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The vagus nerve in appetite regulation, mood and intestinal inflammation

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Abstract

Although the gastrointestinal (GI) tract contains intrinsic neural plexuses that allow a significant degree of independent control over GI functions, the central nervous system provides extrinsic neural inputs that modulate, regulate and integrate these functions. In particular, the vagus nerve (VN) provides the parasympathetic innervation to the GI tract, co-ordinates the complex interactions between central and peripheral neural control mechanisms. This review will discuss the physiological roles of the afferent (sensory) and motor (efferent) vagus in regulation of appetite, mood and the immune system, as well as the pathophysiological outcomes of VN dysfunction resulting in obesity, mood disorders and inflammation. The therapeutic potential of VN modulation to attenuate or reverse these pathophysiological outcomes and restore autonomic homeostasis will also be discussed.

Introduction

The vagus nerve (VN), the longest cranial nerve in the body, not only regulates gut physiology, but is also involved in controlling the cardiovascular, respiratory, immune and endocrine systems. To date, its role in the regulation of appetite and obesity is increasingly recognized, and involves a complex interplay between central and peripheral mechanisms involving both afferent and efferent VN fibers. Similarly, it is increasingly recognized that the VN communicates with the immune system, i.e. inflammation in the periphery is detected by vagal afferents and integrated in the brainstem, affecting appetite, mood and sickness behavior generating, ultimately, an efferent vagal signal modulating the immune response. The “great wandering protector” plays a crucial role in the organism homeostasis,

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and is currently being explored as therapeutic target in a variety of disorders. Undoubtedly, the VN still hides many undiscovered mysteries relevant for better understanding of physiology and pathophysiology and the development of better treatments. In the present review, the current knowledge with respect to appetite regulation, mood and intestinal inflammation will be reviewed.

Vagal anatomy and neuroanatomy

Intrinsic neural networks within the gastrointestinal (GI) tract, including myenteric and submucosal plexuses as well as interstitial cells of Cajal (ICC's), allow a substantial degree of autonomy over GI functions such as motility, secretion and absorption¹. The central nervous system (CNS), however, provides extrinsic neural inputs which integrate, regulate, and modulate these responses. The sympathetic nervous system provides a principally inhibitory influence over GI muscle and mucosal secretion and, at the same time, regulates GI blood flow through neural-dependent vasoconstriction. The parasympathetic nervous system, in contrast, provides both excitatory and inhibitory control over gastric, intestinal and pancreatic functions, suggestive of a more complex homeostatic regulation (see ¹ for review). The stomach and upper GI tract in particular receive an especially dense parasympathetic innervation, the density of which decreases as one progresses distally through the intestine ^{2,3}.

The parasympathetic innervation to the GI tract and pancreas are provided by the VN. As a mixed sensory-motor nerve, the vagus contains approximately 70–80% sensory fibers, depending on the species⁴. The cell bodies of these pseudounipolar sensory neurons are located in the paired nodose ganglia (inferior ganglion of the VN) located in the transverse process of the first cervical vertebra, although some cell bodies in the jugular (superior) ganglion may provide innervation to the GI tract. GI vagal afferents are principally unmyelinated C- or thinly myelinated A δ -fibers and are classified based upon the location of their receptive field (mucosa, muscle or serosa-mesenteric), the region of GI tract innervated, primary stimulus modality (chemical, osmotic, mechanical), or their response to distention or pressure⁵. The majority of vagal afferents are sensitive to low pressure distention, although some vagal afferents can respond to high distention pressures; while the majority of GI vagal afferents traffic is interoceptive, therefore it is also likely that they play a role in nociception, or in the emotional-affective response to pain⁶.

The central terminals of vagal afferents enter the brainstem via the tractus solitarius (TS), and synapse onto neurons of the nucleus of the tractus solitarius (NTS) using glutamate as their principle neurotransmitter⁷. Some vagal afferents also make monosynaptic connections within the dorsal motor nucleus of the vagus (DMV)⁸, or with neurons of the area postrema (AP)⁹. Together, the NTS, DMV and AP, known as the dorsal vagal complex (DVC), function as a critical intersection in the integration of ascending interoceptive signals with descending visceromotor signals. The entire DVC area, which lies ventral to the 4th ventricle, is highly vascularized with fenestrated capillaries and is essentially a circumventricular organ¹⁰. An additional layer of subependymal cerebrospinal contacting neurons (CSF-cNs) are positioned between the CSF and DVC neurons and may integrate the detection of circulating signals with the modulation of autonomic, including GI, functions¹¹.

The NTS appears organized in a viscerotopic manner according to the region of afferent input¹². NTS neurons integrate the vast volume of sensory information together with inputs received from other brainstem and higher CNS nuclei involved in autonomic homeostatic regulation (see later). The integrated response is then relayed to the adjacent DMV which contains the preganglionic parasympathetic motor neurons that send the output response back to the viscera via the efferent VN. The NTS-DMV synapse uses glutamate, GABA or catecholamines as neurotransmitters, although evidence from several groups suggests that, under experimental conditions, GABA is the predominant neurotransmitter^{13–15}. Vagal efferent outflow to the viscera, therefore, appears to be under a tonic inhibitory influence. Dendritic projections of NTS and DMV neurons intermingle within the various subnuclei, however, possibly providing a means by which autonomic reflexes may be integrated across organ system¹⁶.

Unlike the NTS, the DMV does not display a viscerotopic or organotopic organization. Instead, DMV neurons are organized in spindle-shaped ‘columns’ that extend throughout the rostro-caudal extent of the nucleus and innervate the GI tract via one of the five subdiaphragmatic vagal branches¹⁷. In humans, the DMV can be subdivided into nine distinct subnuclei with six distinct morphological and neurochemical neuronal phenotypes¹⁸. Most GI regions receive innervation from more than one subdiaphragmatic vagal branch, however; the stomach is innervated by the anterior and posterior gastric branches as well as the hepatic branch, the duodenum is innervated by all vagal branches, and the colon is innervated by both the celiac and accessory celiac branches³. In humans, the vagus innervates the right two-thirds of the transverse colon, with parasympathetic innervation to the left third of the transverse colon, the descending colon and rectum arising from the pelvic nerves¹⁹. Early neuronal tracing studies showed vagal efferent fibers were present within the celiac ganglion²⁰ suggesting a role in the modulation of splenic nerves and a potential anatomical basis for the cholinergic anti-inflammatory pathway (CAIP). The use of the trans-synaptic, pseudorabies virus (PRV), to map the central neurocircuits innervating the spleen did not, however, find labeling in the DMV²¹. Vagal nerve stimulation clearly modulates splenic function (see later), but the anatomical and mechanistic basis of this action has yet to be clarified. For the purposes of this review, the principle CNS areas and inputs involved in the modulation of GI vagal reflexes by diet, inflammation and mood disorders are outlined in Table 1.

Effects of diet and obesity on vago-vagal functions

The GI tract is one of several organs that contribute to the peripheral signaling of food intake and satiety and, increasingly, vagally-mediated reflexes are recognized as being critical to the neural control of energy homeostasis²² particularly the short-term (homeostatic), rather than longer-term (hedonic), control of appetite and food intake, although clearly the two pathways are inter-related and dependent²³.

Gastric distention activates vagal afferent mechanoreceptors in a dose-dependent manner, suggesting that some mechanosensitive afferents control meal size via signaling of volume or load. In contrast, chemosensitive vagal afferents are activated in response to luminal pH, osmolality, and chemical stimulation, although they can also be activated by light stroking or

compression of the mucosa⁵. Mucosal afferents are most abundant in the upper small intestine, where they innervate the mucosal villae or crypts²⁴. Afferent terminals are found in close proximity to mucosal enteroendocrine cells²⁴ and respond to the mediators they release. Over 30 GI neurohormones have been identified and many play critically important roles in digestion, absorption and satiety signaling²⁵. The majority of these GI neurohormones activate their respective receptors present on vagal afferent nerve endings in a predominantly paracrine manner^{26–28}, increasing vagal afferent firing and resulting, ultimately, in a range of visceral effects including gastric relaxation, reduced gastric emptying, altered motility and pancreatic secretion^{27–29}. Ghrelin, in contrast, is released from the stomach and inhibits vagal afferent firing³⁰ and is, to date, the only GI neurohormone that stimulates feeding directly, in addition to attenuating the anorexigenic actions of CCK and leptin^{31,32}.

Despite being activated principally indirectly, some chemosensitive vagal afferents can respond *directly* to nutrients; discrete subpopulations of gastric vagal afferents are activated³³ while hepatic vagal afferents³⁴ are inhibited, by elevated glucose levels. Hepatic vagal afferents innervating the portal vein are in a unique anatomical position to respond rapidly to nutrients following intestinal absorption; their activity is also modulated by amino acids (increased or decreased, depending on the amino acid)³⁵ suggesting a close correlation between hepatic vagal afferent activity and food ingestion.

Subsequent to their release, GI neurohormones enter the bloodstream from where they may also exert non-paracrine effects on vagal activity. In humans, circulating levels of platelet-free 5-HT increase 3 fold following meal ingestion³⁶ while CCK levels increase 5–8 fold³⁷. Originally thought to have a tight blood-ganglion barrier³⁸, circulating substances³⁹ readily access, hence potentially alter the activity of, vagal afferent somata directly. GI vagal afferent somata display a variety of receptors for neurotransmitters/modulators⁴⁰ and in virtually every instance studied to date, the actions of GI neurohormones on vagal afferent neurons mimic those at their peripheral terminals (see ^{32,33,41,42}).

Several of these GI neurohormones also act centrally to modulate the activity of brainstem vagal neurons. CCK, GLP-1 and 5-HT, for example, also all act as neurotransmitters/modulators within the brainstem to affect vagal afferent and efferent responses either directly or indirectly^{43–46}. We have demonstrated previously that CCK can activate NTS neurons even after selective surgical section of vagal afferent rootlets, implying a direct central action⁴⁴. While the contribution of paracrine vs hormonal modulation of vagal neurocircuits by GI neurohormones is not clear, GI neurohormones appear capable of exerting coordinated, but temporally distinct, actions to regulate vagally dependent functions.

High fat diet and obesity compromise vagal plasticity

Studies from several laboratories have demonstrated that, in both humans and animal models, the ability of vagal afferents to respond to GI neuropeptides is compromised by obesity or exposure to a high fat diet. The actions of CCK, for example, to increase vagal afferent activity is reduced in obese rodents as well as humans^{47–49}. Similarly, subpopulations of vagal afferent neurons from rats fed a high fat diet become leptin resistant

at the onset of hyperphagia⁵⁰. It should also be noted though that, rather than the actions of leptin being attenuated uniformly following conditions of excess energy intake, an action of leptin to inhibit gastric tension receptors in mice only becomes apparent after exposure to a high fat diet. Such changes appear to act in concert, however, to promote increased food intake⁵¹. Several groups have demonstrated that vagal neurocircuits exhibit a significant degree of plasticity and their 'response phenotype', is modulated by ongoing physiological, and pathophysiological, conditions⁵²⁻⁵⁴. Under fasting conditions, when circulating CCK levels are low, the density of cannabinoid (CB1), and melanin concentrating hormone (MCH1) receptors on vagal afferent neurons is increased; this 'orexigenic phenotype' is associated with an increase in food intake. Conversely, following re-feeding, or an increase in circulating CCK levels, Y2 receptor expression increases while CB1 receptor expression decreases; this 'anorexigenic phenotype' is associated with a decrease in food intake⁵². In diet-induced obesity, however, vagal afferent neurons appear fixed in an orexigenic phenotype, regardless of feeding status, supportive of an increased 'drive' for food intake or a decreased satiety response⁵². Furthermore, in diet-induced obese mice, the mechanosensitivity of vagal afferents is reduced⁵⁵ and one recent study suggested that the ghrelin's inhibitory actions on vagal afferent firing are lost⁵⁶, suggestive of a more widespread dysregulation of vagal afferents, not merely a loss of satiety signaling. In contrast, other studies demonstrated that, rather than losing efficacy, in obese mice ghrelin is able to inhibit the activation of both gastric mucosal and tension sensitive vagal afferents, which would be expected to increase its orexigenic actions⁵⁵.

While technical differences between studies may explain some of these differences, clearly the effects of diet and obesity on vagal afferent responsiveness is multifactorial and is likely to be affected by vagal afferent type, sex, time of day and feeding status. In general, both vagal afferent and efferent neurons appear less excitable in diet-induced obese rodent models^{48-50,52-57}; a generalized decrease in neuronal excitability would compromise the ability of vagal afferents and efferents to respond to any signal, mechanical or chemical^{47,50,55,57}.

A decrease in neuronal excitability is unlikely to be the sole mechanism behind loss of vagal afferent responsiveness, however. Gene expression levels of the growth hormone secretagogue (ghrelin) receptor shows a diurnal rhythm⁵⁸ and the mechanosensitivity of gastric vagal afferents shows a striking circadian rhythm, decreasing three-fold during the dark cycle when feeding in mice is prevalent⁵⁹ implying that vagal afferent responsiveness to food ingestion is dictated by feeding cycles and the time of day. This circadian dependency is lost in obesity, however, in concert with a loss in ability of leptin to increase mechanosensitivity, increasing daytime feeding⁶⁰.

It remains to be determined whether these changes in vagal afferent properties with obesity are correlative or causative, however. One recent study suggests that, in rats, the ability of glucose to modulate serotonin signaling in GI vagal afferents is lost after only 3-7 days of exposure to a high fat diet, implying that reduced vagal sensory signaling is affected prior to obesity. Even one day of exposure to a high fat diet increases the recruitment of classically activated macrophages (M1) to the nodose ganglion of mice, suggesting that even short term exposure can induce inflammation in the vagal afferent system⁶¹.

Effects of gut microbiota on vagal afferents

A significant, and growing, body of literature supports the actions of gastrointestinal luminal microorganisms to modulate gut-brain signaling via vagal afferents, the so-called microbiota-gut-brain axis (MGB axis; see reviews⁶²⁻⁶⁴). While gut microbiota might normally be expected to activate vagal afferents directly only under conditions where intestinal permeability is compromised (e.g. following inflammation or stress), luminal bacteria may activate vagal afferents indirectly, subsequent to stimulation and release of neuroactive mediators from enteroendocrine cells or gut associated lymphoid tissue (GALT). In addition to their potential role in the regulation of gut functions including motility and secretion, and activation of immune responses, the gut microbiota have well-described roles in 'higher' CNS functions, including mood, stress-related psychiatric conditions and memory^{63,64}. Administration of *Citrobacter rodentium* to mice, for example, increases anxiety-like behaviors in a vagally-dependent manner⁶⁵ while *Bifidobacterium longum* NC3001 normalized anxiety-like behavior following nematode-induced GI inflammation, again in a manner involving the vagus nerve⁶⁶. Clinical studies have also suggested that ingestion of probiotics may decrease anxiety and depression⁶⁷. Large clinical trials evaluating the efficacy of antibiotic as well as probiotic ("psychobiotic") therapies, as well as in-depth sequencing of the microbiome, is required as a matter of urgency to further elaborate on the role of the MGB axis in several pathophysiological states."

Inflammation and modulation of vagal afferents

Vagal afferents and neurons respond directly to cytokines and endotoxin, not only contributing to the induction of fever and sickness behavior, but also affecting GI function. Indeed, interleukin-1 (IL-1) receptors are expressed on vagal afferents and glomus cells adjacent to the VN^{68,69} while intestinal inflammation, triggered by *Campylobacter jejuni* infection⁷⁰ or intestinal manipulation⁷¹ activates NTS neurons. Inflammation, including intestinal inflammation, and an increase in circulating levels of cytokines are well recognized sequelae of obesity⁷². Indeed, chronic exposure to low doses of the bacterial endotoxin, lipopolysaccharide (LPS), mimics several obesity-associated outcomes including attenuated vagal afferent leptin signaling, hyperphagia, and decreased CCK-induced satiation⁷³ suggesting that inflammation plays a causal role in obesity development. Vagal afferents can not only respond to cytokines and inflammatory mediators⁷⁴, but inflammation induces neuroplasticity, including the upregulation of transient receptor potential (TRP) channels TRPV1 and TRPA1⁷⁵, upregulation of P2X receptors and augmentation of ATP signaling⁷⁶, modulation of sodium ion channel density and function⁵³, reactive gliosis of satellite glial cells⁷⁶, and increased mechanosensitivity⁷⁷ suggesting a means by which vagal afferent signaling and sensitivity may be modulated by inflammation.

Increases in circulating levels of cytokines, including tumor necrosis factor alpha (TNF- α) decreases gastric motility and emptying associated with nausea and vomiting via actions at central vagal neurocircuits. Calcium imaging studies suggest that TNF- α potentiates vagal afferent terminal responses, thus activates NTS neurons⁷⁸, increases DVC microglial activation and induces DMV apoptosis via PAR1 and PAR2 receptor activation^{79,80}. Vagal motoneurons are also inhibited by IL-1 β , partly via local synthesis of prostaglandins⁸¹, suggesting that inflammation-associated GI disorders may result, at least in part, from

dysregulation of both afferent and efferent VN. Finally, inflammation activates DMV neurons as part of the “inflammatory reflex”, which will be discussed in more detail below.

The inflammatory reflex

In 2000, the concept of the cholinergic anti-inflammatory pathway (CAIP) was introduced by Kevin Tracey’s group. In a model of sepsis, VN stimulation (VNS) increased survival by reducing TNF production in the liver, and especially the spleen⁸². This anti-inflammatory effect could be reproduced in vitro using isolated human macrophage cultures; the release of TNF, IL-1b, IL-6 and IL-18 in response to endotoxin was significantly reduced by acetylcholine and nicotine. While searching for a pharmacological target of VNS, Wang et al.⁸³ identified the alpha7 subtype of the nicotinic acetylcholine receptor ($\alpha 7nAChR$) as the main receptor by which splenic macrophages are modulated. Based on these findings, the “cholinergic anti-inflammatory pathway” was introduced whereby the VN exerts its anti-inflammatory effect by direct modulation of macrophages and cytokine production, via $\alpha 7nAChR$, mainly in the spleen. By restraining the magnitude of a potentially fatal peripheral immune response, this mechanism is believed to provide an additional protective mechanism against the lethal effects of cytokines⁸⁴. In comparison with the HPA axis or the local production of anti-inflammatory cytokines, this cholinergic control seems to have several properties favoring a central role in immune homeostasis. Considering the speed of neural conductance, it is capable of providing an instantaneous modulatory input to the inflamed region. Moreover, as the nervous system can adapt its output based on information obtained from different host regions, the modulatory influence of the CAIP is not only fast, but integrated with respect to the general host well-being. This reasoning eventually has led to the introduction of the “inflammatory reflex”⁸⁴.

The Cholinergic Anti-Inflammatory Pathway – controversies and unresolved issues

Although the anti-inflammatory effect of VNS is well accepted and demonstrated in a variety of disease models, the main problem accepting the concept as proposed initially is the absent, or very limited, vagal innervation of the spleen⁸⁵. The adrenergic innervation of the spleen is more abundant, however, arising from prevertebral sympathetic ganglia, particularly from the celiac ganglion^{20,86}. Vagal efferents have been proposed to activate these adrenergic neurons through interaction with $\alpha 7nAChR$ ⁸⁶ affecting cytokine production in the spleen via sympathetic fibers running in the splenic nerve. These fibers are indeed in close proximity to ChAT expressing splenic CD4+ T cells, further characterized as CD44^{high}CD62^{low} memory T cells⁸⁷. These T cells are rather rare in the spleen of naïve mice, however⁸⁵. Nevertheless, VNS in nude mice (deficient in T cells) fails to reduce endotoxin-induced TNF production, while adoptive transfer of memory T cells restored the anti-inflammatory potential of VNS⁸⁷. Based on these findings, the hypothesis was put forward that adrenergic, rather than cholinergic, nerve fibers activate acetylcholine producing memory T cells, via β -adrenoceptor activation, subsequently dampening splenic macrophages and cytokine production via interaction with $\alpha 7nAChRs$ (Figure 1).

Still, several important discrepancies and issues remain. First, using antegrade and/or retrograde tracing, no synaptic connection can be detected between vagal efferents and adrenergic prevertebral neurons innervating the spleen^{88,89}. Second, although splenic

denervation and reserpine-induced depletion of adrenergic nerves abolish the anti-inflammatory effect of VNS, splenic nerve activity cannot be detected in response to vagal efferent nerve stimulation^{89,90}. Third, splenic nerve activation reduces splenic cytokine production in a $\alpha 7nAChR$ *independent* manner⁸⁶. This suggests that $\alpha 7nAChR+$ target cells may be located outside the spleen that migrate to, and are sequestered in, the spleen, especially in conditions of systemic inflammation. In view of the dense innervation of almost all lymphoid structures (lymph nodes, Peyer's patches, thymus), neuromodulation may primarily occur in these structures, after which immune cells travel to the spleen. Clearly, more research is warranted to unravel the exact neuroanatomy of the vagal anti-inflammatory pathway to the spleen.

Another, thus far largely neglected, issue is the fact that electrical stimulation of the entire VN activates not only efferent, but also afferent, nerve fibers leading to stimulation of the contralateral vagus via a central pathway^{86,90}. Indeed, activation of the central end of the cut VN seemingly has the same anti-inflammatory properties as stimulation of the intact or efferent VN^{86,90}. Of note, activation of the intact (hence also afferent) VN recruits additional, distinct neural pathways. For example, stimulation of the intact, but not efferent, VN evokes action potentials in the renal sympathetic nerve⁹⁰ and recruits an $\alpha 7nACh$ -independent anti-inflammatory pathway⁸⁶. Furthermore, electrical stimulation of the central end of the cut VN has a protective effect in a model of renal ischemia, even when the contralateral VN is blocked⁹⁰ suggesting the recruitment of a vago-sympathetic reflex or activation of the hypothalamic-pituitary-adrenal axis. Finally, VNS reduces cytokine production in sepsis via vagal activation of adrenal dopamine production⁹¹. Clearly, many issues remain to be resolved with respect to the neural pathways activated by VNS, including whether, and where exactly, the vagal nerve endings interact with the immune system, at least with respect to the spleen (Figure 1).

The Cholinergic Anti-Inflammatory Pathway in the gut

As the spleen has been repeatedly shown to be the major source of cytokine production in conditions of systemic inflammation (like sepsis), it is certainly a critical site for neural control of distant organ inflammation. In conditions of more subtle or local inflammation, such as in postoperative ileus or mild colitis, however, the spleen is not itself the site of the inflammatory insult, and other anti-inflammatory pathways may be involved. Indeed, we demonstrated recently that VNS prevents inflammation of the muscular externa evoked by intestinal manipulation and improves postoperative ileus⁹², an effect that is independent of T cells and does not involve the spleen⁹³. This inflammatory response was associated with activation of the NTS and DMV neurons innervating the inflamed area, compatible with the existence of a hardwired inflammatory reflex in the gut, independent of the spleen⁸⁸. This observation suggests that the CAIP may comprise two levels of activation; the first involving local modulation of inflammation that is independent of the spleen, whereas vagal modulation of the splenic immune response is recruited when the inflammatory process becomes more generalized.

The GI tract receives the majority of the vagal efferent nerve fibers and harbors the most immune cells in the body. It is to be expected, therefore, that the GI tract may be an

important site of vagal neuroimmune modulation. It is unlikely, however, that vagal efferents interact directly with immune cells; using an anterograde tracing technique, we confirmed that the VN synapses mainly with the myenteric and submucosal plexuses, but maintains no direct contact with the mucosal/submucosal or myenteric/muscular network of immune cells^{88,93}. ChAT+ fibers originating from enteric neurons are, however, present abundantly in the submucosa and lamina propria along the axis of each individual villus. Notably, varicose fibers approach individual immune cells closely (less than 1 μ M)⁸⁵ while resident macrophages in the muscularis externa and the myenteric plexus are in close proximity to cholinergic nerve fibers. These data indicate that vagal neuromodulation in the intestinal wall is indirect, and occurs via the enteric nervous system. At the level of Peyer's patches, ChAT+ fibers are absent from follicle and dome areas, but are consistently present within the interfollicular area where high-endothelial vessels and many lymphocytes are contained⁸⁵.

Before discussing the importance of the CAIP in the GI tract, it is fundamental to underscore that the immune cell population residing in the submucosal/mucosal compartment largely differs from that in the muscularis externa. The latter population is less well studied and consists mainly of resident macrophages located between the longitudinal and circular muscle layer at the level of the myenteric plexus. These resident macrophages have a rather tolerogenic phenotype⁹⁴, play a role in diabetic-induced gastroparesis⁹⁵, postoperative ileus⁹⁶ and LPS-induced septic ileus⁹⁷ and seem to represent the gatekeepers of the enteric nervous system or the "little brain of the gut". Of note, they are in close contact with cholinergic nerve fibers and express α 7nAChRs^{88,93}. As with splenocytes, the α 7nAChR agonists nicotine and choline dampen the activation of this macrophage population, indicating that these immune cells are sensitive to cholinergic modulation (see below).

The mucosal immune system is however more complex, mainly as the constant challenge by the intestinal microbiota requires a perfectly balanced equilibrium between tolerance and defense against foreign antigens. One of the key mechanisms for discrimination of innocent and harmful antigens is oral tolerance, a mechanism orchestrated by the mucosal immune system and dependent on FoxP3+ regulatory T cells (Tregs). We showed recently that vagotomy reduced the ability to develop oral tolerance, a finding that was associated with a reduction in Tregs in the lamina propria and mesenteric lymph nodes⁹⁸. Of note, vagotomized mice were more susceptible to developing colitis following exposure to the mucosal irritant, dextran sulfate sodium (DSS), suggesting that, in addition to TGF- β , retinoic acid and TSLP⁹⁹, vagal input, or cholinergic tone, may be a new player in determining the degree of lamina propria tolerance (Figure 2). This may be relevant for several intestinal, as well as extra-intestinal, immune-mediated diseases; decreased tolerance to microbiota, for example, is proposed as a main pathogenic mechanism in inflammatory bowel disease (Crohn's disease and ulcerative colitis). Food allergy is another well-known example of loss of tolerance, in which the immune system reacts to food antigens leading potentially to severe anaphylactic shock and even death^{100,101}. In both cases, the immune system overreacts to innocent antigens leading to severe tissue damage, morbidity and mortality.

The following paragraphs will discuss the current evidence supporting the relevance of the CAIP in the GI tract. For more information on the CAIP and its physiological importance, the reader is referred to several excellent reviews^{102–104}.

The Cholinergic Anti-Inflammatory Pathway in intestinal inflammatory disorders Preclinical evidence

Colitis—Inflammatory bowel disease (IBD) is a debilitating and chronic inflammatory GI disease. Based on clinical presentation, endoscopic appearance, and histology, two major subtypes of the disease have been identified, i.e. Crohn's disease with transmural inflammation versus ulcerative colitis which involves mostly superficial inflammation confined to the mucosa. Preclinical models provide convincing evidence that the cholinergic innervation of the gut has a major impact on the intestinal immune system. As indicated above, vagotomized animals fail to develop oral tolerance⁹⁸ and develop more severe colitis with increased levels of NF- κ B and cytokines in DSS and hapten-induced (dinitrobenzene sulfonic acid or DNBS) colitis^{98,105,106}. Similarly, mice with depressive-like behavior following chronic reserpine treatment, which is associated with reduced intestinal levels of acetylcholine, developed more severe DSS and DNBS-induced colitis¹⁰⁷. Of interest, treatment with the anti-depressant desimipramine reversed these effects in a vagus-dependent manner. In contrast, vagotomy does not affect T cell transfer-induced colitis^{98,108}, indicating that T cells are less likely to be under direct vagal/cholinergic control. Macrophages or dendritic cells may be more likely to be involved; vagotomy had no effect on colitis in macrophage deficient mice¹⁰⁵, while adoptive transfer of macrophages isolated from vagotomized mice or mice treated with reserpine to macrophage-deficient M-CSF^{-/-} mice produced a modest, but significant, increase in severity of colitis¹⁰⁹. These data may at least partly explain how psychological factors such as depressive mood associated with anxiety predict the onset in IBD patients^{109,110}. Taken together, the above would suggest that the intestinal cholinergic tone, through vagal input, has a significant modulatory effect on the gut immune system, determining the balance between tolerance and inflammation, and affecting the susceptibility to develop colitis. (Figure 2)

Data on VNS in colitis models is rather limited and restricted to rats, principally because the electrodes for chronic VNS available need to be reduced in size to be suitable for implantation in mice. A single episode of VNS significantly improves oxazolone-induced¹¹¹ and DSS-induced (unpublished data) colitis in mice, an effect that is independent of the spleen. In a rat model of TNBS colitis, chronic VNS slightly reduced disease activity index, histological scores, MPO activity, iNOS, TNF- α and IL-6, an effect mediated by disruption of the NF- κ B pathway^{112,113}. A more pronounced reduction in DSS and DNBS-induced colitis was noted using nicotine, an effect resistant to vagotomy¹⁰⁶, and following activation of brainstem vagal neurocircuits using centrally acting drugs like the M1 muscarinic agonist McN-A-343, the M2 muscarinic antagonist methoctramine, or the acetylcholine esterase inhibitor galantamine¹¹⁴. The beneficial effect of central pharmacological activation of the VN was associated with decreased MHC class II expression and reduced cytokine production of splenic CD11c⁺ splenocytes, most likely resulting in decreased priming of CD4⁺CD25⁻ T cells in the spleen. How these findings relate to the reduction in colonic inflammation, however, remains to be explored. As splenic denervation abolished the

beneficial effect of central cholinergic activation, the authors proposed the involvement of a vagal-splenic nerve circuit¹¹⁴. Of note, however, splenic denervation increased colitis to a similar extent as vagotomy. Given that splenic macrophages are mainly modulated by adrenergic input¹¹⁵, with increased splenic macrophage activation and TNF- α production following adrenergic denervation of the spleen, splenic denervation will undoubtedly obscure the beneficial effect of central VN activation. *It is impossible, therefore, to dissect out the extent to which central vagus activation affects splenic function via the splenic nerve. Moreover, as pointed out earlier, no anatomic or electrophysiological evidence is available to support such neurocircuitry.* Direct modulation of the intestinal immune system, especially in view of the dense cholinergic innervation of the intestine, may be an alternative explanation for the beneficial effect of central acting compounds, with entrapment of intestine- or secondary lymphoid tissue-modulated immune cells in the spleen. Clearly, this hypothesis, and the exact role of the spleen in colitis, require further study. Nevertheless, the concept of central activation of the vagal anti-inflammatory pathway is very appealing and may be a promising new approach to treat IBD.

In models of sepsis, $\alpha 7$ nAChRs have been repeatedly shown to mediate the beneficial effect of VNS^{4,83}. Their role in colitis, however, is somewhat controversial. We showed recently the intact development of oral tolerance and lack of increased susceptibility for development of DSS-induced or T cell transfer colitis in $\alpha 7$ nAChR $^{-/-}$ mice⁹⁸, while DSS colitis was worsened by treatment with selective $\alpha 7$ nAChRs agonists (AR-R17779, GSK1345038A)¹¹⁶. Conversely, others reported more severe DSS-induced colitis and increased IL-1b and IL-6 levels in colonic tissue of $\alpha 7$ nAChR $^{-/-}$ mice¹¹⁰ while cytokine production of splenocytes and splenic CD11c+ cells isolated from colitis mice is reduced by the $\alpha 7$ nAChR agonist GTS-21. Such controversies are not restricted to preclinical models since, in IBD patients, it remains to be determined why nicotine and smoking is associated with improvement of disease activity in ulcerative colitis but has an opposite effect in Crohn's colitis. Differences in expression of $\alpha 7$ nAChR, depending on the state of maturation and the inflammatory microenvironment, may partly explain these seemingly contradictory observations. For example, oxazolone-induced colitis was associated with an IL-4 mediated upregulation of $\alpha 7$ nAChR on colonic CD4 T cells which resulted in a beneficial response to nicotine. Conversely, $\alpha 7$ nAChR expression was down-regulated in an IL-12 dependent manner in TNBS-induced colitis and was associated with a worsening of colitis by nicotine¹¹⁷. To what extent this also applies to IBD patients remains however to be confirmed.

Ischemia –reperfusion intestinal damage—Ischemia due to severe blood loss, cardiac arrest or arterial occlusion results in severe cell damage and organ injury. Tissue damage, in addition to endotoxemia resulting from disruption of the intestinal barrier due to ischemia, is a potent trigger for the innate immune system resulting in a systemic inflammatory response. Indeed, hemorrhagic shock increases intestinal mucosal permeability with bacterial translocation and detectable endotoxin levels as well as systemic release of pro-inflammatory cytokines such as TNF- α and IL-6. Of note, high-fat enteral nutrition prevents these changes, an effect mediated via cholecystokinin-induced activation of vagal afferents triggering the CAIP¹¹⁹. In addition to its known effect on macrophages, a recent study showed vagal-mediated expansion of enteric neural stem cells with an increase in

enteric glia and recovery of barrier function¹²⁰. In agreement with these studies, VNS protected against burn-induced intestinal injury and restored barrier function through activation of enteric glia cells, independently from the spleen¹²¹. Of note, and similar to colitis, administration of nicotinic agonists and central activation of the vagal CAIP by ghrelin and melanocortins reduces the inflammatory response and restores organ function^{122,123}.

Postoperative and endotoxin-induced ileus—Every abdominal surgical intervention leads to impaired motility of the entire GI tract lasting several days with symptoms like nausea, vomiting, intolerance to food and absence of defecation, referred to as postoperative ileus (POI). Although some would argue that this represents a physiological response to the surgical insult and should not be regarded as a clinical problem, this iatrogenic condition is a major source of patient morbidity and a significant economic burden to health care^{96,124}. For more than a decade, a subtle microscopic inflammation of the intestinal muscularis, triggered by activation of resident macrophages residing between the longitudinal and circular muscle layer of the intestinal wall, has been identified as key process in the pathophysiology of POI and endotoxin-induced ileus. Activation of these phagocytes results in cytokine and chemokine release, followed by influx of mainly leukocytes and monocytes starting approximately 3–4 hours after surgery. As this inflammatory response has a major impact on neuromuscular function, treatments or interventions hampering this process may be instrumental in reducing POI⁹⁶.

Several lines of preclinical evidence indicate that activation of the CAIP may be an efficient strategy to prevent POI. Stimulation of the vagus, either electrically⁹², via enteral feeding¹²⁵ or central application of semapimod¹¹⁶, all reduced the influx of immune cells into the muscularis, dampened cytokine production, and improved GI transit. The effect of VNS is mediated by vagal activation of cholinergic myenteric neurons, resulting in reduced activation of resident muscular macrophages. The latter express $\alpha 7$ nAChR, explaining the lack of a beneficial effect of VNS in $\alpha 7$ nAChR^{-/-} mice⁹³. Of note, pharmacological stimulation of these neurons with the 5-hydroxytryptamine 4 receptor agonists mosapride or prucalopride mimics the effect of VNS^{126,127}. Finally, the selective $\alpha 7$ nAChR agonist, AR-R17779, improved intestinal transit and reduced intestinal inflammation¹¹⁶. Of note, and in contrast to the neurocircuitry proposed in sepsis models, vagal modulation of intestinal resident macrophages is independent of T cells and results from a direct input to the gut; selective denervation of the spleen leaves the modulatory effect of the VN untouched, arguing against the spleen as site of neuromodulation, at least in this model of subtle intestinal inflammation⁹³.

Human evidence

Accepting that the VN has a major impact on the immune system, one would anticipate increased incidence of intestinal inflammation in patients who undergo vagotomy. To date, however, solid data supporting this assumption are not available. One study reports increased ulcer disease, septicemia and mortality in patients that underwent vagotomy following traumatic injury¹²⁸ although this increased incidence of inflammatory-mediated adverse outcomes may be a consequence of an increased need for gastrectomy and vagotomy in the

more severe cases¹²⁸. One possible explanation for the lack of increased intestinal inflammation may be that cholinergic tone is restored by compensatory increase within the enteric nervous system. In mice, the increased susceptibility to DSS colitis following vagotomy indeed normalizes after several weeks¹²⁹. To what extent similar compensatory mechanisms are activated in other regions of the body remains to be studied.

There is however increasing indirect evidence, based on heart rate variability monitoring, to support an immune-modulatory role of the CAIP in humans (reviewed in ^{130,131}). In healthy subjects, reduced heart rate variability indices (= low vagal tone) are independently associated with increased serum CRP and IL-6 levels and increased TNF and IL-6 production in endotoxin-stimulated whole blood. Also, in immune-mediated diseases like rheumatoid arthritis, VN activity was reduced compared to healthy controls and associated with increased levels of serum HMGB1, while parasympathetic tone is inversely related to inflammatory markers (IL-6 and CRP) in patients with cardiovascular disease. Finally, increased morbidity and mortality following cardiac surgery, myocardial infarction, sepsis, RA, IBD, lupus erythematosus and sarcoidosis has been reported to be associated with decreased VN activity. These data suggest that reduced vagal tone increases the setpoint of the immune response with a subtle rise in pro-inflammatory cytokines and increased disease risk.

The final evidence that activation of the vagal anti-inflammatory pathway is, indeed, a breakthrough for the clinical management of immune-mediated inflammatory diseases will ultimately have to come from clinical trials evaluating specific nicotinic agonists or electrical, pharmacological or nutritional activation of the CAIP. The effect of nicotine (rectal enemas or transdermal application) has been extensively evaluated in patients with ulcerative colitis albeit with controversial results¹³². Side effects due to interaction with several nAChR subtypes and toxicity issues most likely prevent effective dosing with nicotine. Activation of the CAIP by early enteral nutrition improved clinical recovery in patients undergoing major rectal surgery and was associated with less anastomotic leakage¹³³. In contrast, despite being supposed to activate the VN, gum chewing fails to improve postoperative outcome after colorectal surgery¹³⁴. Finally, electrical stimulation of the VN, either in the neck, the abdominal cavity, or the ear, may be considered as treatment for chronic inflammatory diseases such as IBD and rheumatoid arthritis (RA), and will be discussed in more detail in the following paragraph.

Treatment options – VNS in humans

VNS is currently routinely and safely used in patients with intractable epilepsy, depression and migraine¹³⁵. To this end, a coiled electrode is surgically positioned around the cervical VN and connected to a pacemaker. Transcutaneous auricular VNS, contrast, stimulates the auricular branch of the VN using a dedicated intra-auricular electrode. Finally, the cervical VN can be stimulated transcutaneously by low voltage electrical signals delivered by a handheld device (reviewed in¹³⁶).

VNS refers, commonly, to electrical stimulation of the left cervical vagus, the right vagus being thought to be more closely associated with cardiovascular functions. While not

effective acutely, long-term (>9–12 months) VNS has been shown to have clinically relevant outcomes, in both unipolar and bipolar depression, in patients that fail to respond to multiple antidepressant drugs¹³⁷. The mechanism of VNS action to modulate mood is not understood fully, but since the NTS (the principle termination site of vagal afferents) projects to multiple brainstem, midbrain and forebrain nuclei, VNS has the potential to modulate several CNS-dependent functions. Both animal and human studies have suggested that VNS alters neurotransmitter levels centrally; increased serotonin levels¹³⁸, increased locus ceruleus norepinephrine signaling¹³⁹, altered NTS GABA and glutamate signaling¹⁴⁰, and enhanced cortical inhibition¹⁴¹ have all been proposed to be responsible for the efficacy of VNS in treating epilepsy.

The anti-inflammatory effects of VNS were evaluated initially in epilepsy patients; VNS reduced IL-6 and increased IL-10 serum levels^{142,143} and diminished IL-8, TNF, IL-1B and IL-6 production by isolated peripheral blood mononuclear cells^{144,145}. Recently, a small open pilot study in patients with RA provided the first evidence in humans that VNS, indeed, has the potential to improve immune-mediated diseases¹⁴⁵. Cervical VNS improved RA disease activity, reduced TNF production and dampened cytokine production of peripheral blood. Similarly, 5 out of 7 patients with Crohn's disease improved following cervical VNS and remained in remission during the 6 months follow-up¹⁴⁶. Finally, abdominal VNS during abdominal surgery reduced IL-6 and IL-8 production of LPS-stimulated peripheral blood¹⁴⁷. Randomized and sham-controlled trials are ongoing in Crohn's disease (NCT02311660) and postoperative ileus (NCT02524626, NCT02425774) hopefully further confirming the therapeutic potential of VNS.

The use of VNS in the treatment of obesity has also gained some initial attention¹⁴⁸, despite evidence of only a rather modest weight loss; studies in obese minipig models have shown that long-term bilateral VNS prevents further weight gain and decreases food consumption¹⁴⁹ rather than reversing obesity *per se*. Again, the mechanism responsible for these effects is unclear, although activation of the olfactory bulb and its projection pathways, as well as activation of the hippocampus and pallium (involved in assigning hedonic value to ingestive signals) have been reported¹⁴⁹. Shorter periods of VNS have been shown to activate the mesolimbic dopaminergic system suggesting some of the effects on food intake and weight gain may be related to activation of central reward pathways¹⁵⁰.

In contrast to the modest effects of VNS to reduce or reverse obesity, vagal nerve blockade (vBloc), designed as an alternative to standard bariatric surgery, has proven much more efficient in inducing significant weight loss, early and more prolonged satiation and improved glycemic regulation^{151,152}. While this appears initially to directly contradict the actions of VNS to induce weight loss, it is perhaps not that surprising since it has been known for some time that truncal vagotomy improves obesity, including exogenous (hypothalamic) obesity in rodents⁸³ and humans^{153,154}. Vbloc uses a cuff electrode that encircles the VN and employs kilohertz frequency alternating current, with zero net charge delivery, to produce a very localized, true nerve conduction (rather than a neurotransmitter) block, that is rapidly reversible and does not induce any apparent nerve degeneration or damage (reviewed in¹⁵⁵). Biophysical modeling studies suggest that a depolarization induced inactivation of sodium channels is responsible for the nerve block in mammalian

nerves¹⁵⁵ although the contribution of afferent versus efferent VN block still remains to be elucidated.

Conclusion

While a few decades ago, cutting the vagus nerve represented a well accepted treatment for peptic ulcer disease, it is now clear that this nerve is extremely precious not only for the homeostasis of a variety of organ systems, but also for the regulation of appetite, mood and inflammation. The therapeutic potential of stimulating or blocking the VN, either electrically or pharmacologically, is being explored gradually, and the exact pathways and mechanisms of action are becoming understood. Non-invasive devices to stimulate the VN have been developed, although the optimal stimulation parameters and the type of nerve fibers to be stimulated remain to be determined. Clinical studies are ongoing and will hopefully soon confirm that the VN should be handled with great care rather than being cut.

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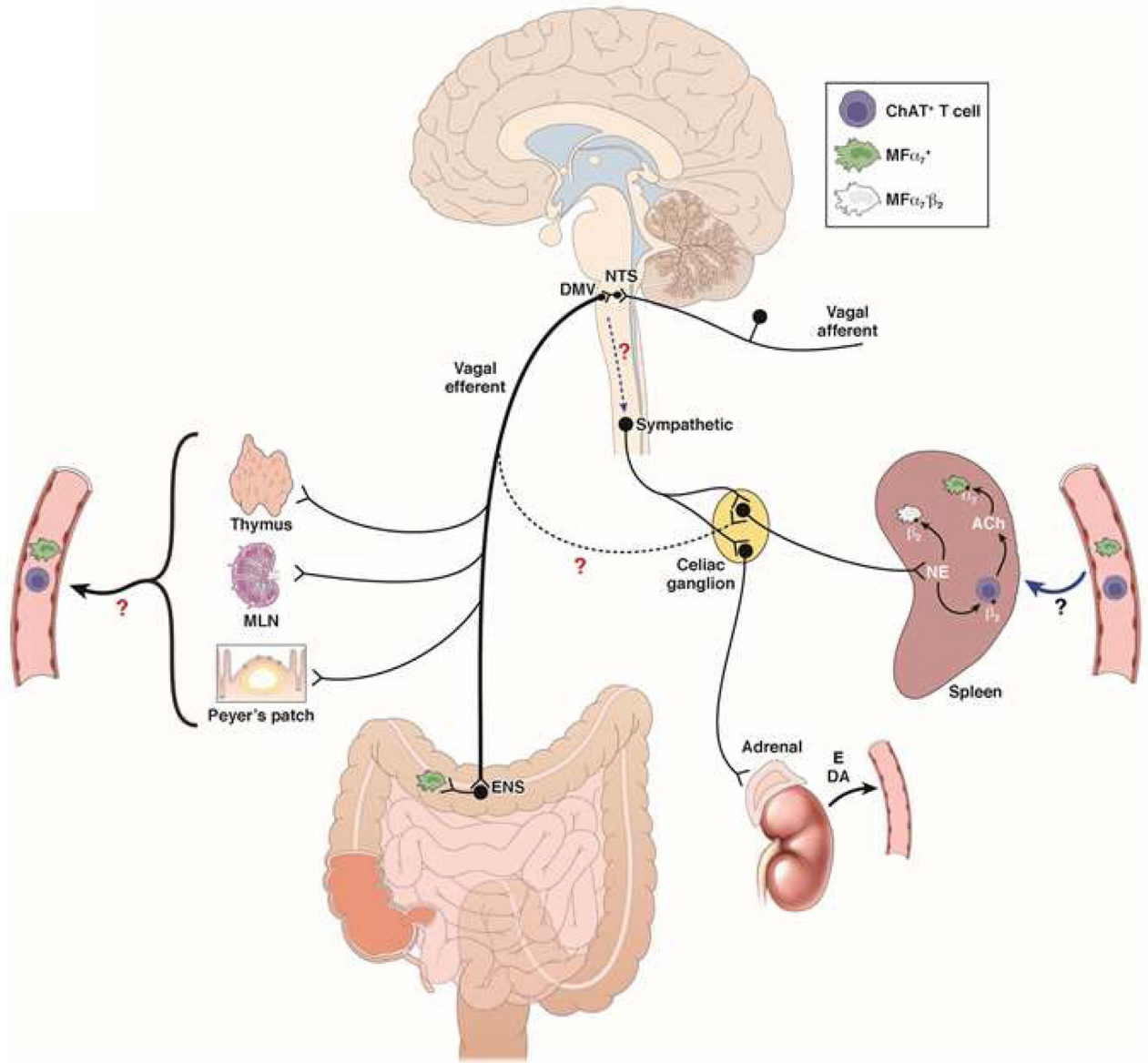


Figure 1. Schematic representation of the cholinergic anti-inflammatory pathway

VNS of the intact vagus nerve stimulates both afferent and efferent fibers. Electrical stimulation of afferent nerve fibers activates neurons in the NTS leading to activation of not only both ipsi- and contralateral efferent vagus nerves, but also of an adrenergic pathway resulting in release of norepinephrine (NE) in the spleen and the production of dopamine (DA) in the adrenal gland. In the spleen, NE reduces TNF production by splenic macrophages both directly, via actions on β_2 adrenoceptor activation, and indirectly, via activation of CHAT+ T cells releasing ACh. Activation of presynaptic α_7 nAChR on adrenergic nerve fibers¹⁵⁶ by choline or other α_7 nAChR agonists may increase NE release contributing to their anti-inflammatory properties. Stimulation of the efferent vagus nerve dampens α_7 nAChR+ resident muscular macrophages in the gastrointestinal tract via activation of cholinergic enteric neurons. The immune cell(s) modulated in the lamina

propria, however, still need to be identified. The extent to which efferent vagus fibers exert an anti-inflammatory effect in the spleen by synapsing with adrenergic postganglionic ($\alpha 7nAChR+$) neurons in the celiac ganglion is a matter of debate since no anatomical or electrophysiological evidence supporting this connection is available. As the vagus nerve innervates the thymus, Peyers' patches and other myeloid organs, one may hypothesize that cholinergic modulation of immune cells ($\alpha 7nAChR+$ macrophages, CHAT+, T cells) occurs in these organs. Under conditions of systemic inflammation, these cells subsequently migrate, or get trapped in the spleen, via the circulation.

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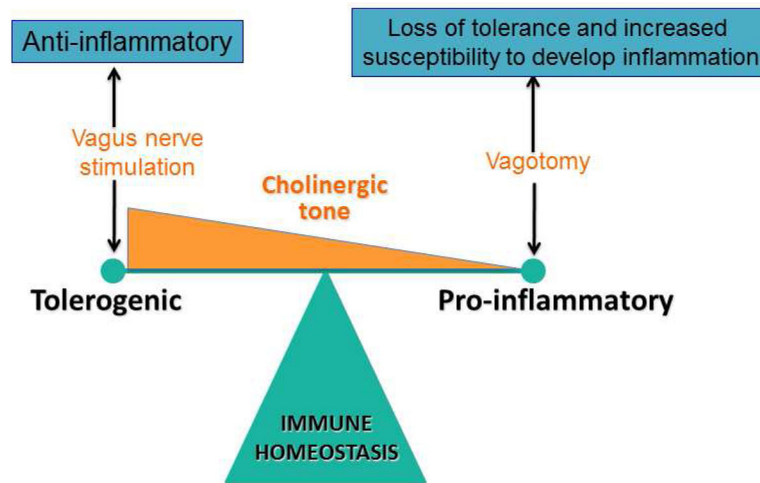


Figure 2. The cholinergic tone determines immune homeostasis either shifting the balance towards tolerance (normal to enhanced tone) or inflammation (decreased tone).

CNS nuclei with *direct* connections with vagal brainstem nuclei involved in the regulation of GI functions¹

Table 1

	Location	Neuroanatomical connections with brainstem vagal nuclei	GI functions
Hindbrain			
Spinal cord	GI afferents terminate within the thoracic (T1-T3) spinal cord	Spinal afferent inputs terminate within the NTS (spinosolitary tract)	Relay nociceptive information; spinal afferents also respond to low-pressure distention and innocuous chemostimulation
Area postrema	Dorsal to the NTS, adjacent to the 4 th ventricle	AP neurons innervate the NTS; NTS and DMV neuronal dendrites reach the AP	Chemoreceptor trigger zone; Contain receptors for, and respond to, a variety of circulating factors including immune and inflammatory mediators
Midbrain			
Paraventricular nucleus of the hypothalamus (PVN)	Hypothalamus; adjacent to 3 rd ventricle within periventricular zone	Reciprocal connections with DVC; release neurotransmitters into neurohypophysis and hormones into hypophysial portal system;	Stress-induced GI responses, including arousal, anxiety and depression
Arcuate nucleus (Arc)	Hypothalamus; adjacent to the 3 rd ventricle, in close apposition to median eminence	Reciprocal connections with DVC	Integration of endocrine and behavioral aspects of GI functions Modulation of food intake and satiety
Barrington's nucleus	Dorsolateral pons	Receives inputs from NTS	Regulation of bladder and colon function; assimilation of emotional and cognitive behaviors with GI functions
Periaqueductal gray (PAG)	Surrounds cerebral aqueduct from dorsal tegmental nucleus to posterior commissure	Dense innervation from NTS	Integration of fear and anxiety; pain modulation
Locus coeruleus (LC)	A6 noradrenergic region; located at rostral pons at lateral floor of 4 th ventricle	Reciprocal connections with DVC	Maintenance of arousal and attention; integration of emotional responses and cognitive inputs with GI functions; stress
Forebrain			
Central nucleus of the amygdala (CeA)	Heterogeneous complex within anteromedial temporal lobe	Reciprocal connections with DVC	Learning and memory; integrative of emotional and aversive inputs with learning, memory and autonomic GI functions; prominent role in anxiety and stress-related GI disorders
Bed Nucleus of the Stria Terminalis (BNST)	Part of the extended amygdala	Reciprocal connections with DVC	Processing and consolidation of behavior and emotions; regulation of HPA axis and autonomic (GI) responses to stress
Cortex		Direct descending connections (infralimbic and prelimbic cortex) to DVC; indirect descending connections via hypothalamus and amygdala	Integration of affect, emotion and memory with autonomic (GI) functions