Neuroscience Letters 459 (2009) 147–151

Contents lists available at ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

Vagus nerve stimulation reduces infarct size in rat focal cerebral ischemia

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ARTICLE INFO

Article history:
Received 12 December 2008
Received in revised form 30 April 2009
Accepted 6 May 2009

Keywords:
Cerebral ischemia
Vagus nerve
Parasympathetic system
Electrical stimulation
Rat

ABSTRACT

Background and purpose: We sought to determine the effect of vagus nerve stimulation (VNS) on infarct size after transient focal cerebral ischemia in rats. *Methods*: Ischemia was produced by transient filament occlusion of the right middle cerebral artery. Stimulating electrodes were implanted on the cervical part of the right vagus nerve. Electrical stimulation was initiated 30 min after the induction of ischemia, and delivered for 30 s at every 30 min for 3 h in experimental group 1 and at every 5 min for 1 h in experimental group 2. All the procedures were duplicated but no stimulus was delivered in the control group. Functional deficit was evaluated and animals were killed to determine the infarct size 24 h after ischemia. *Results*: Ischemic lesion volume was smaller in VNS-treated animals as compared with control animals; the relative percentage of contralateral hemispheric volume that underwent infarction was $16.2 \pm 3.2\%$ in the VNS and $33.0 \pm 5.0\%$ in the control arms in experimental group 1 ($p < 0.05$). The respective values for experimental group 2 were $19.8 \pm 0.5\%$ and $37.9 \pm 2.6\%$ ($p < 0.05$). VNS-treated animals were significantly more likely to have better functional scores at 24 h as compared with control animals. The functional score improved by 50% in experimental group 1 and 44% in experimental group 2 ($p < 0.05$ for both groups). *Conclusion*: VNS appears to offer protection against acute ischemic brain injury.

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Electrical stimulation of afferent vagus nerve fibers in the neck is a safe and effective treatment for refractory partial onset seizures and treatment-resistant depression and is currently under study as a potential therapy for migraine, Alzheimer's disease, traumatic brain injury, and neuropathic pain [6,9,17,19,20]. Recently, it has been suggested that vagus nerve stimulation (VNS) can provide protection against ischemic brain injury as well [16,21]. VNS regulates several pathways that are involved in pathophysiology of ischemic brain injury; it inhibits cytokine synthesis and thereby prevents cytokine-mediated tissue injury in a variety of conditions including experimental models of sepsis, hemorrhagic shock, and ischemia reperfusion [21]. In addition, VNS is associated with reduced neuronal excitability and increased cerebral blood flow (CBF) [12,23]. A previous global ischemia study in gerbils has demonstrated that a brief period of VNS reduces hippocampal injury by approximately 50% following 5 min ischemia [16]. The present study was designed to investigate the effect of VNS on infarct size after transient focal ischemia in rats.

All experiments were performed in accordance with the NIH Guide for the Care and Use of Laboratory Animals and were approved by the Massachusetts General Hospital Subcommittee on Research Animal Care.

Adult male Wistar rats (350–400 g, Charles River Laboratories, Wilmington, MA) were anesthetized by isoflurane (4–5% for induction, 1–2% for maintanance; in 30% oxygen–70% nitrous oxide) and were kept under anesthesia through the experimental period. Rectal temperature was intermittently measured and maintained at 37.5 °C. Right femoral artery was cannulated for continous recording of arterial blood pressure as well as periodic measurements of blood gases, pH, and glucose. Cerebral ischemia was produced by intraarterial filament occlusion of the right middle cerebral artery (MCA) for 2 h followed by reperfusion [15]. Stimulating electrodes were self-constructed by the method of Smith et al. [20]. Electrodes were implanted 15 min after ischemia on the right vagus nerve due to surgical ease of electrode implantation on the the right side. Animals were assigned to the treatment or control groups by computer-generated random sequence. The sample size calculation ($n = 6$) was based on a 40% anticipated difference in mean infarct volume by treatment at a power of 95% at an alpha level of 0.05. According to our prior experience, the untreated group mean was considered to be 32% of total hemispheric volume with a standard deviation of 6%. Animals that failed to develop significant neurological deficit (functional score <2) 4 h after ischemia or developed infarction limited to only the striatum or intracranial bleeding were considered a technical failure and replaced by another animal to meet the calculated sample size.

At the end of the experimental period, stimulating electrodes and arterial catheters were removed, and incisions were sutured. Before the incision and at the end of surgery 0.25% bupivacaine was topically applied to the wounds to alleviate surgical pain. Also,

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doi:10.1016/j.neulet.2009.05.018

buprenorphine HCl (0.05 mg/kg; sc) was injected, animals were allowed to awaken, behavioral testing was performed and animals were returned to their home cages.

In animals receiving the treatment, VNS was initiated 30 min after the induction of ischemia. Square pulses were delivered using Grass Model S48 stimulator and constant current unit (Grass Instruments, West Warwick, RI) at 0.5 mA, 30 s train of 0.5 ms pulses delivered at 20 Hz [20]. We tested the VNS at two different stimulation frequency and duration parameters; one based on an earlier study of VNS in rat traumatic brain injury [20] (experimental group 1) and the other one based on parameters approved by the FDA for use in humans (experimental group 2). In experimental group 1, electrical stimulation was repeated at every 30 min for 3 h in VNS-treated animals (Fig. 1). Thus, animals received electrical stimulation during the last 90 min of ischemia and the first 90 min of reperfusion. In experimental group 2, electrical stimulation was repeated at every 5 min for 1 h in VNS-treated animals (Fig. 1). In the control animals, all procedures were duplicated including implantation of electrodes on the vagus nerve but no stimulus was delivered.

Neurological deficit was evaluated on a five-point scale (0 = no deficit - 4 = no spontaneous walking) [3,15] both at the end of the last stimulation and 24 h later. Briefly, rats were (1) held by the tail, suspended on air, and observed for forelimb flexions; (2) placed on an underpad, held by the tail, and laterally pushed to slide the forelimbs on each side separately to observe resistance; (3) allowed to move freely to observe circling behavior. Animals were killed by sodium pentobarbital injection (200 mg/kg; i.p.) and the brain was rapidly removed. Starting from the frontal, the brain was immediately sliced into seven 2-mm thick sections using brain matrix and these were incubated with 2,3,5-triphenyltetrazolium chloride (TTC) at room temperature for 30 min. The sections then transferred into 10% formalin and kept at 4 °C for 48 h. Images of these sections were then obtained by a digital camera. One of the investigators (JL) blinded to treatment groups manually outlined infarct area as well as ipsilateral non-infarct area and controlateral hemispheric area using Image J (NIH) in all of the sections. Infarct volume was calculated by multiplying infarct area (contralateral hemispheric area minus ipsilateral non-infarct area) by slice thickness and expressed as a percentage of contralateral hemispheric volume.

Data were expressed as mean ± S.E.M. Physiological measurements [mean arterial blood pressure (MABP), heart rate (HR), blood gases and pH, blood glucose, and rectal temperature] were analyzed by repeated measures ANOVA followed by Student–Newman–Keuls test. When calculating the effect of VNS on transient changes in MABP and HR, data from the first stimulation were not included. This is because electrode's position was readjusted during the first stimulation in some animals and that resulted in artifact in recordings. Infarct volumes were compared using unpaired *t*-test. Neurological scores were compared using repeated measures ANOVA followed by Mann–Whitney *U*-test. *p*-Value of <0.05 was considered statistically significant.

There was no difference in body temperature, arterial blood gases, pH, and glucose between control and treatment groups. Two animals (one in control and treatment groups each) were excluded as a result of infarction limited to only the striatum.

Electrical stimulation of the right cervical vagus nerve caused an immediate and transient decrease in systolic and diastolic blood pressure and HR (Fig. 2). The amplitude of decrease in MABP was 20.944 ± 1.757 mm Hg ($n = 36$) in experimental group 1 and 49.63 ± 7.945 mm Hg ($n = 72$) in experimental group 2. The amplitude of decrease in HR was 290.79 ± 11.49 beats/min ($n = 36$) in experimental group 1 and 263.268 ± 26.05 beats/min ($n = 72$) in experimental group 2. The stimulation-induced reduction in MABP and HR lasted for only 10–30 s and completely returned back to normal following stimulation. An assessment for the entire period of stimulation did not reveal a significant difference in MABP and HR by the VNS as compared with the control group (repeated measures of ANOVA, $p = 0.1595$ for MABP and $p = 0.2644$ for HR in experimental group 1, $p = 0.1073$ for MABP and $p = 0.6077$ for HR in experimental group 2).

Transient occlusion of the right MCA for 2 h resulted in infarct in the ipsilateral cerebral cortex and underlying striatum (Fig. 3A and E). In VNS-treated animals, the infarct involved the striatum and an area of overlying cerebral cortex that was smaller as compared with untreated animals (Fig. 3B and F). In the experimental group

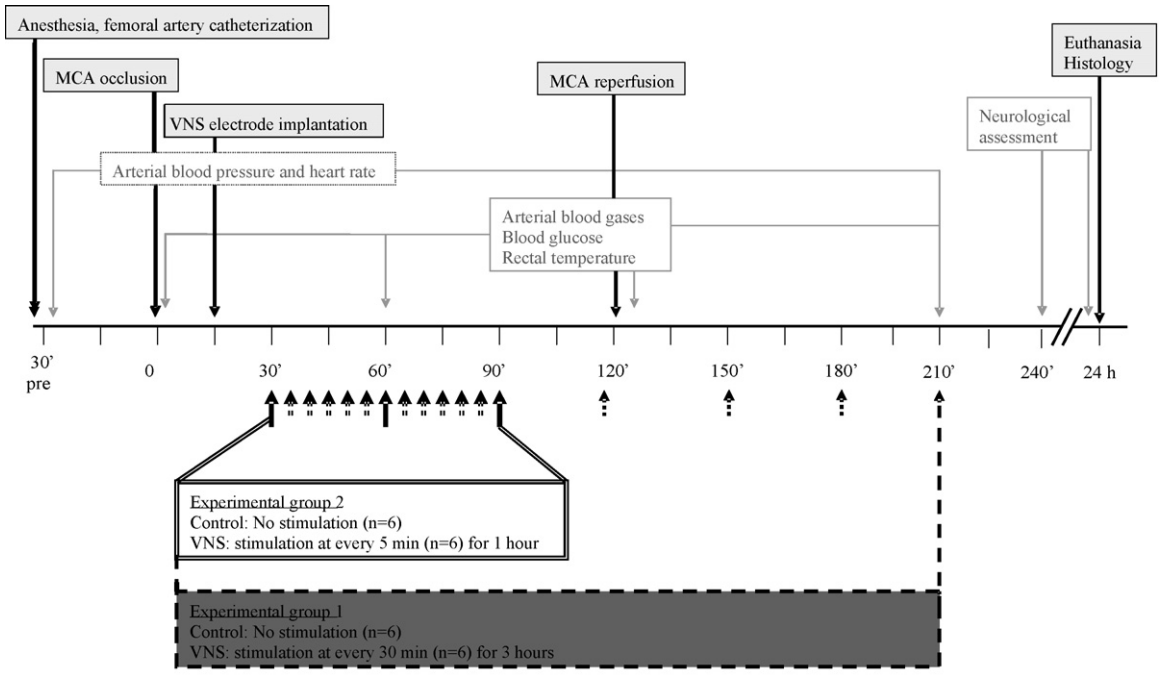

Fig. 1. The experimental protocol. At 120 min time point, electrical stimulation was given just before MCA reperfusion. MCA: middle cerebral artery, VNS: vagus nerve stimulation.

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