Selective Stimulation of Parasympathetic Nerve Fibers to the Human Sinoatrial Node

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Background. In animals, parasympathetic nerve fibers that innervate the sinoatrial node can be selectively stimulated to increase atrial cycle length. These nerve fibers course through an epicardial fat pad at the margin of the right superior pulmonary vein, the superior vena cava, and the right atrium. We hypothesized that similar nerves exist and can be selectively stimulated in humans.

Methods and Results. Microscopic examination of fat pads excised from the margin of the right superior pulmonary vein, the superior vena cava, and the right atrium during two human autopsies revealed the presence of nerve fibers and ganglia. We electrically stimulated this epicardial fat pad in 16 patients during cardiac surgery. The fat pads were stimulated with continuous-pulse trains for 15 seconds via a hand-held bipolar electrode using constant current (10–15 mA), constant pulse width (0.02–0.05 msec), and at 6.6, 10, 20, 25, and 30 Hz. The mean atrial cycle length ± 1 SEM increased from 734±34 msec at baseline to a maximum of 823±61 msec at 6.6 Hz, 1,167±125 msec at 10 Hz, 1,734±281 msec at 20 Hz, 2,993±661 msec at 25 Hz, and 2,461±668 msec at 30 Hz during nerve stimulation. Linear regression analysis showed that the response of atrial cycle length to sinoatrial parasympathetic nerve stimulation was frequency dependent. The maximum response and complete decay of the response occurred within 4–8 seconds of initiation or termination of sinoatrial parasympathetic nerve stimulation. Atrioventricular conduction time and the PR interval did not change during sinoatrial parasympathetic nerve stimulation, even when the atrial were paced at the baseline heart rate.

Conclusions. Electrical stimulation of parasympathetic nerve fibers in a fat pad near the sinoatrial node increased atrial cycle length without affecting atrioventricular nodal conduction. This is the first study in which such nerve fibers that innervate the sinoatrial node have been selectively stimulated in humans.

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Key Words • sinoatrial node • nervous system, parasympathetic • nervous system, autonomic

The parasympathetic innervation of the canine heart has been described by several investigators. Furukawa et al.1,2 used an isolated perfused canine right atrium to study the negative chronotropic and inotropic responses to intracardiac parasympathetic nerve stimulation. Randall and associates3–7 have used ablative techniques in dogs to demonstrate that parasympathetic nerve fibers selectively innervate either the sinoatrial or the atrioventricular nodal areas via nerve fibers located in discrete epicardial fat pads. Lazarra et al.8 elicited negative chronotropic or dromotropic responses in anesthetized dogs by selectively electrically stimulating intracardiac parasympathetic nerve fibers to the sinoatrial or atrioventricular nodal areas. Using similar techniques, Furukawa et al.9,10 demonstrated in dogs that the chronotropic response to continuous stimulation of intracardiac parasympathetic nerve fibers depended on the stimulation frequency.

Billman et al.11 recently demonstrated that distinct parasympathetic efferent pathways to the sinoatrial node and atrioventricular node also exist in the nonhuman primate. Thus, in dogs and monkeys, parasympathetic nerve fibers in an epicardial fat pad at the margin of the right pulmonary vein and right atrium innervate the sinoatrial node. Another fat pad, located at the margin of the inferior vena cava and the left atrium, contains parasympathetic nerve fibers that innervate the atrioventricular node with little or no innervation of the sinoatrial node.

In addition to providing a method by which to study the innervation of the sinoatrial and atrioventricular nodes, selective cardiac parasympathetic nerve stimulation has been used in dogs to study neural control of the heart more effectively. By use of these techniques, the responses of the sinoatrial and atrioventricular nodes to parasympathetic nerve stimulation have been determined in dogs independent of the global cardiovascular

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effects of cervical vagal nerve stimulation and independent of the confounding effects of reflex activation of efferent sympathetic nerves to these structures.

The parasympathetic innervation of the sinoatrial node, however, has not been described in humans. Because of the variability of function and anatomy of parasympathetic and sympathetic nerves in different mammals, one cannot assume that data obtained in animal studies apply to humans. Thus, selective stimulation of cardiac parasympathetic nerves to the sinoatrial node would provide a useful technique to study neural control of the human heart. If cardiac parasympathetic nerves to the sinoatrial node could be stimulated selectively in humans, then this could be used to investigate the neural control of the sinoatrial node in humans.

We hypothesized that in humans, parasympathetic nerves that selectively innervate the sinoatrial node course through a fat pad at the margin of the right superior pulmonary vein and the right atrium. The purpose of this study, therefore, was to determine whether 1) such a fat pad can be identified in humans intraoperatively, 2) parasympathetic nerve fibers in the fat pad can be selectively stimulated to increase atrial cycle length, and 3) the response to stimulation is frequency dependent.

Methods

Sixteen patients were studied under general anesthesia during cardiac surgery. The protocol was approved by the Investigational Review Board at University Hospitals of Cleveland. Written informed consent was obtained from all patients. The mean age of the 16 patients who underwent nerve stimulation was 57±5 years. Fifteen patients were male and one was female. Ten patients had coronary artery disease, four patients had the Wolff-Parkinson-White syndrome, one patient had dilated cardiomyopathy, and one patient had aortic stenosis. Four patients were taking digoxin; none were taking a β-blocking agent at the time of surgery. Six patients underwent coronary artery bypass surgery, one patient underwent left ventricular aneurysmectomy, four patients underwent ablation of an accessory atioventricular connection, five patients had automatic defibrillators implanted, and in one patient, the aortic valve was replaced. In each patient, nerve stimulation was performed at the beginning of the operation, before the therapeutic procedure was performed and before the patient was placed on heart–lung bypass. Thirteen patients received intravenous vecuronium for neuromuscular blockade, and three received intravenous pancuronium anesthesia. Pancuronium increases heart rate by causing postganglionic vagal blockade and by blocking noradrenaline reuptake. Vecuronium, a pancuronium analogue, does not interfere with vagal nerve activity and has few cardiovascular effects. The experiments were performed in the absence of β-blocking drugs, atropine, exogenous catecholamines, or other drugs known to affect the autonomic nervous system. After median sternotomy and pericardiotomy, multipolar plaque electrodes were sown onto the right atrial and right ventricular epicardium. Surface ECG leads I, II, aVF, and V5, as well as right atrial and right ventricular bipolar electrograms, were monitored continuously on a multichannel oscilloscope (model VR-16, Electronics-for-Medicine). The atrial and ventricular electrograms were filtered with a band pass at 30–500 Hz. A hand-held bipolar electrode probe (interelectrode distance, 2 mm) was used to stimulate parasympathetic nerves in an epicardial fat pad at the margin of the right superior pulmonary vein, right atrium, and superior vena cava. Nerve stimulation was performed with a Grass S-88 stimulator via an SIU7 stimulus isolation unit.

Cardiac parasympathetic nerves were stimulated with continuous electrical pulse trains for 15 seconds. In each patient, nerves were stimulated at a constant pulse width (0.02–0.05 msec), constant current (10–15 mA), and at 6.6, 10, 20, 25, and 30 Hz. These parameters were chosen because in dogs, they stimulate cardiac parasympathetic nerves but do not capture the myocardium. In each patient, the experiment required between 10 and 20 minutes to complete.

Atrial cycle length and atioventricular conduction time were measured before, during, and after parasympathetic nerve stimulation. Atrioventricular conduction time was determined by measuring the time between the onset of the atrial and ventricular electrograms. The PR interval was measured from surface ECG lead I. Atrioventricular conduction time and the PR interval were measured during sinus rhythm and during atrial pacing. We determined the maximum atrial cycle length and time to the maximum atrial cycle length at each nerve stimulation frequency in all patients. When sinoatrial arrest occurred, the atrial cycle length immediately preceding sinoatrial arrest was recorded as the maximum. We also determined the time from termination of stimulation to return of the baseline atrial cycle length.

Microanatomy

The sinoatrial fat pad was located and excised with the underlying myocardium during two randomly chosen human autopsies from patients otherwise unrelated to this study. These were fixed in 10% formalin and embedded in paraffin. Three sections were cut from each tissue block and stained with hematoxylin and eosin.

Statistics

Data were analyzed by one-way ANOVA and linear regression analysis, as indicated under “Results.”

Results

Anatomy

A fat pad approximately 1 cm in diameter was identified at the margin of the right superior pulmonary vein, the superior vena cava, and the right atrium in each patient and in the two autopsy specimens (Figure 1). Light microscopic examination of the sinoatrial fat pad autopsy specimens revealed numerous bundles of nerve fibers measuring from 30 to more than 200 μm in diameter within the epicardial adipose tissue, situated from close to the visceral pericardium and coursing between the atrial myocytes (Figure 2). Associated with these nerve fibers were scattered ganglia containing up to 20 cell bodies per section.
Atrial Cycle Length During Sinoatrial Fat Pad Stimulation

The atrial cycle length did not change in the three patients who received pancuronium anesthesia. Therefore, the patients who received pancuronium were excluded from further analysis. The atrial cycle length increased significantly during fat pad stimulation in all 13 patients who received vecuronium.

Transient sinoatrial arrest occurred in two patients during parasympathetic nerve stimulation (Figure 3). The response to nerve stimulation began immediately; atrial cycle length increased on the first or second beat after the onset of nerve stimulation. The time from the onset of sinoatrial parasympathetic nerve stimulation to the maximum atrial cycle length varied between 4 and 8 seconds and was frequency dependent (Figure 4). Linear regression analysis of the effect of nerve stimulation frequency (6.6–30 Hz) on time to maximum atrial cycle length showed that the slope of this line was not zero ($p<0.05$).

The mean atrial cycle lengths±1 SEM before and after recovery from sinoatrial parasympathetic nerve stimulation were not significantly different (734±34 versus 746±44 msec). The mean maximum atrial cycle lengths during sinoatrial parasympathetic nerve stimulation were 823±61 msec at 6.6 Hz, 1,167±125 msec at 10 Hz, 1,734±281 msec at 20 Hz, 2,993±661 msec at 25 Hz, and 2,461±668 msec at 30 Hz (Figure 5). The mean maximum atrial cycle lengths during sinoatrial parasympathetic nerve stimulation at 10, 20, 25, and 30 Hz were greater than the mean baseline atrial cycle length ($p<0.05$). The atrial cycle length during nerve stimula-
tion at 6.6 Hz was not statistically different from the baseline atrial cycle length.

The response to nerve stimulation was monotonically frequency dependent up to 25 Hz. Linear regression analysis of the effect of nerve stimulation frequency (6.6–30 Hz) on atrial cycle length indicated that the slope of this line was not zero (p<0.001).

The decay of the response of atrial cycle length to parasympathetic nerve stimulation began immediately upon cessation of nerve stimulation as well. The mean time from cessation of nerve stimulation to return of the baseline atrial cycle length was between 4 and 6 seconds (Figure 6). Most of the recovery occurred during the three or four beats that followed cessation of stimulation. Regression analysis of the effect of nerve stimulation (6.6–30 Hz) on time to baseline atrial cycle length showed that the slope of this line was not significantly different from zero.

**Figure 4.** Graph of time to maximal atrial cycle length during sinoatrial parasympathetic nerve stimulation at each nerve stimulation frequency. The slope of the relation between nerve stimulation frequency and time to return of baseline cycle length was not equal to zero.

**Figure 5.** Graph of atrial cycle length (CL) at baseline and during sinoatrial parasympathetic nerve stimulation at 6.6, 10, 20, 25, and 30 Hz. The atrial cycle length at 10, 20, 25, and 30 Hz was different from that at baseline (p<0.05).

**Effect of Fat Pad Stimulation on Atrioventricular Conduction Time**

In the 10 patients without Wolff-Parkinson-White syndrome, the atrioventricular conduction time was the same at baseline (168±14 msec) and during nerve stimulation (169±14 msec). Likewise, the mean PR interval was not different at baseline (204±16 msec) and during nerve stimulation (196±14 msec). Because the atrioventricular conduction time during sinoatrial parasympathetic nerve stimulation may have been influenced by changes in atrial cycle length, we paced the atria at cycle lengths identical to the patient's baseline atrial cycle length during sinoatrial parasympathetic nerve stimulation. The atrioventricular conduction time and PR interval did not change during simultaneous atrial pacing and parasympathetic nerve stimulation.

**Discussion**

The anatomic distribution of sympathetic and parasympathetic nerves that innervate the heart has been described in animals by several investigators. Randall et
al3-7 and others ablated and/or stimulated sympathetic and parasympathetic nerves to determine the chronotropic and inotropic effects at various sites and, thus, the distribution of the nerves in dogs. These investigators found that parasympathetic nerve fibers that selectively innervate the sinoatrial node course through an epicardial fat pad at the margin of the superior vena cava, the right pulmonary vein, and the right atrium. Lazarra et al8 demonstrated in dogs that parasympathetic nerves in this fat pad could be selectively stimulated to prolong the atrial cycle length without affecting atrioventricular conduction time.

Kountz et al15 demonstrated that stimulation of the vagus trunk modified heart rate in the excised human heart. Murphy et al16 stimulated dorsal mediastinal cardiac nerves in 20 patients during coronary artery bypass surgery. Nerve stimulation decreased heart rate in eight patients and increased heart rate in 12 patients. Furthermore, the authors noted an increase in mean aortic pressure in 12 patients and decreased coronary flow in eight patients during nerve stimulation. These results suggest that dorsal mediastinal nerve stimulation elicited a nonspecific autonomic response and that fibers in this nerve may innervate the coronary and peripheral arteries as well as the sinoatrial node. Our study is the first to demonstrate that cardiac parasympathetic nerve fibers selectively innervate the sinoatrial node and can be electrically stimulated in humans.

In humans, as in animals, parasympathetic nerve ganglia are located in an epicardial fat pad at the margin of the right atrium, the superior vena cava, and the right superior pulmonary vein. Postsynaptic nerve fibers from these ganglia selectively innervate the sinoatrial node. We selectively electrically stimulated these nerves to increase the atrial cycle length without affecting atrioventricular conduction time. Although data from dogs suggest that the response to sinoatrial parasympathetic nerve stimulation results mainly from stimulation of preganglionic nerve fibers, it is possible that in this experiment, both preganglionic and postganglionic nerve fibers were activated. Further studies using ganglionic blocking agents will be necessary to determine whether this is so.

The response to parasympathetic nerve stimulation was almost immediate. Prolongation of the atrial cycle length occurred within one or two atrial beats. The maximal response to sinoatrial parasympathetic nerve stimulation occurred within 4–8 seconds. Similarly, the response resolved within 4–6 seconds after cessation of sinoatrial parasympathetic nerve stimulation. Thus, the time course of the response and resolution of the response to cardiac parasympathetic nerve stimulation was similar to that which has been reported in animal studies.9,10,17,18 The response of atrial cycle length and atrioventricular conduction to vagal stimulation and to selective stimulation of cardiac parasympathetic nerves occurs immediately in animals. Furthermore, the decay of the response to vagal or cardiac parasympathetic nerve stimulation occurs within seconds in these animal models.

The response to sinoatrial parasympathetic nerve stimulation behaved in a frequency-dependent manner between 6.6 and 25 Hz. The magnitude of the response to nerve stimulation at 30 Hz, however, did not increase, for reasons that cannot be determined from our experiment. This may have been a result of one of several mechanisms. First, stimulation at 30 Hz may have activated sympathetic afferent and efferent nerve fibers. Direct or reflex activation of sympathetic nerves could have modulated the response to parasympathetic stimulation. Second, stimulation frequencies above 25 Hz may not further increase the quantity of acetylcholine released from cardiac parasympathetic nerves. If this is the case, the response to parasympathetic nerve stimulation at frequencies greater than 25 Hz would be expected to plateau. Finally, the magnitude of the response to sinoatrial parasympathetic nerve stimulation has finite bounds. Increasing nerve stimulation frequency increases atrial cycle length to a certain point above which sino arrest may occur. Nerve stimulation at higher frequencies in such cases would not yield a measurable increased response. This phenomenon was noted in the two patients who experienced transient sinoatrial arrest in the study.

The absence of a response to sinoatrial fat pad stimulation in patients treated with pancuronium supports the hypothesis that the increase in cycle length during sinoatrial fat pad stimulation was caused by stimulation of parasympathetic efferent fibers to the sinoatrial node. The lack of a response was probably because of the vagolytic effect of pancuronium.13 Vecuronium, unlike pancuronium, does not block postganglionic vagal efferent activity.14 Thus, the response to the sinoatrial fat pad stimulation was not inhibited in patients who received vecuronium.

The data from our study are consistent with those from previous animal studies. Rosenblau et al19 reported that the change in heart rate caused by vagal stimulation in cats was a hyperbolic function of stimulation frequency. We found previously that in dogs, the response of atrial cycle length to sinoatrial parasympathetic nerve stimulation is frequency dependent.10 The nerve stimulation frequencies required to demonstrate a significant change in atrial cycle length in this human study, however, were greater than those used in either cats or dogs. In cats, the response to nerve stimulation peaked between 5 and 10 Hz.19 In dogs, significant changes in atrial cycle length were noted at a nerve stimulation frequency of 4 Hz.10 The lack of a response at nerve stimulation frequencies below 10 Hz in humans cannot be explained by the data from this study. Direct or reflex activation of sympathetic nerve fibers may have antagonized the parasympathetic response at lower nerve stimulation frequencies.

Limitations

In contrast to previous animal studies, and for obvious reasons, central cardiac denervation was not performed on the patients in this study. It is possible that cardiac parasympathetic nerve stimulation activated local or central vagal sympathetic reflexes that modulated the results. Nerve stimulation may have directly stimulated afferent nerve fibers, thereby inducing reflex effects on atrial cycle length. Furthermore, a decrease in arterial pressure associated with the bradycardic re-
sponse to sinoatrial parasympathetic nerve stimulation may have increased sympathetic efferent nerve activity to the sinoatrial node via arterial baroreflexes. Because parasympathetic responses tend to dominate responses to sympathetic nerve activation, however, direct or reflex activation of efferent sympathetic nerves would be expected to have a negligible effect relative to those caused by direct stimulation of parasympathetic nerve fibers. This is particularly true because the parasympathetic nerve stimulation in this study was supramaximal.

The data on the time course of the response to parasympathetic nerve stimulation suggest that sympathetic nerves were not significantly activated by this stimulation. The response to parasympathetic nerve stimulation has been shown to be far more rapid than the response to sympathetic stimulation. Therefore, the maximal response to parasympathetic nerve stimulation would be expected to occur before a significant sympathetic effect was observed. More importantly, the decay of the response after termination of parasympathetic stimulation is also more rapid than the decay after termination of sympathetic stimulation. Had sympathetic nerves been stimulated at 30 Hz, a relative tachycardia would be expected to occur soon after cessation of nerve stimulation. The absence of a relative tachycardia after cessation of nerve stimulation at any frequency suggests that sympathetic nerve fibers were not stimulated. Further studies with adrenergic-receptor–blocking agents will be necessary to determine with certainty that sympathetic nerve fibers are not stimulated by these techniques.

The requirement of higher nerve stimulation frequencies to demonstrate a response in humans relative to animals may have resulted from the anesthetic or neuromuscular blocking agents used in our study. These agents may have modulated the response to cardiac parasympathetic nerve stimulation. Indeed, no response to parasympathetic nerve stimulation was observed in the three patients who received pancuronium. Vecuronium, although it did not block the response, may have decreased the response to cardiac parasympathetic nerve stimulation. Neuromuscular blocking agents may decrease the response to parasympathetic nerve stimulation by competitively antagonizing the response to acetylcholine. Further studies are planned to determine whether and how these agents affect the response to parasympathetic nerve stimulation.

Significance

Selective stimulation of parasympathetic nerves to the sinoatrial node may prove to be a useful technique for studying neural control of the heart. This technique may also provide a better method by which to study the anatomy and physiology of the human sinoatrial node. Nerve stimulation techniques have diagnostic and therapeutic implications as well. Sinoatrial parasympathetic nerve stimulation may provide a method to differentiate between sinus and other supraventricular tachycardias. The technique may also allow for better heart rate control during or after cardiac surgery. Finally, knowledge of the anatomy of these nerve fibers will allow surgeons to avoid ablating nerves that may be required to maintain normal neural control of the heart rate.

Summary

This is the first study in which cardiac parasympathetic nerve fibers that selectively innervate the sinoatrial node have been selectively stimulated in humans. Stimulation of parasympathetic nerve fibers in the sinoatrial fat pad caused an immediate increase in sinoatrial cycle length without affecting atrioventricular nodal conduction. Furthermore, the response to stimulation of sinoatrial parasympathetic nerve fibers was frequency dependent.

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