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REVIEW ARTICLE

Vagus nerve stimulation: from epilepsy to the cholinergic anti-inflammatory pathway

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Abstract

Background *The brain and the gut communicate bidirectionally through the autonomic nervous system (ANS). The vagus nerve (VN), a major component of the ANS, plays a key role in the neuro-endocrine-immune axis to maintain homeostasia through its afferents (through the activation of the hypothalamic pituitary adrenal axis and the central ANS) and through its efferents (i.e. the cholinergic anti-inflammatory pathway; CAP). The CAP has an anti-TNF effect both through the release of acetylcholine at the distal VN acting on macrophages and through the connection of the VN with the spleen through the splenic sympathetic nerve. Vagus nerve stimulation (VNS) of vagal afferents at high frequency (20–30 Hz) is used for the treatment of drug-resistant epilepsy and depression. Low-frequency (5 Hz) VNS of vagal efferents activates the CAP for an anti-inflammatory effect that is as an anti-TNF therapy in inflammatory diseases were TNF is a key cytokine as represented by experimental sepsis, postoperative ileus, burn-induced intestinal barrier injury, colitis. However, both vagal afferents and efferents are activated by VNS.*

Purpose *The objective of this review was to explore the following: (i) the supporting evidence for the importance of VNS in epilepsy (and depression) and its mechanisms of action, (ii) the anti-inflammatory characteristics of the VN, (iii) the experimental evidence that VNS impact on inflammatory disorders focusing on the digestive tract, and (iv) how VNS could potentially be harnessed therapeutically in human inflammatory disorders such as inflammatory bowel diseases, irritable bowel syndrome, postoperative ileus, rheumatoid arthritis as an anti-inflammatory therapy.*

Keywords *autonomic nervous system, brain–gut interactions, inflammation, inflammatory bowel diseases, vagus nerve, vagus nerve stimulation.*

Abbreviations: βAR, beta adrenergic receptor; ACh, acetylcholine; AChR, acetylcholine receptor; ACTH, adrenocorticotrophin; ANS, autonomic nervous system; BOLD, blood oxygen level dependent; CAP, cholinergic anti-inflammatory pathway; CCK, cholecystokinin; CNS, central nervous system; CRF, corticotrophin-releasing factor; DMNV, dorsal motor nucleus of the vagus; DVC, dorsal vagal complex; fMRI, functional magnetic resonance imaging; HPA, hypothalamic pituitary adrenal; HRV, heart rate variability; IBD, inflammatory bowel diseases; IBS, irritable bowel syndrome; IL, interleukin; JAK, Janus Kinase; LC, locus coeruleus; LPS, lipopolysaccharides; nAChR, nicotinic acetylcholine receptor; NE, norepinephrine; NFκB, nuclear factor kappa B; NTS, nucleus tractus solitarius; PB, parabrachial nucleus; POI, postoperative ileus; PVN, paraventricular nucleus; RA, rheumatoid arthritis; STAT, signal transducer and activator of transcription; TNBS,

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trinitrobenzene sulfonic acid; TNFα, tumor necrosis alpha; VN, vagus nerve; VNS, vagus nerve stimulation

INTRODUCTION

Inflammation plays a major role in many chronic and autoimmune diseases involving a complex reaction between proinflammatory cytokines, chemokines, neuromediators, and other signaling molecules initiating and perpetuating the inflammatory reaction.

Tumor necrosis factor alpha (TNFα) is a key cytokine involved in the pathobiology of inflammatory digestive disorders, such as inflammatory bowel diseases (IBD, Crohn's disease, and ulcerative colitis), as well as extra-digestive inflammatory disorders such as rheumatoid arthritis (RA).[1] Anti-TNF therapy is a gold standard in such inflammatory diseases although not devoid of side-effects and treatment resistance.[2,3]

The brain and the gut communicate bidirectionally through the brain–gut axis. A dysfunction of this axis is classically evoked in the pathobiology of irritable bowel syndrome (IBS)[4] and IBD.[5] The autonomic nervous system (ANS), represented by the sympathetic and parasympathetic nervous systems, is a key element in brain–gut interactions. Stress is involved in the pathogeny of such disorders and is known to inhibit the parasympathetic while increasing the sympathetic nervous system.[5] An imbalanced ANS is observed in IBS and IBD[6] as well as in RA.[7] Consequently, restoring the balance of the ANS should be an innovative approach in the treatment of IBD, IBS, RA, and others related diseases.

The vagus nerve (VN) is a major component of the ANS (i.e. the parasympathetic nervous system)[8] and plays a key role in the neuro-endocrine-immune axis to maintain homeostasis through the activation of the hypothalamic pituitary adrenal (HPA) axis by its afferents[9] and in the newly discovered 'cholinergic anti-inflammatory pathway' (CAP) through its efferents.[10] The CAP has an anti-TNF action both through the effect of acetylcholine (ACh), released at the distal VN, on peripheral macrophages[10] and the connections of the VN with the spleen.[11]

Consequently, potential anti-inflammatory strategies targeting these autonomic pathways are of interest. The idea being therefore to 'activate' the parasympathetic nervous system through the VN to improve the CAP in numerous acute or chronic inflammatory processes such as sepsis, postoperative ileus (POI), IBD, RA, autoimmune diseases where the inflammation is speeded up.

Among these new therapeutic strategies, VN stimulation (VNS), classically used in drug-resistant epilepsy and depression,[12] should be of interest. If activation of vagal afferents is the mechanism of action of VNS in the treatment of epilepsy and depression, activation of vagal efferents by VNS would be the objective to target the CAP although both activation of VN afferents (through the effect on the HPA axis and other components of the central ANS) and efferents are of interest.

In this review, we will explore the supporting evidence for the importance of VNS in epilepsy (and depression) and its mechanisms of action and the experimental evidence that VNS impact on inflammatory disorders focusing on the digestive tract and how VNS could potentially be harnessed therapeutically in humans with such disorders.

FUNCTIONAL ANATOMY OF THE VAGUS NERVE

The VN (tenth cranial nerve) is the longest of the cranial nerves (from the brainstem to the abdomen) and a major component of the parasympathetic nervous system providing innervation of several organs of the neck, thorax, and abdomen,[8] including organs of the reticulo-endothelial system, such as the spleen and liver that are major sources of damaging cytokines.[10] The right and left cervical VN emerge from the brain medulla at the jugular foramen, extend via the nodose ganglia into the neck, course along the esophagus and then enter the abdomen as two trunks (i.e. the dorsal and ventral trunks) dividing into four or five distinct primary branches at the subdiaphragmatic esophageal level: the ventral and dorsal gastric branches, the ventral and dorsal celiac branches, and a single hepatic branch derived from the ventral trunk.[13] The ventral gastric branch of the VN supplies the ventral part of the stomach, the pyloric sphincter, and also the proximal duodenum. The dorsal gastric branch enters near the cardia and innervates the dorsal part of the stomach as well as the proximal duodenum through transpyloric fibers. The ventral and dorsal celiac branches of the VN course along the celiac artery and near the celiac ganglia distribute to innervate the small intestine and the proximal and descending colon by traveling along the superior mesenteric artery (Fig. 1). The common hepatic branch divides into the hepatic branch proper that innervates the liver and the gastroduodenal/pyloric branch that innervates the gastric antrum, pylorus, duodenum, and pancreas. In rats, all regions of the colon, except the rectum, are innervated by the celiac and accessory celiac branches of the VN but it is largely similar across species, including humans.[14] The gastric branches (composed of over half of all abdominal vagal

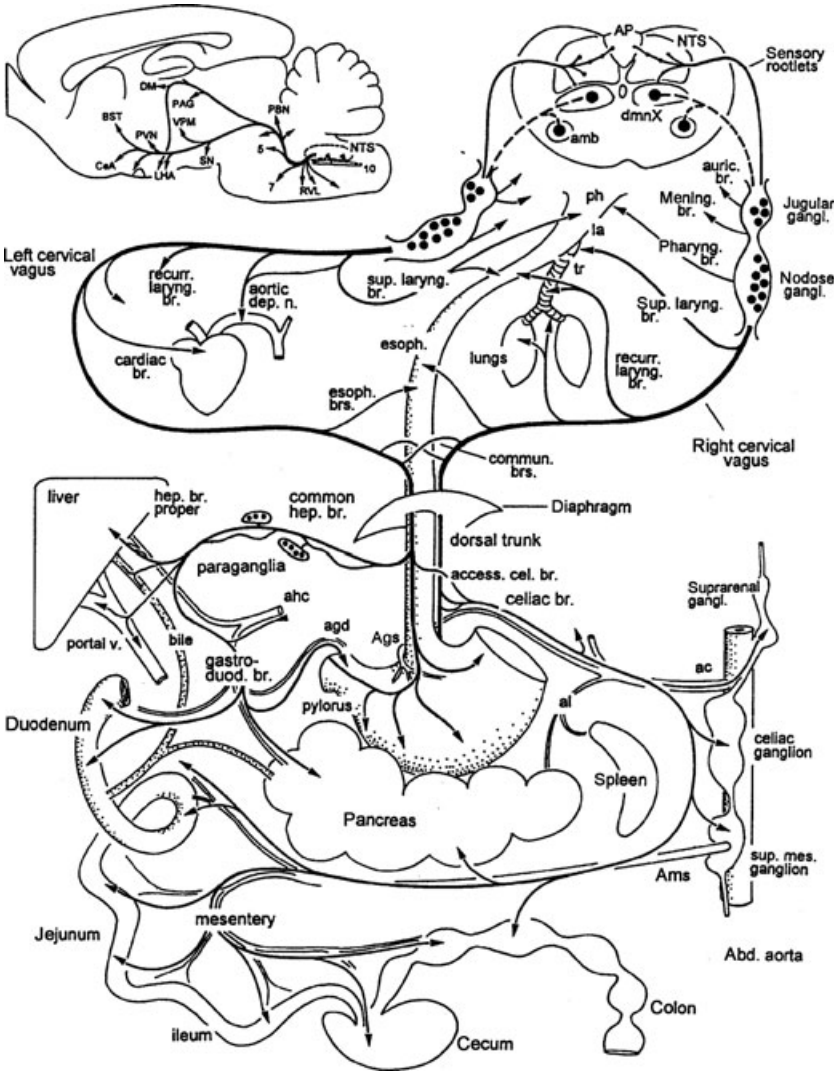

Figure 1 Schematic representation of the vagal innervation of the gastrointestinal tract and central distribution of vagal afferent information from the nucleus tractus solitarius (from Ref. 13). Abbreviations: ac, celiac artery; agd, right gastric artery; ags, left gastric artery; ahc, common hepatic artery; al, splenic artery; Amb, nucleus ambiguus; ams, superior mesenteric artery; AP, area postrema; BST, bed nucleus of stria terminalis; DM, dorsomedial nucleus of thalamus; CeA, central nucleus of amygdala; la, larynx; LHA, lateral hypothalamic area; NTS, nucleus tractus solitarius; PAG, periaqueductal gray; ph, pharynx; tr, trachea. PVN, paraventricular nucleus of hypothalamus; PBN, parabrachial nucleus; RVL, rostroventrolateral medulla; SN, substantia nigra; VPM, ventral posteriomedial nucleus of thalamus; five, trigeminal nucleus; seven, facial nucleus; 10 (dmnX), dorsal motor nucleus of the vagus.

fibers) control stomach acid secretion; the hepatic branch influences the motility of the gall bladder and biliary tract, and the motility of the distal intestine and colon is mediated by the celiac branches.

The VN regulates heart rate and blood pressure. The right VN innervates the sinoatrial node (involved in the pace-maker function of the heart), whereas the left VN innervates the atrioventricular node (regulating the force of contraction of the heart muscle with less influence over heart rate). VNS of the right, compared to the left VN, caused a greater reduction in heart rate whereas stimulation of the left VN had no effect on heart rate.[15] Consequently, in experimental as well as clinical conditions VNS is classically performed on the left VN.

The vagal trunk innervating the gut is composed mainly of unmyelinated fibers with afferent fibers being the major component (over 80%) while vagal efferents represent less than 20%.[14] Vagal afferents vehiculate information to the brain from the head, neck, thorax, and abdomen that mediate vital digestive reflexes and influence ingestive behavior. Vagal efferents originate from cell bodies located in specific brainstem nuclei, namely the dorsal motor nucleus of

the vagus (DMNV) and nucleus ambiguus (Fig. 1) and control autonomic functions like heart rate, blood pressure and gastrointestinal motility and secretion. Within the DMNV, the preganglionic neurons are organized into longitudinal columns, corresponding to a different abdominal branch. The sensory afferent cell bodies reside in the nodose ganglia and relay information to the nucleus tractus solitarius (NTS) which is in close relation with the DMNV to compose the dorsal vagal complex (DVC). The NTS is divided into various subnuclei, partly correlated with the areas of projection of peripheral afferent endings,[16] relays sensory information to the forebrain through the parabrachial nucleus (PB), and sends direct projections to the amygdala and the hypothalamus (e.g. paraventricular nucleus, PVN) (Fig. 1).

The VN contains A-, B-, and C-fibers, defined in accordance to their conduction velocity, which, in myelinated fibers, is proportional to their size.[17] The most numerous fibers in the VN are the afferent C-fibers (~65–80% of the total numbers of the fibers in the cat).[15] The types of fibers which form the VN play different physiological roles: (i) the vagal A-fibers are the largest and myelinated fibers and carry afferent visceral information and motor input, (ii) the vagal B-fibers are small and myelinated fibers carrying parasympathetic input, and (iii) the vagal C-fibers are small and unmyelinated and carry afferent visceral information.

Vagus nerve afferents to the central nervous system (CNS) have been used as a target for the treatment of epilepsy (and depression).

VNS IN EPILEPSY: STIMULATION OF VAGAL AFFERENTS

Vagus nerve stimulation for suppression of seizures was first performed by J. L. Corning, in the early 1880s using an 'electrocompressor' for transcutaneous VNS of the VN cervical trunk based on the idea that seizures may be due to alterations in cerebral blood flow. However, this treatment did not produce consistent positive effects, and was not widely adopted by his contemporaries and largely forgotten about for a century.[18] As early as 1938, Bailey and Bremer[19] reported that VNS in the cat elicited synchronized activity in the orbital cortex. The first implant of a VNS device into a human for chronic VNS for the treatment of drug-resistant epilepsy was reported in 1988.[20] Vagus nerve stimulation was approved in the US in 1997 by FDA as an adjunctive treatment for partial onset seizures refractory to drugs, and then in 2005 for chronic or recurrent depression therapy based on its improvement in mood, independent of seizure activity, in patients with epilepsy receiving VNS.[21] Vagus nerve stimulation is also currently approved as a treatment for epilepsy and depression since 2001 in the European Union and in Canada. The long-term results have shown that patients reached a 50% seizure reduction after 2 and 3 years [22] although insufficient data are available to describe VNS as effective in the treatment of depression.[23]

Vagus nerve stimulation is applied through a spiral electrode wrapped around the left VN in the neck.[24] The connected cable is tunneled subcutaneously to and connected with a pulse generator that is placed in a subcutaneous pocket in the left chest wall (Fig. 2). Neurosurgeons are ideally suited because of the familiarity with epilepsy. The duration of the surgical implantation is about 1 h. The current device is manufactured by Cyberonics (Houston, TX, USA) and includes a pair of helical electrodes (2 or 3 mm diameter), a battery-powered generator, a tunneling tool, software and programming tools, and supplies for the patient (http://us.cyberonics.com/en/).

Zanchetti *et al.*[25] clearly demonstrated the role of central vagal afferents in modulating directly cortical activity. They showed that a broad range of frequency of stimulation of the VN (2–300 Hz) produced electroencephalographic desynchronization of the 'encéphale isolé' cat. The effect was abolished if vagal impulses were blocked by a tight ligature placed on the proximal end of the vagal trunk. Antiepileptic potency of VNS

Figure 2 The Vagus nerve stimulation system (from Cyberonics) with the pulse generator that is placed in a subcutaneous pocket in the left chest wall[1] and a spiral electrode wrapped around the left vagus nerve in the neck.[2]

was suggested to be directly related to vagal C-fibers, but their destruction does not alter subsequent VNS-induced seizure suppression in rats[26] suggesting that seizure suppression results from activation of vagal A- and B-fibers. Vagal A-fibers have the lowest amplitude-duration threshold required for VNS to excite action potentials (ranging from 0.02 to 0.2 mA). The B-fibers have higher excitation thresholds (ranging from 0.04 to 0.6 mA), whereas the highest excitation thresholds (more than 2.0 mA) belong to the narrow, unmyelinated C-fibers.[15,21] Vagus nerve stimulation can be administered with a range of at least five different use parameters (intensity, frequency, pulse width, on-time, and off-time). Therapeutic stimulation parameters for epilepsy and depression consist of output current 0.5–3.5 mA, frequency 20–30 Hz, pulse width 500 μs, and stimulation on-time of 30–90 s followed by off-time of 5 min. Frequencies of 50 Hz and above caused major irreversible damage to the VN.[21]

The exact mechanism of VNS effect in epilepsy and depression is not known, but many argue that an afferent polysynaptic pathway from the NTS to cortical regions mediates its anticonvulsant action through: (i) an increased synaptic activity in the thalamus and thalamo-cortical projection pathways, (ii) an increased synaptic activity in the components of the central ANS, (iii) a decreased synaptic activity in the limbic system (amygdala and hippocampus), and (iv) an increased release of norepinephrine (NE) and serotonine over widespread cerebral regions.[12] All these regions are innervated directly and indirectly by the VN through the projection of the NTS.[27] The locus coeruleus (LC), the principal brain noradrenergic nucleus, which is directly connected to the NTS, mediates at least some of the effects of VNS in attenuating seizures.[28]

The *C-fos* studies in rats during VNS at antiepileptic stimulation reveal increased activity in many brain structures important for genesis or regulation of seizures in the forebrain such as the amygdala, cingulate, LC, and hypothalamus.[29] In humans *in vivo*, functional neuroimaging studies of VNS have shown longer term changes in brain regions with VN innervations including the thalamus, cerebellum, orbitofrontal cortex, limbic system,hypothalamus, and medulla.[30,31]

Common side effects reported for VNS of afferents (20–30 Hz) in epilepsy and depression are as follows: cough, hoarseness, voice alteration, and paresthesias; they are generally well tolerated and usually related to the 'on' phase of stimulation, often fade with time[32] and easy to control by reducing the stimulation intensity. No significant impact on heart rate has been identified. Vagus nerve stimulation at low frequency (1 Hz) has been used as a group control in epilepsy and was well tolerated; patients reported fewer side effects than in the 30 Hz stimulated group[33]; VNS at 10 Hz did not also induce deleterious side effects.[30,31]

Besides its role in the treatment of epilepsy through its afferents, the VN has also anti-inflammatory properties both through its afferents and, more recently described, through its efferents.

ANTI-INFLAMMATORY PROPERTIES OF VAGUS NERVE AFFERENTS

In addition to its role in the control/modulation of motility and secretion of the digestive tract,[34] the VN has an anti-inflammatory effect through its afferents which are classically involved in the activation of the HPA axis to release corticosteroids by the adrenal glands with well-known anti-inflammatory properties[9] (Fig. 3A). An impaired HPA axis is an important risk factor for susceptibility to and severity in numerous animal models and human inflammatory disorders and autoimmune diseases including RA, IBD, multiple sclerosis, and the allergic conditions, asthma and dermatitis.[5,35] The VN is an important structure for communication of the immune system with the brain. Stimulation of toll like receptors expressed on immune cells by pathogen-associated molecular patterns or disease-associated molecular pattern induces an increase of proinflammatory cytokines [i.e. interleukin (IL)-1β, IL-6, TNFα], which are able to communicate with the brain through neural and humoral (i.e. circumventricular organs which lack the blood-brain barrier) pathways[9] (Fig. 3A). The neural pathway involves VN afferents locally stimulated by cytokines, through receptors on dendritic immune cells, which are located within the VN and associated paraganglia, expressing IL-1 receptors.[36] Thus, systemic IL-1β may bind directly to cells in the vagal paraganglia that subsequently activate vagal afferents, which terminate in the DVC (i.e. in the NTS) of the caudal medulla. Indeed, peripheral administration of IL-1β and lipopolysaccharides (LPS) produce *c-fos* activation in the NTS, which is the predominant termination site of afferent VN.[37] IL-1β increases the number of sensory neurons in the nodose ganglion that express *c-fos*; this response is attenuated in animals pretreated with the cyclooxygenase inhibitor indomethacin thus suggesting partial mediation by prostaglandins.[38] Glutamate is the neurotransmitter for a majority of vagal afferents in terminal fields of the NTS.[39] The DVC integrates sensory signals with descending neural inputs to control visceral reflexes and to relay visceral sensory information to nuclei of the central ANS.[40] In particular, the NTS, through its noradrenergic (i.e. from the A2 group located in the

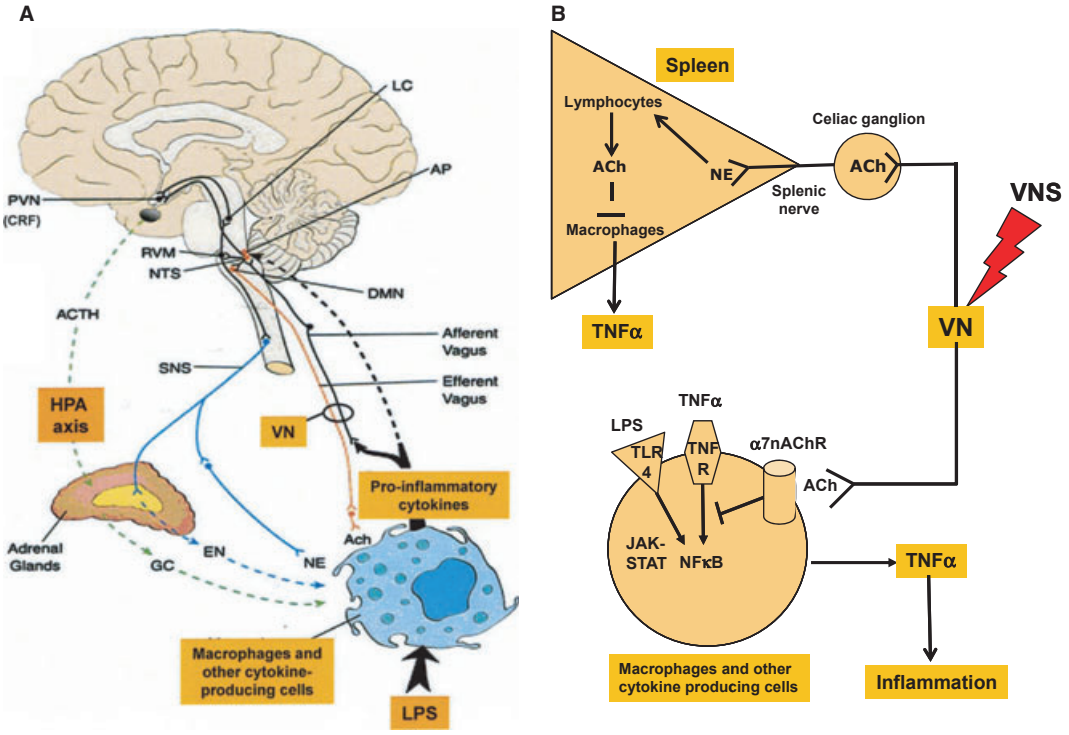

Figure 3 (A) The neuro-endocrine-immune axis. ACh, acetylcholine; ACTH, adrenocorticotropin hormone; AP, area postrema; CRF, corticotrophin-releasing factor; DMN, dorsal motor nucleus of the vagus; EN, epinephrine; GC, glucocorticoids; HPA, hypothalamic pituitary adrenal; LC, locus coeruleus; LPS, lipopolysaccharides; NE, norepinephrine; NTS, nucleus tractus solitarius; PVN, paraventricular nucleus of the hypothalamus; RVM, rostral ventrolateral medulla; SNS, sympathetic nervous system; VN, vagus nerve (From Pavlov *et al.*[53]). (B) The anti-inflammatory effect of the vagus nerve at the level of macrophages and other cytokine-producing cells as well as at the level of the spleen through an interaction of the vagus nerve with the splenic sympathetic nerve. ACh, acetylcholine; α7nAChR, alpha7 nicotinic ACh receptor; JAK-STAT, janus kinase-signal transducer and activator of transcription pathway; LPS, lipopolysaccharides; NE, norepinephrine; NFkB, nuclear factor kappa B; TLR 4; Toll like receptor 4; TNFα, tumor necrosis factor α; TNFR, TNF receptor; VN, vagus nerve; VNS, vagus nerve stimulation.

NTS) projections to the parvicellular division of the PVN,[41] a region known to contain a prominent population of corticotropin-releasing factor (CRF)-immunoreactive neurons, is able to activate the HPA axis response to a peripheral inflammatory signal.[9] Lesions of the noradrenergic projections to the hypothalamus in rats impair the increase in plasma corticosterone induced by IL-1β.[42] Subdiaphragmatic vagotomy prevents the decrease in hypothalamic NE in response to IL-1β injection in the rat[43] and inhibits activation of PVN neurons and subsequent secretion of adrenocorticotropin (ACTH).[44] Chronic depression increases susceptibility to IBD through an impaired parasympathetic function in a mouse model of depression.[45] Hypersecretion of ACTH after CRF challenge in chronically depressed patients is compatible with a state of chronic hypersecretion of CRF leading to increased synthesis of ACTH and these responses are reduced after 3 months of treatment with VNS.[46] Electrical stimulation of the central end of the left VN at 10 Hz in rats induced an increase in the expression of IL-1β mRNA in the hypothalamus and hippocampus as well as an increase in the expression of CRF mRNA in the hypothalamus and an increase in plasma levels of both ACTH and corticosterone.[47] Such modifications in relation with IL-1β, the VN, and CRF are able to either counterbalance or favor the anti-inflammatory properties of vagal afferents depending on the activation or disruption of the neuro-endocrine-immune axis.

ANTI-INFLAMMATORY PROPERTIES OF VAGUS NERVE EFFERENTS

Vagal efferents have an anti-inflammatory property either directly on immune cells (e.g. macrophages) or through the splenic sympathetic nerve.

THE CHOLINERGIC ANTI-INFLAMMATORY PATHWAY

If HPA axis activation through vagal afferents stimulation by endotoxin or cytokines was well established, little was known about the role of vagal efferents in the modulation of inflammation until the work of the

group of Tracey which first reported an anti-inflammatory role of VN efferents through the CAP.[48] This pathway is the efferent arm of an 'inflammatory reflex', which can be activated by inflammatory mediators (e.g. cytokines) in peripheral tissues that activate firing of afferent signals in the VN which, in effect, 'notify' the CNS about the presence of inflammation in the body. This, in turn, activates an opposing efferent VN response via the CAP which inhibits inflammation through the suppression of cytokine production and prevents damage[10,49] (Fig. 3A). Thus, the VN possesses a double role within the framework of inflammation: informing the CNS via its afferents of the presence of inflammation and modulating inflammation via its efferents.[10] The cellular molecular mechanism for inhibition of cytokine synthesis is attributable to ACh, the major VN neurotransmitter. Macrophages and other cytokine-producing cells express ACh receptors (AChRs) which transduce an intracellular signal that inhibits cytokine synthesis. The α7 subunit of the nicotinic AChR (α7nAChR) is the best characterized of these cholinergic receptors that suppress cytokines. α7nAChR and other sub-units of nAChR are expressed on macrophage surface.[50] Others immune cells like B lymphocytes express α7nAChR and α4β2 nAChR.[51] Muscarinic as well as nicotinic receptors are also found on T lymphocytes.[52] ACh, released at the distal end of VN efferents, dose dependently decreases the production of proinflammatory cytokines such as TNFα by human macrophages *in vitro* stimulated by endotoxins through α7nAChR expressed by macrophages[53] (Fig. 3B). Vagus nerve stimulation in α7nAChR-knockout animals failed to suppress cytokine synthesis whereas it significantly inhibited cytokine release in wild-type littermates.[54] However, α7nAChR properties remain unclear. On the one hand, nicotine and cholinergic agonists had no clinical effects in TNBS rodent model, on the other hand, the use of cholinergic agonists in dextran sodium sulfate model worsened sickness parameters and increased proinflammatory cytokines; only high doses improved the clinical status but had no effect on inflammatory parameters.[55] The vagal integrity is an important protective factor and vagotomy has deleterious effects on experimental colitis[56] but HPA activation plays this role when vagal integrity is compromised beyond 2 weeks.[57]

THE SPLEEN: AN EFFECTOR OF THE CHOLINERGIC ANTI-INFLAMMATORY PATHWAY

The spleen, a secondary lymphoid organ, is a major source of systemic TNFα during endotoxemia.[58] Prevertebral sympathetic ganglia associated with the celiac-mesenteric plexus provide a major sympathetic input to the spleen. The splenic nerve is the final common pathway for neural input to the spleen. The parasympathetic innervation of the spleen remains controversial because some studies have shown a direct innervation of the spleen by the VN[59] whereas others did not show connection.[60] In addition, choline acetyltransferase, a more specific marker of cholinergic nerve fibers than acetylcholine-esterase, is absent in the spleen.[61] Splenectomy inactivates the CAP during a septic shock in animals and specific lesions of the celiac ganglia suppress the decrease of TNFα induced by VNS.[58,62] These data argue for a link between the spleen and the CAP via the VN through the splenic sympathetic nerve. Indeed, if classically sympathetic and parasympathetic systems act in opposition to maintain physiological homeostasis, the VN is connected to the splenic sympathetic nerve and controls systemic inflammation through the sympathetic noradrenergic splenic nerve via the α7nAChR. ACh released by the VN in the celiac-mesenteric ganglia activates postsynaptic α7nAChR of the splenic nerve, leading to the release of NE in the spleen thus inhibiting the production and secretion of TNFα by splenic macrophages in response to LPS via beta adrenergic receptor (βAR)[11] (Fig. 3B). The β2AR subtype is the primary receptor that is expressed on immune cells in both rodents and humans[63] and β2AR stimulation increases the intracellular level of cAMP and protein kinase A activation as well as mitogen-activated protein kinase. T and B cells involved in adaptive immunity express the β2AR subtype exclusively. Populations of CD8 + and CD4 + T cells express the β2AR, as do naive CD4 + T cells and murine Th1 cells, while clones of murine Th2 cells do not. Norepinephrine causes a shift to a Th2-like cytokine environment. The role of the VN in the regulation of inflammation is not limited to the macrophages, but could also have an important role on splenic lymphocytes. Indeed, subdiaphragmatic vagotomy in mice induced an increase in proliferation of splenic T CD4 lymphocytes and of proinflammatory cytokines secretion after *in vitro* stimulation[64]; thus the VN exerts a tonic inhibition on lymphocytes TCD4 + activity. Norepinephrine binds itself on βAR of splenic T lymphocytes. This binding induces a secretion of ACh by T lymphocytes, which then binds to the α7nAChR receptors of splenic macrophages to inhibit the release of TNFα[65] (Fig. 3B). Activation of the splenic nerve also arrests B-cell migration and inhibits antibody production.[49]

This CAP could be dampened in stress conditions thus explaining the role of stress in inflammatory

conditions such as IBD.[5] Indeed, stress decreases VN efferent outflow and increases sympathetic outflow and adrenomedullary activity leading to increased NE and epinephrine levels thereby inhibiting immune cell functions and favoring intestinal inflammation.[66–68]

VAGUS NERVE STIMULATION TO ACTIVATE THE CHOLINERGIC ANTI-INFLAMMATORY PATHWAY

The CAP appears as an interesting target to treat inflammatory disorders of the digestive tract, such as IBD, as well as extra-digestive inflammatory disorders such as RA.

Besides its first description in the attenuation of the systemic inflammatory response to endotoxin,[48] the CAP may be activated to dampen inflammatory conditions of the gastrointestinal tract as well as of liver and pancreas. This anti-inflammatory effect could be driven as follows: (i) pharmacologically, using selective alpha7nAChR agonists as observed in experimental model of pancreatitis,[69] POI,[70,71] central injections of CNI-1493 (a tetravalent guanylhydrazone, which inhibits production of proinflammatory cytokines in macrophages) in a model of acute inflammation in rats,[48] as well as peripheral or central cholinesterase inhibitors in models of colitis [72] or murine endotoxemia,[73] (ii) through high-fat enteral nutrition-induced release of cholecystokinin (CCK) which stimulates CCK receptors on vagal afferents and attenuates the inflammatory response in a model of hemorrhagic shock-induced TNF and IL-6 release,[74] (iii) through physical activity or exercise training which may favorably affect the CAP,[75,76] (iv) through complementary alternative medicine as there is cumulative evidence that meditation, cognitive and relaxation therapies, acupuncture, hypnosis and tai-chi may increase the high frequency power of heart rate variability (HRV), a marker of the sympatho-vagal balance,[5,6] suggesting enhancement of VN activity,[5,77] (v) and finally through VNS.

The use of VNS as an anti-inflammatory tool was first reported, after the approval of VNS for epilepsy, in the work of Borovikova *et al.*[48] To determine whether direct stimulation of efferent VN activity might suppress the systemic inflammatory response to endotoxin, they subjected adult male Lewis rats to bilateral cervical vagotomy and efferent VN activity was stimulated by application of constant voltage pulses (5 V, 2 ms, 1 Hz) to the distal end of the divided VN 10 min before and again 10 min after the administration of a lethal LPS dose. Electrical stimulation of the efferent VN significantly decreased the amounts of TNFα in the serum. In contrast, vagotomy without VNS significantly increased peak serum TNFα amounts compared to sham-operated controls. Electrical stimulation of the distal VN decreased hepatic LPS-stimulated TNFα synthesis compared to sham-operated controls. Vagotomy without stimulation was associated with increased TNFα synthesis in liver. These data directly implicate efferent VN signaling in the regulation of TNFα production *in vivo*. Direct electrical stimulation of the peripheral VN did not stimulate an increase in either the corticosteroid or the IL-10 responses thus the authors concluded that suppressed TNFα synthesis in the serum and liver after VNS could not be attributed to the activity of these humoral anti-inflammatory mediators although VNS was performed in vagotomized (i.e. disconnected from the brain) animals. In a comparable study but performed in anesthetized Lewis rats with intact vagi, Bernik TR *et al.*[78] performed VNS with constant voltage at either 1V (2 ms, 5 Hz) or 5V (2 ms, 5 Hz) for 10 min intervals before and after LPS injection, for a total of 20 continuous min to either the left or right VN. They showed that intact VNS protected against endotoxin-induced hypotension and endotoxin-induced shock. Electrical stimulation of intact VN also recapitulated the anti-inflammatory action of CNI-1493, attenuated LPS-induced systemic TNFα release and TNFα synthesis in the heart and prevented the development of endotoxin-induced shock. The protective effects of VNS were partially dependent on stimulus voltage but not nerve laterality. Indeed, no significant differences were observed between left or right VNS with regard to protection against endotoxin-induced shock, demonstrating that stimulation of either VN is effective. VNS (5 V, 2 ms, 5 Hz) during lethal endotoxemia significantly attenuated cardiac TNFα levels, but failed to inhibit pulmonary TNFα levels thus indicating that the CAP inhibition of TNFα in liver and heart was specific. Consequently, the inhibition of cardiac TNFα by VNS has potential therapeutic implications in heart diseases.

When applied to the digestive tract, de Jonge *et al.*[71] performed VNS in a mouse model of intestinal manipulation known to induce POI. The role of splanchnic capsaicin-sensitive and brain CRF pathways are proposed as part of a splanchnic-NTS/LC-hypothalamus reflex circuitry subserving the inhibition of gastric ileus immediately postsurgery[79,80] as well as inflammation of the intestinal muscularis due to activation of resident macrophages that are triggered by bowel manipulation.[81] The authors studied the effect of cholinergic inhibition of macrophage activity *in vivo* on the occurrence of postsurgical intestinal inflammation. They stimulated the distal part of the ligated left VN trunk or the VN was transected and the distal part was stimulated thus activating vagal efferents. Voltage

stimuli (5 Hz for 2 ms at 1 or 5 V) were applied for 5 min before and for 15 min after intestinal manipulation. They showed that intestinal manipulation-induced inflammation of the muscularis externa in mice that received VNS was reduced in a voltage-dependent way compared with that of mice that received intestinal manipulation plus sham stimulation. They confirmed the results of Borovikova *et al.*[48] showing that prior vagotomy of the proximal end of the stimulated VN did not affect these results, indicating that the anti-inflammatory effect of VNS was not dependent on the activation of central nuclei. In intestinal segments treated with hexamethonium, a nAChR antagonist, VNS failed to prevent inflammation, in contrast to incubation with vehicle, demonstrating that the anti-inflammatory effect of VNS acted through local activation of nicotinic receptors. Signal transduction of α7nAChR in neurons is modulated by ligand-gated ion channel functionality. Ligand-receptor interaction on cytokine-expressing cells culminates in decreased nuclear translocation of nuclear factor kappaB (NFκB) as well as activation of the transcription factor signal transducer and activator of transcription 3 (STAT3) via phosphorylation by Janus Kinase 2 (JAK2), which is recruited to the α7nAChR. The authors showed that the intracellular signaling pathways activated by α7nAChR involved JAK2 and STAT3 proteins and the transcription factor NFκB in intestinal macrophages in response to VNS which indicates activation of STAT3 induced by ACh derived from vagal efferents.[10] Acetylcholines binding on its receptor activates STAT3 which inhibits the translocation of NFκB.[54,71] Activation of the STAT3 cascade after nAChR ligation is fully consistent with the observed inhibition of proinflammatory cytokine release by macrophages, because STAT3 is a negative regulator of the inflammatory response.[82] In another study, Costantini *et al.*[83] demonstrated that in anesthetized male BALB/c mice VNS of the intact right VN at 1 Hz (2 mA) to assess the role of the parasympathetic signaling on enteric glia activation, as observed by an increase of mRNA expression of glial fibrillary acidic protein, was associated with increased activation of enteric glia cells and resulted in attenuation of burn-induced intestinal barrier injury. Vagus nerve stimulation 10 min prior to injury maintained intestinal barrier integrity, significantly reducing burn-induced intestinal permeability. They showed that abdominal vagotomy at the gastroesophageal junction prior to VNS prevented the protective effects of VNS, thus confirming that efferent VN signaling modulates gut barrier integrity following injury. Animals undergoing abdominal vagotomy prior to VNS had intestinal permeability equivalent to animals subjected to burn, eliminating the protective effects of VNS. The ability of VNS to activate enteric glia cells may give insight into the signaling that occurs from the CNS to the enteric nervous system following gut injury. They showed that the protective effects of VNS on the gut were not due to modulation of splenic production of circulating TNFα indicating a local, but not systemic inflammatory response. The authors proposed that the response of the enteric glia to intestinal injury may be modulated by the CNS via the VN. Enteric glia is known to improve intestinal barrier function by increasing the expression of tight junction proteins, resulting in improved intestinal barrier integrity.[84] Exploiting the barrier inducing effects of enteric glia activation may prevent or limit intestinal barrier breakdown and may be a therapeutic target in diseases causing intestinal inflammation.

Most of the studies using VNS to activate the CAP were performed in vagotomized anesthetized animals with VNS of the distal end of the cut VN or in animals with stimulation of the distal part of the ligated VN trunk[71] thus stimulating selectively vagal efferent fibers without any effect on afferents. When performed in intact vagi, VNS was generally performed in anesthetized animals[78,83] and it is known that anesthesia can change the threshold for activation of different types of fibers in the vagal bundle.[15] In addition, some anesthetics (i.e. lidocaine and isoflurane) are reported to reduce inflammatory markers, including cytokines and chemokines.[85] Most of these studies were also performed with acute but not chronic VNS. One might expect that VNS chronically performed in awakened animals would be of interest to extrapolate experimental data to clinical data, as observed for VNS in epilepsy or depression, for a translational therapeutic approach in inflammatory digestive as well as extra-digestive disorders in human. For this purpose, we have studied the anti-inflammatory effect of a chronic VNS in a model of colitis in non-anesthetized rats.[86] Colitis was induced by intracolonic instillation of trinitrobenzene sulfonic acid (TNBS, 10 mg rat^{-1} in 50% ethanol; total volume, 0.25 mL), which shares many of the clinical, histopathological, and immunological features of Crohn's disease.[87] Vagus nerve stimulation of the left cervical VN was performed in freely moving animals 3 h per day for five consecutive days, with stimulation parameters (1 mA, 5 Hz, pulse width of 500 μs; 10 s ON, 90 s OFF; continuous cycle) adapted from previous studies.[29,78] For this purpose, an electrode (Cyberonics, Lyon, France) was gently wrapped around the left VN and linked to a connector fixed to the rat's head with dental cement. The connector was linked to a stimulator chain through a slip ring. The first day, VNS

started 1 h before TNBS colitis. Control rats implanted according to the same procedure were not stimulated. Assessment of colonic inflammation was obtained using physiological (e.g. body weight, temperature and locomotor activity), macroscopical (area of lesions), histological, and biological (e.g. myeloperoxidase activity, cytokine and cytokine-related mRNAs) parameters both at the level of the damaged colon and the part immediately above the lesion, without macroscopic lesion, a key target for VNS therapy, that is to have a preventive effect or to limit the extension of the lesion. A global multivariate index of colitis (including body weight, myeloperoxidase quantification, telemetric data, and areas of lesion, cytokine and cytokine-related mRNAs) was then generated for a better characterization of colonic inflammation. We showed that VNS reduced the degree of body weight loss, a classical parameters of inflammation in IBD patients, and inflammatory markers as observed above the lesion by histological score and myeloperoxidase quantification. This anti-inflammatory effect was also demonstrated by the improvement of the multivariate index of colitis. These data argue for an anti-inflammatory role of VNS chronically performed in freely moving rats with TNBS colitis and provide potential therapeutic applications for IBD patients. Indeed, we are currently running a pilot study of VNS in patients with moderate to severe Crohn's disease (ClinicalTrials.gov identifier: NCT01569503).

High-frequency (20–30 Hz) stimulation is generally used to activate vagal afferents for the use of VNS in epilepsy and depression whereas it is thought that, in animal models, low-frequency (1–10 Hz) stimulation activates preferentially vagal efferents and consequently the CAP.[48,71,78] Dorsal motor nucleus of the vagus neurons that project to the digestive tract are remarkable in that they exhibit slow (1–2 Hz) spontaneous pacemaker-like activity *in vitro* as well as *in vivo*[88,89] the rate of which can be modulated by synaptic inputs. Consequently, stimulation frequency of 5 Hz, classically used in experimental VNS of vagal efferents, is in the range of normal nerve traffic in the VN. In contrast, the majority of NTS neurons do not possess pacemaker activity; their inputs onto DMV neurons must be driven and are modulated by synaptic activity, either from the afferent VN, from other CNS areas, or via circulating hormones.[90]

The central effects of VNS at a low frequency of stimulation have been rarely explored. Lomarev *et al.*[30] examined whether or not different frequencies of VNS have differing brain effects. They used interleaved VNS and blood oxygen level dependent (BOLD) functional magnetic resonance imaging (fMRI) to measure the regional cerebral blood flow related changes resulting from intermittent applications either 20 or 5 Hz VNS in patients with major depression. 20 Hz VNS increased BOLD-fMRI response in the orbitofrontal cortex, frontal pole, hypothalamus, left pallidum, and, less significantly, the thalamus while lower frequency stimulation at 5 Hz was associated with reduced brain stimulation. Consequently, there appears to be a frequency/dose-effect of VNS on acute blood flow changes. Osharina *et al.*[91] performed VNS of the central cut left VN. They showed a gradual increase in *c-fos* expression in the brain of animals with VNS from 1 to 10 Hz; 1 Hz VNS only discretely affected the level of *c-fos* expression in the NTS, compared to sham-operation, while in contrast, *c-fos* expression was markedly above sham-operation levels in the NTS following 10 Hz stimulation. We have performed the first fMRI study of acute (1 h) VNS in anesthetized rodents[92] to see if VNS at low-frequency stimulation (5 Hz), known to activate vagal efferents, is also able to activate the CNS in conditions that we have shown to attenuate TNBS colitis.[86] The fMRI experiments were performed on a 4.7 T Bruker Avance III horizontal animal scanner. Highly significant VNS-related deactivations were observed in large portions of the brain, and particularly in the NTS and closely connected structures, such as the PB, the LC and the hippocampus.[27] Significant deactivations were also reported in the prefrontal cortex and retrosplenial cortex, regions which are known to express *c-fos* after continuous 30 Hz VNS.[29] The most significantly deactivated structures belonged to the central ANS.[40] No brain activation was found when the distal end of the VN (below VN section, i.e. disrupted vagal afferents) was stimulated, whereas brain activations remained unchanged when vagal efferents were disrupted (i.e. stimulation of the proximal end of the VN, just above VN section). Consequently, even low-frequency stimulation at 5 Hz, known to theoretically activate vagal efferents, is also able to have central effects. These data suppose that the anti-inflammatory effect of low-frequency stimulation of the intact VN could both involve a dual peripheral (i.e. the CAP) and central effect (through a vago-vagal positive loop and/or a stimulation of the HPA axis and/or a modification of the central ANS) to modulate the ANS (i.e. the sympatho-vagal balance) and thus inflammation.[5]

POTENTIAL THERAPEUTIC APPLICATIONS OF VNS IN HUMANS

Besides its role in the treatment of epilepsy and depression, VNS at low-frequency stimulation (5–10 Hz)

appears as an interesting tool to activate the CAP in the treatment of inflammatory digestive disorders as represented by IBD and POI.[93] Irritable bowel syndrome, characterized by a low grade inflammation,[94] should also be a target; in addition, low intensity VNS has shown to decrease visceral pain in response to colorectal distension,[95] a classical marker of IBS. Rheumatoid arthritis and psoriasis, where proinflammatory cytokines such as TNFα play a critical role and where a strong expression of α7 nAChR is observed in the synovium of RA and psoriatic arthritis patients,[96] are also potential targets. Indeed, nicotine, which has demonstrated efficacy in active ulcerative colitis,[97] induces modulation of experimental models of arthritis[98,99] and inhibits the TNFα dependant inflammatory pathway in synoviocytes by suppressing the activation of the NFκB pathway.[100]

Vagus nerve stimulation is a safe technique with even less side effects at low frequency classically used in inflammatory conditions. The use of VNS in human inflammatory disorders is attractive because: (i) it uses a physiological anti-inflammatory pathway (i.e. the CAP) as an anti-TNFα therapy and could thus be an alternative to classical anti-TNF treatments, (ii) it has both central and peripheral effects at low-frequency stimulation (1–10 Hz), (iii) it should be of interest for restoring an equilibrated sympatho-vagal balance as a dysautonomia, as observed with HRV, is often observed in IBD/IBS[6] and RA[7] patients, (iv) as overall intentional non-adherence is reported by 39% of IBD patients,[101] it is of interest because it is independent of patient compliance, and (v) in addition, the development of less invasive ways of delivering VNS at externally stimulating the VN via the noninvasive transcutaneous VNS stimulation,[102] a safe and well-tolerated method for relatively long periods, might be of interest.

At the present time ≈ 45 clinical studies on VNS are registered on ClinicalTrials.gov, a service of the US National Institutes of Health, with 13 studies on epilepsy, 13 on depression, four on RA, one on Crohn's disease, and one on postoperative ileus, thus demonstrating the interest for such a procedure in various health domains.

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DISCLOSURES

Pr Bruno Bonaz has served as consultant to Abbott France, Almirall France, Cephalon France, Ferring France, MSD France, Otsuka Pharmaceutical France. The others authors have no disclosures.

AUTHOR CONTRIBUTIONS

BB wrote the paper as a review, the other authors have contributed to the scientific revision and redaction of the paper; CP was involved in preclinical studies on vagus nerve stimulation in rats with experimental colitis and has sustained her PhD thesis on experimental vagus nerve stimulation on June 29, 2012. The other authors were involved in the design of the research study, they also performed the research and analysis of the data.

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