

Effect of parasympathetic system stimulation on infarct size in rat focal cerebral ischemia



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BACKGROUND

Parasympathetic system:

Extensively innervates cerebral vascular system and increases cerebral blood flow (CBF):

- Fibers originate from the superior salivatory nucleus in the pons,
- Run along the facial nerve until they synapse in the sphenopalatine ganglion,
- Innervate blood vessels via the greater superficial petrosal nerve.

Regulates several pathways that are involved in pathophysiology of ischemic brain injury other than blood flow:

- Inhibits cytokine release and thereby prevents cytokine-mediated tissue injury,
- Reduces neuronal excitability.

Hypothesis: Modulation of brain's endogenous parasympathetic system may provide the opportunity to gain access to the region of ischemic but still viable tissue with critically impaired blood flow.

Electrical stimulation of unilateral sphenopalatine ganglion (SPG) in rat permanent middle cerebral artery (MCA) occlusion is associated with reduced infarct size (Henninger and Fisher, 2007).

SPG's intracranial location may limit the feasibility of its stimulation in humans, whereas vagus nerve is easily accessible in the neck.

Vagus nerve stimulation (VNS):

- Stimulates superior salivatory nucleus, the key structure for cerebrovascular parasympathetic innervation.
- Safe and effective treatment for refractory partial onset seizures and treatment-resistant depression (George et al., 2002).
- Under study as a potential therapy for migraine, Alzheimer's disease, traumatic brain injury, and neuropathic pain (George et al., 2002; Smith et al., 2005).
- Decreases neuronal cell death in gerbil forebrain ischemia (Masada et al., 1996).

Aim: To investigate the effect of VNS on infarct size after transient focal cerebral ischemia in rats.

METHODS

Surgical procedures:

- Adult male Wistar rats (350-400 g, n=12; Charles River Laboratories) were anesthetized by isoflurane.
- Rectal temperature was maintained at 37.5 °C.
- Arterial blood pressure and heart rate were continuously monitored.
- Arterial blood gases and blood glucose were measured intermittently.
- rCBF was continuously monitored via laser Doppler flowmeter in a subgroup of animals (n=4) from each experimental group.
- Cerebral ischemia was produced by intraarterial filament occlusion of the right MCA for two hours (Longa et al., 1989).
- Stimulating electrodes were self-constructed by the method of Smith et al. (2005).
- Electrodes were implanted on the right vagus nerve, following ischemia.

VNS:

- Initiated 30 minutes after the induction of ischemia
- Repeated at every 30 minutes for 3 hours
- Stimulation parameters: 0.5 mA, 30 sec train of 0.5 msec pulses delivered at 20 Hz

Experimental groups:

- Treatment (n=10)
- Control (n=10): all procedures were duplicated but not stimulus was delivered

Neurological assessment:

- Four-point scale (0=no deficit – 4=no spontaneous walking) evaluation
- At the end of the last stimulation and 24 hours after MCA occlusion

METHODS cont'd

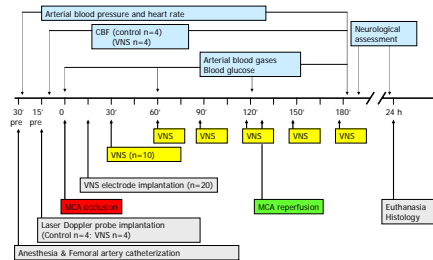
Determination of infarct size:

- 2 mm thick coronal brain sections were incubated with 2,3,5-triphenyltetrazolium chloride
- Infarct, ipsilateral non-infarct, and contralateral hemispheric areas were manually outlined by an investigator blinded to the treatment groups using Image J
- Infarct volume was calculated by multiplying infarct area (contralateral hemispheric area subtracted by ipsilateral non-infarct area) by slice thickness
- Infarct volume was expressed as a percentage of contralateral hemispheric volume

Data analysis:

- Data were expressed as mean ± SD.
- Analyzed by repeated measures ANOVA or unpaired t-test
- P < 0.05 were considered statistically significant

Experimental Design



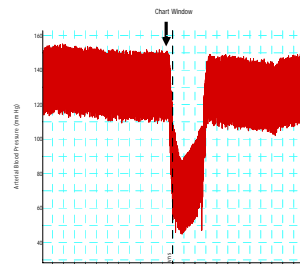
1 VNS has no effect on arterial blood gases and blood glucose before and after the MCA occlusion in rats.

ABGs		Before the MCA occlusion	60' after the MCA occlusion	After the reperfusion	180' after the MCA occlusion
pH	Control	7.39 ± 0.01	7.41 ± 0.02	7.40 ± 0.01	7.42 ± 0.01
	VNS	7.39 ± 0.01	7.40 ± 0.01	7.40 ± 0.01	7.42 ± 0.01
pCO2 (mmHg)	Control	43.5 ± 2.58	41.1 ± 5.78	40.0 ± 6.78	36.4 ± 2.13
	VNS	45.7 ± 1.10	41.8 ± 2.20	38.8 ± 5.65	35.8 ± 2.01
pO2 (mmHg)	Control	87.6 ± 5.71	89.2 ± 4.32	96.1 ± 4.65	102.6 ± 3.06
	VNS	81.5 ± 4.01	84.7 ± 2.87	89.5 ± 7.43	101.1 ± 3.92
Blood glucose (mg/dL)	Control	123 ± 4.65	122 ± 8.56	120 ± 5.98	119 ± 4.86
	VNS	121 ± 6.23	121 ± 2.85	119 ± 2.43	116 ± 5.42

2 VNS causes an immediate and transient decrease in both systolic and diastolic blood pressure in rats.

The decrease was observed in all the VNS-treated animals. The duration of this effect was around 30 seconds and the amplitude of decrease in mean blood pressure was 32.83 ± 5.13 mm Hg (n=60).

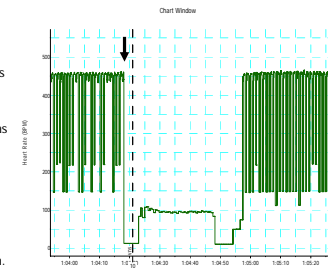
Representative arterial blood pressure tracing from a VNS-treated animal. Arrow shows the time of electrical stimulation.



3 VNS causes an immediate and transient decrease in heart rate in rats.

The decrease was observed in all the VNS-treated animals. The duration of this effect was around 30 seconds and the amplitude of decrease in heart rate was 22.82 ± 5.13 beats/min (n=60).

Representative heart rate tracing from a VNS-treated animal. Arrow shows the time of electrical stimulation.



4 There was no difference between mean arterial blood pressure and heart rate of control and VNS-treated animals before and after the MCA occlusion in rats.

		MABP (mmHg)	HR (beats/min)
Control	Before MCA occlusion	127.96 ± 7.92	365.98 ± 11.84
	3 hours after MCA occlusion	129.46 ± 4.85	397.34 ± 12.01
VNS	Before MCA occlusion	128.71 ± 8.69	371.42 ± 12.74
	3 hours after MCA occlusion	131.12 ± 6.71	386.83 ± 21.85

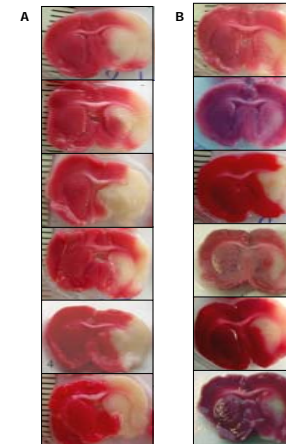
Repeated measures ANOVA P=0.1921 P=0.2644

5 VNS treatment decreases cell death in the penumbra in rat transient MCA occlusion.

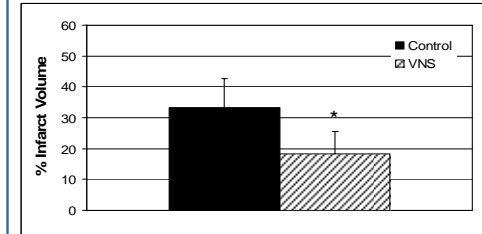
Transient occlusion of the right MCA for 2 hours resulted in infarct in the ipsilateral cerebral cortex and underlying striatum (A).

In VNS-treated animals, the infarct area was limited to striatum and involved a smaller area of cerebral cortex as compared with untreated animals (B).

Images of TTC-stained sections at the level of bregma 0.4 mm from control (A) and VNS-treated (B) animals.



6 VNS treatment decreases infarct volume in rat transient MCA occlusion.



The mean infarct volume and SD was 33.30 ± 9.27% of the contralateral hemispheric volume in control animals (n=10). This was significantly larger than the mean infarct volume in VNS-treated animals (18.22 ± 7.29%, n=10; unpaired t-test: t=4.044, p=0.0008)

7 VNS has no effect on rCBF in rat transient MCA occlusion.

rCBF (%)	Pre-MCA occlusion	20 min after MCA occlusion	After 1 st stimulation (occlusion)	After 3 rd stimulation (occlusion)	After 5 th stimulation (reperfusion)
Control	100	20.81 ± 17.42	21.63 ± 18.01	43.10 ± 15.27	103.26 ± 24.46
VNS	100	23.68 ± 14.65	22.63 ± 18.33	45.26 ± 13.07	94.73 ± 21.05

8 VNS-treated animals have better neurological scores after cerebral ischemia/reperfusion.

	4 h after MCA occlusion	24 h after MCA occlusion
Control	2.17 ± 0.18	2.8 ± 0.21
VNS	2.00 ± 0.0	1.2 ± 0.10 *

CONCLUSIONS

In rat transient MCA occlusion, stimulation of right cervical vagus nerve:

- has no effect on arterial blood gases and blood glucose,
- causes transient decrease in arterial blood pressure and heart rate,
- has no effect on cerebral blood flow,
- decreases infarct size,
- improves motor function of animals.

References:

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*A full listing of A.G.S.'s competing interests is available at www.biomarkers.org.