Central-peripheral neural network interactions evoked by vagus nerve stimulation: functional consequences on control of cardiac function

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1Neurocardiology Research Center of Excellence, David Geffen School of Medicine, University of California-Los Angeles, Los Angeles, California; 2Cardiac Arrhythmia Center, David Geffen School of Medicine, University of California-Los Angeles, Los Angeles, California; 3Molecular, Cellular, and Integrative Physiology Program, University of California-Los Angeles, Los Angeles, California; 4Department of Biomedical Sciences, Quillen College of Medicine, East Tennessee State University, Johnson City, Tennessee; and 5Cyberonics Inc., Houston, Texas

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Ardell JL, Rajendran PS, Nier HA, KenKnight BH, Armour JA. Central-peripheral neural network interactions evoked by vagus nerve stimulation: functional consequences on control of cardiac function. Am J Physiol Heart Circ Physiol 309: H1740–H1752, 2015. First published September 14, 2015; doi:10.1152/ajpheart.00557.2015.—Using vagus nerve stimulation (VNS), we sought to determine the contribution of vagal afferents to efferent control of cardiac function. In anesthetized dogs, the right and left cervical vagosympathetic trunks were stimulated in the intact state, following ipsilateral or contralateral vagus nerve transection (VNTx), and then following bilateral VNTx. Stimulations were performed at currents from 0.25 to 4.0 mA, frequencies from 2 to 30 Hz, and a 500-μs pulse width. Right or left VNS evoked significantly greater current- and frequency-dependent suppression of chronotropic, inotropic, and lusitropic function subsequent to sequential VNTx. Bradycardia threshold was defined as the current first required for a 5% decrease in heart rate. The threshold for the right vs. left vagus-induced bradycardia in the intact state (2.91 ± 0.18 and 3.47 ± 0.20 mA, respectively) decreased significantly with right VNTx (1.69 ± 0.17 mA for right and 3.04 ± 0.27 mA for left) and decreased further following bilateral VNTx (1.29 ± 0.16 mA for right and 1.74 ± 0.19 mA for left). Similar effects were observed following left VNTx. The thresholds for afferent-mediated effects on cardiac parameters were 0.62 ± 0.04 and 0.65 ± 0.06 mA with right and left VNS, respectively, and were reflected primarily as augmentation. Afferent-mediated tachycardias were maintained following f-blockade but were eliminated by VNTx. The increased effectiveness and decrease in bradycardia threshold with sequential VNTx suggest that 1) vagal afferents inhibit centrally mediated parasympathetic efferent outflow and 2) the ipsilateral and contralateral vagi exert a substantial buffering capacity. The intact threshold reflects the interaction between multiple levels of the cardiac neural hierarchy.

vagus nerve stimulation; autonomic nervous system; parasympathetic; afferent; intrinsic cardiac nervous system

NEW & NOTEWORTHY

Vagus nerve stimulation-evoked changes in cardiac function reflect the dynamic interplay between direct activation of descending efferents and afferent-induced decreases in central parasympathetic drive to the heart. With increasing current, vagus nerve stimulation first activates afferent fibers and then descending parasympathetic efferent fibers, interactions that maintain cardiac stability.

CARDIAC CONTROL IS A MANIFESTATION of a neural hierarchy that may be considered in three levels (9, 21, 33). Level 1 comprises the spinal cord and medulla as modulated by higher centers (1, 16, 27, 38). Level 2 comprises extracardiac-intrathoracic neurons, including the stellate, middle cervical, and mediastinal ganglia (4, 6, 8). Level 3 involves the intrinsic cardiac nervous system (ICNS) (9). The peripheral levels (2 and 3) form cardiocentric control loops, while the central nervous system (level 1) engages neural mechanisms for regulation of both cardiac and peripheral vasculature (33, 60). Acting together, these hierarchical populations coordinate and regulate regional cardiac electrical, mechanical, and metabolic indexes throughout each cardiac cycle (7, 9, 12). Endogenous or exogenous stresses have the potential to impact multiple levels of this hierarchy (19, 21, 32, 60). It is through the understanding of such hierarchical control and how it adapts to acute and chronic stress that rational, mechanistic-based approaches can be devised to target the cardiac neural hierarchy to manage cardiovascular pathology (14, 19, 21).

The vagus nerve is a complex neural structure containing descending efferent parasympathetic fibers and ascending afferent fibers. Efferent parasympathetic fibers modulate several cardiac indexes, including chronotropy, dromotropy, inotropy, and lusitropy (46, 48). The majority (~80%) of fibers contained within the vagus are afferent (sensory) in nature (13, 42). Thus the vagus nerve is an important pathway that carries sensory information from visceral organs, including the heart, to the central nervous system. Also, structural and functional data suggest that the cervical vagus trunk contains a small population of sympathetic fibers (41, 47).

For any bioelectronic approach for therapeutic neuro modulation, one must consider both direct and reactive (reflex) responses (14). The vagus can be stimulated in many different ways, at a number of different levels, and for multiple pathologies. As such, the anatomic characteristics of the nerves being stimulated (afferent/efferent) and the functional impact of stimulation parameters (current, frequency, pulse width, waveform, and duty cycle) must be considered (13, 14). Ultimately, these factors influence off-target adverse effects and, more importantly, the acute and chronic efficacy of the applied therapy. In most clinical applications for cardiovascular pathologies, electrical vagus nerve stimulation (VNS) is imposed unilaterally to either the right or left cervical vagosympathetic trunk (17, 18, 43).

While preclinical and clinical studies have yielded encouraging results for the safety and efficacy of VNS for cardiac
therapeutics (14, 50), there is a major information deficit in understanding how VNS impacts central and peripheral aspects of the cardiac nervous system to exert its influence on cardiac control. The objectives of this study were, therefore, to investigate 1) the functional role of VNS-evoked changes in afferent vs. efferent activation in integrated efferent control of cardiac function and 2) the potential for hierarchical interactions within the cardiac nervous system to impact VNS-evoked responses.

METHODS

Mongrel dogs (n = 26, male or female, 22.2 ± 0.5 kg body wt) were used in this study. All experiments were performed in accordance with the guidelines set forth by the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the East Tennessee State University Animal Care and Use Committee.

Animal Preparation

Animals were sedated with propofol (3–8 mg/kg iv) and then subjected to endotracheal intubation and mechanical ventilation. General anesthesia was maintained with isoflurane (1–2%, inhalation). Depth of anesthesia was assessed by monitoring corneal reflexes, jaw tone, and hemodynamic indexes. Left femoral venous access was obtained for maintenance fluid and drug administration. Right femoral arterial access was obtained for monitoring aortic pressure. A pressure transducer catheter (Mikro-Tip, Millar Instruments, Houston, TX) was inserted into the left ventricle (LV) chamber via the left femoral artery. Lateral incisions of the neck were made bilaterally to expose cervical vagosympathetic nerve trunks. At the completion of the surgery, general anesthesia was switched to isoflurane (1–2%, inhalation). A pressure transducer catheter was inserted into the LV chamber and connected to a control unit (model PCU-200, Millar Instruments). A lead II electrocardiogram was inserted into the LV chamber and connected to a control unit (model PCU-200, Millar Instruments). A lead II electrocardiogram was recorded via needle electrodes and amplified by a preamplifier (model PS11, Grass Technologies). Acid-base status was evaluated hourly (IRMA TruPoint, ITC, Edison, NJ); respiratory rate and tidal volume were adjusted and/or sodium bicarbonate was infused as necessary to maintain blood gas homeostasis. At the completion of the experiments, animals were humanely euthanized under deep anesthesia and by induction of ventricular fibrillation via direct current stimulation.

Vagus Nerve Stimulation

Bipolar stimulating helical cuff electrodes (PerenniaFlex model 304, Cyberonics; Houston, TX) were placed around the right and left cervical vagosympathetic trunks, with the anodes positioned cephalad to the cathode. A stimulator with a photoelectric constant-current isolation unit (models S88 and PSIU6, Grass Technologies, Warwick, RI) was used to deliver square pulses to these electrodes. Bradycardia threshold was defined as the current required to first produce a 5% decrease in heart rate (HR) at a frequency of 10 Hz and a pulse width of 500 µs in the intact state. Tachycardia threshold was defined as the current required to first produce a 5% increase in HR at a frequency of 10 Hz and a pulse width of 500 µs in the intact state. The effects of VNS on chronotropic, LV inotropic, and LV lusitropic function were evaluated over a range of currents (0.25–4.0 mA) with 10-Hz frequency and 500-µs pulse width. The effects of VNS on these cardiac indexes were also evaluated over a range of frequencies (2, 5, 10, 15, 20, and 30 Hz) delivered at 1.2 times the bradycardia threshold, determined in the intact state at 10 Hz and with a 500-µs pulse width. VNS was performed for 14 s followed by a 66-s off-phase. This time period was sufficient for return of cardiac indexes to baseline values, with no degradation in the responses to VNS over the duration of the experiments. After stimulations in the intact state, the vagus nerve was transected central to the stimulating electrode, allowing for the stimulation of only efferent vagal fibers in subsequent parts of the protocol.

Hemodynamic Assessment

A pressure transducer catheter (Mikro-Tip, Millar Instruments) was inserted into the LV chamber and connected to a control unit (model PCU-200, Millar Instruments). A lead II electrocardiogram was recorded via needle electrodes and amplified by a preamplifier (model PS11, Grass Technologies). Hemodynamic data were acquired with a data acquisition system (Power1401, Cambridge Electronic Design, Cambridge, UK) and analyzed offline with Spike2 (Cambridge Electronic Design). Derived indexes included HR, aortic blood pressure, LV end-systolic pressure, maximum rate of change in LV pressure (LV dp/dt maximum), and minimum rate of change in LV pressure (LV dp/dt minimum). Offline
analysis was used to determine the average response for each of the parameters at baseline and during the 14-s VNS on-phase.

**Experimental Protocol**

Animals were divided into three experimental groups. In group 1 (n = 10), the right and left vagus nerves were individually stimulated in the following three states: 1) intact, 2) after right vagus nerve transection (VNTx), and then 3) after left VNTx (bilateral VNTx). In group 2 (n = 10), the right and left vagus were individually stimulated in the following three states: 1) intact, 2) after left VNTx, and then 3) after right VNTx (bilateral VNTx). In group 3 (n = 6), the right and left vagus nerves were stimulated individually in the following four states: 1) intact, 2) intact with the nonselective β-blocker timolol (1 mg/kg iv bolus with 0.5 mg/kg iv bolus every 90 min as needed), 3) after bilateral VNTx with timolol, and then 4) after bilateral VNTx with timolol and atropine (1 mg/kg iv bolus).

Statistics

Data are represented as means ± SE. A repeated-measures mixed analysis of variance model was used for comparisons of mean current and frequency curves generated in the different states. A repeated-measures analysis of variance model with Tukey’s multiple comparison was used for analysis of threshold. P < 0.05 was considered to be statistically significant. Statistical analyses were performed with JMP Pro v11.2 (SAS Institute, Cary, NC).

**RESULTS**

VNS impacted multiple indexes of cardiac electrical and mechanical function. Figure 1 shows a typical example of the response to right VNS at intensity sufficient to impose acute changes in cardiovascular function. Note the VNS-evoked decrease in HR, systemic pressure, LV pressure, LV dp/dt maximum, and LV dp/dt minimum. At VNS offset, all the indexes recovered rapidly to baseline values, with a potential to transiently overshoot baseline values, especially at higher current and frequency levels.

**Central-Peripheral Neural Network Interactions With VNS**

**Effects on current intensity.** Figure 2 shows percent changes in chronotropy (Fig. 2, A and B), as well as LV inotropy and lusitropy (Fig. 2, C–F), in response to 10-Hz right-sided VNS across different currents in the intact state, as well as following right (ipsilateral, C and E) or left (contralateral, D and F) VNTx and then bilateral VNTx. Figure 3 shows the corre-
sponding HR levels at baseline and in response to right VNS following ipsilateral (Fig. 3A) or contralateral (Fig. 3B) VNTx and then bilateral VNTx. In the intact state, right VNS produced tachycardia at lower currents (starting at ~0.25–0.50 mA) and bradycardia at higher currents (Fig. 2, A and B, and Fig. 3). The augmentor effects on HR produced at lower currents were eliminated following ipsilateral, but not contralateral, VNTx (Fig. 2, A and B, and Fig. 3). Right-sided VNS following ipsilateral or contralateral VNTx resulted in greater reductions in HR, LV dp/dt maximum, and LV dp/dt minimum than in the intact state. Right-sided VNS following ipsilateral or contralateral VNTx resulted in greater reductions in HR, LV dp/dt maximum, and LV dp/dt minimum than in the intact state. Right-sided VNS following bilateral VNTx resulted in further reduction in these indexes for bilateral VNTx vs. intact (P < 0.004). The suppressing effects of left VNS were further enhanced following bilateral VNTx for bilateral VNTx vs. intact (P < 0.004) or bilateral VNTx vs. unilateral VNTx (P < 0.0001).

**Effects on threshold.** Bradycardia threshold was defined as the current required to first produce a 5% decrease in mean HR during the 14 s of VNS. VNS was delivered at 10 Hz and pulse width of 500 μs. In the right-sided protocol in the intact state, threshold was 2.91 ± 0.18 mA for the right vagus nerve and 3.47 ± 0.20 mA for the left vagus nerve (Fig. 5A). After right VNTx, thresholds for the right and left vagus nerve decreased: 1.69 ± 0.17 mA (P < 0.001) for right and 3.04 ± 0.27 mA (P < 0.04) for left. Thresholds decreased further following bilateral VNTx: 1.29 ± 0.16 mA for right and 1.74 ± 0.19 mA for left (P < 0.001 vs. intact and P < 0.002 vs. right VNTx). Figure 5B displays the percent change in bradycardia threshold for right and left VNS following right and bilateral VNTx.
compared with the intact state. A similar pattern was observed in the left-sided protocol. Threshold was 3.03 ± 0.24 mA for the right vagus nerve and 2.99 ± 0.15 mA for the left vagus nerve in the intact state (Fig. 5C). The thresholds for the right and left vagus nerve decreased following left VNTx: 2.56 ± 0.25 mA (P < 0.001) for right and 1.81 ± 0.22 mA (P < 0.001) for left. The thresholds decreased further following bilateral VNTx: 1.64 ± 0.21 mA for right and 1.32 ± 0.22 mA for left (P < 0.001 vs. intact and P < 0.002 vs. left VNTx).

Figure 5D displays the percent change in bradycardia threshold for right and left VNS following left and bilateral VNTx compared with the intact state. Taken together, the bradycardia threshold following bilateral VNTx was ~50% lower than that established in the intact state for either right or left vagus stimulation.

Tachycardia threshold was defined as the current required to first produce a 5% increase in mean HR during the 14 s of VNS. VNS was delivered at 10 Hz and pulse width of 500 µs. Across all animals in the intact state, the tachycardia threshold was 0.62 ± 0.04 mA for right VNS and 0.65 ± 0.06 for left VNS. The potential for VNS to augment chronotropic function at the lower ranges of current was maintained following contralateral VNTx (Figs. 2B, 3B, and 4B) but eliminated by ipsilateral VNTx (Figs. 2A, 3A, and 4A). VNS-evoked changes in HR occurred against a significant background of parasympathetic central drive, as evidenced by elevations in baseline HR in intact (~60 beats/min) vs. bilateral VNTx (~110 beats/min) states (Table 1, Fig. 3).

Effects of frequency. After determination of thresholds in the intact state, the effects of VNS on chronotropy, as well as LV inotropy and lusitropy, were evaluated over a range of frequencies (2–30 Hz) while the same current (1.2 times bradycardia threshold determined at 10 Hz) and pulse width (500 µs) were maintained. Figure 6 shows the percent changes in chronotropy, LV inotropic and lusitropic function in response to left VNS across a range of frequencies in the intact state, following left VNTx or right VNTx, and then following bilateral VNTx. In all states, increasing frequency resulted in greater reductions in HR, LV dp/dt maximum, and LV dp/dt minimum. Left VNS following either ipsilateral or contralateral VNTx resulted in a greater reduction in all measured indexes than the intact state (P < 0.0001), with the exception of LV dp/dt maximum.
following ipsilateral VNTx. There was even a further reduction in these indexes with left VNS following bilateral VNTx compared with the intact state \( (P < 0.0001) \), as well compared with ipsilateral \( (P < 0.0001) \) or contralateral \( (P < 0.0001) \) VNTx.

Figure 7 shows the analogous percent changes in chronotropic and LV inotropic function in response to right-sided VNS across different frequencies in the intact state, following left or right VNTx, and then following bilateral VNTx. In all these states, increasing frequency suppressed chronotropic and LV inotropic function. Ipsilateral (right-sided) VNTx exerted a predominant shift in the evoked functional response surface to right-sided VNS; contralateral VNTx resulted in a significant shift in the response surface only in LV lusitropy (Fig. 7F). After bilateral VNTx, there was a further enhancement in these negative chronotropic and LV inotropic effects compared with the intact state \( (P < 0.0001) \), as well compared with ipsilateral \( (P < 0.0001) \) or contralateral \( (P < 0.0001) \) VNTx.

**Effects of autonomic blockade.** Changes in cardiac function mediated by the autonomic nervous system are manifest, in part, by changes in parasympathetic and/or sympathetic outflows. While timolol exerted minimal effects on hemodynamic function (Table 2) and unilateral VNTx was likewise associated with minimal changes (Table 1), bilateral VNTx was associated with ~55% increase in HR (Tables 1 and 2). Figures 8 and 9 summarize VNS-evoked effects on chronotropic function (Figs. 8A and 9A), LV end-systolic pressure (Figs. 8B and 9B), and LV contractility and LV relaxation (Fig. 8, C and D, and Fig. 9, C and D) in the control state, following \( \beta \)-adrenergic blockade (timolol), subsequent bilateral VNTx (timolol + bilateral VNTx), and following subsequent muscarinic blockade (timolol + bilateral VNTx + atropine). While timolol by itself exerted no significant effects on right VNS chronotropic or LV inotropic function (Fig. 8), it did alter chronotropic, inotropic, and lusitropic responses to left VNS (Fig. 9). Subsequent VNTx substantially enhanced VNS-induced negative chronotropic and LV inotropic effects, which were abolished by atropine.

**DISCUSSION**

The aim of the present study was to determine the role of cervical VNS-evoked afferent vs. efferent axon activation in integrated control of regional cardiac functions. The major findings of this study are as follows. 1) VNS-evoked changes in cardiac function, delivered to an intact vagus nerve, reflect the dynamic interplay between direct activation of descending afferents against afferent-induced changes in central drive to the heart. 2) The functional threshold for activation of vagal afferent fibers is lower than that for activation of efferent

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**Table 1. Hemodynamic effects of sequential cervical vagus transection**

<table>
<thead>
<tr>
<th>State</th>
<th>LV +dp/dt, mmHg/s</th>
<th>LV −dp/dt, mmHg/s</th>
<th>LVSP, mmHg</th>
<th>Aortic BP, mmHg</th>
<th>HR, beats/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact</td>
<td>2.011 ± 130</td>
<td>−2.402 ± 175</td>
<td>152.5 ± 6.3</td>
<td>127.4 ± 4.0</td>
<td>59.3 ± 3.6</td>
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<tr>
<td>Right vagus cut</td>
<td>1.819 ± 123</td>
<td>−2.334 ± 196</td>
<td>138.4 ± 8.2</td>
<td>119.8 ± 6.2</td>
<td>69.8 ± 4.0</td>
</tr>
<tr>
<td>Both vagi cut</td>
<td>1.914 ± 164</td>
<td>−2.234 ± 201</td>
<td>121.5 ± 9.4*</td>
<td>111.5 ± 8.9*</td>
<td>112.8 ± 7.7*#</td>
</tr>
<tr>
<td>Intact</td>
<td>1.956 ± 153</td>
<td>−2.451 ± 176</td>
<td>149.5 ± 6.8</td>
<td>127.5 ± 6.3</td>
<td>62.0 ± 2.7</td>
</tr>
<tr>
<td>Left vagus cut</td>
<td>1.751 ± 140</td>
<td>−2.340 ± 163</td>
<td>150.0 ± 9.2</td>
<td>131.3 ± 7.6</td>
<td>69.2 ± 6.5</td>
</tr>
<tr>
<td>Both vagi cut</td>
<td>1.751 ± 152*</td>
<td>−2.180 ± 222</td>
<td>128.7 ± 12.3*</td>
<td>119.9 ± 13.2</td>
<td>106.0 ± 5.4*#</td>
</tr>
</tbody>
</table>

Values are means ± SE. LV, left ventricular; dp/dt, 1st derivative of LV pressure; LVSP, LV end-systolic pressure; BP, blood pressure; HR, heart rate. \( *P \leq 0.05 \), intact vs. unilateral or bilateral cervical vagus transection. \( #P \leq 0.05 \), unilateral vs. bilateral cervical vagus transection.
fibers. 3) Activation of vagal afferents is primarily reflected as withdrawal of central parasympathetic drive. 4) The potential exists for low-level sympathoexcitation as a result of bioelectric activation by vagal afferents.

Structure/Function of the Cervical Vagosympathetic Nerves: Relationship to the Neural Hierarchy for Cardiac Control

The cervical vagosympathetic nerve is a mixed nerve containing both ascending (afferent) and descending (efferent) axons (13, 28). Figure 10 schematically represents the relationship of these axonal projections within the framework of the hierarchy for cardiac control. The axons themselves are ~80% afferent, including both myelinated and unmyelinated fibers (13, 42). Cardiac-related vagal afferent neurons have cell bodies located in the nodose ganglia that project sensory information onto secondary afferent neurons in the nucleus tractus solitarii located in the medulla (23, 28, 52). Nucleus tractus solitarius secondary neurons project primarily to neuronal somata in the nucleus ambiguus for control of parasympathetic preganglionic efferent neuronal activity and, via brain stem reticulospinal projections, to the spinal cord intermedio-
lateral cell column for control of preganglionic sympathetic neuronal activity (1, 23, 29, 60). Sympathetic afferents, arising from the dorsal root ganglia, likewise input to the nucleus tractus solitarius via spinoreticular projections to contribute to reflex control of cardiovascular function (1, 20, 60).

Cardiac-related efferent projections contained within each cervical vagosympathetic trunk are predominantly parasympathetic preganglionic axons (46, 47). Parasympathetic preganglionic projections arising bilaterally from nucleus ambiguus neurons innervate multiple intrinsic cardiac ganglionic plexuses located within atrial and ventricular tissues, making direct contact with parasympathetic postganglionic neurons (Fig. 10). They also modulate the activity of local circuit neurons (LCNs) therein (9, 12). The LCNs represent the predominant neuronal population (~80%) of the ICNS, providing the primary integrating function for local reflex control of cardiac performance (3, 9, 12). The direct parasympathetic projection pathway, pre-to postganglionic neurons, represents ~15% of the ICNS (10, 12, 22). The remaining 5% of ICNS neurons are afferent (3, 12). There is anatomic and functional evidence to indicate that the vgosympathetic trunk also contains a small number of
sympathetic efferent fibers (41, 47). These sympathetic fibers have been described to originate from sympathetic efferent parasympathetic somata in the superior and middle cervical ganglia (29, 41, 48). Sympathetic projections to the heart can either directly project to cardiac myocyte end-effectors or modulate cardiac function via network sympathetic-parasympathetic interactions mediated by the ICNS (12, 39, 45, 55).

VNS-Induced Changes in Regional Cardiac Function

Hierarchical interactions. Any bioelectronic approach for neuromodulation needs to be considered in the context of the cardiac nervous system as a whole, as both direct and reactive (reflex) responses are evoked (14). Thus, to understand the central and peripheral neural network interactions within the cardiac neuronal hierarchy evoked by VNS, we studied the effects of VNS I in the intact state, 2) following sequential cervical VNTx, and 3) in the presence of autonomic blockade.

Chronotropic control. In the intact state, tachycardias evoked at low stimulus intensities (~0.6 mA) transitioned to bradycardias at higher stimulus intensities (~3.0 mA). VNS-induced tachycardias were maintained after contralateral VNTx but were eliminated by ipsilateral VNTx. Pretreatment

<table>
<thead>
<tr>
<th>State</th>
<th>LV ( +\mathrm{dp/dt} ), mmHg/s</th>
<th>LV ( -\mathrm{dp/dt} ), mmHg/s</th>
<th>LVSP, mmHg</th>
<th>Aortic BP, mmHg</th>
<th>HR, beats/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact</td>
<td>2.045 ± 165</td>
<td>−2.700 ± 189</td>
<td>154.6 ± 13.0</td>
<td>131.7 ± 6.7</td>
<td>74.8 ± 4.6</td>
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<tr>
<td>Timolol</td>
<td>1.791 ± 52</td>
<td>−2.302 ± 117</td>
<td>143.4 ± 9.2</td>
<td>124.6 ± 3.6</td>
<td>67.7 ± 3.5</td>
</tr>
<tr>
<td>Timolol + vagi cut</td>
<td>2.060 ± 108</td>
<td>−2.375 ± 101</td>
<td>165.2 ± 16.5</td>
<td>143.9 ± 13.8</td>
<td>111.7 ± 6.0*#</td>
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<tr>
<td>Timolol + vagi cut + atropine</td>
<td>1.911 ± 110</td>
<td>−2.348 ± 109</td>
<td>142.9 ± 8.1</td>
<td>129.7 ± 7.2</td>
<td>114.2 ± 7.8*#</td>
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</table>

Values are means ± SE. *\( P \leq 0.05 \) vs. intact. \( \# P \leq 0.05 \) vs. timolol.
with a β-adrenoceptor blocker did not affect VNS-evoked positive chronotropic effects. The positive chronotropic responses were also manifest at stimulus intensities below that required to evoke bradycardias following bilateral VNTx. Moreover, VNS-evoked changes in HR in the intact state were elicited against a significant background of parasympathetic central drive, as evidenced by elevations in baseline HR in intact (∼60 beats/min) vs. bilateral VNTx (∼110 beats/min) states (Table 1, Fig. 3). Taken together, these data support the hypothesis that vagal afferent axons are activated at lower VNS current densities and that the primary effect of stimulation of these afferents is transient withdrawal of central parasympathetic drive.

In the present study we also demonstrate that the VNS bradycardia threshold, specifically the output current required to first produce a minimum defined (5%) decrease in HR, reflects the interdependent interactions between central and peripheral neural network aspects of the cardiac nervous system. This threshold progressively decreased following sequential VNTx, regardless of whether the right or left vagus nerve was transected first. Compared with the intact state, the bradycardia threshold was reduced by ∼50% when the peripheral ends of the cervical vagosympathetic trunks were stimulated in decentralized preparations.

Efferent outflows from the ICNS to the cardiac tissues reflect the dynamic network interactions between central drive and local afferent feedback as modulated by LCNs. The LCNs subserve intra- and interganglionic coordination, thereby acting as primary coordinators of regional function (3, 9, 12). When intrinsic cardiac ganglion function is evaluated in vitro, the principal synapse between pre- and postganglionic parasympathetic fibers is primarily obligatory in nature (24–26). Thus the evoked response to VNS when delivered in the bilateral decentralized state in vivo most closely approximates the in vitro condition. The differences in VNS-evoked responses between bilateral VNTx, unilateral VNTx, and intact conditions reflect the hierarchical organization of the interdependent reflex control circuits, the ultimate function of which is to stabilize and optimize cardiac electrical, mechanical, and metabolic function (12).

There are multiple hypotheses to explain decreases in threshold. The first reflects the role of vagal afferent axons in reflexly modulating parasympathetic efferent outflows to the heart. Our data indicate that activated vagal afferents initiate centrally mediated reflexes that inhibit parasympathetic efferent outflows to the heart. As such, the level of activity on a given parasympathetic postganglionic neuron would reflect not only diminished endogenous central drive, but also inputs arising from direct electrical activation of parasympathetic efferent fibers. Since both vagi project to neurons throughout the ICNS (5, 9), this would have the net effect of shifting the VNS chronotropic response surface/cuve to the right. A second
hypothesis is that bioelectrical stimulation of the cervical vagosympathetic trunk activates axons projecting to the LCNs in the ICNS that, in turn, inhibit the direct preganglionic-to-postganglionic parasympathetic neuron pathways (31). We previously demonstrated that similar inputs to the ICNS can initiate directionally opposite responses from adjacent intrinsic cardiac neurons that reflect this potential (12). In the context of the current study, this proposed descending direct inhibitory mechanism is unlikely to be functionally important, since it should also occur following unilateral VNTx, and, as such, response surfaces would not have shifted, as was observed in this study. A third hypothesis involves induced alterations in sympathetic-parasympathetic interactions both within the ICNS and at the end-effectors (39, 45). Such a mechanism would, at best, represent a minor component, as reflected in the minimal changes observed with VNS in the presence of $\beta$-adrenergic blockade. However, the fall in LV and systemic blood pressure following bilateral VNTx may be reflective of a tonic sympathetic component for cardiovascular control mediated by axonal projections contained within the cervical vagosympathetic complex or alterations in integrated reflex control resulting from the loss of afferent inputs. A fourth possibility is that VNTx alters the neural interactions/balance for control of the sinoatrial nodal complex (39). In this regard, a similar mechanism could also occur within other intrinsic cardiac LCN populations regulating atrial and/or ventricular contractile function (15, 56). Future experiments are required to explore these hypotheses.

**Inotropic and lusitropic control.** Integrated parasympathetic control of atrial, atrioventricular, and ventricular function is mediated via parasympathetic preganglionic inputs to the distributed network of interdependent intrinsic cardiac ganglionated plexi (9, 15, 57). While earlier work suggests that vagal restraint on inotropic function is manifest against an enhanced sympathetic background (e.g., accentuated antagonism) (34, 35), more recent work demonstrates that activation of the ICNS, either endogenously or exogenously, impacts cardiac contraction and relaxation directly (15, 44, 56). As is evident from the VNS intensity plots depicted in the present study (Figs. 2–4), with intact vagi the magnitude of the evoked inotropic and lusitropic responses is moderate (<5% of baseline). Subsequent to VNTx, responses evoked in ventricular contractility were shifted to the left, to substantially greater levels. This shift is likewise evident when a fixed output current is applied (Figs. 6 and 7), where, even at the lowest frequency (2 Hz), the minimal change in contractility evoked in the intact state transitioned to a 15–25% decrease following bilateral VNTx. These data demonstrate that in the intact state the neuronal hierarchy for cardiac control has a substantial buffering capacity that acts reflexly to maintain cardiac stability in the face of endogenous and exogenous stresses, including those induced by VNS itself.
Differential Effects of VNS on Autonomic Efferent Outflows

Parasympathetic and sympathetic divisions of the autonomic nervous system cannot be considered in isolation, as they function as interdependent components of the cardiac neuronal hierarchy (9, 21, 59). As such, changes in autonomic efferent outflow in response to VNS therapy reflect 1) direct activation of autonomic efferent axons, 2) central reflex-induced changes in efferent activity in response to bioelectrical activation of vagal afferent axons, and 3) reflex-induced changes in autonomic function as cardiovascular afferents transduce altered mechanical stress in both cardiac and extracardiac (e.g., arterial baroreceptors) sites accompanying VNS-induced effects on cardiac function (11, 37, 60). Data presented in Figs. 8 and 9 demonstrate the minor contribution to cardiac control evoked by sympathetic efferent axons contained within the vagosympathetic complex in response to VNS, as delineated from the minimal changes in VNS-induced effects on regional cardiac indexes after timolol infusion. In contrast, atropine blocked induced changes in chronotropic, inotropic, and lusitropic function, even against an enhanced sensitivity imposed by bilateral VNTx.

VNS and the Neural Fulcrum

When considering stimulation parameters necessary for VNS therapy, one must be cognizant of not only the anatomic/functional characteristics of the axons (afferent/efferent) being stimulated, but also the effects of direct vs. reactive changes that result from the whole cardiac neuronal hierarchy attempting to maintain stability of cardiovascular function (14). Since the cardiac hierarchy normally acts as a negative-feedback system, perturbing the system in one direction evokes a corresponding reflex response in the opposite direction to maintain stability. The greater the perturbation, the greater is the reflex response and the greater the instability of the entire system.

We propose that the optimum therapeutic parameters for cervical VNS therapy are at the point at which afferent and efferent fibers are activated in a balanced manner, that is, when afferent-driven decreases in central parasympathetic drive are counteracted by direct activation of the cardiac parasympathetic efferent projections to the ICNS and heart, the net result of which is a null HR response (2, 31). We define this point as the neural fulcrum. VNS performed near this neural fulcrum operates within the normal constraints of the cardiac neuronal hierarchy, without evoking reactive changes that occur when high currents and/or frequencies are utilized (31). At this neural fulcrum, it should be recognized that both central and peripheral aspects of the cardiac nervous system become engaged, such that the reflex hierarchy maintains its capability to respond to stressors.

Clinical Perspectives

Autonomic imbalance plays an important role in the genesis of cardiac arrhythmias and progression of heart failure (21, 32, 51, 54). Sympathetic activation and parasympathetic withdrawal are not only proarrhythmic (21), they also accelerate progression of heart failure (19, 30, 58, 60). Furthermore, heart failure patients with poor vagal tone are known to have a worse prognosis (40). In animal models of chronic heart failure, VNS has been shown to decrease resting HR, improve LV function, and decrease mortality, presumably by preventing adverse cardiac remodeling (36). Given the promising results from preclinical studies, VNS is currently being evaluated in multiple clinical trials, including CardioFit-HF, NECTAR-HF, and ANTHEM-HF trials (17, 18, 43), for reduced ejection fraction and decrease mortality, presumably by preventing adverse cardiac remodeling (36). Given the promising results from preclinical studies, VNS is currently being evaluated in multiple clinical trials, including CardioFit-HF, NECTAR-HF, and ANTHEM-HF trials (17, 18, 43), for reduced ejection fraction heart failure. Initial results have been positive in the CardioFit-HF and ANTHEM-HF trials; neutral effects were reported for the NECTAR-HF trial after the first 6 mo of follow-up. One of the key differences between these trials is the choice of stimulation parameters (current, frequency, pulse...
width, and duty cycle) and, in the case of the CardioFit-HF trial, a proposed methodology to induce transient vagal afferent block during VNS. On the basis of data presented here, for proposed application of afferent blockade, one should consider potential deleterious effects on the effective gain of VNS therapy (17). Nevertheless, it is obvious from these ongoing trials that VNS is safe and feasible in the setting of reduced ejection fraction heart failure. Data from randomized, controlled studies are required to elucidate the impact of VNS on morbidity and mortality in patients with chronic heart failure syndrome.

In view of the data presented here, future studies should consider what is “subthreshold” VNS. Multiple studies have recently evaluated the effects of low-level VNS for treatment of atrial arrhythmias and heart failure (49, 53). It is obvious from data presented here that central and peripheral elements of the hierarchy for cardiac control become engaged by output current levels well below those current density applications that suppress chronotropism. Thus these data lead us to propose that J) the chronic effects of VNS therapy rest primarily on the indirect pathways that target intrinsic cardiac LCNs and 2) the optimal VNS stimulus parameters are coincident with the neural fulcrum (31). Future studies on the efficacy of VNS therapy for heart failure should focus on optimization of stimulation parameters, along with patient selection and therapeutic transition where indicated in the standard of care. As such, future preclinical and clinical studies should be designed to employ the entire cardiac nervous system to achieve long-term therapeutic benefits while minimizing off-target side effects of VNS.

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**AUTHOR CONTRIBUTIONS**


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