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Review Article

The parasympathetic nervous system in the quest for stroke therapeutics

Cletus Cheyuo[1,2,3], Asha Jacob[1,3], Rongqian Wu[1,3], Mian Zhou[1,3], Gene F Coppa[3] and Ping Wang[1,2,3]

[1]*Center for Immunology and Inflammation, The Feinstein Institute for Medical Research, Manhasset, New York, USA;* [2]*Elmezzi Graduate School of Molecular Medicine, Manhasset, New York, USA;* [3]*Department of Surgery, North Shore University Hospital and Long Island Jewish Medical Center, Manhasset, New York, USA*

Stroke is a devastating neurovascular disease with limited therapeutic options. The pathogenesis of stroke involves complex interrelated molecular mechanisms including excitotoxicity, oxidative and nitrosative stress, cortical spreading depolarizations, inflammation, necrosis, and apoptosis. Successful development of stroke therapeutics depends on understanding these molecular mechanisms and how to counteract them to limit tissue damage during stroke. Activation of the parasympathetic nervous system (PNS) has been shown to antagonize a multiplicity of pathologic mechanisms. Elements of parasympathetic activation such as vagus nerve stimulation have already been used successfully in treating brain disorders such as epilepsy and depression. This review discusses the anatomical basis and molecular mechanisms involved in activation of the PNS, and assesses the strength of available evidence for the further development of this modality into a stroke therapy.
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Introduction

Stroke is a devastating neurovascular disease with high mortality and a staggering economic burden on the United States, estimated at $73.7 billion for the year 2010 (Lloyd-Jones *et al*, 2010). With recombinant tissue plasminogen activator as the only available treatment for ischemic stroke, the need to develop new stroke therapies is imperative. Neuroprotection is an experimental approach aimed at developing therapies that counteract the molecular mechanisms of stroke, which include excitotoxicity, oxidative and nitrosative stress, cortical spreading depolarizations, inflammation, necrosis and apoptosis (for review see Moskowitz *et al*, 2010; Furlan *et al*, 2003). However, in spite of impressive neuroprotection in preclinical stroke models, many neuroprotective agents have failed in clinical trials. These failures have been blamed on poor research design, inadequate preclinical testing, and redundancy in the mechanisms of cerebral ischemia (for review see Ginsberg, 2008; Savitz, 2007). Given that the relative contributions of individual molecular mechanisms to the severity of stroke remain unknown, the successful development of stroke therapy may hinge on exploring treatment modalities with multiple mechanisms of action.

Activation of various aspects of the parasympathetic nervous system (PNS) has shown multiple therapeutic benefits in brain diseases. Examples include vagus nerve stimulation (VNS) for epilepsy (Ardesch *et al*, 2007) and cholinesterase inhibitors for Alzheimer's disease (López-Pousa *et al*, 2010). However, PNS activation has not yet been successfully developed as a therapy for stroke. Optimistically, various forms of PNS activation, namely VNS (Ay *et al*, 2009; Miyamoto *et al*, 2003), sphenopalatine ganglion (SPG) stimulation (Yarnitsky *et al*, 2006), cholinesterase inhibitors (Akasofu *et al*, 2003), and α7 nicotinic acetylcholine receptor (nAChRα7) agonists (Parada *et al*, 2010) have shown

Correspondence: Dr P Wang, Laboratory of Surgical Research, The Feinstein Institute for Medical Research, 350 Community Drive, Manhasset, NY 11030, USA.
E-mail: pwang@nshs.edu
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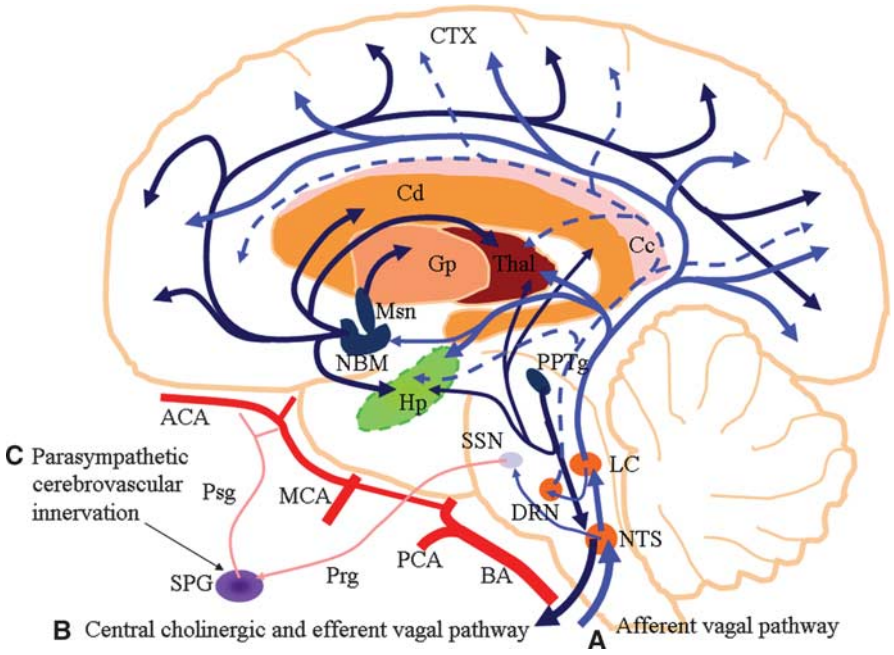

Figure 1 Schematic representation of a parasagittal section of the human brain, showing the neuroanatomical projections involved in parasympathetic nervous system activation for neuroprotection. (**A**) Afferent vagus nerve pathway (represented by deep blue color ➡): Afferent vagus nerve neurons synapse bilaterally on the nucleus tractus solitarius (NTS) in the medulla oblongata. The NTS makes projections to various brain structures including the locus coeruleus (LC), which is the main source of norepinephrine for the entire brain. The LC provides noradrenergic innervation to various parts of the brain, including the thalamus (Thal), hippocampus (Hp), cerebral cortex (CTX), and the forebrain cholinergic system, of which the nucleus basalis of Meynert (NBM) is a principal component. The LC also innervates the dorsal raphe nucleus (DRN). The DRN provides extensive serotonergic innervation to many brain structures including the thalamus, cerebral cortex, and hippocampus. (**B**) Central cholinergic and efferent vagal pathway (represented by dark blue color ⬅): The central cholinergic system consists mainly of projections from the nucleus basalis of Meynert, the medial septal nuclei (Msn), and the pedunculopontine tegmental nucleus (PPTg). These provide cholinergic innervation to brain structures including the caudate nucleus (Cd), globus pallidus (Gp), thalamus, hippocampus, and cerebral cortex and brainstem structures. The central cholinergic system drives the efferent vagal output. (**C**) Parasympathetic cerebrovascular innervation (represented by pink color ⬅): The sphenopalatine ganglion (SPG), which is a parasympathetic ganglion found in the pterygopalatine fossa, receives preganglionic neurons (Prg) from the superior salivatory nucleus (SSN). Postganglionic neurons (Psg) from the SPG enter the cranial cavity, through the ethmoidal foramina, where they provide nitroxidergic-cholinergic innervation to the major cerebral blood vessels; anterior cerebral artery (ACA), middle cerebral artery (MCA), posterior cerebral artery (PCA), and basilar artery (BA), and the communicating arteries of the circle of Willis. Cc, corpus callosum.

neuroprotection in both preclinical models of cerebral ischemia and *in vitro* neuronal hypoxia. The mechanisms involved in these neuroprotective effects remain unclear. This review discusses the neuroanatomical (Figure 1) and molecular basis (Figure 2) for the beneficial effects of PNS activation, and provides an assessment of the strength of current available data on its applications in ischemic stroke. Using the updated recommendations of Stroke Therapy Academic Industry Roundtable (Fisher *et al*, 2009) as a yardstick, we make suggestions for further studies aimed at developing this therapeutic approach.

Anatomy of the vagus nerve

To understand the mechanisms of vagus nerve activation, it is important to know both its peripheral innervation and central projections. The vagus nerve is the main parasympathetic innervation for the thoracic and abdominopelvic viscera. The vagus nerve is composed of predominantly afferent fibers (80%), which originate from cell bodies in the superior (jugular) vagal ganglion and the larger inferior (nodose) vagal ganglion situated just below the jugular foramen. The efferent arm of the vagus nerve consists of preganglionic visceromotor fibers, originating from the dorsal motor nucleus of the vagus nerve and the nucleus ambiguus in the medulla oblongata. The preganglionic fibers synapse with postganglionic fibers located in the parasympathetic ganglia, close to the viscera they innervate (for review see Henry, 2002).

Central Projections of Afferent Vagus Nerve Fibers

Majority of the afferent vagus nerve fibers project bilaterally to the nucleus of the tractus solitarius (NTS) in the medulla oblongata. The rest of the fibers project ipsilaterally to nucleus of the spinal tract of

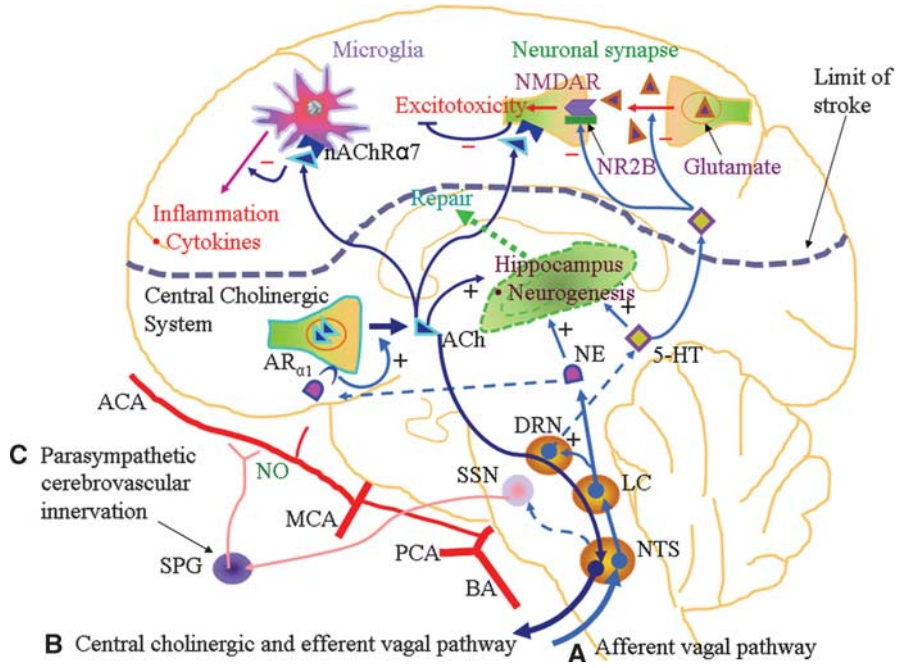

Figure 2 The neuroprotective mechanisms of parasympathetic nervous system activation. (**A**) Afferent vagal pathway (represented by deep blue color ➡): Afferent vagus nerve signals project to the dorsal vagal complex, where majority of the fibers synapse on the nucleus tractus solitarious (NTS). Through the NTS projections to the locus coeruleus (LC), vagus nerve stimulation (VNS) causes the release of norepinephrine (NE). Norepinephrine subsequently stimulates the release of serotonin (5-hydroxytryptamine, 5-HT) from the dorsal raphe nucleus (DRN). Norepinephrine stimulates neurogenesis as well as excitation of cholinergic neurons leading to acetylcholine (ACh) release and inhibition of inflammation. 5-HT stimulates neurogenesis and suppresses excitotoxicity by inhibiting glutamate release and downregulating NR2B. (**B**) Central cholinergic and efferent vagal pathway (represented by dark blue color ⬅): The central cholinergic system releases ACh, which inhibits inflammation in the brain by activating α7 nicotinic acetylcholine receptor (nAChRα7), leading to suppression of cytokine release. The central cholinergic system also drives the cholinergic antiinflammatory pathway systemically via the efferent vagus nerve. (**C**) Parasympathetic cerebrovascular innervation (represented by pink color ⬅): The sphenopalatine ganglion (SPG) receives preganglionic neurons from the superior salivatory nucleus (SSN). Postganglionic neurons from the SPG provide nitroxidergic innervation to the cerebral vaculature: anterior cerebral artery (ACA), middle cerebral artery (MCA), posterior cerebral artery (PCA), and basilar artery (BA). Through the release of nitric oxide (NO) stimulation of the SPG causes vasodilation and increased perfusion leading to neuroprotection. AR$_{α1}$, $α_1$-adrenergic receptor; NMDAR, *N*-methyl-D-aspartate receptor; '+' denotes stimulation, '−' denotes inhibition.

the trigeminal nerve, medial reticular formation of the medulla, area postrema, dorsal motor nucleus of the vagus, and nucleus ambiguus (Kalia and Sullivan, 1982). The NTS projects directly to the parabrachial nuclei, the cerebellum, the raphe, the periaqueductal gray, and the locus coeruleus (LC). Through the NTS, vagus nerve also makes extensive polysynaptic projections to the thalamus, hypothalamus, the limbic system, and the cerebral cortex (Figure 1; Pathway A) (for review see Henry, 2002).

Central Vagal-Mediated Neurotransmission

The NTS is involved in the processing and integration of a wide range of visceral and somatic sensory information (Bailey *et al*, 2008). The neurotransmission involved in this function includes excitatory components such as glutamate and the *N*-methyl-D-aspartate receptor (Lam *et al*, 2010), as well as inhibitory components such as α-aminobutyric acid (Bailey *et al*, 2008). The NTS makes projections to the LC (Van Bockstaele *et al*, 1999), which is the major source of the neurotransmitter, norepinehrine, for the entire brain, including the cerebral cortex (Levitt and Moore, 1978). It has been shown electrophysiologically that VNS initially causes increased firing of the LC leading to the release of norepinephrine. The norepinephrine then stimulates $α_1$-adrenergic receptors in the dorsal raphe nucleus, resulting in serotonin (5-hydroxytryptamine, 5-HT) release (Manta *et al*, 2009). This explains the finding that VNS caused the release of norepinephrine from the LC as early as 1 hour after stimulation, whereas 5-HT was released from the dorsal raphe nucleus only at day 14 of stimulation (Dorr and Debonnel, 2006). Florin-Lechner *et al* (1996) showed that the LC can release norepinephrine in both tonic and phasic modes and that the level of cortical norepinephrine depends on the frequency of LC stimulation. Cortical norepinephrine has been shown to either tonically suppress glutamate-evoked discharge or initially facilitate glutamate-evoked discharge followed by suppression, depending on whether it stimulates β-adrenergic receptors or $α_1$-adrenergic receptors, respectively (Devilbiss and Waterhouse, 2000). The dorsal raphe

nucleus projects serotonergic neurons extensively to many parts of the brain including the cerebral cortex (Figure 1; Pathway A) (Van Bockstaele *et al*, 1993).

Cholinergic system

Acetylcholine (ACh) is a neurotransmitter at the neuromuscular junction, in autonomic ganglia, and in postganglionic parasympathetic nerve-target organ junctions and some postganglionic sympathetic nerve-target junctions (Barrett *et al*, 2010). There is also an intercommunicating central cholinergic network involving the medial prefrontal cortex, anterior cingulate cortex, insular cortex, paraventricular nucleus, central nucleus of the amygdala, and lateral hypothalamic area. This network sends signals via the periaqueductal gray in the midbrain, through the parabrachial nuclei in the pons to medullary nuclei including NTS, nucleus ambiguus, and ventrolateral medulla. The main output of the central cholinergic system is through the vagus nerve (Figure 1; Pathway B) and the stellate ganglia (for review see Benarroch, 1993). This is in agreement with the finding of Pavlov *et al* (2006) that intracerebroventricular administration of a selective muscarinic agonist activated the cholinergic anti-inflammatory pathway peripherally, resulting in decreased tumor necrosis factor-α production in endotoxemia. The cholinergic antiinflammatory pathway is a product of the inflammatory reflex, the afferent arm of which senses tissue damage and sends signals via the afferent vagus nerve to the brain. The efferent arm consists of efferent vagal signals that decrease cytokine expression through the binding of ACh to nAChRα7 expressed on macrophages (Borovikova *et al*, 2000). Microglia also express nAChRα7, activation of which has been shown to attenuate inflammatory response in the brain (Shi *et al*, 2009; Shytle *et al*, 2004). Cholinergic signaling is also present in a variety of non-neuronal cells such as endothelial cells (Heeschen *et al*, 2002). Cholinergic signaling in endothelial cells has been shown to inhibit expression of adhesion molecules (Saeed *et al*, 2005).

Parasympathetic cerebrovascular innervation

The SPG is a parasympathetic ganglion that receives preganglionic neurons from the superior salivatory nucleus (Agassandian *et al*, 2002). Postganglionic neurons from the SPG, which join the ethmoidal nerve, have been shown to enter the cranial cavity via ethmoidal foramina where they innervate the cerebral blood vessels (Hara and Weir, 1986; Hara *et al*, 1993). These postganglionic neurons contain nitric oxide synthase (Yoshida *et al*, 1993), ACh (Kimura *et al*, 1997) and vasoactive intestinal peptide (Suzuki *et al*, 1988). Agassandian *et al* also showed that the cerebrovascular part of NTS connects directly with the parasympathetic preganglionic neurons at the superior salivatory nucleus (Figure 1; Pathway C). Through this NTS-superior salivatory nucleus pathway, the baroreceptors participate in the control of cerebrovascular tone (Agassandian *et al*, 2002, 2003).

Neuroprotective effects of parasympathetic activation

Vagus Nerve Stimulation—Effect of Neurotransmitters

Electrical VNS has been shown to attenuate cerebral ischemic injury (Ay *et al*, 2009; Miyamoto *et al*, 2003). In a transient model of focal cerebral ischemia (2 hours of ischemia followed by reperfusion) in rats, Ay *et al* (2009) found that VNS significantly decreased infarct size and neurologic deficit at 24 hours after ischemia/reperfusion. In a mechanistic approach, Miyamoto *et al* (2003) found that VNS significantly decreased extracellular glutamate levels between 15 and 20 minutes after 5 minutes of transient global ischemia model in Mongolian gerbils. Excessive glutamate release has a role in excitotoxicity during cerebral ischemia through the activation of *N*-methyl-D-aspartate receptor (Bosel *et al*, 2005; Choi, 1985). The vagus nerve may also be stimulated pharmacologically to ameliorate ischemic stroke injury. We have previously shown that ghrelin treatment caused various vagus nerve-mediated antiinflammatory effects such as suppression of neutrophil infiltration and proinflammatory cytokine levels in cerebral ischemia (Cheyuo *et al*, 2010). Similarly, the melanocortins, an endogenous group of peptides, were shown to mediate a vagus nerve-dependent downregulation of plasma and brain tumor necrosis factor-α levels after ischemic stroke (Ottani *et al*, 2009). The receptors of both ghrelin (Zhang *et al*, 2004) and melanocortins (Wan *et al*, 2008) are expressed at the NTS. The mechanisms for the beneficial effects of VNS in ischemic stroke remain largely unknown.

However, one may speculate on possible mechanisms of action based on the effects of neurotransmitters, which are released by VNS. It has been clearly shown that VNS leads to an early release of norepinephrine from the LC (Dorr and Debonnel, 2006), which projects to various parts of the brain including the cerebral cortex (Levitt and Moore, 1978). Various studies have shown neuroprotective effects of norepinephrine, including antiinflammatory effects via α_1-adrenergic receptors (Dello *et al*, 2004; Kalinin *et al*, 2006). Moreover, lesions of the LC worsened cerebral ischemia (Blomqvist *et al*, 1985; Nishino *et al*, 1991). The norepinephrine released through VNS also stimulates the release of 5-HT from the dorsal raphe nucleus (Manta *et al*, 2009). 5-Hydroxytryptamine has been shown to antagonize excitotoxicity by inhibiting glutamate

release via 5-HT_{1A} receptors (Marcoli *et al*, 2004). In addition, 5-HT selectively downregulates the NR2B subunit of the *N*-methyl-D-aspartate receptor (Yuen *et al*, 2005), which is associated with excitotoxicity in contrast to the survival-promoting NR2A subunit (Chen *et al*, 2008). Taken together, VNS may suppress inflammation and excitotoxicity through the release of norepinephrine and 5-HT, respectively (Figure 2; Pathway A).

Cholinergic Antiinflammatory Pathway

Brain parenchymal inflammation (Dawson *et al*, 1996) and systemic inflammation (McColl *et al*, 2007) both have deleterious roles in ischemic stroke. The cholinergic antiinflammatory pathway suppresses inflammation through nAChRα7-mediated suppression of the nuclear factor-κB pathway (Borovikova *et al*, 2000). The cholinergic antiinflammatory pathway is driven by the central cholinergic system in the brain, whose peripheral output is through the efferent vagus nerve (Pavlov *et al*, 2006). The effector receptor, nAChRα7, is expressed on peripheral blood macrophages as well as residential macrophages such as microglia in the brain (Shytle *et al*, 2004). The LC, which is the source of norepinephrine, has a direct adrenergic connection to the forebrain basal cholinergic system (Smiley *et al*, 1999; Zaborszky and Cullinan, 1996), and norepinephrine has been shown to stimulate excitation of cholinergic neurons via α_1-adrenergic receptors (Fort *et al*, 1995). Thus, VNS may activate the cholinergic antiinflammatory response within the brain by stimulating this pathway to release ACh, which then activates nAChRα7 on microglia (Figure 2; Pathway B). Stimulation of nAChRα7 on microglia has been shown to attenuate inflammatory responses in the brain (Shi *et al*, 2009; Shytle *et al*, 2004). Similarly, the nAChRα7 agonist, PNU282987, was shown to protect neuronal cells from oxidative stress by stimulating a JAK2/PI3K/Akt cascade (Parada *et al*, 2010). The cholinergic antiinflammatory pathway may also improve the outcome of ischemic stroke by suppressing systemic inflammation.

Stroke stimulates a robust peripheral inflammatory response (Offner *et al*, 2006). C-reactive protein is a marker of peripheral inflammation in stroke, and elevation of C-reactive protein has been associated with poor outcomes in stroke (Welsh *et al*, 2009). Other systemic inflammatory responses to cerebral ischemia that worsen the overall outcome of stroke include intestinal barrier dysfunction, bacterial translocation, and sepsis (Caso *et al*, 2009; Schulte-Herbruggen *et al*, 2009; Tascilar *et al*, 2010). Vagus nerve stimulation, which activates the cholinergic antiinflammatory pathway, suppresses systemic inflammation (Pavlov *et al*, 2006), decreases C-reactive protein levels in heart failure (Zhang *et al*, 2009), and also prevents increase in intestinal barrier dysfunction after brain injury (Bansal *et al*, 2010). Thus, VNS, through the activation of the cholinergic antiinflammatory pathway, can suppress both brain parenchymal inflammation and peripheral inflammation leading to neuroprotection in ischemic stroke. In addition, cholinergic signaling in the brain may also suppress excitotoxicity by inhibiting glutamate-induced p38 MAPK signaling (Asomugha *et al*, 2010) (Figure 2; Pathway B).

Parasympathetic Modulation of Cerebral Blood Flow

The cerebral blood vessels are innervated by nitroxidergic-cholinergic neurons from the SPG, a parasympathetic ganglion (Kimura *et al*, 1997; Nozaki *et al*, 1993; Suzuki *et al*, 1990). Stimulation of this SPG-nitroxidergic-cholinergic system has been shown to increase cerebral blood flow through vasodilation mediated by nitric oxide release (Toda *et al*, 2000) (Figure 2; Pathway C). Sphenopalatine ganglion stimulation in a rat model of cerebral ischemia resulted in reduction in infarct size and neurologic deficit (Yarnitsky *et al*, 2006). Importantly, unlike other experimental stroke therapies that exert neuroprotective effects within a narrow therapeutic time window, SPG stimulation starting at 24 hours after cerebral ischemia still significantly decreased mortality and improved long-term neurologic outcome (Solberg *et al*, 2008). Using imaging studies, Henninger and Fisher (2007) showed that SPG stimulation resulted in increased perfusion of the penumbra, thus correcting diffusion–perfusion mismatch, leading to reduction in infarct size. Conversely, parasympathetic denervation of cerebral blood vessels (Kano *et al*, 1991) and resection of nerve bundles from the SPG (Diansan *et al*, 2010) worsened cerebral ischemia. Interestingly, SPG stimulation has also been shown to increase blood–brain barrier (BBB) permeability (Yarnitsky *et al*, 2004*a*, *b*). Even though the BBB permeability is transiently increased in ischemic stroke (Belayev *et al*, 1996), it is likely that the delivery of some therapeutic molecules could still be impaired. Thus, administration of neuroprotective agents together with SPG stimulation in cerebral ischemia could lead to synergistic beneficial effects. However, some investigators also associate deleterious effects such as hemorrhage in thrombolytic therapy to the opening of the BBB in stroke (Kastrup *et al*, 2008). Thus, further studies are needed to assess whether the increased BBB permeability in SPG stimulation has any independent deleterious effects in the stroke setting.

Parasympathetic activation enhances neurogenesis

Neurogenesis occurs in the adult brain in two regions, namely the subgranular zone of the hippocampus and the subventricular zone of the lateral

ventricles. Under normal physiological conditions, the neural stem cells in the subgranular zone and subventricular zone produce neuroblasts, which migrate to the dentate gyrus and olfactory bulb, respectively (Altman and Das, 1965; Lois and Alvarez-Buylla, 1994). Neural stem cell proliferation is increased in conditions of brain injury such as stroke (Arvidsson *et al*, 2002). Vagus nerve stimulation has been shown to increase proliferation of neural progenitor cells in the rat hippocampus (Revesz *et al*, 2008). Vagus nerve stimulation releases norepinephrine, basic fibroblast growth factor and brain derived neurotrophic factor (Follesa *et al*, 2007), and 5-HT (Dorr and Debonnel, 2006). Norepinehrine (Bauer *et al*, 2003; Jhaveri *et al*, 2010), basic fibroblast growth factor (Maric *et al*, 2007), brain derived neurotrophic factor (Choi *et al*, 2009), and 5-HT (Brezun and Daszuta, 1999) have been shown to promote neurogenesis (Figure 2; Pathway A).

5-Hydroxytryptamine and norepinephrine together have been shown to regulate hippocampal neurogenesis by stimulating the sonic hedgehog pathway (Rajendran *et al*, 2009). The hippocampus also has an extensive cholinergic innervation from the medial septal nucleus, which is part of the basal forebrain cholinergic system (Mohapel *et al*, 2005). Cholinergic signaling through nAChRα7 promotes survival, maturation, and integration of newborn neurons in the hippocampus (Campbell *et al*, 2010). Neurogenesis in the hippocampus was impaired by forebrain lesions disrupting the basal forebrain cholinergic network (Figure 2; Pathway B) (Campbell *et al*, 2010; Cooper-Kuhn *et al*, 2004; Mohapel *et al*, 2005). Thus, PNS activation by VNS or the use of nAChRα7 agonists may enhance neurogenesis in the brain, leading to improved functional recovery.

Perspectives and future directions

The data on the neuroprotective roles of VNS, the cholinergic antiinflammatory pathway, and parasympathetic modulation of cerebrovascular tone are promising. However, using the updated recommendations of Stroke Therapy Academic Industry Roundtable (Fisher *et al*, 2009) as a yardstick of quality and extent of preclinical testing, further progress is required before individual components or combinations of PNS activation could be translated into clinical testing.

The effect of VNS on ischemic stroke was investigated in two different laboratories using rodent transient cerebral ischemia models, with some form of physiological monitoring during experiments (Ay *et al*, 2009; Miyamoto *et al*, 2003). In one study (Ay *et al*, 2009), animal allocation was randomized and assessment of outcome blinded, in contrast to the other study (Miyamoto *et al*, 2003). Overall, the effect of VNS on ischemic stroke needs to be more rigorously assessed using both permanent and transient cerebral ischemia models in both rodents and gyrencephalic species such as primates, with randomization and blinding of outcome assessment. In the treatment of epilepsy, VNS over broad stimulation parameters (frequency 2 to 10 Hz and higher amplitudes 2.75 to 3.75 mA) have shown no significant cardiorespiratory effects (Binks *et al*, 2001). In order to develop VNS as a stroke therapy, it is also important to assess its efficacy and safety profile over a broad range of stimulation parameters at different time points after stroke onset. The evidence of neuroprotective effects of SPG stimulation indicates an impressive long therapeutic time window of 24 hours (Solberg *et al*, 2008). However, this therapeutic modality also needs to be extensively tested and the range of stimulation frequencies, and side effects defined.

In summary, activation of the PNS exerts a broad range of neuroprotective mechanisms in ischemic stroke (Figure 2). This review shows that the PNS can be activated by various methods to provide neuroprotection. Some of the experimental methods of PNS activation such as VNS and SPG stimulation are invasive and may not be very practical for the patient who presents with acute stroke. In developing PNS activation as a stroke therapy, it will be important to assess the efficacy of less invasive and more easily applicable methods of PNS activation such as transcutaneous VNS. In addition, in order for PNS activation to antagonize multiple mechanisms of cerebral ischemia for optimal efficacy, it may need the combination of different modes of PNS activation. For example, as SPG stimulation increases BBB permeability (Yarnitsky *et al*, 2004*a*, *b*), it may act synergistically with nAChRα7 agonists or cholinesterase inhibitors to produce neuroprotection. In conclusion, PNS activation is a promising therapeutic modality for acute ischemic stroke that needs further development.

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Disclosure/conflict of interest

The authors declare no conflict of interest.

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