Brain Stimulation 10 (2017) 1045–1054

Contents lists available at ScienceDirect

Brain Stimulation

journal homepage: http://www.journals.elsevier.com/brain-stimulation

Vagus nerve stimulation improves locomotion and neuronal populations in a model of Parkinson's disease

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ARTICLE INFO

Article history:
Received 13 March 2017
Received in revised form
15 July 2017
Accepted 22 August 2017
Available online 24 August 2017

Keywords:
Parkinson's disease
Vagus nerve
Dopamine
Substantia nigra
Locus coeruleus
Norepinephrine

ABSTRACT

Background: Parkinson's disease (PD) is a progressive, neurodegenerative disorder with no disease-modifying therapies, and symptomatic treatments are often limited by debilitating side effects. In PD, locus coeruleus noradrenergic (LC-NE) neurons degenerate prior to substantia nigra dopaminergic (SN-DA) neurons. Vagus nerve stimulation (VNS) activates LC neurons, and decreases pro-inflammatory markers, allowing improvement of LC targets, making it a potential PD therapeutic.
Objective: To assess therapeutic potential of VNS in a PD model.
Methods: To mimic the progression of PD degeneration, rats received a systemic injection of noradrenergic neurotoxin DSP-4, followed one week later by bilateral intrastriatal injection of dopaminergic neurotoxin 6-hydroxydopamine. At this time, a subset of rats also had vagus cuffs implanted. After eleven days, rats received a precise VNS regimen twice a day for ten days, and locomotion was measured during each afternoon session. Immediately following final stimulation, rats were euthanized, and left dorsal striatum, bilateral SN and LC were sectioned for immunohistochemical detection of monoaminergic neurons (tyrosine hydroxylase, TH), α-synuclein, astrocytes (GFAP) and microglia (Iba-1).
Results: VNS significantly increased locomotion of lesioned rats. VNS also resulted in increased expression of TH in striatum, SN, and LC; decreased SN α-synuclein expression; and decreased expression of glial markers in the SN and LC of lesioned rats. Additionally, saline-treated rats after VNS, had higher LC TH and lower SN Iba-1.
Conclusions: Our findings of increased locomotion, beneficial effects on LC-NE and SN-DA neurons, decreased α-synuclein density in SN TH-positive neurons, and neuroinflammation suggest VNS has potential as a novel PD therapeutic.

Introduction

Parkinson's disease (PD) is a progressive, neurodegenerative disorder characterized clinically by tremor, bradykinesia, rigidity, and non-motor symptoms [1]. The pathological process underlying PD begins in the peripheral nervous system, and progresses through the vagal nerve to the brainstem, before reaching the substantia nigra (SN) and cortex [2,3]. The loss of locus coeruleus noradrenergic (LC-NE) neurons is significant in PD, and occurs prior to the loss of SN dopaminergic (SN-DA) neurons [4,5]. Degeneration of these LC-NE neurons is implicated in the early cognitive dysfunction in PD and increases sensitization of SN-DA neurons [6,7], providing evidence for a link between LC-NE and SN-DA systems. One major neurodegenerative mechanism in PD is neuroinflammation, which is associated with increases in astrocytes and increased activation of microglia [8]. While it remains unknown when neuroinflammation begins in PD relative to degeneration, prolonged neuroinflammation can exacerbate neurodegeneration [9].

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http://dx.doi.org/10.1016/j.brs.2017.08.008
1935-861X/

Existing treatment strategies for PD are purely symptomatic and incompletely address symptoms, often with intolerable side effects [10,11]. New strategies are vital to develop more effective treatments for PD. Therefore, here we study the effects of vagus nerve stimulation (VNS) on motor function, LC-NE and SN-DA systems, α-synuclein, and neuroinflammation in a rat model of PD. Rodent models of cerebral ischemia and depression show that VNS has beneficial effects on LC neurons, decreases both inflammation and oxidative stress, and increases neurotrophic factors [12–14]. Because dysfunction in these mechanisms is known to contribute to degeneration in PD, a multi-modal approach like VNS may be beneficial in PD patients, where pharmaceutical trials targeting one mechanism at a time have failed, including a meta-analysis examining the impact of NSAIDs on the risk of developing PD [15].

In rodents, LC-NE degeneration can be induced using the noradrenergic neurotoxin, N-Ethyl-N-(2-chloroethyl)-2-bromobenzylamine hydrochloride (DSP-4). DSP-4 is NE specific, and can readily cross the blood brain barrier [16]. While it has transient effects on peripheral NE, effects in the CNS are long-lasting [17]. NE depletion with DSP-4 potentiates memory deficits and motor symptoms in conjunction with DA lesions caused by 6-hydroxydopamine (6-OHDA) in both the striatum and the medial forebrain bundle [7,18,19]. A double lesion of LC and SN neurons can result in a greater loss of SN-DA neurons and motor function than 6-OHDA alone, providing further evidence for a link between the two populations [7,18]. Thus, the use of DSP-4 with intrastriatal 6-OHDA in this model allows for better representation of the progression from lower brain stem to higher brain regions previously proposed by Braak et al. (2003).

VNS is approved by the Food and Drug Administration for treatment-resistant forms of epilepsy (1997) and depression (2005) by modifying brain activity through the solitary nucleus (NTS) and its projections [20]. The vagus nerve is essential for parasympathetic nervous system function, and descending fibers innervate major internal organs including the GI tract, heart, lungs, and kidneys. Ascending fibers of the vagus nerve project to brainstem nuclei, including the NTS, which projects to the LC, and from LC to higher regions including the caudate/putamen and cortex. In rats, the right vagus is mostly efferent, while the left vagus is mostly afferent; thus, by targeting the left vagus as we are doing in the current study, effects are induced primarily in the brain rather than the periphery [21]. Chronic VNS in rats significantly increases firing rates of LC-NE neurons and increases NE levels in LC target regions [22–24]. In addition, increased NE induces neuroprotection in the hippocampus and cortical neurons [25–27]. While the effects of VNS on NE systems in non-PD models are well established, no published studies have looked at the effects of VNS on the SN-DA system and its relation to motor function. By using the more representative LC-NE lesion with the DAergic lesion in rats, the relationship between these neurotransmitter systems, as well as the therapeutic potential for VNS in PD, are more closely examined.

Materials and methods

Animals

Adult male Long Evans rats (200–225 g, Charles River) were randomly divided into four treatment groups: saline non-VNS (n = 8), saline VNS (n = 5), lesion non-VNS (n = 7), lesion VNS (n = 6). These rats were housed in an AAALAC-accredited facility at the Medical University of South Carolina (MUSC), with two rats per cage until surgery, and single-housed post-surgery. The facility was kept at 20–22 °C with a 12 h light:dark cycle, and food and water provided ad libitum. All procedures were approved by MUSC's Institutional Animal Care and Use Committee.

Surgical procedures

A timeline of experimental design for these experiments is depicted in Fig. 1. To induce NE lesions in the LC, rats were injected with DSP-4 (50 mg/kg, i. p., Sigma, St Louis, MO) or with sterile saline (0.9% NaCl, i. p., Hospira, Lake Forest, IL, Fig. 1). Seven days later, rats were deeply anesthetized using chloral hydrate (400 mg/kg, i. p.) and administered bupivacaine at incision sites (1 mg/kg, s. c.; Hospira). VNS cuff placement was conducted as previously described [28,29]. Briefly, an incision was made on top of the skull and in the left ventral cervical region for VNS animals only. Muscle groups were bluntly dissected, the left cervical vagus nerve was isolated, and a bipolar platinum-iridium cuff electrode (impedance <10 kOhms) was implanted around the isolated nerve. Leads from the cuff were tunneled subcutaneously behind the ear through the head incision, and the neck incision sutured. Both non-VNS and VNS rats were then placed in a stereotaxic frame (Stoelting, Wood Dale, IL), and four burr holes made in the skull above the striatum (two per hemisphere) using a Dremel (Dremel, Racine, WI) at the following coordinates: Hole 1: AP:+1.6, ML:±2.4, DV: −4.2; Hole 2: AP:+0.2, ML:±3.7, DV: −5.0 [30]. For lesion rats, 6-OHDA (5μg/μl; 2μl/site, containing sterile saline and 0.02% ascorbate, Sigma) was injected into each striatal site via Hamilton syringe [19] (SGE, Melbourne, Australia), while saline-treated rats received sterile saline (2μL/site). After each injection, the needle was left in place for 5 min before retracting slowly. Leads from the vagus cuff in VNS rats were then coupled to a two-channel connector headcap. Four sterile bone screws were screwed into the skull and covered with acrylic (Lang Dental, Wheeling, IL) to secure the headcap.

VNS stimulation

VNS was administered to freely-moving, unanesthetized rats in a locomotor box beginning eleven days post-surgery (to allow for 6-OHDA lesion development) after confirming successful lesion via locomotor assessment. Each rat's headcap was plugged into the stimulator (A-M Systems, Carlsborg, WA) and received two thirty-minute sessions of VNS per day for ten days, with daily sessions separated by 4 h. Stimulation parameters delivered a 500 ms train of 15 biphasic pulses at 30 Hz every 30 s. Each pulse gave 0.8 mA of current and lasted 100 μs. The time course and stimulation parameters were chosen based on previous studies that both demonstrated sufficient timing to produce therapeutic effects [28,31] and confirmed successful stimulation using neuronal spiking synchrony and electroencephalographic measures during stimulation [29,32]. Non-VNS rats were also placed in locomotor boxes for two thirty-minute sessions each day.

Locomotor activity

Locomotor activity (total distance traveled) was measured each stimulation day during the afternoon session in a darkened environment using Digiscan photobeam chambers (Omnitech, Columbus, OH) [33]. Beam breaks were recorded every five minutes and reported in centimeters throughout the thirty-minute session, and the total distance traveled across the ten stimulation days was averaged for each animal to obtain an individual mean distance traveled in a blinded version of the data set.

Brain preparation

Immediately following the afternoon locomotor session on day ten, rats were deeply anesthetized using isoflurane (Piramal Healthcare, Andhra Pradesh, India) and decapitated. The left striatum, bilateral SN, and bilateral LC were blocked and preserved in 4%