitudinally to follow pharmacologically induced changes in electrophysiological parameters, we studied a series of mice before and after a course of treatment with amiodarone. After initial studies, chronic amiodarone treatment was initiated in 12 mice. Four of the mice died within 3 days of initiation of treatment, whereas the remainder survived to follow-up studies after 2 wk of daily amiodarone administration. All of the nine matched controls (injected with D5W daily) survived to follow-up studies. Treatment with amiodarone was associated with significant prolongations in QRS duration and RR interval on follow-up testing (Table 3). In addition, VERP at three different pacing cycle lengths (80, 100, and 120 ms) was markedly prolonged after treatment with amiodarone (P ≤ 0.001). There were no significant changes in any of the tested electrocardiographic or electrophysiological parameters in the matched controls from initial to follow-up studies (data not shown). Only one of the control mice had pacing induced nonsustained ventricular tachycardia during initial studies and none of the mice had induced arrhythmias on follow-up testing.

Subdiaphragmatic PES induces lethal ventricular arrhythmias in Cx43 CKO mice. To determine the predictive value of the subdiaphragmatic approach for PES in mouse models of arrhythmic sudden death, we tested this procedure on three cardiac-specific Cx43 CKO mice (9). All three mice were induced with either single or double extrastimuli into sustained ventricular tachyarrhythmias, which degenerated into ventricular fibrillation and ultimately asystole despite attempts at pace termination (Fig. 5). None of the three littermate controls was induced into sustained arrhythmias. Induced arrhythmias were polymorphic and incessant in the Cx43 CKO mice, in contrast to the wild-type 129/Sv mice, in which induced arrhythmias appeared monomorphic and were all self-terminating.

DISCUSSION

In this study, we present a protocol that allows, for the first time, the performance of repeat electrophysiological testing in the mouse. Previously, electrophysiological testing in the mouse has involved either the jugular transvenous approach, or an open-chest approach in an intubated animal (2, 12). However, neither of these other approaches allows for repeat testing. The right jugular vein must be ligated after a transvenous study, precluding a subsequent transvenous procedure. Electrophysiological testing via sternotomy, while providing the ability to test supraventricular indexes, requires endotracheal intubation, and because of the morbidity involved, should not be applied more than once in the same animal.

Because our approach does not require mechanical ventilation and entails no manipulation of the vasculature in the neck, it may be combined easily with invasive hemodynamics. With the use of the subdiaphragmatic approach, PES may be performed with a hemodynamic catheter in the left ventricle or aorta to monitor pressures during an induced arrhythmia. Because the procedure may be performed in a spontaneously breathing, closed-chest mouse, hemodynamic measurements will not be influenced by positive-pressure ventilation from a mechanical ventilator. Furthermore, the short procedure time allows for high throughput testing.

Table 3. Electrocardiographic and electrophysiological data in wild-type mice treated with amiodarone

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Postamiodarone</th>
<th>P Value</th>
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<tbody>
<tr>
<td>P wave duration, ms</td>
<td>23.4 ± 0.3</td>
<td>24.0 ± 0.7</td>
<td>0.41</td>
</tr>
<tr>
<td>PR interval, ms</td>
<td>40.9 ± 2.6</td>
<td>43.1 ± 1.0</td>
<td>0.32</td>
</tr>
<tr>
<td>QRS duration, ms</td>
<td>10.8 ± 0.4</td>
<td>11.6 ± 0.4*</td>
<td>0.02</td>
</tr>
<tr>
<td>R-R interval, ms</td>
<td>129.9 ± 1.9</td>
<td>150.0 ± 6.2*</td>
<td>0.03</td>
</tr>
<tr>
<td>QRS amplitude, µV</td>
<td>89.2 ± 10.2</td>
<td>82.9 ± 11.0</td>
<td>0.54</td>
</tr>
<tr>
<td>Stimulating threshold, µA</td>
<td>175.0 ± 9.4</td>
<td>200.0 ± 14.4</td>
<td>0.26</td>
</tr>
<tr>
<td>VERP120, ms</td>
<td>33.1 ± 1.7</td>
<td>56.4 ± 3.0†</td>
<td>0.0001</td>
</tr>
<tr>
<td>VERP100, ms</td>
<td>34.6 ± 1.8</td>
<td>58.8 ± 3.7†</td>
<td>0.0003</td>
</tr>
<tr>
<td>VERP90, ms</td>
<td>35.9 ± 2.1</td>
<td>61.4 ± 3.6†</td>
<td>0.0002</td>
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</table>

Values are group means ± SE. Paired statistical analyses were used to determine significance between baseline and postamiodarone trials in individual amiodarone-treated mice (n = 8). QT interval was detectable in an insufficient number of mice to allow for comparison. *P < 0.05; †P < 0.001, compared with matched baseline.
In comparison with other reported protocols for in situ electrophysiological testing, the subdiaphragmatic approach consistently yielded lower values for VERPs with less variability. For example, electrophysiological testing using an open-chest approach in intubated wild-type mice with epicardial leads yielded VERP values as high as 61.0 ± 20.2 ms for the right ventricle and 62.5 ± 19.8 ms for the left ventricle (mean ± SD) (2, 3). VERP values obtained by transvenous PES in wild-type mice ranged from 46.0 ± 5 ms to 63.0 ± 7.6 ms (4, 5, 12, 18). Several factors may account for the differences in VERP values. PACing site is an important factor in the determination of ventricular refractoriness (13), with apical pacing as performed in this study resulting in shorter VERP values than pacing at the base of the heart (1). Technical issues, such as differences in depth of anesthesia, decreased procedure time, or decreased total pacing duration per trial as well as strain differences may also contribute to the difference in VERP results between these protocols. It is worth noting that our values correspond well with monophasic action potential recordings from the epicardial surface of the left ventricle in an open-chest model (6).

Sequential electrophysiological studies in genetically manipulated mice may help to address important issues related to formation of arrhythmogenic substrate. With this method, longitudinal studies can be designed to test the increased arrhythmogenicity associated with the normal growth of the animal or with interventions that cause hypertrophy. Additionally, studies comparing the efficacy of pharmacological interventions can now be performed on genetic models of arrhythmia (12, 14, 15). Studies employing amiodarone are particularly amenable to the subdiaphragmatic approach for PES. Because the prolongation of ventricular repolarization by amiodarone is more pronounced after chronic treatment than acute administration (8, 10, 17), subdiaphragmatic electrophysiological study is an ideal way to follow the time-dependent electrophysiological effects of amiodarone.

A limitation to the subdiaphragmatic approach that we describe is that at present atrial pacing is not possible, and thus supraventricular electrophysiological data cannot be obtained. We are presently redesigning the stimulating electrode to allow for atrial pacing protocols.

In conclusion, we have developed a novel technique for electrophysiological testing in the mouse, which is safe and reproducible and allows for longitudinal investigation in the same mouse. This approach should help in the investigation of mechanisms and treatment of arrhythmias in mouse models of human disease.

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