Neuroimmune Circuits Activated by Vagus Nerve Stimulation

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Abstract

The interaction between the nervous system and the immune system has recently been well-recognized. Vagus nerve stimulation (VNS) presents potential as an anti-inflammatory therapy through activation of neuroimmune pathways. Detailed understanding of the neuroimmune pathways VNS evokes is critical in order to successfully use it in the clinic for the treatment of acute kidney injury, in which inflammation plays an important role. In this review, we describe recent findings regarding VNS-induced neuroimmune pathways responsible for anti-inflammation and tissue protection.

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Introduction

Inflammatory responses play a critical role in the maintenance of tissue homeostasis [1]. However, excessive and inappropriate activation of the immune system contributes to the pathogenesis and exacerbation of various inflammatory diseases. Recently, the interaction between the nervous system and the immune system has been recognized. Vagus nerve stimulation (VNS) has potential as an anti-inflammatory therapy by activating a neuroimmune circuit, the cholinergic anti-inflammatory pathway (CAP), which has been extensively reviewed [2–4]. In brief, the CAP is initiated by firing of the efferent vagus nerve. The signal is transmitted to the splenic nerve, resulting in the release of norepinephrine. Norepinephrine binds to β_2 -adrenergic receptors expressed on a choline acetyltransferase-positive T-cell subpopulation in the spleen, which leads to the release of acetylcholine from these cells. Acetylcholine then binds to α_7 nicotinic acetylcholine receptors (α_7 nAChRs) on macrophages, resulting in the suppression of pro-inflammatory cytokine production in the spleen. Thus, the canonical CAP involves the efferent vagus nerve, splenic nerve, β_2 -adrenergic receptors on choline acetyltransferase-positive T cells, and α_7 nAChRs on macrophages in the spleen.

Fig. 1. Illustration depicting potential pathways in the cervical vagus activated by electrical stimulation. **a** Four distinct pathways in the cervical vagus are activated upon electrical VNS. Action potentials elicited by VNS are transmitted in 2 directions (anterograde and retrograde) in 2 types of fibers (efferent and afferent). **b** Two distinct pathways in the cervical vagus activated by “efferent VNS” and “afferent VNS.” A local anesthetic can also be used to block nerve conduction. VNS, vagus nerve stimulation.

Recent studies have shown that VNS can also activate other neuroimmune pathways. In this review, we describe those recent findings, focusing on neural circuits responsible for anti-inflammation and tissue protection.

Anti-Inflammatory Effects of Afferent VNS

Electrical stimulation of the vagus (usually at the cervical vagus nerve trunk) activates not only efferent fibers but also afferent fibers. Indeed, the vagus nerve is composed of about 80% sensory afferent fibers and about 20% motor efferent fibers [5]. Emerging evidence suggests that afferent VNS also has an anti-inflammatory effect. In an arthritis model, afferent VNS activated the sympathetic nervous system through the central nervous system, which increased the release of norepinephrine from local sympathetic nerve terminals within inflamed joints to suppress the innate immune response [6]. The protective effect of afferent VNS was also observed in a lipopolysaccharide-induced endotoxemia model [7]; plasma levels of

tumor necrosis factor were decreased by stimulation of abdominal vagal afferent fibers, and the anti-inflammatory effect was abolished when the splanchnic nerves were transected. These findings indicate that afferent VNS can elicit anti-inflammatory neuroimmune pathways that are distinct from the canonical CAP.

Optogenetics: A Useful Tool to Dissect Neural Circuits

Optogenetics enables us to manipulate the activity of neurons in a selective manner [8]. For example, channel-rhodopsin-2 (ChR2), which is an excitatory light-sensitive opsin, is a nonselective cation channel; its gate is in open configuration only during blue light application. When blue light is applied to neurons that have been genetically modified to express ChR2, these neurons are depolarized via Na^+ entry. There are also inhibitory light-sensitive opsins that promote hyperpolarization of neurons. Halorhodopsin and archaerhodopsin function as an

Fig. 2. Hypothetical neuroimmune interactions involving the splenic nerve branches in efferent/afferent VNS. Stimulation of the arterial splenic nerve, composed of catecholaminergic fibers, requires T cells to exert an anti-inflammatory effect. In contrast, stimulation of the apical splenic nerve, composed of both catecholaminergic and cholinergic fibers, does not require T cells but re-

quires both β_2 -adrenergic receptors and α_7 nAChRs on macrophages to exert an anti-inflammatory effect, with these 2 pathways acting in parallel. It remains unclear which component of the splenic nerve is activated by efferent/afferent VNS. VNS, vagus nerve stimulation.

inward Cl^- pump and an outward H^+ pump when illuminated with yellow and green light, respectively. Thus, optogenetics is a powerful tool to selectively activate or inhibit neurons of interest and to dissect neural circuits responsible for the protective effect of VNS.

Identifying Neural Circuits Responsible for VNS-Induced Kidney Protection

In the mouse kidney ischemia-reperfusion injury (IRI) model, prior electrical stimulation of the cervical vagus nerve significantly ameliorated acute kidney injury and the kidney protection was mediated through α_7 nAChR⁺ splenocytes [9]. However, the precise neural circuit involved in kidney protection by VNS was unknown. When the vagus is electrically stimulated, action potentials are transmitted in 2 directions (anterograde and retrograde) in 2 types of fibers (efferent and afferent) (Fig. 1a). Thus, 4 distinct neural pathways can be activated by electrical

VNS. Retrograde stimulation, especially in sensory afferent fibers, could contribute to anti-inflammatory and protective effects because it causes the release of various neuropeptides (such as substance P and calcitonin gene-related peptide) at nerve terminals in thoracic and abdominal organs [10], and those peptides have immunomodulatory effects on immune cells [11, 12]. However, due in part to prior methodological constraints, this concept had not yet been explored. In previous studies, “efferent VNS” or “afferent VNS” was performed by transecting or applying a local anesthetic to the vagus, in order to block nerve conduction prior to electrical VNS; however, this strategy still elicits a combination of anterograde and retrograde stimulation in efferent and afferent fibers (Fig. 1b).

Recently, we dissected out these 4 neural pathways in VNS by using optogenetics to identify neural circuits responsible for kidney protection [13]. We used *Chat-ChR2* and *Vglut2-ChR2* mice in which ChR2 is expressed in vagal efferents and vagal afferents, respectively. By

combining selective stimulation of vagal efferents versus afferents with nerve conduction blockade, we demonstrated that anterograde efferent or afferent fiber stimulation was sufficient to protect the kidneys against IRI, while retrograde activation of either fiber type had no effect. We further identified the C1 neurons (in the medulla oblongata) – sympathetic nervous system – the splenic nerve – spleen – kidney axis as the downstream target of vagal afferent stimulation [13]. The importance of C1 neurons in kidney protection is consistent with a previous study in which optogenetic stimulation of C1 neurons was sufficient to protect the kidneys [14]. Adoptive transfer of splenocytes, but not lymph node cells or bone marrow cells, isolated from mice that underwent VNS protected recipient mice from renal IRI, which further supports a critical role of spleen/splenocytes in this context [13]. The signal from the splenic nerve seems to alter the phenotype of splenocytes, which promotes kidney protection; however, the mechanism for this has yet to be determined.

Other Potential Neuroimmune Pathways Induced by VNS

Recently, Guyot et al. [15] reported that there were 3 nerve branches projecting to the spleen in mice. Two of these ran in parallel with an artery and contained catecholaminergic fibers (“arterial splenic nerve”), whereas the other branch was located at the apex of the spleen and contained both catecholaminergic and cholinergic fibers (“apical splenic nerve”) [15]. Arterial nerve stimulation required T cells to exert an anti-inflammatory effect, which is consistent with the canonical CAP. In contrast, an anti-inflammatory effect by apical nerve stimulation did not require T cells but required parallel activation of both β_2 -adrenergic receptors and α_7 nAChRs expressed on macrophages. It is unclear whether the cholinergic fibers in the apical nerve contain vagal preganglionic axons or are activated by vagal efferents. On the other hand,

Murray et al. [16] showed that an anti-inflammatory effect by efferent VNS required both T-cell-derived acetylcholine and β_2 -adrenergic receptors, whereas afferent VNS required β_2 -adrenergic receptors but not T cell-derived acetylcholine. Taken together, these findings may suggest that vagal efferent stimulation activates the arterial splenic nerve and requires T-cell-derived acetylcholine to exert an anti-inflammatory effect (canonical CAP), while vagal afferent stimulation activates catecholaminergic fibers in the apical splenic nerve (and arterial splenic nerve) and is independent from T cells (Fig. 2), which clearly warrants further investigation.

Conclusion and Future Perspectives

VNS has been explored as a promising strategy for the treatment of many inflammatory diseases because it activates the CAP. Notwithstanding, recent studies have revealed that VNS can also activate other anti-inflammatory neuroimmune pathways. One key question that remains in the kidney protection by VNS is how splenocytes with altered phenotypes after VNS protect the kidney. Precise understanding of the neuroimmune pathways evoked by VNS is critical for the successful clinical application of VNS, including noninvasive VNS [17], for the treatment of inflammatory diseases including acute kidney injury.

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Conflict of Interest Statement

The authors report no conflicts of interest.

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