Vagal nerve stimulation activates vagal afferent fibers that reduce cardiac efferent parasympathetic effects

Kentaro Yamakawa,2,3 Pradeep S. Rajendran,1,2 Tatsuo Takamiya,2,3 Daigo Yagishita,1,2 Eileen L. So,1,2 Aman Mahajan,1,2,3 Kalyanam Shivkumar,1,2 and Marmar Vaseghi1,2

1University of California Los Angeles Cardiac Arrhythmia Center, Los Angeles, California; 2University of California Los Angeles Neurocardiology Research Center of Excellence, Los Angeles, California; and 3Department of Anesthesiology, David Geffen School of Medicine at the University of California Los Angeles, Los Angeles, California

Submitted 15 July 2015; accepted in final form 10 September 2015

Yamakawa K, Rajendran PS, Takamiya T, Yagishita D, So EL, Mahajan A, Shivkumar K, Vaseghi M. Vagal nerve stimulation activates vagal afferent fibers that reduce cardiac efferent parasympathetic effects. Am J Physiol Heart Circ Physiol 309: H1579–H1590, 2015. First published September 14, 2015; doi:10.1152/ajpheart.00558.2015—Vagal nerve stimulation (VNS) has been shown to have antiarrhythmic effects, but many of these benefits were demonstrated in the setting of vagal nerve decentralization. The purpose of this study was to evaluate the role of afferent fiber activation during VNS on efferent control of cardiac hemodynamic and electrophysiological parameters. In 37 pigs a 56-electrode sock was placed over the ventricles to record local activation recovery intervals (ARIs), a surrogate of action potential duration. In 12 of 37 animals atropine was given systemically. Right and left VNS were performed under six conditions: both vagal trunks intact (n = 25), ipsilateral right (n = 11), ipsilateral left (n = 14), contralateral right (n = 7), contralateral left (n = 10), and bilateral (n = 25) vagal nerve transaction (VNTx). Unilateral VNTx significantly affected heart rate, PR interval, Tau, and global ARIs. Right VNS after ipsilateral VNTx had augmented effects on hemodynamic parameters and increase in ARI, while subsequent bilateral VNTx did not significantly modify this effect (% change in ARI in intact condition 2.2 ± 0.9% vs. ipsilateral VNTx 5.5 ± 1.7% and bilateral VNTx 5.3 ± 0.8%, P < 0.05). Left VNS after left VNTx tended to increase its effects on hemodynamics and ARI response (P = 0.07), but only after bilateral VNTx did these changes reach significance (intact 1.1 ± 0.5% vs. ipsilateral VNTx 3.6 ± 0.7% and bilateral VNTx 6.6 ± 1.6%, P < 0.05 vs. intact). Contralateral VNTx did not modify VNS response. The effect of atropine on ventricular ARI was similar to bilateral VNTx. We found that VNS activates afferent fibers in the ipsilateral vagal nerve, which reflexively inhibit cardiac parasympathetic efferent electrophysiological and hemodynamic effects.

afferent cardiac neurotransmission; vagotomy; parasympathetic; electrophysiology

NEW & NOTEWORTHY

This study demonstrates that vagal nerve transaction at parameters designed to influence parasympathetic efferent outflow also activates afferent neural fibers in the stimulated trunk. The activation of these afferent fibers reduces the overall effects of vagal nerve transaction, as manifested by the electrophysiological and hemodynamic parameters before and after vagal nerve transaction. These findings are directly applicable to clinical studies performing vagal nerve transaction of the intact trunk in humans, as many of the studies demonstrating benefit in animal models were previously performed in the decentralized/vagotomized state, removing the influences of afferent activation.

The autonomic nervous system plays a central role in the initiation and maintenance of ventricular arrhythmias (41, 47). Parasympathetic withdrawal, as manifested by decreased heart rate (HR) variability and baroreflex sensitivity, is proarrhythmic, whereas increasing parasympathetic input to the heart via vagal nerve stimulation (VNS) is thought to be cardioprotective (10, 16, 23, 25, 55). Specifically, VNS has been shown to decrease infarct size (21, 42), reduce ischemia-related ventricular arrhythmias (38, 42), and improve survival in animal models of heart failure (35). The electrophysiological effects of stimulation of the intact right and left vagosympathetic trunk appear to be similar in a porcine model, without significant global or regional differences (52). Although the vagosympathetic trunk provides important cardiomotor efferent fibers to the heart, >80% of the fibers within the vagal nerve are afferent neural fibers, transducing information from visceral organs, including the heart, to the central nervous system (14, 32, 49). VNS likely leads to activation of both afferent and efferent fibers and may cause reflex autonomic activation through the contralateral trunk and via the sympathetic chain and dorsal root ganglia. However, the role of cardiac afferent fibers on efferent parasympathetic outflow during VNS remains unknown. Furthermore, whether VNS elicits primarily activation of afferent fibers in the stimulated trunk or activation of afferent (and efferent) fibers in the contralateral trunk due to reflex mechanisms remains to be elucidated. This is especially important, since many of the studies that demonstrated an antiarrhythmic benefit from VNS were performed in the decentralized state, after transection of the vagal trunk, stimulating only the efferent fibers (2, 8, 18, 24, 27, 34, 45, 50, 53, 56). Other studies have used an isolated innervated preparation, where cardiac afferent fibers no longer play an important role (6, 7, 29). Meanwhile, the majority of the studies showing pro-arrhythmic effects were done in the intact state (17, 22, 36, 37, 46). Furthermore, a large-scale human clinical trial of VNS for the management of heart failure did not reproduce the expected benefit noted in animal studies (54). These conflicting results are likely due to a lack of understanding of the contribution of afferent fibers to efferent control during VNS. The purpose of this study was to evaluate the effect of vagal nerve transection (VNTx) on modulation of cardiac hemodynamic and electrophysiological parameters by VNS, to delineate influences of afferent activation.
METHODS

All procedures were approved and performed in accordance with guidelines of the University of California, Los Angeles Institutional Animal Care and Use Committee and the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Anesthesia and Surgical Preparation

Yorkshire pigs (n = 37, weighing 50 ± 3 kg, male or female) were sedated with intramuscular telazol (6–8 mg/kg), followed by endotracheal intubation, mechanical ventilation, and anesthesia with isoflurane (1–1.5%, inhalation). Intermittent intravenous boluses of fentanyl (50–100 μg) were given for analgesia during surgical preparation. A surface 12-lead electrocardiogram was obtained via the Prucka Cardio Lab System (GE Healthcare, Fairfield, CT). Arterial blood pressure monitoring was performed via a 5-Fr sheath in the femoral artery, and saline and medications were infused via a 5-Fr sheath in the femoral vein. Bilateral cervical vagal nerves were isolated carefully at the cricoid level, and the heart was exposed via median sternotomy. Arterial blood gas levels were measured hourly. The ventilator settings were adjusted, or sodium bicarbonate was administered to maintain acid-base homeostasis. After completion of surgical exposure, anesthetics were switched from isoflurane to α-chloralose (50 mg/kg initial bolus, subsequently 20–30 mg·kg⁻¹·h⁻¹, continuous infusion), followed by a stabilization period of 1 h. Animals were killed by an overdose of intravenous anesthesia (pentobarbital sodium) followed by intravenous saturated potassium chloride to arrest the heart.

Electrophysiological Recordings and Analysis

Multiple unipolar epicardial electrograms were continuously recorded from a 56-electrode sock placed over the ventricles, connected to a Prucka Cardio Lab System (GE Healthcare). The activation recovery interval (ARI) from each electrode was analyzed using iScaldyn (University of Utah, Salt Lake City, UT) (1, 48, 52). Briefly, activation time (AT) was defined from the origin of the activation wavefront, and recovery time (RT) was defined as the time to the maximum dV/dt. ARI has been shown to correlate well with local action potential duration (11, 28). For purposes of this manuscript, anterior refers to the ventral aspect and posterior refers to the dorsal aspect of the animal. Mean ARIs in the following regions were quantified: left ventricle (LV) anterior, lateral, posterior, and apex, right ventricle (RV) anterior, lateral, posterior, and RV outflow tract. The median number of electrodes in each region was four (range of 3–6). Polar maps were also generated from the sock electrode to assess regional ARI values qualitatively (Fig. 1).

The PR interval was measured as the interval from the beginning of the P wave to the start of the Q wave on the surface electrocardiogram. Either lead II or AVF was used, whichever provided the clearest P wave. The same lead was used under all conditions in each animal.

Vagal Nerve Stimulation

Helical bipolar VNS electrodes (Cyberonics, Houston, TX) were placed around the cervical vagal trunks and connected to a Grass S88 stimulator (Grass Technologies, Warwick, RI) via photoelectric current isolation units for subsequent stimulation. VNS was performed with square pulses (10 Hz, 1 ms). Bradycardia threshold for each vagus nerve was defined as the current required to achieve a 10% decrease in HR. VNS was performed for 20 s at 1.2 times threshold.

Hemodynamic Assessment

A 12-pole conductance pressure catheter (5 Fr) was placed in the LV via the left carotid artery and connected to a MPVS Ultra Pressure Volume Loop System (Millar Instruments, Houston, TX). Appropriate catheter placement was confirmed by cardiac ultrasound (GE Healthcare) and via the pressure-volume loops recorded. Tau was calculated using the method defined by Weiss et al. (51) from the pressure-volume loop as a parameter describing the time course of the exponential decay in LV pressure during isovolumic relaxation. The following equation was used to calculate Tau:

$$P(t) = A \cdot e^{-t/Tau}.$$ 

where P is left ventricular pressure, A is a constant, and t is time from $-dP/dt_{max}$.

Experimental Protocol

The experimental protocol and the number of animals used in each condition are shown in Fig. 2. 

Intact vagal nerve stimulation. Unilateral right and left VNS were performed randomly (10 Hz, 1 ms, 1.2 times threshold) in 25 animals with both vagal trunks intact.
Ipsilateral vagal nerve transection. Subsequently, the right vagus nerve (n = 11) or the left vagus nerve (n = 14) was transected 2 cm above the stimulation probe. At least 20 min of observation were used to allow for hemodynamic and ARI parameters to return to stable values. Next, the threshold test was repeated, and the current required to achieve a 10% decrease in HR was remeasured. After the new threshold was obtained, ipsilateral efferent stimulation (of the distal/caudal end of the vagal trunk) was performed using the same current as the intact condition.

Contralateral vagal nerve transection. Right VNS was performed in the setting of left (contralateral) VNTx in 10 animals, and left VNS was performed in the setting of right (contralateral) VNTx in 7 animals. After contralateral VNTx, at least 20 min of observation were allowed for stabilization of hemodynamic and ARI parameters. Next, the threshold current required for a 10% decrease in HR was reevaluated in the setting of contralateral VNTx. Right VNS (in the setting of left VNTx) or left VNS (in the setting of right VNTx) was then performed using the same current as the intact condition, and ARI and hemodynamic parameters were analyzed.

Bilateral vagal nerve transection. After right or left ipsilateral VNTx, the remaining intact vagal trunk was transected to study the effect of bilateral VNTx on hemodynamic and ARI parameters. The new threshold current for either right or left VNS was remeasured after bilateral transection. Subsequently, right or left VNS (stimulation of the distal end of the vagal trunk) was performed in the setting of bilateral VNTx at 1.2 times the intact threshold current.

Atropine infusion. To compare electrophysiological effects of efferent muscarinic blockade with bilateral transection, atropine was administered as an intravenous bolus (0.04 mg/kg) in 12 animals with both vagi intact. The HR, systolic blood pressure (SBP), and ARI values were measured before and after 5 min of infusion. These parameters were compared with changes in ARI pre- and postbilateral VNTx. Comparison of muscarinic efferent blockade with atropine to bilateral VNTx was performed to provide insight as to whether the hemodynamic and electrophysiological effects observed after transection were due to sympathectomy or parasympathetic efferent tone withdrawal.

Statistical Analysis

Data are presented as means ± SE. Comparison of the change in ARI with atropine to bilateral VNTx was performed using the Wilcoxon Rank Sum Test. Baseline values and percent changes from baseline after stimulation were compared between various experimental conditions (intact, ipsilateral, contralateral, and bilateral transection) using separate linear mixed-effects models, including random animal effects to account for repeated measurements. Comparisons between pairs of conditions were performed using model contrasts. P values <0.05 were considered statistically significant. To account for multiple comparisons within each experiment, we report which differences remained significant after controlling for the false discovery rate at 5%. The Benjamini-Hochberg procedure was used to control the false discovery rate. All analyses were performed using SAS version 9.4 (SAS Institute, Cary, NC).

RESULTS

Effect of Vagal Nerve Transection on Hemodynamic and Electrophysiological Parameters

Hemodynamic and electrophysiological responses to ipsilateral and bilateral VNTx are shown in Table 1. Right VNTx increased HR, decreased SBP, and decreased Tau (Table 1). Left VNTx also affected HR and Tau but did not significantly affect blood pressure or dP/dmax. There were no statistically significant differences in hemodynamic parameters after bilateral transection compared with ipsilateral transection.

Both right and left VNTx shortened PR interval. Global ventricular ARI was decreased by right or left VNTx (right VNTx from 338 ± 15 to 298 ± 20 ms, P < 0.01; left VNTx from 361 ± 14 to 330 ± 13 ms, P < 0.01) (Figs. 2 and 3). Hemodynamic and electrophysiological influences, however, stabilized at ~5 min after VNTx and remained stable at 20 min (Table 2). Immediately after right VNTx, a rise in SBP and dP/dt was noted that returned to baseline after 5 min. This rise in blood pressure persisted after left VNTx.

<table>
<thead>
<tr>
<th>Condition</th>
<th>HR (bpm)</th>
<th>SBP (mmHg)</th>
<th>dP/dt (mmHg/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ipsilateral Right VNTx</td>
<td>120 ± 5</td>
<td>120 ± 10</td>
<td>1500 ± 200</td>
</tr>
<tr>
<td>Ipsilateral Left VNTx</td>
<td>130 ± 6</td>
<td>130 ± 15</td>
<td>1600 ± 250</td>
</tr>
<tr>
<td>Bilateral VNTx</td>
<td>140 ± 7</td>
<td>140 ± 20</td>
<td>1700 ± 300</td>
</tr>
</tbody>
</table>

Fig. 2. Flow chart of the experimental protocol: 25 of 37 animals underwent vagal nerve stimulation (VNS) followed by vagal nerve transection (VNTx), and 12 of 37 animals received only iv infusion of atropine. Animals with ipsilateral transection also underwent bilateral transection after right or left VNS. RVNS, right vagal nerve stimulation; LVNS, left vagal nerve stimulation.
After bilateral transection, differences in electrophysiological and hemodynamic parameters remained significant compared with the intact condition but were not statistically different from ipsilateral transection. The mean global ventricular ARI in all animals \(n = 25\) decreased from 351 ± 10 ms in the intact condition to 303 ± 13 ms after bilateral VNTx \(\Delta\text{ARI} = 47 ± 9\) ms, \%change in ARI = 14 ± 3\%, \(P < 0.001\) compared with intact condition). AT was not influenced by right or left VNTx.

**Effect of Atropine on ARI**

Atropine increased HR (from 75 ± 3 to 91 ± 3 beats/min, \(P < 0.05\)) and SBP (from 124 ± 5 to 130 ± 6 mmHg, \(P < 0.05\)). Atropine shortened ARI from 365 ± 20 to 319 ± 15 ms \(P < 0.05\). Furthermore, the change in ARI \((\Delta\text{ARI} = 46 ± 12\) ms, \%change of 11 ± 2\%) was not statistically different from the change in ARI after bilateral VNTx \((\Delta\text{ARI} = 51 ± 10\) ms, \%change in ARI of 15 ± 13\%, \(P = 0.6\) compared with atropine).

**Hemodynamic Response to Right and Left VNS in the Setting of Intact, Ipsilateral, Contralateral, and Bilateral VNTx**

**Intact Vagal nerve stimulation.** With both vagi intact, right and left VNS decreased HR, SBP, and dP/dt\(\max\) \((P < 0.05, \text{Table } 3)\). Diastolic function worsened with VNS with a rise in dP/dt\(\min\). Tau was increased with left VNS (Table 3).

**Ipsilateral and bilateral vagal nerve transaction and VNS.** The effects on HR, SBP, dP/dt\(\max\), dP/dt\(\min\), and Tau in response to right VNS were significantly augmented after ipsilateral VNTx (Table 1). Right VNS after bilateral VNTx had no significant additional effects (Table 1).

An augmentation of the effects of left VNS on the change in HR and SBP was observed after left VNTx. Left VNS after left VNTx did not significantly affect the change in

---

**Table 1. Effect of VNS on hemodynamic parameters after ipsilateral and bilateral vagal stimulation**

<table>
<thead>
<tr>
<th></th>
<th>BL</th>
<th>VNS</th>
<th>%Change</th>
<th>BL</th>
<th>VNS</th>
<th>%Change</th>
<th>BL</th>
<th>VNS</th>
<th>%Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR, beats/min</td>
<td>82 ± 4</td>
<td>74 ± 4*</td>
<td>−10 ± 1</td>
<td>96 ± 5*</td>
<td>82 ± 5*</td>
<td>−15 ± 2*</td>
<td>101 ± 5*</td>
<td>87 ± 6*</td>
<td>−14 ± 2*</td>
</tr>
<tr>
<td>SBP, mmHg</td>
<td>74 ± 3</td>
<td>67 ± 3*</td>
<td>−10 ± 1</td>
<td>84 ± 2*</td>
<td>72 ± 3*</td>
<td>−15 ± 1</td>
<td>91 ± 4*</td>
<td>74 ± 3*</td>
<td>−18 ± 2*</td>
</tr>
<tr>
<td>dP/dt(\max), mmHg/s</td>
<td>117 ± 6</td>
<td>112 ± 7*</td>
<td>−5 ± 2</td>
<td>104 ± 7*</td>
<td>95 ± 6*</td>
<td>−9 ± 1*</td>
<td>96 ± 7*</td>
<td>86 ± 6*</td>
<td>−11 ± 1*</td>
</tr>
<tr>
<td>Tau, ms</td>
<td>39 ± 2</td>
<td>41 ± 2*</td>
<td>4 ± 2</td>
<td>35 ± 3*</td>
<td>39 ± 4*</td>
<td>10 ± 3*</td>
<td>33 ± 3*</td>
<td>37 ± 4*</td>
<td>10 ± 3*</td>
</tr>
<tr>
<td>PR interval, ms</td>
<td>118 ± 5</td>
<td>135 ± 5*</td>
<td>13 ± 3</td>
<td>109 ± 5*</td>
<td>133 ± 5*</td>
<td>23 ± 5*</td>
<td>102 ± 3*</td>
<td>128 ± 7*</td>
<td>26 ± 5*</td>
</tr>
</tbody>
</table>

Note: Values are means ± SE; n, no. of pigs. BL, baseline; VNS, vagal nerve stimulation; HR, heart rate; SBP, systolic blood pressure. \*\(P < 0.05\) compared with baseline before VNS (*), compared with intact baseline (#), and compared with percentage change during VNS with both vagal nerves intact (§).

---

**Table 2. Hemodynamic and electrophysiological responses to right and left VNTx**

<table>
<thead>
<tr>
<th></th>
<th>BL</th>
<th>Post 1 min</th>
<th>Post 5 min</th>
<th>Post 10 min</th>
<th>Post 20 min</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Right VNTx ((n = 8))</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>81 ± 6</td>
<td>91 ± 6*</td>
<td>91 ± 6*</td>
<td>92 ± 6</td>
<td>94 ± 6</td>
</tr>
<tr>
<td>SBP, mmHg</td>
<td>108 ± 6</td>
<td>114 ± 7*</td>
<td>109 ± 8*</td>
<td>107 ± 8</td>
<td>104 ± 8</td>
</tr>
<tr>
<td>dP/dt(\max), mmHg/s</td>
<td>1,837 ± 166</td>
<td>1,894 ± 145*</td>
<td>1,883 ± 135</td>
<td>1,875 ± 130</td>
<td>1,833 ± 130</td>
</tr>
<tr>
<td>dP/dt(\min), mmHg/s</td>
<td>−2,697 ± 478</td>
<td>−2,931 ± 430*</td>
<td>−2,927 ± 460</td>
<td>−2,832 ± 465</td>
<td>−2,744 ± 448</td>
</tr>
<tr>
<td><strong>Left VNTx ((n = 13))</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>73 ± 4</td>
<td>81 ± 4*</td>
<td>83 ± 5</td>
<td>85 ± 5</td>
<td>84 ± 5</td>
</tr>
<tr>
<td>SBPP, mmHg</td>
<td>125 ± 6</td>
<td>136 ± 7*</td>
<td>134 ± 7</td>
<td>135 ± 7</td>
<td>133 ± 7</td>
</tr>
<tr>
<td>dP/dt(\max), mmHg/s</td>
<td>1,682 ± 130</td>
<td>1,768 ± 131*</td>
<td>1,800 ± 137</td>
<td>1,812 ± 136</td>
<td>1,806 ± 145</td>
</tr>
<tr>
<td>dP/dt(\min), mmHg/s</td>
<td>−2,933 ± 226</td>
<td>−3,184 ± 243*</td>
<td>−3,219 ± 267</td>
<td>−3,236 ± 251</td>
<td>−3,249 ± 275</td>
</tr>
</tbody>
</table>

Note: Values are means ± SE; n, no. of pigs. VNTx, vagal nerve transection. Both hemodynamic and electrophysiological properties were stabilized 5 min after right or left VNTx. \*\(P < 0.05\) vs. prior condition.
**Table 3. Effect of VNS on hemodynamic parameters after contralateral vagal nerve transection**

<table>
<thead>
<tr>
<th>HR, beats/min</th>
<th>Intact</th>
<th>VNS</th>
<th>%Change</th>
<th>BL</th>
<th>VNS</th>
<th>%Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Right (n = 10)</td>
<td>79 ± 4</td>
<td>69 ± 3*</td>
<td>−11 ± 2</td>
<td>86 ± 4*</td>
<td>73 ± 2*</td>
<td>−15 ± 2</td>
</tr>
<tr>
<td>Left (n = 8)</td>
<td>82 ± 5</td>
<td>75 ± 5°</td>
<td>−9 ± 1</td>
<td>94 ± 6*</td>
<td>83 ± 5°</td>
<td>−11 ± 2</td>
</tr>
<tr>
<td>SBP, mmHg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right (n = 10)</td>
<td>133 ± 8</td>
<td>123 ± 7°</td>
<td>−7 ± 1</td>
<td>130 ± 8°</td>
<td>118 ± 8°</td>
<td>−10 ± 1</td>
</tr>
<tr>
<td>Left (n = 8)</td>
<td>119 ± 7</td>
<td>115 ± 8°</td>
<td>−4 ± 1</td>
<td>101 ± 9°</td>
<td>94 ± 9°</td>
<td>−8 ± 2</td>
</tr>
<tr>
<td>dP/dt max, mmHg/s</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right (n = 7)</td>
<td>1,673 ± 102</td>
<td>1,576 ± 96°</td>
<td>−6 ± 1</td>
<td>1,613 ± 99</td>
<td>1,475 ± 82°</td>
<td>−8 ± 2</td>
</tr>
<tr>
<td>Left (n = 7)</td>
<td>2,060 ± 191</td>
<td>1,904 ± 173°</td>
<td>−7 ± 1</td>
<td>1,815 ± 159</td>
<td>1,678 ± 161°</td>
<td>−8 ± 2</td>
</tr>
<tr>
<td>dP/dt min, mmHg/s</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right (n = 7)</td>
<td>−2,916 ± 373</td>
<td>−2,705 ± 368°</td>
<td>8 ± 1</td>
<td>−3,030 ± 334</td>
<td>−2,704 ± 321°</td>
<td>11 ± 2</td>
</tr>
<tr>
<td>Left (n = 7)</td>
<td>−3,281 ± 509</td>
<td>−2,981 ± 468°</td>
<td>10 ± 3</td>
<td>−2,832 ± 534</td>
<td>−2,565 ± 554°</td>
<td>14 ± 4</td>
</tr>
<tr>
<td>Tau, ms</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right (n = 7)</td>
<td>37 ± 2</td>
<td>41 ± 3°</td>
<td>10 ± 3</td>
<td>35 ± 2°</td>
<td>38 ± 3°</td>
<td>6 ± 2</td>
</tr>
<tr>
<td>Left (n = 7)</td>
<td>39 ± 5</td>
<td>43 ± 5°</td>
<td>10 ± 3</td>
<td>31 ± 2°</td>
<td>35 ± 2°</td>
<td>13 ± 3</td>
</tr>
<tr>
<td>PR interval, ms</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right (n = 10)</td>
<td>115 ± 4</td>
<td>131 ± 5°</td>
<td>14 ± 2</td>
<td>101 ± 4°</td>
<td>127 ± 5°</td>
<td>27 ± 6</td>
</tr>
<tr>
<td>Left (n = 8)</td>
<td>115 ± 4</td>
<td>141 ± 11°</td>
<td>22 ± 8</td>
<td>105 ± 3°</td>
<td>132 ± 9°</td>
<td>25 ± 6</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of pigs. *P < 0.05 compared with baseline before VNS (*) and compared with intact baseline (#).

**Impact of Ipsilateral, Contralateral, and Bilateral VTNx on Stimulation Threshold During Right and Left VNS**

Bradycardia threshold with right VNS was reduced after ipsilateral and bilateral VTNx (Fig. 3). A significant reduction in threshold was also observed for left VNS after ipsilateral and bilateral VTNX (Fig. 4). There was no significant difference in the threshold current between ipsilateral VTNx and bilateral VTNx for right or left VNS. The right VNS threshold decreased after contralateral VTNx, whereas left VNS threshold was unchanged by contralateral right VTNx (Fig. 5).

**Effect of Ipsilateral, Contralateral, and Bilateral VTNx on Electrophysiological Parameters During Right and Left VNS**

Using the same current as the intact condition, right VNS after ipsilateral (right) transection augmented the percentage change in ARI from 2.2 ± 0.9 to 5.8 ± 1.7% (P < 0.01, Fig. 3). Subsequent right VNS after bilateral VTNx did not demonstrate significant additional effects. Left VNS after left VTNx demonstrated a trend for augmented ARI effects (intact 1.1 ± 0.5 vs. 3.6 ± 0.7%), but these differences did not reach statistical significance (P = 0.07, Fig. 4). After subsequent right-sided transection (i.e., after bilateral transection), left VNS had a significantly greater effect on ARI compared with the intact condition (bilateral VTNx 6.6 ± 1.6% vs. intact 1.1 ± 0.5%, P < 0.01).

In the setting of ipsilateral VTNx, both right and left VNS significantly prolonged PR interval more than the intact condition. During right VNS after right VTNx, the PR interval increased by 23 ± 5% compared with 13 ± 3% before transection (P = 0.036, Table 1). During left VNS after left VTNx, PR interval increased by 23 ± 5% compared with 13 ± 3% before transection (P < 0.05, Table 1). After bilateral transection, the increase in PR interval remained significant compared with the intact condition, but was not different from ipsilateral transection.

Right VNS in the setting of contralateral VTNx (ipsilateral side intact and stimulated) led to a decrease in bradycardia threshold, but effects on ARI during VNS were not significantly augmented (intact 2.8 ± 1% vs. contralateral VTNx 3.3 ± 1%, P = 0.53). Left VNS after contralateral (right-sided) VTNx did not alter threshold (Fig. 5) or effects on ARI (intact 4 ± 1% vs. contralateral VTNx 6 ± 2%, P = 0.15, Fig. 5).

AT was not affected by VNS before transection (24 ± 2 vs. 25 ± 2 ms for right VNS and 22 ± 1 vs. 21 ± 1 ms for left VNS). Furthermore, ipsilateral, contralateral, and bilateral VTNx did not affect AT.

**The Impact of Vagal Nerve Transection on Regional ARI**

After ipsilateral (right) VTNx, regional ARI shortened in all regions (Fig. 6). Right VNS after right VTNx had an augmented effect on ARI across all regions (P < 0.05 for the %change in all regions, right VTNx vs. intact). Right VNS after bilateral VTNx did not show significant additional prolongation beyond ipsilateral VTNx. Left VTNx also shortened ARI in all regions compared with the intact condition (Fig. 7). The additional change in ARI after bilateral VTNx was not statistically significant compared with ipsilateral VTNx. Left VNS after left VTNx showed a trend for greater prolongation in ARI across all regions, but...
these differences did not reach statistical significance after controlling for the false discovery rate. However, after subsequent right VNTx, left VNS significantly augmented the effects on regional ARI (P < 0.05 for the %change in all regions, bilateral VNTx vs. intact).

**DISCUSSION**

**Major Findings**

In this study, a comprehensive evaluation of the electrophysiological (global and regional) and hemodynamic effects of VNS before and after vagotomy was undertaken to assess the role of vagal afferent fiber activation on cardiac efferent parasympathetic control. The major findings are as follows: 1) activation of afferent fibers with VNS reduces its efferent effects and diminishing parasympathetic drive. Therefore, the effect of VNS on HR, sinus cycle length, and atrio-his interval had been previously reported (13, 19, 26, 31), but no further delineations of the reason for these effects were undertaken. In addition, MacCanon and Horvath (26) in 1957 performed bilateral chronic vagotomy in a canine model and, in the animals that survived, noted an initial increase in HR and SBP that returned to baseline levels within 15–20 min. They further observed a chronic decrease in cardiac output over time

**Impact of Vagal Nerve Transection on Global Ventricular Repolarization**

Despite the fact that the vagal trunk is a complex nerve, consisting of predominantly afferent fibers (14, 49), the detailed effects of vagotomy on cardiac function and electrophysiological parameters in the resting state have not been elucidated. The effect of vagotomy on HR, sinus cycle length, and atrio-his interval had been previously reported (13, 19, 26, 31), but no further delineations of the reason for these effects were undertaken. In addition, MacCanon and Horvath (26) in 1957 performed bilateral chronic vagotomy in a canine model and, in the animals that survived, noted an initial increase in HR and SBP that returned to baseline levels within 15–20 min. They further observed a chronic decrease in cardiac output over time.
In the porcine model, unilateral and bilateral vagotomy caused significant hemodynamic and electrophysiological effects that were maintained at 20 min. Unlike our study that shows that unilateral vagotomy causes a significant withdrawal of parasympathetic tone, in canines with normal hearts, unilateral vagotomy did not affect HRs significantly, and only after bilateral vagotomy was significant tachycardia observed, suggesting significant interspecies differences (19). Brooks and colleagues showed a decrease in repetitive extrasystole threshold, a surrogate of ventricular vulnerability to ventricular fibrillation, after either right or left vagotomy in canines with normal hearts, but the effect on VNS or other cardiac parameters after vagotomy was not reported (8). They did note that bilateral vagotomy had minimal additional effects to unilateral vagotomy on repetitive extrasystole threshold. Schwartz and colleagues assessed the effects of vagotomy and atropine on ventricular refractory periods. Unlike our findings, this study demonstrated a modest effect of bilateral VNTx on refractory period (2–3 ms) after atropine infusion (40). Possible explanations for the differences observed are due to the fact that, in the study by Schwartz and colleagues, ventricular refractory periods were measured with ventricular pacing/extrastimulus testing, which is known to cause an enhanced sympathetic tone (12) that could additionally shorten the refractory period, diluting the results. Furthermore, spatial heterogeneities across the RV and LV have not been previously assessed. In this study, we show that bilateral vagotomy has very significant effects on HR, PR interval, ARI, and Tau. Regional ARI effects were similar. Possible explanations for these findings are loss of inhibition of sympathetic tone by vagal afferent fibers and, therefore, occurrence of sympathoexcitation post-transection or withdrawal of efferent parasympathetic tone after transection. The effects of bilateral transection on ARI were similar to atropine infusion, suggesting, from the results of this study, that the majority of the electrophysiological effects of transection in the resting state are due to withdrawal of efferent parasympathetic tone.

Fig. 4. Effect of ipsilateral (left) VNTx on stimulation threshold and global ventricular ARI pre- and post-LVNS. Top: representation of polar maps obtained from the sock electrode at baseline and during left VNS before and after transection in one animal. The polar maps demonstrate that regional ARIs decrease uniformly across all epicardial regions after transection. The magnitude of increase in ARI from baseline with left VNS in all regions appears to be greatest after bilateral VNTx. Bottom: mean data from all animals that underwent LVNS after left VNTx, combined. Stimulation threshold was decreased by ipsilateral and bilateral VNTx. Ipsilateral and bilateral VNTx decreased baseline mean global ventricular ARIs. The change in ARI and dP/dt max during LVNS was somewhat augmented by ipsilateral VNTx, but this difference did not reach statistical significance until after bilateral VNTx. *P < 0.05 for pre- vs. post-VNS (*) and vs. the intact condition (#).
vagal ganglia) and synapse in the nucleus tractus solitarius (NTS). Efferent vagal fibers originate in the nucleus ambiguus and dorsal motor nucleus (20, 43), innervating the sinoatrial node, atrioventricular node, and atrial and ventricular myocardium through the intrinsic cardiac nervous system (3, 4). We had previously shown that stimulation of the intact right or left vagal nerves had similar hemodynamic and electrophysiological effects (52), since both of these nerves provide preganglionic efferent fibers that synapse in the intrinsic cardiac ganglia. Because the vagal trunk consists primarily of afferent fibers, the role of these fibers, particularly during stimulation, needs to be clearly assessed. Multiple studies have shown beneficial effects of VNS in preventing arrhythmias, but the large majority of these studies were performed in animal models in the decentralized state, after VNTx (2, 8, 15, 18, 24, 27, 34, 45, 50, 53, 56) or in isolated innervated hearts (6, 7, 30), where afferent activation was not possible. Other studies that demonstrated some benefit in the intact state often had mixed results, showing a reduction of polymorphic ventricular arrhythmias, but an increase monomorphic ventricular tachycardia (44). Still, other studies have reported proarrhythmic effects, or greater burden of ventricular arrhythmias during intact stimulation with or without ischemia (17, 22, 36, 37, 46). Meanwhile, a large randomized prospective clinical trial, Neural Cardiac Therapy for Heart Failure, failed to demonstrate the benefits of VNS in heart failure patients that had been demonstrated in animal models (54). The mixed results of these studies may be due to the method of stimulation, neglecting the role of afferent fiber activation (15). In our study, afferent vagal fibers did not play a significant role on cardiac electrophysiology and hemodynamic parameters in the resting state, and the primary effects of vagal transection could be explained by the removal of efferent parasympathetic tone. However, during stimulation, afferent fibers were activated and played a significant role in reducing the effects of efferent VNS. Transection of the ipsilateral and bilateral vagosympathetic trunks followed by stimulation significantly augmented electrophysiological and hemodynamic effects of VNS. Transection of the contralateral vagosympathetic trunk and removal of the contralateral vagal afferents did not have as great of an effect, since this was not the trunk that was being actively stimulated. However, the inhibitory role of even these contralateral afferents at modest stimulation levels, as demonstrated by the decrease in threshold of right VNS after left VNTx, suggests that the autonomic nervous system is an integrated network that senses hemodynamic changes acutely and acts to return these changes to the baseline state. Direct stimulation of afferent fibers and hemodynamic changes transduced by contralateral vagal afferent fibers can lead to activation of neurons in the NTS that subsequently could inhibit parasympathetic
outflow from the dorsal motor nucleus or nucleus ambiguous and may even cause sympathoexcitation. It is also possible that activation of these fibers could lead to sympathoexcitation either via reflex activation or by direct activation of these fibers in the vagal trunk. It has been reported that the vagus nerve is a complex nerve that contains sympathetic fibers, particularly at the level of the thorax (33). Therefore, the possibility of activating these fibers also exists with electrical stimulation. However, in this study, the role of these sympathetic fibers is likely to be more negligible. If these fibers were responsible for “sympathoexcitation,” then, upon transection, similar sympathetic effects would have been observed. Instead, an increase in parasympathetic effects was found.

Previous studies had also suggested an inhibitory role of vagal afferent fibers on reflex sympathetic outflow (5, 39). Based on these studies, we would have expected a decrease in the magnitude of the effects of VNS after vagal transection, rather than augmentation, since the inhibitory input of vagal afferents on sympathetic efferents would be removed. The results of our study were surprising but do show that activation...
of afferent fibers by electrical stimulation of the vagus nerve reduces parasympathetic efferent outflow and may cause reflex sympathoexcitation, counteracting or reducing the beneficial effects of VNS. Therefore, the role of these afferent fibers must be remembered when performing VNS in the intact state.

**Right vs. Left VNTx and Effects of VNS**

Compared with right VNS, the effect of left VNS on ARI was more significant after bilateral VNTx compared with ipsilateral VNTx. This may suggest that the right vagal trunk may contain greater cardiac afferent mechanoreceptor fibers. These fibers are intact in the setting of ipsilateral (left) VNTx, and may be activated by the drop in SBP and dP/dt max during VNS, causing reflex sympathetic activation or greater withdrawal of parasympathetic tone through this intact nerve, and subsequently inhibiting to some degree the increase in ventricular ARI observed during left VNS. Our results support those of Hirota and Ishiko (13), who performed vagotomy pre- and
postbilateral IXth nerve stimulation to assess the effect of afferent activation using gustatory stimuli. As in our study, Hirota and Ishiko also noted subtle differences in HR and blood pressure, depending on the order that the right or left vagal trunk was transected, with less tachycardia after left-sided transection compared with right (13). They also showed that the tachycardia response to gustatory stimuli was reduced more after right vagotomy than left vagotomy, again suggesting that the right vagal trunk may contain more cardiac afferents fibers (13). Chen et al. showed an increase in HR, blood pressure, LV systolic pressure, and dP/dt_{min} after both unilateral left and unilateral right vagotomy (9). However, baroreflex sensitivity was increased only after right vagotomy, also suggesting a greater role for cardiac vagal afferent fibers in the right vagus.

Limitations

General anesthesia can cause suppression of nerve activity. However, we were able to reliably record a cardiomotor response during VNS. To reduce the effect of inhaled anesthetics, \alpha\text{-}chloralose infusion was used during ARI and hemodynamic evaluation. For global and regional analysis, ARIs were not corrected for HR, since any HR effects, particularly with regard to assessment of parasympathetic tone, are physiologically important. Furthermore, HR would not affect regional differences, since these were assessed at the same HR and percentage changes were used to assess regional differences. Atrial and ventricular pacing was not performed to correct for HR, given the effect of pacing on altering autonomic tone (12). Effects of VNS were evaluated at one frequency and pulse width. This frequency and pulse width were chosen to avoid aggressive stimulation. Higher frequencies that are thought to specifically and only activate afferent fibers in epilepsy studies were avoided. Additionally, narcotics, such as fentanyl, can cause central modulation of parasympathetic tone through interaction with the opioid mu in the nucleus ambiguus. However, the use of fentanyl was standardized across all animals, and recordings were performed after initiation of \alpha\text{-}chloralose during steady state. Finally, the vagal trunk contains afferent fibers from many visceral organs. Therefore, it is not clear from this study activation of which afferent fibers may have led to a reduction in cardiac parasympathetic efferent outflow or sympathoexcitation.

In conclusion, parasympathetic efferent cardiomotor fibers in both the right and left vagal trunks are required for maintaining the resting basal parasympathetic tone. Vagal afferents are activated during VNS and decrease efferent parasympathetic electrophysiological and hemodynamic effects of electrical stimulation. The activation of both ipsilateral afferent fibers and reflex activation of the autonomic nervous system must be considered when applying VNS.

ACKNOWLEDGMENTS

We thank Jonathon Hoang for contributions to this study.

GRANTS

This study was supported by American Heart Association Grant 11FFTP75500 to M. Vaseghi and by National Heart, Lung, and Blood Institute Grant RO1-HL-084261 to K. Shivkumar.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

REFERENCES


