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Excitation properties of the right cervical vagus nerve in adult dogs

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ABSTRACT

Vagus nerve stimulation (VNS) is an approved treatment for epilepsy and depression, and it is currently under investigation for applications in Alzheimer's disease, anxiety, heart failure, and obesity. However, the mechanism(s) by which VNS has its effects are not clear, and the stimulation parameters for obtaining therapeutic outcomes appear highly variable. The purpose of this study was to quantify the excitation properties of the right cervical vagus nerve in adult dogs anesthetized with propofol and fentanyl. Input–output curves of the right cervical vagus nerve compound action potential and laryngeal muscle electromyogram were measured in response to VNS across a range of stimulation parameters: amplitudes of 0.02–50 mA, pulsewidths of 10, 50, 100, 200, 300, 500, and 1,000 μs, frequencies of 1–2 Hz, and train lengths of 20 pulses with 3 different electrode configurations: monopolar cathode, proximal anode/distal cathode, and proximal cathode/distal anode. Electrode configuration and stimulation waveform (monophasic vs. asymmetric charge-balanced biphasic) did not affect the threshold or recruitment of the vagal nerve fibers that were activated. The rheobase currents of A- and B-fibers were 0.4 mA and 0.7 mA, respectively, and the chronaxie of both components was 180 μs. Pulsewidth had little effect on the normalized threshold difference between activation of A- and B-fibers. The results provide insight into the complement of nerve fibers activated by VNS and guidance to clinicians for the selection of optimal stimulation parameters.

Introduction

The vagus nerve represents a large portion of the peripheral autonomic nervous system, and many chronic diseases are thought to involve an imbalance between the parasympathetic and sympathetic components (Tracey, 2002). Vagus nerve stimulation (VNS) was approved by the Food and Drug Administration for the treatment of epilepsy (1997) and depression (2005), and is currently under investigation for applications in Alzheimer's disease, anxiety, heart failure, inflammatory disease and obesity (Groves and Brown, 2005; Li et al., 2004; Milby et al., 2008; Schwartz, et al., 2008; Tracey, 2002; Vanoli et al., 1991). However, the mechanism(s) by which VNS has its effects are not clear, and the stimulation parameters for obtaining therapeutic outcomes appear highly variable. The purpose of this study was to quantify the excitation properties of the right cervical vagus nerve, which represents a less common but effective site for therapeutic electrical stimulation (Navas et al., 2010; Schwartz et al., 2008; Spuck et al., 2008). Specifically, we measured the relationship between the amplitude and pulsewidth of stimulation using different electrode configurations and the activation of different diameter nerve fibers.

The vagus nerve originates in the medulla and innervates organs of the neck, thorax, and abdomen. The cervical vagus nerve of an adult dog contains approximately 20,000 myelinated nerve fibers (Satchell et al., 1982), and an even greater number of unmyelinated nerve fibers. Studies in rabbits and cats report unmyelinated nerve fiber population estimates of 65–85% (Evans and Murray, 1954; Mei et al., 1980). Afferents from the esophagus, gastrointestinal tract, heart, and lungs outnumber somatic efferents of the voluntary muscles of the neck and parasympathetic efferents of the visceral organs by a ratio of 4:1 (Brodal, 1981; Foley and DuBois, 1937; Paintal, 1973; Rutecki, 1990). Cardiac efferents in the left vagus nerve are associated with the atrioventricular node, regulating cardiac contractility, while cardiac efferents in the right vagus nerve are associated with the sinoatrial node, regulating heart rate (Saper et al., 1990; Schachter and Saper, 1998). Mammalian vagal nerve fibers are divided into 3 populations: A, B, and C, whose characteristics are summarized in Table 1 (Berthold, 1978; Erlanger and Gasser, 1930; Groves and Brown, 2005; Woodbury and Woodbury, 1990).

Abbrevations: CAP, Compound action potential; I_r, Rheobase current; MonoC, Monopolar cathode; PADC, Proximal anode/distal cathode; PCDA, proximal cathode/distal anode; rCAP, Right cervical vagus nerve compound action potential; RLN, Recurrent laryngeal nerve; T_c, Chronaxie time; tVN, thoracic component of the vagus nerve; VNS, Vagus nerve stimulation.

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Table 1
Populations of mammalian vagal neurons.

	A-fibers	B-fibers	C-fibers
Diameter	5–20 μm	1–3 μm	0.2–2 μm
Myelinated	Yes	Yes	NO
Conduction velocity	30–120 m/s	3–20 m/s	0.3–2 m/s
Latencies	0.08–0.33 ms/cm	0.5–3.33 ms/cm	5–33.33 ms/cm

Latencies refer to the distance between the distal stimulating electrode and the recording electrode (Fig. 1). Therefore, for a 10-cm separation, the expected latency of A-fibers would be 0.8–3.3 ms. A-fibers refer to large diameter components (A-α, A-β, and A-γ). As a result, the diameter and conduction velocity of smaller A-components (A-δ) might overlap with B-fibers.

The relationship between stimulation parameters and activation of different populations of fibers within the cervical vagus nerve is not clear, and the stimulation parameters appropriate to maximize intended therapeutic effects and mitigate side effects have not been identified. Recommended stimulation parameters from previous preclinical studies to suppress or terminate seizures using VNS are wide ranging: 2–20 mA, 200 μs, 20–30 Hz, and 30 s on/variable off (Zabara, 1992); 0.2–0.5 mA/mm^2 of nerve cross-section, 500–1,000 μs, 10–20 Hz, and 30 s on/variable off (Woodbury and Woodbury, 1990). Similar variability is also observed from previous clinical studies: 0.25–3.5 mA, 130–500 μs, 20–30 Hz, and variable duty trains (Koo et al., 2001); ≤ 3.5 mA, 500 μs, 20–50 Hz, and 30–90 s on/5–10 min off (Ben-Menachem et al., 1994; George et al., 1994; Handforth et al., 1998; Penry and Dean, 1990; Ramsay et al., 1994; Uthman et al., 1993).

Experiments were conducted in adult dogs to record the neural and laryngeal muscle responses to graded stimulation of the right cervical vagus nerve using different electrode configurations. Since conduction velocity (θ) is proportional to fiber diameter (d) for myelinated fibers, $\theta/d \approx 6$ m/s/μm (Hursh, 1939), and proportional to the square root of fiber diameter for unmyelinated fibers, $\theta(\text{m/s}) \approx [d(\mu\text{m})]^{1/2}$ (Pumphrey and Young, 1938), the latency of the components of the compound action potential (CAP) are correlated with fiber diameter (Braund et al., 1988). This allowed us to differentiate the contributions of A-, B-, and C-fibers to the overall CAP, and generate input–output curves of the proportion of each evoked component as a function of stimulation intensity. The specific aims of this study were to characterize the relationships between stimulation intensity, electrode configuration, and waveform (monophasic vs. asymmetric charge-balanced biphasic) on the population of vagal nerve fibers that were activated, to measure the strength–duration properties of the CAP components, and to determine the effect of pulsewidth on the normalized threshold difference between activation of A- and B-fibers.

Materials and methods

Preparation and instrumentation

This study was conducted on 7 female and 2 male adult dogs weighing 18–21 kg. All procedures were reviewed and approved by the Institutional Animal Care and Use Committee at Duke University. Animals were fasted (12–24 h) and a fentanyl transdermal patch (50 μg/h for dogs < 20 kg and 75 μg/h for dogs ≥ 20 kg) was applied to the back of the neck prior to surgery. At the beginning of the experiment, animals were sedated with thiopental sodium (20 mg/kg, i.v.), and anesthesia was induced by inhalation of isoflurane (1–5%). Once the animal was intubated and placed on a ventilator, the isoflurane (1–5%) was discontinued and replaced with propofol (initial bolus of 2 mg/kg, i.v., followed by 0.2–0.8 mg/kg/min, i.v.). Thirty to 60 min before incisions were made, fentanyl (initial bolus of 5 μg/kg, i.v., followed by 10–15 μg/kg/h, i.v.) was administered. Heart rate and blood pressure were monitored to assess the depth of anesthesia, and arterial blood samples were drawn every 30–60 min for blood gas and electrolyte analysis. A continuous infusion of lactated ringer's solution (10 mL/kg/h, i.v.) was maintained throughout the experiment. At the conclusion of the study, the animals were euthanized with euthasol (0.2 ml/kg, i.v.).

The right cervical vagus nerve was exposed via a longitudinal incision along the ventral aspect of the neck. Bipolar stimulating helical nerve cuff electrodes with an inter-electrode spacing of 5–10 mm were placed on the right cervical vagus nerve. The leads were connected either directly to an external stimulator (Pulsar-6 bp, FHC, Inc.) for monophasic stimulation or a V-to-I converter (Analog Stimulus Isolator 2200, A-M Systems, Inc.) in series with the output of a computer D/A converter programmed with LabView (National Instruments) for biphasic stimulation. A tripolar recording nerve cuff electrode with an inter-electrode spacing of 6–8 mm was implanted 5–13.5 cm distal to the closest stimulating electrode (Fig. 1). Evoked CAPs were filtered (100 Hz–30 kHz) and amplified (100,000) using a low-noise voltage preamplifier (SR560, Stanford Research Systems, Inc.). Bipolar intramuscular wire electrodes were implanted into the laryngeal muscles to monitor the electromyogram (EMG). EMG signals were filtered (30 Hz–3 kHz) and amplified (1,000). Surface electrodes were used to record the electrocardiogram (ECG). Arterial blood pressure was measured by a catheter placed within the femoral artery, and left ventricular pressure was measured by endoventricular catheterization (SPC-454F Mikro-Tip Catheter Pressure Transducer, Millar Instruments, Inc.). All signals were sampled at 20 kHz and recorded (DASH, Astro-Med, Inc.).

Data collection

Input–output curves of the right cervical vagus nerve CAP (rCAP) and EMG were measured using monophasic pulses with amplitudes of 0.02–50 mA, pulsewidths of 10, 50, 100, 200, 300, 500, and 1,000 μs, frequencies of 1–2 Hz, and train lengths of 20 pulses. Three different electrode configurations were used: monopolar cathode (MonoC), proximal anode/distal cathode (PADC), and proximal cathode/distal anode (PCDA). During MonoC stimulation, a subcutaneous needle served as the return electrode. Input–output curves were also measured using asymmetric charge-balanced biphasic pulses consisting of a 100 μs primary phase followed by a 1 ms secondary phase or a 300 μs primary phase followed by a 3-ms secondary phase, both with a 100 μs delay between the primary and secondary phases.

In 4 experiments, the distal branches of the right vagus nerve were exposed via median sternotomy. The recurrent laryngeal (RLN) and thoracic component (tVN) of the vagus nerve were isolated by blunt dissection and a bipolar stimulating nerve cuff electrode was placed on each branch. The rCAPs evoked by individual stimulation of the RLN and tVN were recorded.

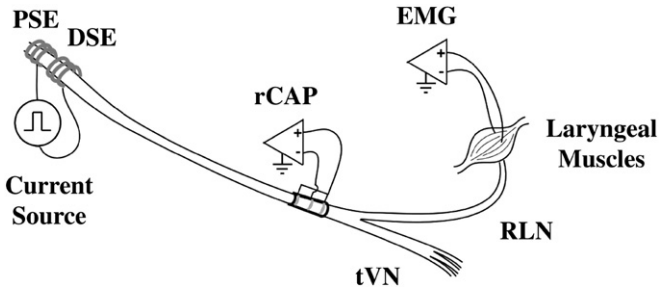

Fig. 1. Diagram of the right cervical vagus nerve and experimental setup. DSE, distal stimulating electrode; EMG, electromyogram; PSE, proximal stimulating electrode; rCAP, right cervical vagus nerve compound action potential; RLN, recurrent laryngeal nerve; tVN, thoracic component of the vagus nerve.