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Electrical Stimulation of the Vagus Nerve Dermatome in the External Ear is Protective in Rat Cerebral Ischemia

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ABSTRACT

Background: Although cervical vagus nerve stimulation is effective for reducing infarct volume in rats, it is not feasible for acute human stroke as it requires surgical incision of the neck. We hypothesized that stimulation of the dermatome in the external ear innervated by the vagus nerve (auricular vagus nerve stimulation; aVNS) reduces infarct volume after transient focal ischemia in rats.
Methods: Animals were randomized to active aVNS or sham stimulation. For aVNS, electrical stimulation of the left cavum concha (1 h duration) using percutaneous needles was initiated 30 min after induction of ischemia. Behavioral and tissue outcome were measured 24 h after induction of ischemia. In a separate experimental dataset, c-Fos immunohistochemistry was performed to identify the brain regions activated after the stimulation.
Results: Stimulation of the left cavum concha resulted in bilateral c-Fos staining in the nuclei tractus solitarii and the loci coerulei in all animals. There was no c-Fos staining in any part of the brainstem in sham control animals. The mean infarct volume (SD) as calculated by indirect method was 44.20 ± 7.58% in controls and 31.65 ± 9.67% in treated animals ($P < 0.0001$). The effect of aVNS on tissue outcome was associated with better neurological scores at 24 h after ischemia ($P < 0.0001$).
Conclusions: Electric stimulation of the vagus nerve dermatome in the external ear activates brainstem afferent vagal nuclei and reduces infarct volume in rats. This finding has potential to facilitate the development of treatments that leverage the brain's endogenous neuroprotective pathways at the setting of acute ischemic stroke.

Introduction

Cervical vagus nerve stimulation (cVNS), initiated 30 min after middle cerebral artery occlusion (MCAO), reduces infarct volume by up to 50% in rats [1,2]. The effect of cVNS on tissue outcome is associated with significant and persistent improvement in functional outcome [3]. Although cVNS is effective in experimental models of cerebral ischemia, the procedure itself is not feasible in the setting of acute human stroke as it requires surgical incision of the neck and implantation of stimulation electrodes and a pulse generator. A simple and non-invasive means of stimulation is needed to develop vagus nerve stimulation as a viable treatment for acute human stroke.

While the vagus nerve is primarily a viscero-motor and viscero-sensory nerve, it has also somatic-sensory function where it conducts sensation from the larynx, pharynx, external auditory canal, external surface of the tympanic membrane, and the meninges of the posterior cranial fossa [4]. It has been suggested that stimulation of the dermatome corresponding to the auricular branch of the vagus nerve (Arnold's nerve) in the external ear may be a non-invasive alternative to cVNS (Fig. 1) [5–8]. This is the region where the familiar "ear-cough reflex" can be elicited upon cutaneous stimulation and the dermatome is quite similar between rats

Abbrevitions: MCAO, middle cerebral artery occlusion; cVNS, cervical vagus nerve stimulation; aVNS, auricular vagus nerve stimulation; ABP, arterial blood pressure; HR, heart rate; rCBF, regional cerebral blood flow; TTC, 2,3,5-triphenyltetrazolium chloride.

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and humans [9,10]. Stimulation of this region in the ear provides seizure control in animals and induces antinociceptive effects, similar to cVNS, in humans [9,11,12]. In the present study, we sought to determine whether auricular vagus nerve stimulation (aVNS) activated the same vagal nuclei activated by the gold standard cVNS and whether this was associated with a reduction in infarct volume and improvement in functional outcomes in a model of MCAO in rats.

Material and methods

The effect of aVNS on infarct size

Thirty four adult male Wistar rats (350–400 g, 10–13 weeks of age, Charles River Laboratories, Wilmington, MA) were used. All studies were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals and the regulations of the Massachusetts General Hospital. Physiological monitoring, ischemia surgery, and neurological assessment were performed as previously reported [2]. In brief, animals were anesthetized by isoflurane (4–5% for induction, 1–2% for maintenance in 30% oxygen and 70% nitrous oxide) and rectal temperature was maintained at 37–37.5 °C using a homeothermic pad. As an indirect measure of brain temperature, the left temporalis muscle temperature was measured continuously throughout the study using a T-type implantable thermocouple probe and kept constant at 37–37.5 °C using an incandescent lamp. A PE-50 catheter was inserted into the right femoral artery to monitor arterial blood pressure (ABP), heart rate (HR), blood gases, and pH. A burr hole was drilled 5 mm right lateral and 1 mm posterior to bregma and the laser Doppler flowmeter probe was placed above the dura to continuously record regional cerebral blood flow (rCBF) starting from prior to the onset of ischemia until early after reperfusion (Blood Flow Meter, ADInstruments, Colorado Springs, CO) [13–15]. Transient right MCAO was induced by an intraarterial filament (diameter: 0.39 ± 0.02 mm; Doccol Corporation, Redlands, CA) and lasted for 2 h [1,16].

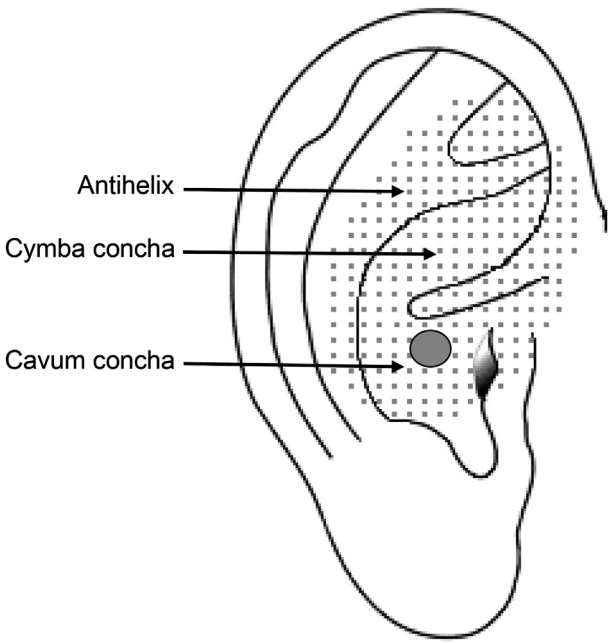

Figure 1. Schematic representation of the ear. Shaded area shows the dermatome corresponding to the auricular branch of the vagus nerve. Circle illustrates the electrode implantation site.

Animals were randomly assigned to aVNS treatment ($n = 15$) or control group ($n = 16$) by computer generated algorithm. aVNS was performed using two acupuncture needles (38 gauge, stainless steel with silver handle; Cloud Dragon, China) connected to a Grass Model S48 stimulator and constant current unit (Grass Instruments, West Warwick, RI). The needles were inserted 0.5–1 mm under the skin over the left cavum concha 15 min after MCAO (Fig. 1). A 30 s train of stimulation consisting of 0.5 ms square pulses (0.5 mA) delivered at 20 Hz was initiated 30 min after MCAO. Stimulation was repeated at every 5 min for 1 h [1,17]. All the procedures including electrode implantation were replicated in the control animals except delivery of electrical stimulation.

At the end of the ischemic period, the intraarterial filament was removed and reperfusion was instituted. All catheters and electrodes were removed, and incisions were sutured 20 min after reperfusion. Bupivacaine (0.25%; topical) and buprenorphine HCl (0.05 mg/kg; sc) was given to ameliorate surgical pain, and animals were allowed to awaken from anesthesia. Functional outcome was assessed using a five-point scale that measures forelimb flexion, resistance, and circling behavior at 3 h and 24 h after ischemia [16,18].

Animals were euthanized by CO_2 inhalation 24 h after ischemia. The brain was removed and the cerebrum was sliced into seven 2 mm-thick coronal sections. After incubation with 2,3,5-triphenyltetrazolium chloride (TTC; 2%, 30 min at room temperature) followed by 10% formalin (48 h at 4 °C), digital images of the sections were acquired. Areas of interest (infarct area, ipsilateral non-infarct area, and contralateral hemispheric area) were manually outlined in a blinded fashion, using Image J (NIH). Infarct volume of each brain was determined using both direct and indirect methods. For this, the infarct area (direct method) or the corrected infarct area (contralateral hemispheric area minus ipsilateral non-infarct area; indirect method) was multiplied by slice thickness and the volume was expressed as a percentage of the contralateral hemispheric volume.

Brain regions activated by aVNS

We performed c-Fos immunohistochemistry in order to identify the brain regions activated after the stimulation in a separate set of animals ($n = 12$). We used the same ischemia − stimulation protocol outlined before. There were 4 experimental groups: 1) *aVNS group* ($n = 3$): 1 h electrical stimulation of the cavum concha was performed, 2) *control group* ($n = 3$): electrodes were implanted into the cavum concha but no stimulus was delivered, 3) *cVNS group* ($n = 3$): self-constructed stimulation electrodes were implanted into the right cervical vagus nerve and 1 h electrical stimulation was performed (positive control), 4) *trigeminal nerve stimulation group* ($n = 3$): the acupuncture electrodes were implanted into the left lower side of the mouth and 1 h electrical stimulation was performed (negative control). In this experiment, animals were euthanized and transcardially perfused with 4% formaldehyde in phosphate buffered saline (pH 7.4) 30 min after the end of the electrical stimulation or 2 h after MCAO (in contrast to 24 h after MCAO in the prior experiment). The brains were removed and cryoprotected in 30% sucrose at 4 °C for 3 days. Using a cryostat, 30 μm-thick coronal sections with a 500 μm interslice gap in the brainstem and cerebellum and 1500 μm gap in the cerebrum were obtained. Sections were stored in cryoprotectant solution at −20 °C. After quenching endogenous peroxidase activity with 0.3% H_2O_2 (30 min at room temperature) and blocking with 3% normal horse serum in 0.25% Triton X-100 (2 h at room temperature), free floating sections were incubated with polyclonal anti-cFos antibody (Ab-5; 1:30,000; Calbiochem, San Diego, CA) for 72 h at 4 °C [19]. This was followed by incubation in biotinylated horse anti-rabbit IgG (1:200;