MINI REVIEW

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MINI REVIEW

Anti-inflammatory effects of vagal nerve stimulation with a special attention to intestinal barrier dysfunction

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Abstract
The vagus nerve (VN), the longest nerve of the organism innervating the gastrointestinal tract, is a mixed nerve with anti-inflammatory properties through its afferents, activating the hypothalamic-pituitary adrenal axis, and its efferents through the cholinergic anti-inflammatory pathway inhibiting the release of pro-inflammatory cytokines (e.g., TNFα) by splenic and gut macrophages. In addition, the VN is also able to modulate the permeability of the intestinal barrier although the VN does not innervate directly the intestinal epithelium. Targeting the VN through VN stimulation (VNS) has been developed in experimental model of intestinal inflammation and in inflammatory bowel disease (IBD) and might be of interest to decrease intestinal permeability in gastrointestinal disorders with intestinal barrier defect such as IBD, irritable bowel syndrome (IBS), and celiac disease. In this issue of neurogastroenterology and motility, Mogilevski et al. report that a brief non-invasive transcutaneous auricular VNS in healthy volunteers consistently reduces the permeability of the small intestine induced by intravenous administration of the stress peptide corticotropin releasing hormone, known to increase intestinal permeability and to inhibit the VN. In this review, we outline the mechanistic underpinning the effect of stress, of the VN and VNS on intestinal permeability. In particular, the VN can act on intestinal permeability through enteric nerves, and/or cells such as enteric glial cells. We also review the existing evidence of the effects VNS on intestinal permeability in models such as burn intestinal injury and traumatic brain injury, which pave the way for future clinical trials in IBD, IBS, and celiac disease.

KEYWORDS
cholinergic anti-inflammatory pathway, enteric glial cells, inflammation, intestinal barrier, stress, vagal nerve stimulation, vagus nerve

Abbreviations: ACh, acetylcholine; CAP, cholinergic anti-inflammatory pathway; CRH, corticotropin releasing hormone; EGCs, enteric glial cells; IBD, inflammatory bowel disease; IBS, irritable bowel syndrome; I-FABP, intestinal fatty-acid binding protein; nAChR, nicotinic acetylcholine receptor; TJ, tight junctions; Ucn, urocortin; VN, vagus nerve; VNS, vagal nerve stimulation.

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In an original manuscript published in the present issue of neurogastroenterology and motility, Mogilevski et al. aimed to determine the effect of transcutaneous auricular vagal nerve stimulation (VNS) on intestinal permeability in adults receiving a bolus dose of intravenous corticotropin releasing hormone (CRH) known to increase small intestinal permeability in healthy human subjects. They report that a brief non-invasive transcutaneous auricular VNS consistently reduces paracellular permeability of the small intestine after CRH administration but does not mitigate the release of intestinal fatty-acid binding protein (I-FABP), an intracellular protein released from the epithelium at times of epithelial injury without cellular disruption. They conclude that studies of VNS in disease states are warranted.

In this Mini Review, based on these data, we will make a reminder on the concept of the intestinal barrier, the cholinergic anti-inflammatory pathway (CAP), the role of stress, and the VN as well as the effect of VNS in different models of intestinal injury and how the VN, and thus VNS, can improve intestinal permeability. Targeting the intestinal barrier through VNS opens new therapeutic avenues in gastrointestinal diseases characterized by an increased intestinal permeability such as irritable bowel syndrome (IBS), inflammatory bowel disease (IBD), and celiac disease.

2 | THE INTESTINAL BARRIER

The role of the intestinal barrier is to protect the host against gut microbes, food antigens, and toxins present in the gastrointestinal tract. A dysfunction or a disruption of this barrier, defined as a “leaky gut,” can favor a translocation of microbial components into the body, inducing systemic, low-grade inflammation. Consequently, innovative interventions able to restore and/or maintain gut barrier integrity, such as VNS, are of interest.

Tight junctions (TJ) are the major complexes responsible for the adherence of intestinal epithelial cells to each other and thus are an important part of the intestinal barrier. Occludins and members of the claudin family are the major sealing proteins building TJ. These proteins interact with cytoplasmic proteins, including zonaoccludin proteins, functioning as adaptors between the TJ proteins and actin and myosin contractile elements within the cells. A perturbation of intestinal barrier function is reported in gastrointestinal disorders such as IBS, IBD, and celiac disease. The paracellular route and the transcellular endocytic route are the two relevant pathways in the context of the leaky gut but most of the in vivo studies assessing human intestinal permeability mainly target the paracellular pathway. The most commonly used test evaluates the urinary ratio of lactulose to mannitol in order to correct for differences in absorption kinetics through gastrointestinal motility and alterations in renal excretion. New strategies include the detection in the blood of molecules normally present in the intestinal lumen as a sign of impaired barrier function (e.g., lipopolysaccharide: LPS) or the detection of increased levels of proteins which are components of the intestinal barrier (I-FABP or TJ molecules) signaling damage to the intestinal wall or higher blood concentration of barrier-regulating proteins, that is, zonulin. I-FABP, a cytosolic protein present in differentiated enterocytes of the small intestine and to a lesser extent in the colon, is present in low amounts in the circulation in normal conditions but is released into the bloodstream after damage of the intestinal barrier.

3 | THE VAGUS NERVE AND THE CHOLINERGIC ANTI-INFLAMMATORY PATHWAY

The mechanisms through which the brain regulates intestinal barrier function are still a matter of debate. The VN, the longest nerve of the human organism, innervating the digestive tract, is a key component of the gut-brain axis. The VN has anti-inflammatory properties both through its afferents, activating the hypothalamic–pituitary adrenal (HPA) axis, and efferents via the CAP, putting the VN at the interface of the neuro–endocrine–immune axis. The CAP, first described in 2000 by Tracey’s group, is mediated by the release of acetylcholine (ACh), binding to α7 nicotinic acetylcholine receptor (α7nAChR) of macrophages, thus inhibiting the release of TNFα by these cells. In the gut, the VN does not interact directly with macrophages but with nNOS–VIP–ACh enteric neurons that relate the anti-inflammatory signal. Intestinal muscularis resident macrophages expressing α7nAChR are most likely the ultimate target of the gastrointestinal CAP. The VN is also connected with the splenic sympathetic nerve, releasing norepinephrine that binds to β2 receptors of splenic T-lymphocytes releasing ACh, leading to the inhibition of TNFα release by splenic macrophages through α7nAChR, that is, the non-neuronal cholinergic pathway. Alternative pathways involve the greater splanchnic nerves, which are activated in response to an immune challenge and, in turn, drive postganglionic sympathetic neurons to inhibit inflammation through splenic sympathetic nerve terminals. The thoracolumbar spinal cord at the origin of the splenic nerve is activated by brain descending pathways, which are connected to vagal afferents. More recently, Zhang X et al. have shown that splenic nerve activity enhances plasma cell production.

Key Points

- The vagus nerve has a dual anti-inflammatory property through its afferents and efferents.
- The vagus nerve is also able to modulate the permeability of the intestinal barrier through enteric nerves and/or cells such as enteric glial cells.
- Stress increases intestinal permeability and is involved in the pathogenesis of inflammatory bowel disease (IBD) and irritable bowel syndrome (IBS).
- Vagal nerve stimulation (VNS) can improve intestinal permeability. Targeting the intestinal barrier through VNS opens new therapeutic avenues in IBD and IBS.
in a manner that requires B-cell responsiveness to ACh mediated by the α9nAchR, and T cells that express choline acetyl transferase probably act as a relay between the noradrenergic nerve and ACh-responsing B cells. They showed that neurons in the central nucleus of the amygdala and paraventricular nucleus of the hypothalamus that express CRH are connected to the splenic nerve.

4 | STRESS AND INTESTINAL PERMEABILITY

Stress is well known to induce perturbations of the digestive tract both in animals and humans.11 CRH, the principal neuromediator of stress, first isolated in the hypothalamus, is a member of a family of mammalian CRH-related peptides including urocortin (Ucn) 1, Ucn 2, and Ucn 3. These peptides exert their biological actions on target cells through activation of two receptors, known as CRH receptor type 1 (CRH1) and type 2 (CRH2). The CRH system is present both in the central nervous system and in the digestive tract. The pattern of distribution of CRH and Ucn 1 and 2 in the brain encompasses several stress-related neuronal groups involved in the modulation of gastrointestinal functions and the integration of afferent signals from visceral origin, namely the paraventricular nucleus of the hypothalamus and Barrington nucleus/locus ceruleus.11 CRH ligands and receptors are expressed in the gastrointestinal tract in animals and humans giving rise to a local CRF signaling pathway that can act directly on the gut in either a paracrine or an autocrine manner.11

We have shown that Ucn3, a selective CRH2 agonist, alters both para- and trans-cellular permeability of differentiated HT-29 and Caco-2 cells. These effects were partly mediated by Ucn3-induced morphological changes associated with the disruption of mature adherens junctions in HT-29 cells and TJ in Caco-2 cells.12 Stress is known to stimulate the sympathetic nervous system while inhibiting the VN,11 consequently stress may have a proinflammatory role and increases intestinal permeability. The role of CRH in the cholinergic control of permeability has been explored in rats. Gareau et al.13 showed that neonatal maternal separation predisposes adult rats to develop stress-induced mucosal barrier dysfunction and provided strong evidence that CRH, via CRH2, acts on cholinergic nerves to induce epithelial barrier dysfunction. Overman et al.14 showed that CRH triggers an increase in intestinal paracellular permeability via mast cell dependent release of TNF-α and proteases, requiring critical input from the enteric nervous system. Moreover, CRH-induced activation of the myenteric cholinergic neurons assessed by the marker of neuronal activity Fos supports these observations. When injected intraperitoneally, CRH only seems to activate colonic myenteric neurons expressing CRH1 (but not in submucosal plexus), but not those in the small intestine or stomach.15 Since the physiological changes in barrier function described above were occurring in the small bowel, it remains to be determined what is happening pathophysiologically in various bowel regions in animals under stress conditions involving the release of CRH and under baseline conditions. However, it seems appropriate to conclude that enteric nerves regulate epithelial barrier function either directly or indirectly, and in the case of an indirect effect, mast cell products may be an important source of mediators regulating TJ. The overall pathophysiologic importance of these data relates to the control of permeability during stress conditions. Yu Y et al.16 have reported that psychological stress-derived CRH can break the established endotoxin tolerance in the intestinal mucosa leading to the development of clinical or subclinical inflammation and associated conditions of IBS and IBD.

5 | VAGUS NERVE STIMULATION

VNS is approved for the treatment of drug refractory epilepsy and depression.17 In these conditions, invasive VNS was performed through an electrode wrapped around the left VN in the neck, near the carotid artery, tunneled under the skin, and connected to a bipolar pulse generator implanted subcutaneously in the left chest wall. The classical stimulation parameters are as follows: 0.25–1.5 mA intensity, pulse width 500 μs, frequency 20–30 Hz, 30s ON, and 5 min OFF performed continuously. However, non-invasive transcutaneous auricular VNS has been developed since the auricular branch of the VN innervates 100% of the cymba concha and 45% of the cavity of concha and tragus.18 However, there are contradictions between the outcomes described in the main text and those listed in the manuscript’s table regarding the innervation of the anthelix, the tragus, and the cavity of the concha.19 Stimulating this part of the ear with an electrode and a neurostimulator activates the same brain loci than invasive VNS and thus the CAP through a vago-vagal reflex.6 This non-invasive approach is the subject of a growing number of clinical trials in various clinical conditions (gastro-intestinal and others) with less side effects than invasive VNS and easier to perform since it does not require surgery. The anti-inflammatory effect of VNS has been used in experimental models of colitis20 and in IBD.21

6 | HOW THE VAGUS NERVE CAN MODULATE INTESTINAL PERMEABILITY?

Besides its effects on macrophage cytokine release, the VN is also able to modulate intestinal permeability. The VN interacts with the enteric nervous system but does not innervate directly the intestinal epithelium since vagal extension beyond the myenteric plexus to the intestinal mucosa has not been identified. Consequently, the question is how the VN can modulate intestinal permeability. Indeed, characterizing the interactions between the VN, the enteric nervous system, and the intestinal epithelium may open therapeutic avenues at reducing gut barrier failure and intestinal inflammation. One hypothesis is that the VN can act on intestinal permeability through enteric nerves and/or cells such as enteric glial cells (EGCs), the predominant cell type in the enteric nervous system which are key regulators of intestinal barrier function.22 One of the mechanisms could be the connection of the VN with the enteric nervous system, which communicates with EGCs through nicotinic cholinergic
signaling. 22 EGCs are phenotypically like astrocytes of the central nervous system, recognized as key regulators of the blood–brain barrier and involved in the response to inflammation. EGCs are positioned throughout the intestine, in both the myenteric and submucosal plexuses and intramuscularly with projections extending to villus tips. There is also a population of cells with the phenotypic characteristics of enteric glia that lie just beneath the epithelium. 24 These subepithelial enteric glia have a close connection with epithelial cells and play a role in the maintenance of barrier function. EGCs form a rich network surrounding both enteric neurons and efferent vagal fibers. 25 They can receive and transmit numerous signals from neighboring cells, and thus are prime candidates to modulate the gut inflammatory response following injury. 24 EGCs are of critical importance to both immune cell recruitment and gut barrier integrity after injury. 25 Leukocyte infiltration is increased in EGC-ablated segments of the bowel following injury compared with segments with intact EGCs.

Macrophage infiltration was decreased when VNS was applied after ischemia–reperfusion injury but only in segments with an intact EGCs. These findings suggest that EGCs play an important role in modulating the immune cell response following injury and that vagal anti-inflammatory signals may be transmitted to resident immune cells through EGCs. 23 Matteoli G et al. 7 also reported the transmission of vagal anti-inflammatory signals to resident immune cells in the gut through the enteric nervous system. Mucosal EGC bodies lie <1 μM from the epithelial border and have terminals that appear to contact the basement membrane. 26 The close proximity of EGCs to epithelial cells permits for signal transmission through secreted factors. Regulation of mucosal barrier and secretory function by a neuronal–glial circuit may confer gut protection and represent potentially important target cells in gastrointestinal inflammatory and permeability disorders.

Studies supporting the spleen-independent pathway showed decreased immune cell infiltration and cytokine production, prevention of TJ loss, and maintenance of barrier function by VNS following injury in splenectomized animals. 27 Mechanistic studies are ongoing to determine the pathway by which VNS alters gut barrier function in light of the absence of cholinergic vagal fibers in close proximity to epithelial cells and resident immune cells. Thus, there is an alternate mechanism for the spleen-independent vagal-mediated anti-inflammatory pathway, namely through activation of EGCs. Ablation of EGCs in a segment of bowel prevents the protective effects of VNS on immune cell infiltration and gut barrier integrity. 25 EGCs are near vagal terminals in vivo 24 and the α7nAChR involved in vagal signaling has been reported in culture EGCs. 28 EGCs preserve epithelial barrier against intestinal bacteria insult by increasing the expression of TJ proteins such as occludin and ZO-1 through the secretion of S-nitrosoglutathione. 24 The presence of an epithelial cholinergic system (muscarinic receptors and ACh) has been reported in both the small and large intestine of animals and humans. 29 During intestinal inflammation, epithelial choline acetyltransferase expression is reduced. 29 EGCs secrete numerous molecules when activated, several of which improve gut barrier function and limit mucosal inflammation, thus having barrier-protective properties. 30 These glia cells secrete S-nitrosoglutathione, which improves the expression of TJ proteins including occludin, ZO-1, and phosphorylated myosin light chain thus preserving epithelial barrier against intestinal bacteria insult. 21 EGCs also secrete transforming growth factor, another molecule that improves intestinal barrier integrity. 22 Glia cells express glial fibrillary acidic protein upon activation, increasing the number of glial fibrillary acidic protein expressing cells following injury. Proinflammatory cytokines (IL-1β, TNFα) and LPS cause a significant increase in glial fibrillary acidic protein-positive enteric glia. This suggests that cytokines play an important role in controlling glial fibrillary acidic protein-positive enteric glia, which in turn might modulate the integrity of the bowel during inflammation. 33

7 | EFFECT OF VAGUS NERVE STIMULATION ON INTESTINAL PERMEABILITY

Consequently, therapies that increase EGC activation, such as VNS, may be a novel strategy to limit barrier failure in patients following severe injury. Costantini et al. 27 have shown that VNS protects against burn-induced intestinal injury through activation of EGCs, by modulating the response to injury at the level of the gut tissue, instead of altering systemic inflammation, and gut protection was independent of splenic cytokine production. Injection of S-nitrosoglutathione attenuates burn-induced intestinal barrier injury with results similar to animals undergoing VNS. Thus, VNS may maintain barrier integrity and intestinal TJ protein expression through the ability of activated EGCs to produce S-nitrosoglutathione. 27 The same group reported that treatment with nicotine, a pharmacological agonist of α7nAChR, after severe burn injury prevented intestinal barrier integrity breakdown and limited histological gut injury. Nicotine prevented decreased expression and altered localization of occludin and ZO-1. The barrier-protective effects of nicotine were lost in proinflammatory cytokine-stimulated intestinal epithelial cells when EGCs were retrieved from the culture, suggesting that the effects of nicotine may be due to activation of EGCs. 28 Similar protective effects were observed in injured animals who underwent VNS. 27 Indeed, severe burn injury increases the activation of EGCs, increasing intestinal glial fibrillary acidic protein mRNA expression. VNS alone induces increased expression of intestinal glial fibrillary acidic protein, prevents burn-induced intestinal permeability, and attenuates histological gut injury. Vagotomized animals before VNS had intestinal permeability similar to animals subjected to burn alone, thus confirming the protective effects of efferent vagal nerve signaling. VNS prevented the burn-induced increase in intestinal TNFα, attenuated the increase in intestinal TJ proteins myosin light chain kinase, and phosphorylated myosin light chain, which play an important role in the loss of TJ integrity. These results pave the way of the mechanism by which VNS prevents intestinal barrier loss and subsequent intestinal inflammation. 27 The importance of
enteric glia was reported in transgenic mice with targeted ablation of EGCs causing a fulminating and fatal jejuno-ileitis.\textsuperscript{34} Likewise, immune-mediated damage to enteric glia may participate in the initiation and/or the progression of human IBD.\textsuperscript{35}

An increased intestinal permeability can occur as early as 6 h after traumatic brain injury leading to bacterial translocation and subsequent sepsis.\textsuperscript{36} The mechanism is multifactorial and most likely include an unchecked inflammatory cytokine milieu, altered intestinal cellular architecture, epithelial cell apoptosis, and changes in TJ integrity.\textsuperscript{36} In a mouse model of traumatic brain injury, these authors showed that VNS prevents traumatic brain injury-induced intestinal permeability and intestinal injury, and significantly reduces intestinal TNFα. VNS also increased enteric glial activity as measured by an increase of enteric glial fibrillary acidic protein and may represent a pathway for central nervous system regulation of intestinal barrier dysfunction.\textsuperscript{36}

Intestinal barrier integrity is preserved when VNS is performed before severe burn injury, through an efferent signaling pathway associated with improved expression and localization of occludin.\textsuperscript{37} Decreasing alterations in intestinal TJ protein expression and subsequent gut barrier breakdown may limit the systemic inflammatory response syndrome and improve outcomes in patients after severe injury. Vagal nerve signaling needs to occur very early after injury since increased expression of the TJ protein myosin light chain kinase is observed 2h after burn.\textsuperscript{38} The breakdown of the epithelial barrier and changes of TJ protein expression in response to inflammation are due, in part, to the activation of myosin light chain kinase, phosphorylation of myosin II light chain, and contraction of the actin-myosin ring.\textsuperscript{39} Following burn injury, a significant inflammatory reaction in the gut upregulates myosin light chain kinase and increases phosphorylation of myosin II light chain, leading to TJ separation.\textsuperscript{38} VNS before thermal injury has protective effects against gut epithelial barrier breakdown.\textsuperscript{27} VNS performed within 90 min after the initial burn insult similarly protects the intestinal epithelial barrier and maintain its integrity thus demonstrating a “therapeutic window” for intervention and responding to the question as to whether VNS is preventing barrier breakdown or speeding barrier recovery. The results show clearly that when VNS is performed within 90 min of burn injury there is consistency in appearance, permeability, and protein expression.\textsuperscript{40} A decrease in inflammation was also observed in VNS-treated animals with low levels of local TNFα production from the gut, similar to sham. In addition, a reduction in the activation of myosin light chain kinase was observed. An increase in myosin light chain kinase is observed after activation of the nuclear factor-κB pathway, of which TNFα is a known potent activator.\textsuperscript{41} Thus, there is a “therapeutic window” for mucosal barrier protection via VNS in a postinjury model and its protective effect is lost when VNS is performed after 90 min from injury. One hypothesis may be that the local inflammatory reaction generated within the gut is too robust or too organized at this time point for VNS to rise above. There may be an inflammatory threshold beyond which VNS is no longer effective.

To the best of our knowledge, there are presently no published data regarding the effect of VNS on intestinal permeability in IBS, IBD, and celiac disease. Consequently, the work of Mogilevski et al.\textsuperscript{1} as well as the data presented above on the effects of VNS on intestinal barrier function in other models of intestinal injury pave the way for future clinical trials in such gastro-intestinal disorders.

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Bruno Bonaz contributed to the conception, drafting, and editing of the manuscript.

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