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Stimulation Along the Vagal Pre and Postganglionic Pathway to Selectively Enhance Vagal Tone to the Heart

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Abstract: *Objectives:* Sympathetic overactivity and vagal withdrawal are cardinal presentations in patients with heart failure that predisposes them to malignant arrhythmias and sudden cardiac death. To develop therapy to enhance vagal tone and regain autonomic balance we conducted stimulation recruitment tests at three locations along the cardiac vagal pathway.

Materials and Methodology: In anesthetized canines, small electrodes were placed against the vagus nerve (n=4) or in epicardial fat containing the sinoatrial or ventricular cardiac ganglia (n=8).

Results: Stimulation of the vagus nerve induced vocal cord evoked potentials at 0.5 mA and increases in heart rate at 1.3 ± 0.3 mA. Higher currents were needed to induce parasympathetic effects of decreased heart rate and blood pressure, 3.7 ± 1.0 mA. Stimulation of the sinoatrial node ganglia decreased heart rate at a threshold current of 4.4 ± 2.1 mA. Stimulation of the ventricular ganglia, produced sympathetic effects at 9.3 ± 0.7 mA of current and ventricular arrhythmias or pacing at 10.7 ± 0.7 mA; using small wire electrodes unwanted sympathetic and ventricular pacing effects occurred at even lower stimulating currents.

Conclusion: Our results show that the effects of stimulation are highly dependent upon the site of stimulation and stimulation currents. Further, given the significant side effects of whole nerve stimulation an important need exists for more selective vagal and cardiac ganglia stimulation technology as offered by microelectrode arrays.

INTRODUCTION

Heart failure leads to a sustained, decreased cardiac output due to compromised ventricular function secondary to ischemia, increased afterload, infection, and other etiological factors. Patients with heart failure present with parasympathetic withdrawal and increased sympathetic activity, autonomic imbalances that predisposes them to cardiac arrhythmias and sudden cardiac death [1,2]. Enhanced vagal tone leads to negative inotropic, chronotropic, and dromotropic effects and is cardioprotective against arrhythmias and sudden death [3]. Further, vagal activity reduces oxidative stress and inflammation whereas sympathetic activity is pro-inflammatory [4]. Thus, enhancing vagal activity will balance autonomic tone and reduce the morbidity and mortality associated with heart failure.

The mechanisms of vagal withdrawal in heart failure have only recently come to light. Bibevski and Dunlap have shown that the site of defective neurotransmission in heart failure is within cardiac ganglia; they found that preganglionic stimulation did not overcome the parasympathetic withdrawal but postganglionic stimulation did, establishing cardiac ganglia as the site for the withdrawal [5]. We have shown that nerve growth factor is increased in heart failure and leads to hypertrophy of cardiac neurons in ganglia. Hypertrophied neurons are less excitable based on the size principal; larger neurons have greater membrane capacitance and decreased input resistance and, thus, they need greater current to reach threshold [2].

Pharmacotherapy for augmenting parasympathetic effects on the heart is not available because of the adverse side effects associated with cholinergic muscarinic agonists [6]. Electrical stimulation of the vagus, though, has shown some promising benefits. Schwartz *et al.* implanted a cuff electrode on the right vagus nerve in heart failure patients and reported improvements in walking distances and NYHA functional class [7,8]. Unfortunately, given that the whole vagus is stimulated there were

significant side effects including unwanted vocal cord activation and sufficient pain and discomfort such that treatments had to be limited to currents below desirable levels and heart rate reduction goals.

Elucidation of effective stimulation sites along the vagal pre and postganglionic pathway as well as optimal stimulation parameters to maximize vagal effects and minimize side effects remains essential Fig. (**1**). Therapuetic goals of vagal stimulation include selective activation of small 'B' fibers producing parasympathetic activation of the heart while avoiding stimulation of sensory, vocal cord, and sympathetic fibers [8-10]. Further, with stimulation of ganglia located on the epicardial surface, it is important to avoid pacing of the heart [11].

With these therapeutic aims, we conducted recruitment tests with small electrodes placed on the vagus nerve or at two distinct populations of cardiac ganglia, one near the sinoatrial node and the other at the base of the great vessels on the ventricular surface Fig. (**1**). Vocal cord evoked potentials and cardiovascular consequences of vagal stimulation were recorded including changes in blood pressure, heart rate, contractility, and atrioventricular conduction.

MATERIALS AND METHODOLOGY

This study was approved by the Institutional Animal Care and Use Committee at the Hines VA Hospital. Terminal studies were conducted in 14-23 kg (mean weight: 17.5 $\pm$ 0.6 kg; n=11) canines of either sex (Oak Hills Genetics, Inc; St. Louis, MO). Anesthesia was initiated with intravenous propofol (3-6 mg/kg, IV to effect) in the cephalic vein prior to intubation; next isoflurane (0.5% to effect) was delivered through an endotracheal tube in combination with continuous IV infusion of fentanyl (5-10 mcg/kg/hr) [12]. During surgery, animals were artificially ventilated to maintain an

end-tidal CO_2 partial pressure of 35 mmHg. Body temperature was maintained at 37^0C and intravenous isotonic saline (0.9%) administered at a rate of 10 ml/kg/h.

Intramuscular barb electrodes were used to record a lead II electrocardiogram (MedWire, Cooner Wire, Inc; Chatsworth, CA). Dromotropic responses to stimulation reflected by the P-R interval (ms) were obtained from the electrocardiogram. A catheter with pressure transducer was placed in the femoral artery for recording arterial blood pressure (SPC-350; Millar; Houston, TX). Heart rate in beats per minute (bpm) was obtained from pressure recordings and displayed as a running average. A 10 cm midline incision was made at the fourth cervical vertebra in the neck and the larynx exposed. Bilateral sets of two small hook electrodes (EMG, Life-Tech, Inc; Stafford, TX) were inserted under the ventrolateral edge of the larynx and into the vocal cords to record laryngeal evoked potentials. An eight-channel data acquisition system (PowerLab, ADI Instruments, Inc; Colorado Springs, CO) was used to record cardiovascular and vocal cord responses.

Testing was conducted at three sites along the vagal pre and postganglionic pathway with different electrode combinations to selectively enhance parasympathetic tone to the heart Fig. (**1**). Since multiple electrodes and test sites were examined, the sample size with any given combination of electrode and test sites was somewhat limited. In ten animals only one test site was studied: in three animals the vagus nerve, in four the sinoatrial node ganglia, and in three the ventricular ganglia. In an eleventh animal, both the vagus nerve and sinoatrial node ganglia were tested.

Vagus Nerve

The vagosympathetic trunk was exposed on the right side of the neck at the fourth cervical level and separated from the fascia surrounding the carotid artery. The nerve was electrically isolated

with a 3 cm wide Silicone insulating pad (McMaster-Carr; Chicago, IL; Fig. (**1**). Three small needle electrodes (surface area: 0.34 mm^2, TECA Model 902-DMF37-TP; CareFusion; San Diego, CA) separated by 5 mm were placed longitudinally on the ventral side of the nerve without penetrating the nerve; the two outer electrodes were positive and middle negative.

Pulse stimulators (S-88, Astromed, Inc; West Warwick, RI) with capacitor-coupled charge balancing were used at 20 Hz frequency and 1 ms pulse duration [12]. Recruitment testing was conducted with stepwise increases in the current from 0.5 mA to 6 mA every 5 to 10 s. Currents were increased until there was a heart rate slowing of 40 bpm. Threshold cardiovascular effects were determined as the first observed sustained increases or decreases in heart rate of 3 bpm or more during recruitment tests. At each stimulation current two to six recruitment tests were conducted in each animal and data averaged (mean $\pm$ SEM).

Sinoatrial Node Ganglia

A 20 cm long incision was made in the skin at the fourth intercostal space on the right side; the pericardium was exposed and a hammock to support the heart made with multiple sutures extending from the pericardium to the chest wall. The sinoatrial node fat pad, which includes ganglia that innervates the sinoatrial node and atrial myocardium, is lateral to the right pulmonary veins Fig. (**1**) [13,14]. Small cylindrical electrodes (0.7 mm diameter, 3.5 mm length, 7.7 mm^2 surface area - Streamline^R Model 6500, Medtronic, Inc; Minneapolis, MN) separated by 5 mm were inserted into the middle of the fat pad with the suture attached to the end of the electrode Fig. (**2**).

Recruitment testing for the sinoatrial node ganglia was similar to that for the vagus nerve with three minor changes. One, shorter pulse durations of 50 μs (n=3) or 100 μs (n=2) were used to limit pacing of the heart [11,13,14]. Two, stepwise increases in current were used in the first three

animals but in the last two, tests were conducted with single-currents. Single stimulation currents were applied for 5 to 10 s with 1 to 2 min rest periods between tests. Three, higher stimulating currents were needed to meet the preestablished criteria of 40 bpm slowing of the heart. Either one or two recruitment tests were conducted in each animal and average values for the pooled data are presented. Cardiovascular changes elicited using the two different pulse durations of 50 and 100 μs, and the two different recruitment methods (stepped ramp or single-current) induced responses in the same physiological range and, therefore, results were averaged.

Ventricular Ganglia

A second pressure catheter inserted through the right carotid artery into the left ventricle was used to measure ventricular contractility reflected by the peak value of the first derivative of the ventricular pressure (dP/dt). Ventricular cardiac ganglia are located at the base of the great vessels on the wall of the left ventricle and surrounded by epicardial fat; these ganglia extend some distance along the left coronary artery Fig. (**1**) [15,16]. The base of the great vessels was exposed after separation of overlying connective tissue and fat. Eight small cylindrical electrodes (Streamline^{R} Model 6500, Medtronic, Inc) were implanted with two sets ventrolateral and two sets dorsolateral to the aorta Fig. (**1**). For each of the four bipolar electrode sets there was 5 mm separation within the set and 5 to 8 mm separation between sets. For each test two stimulators and two bipolar set of electrodes were used. In the first two animals all six possible combinations of two sets of bipolar electrodes were tested. In a third animal, small wire hook electrodes (75 μm diameter wire, 5 mm long, 1.18 mm^2 surface area, EMG, LifeTech, Inc) were implanted at the same locations, but only two combinations of two bipolar sets were tested that included four electrodes in the first test and the remaining four electrodes in the second test. Stimulation parameters were the same as for the

sinoatrial node ganglia and included 100 μs pulse durations and recruitment testing using single-current stimulation.

RESULTS

Vagus Nerve Stimulation

Stimulation of the vagus nerve Fig. (**1**) with small needle electrodes induced cardiovascular responses that depended on the stimulating current as shown in one animal Fig (**3**). Vocal cord activation occurred at the lowest stimulating currents of 0.5 mA (data not shown). Small increases in heart rate and blood pressure occurred with 1.0-2.0 mA. Decreased heart rate and blood pressure first occurred at 4.0 mA and even greater vagal effects occurred at 6.0 mA. When stimulation was turned off there was a strong rebound increase in heart rate and blood pressure suggesting a baroreceptor mediated negative feedback response to the hypotension induced by the vagal stimulation.

Average recruitment data for vagal stimulation are shown in Table Sections **1A**, **2A**, and **2D** (n=4). The threshold for vocal cord evoked potentials occurred at 0.5 mA (**1A**, n=2). At these lower currents significant vocal cord activation occurred suggesting that the actual threshold for vocal cord evoked potentials would have been even lower than 0.5 mA. In a third animal, the lowest current tested was 1.0 mA and this also induced large vocal cord evoked potentials.

Unexpected increases in heart rate with vagal stimulation first occurred at 1.3 ± 0.3 mA in three out of four animals (**1A**); at 2.7 ± 0.3 mA even greater increases in heart rate and blood pressure occurred (**2A**). Given the almost immediate response to stimulation, which is characteristic of vagal rather than sympathetic responses, the increased heart rate may have been due to vagal inhibition mediated by vagal afferent activation of brainstem reflex pathways.

Parasympathetic effects of vagal stimulation occurred in all four animals at threshold currents of 3.7 ± 1.0 mA and included decreases in heart rate and blood pressure (**1A**). At higher stimulating currents (5.3 ± 0.8 mA; **2A**) heart rate decreased even more by 51.3 ± 6.5 bpm and systolic blood pressure decreased by 25.0 ± 5.1 mmHg (**2A**). Dromotropic changes during vagal stimulation were small (**2D**). The mean heart rate during vagal stimulation protocols remained stable between tests at 80.3 ± 8.8 bpm and the mean systolic blood pressure at 99.8 ± 2.3 mmHg.

Sinoatrial Node Ganglia Stimulation

Stimulation in this area Fig (**1**) with two cylinderical electrodes (Streamline^{R}) induced significant parasympathetic effects Fig. (**4**). Heart rate slowing first occurred at 2.0 mA and even greater reductions in heart rate and blood pressure occurred with 3.0 and 4.0 mA. Similar to vagal nerve stimulation in the neck, cessation of stimulation of the sinoatrial node ganglia led to rebound increases in heart rate and blood pressure.

Averarge recruitment results for sinoatrial node ganglia stimulation are summarized in Table Sections **1B**, **2B**, and **2E** (n=5). Threshold for parasympathetic effects was 4.4 ± 2.1 mA in four out of five animals (Table **1B**). At higher currents of 5.8 ± 1.4 mA there was a greater decrease in heart rate by 12.8 ± 4.0 bpm (**2B**, n=4) which was well below the criteria of 40 bpm slowing. Sympathetic effects were first observed at higher currents of 10.0 ± 2.9 mA (**1B**, n=3) with increases in heart rate and blood pressure; further increases were seen at 13.7 ± 4.2 mA (**2B**, n=3). In our fifth animal, stimulation did not induce parasympathetic or sympathetic effects but only atrial pacing that occurred at 10.0 mA. Such variations in testing among animals were most likely due to variability in placement of the electrode and the physiological status of the animal during the extended surgical procedure. Dromotropic changes during sinoatrial node ganglia

stimulation were small (**2E**). Mean heart rate remained stable during sinoatrial node ganglia stimulation between tests at 106.4 ± 4.1 bpm and mean systolic blood pressure at 77.0 ± 6.8 mmHg; note, baseline heart rates were higher and blood pressure lower during open chest procedures than during vagal stimulation in the neck that occurred prior to opening the thoracic cavity.

Ventricular Ganglia Stimulation

Sample results from ventricular ganglia stimulation using four Streamline^{R} electrodes is shown in Fig. (**5**). Ventricular pacing occurred at the onset of stimulation evidenced by rapid ventricular contractions and significantly reduced blood pressures. At lower stimulating currents of 2.0 and 5.0 mA no detectable cardiovascular changes occurred.

All six possible combinations of two bipolar sets of electrodes among the four bipolar sets implanted in the ventricular ganglia Fig. (**1**) were tested and results are shown in Table Sections **1C**, **2C**, and **2F** (n=2). Large increases in heart rate were first observed with 9.3 $\pm$ 0.7 mA of current (**1C** and **2C**) and were 43.8 ± 13.8 bpm (**2C**); only small increases in blood pressure occured. Increases in heart rate occurred with five of six combinations of electrodes in one animal and two of six combinations in a second animal. Cardiovascular changes occurred with a 3 to 11 second delay after onset of stimulation suggesting activation of sympathetic fibers [17] that are known to course along the base of the great vessels. Large dromotropic changes indicated by shortening of the P-R interval (22.5 ± 10.5 ms) occurred with ventricular ganglia stimulation and concomitant large increases in heart rate (**2F**).

During ventricular ganglia stimulation, arrhythmias or pacing occurred in all cases; threshold currents for these unwanted effects were 10.7 ± 0.7 mA (**1C**). Arrhythmias and pacing alone or

in conjunction with sympathetic effects occured at four of the six locations tested in one animal; atrial or ventricular fibrillation occurred in four of the six stimulation sites in a second animal. Cardioversion was required in one animal with persistant atrial fibrillation to restore normal sinus rhythm.

Due to the inability of the Streamline^{R} electrodes to induce parasympathetic effects, small wire EMG hook electrodes were tested in a third animal. With the two bipolar sets of electrodes located ventrolateral to the base of the aorta, vagal effects were not induced and stimulation at 0.5 mA induced ventricular pacing. With the bipolar sets of electrodes dorsolateral to the base of the aorta, irregular sinoatrial node slowing occurred at 7.0 mA. Mean heart rate remained stable during these tests at 101.0 ± 5.7 bpm and mean systolic blood pressure was stable at 77.7 ± 8.2 mmHg (n=3).

DISCUSSION

Evidence is accumulating that vagal stimulation is therapeutic for patients with heart failure; however, side effects of whole-vagal nerve stimulation remain a serious concern [7,8]. To promote vagal effects while trying to limit side effects we conducted tests along the vagal pre and postganglionic pathway. With stimulation of the isolated vagus using small electrodes side effects could not be reduced. Threshold currents for vocal cord evoked potentials and parasympathetic slowing were similar to threshold currents reported by others [8,9,18,19].

Unexpected increases in heart rate occurred with neck vagus nerve stimulation at low stimulating currents that were observed in almost all the animals. Increases in heart rate during vagal stimulation have only been reported under special conditions including administration of muscarinic blockers or during vagal stimulation concomitant with cardiac pacing, which leads to post-

stimulation tachycardia [20]. Our unexpected observations could have resulted from stimulation of sympathetic fibers in the vagus nerve that arise from the superior cervical ganglia [21]. The functional role of these fibers, however, is thought to be primarily respiratory and their role in increased heart rates has not been previously described. Another explanation for the observed tachycardia is activation of vagal afferents with reflex vagal inhibition mediated through brainstem neural circuits. Afferent fibers have low stimulating thresholds which is consistant with the tachycardia occurring at low stimulating currents. Further, typical of parasympathetic responses, the onset of the tachycardia occurred without significant delay after the onset of stimulation. Our observations suggest the use of higher stimulating currents (3.7 ± 1.0 mA) to elicit parasympathetic responses and avoid increases in heart rate.

The anesthetic used in different studies may also help explain the tachycardia induced with vagal stimulation. We used isoflurane and fentanyl, cardiac anesthetics known to maintain near normal cardiovascular variables including heart rate and blood pressure. With these agents the resting heart rate was 80.3 ± 8.8 bpm. In contrast, two anesthetics commonly used in many cardiovascular animal studies, nembutal or alpha-chloralose, produce high resting heart rates, up to 180 bpm, through sympathetic activation and vagal inhibition [13,22]. Such high baseline or "resting" heart rates would mask any tachycardia that occurred during vagal stimulation in previous studies, whereas the anesthetics we used are more amenable to revealing sympathetic activity during vagal stimulation.

Sinoatrial Node and Ventricular Ganglia Stimulation

Parasympathetic ganglia located in epicardial fat pads are promising vagal stimulation sites because they are associated with selective functions on the heart including control of chronotropic and dromotropic responses. During sinoatrial node ganglia stimulation with Streamline^{R} electrodes,

threshold currents were similar to those reported previously [11,13,14,23]. Maximal heart rate slowing with sinoatrial node ganglia stimulation was less than with vagal stimulation (*i.e.*, did not meet the criteria of 40 bpm slowing). These differences are likely due to the fact that vagus nerve stimulation activates all cardiac ganglia simultaneously, whereas selective sinoatrial node ganglia stimulation only activates a small fraction of the cardiac ganglia.

While prior anatomical and pharmacological studies have shown that ventricular ganglia mediate negative inotropic and membrane stabilizing effects of vagal stimulation [16,24,25], to our knowledge, studies of direct ventricular ganglia stimulation while recording cardiovascular responses do not exist in the literature. We observed only non-vagal effects with ventricular ganglia stimulation even at high stimulating currents; these effects included sympathetic responses, arrhythmias, or cardiac pacing. Using small wire electrodes pacing occurred at even lower thresholds in one animal. High charge injection densities associated with small electrodes probably contributed to this low-current pacing effect. Although a large number of electrodes were implanted at the locations where the majority of ventricular ganglia are found, future work should include histological analysis to confirm the presence of ganglia near the stimulating electrodes.

Non-vagal effects during ventricular ganglia stimulation may have been due to the following reasons: one, the electrodes may have pierced the myocardium thereby reducing the threshold for ventricular pacing [16]. Two, ventricular ganglia are more diffuse and lightly populated than at other ganglionic sites like the sinoatrial or atrioventricular node ganglia and depending on the location of the electrodes only a few ganglia may have been recruited [15,16]. Three, sympathetic fibers which course along the base of the aorta and ventricular ganglia may have been stimulated to produce the sympathetic effects observed with stimulation [16].

Future Directions and Clinical Implications

The results of the vagal stimulation sites tested here provide further insight into the goal of promoting vagal effects while limiting side effects on the heart as a theraphy for heart failure patients. Stimulation of the vagus nerve in the neck with an isolating pad and small needle (TECA) electrodes was not promising for clinical application as additional side effects of increased heart rate at low stimulating current and neck muscle contractions were observed. Stimulation of the sinoatrial node ganglia with Streamline^{R} electrodes was effective. This type of stimulation is currently in clinical investigations at the atrioventricular ganglia as a rate control intervention for refractory atrial arrhythmia. Ventricular ganglia stimulation tested here, however, were not promising for clinical applications as pacing or sympathetic effects were observed.

There are alternatives to the current methods tested here that should be investigated. A microelectrode array implanted in the vagus could be selective for stimulating cardiovascular nerve fascicles and minimize or eliminate the non-cardiac side effects of vagal stimulation [26]. Electrodes placed on the vagus in the superior thoracic cavity distal to the bifurcation of the recurrent laryngeal nerve from the vagus should eliminate vocal cord activation side effects [21]. Stimulation of ventricular ganglia should continue to be investigated as it is a direct target for selective vagal effects on the ventricles.

CONCLUSIONS

Stimulation of the vagus nerve in the neck induces vocal cord activation and increased heart rates at stimulating currents below those for the desired parasympathetic effects. Stimulation of sinoatrial node ganglia consistently induced parasympathetic effects; given the low threshold for stimulation and specificity for negative chronotropic effects, the sinoatrial node ganglia hold promise as

therapeutic targets in heart failure. Stimulation of the ventricular ganglia only produced non-vagal effects; however, given the difficulty in visually identifying these ganglia at the aortic base, further refinement of stimulating procedures are warranted. Our findings reveal that vagal and non-vagal effects of stimulation along the vagal cardiac pathway are highly dependent upon the test site and magnitude of the stimulating current. Alternatively, microelectrode arrays implanted in the vagus or in cardiac ganglia could allow for more selective vagal stimulation and may offer the most targeted approach to enhancing vagal tone in heart failure with reduced side effects.

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Figure Legends

Fig. (1). Vagal pre and postganglionic fibers (solid lines) and sympathetic postganglionic fibers (dashed lines) innervating the sinoatrial node, ventricular cardiac ganglia, and myocardium. Shaded areas indicate fat pads containing vagal pre and postganglionic fibers. Sympathetic fibers from the superior cervical ganglia to cardiac ganglia are not illustrated in the figure for simplicity. SVC, superior vena cava; PArt, pulmonary artery; modified from Singh *et al.* [15].

Fig. (2). Sinoatrial node fat pad containing ganglia with two implanted bipolar Streamline^{R} electrodes (Medtronic, Inc). Electrode leads (left) and helical anchoring polypropylene sutures (right) are shown. (SVC superior vena cava, IVC inferior vena cava, RSPV right superior pulmonary vein, RIPV right inferior pulmonary vein).

Fig. (3). Stimulation at 20 Hz, 1 ms, with three TECA electrodes placed longitudinally along the right vagus nerve; heart rate and blood pressure changes shown. With 0.5 mA of current vocal cord evoked potentials occurred (not shown). Increases in heart rate and blood pressure occurred with 1.0 and 2.0 mA of current (solid arrow heads); higher currents induced parasympathetic effects (open arrows). A rebound increase in heart rate and blood pressure occurred after stimulation was stopped most likely due to barroreceptor mediated reflex compensation for the decreased blood pressure due to vagal induced parasympathetic effects. Horizontal dashed lines show baseline heart rate and blood pressure before stimulation.

Fig. (4). Stimulation of sinoatrial node ganglia with bipolar cylindrical electrodes (Streamline^{R}, Medtronic, Inc; 20 Hz and 100 μs pulse duration) and inserted into the epicardial fat pad containing cardiac ganglia and separated by 5 mm. Horizontal dashed lines show baseline heart rate and blood pressure before stimulation. Open arrows represent parasympathetic cardiovascular changes from baseline stimulations with 2.0 to 4.0 mA of current; sympathetic side effects did not occur with stimulation of the sinoatrial node ganglia.

Fig. (5). Stimulation of ventricular ganglia with two sets of bipolar cylindrical electrodes (Streamline,^{R} Medtronic, Inc; 20 Hz and 100 μs pulse duration) led to ventricular pacing at 10.0 mA of current. Pacing is evidenced by irregular and reduced ventricular contractility. As a consequence of ventricular pacing and concomitant decrease in cardiac output, blood pressure plummeted though heart rate was significantly elevated.