Sympathetic nerve fibers in human cervical and thoracic vagus nerves

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BACKGROUND Vagus nerve stimulation (VNS) therapy has been used for chronic heart failure and is believed to improve imbalance of autonomic control by increasing parasympathetic activity. Although it is known that there is neural communication between the VN and the cervical sympathetic trunk, there are few data regarding the quantity and/or distribution of the sympathetic components within the vagus nerve (VN).

OBJECTIVE To examine the sympathetic components within the human VN and correlate them with the presence of cardiac and neurologic diseases.

METHODS We performed immunohistochemistry on 31 human cervical and thoracic VNs (total 104 VNs) from autopsies and reviewed the patients' records. We correlated the quantity of sympathetic nerve fibers within the VNs with cardiovascular and neurologic disease states.

RESULTS All 104 VNs contain tyrosine hydroxylase (TH)–positive (sympathetic) nerve fibers; the mean TH-positive areas were 5.47% in the right cervical VN, 3.97% in the left cervical VN, 5.11% in the right thoracic VN, and 4.20% in the left thoracic VN. The distribution of TH-positive nerve fibers varied from case to case: central, peripheral, or scattered throughout nerve bundles. No statistically significant differences in nerve morphology were seen between diseases in which VNS is considered effective (depression and chronic heart failure) and other cardiovascular diseases or neurodegenerative disease.

CONCLUSION Human VNs contain sympathetic nerve fibers. The sympathetic component within the VN could play a role in physiologic effects reported with VNS. The recognition of sympathetic nerve fibers in the VNs may lead to better understanding of the therapeutic mechanisms of VNS.

KEYWORDS Cervical vagus nerves; Sympathetic nerves; Ganglion cells; Heart failure; Vagal nerve stimulation

ABBREVIATIONS ChAT = choline acetyltransferase; **CHF** = chronic heart failure; **TH** = tyrosine hydroxylase; **VN** = vagus nerve; **VNS** = vagal nerve stimulation

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Introduction

Cardiovascular abnormalities are associated with an imbalance in the cardiac autonomic nervous system,[1–4] typically characterized by increased sympathetic activity and decreased parasympathetic (vagal) activity.[2–6] Chronic vagal nerve stimulation (VNS) therapy is believed to improve the imbalance of cardiac autonomic control by increasing parasympathetic activity. Accordingly, vagus nerve stimulation (VNS) therapy has been introduced as a novel therapy for chronic heart failure (CHF)[1,4–9] and has demonstrated improvement in symptoms.[4,6,9]

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The anatomy of the vagus nerve (VN) is variable between species.[10,11] In humans, the VN is largely anatomically separated from sympathetic nerves.[10,12] However, there is communication between the VN and the cervical sympathetic trunk.[10,12] Onkka et al[13] performed immunohistochemical studies in dogs and reported the percentage and the distribution of sympathetic nerve fibers within the cervical VN. Kawagishi et al[12] using immunohistochemistry showed that catecholaminergic fibers were present in the human VN. Sympathetic components within the VN may contribute to both therapeutic and adverse events associated with VNS therapy. However, there are limited data regarding the location and quantity of the sympathetic nerve component within the human VN. There is no information on how any sympathetic component in the VN might correlate with clinical disease. We examined the VNs from 31 patients at autopsy, described the sympathetic component, and correlated the structural components of the VNs with the presence of major cardiac and neurologic diseases.

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Table 1 Disease states

Total number of cases	31
Cardiovascular diseases	20 cases (64.5%) including 12 diagnoses of hypertension, 10 coronary artery disease or myocardial infarction, 4 atrial fibrillation, 3 sudden cardiac death, and 2 ventricular tachycardia
Neurodegenerative disease	12 cases (38.7%) including 11 diagnoses of Alzheimer pathology, 2 Parkinson disease, 1 Huntington disease, and 1 multiple system atrophy
Diseases related to vagus nerve stimulation therapy: chronic heart failure and/or depression	10 cases (32.3%) including 4 diagnoses of chronic heart failure and 7 depression

Methods

Histological study for VNs

At autopsy, we harvested left and right cervical and thoracic VNs from 20 men and 11 women (Table 1). In all cases, consent for research was included with consent for autopsy. Cervical VNs were identified deep to the jugular vein and common carotid artery. Then, the dissection proceeded into the more distal parts of the VNs that entered the thoracic cavities. The right and left cervical VNs were sampled from the region where VNS therapy is applied. The right and left thoracic VNs were sampled 5 cm inferior to the aortic arch. Nerves were fixed in 10% formalin for 1 hour and then transferred to 70% ethyl alcohol. Tissues were processed routinely in graded alcohols, cleared in xylol, and paraffin embedded. Paraffin blocks were cut into 5-μm-thick sections. Samples were cut to obtain both cross sections and longitudinal sections. Sections were stained with hematoxylin and eosin. Immunohistochemical staining was also performed on paraffin-embedded cross sections. We used tyrosine hydroxylase (TH) antibodies (mouse monoclonal anti-TH antibody, T1299, Sigma; St. Louis, MO) to identify adrenergic nerve fibers and sympathetic ganglion cells. Anti–choline acetyltransferase (ChAT) antibodies (rabbit polyclonal antibody to ChAT, ab68779, Abcam, Cambridge, MA, USA) were used to identify parasympathetic ganglion cells. Glass slides stained with TH were scanned with a digital slide scanner at 20× (Aperio XT scanner, Aperio Technologies, Vista, CA). Digital image analysis software (Definiens' Tissue Studio, Definiens Inc, Parsippany, NJ) was used to calculate the cross-sectional area of the nerve and TH-positive areas within the VNs. The TH-positive area was divided by the cross-sectional area. Figure 1 shows the methods that were used to quantify the TH-positive area.

Sympathetic nerve fibers and clinical disease

In order to determine whether there was any correlation of the presence of sympathetic nerve fiber with clinical disease, we reviewed the records from the 31 patients. Demographic data included sex, age, ethnicity, and history of cardiovascular and neurologic diseases.

Statistical analysis

The groups were evaluated for statistically significant differences using the nonparametric exact Wilcoxon rank-sum (Mann-Whitney) test for 2 groups and the Kruskal-Wallis rank-sum test for more than 2 groups. Nonparametric correlations were performed using the Spearman's rank correlation test. A total of 21 tests for statistical significance were performed. The resulting P values were adjusted for multiple testing using a Bonferroni correction factor of 21.

Results

The median age for these 31 cases was 64 years (34–99 years). Twenty patients (64.5%) were Caucasian, 4 (12.9%) Hispanic, 1 (3.2%) Asian, and 1 (3.2%) Native American, and 5 (16.1%) were of unknown ethnicity. Disease status in these patients is given in Table 1. Data regarding histologic findings are presented in Table 2. No statistically significant differences were identified between the 4 VN groups for bundle number (uncorrected P value = .125; corrected P value = 1) or cross-sectional area (uncorrected P value = .008; corrected P value = .178).

TH-positive nerve fiber areas within the VNs varied from patient to patient. However, no statistically significant

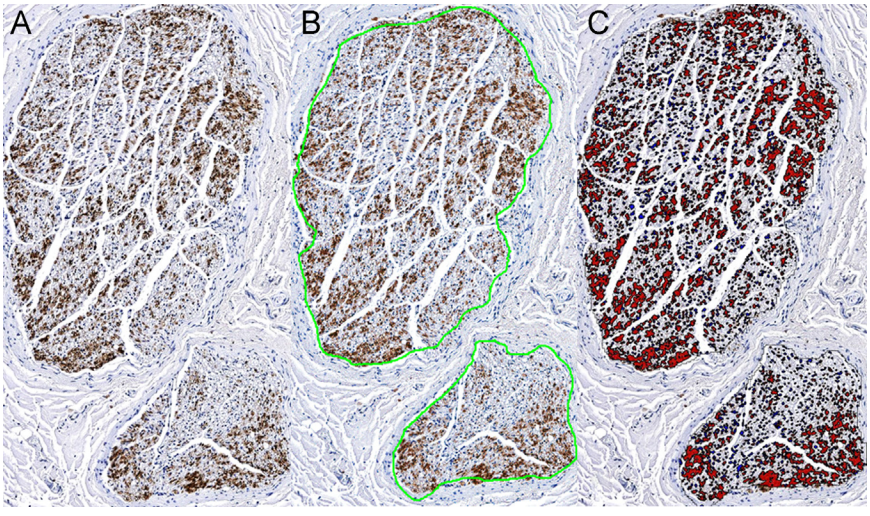

Figure 1 Quantification of cross-sectional total nerve and tyrosine hydroxylase (TH)–positive nerve fibers within the vagus nerves. **A:** The cross section of vagus nerve fibers with TH staining. **B:** The nerve bundles were manually outlined (green). Areas of interest were calculated by using digital image analysis software. **C:** TH-positive nerve fibers were identified by an automated image analysis system.