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Noninvasive vagus nerve stimulation as treatment for trigeminal allodynia

Michael L. Oshinsky [a,*], Angela L. Murphy [a], Hugh Hekierski Jr. [a], Marnie Cooper [a], Bruce J. Simon [b]

[a] Thomas Jefferson University, Department of Neurology, Philadelphia, PA, USA
[b] Electrocore, LLC, Basking Ridge, NJ, USA

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ABSTRACT

Implanted vagus nerve stimulation (VNS) has been used to treat seizures and depression. In this study, we explored the mechanism of action of noninvasive vagus nerve stimulation (nVNS) for the treatment of trigeminal allodynia. Rats were repeatedly infused with inflammatory mediators directly onto the dura, which led to chronic trigeminal allodynia. Administration of nVNS for 2 minutes decreased periorbital sensitivity in rats with periorbital trigeminal allodynia for up to 3.5 hours after stimulation. Using microdialysis, we quantified levels of extracellular neurotransmitters in the trigeminal nucleus caudalis (TNC). Allodynic rats showed a 7.7 ± 0.9-fold increase in extracellular glutamate in the TNC after i.p. administration of the chemical headache trigger glyceryl trinitrate (GTN; 0.1 mg/kg). Allodynic rats that received nVNS had only a 2.3 ± 0.4-fold increase in extracellular glutamate after GTN, similar to the response in control naive rats. When nVNS was delayed until 120 minutes after GTN treatment, the high levels of glutamate in the TNC were reversed after nVNS. The nVNS stimulation parameters used in this study did not produce significant changes in blood pressure or heart rate. These data suggest that nVNS may be used to treat trigeminal allodynia.

1. Introduction

The vagus nerve innervates many major organs of the body and relays sensory information about the state of the viscera to the central nervous system [5,8]. The vagus nerve is composed of 80% afferent and 20% efferent fibers. Vagal afferents convey physiological information from these organs to the central nervous system. Vagus nerve stimulation (VNS) has been approved in the United States for treatment of epilepsy and depression [9,12]. During clinical trials of VNS for the treatment of epilepsy, patients reported a reduction in headache frequency and intensity [10,25] and a prophylactic effect of VNS [13]. These observations suggest that VNS may be a novel nonpharmacologic treatment for migraine. In this study, we stimulated the vagus nerve noninvasively through the skin on the ventral surface of the neck using a noninvasive vagus nerve stimulator. This allowed us to stimulate the vagus nerve without the surgical implantation of stimulation electrodes in the neck of the rat.

VNS reduces tail-flick reflex elicited by noxious heat and formalin-induced nociceptor activation in rodent models [2]. VNS also reduces c-Fos expression in the ipsilateral TNC when applied at the same time as an injection of formalin into the facial vibrissae [2]. Another study found that chronic interruption of the vagal afferent input to the abdominal viscera halted antinociceptive responses to abdominal pain in rats [9].

Our rodent model of trigeminal allodynia relies on repeated activation of trigeminal nociceptive circuits through repeated infusions of prostglandin E2 onto the dura, which leads to chronic trigeminal allodynia similar to the hypersensitivity found in patients with migraine [18,22,23]. Using this model, this study aims to examine the mechanism of action of acute noninvasive vagus nerve stimulation (nVNS) for the treatment of trigeminal allodynia.

2. Methods

2.1. Ethics statement

All procedures performed on the animals were reviewed and approved by the Thomas Jefferson University Institutional Animal Care and Use Committee before initiating research. All surgeries

* Corresponding author. Address: Department of Neurology, Thomas Jefferson University, 1020 Locust Street, Suite 398 JAH, Philadelphia, PA 19107, USA. Tel.: +1 (215) 955 0433; fax: +1 (215) 955 4878.
E-mail address: michael.oshinsky@jefferson.edu (M.L. Oshinsky).

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were performed under isoflurane anesthesia, and all efforts were made to reduce animal numbers and suffering.

2.2. Experimental groups

Male Sprague-Dawley rats (350–375 g, Charles River, Malvern, PA) were divided into naive and prostaglandin-infused allodynic rats. These groups were further divided by treatment during microdialysis: no treatment, glyceryl trinitrate (GTN), nVNS, and nVNS with GTN.

2.3. Periorbital von Frey sensory testing

Rats were trained to enter a plastic tube restraint uncoaxed for periorbital sensory testing. Sensory testing occurred during the day using von Frey filaments with reproducible calibrated buckling forces varying from 10 to 0.07 g in descending sequential order [22]. von Frey monofilaments were required to make firm, perpendicular contact with the skin in the periorbital region, causing the filament to bend. The right and left periorbital threshold data were recorded separately. A positive response was characterized by several behavioral criteria: stroking the face with a forepaw, head withdrawal from the stimulus, and head shaking. The threshold was defined as a positive response to 2 of 3 trials. A normal periorbital threshold for rats was defined as 6 g or more; allodynic rats were defined as having periorbital thresholds of 4 g or less [22].

2.4. Cannula implantation and infusion

After 2 weeks of habituation and training, the rats were fitted with a stainless steel cannula (26-gauge, Plastics One Inc., Roanoke, VA) under isoflurane anesthesia (3% induction, 1.5% maintenance). First, a ~3-mm-diameter craniotomy was performed above the junction of the superior sagittal sinus and transverse sinus to expose the dura. The cannula was fixed to the bone with small screws and dental cement. An obdurator that extended just past the end of the cannula was inserted to prevent scar tissue from forming and thus clogging the cannula. Animals were allowed at least 1 week of recovery. Periorbital thresholds were monitored during the recovery period to ensure that the thresholds returned to presurgery baselines. If animals did not return to baseline, they were excluded from the study.

Rats were permitted free movement within their cages during infusions of sterile saline solution or 0.1 μmol/L prostaglandin E2 in 0.9% sterile saline solution. Polyethylene tubing (PE20) was connected to a Hamilton syringe, as well as the top of the cannula. A 15-μL quantity of saline solution or prostaglandin E2 was infused over 3 minutes while the rats moved freely. Visual inspection of the area around the cap was done to verify that there was no leakage of fluid. Obdurators were replaced after the last sensory test of the day, roughly 5 hours after the infusion. Rats were infused 3 times a week over the course of 3 to 4 weeks.

The cannula position was verified post mortem via visual assessment to ensure placement over the superior sagittal and transverse sinuses.

2.5. Trigeminal von Frey thresholds

To test the effect of nVNS on periorbital von Frey thresholds, an Elizabethan collar with electrodes and an electrolyte gel (Signa gel; Parker Laboratories, Inc., Fairfield, NJ) was placed on the neck lateral to the trachea (Fig. 1A). The electrodes were placed on the shaved skin of the neck, parallel to and over the vagus nerve (Fig. 1B). nVNS stimulation consisted of a 1-millisecond pulse of 5-kHz sine waves, repeated at 25 Hz, for 2 minutes. Periorbital thresholds were taken before stimulation, then 5, 30, 90, 150, and 210 minutes and 24 hours after nVNS stimulation.

2.6. Microdialysis of the trigeminal nucleus caudalis

CMA 12MD Elite probes (CMA Microdialysis Inc., Solna, Sweden) with a 1-mm PAES membrane length with a 20,000-Dalton cutoff and a 0.5-mm outer diameter was placed into the trigeminal nucleus caudalis (TNC) −2.6 to −2.9 mm from the obex and 1.7 to 1.9 mm lateral to the midline. PE10 tubing connected the probe to a 2.5-mL glass syringe mounted on a CMA/100 microinjection pump (CMA Microdialysis AB, North Chelmsford, MA). The dialysis system was perfused at 2.0-μL/min with sterile, pyrogen-free artificial extracellular spinal fluid (aCSF; composition in mM: 135 NaCl; 3 KCl; 1 $MgCl_2$; $CaCl_2$; 2-sodium phosphate mono- and dibasic; pH = 7.4). Probes were inserted 2 to 3 hours before stimulation to allow the transmitter levels to settle to baseline levels. Samples were collected continuously every 15 minutes and began immediately after probe insertion. Sample collection continued for at least 3.5 hours after stimulation.

At the end of the experiment, animals were administered 0.5 mL of Euthasol via i.p. injection. The probe was then removed and stored in distilled water. The brain and spinal cord were removed and stored in 4% paraformaldehyde for later analysis to check the position of the probe via sectioning and staining.

2.7. HPLC measurement of amino acids

The amino acid content (specifically, glutamate, norepinephrine, glycine, and gamma aminobutyric acid (GABA)) of each sample was analyzed via high-performance liquid chromatography (HPLC) using a binary gradient and pre-column derivatization of O-phthal aldehyde (OPA) with fluorescence detection.

Samples were diluted (5 μL aCSF + 5 μL dialysate), and a 1:2 sample-to-reagent ratio was used (10 μL sample + 20 μL OPA). After a 60-second reaction, 20 μL of the sample–OPA mixture was auto-injected into an Agilent Zorbax Eclipse AAA column (150 × 4.6 mm; 5-μm particle size). A binary gradient of mobile phase A (40 mmol/L sodium phosphate monobasic; pH = 7.4) and mobile phase B (45% acetonitrile; 45% methanol; 10% water) with a flow rate of 1.5 mL/min was used for separation. The column temperature was maintained at 30 °C. EZChrom Elite version 3.1.6 software was used to determine concentrations of extracellular neurotransmitters.

2.8. HPLC measurement of monoamines

Measurement of monoamine neurotransmitters, particularly norepinephrine, serotonin (5-HT) and 5-hydroxyindoleacetic acid,

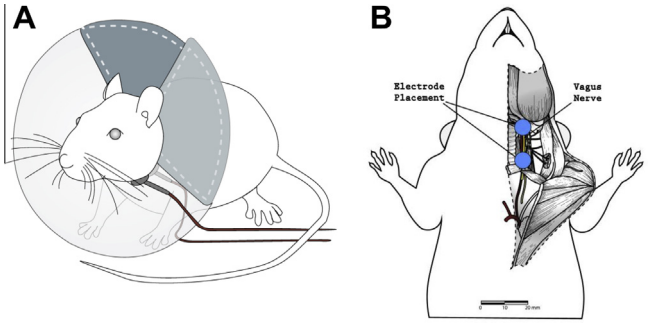

Fig. 1. (A) An Elizabethan collar with 2 silver-coated electrodes (0.8-cm diameter) was placed on conscious naive and allodynic rats. The electrodes were connected to an amplifier that produced a 1-millisecond pulse of 5-kHz sine waves, repeated at 25 Hz. The electrodes were positioned on the skin of the rat, lateral to the rat's trachea. (B) Although in the experiments in this study vagus nerve stimulation was achieved through stimulation of the shaved skin on the neck over the vagus nerve, a diagram of the anatomy of the neck of the rat is included to illustrate the placement of electrodes with respect to the vagus nerve. This diagram is a cutaway of the rat's neck that shows the trachea (white), carotid artery (red), and vagus nerve (yellow) in relation to the placement of the electrodes (blue).