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SYMPOSIUM REVIEW

Anti-inflammatory properties of the vagus nerve: potential therapeutic implications of vagus nerve stimulation

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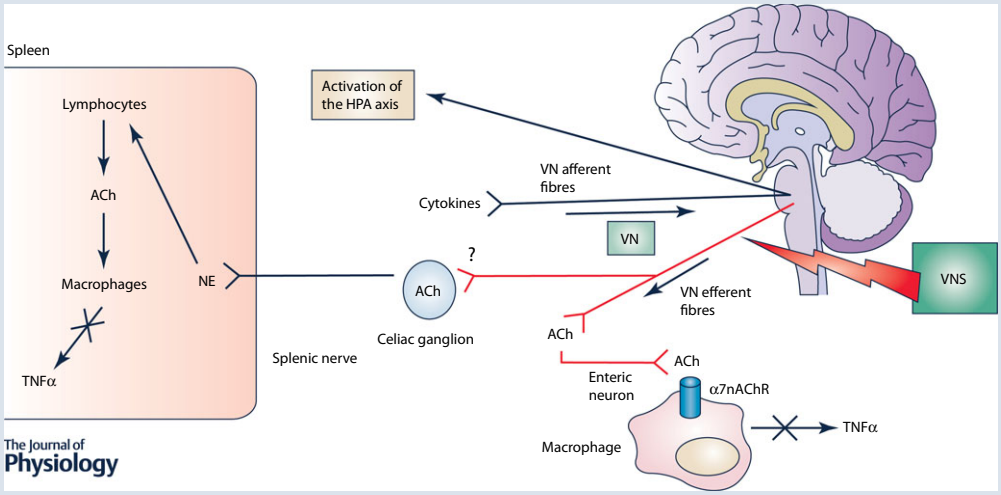

Abstract Brain and viscera interplay within the autonomic nervous system where the vagus nerve (VN), containing approximately 80% afferent and 20% efferent fibres, plays multiple key roles in the homeostatic regulations of visceral functions. Recent data have suggested the anti-inflammatory role of the VN. This vagal function is mediated through several pathways, some of them still debated. The first one is the anti-inflammatory hypothalamic–pituitary–adrenal axis which is stimulated by vagal afferent fibres and leads to the release of cortisol by the adrenal glands. The second one, called the cholinergic anti-inflammatory pathway, is mediated through vagal efferent fibres that synapse onto enteric neurons which release acetylcholine (ACh) at the synaptic junction with macrophages. ACh binds to α-7-nicotinic ACh receptors of those macrophages to inhibit the release of tumour necrosis (TNF)α, a pro-inflammatory cytokine. The last pathway is the splenic sympathetic anti-inflammatory pathway, where the VN stimulates the splenic sympathetic nerve. Norepinephrine (noradrenaline) released at the distal end of the

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splenic nerve links to the β2 adrenergic receptor of splenic lymphocytes that release ACh. Finally, ACh inhibits the release of TNFα by spleen macrophages through α-7-nicotinic ACh receptors. Understanding of these pathways is interesting from a therapeutic point of view, since they could be targeted in various ways to stimulate anti-inflammatory regulation in TNFα-related diseases such as inflammatory bowel disease and rheumatoid arthritis. Among others, VN stimulation, either as an invasive or non-invasive procedure, is becoming increasingly frequent and several clinical trials are ongoing to evaluate the potential effectiveness of this therapy to alleviate chronic inflammation.

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Abstract figure legend The vagus nerve and the neuroendocrine–immune axis. The vagus nerve (VN) is a mixed nerve which is a key component of the neuroendocrine–immune axis. Cytokines, released at the periphery in inflammatory conditions, activate vagal afferent fibres then activating the anti-inflammatory hypothalamic–pituitary–adrenal (HPA) axis to release cortisol by the adrenal glands. In an inflammatory reflex activating vagal efferent fibres, acetylcholine (ACh) is released at the distal end of the VN and activates enteric neurons that release ACh interacting with resident macrophages to inhibit the release of tumour necrosis (TNF)α, a pro-inflammatory cytokine, by macrophages through α-7-nicotinic ACh receptors (α7nAChR): i.e. the cholinergic anti-inflammatory pathway (CAP). Consequently, the VN does not interact directly with resident macrophages. The VN could also stimulate the splenic nerve, through a vagosympathetic interaction involving ACh at the level of the coeliac ganglion, thus releasing noradrenaline at the distal end of the splenic nerve to inhibit the release of TNFα by spleen macrophages through an interaction of norepinephrine (NE) with spleen lymphocytes that release ACh. However, this pathway is still debated. VN stimulation (VNS) activates the CAP and is thus a potential anti-TNFα therapy that could be used as a non-drug treatment of TNFα-related diseases such as inflammatory bowel disease, rheumatoid arthritis, and others.

Abbreviations ACh, acetylcholine; α7nAChR, α-7-nicotinic acetylcholine receptor; ANS, autonomic nervous system; CAN, central autonomic network; CAP, cholinergic anti-inflammatory pathway; CD, Crohn's disease; CRF, corticotrophin-releasing factor; DMN, dorsal motor nucleus of the vagus; EEG, electroencephalographic; fMRI, functional magnetic resonance imaging; HPA, hypothalamic–pituitary–adrenal; HRV, heart rate variability; IBD, inflammatory bowel disease; IL, interleukin; LPS, lipopolysaccharide; NTS, nucleus tractus solitarii; PVH, paraventricular nucleus of the hypothalamus; RA, rheumatoid arthritis; TNFα, tumour necrosis factorα; tVNS, transauricular vagus nerve stimulation; VN, vagus nerve; VNS, vagus nerve stimulation.

Introduction

The vagus nerve (VN), the longest nerve of the human body (from the brainstem to the abdomen), innervates most organs especially in the gastro-intestinal tract. The VN is a key component of the autonomic nervous system (ANS). It is a mixed nerve (i.e. essentially sensitive) containing 80% afferent fibres that convey visceral, somatic and taste sensations and 20% efferent fibres representing the parasympathetic branch of the ANS that leads to the release of acetylcholine (ACh) at the synaptic junction with smooth muscles, intrinsic nervous fibres or secreting cells. The VN modulates gastro-intestinal motility and secretion at the digestive tract level. The VN is considered as the sixth sense of the body by some authors (Zagon, 2001).

The vagus nerve anatomy

Vagal efferent fibres originate in the dorsal motor nucleus (DMN) of the VN located in the medulla and, in humans, innervate the digestive tract from the oesophagus to the splenic flexure while the rest of the gut, i.e. the left colon and rectum, is innervated by the sacral (S2–S4) parasympathetic nucleus (Netter, 1989). However, for some anatomists, the VN innervates all of the digestive tract in humans (Delmas & Laux, 1933). In the rat, the VN innervates all of the digestive tract except for the rectum (Altschuler *et al.* 1993). Vagal efferent fibres do not reach the intestinal lamina propria directly (Berthoud *et al.* 1991) but synapse onto enteric neurons that innervate the lamina propria where they release ACh acting on nicotinic or muscarinic receptors.

Vagal afferent fibres originate from the mucosa to the muscle layers of the digestive tract. The sensory afferent cell bodies are located in nodose ganglia and relay information to the nucleus tractus solitarii (NTS) (Cechetto, 1987) and the area postrema, in close relation with the DMN of the VN to form the dorsal vagal complex. This complex is involved in the autonomic, endocrine, and limbic responses of the 'inner medium' (Fig. 1). Visceral information is subsequently sent to areas of

the forebrain such as the hypothalamus, the amygdala, and the cortex, via a relay through the parabrachial nucleus, the hypothalamic–pituitary–adrenal (HPA) axis, the thalamus before final visceral afferent inputs in the insular cortex, the anterior cingulate and prefrontal cortices corresponding to the central autonomic network (CAN) (Benarroch, 1993). The CAN, in turn, is able to modulate the ANS especially through projections of: (i) the paraventricular nucleus of the hypothalamus (PVH) to the DMN and preganglionic neurons of the sympathetic nervous system at the spinal cord level, (ii) the amygdala to the DMN, (iii) the Barrington nucleus to the sacral parasympathetic nucleus, (iv) the A5 noradrenergic group to spinal preganglionic sympathetic neurons (Ricardo & Koh, 1978).

The vagus nerve and the immune system

The afferent vagus nerve and the anti-inflammatory hypothalamic–pituitary–adrenal axis pathway. The VN is a major component of the neuroendocrine–immune axis which is involved in coordinated neural, behavioural, and endocrine responses that provide an important first-line innate defense against infection/inflammation and help to restore homeostasis in the body (Johnston & Webster, 2009). In particular, the VN is sensitive to peripheral pro-inflammatory cytokines, such as interleukin (IL)-1, Il-6 and tumour necrosis factor (TNF)α, that are released by macrophages and other immune cells in, for example, the case of septic shock as observed after peripheral (I.V. or I.P.) injection of

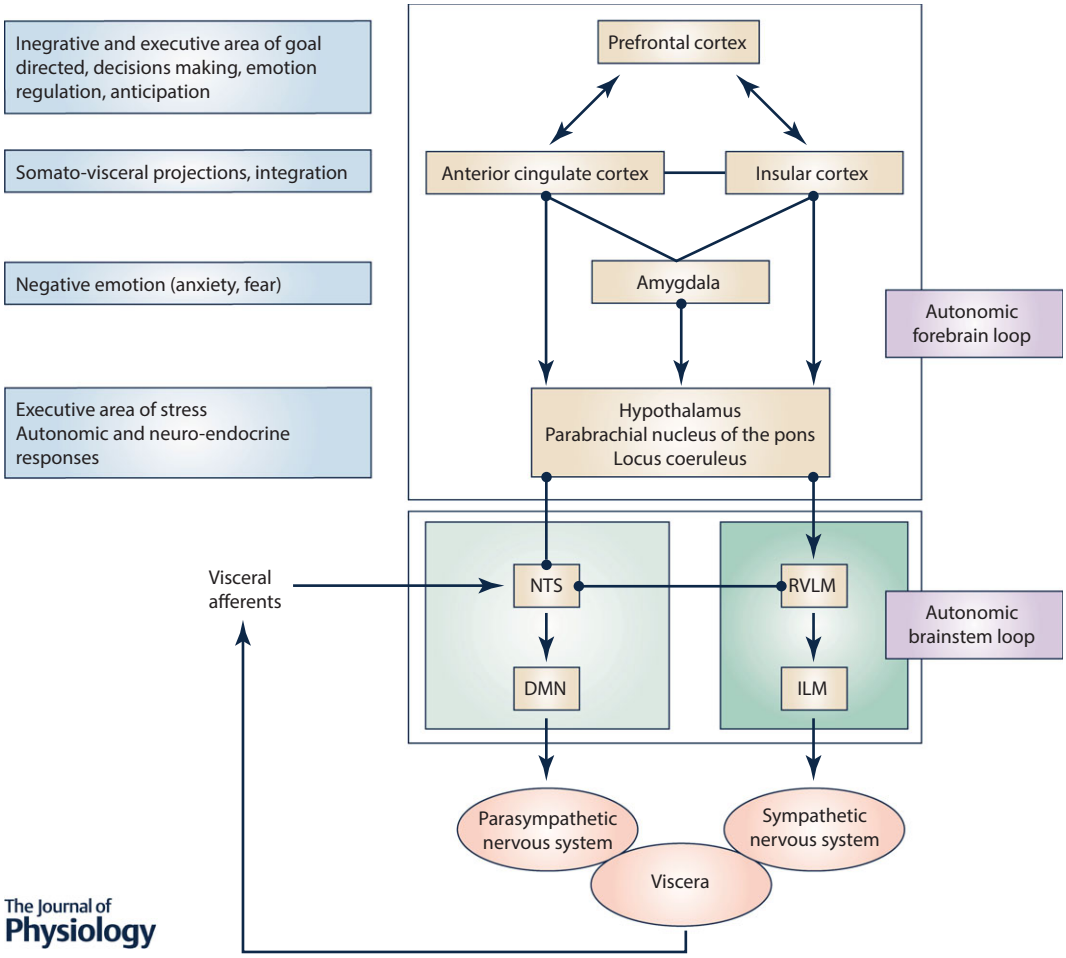

Figure 1. Schematic diagram showing the central autonomic network modulation of visceral activity
An autonomic vagovagal loop includes visceral inputs to the nucleus of the solitary tract (NTS) that sends outputs to the dorsal motor nucleus (DMN), to the rostral ventrolateral medullary (RVLM) and to the intermediate lateral medulla (ILM) to adapt the balance between the sympathetic and parasympathetic activities to body constraints. This autonomic forebrain loop is modulated by a forebrain autonomic loop, through cross-talk between the NTS and brain areas (hypothalamus, amygdala, cingulate cortex, insula, prefrontal cortex) that are also involved in neuroendocrine, emotional and cognitive controls of behaviour. Figure adapted from Thayer & Lane (2009).

lipopolysaccharide (LPS) (Werner *et al.* 2003; Hosoi *et al.* 2005). In particular, vagal afferents are equipped with IL-1β receptors at the paraganglia level that convey the information to the NTS where neurons located in the A2 noradrenergic group are activated and then project information to the parvo-cellular zone of the PVH around corticotrophin-releasing-factor (CRF)-containing neurons. These CRF neurons then activate the release of adreno-corticotrophin hormone by the hypophysis that will finally stimulate the release of glucocorticoids by the adrenal glands to decrease peripheral inflammation, i.e. the HPA axis. Thus the VN has an anti-inflammatory role through the activation of the HPA axis via vagal afferent fibres. Vagotomy disrupts this anti-inflammatory pathway and sensitizes animals to inflammation as observed in experimental colitis models (Ghia *et al.* 2006). Likewise, Lewis rats that display a blunted HPA axis response to stress, related to a lower hypothalamic CRF release, are more sensitive to inflammation while Fischer rats, that overexpress CRF, are more resistant (Calogero *et al.* 1992). Furthemore, this HPA axis is also activated by circulating pro-inflammatory cytokines on circumventricular organs, located outside the blood–brain barrier, that stimulate neurons located in close by and which project to CRF neurons of the PVN, thus activating the HPA axis (Buller, 2001).

The efferent vagus nerve and the cholinergic anti-inflammatory pathway. More recently, an 'inflammatory' reflex was described by Tracey's group. This involves an anti-inflammatory vagovagal reflex where vagal afferent fibres activate vagal efferent fibres. These authors reported that, in a model of septic shock in rodents, following peripheral (I.V.) injection of LPS, septic shock was prevented by VN stimulation (VNS) of the distal end cut VN and thus of vagal efferent fibres (Borovikova *et al.* 2000*b*). This effect is due to the release of ACh at the distal end of the VN that inhibits the release of pro-inflammatory cytokines such as TNFα by macrophages. This inflammatory reflex is mediated through the link of ACh with α-7-nicotinic ACh receptors (α7nAChR) of macrophages (Wang *et al.* 2003) and is called the cholinergic anti-inflammatory pathway (CAP) (Pavlov *et al.* 2003; Pavlov & Tracey, 2015). Indeed, this effect is suppressed in α7nAChR knockout animals (Wang *et al.* 2003) and is mediated intracellularly through the activation of the JAK2–STAT pathway (de Jonge *et al.* 2005; Pena *et al.* 2010). However, the exact anatomical interaction between the VN and the intestinal immune system is still a matter of debate since the VN does not directly interact with resident macrophages in the gut. Instead, the VN preferentially interacts with nNOS, VIP and ChAT enteric neurons located within the gut muscularis. The nerve endings of these enteric neurons are located close to resident macrophages (Cailotto *et al.* 2014). The vagal modulation of intestinal resident macrophages is indirect, most likely through these enteric neurons rather than by direct vagal nerve fibre interaction with resident macrophages. The use of anterograde labelling failed to detect vagal efferent fibres in contact with resident macrophages, but proved close contacts between cholinergic myenteric neurons and intestinal muscularis CX3CR1 expressing macrophage-like cells, originally described by Mikkelsen *et al.* (1985), but not with the much more abundant mucosal macrophages (Matteoli *et al.* 2014). The latest development in the understanding of the cholinergic anti-inflammatory pathway has been contributed by Tracey's team; they suggested that the VN was able to activate the splenic sympathetic nerve through a vagosympathetic synergistic effect (Rosas-Ballina *et al.* 2008) (Fig. 2). Norepinephrine released at the distal end of the splenic nerve binds to the β2 adrenergic receptor of splenic lymphocytes which release ACh which in turn binds to α7nAChRs of splenic macrophages to finally inhibit the release of TNFα by the spleen (Olofsson *et al.* 2012). So the VN could have an anti-TNFα effect either at the level of peripheral macrophages or at the level of the secondary lymphoid organ, namely the spleen. However, this theory is still debated because other investigators have not been able to find innervation of the spleen by the VN either directly or indirectly through a connection with the spleen (Bratton *et al.* 2012), while it has been shown that VN efferent fibres innervate the coeliac ganglia and the superior mesenteric ganglia in the coeliac plexus (Berthoud & Powley, 1993, 1996). Cholinergic nerve fibres have been shown to surround catecholaminergic neurons in the coeliac ganglia in mice (Downs *et al.* 2014) and the coeliac ganglia are at the origin of the sympathetic spleen innervation (Bellinger *et al.* 1989). Buijs *et al.* (2008) showed that the spleen receives not only a sympathetic input but also a parasympathetic input; the sympathetic input reaches the spleen via the arteries while the parasympathetic input reaches the spleen via both tips of the spleen. According to Gautron *et al.* (2013), cholinergic fibres found in the spleen come from cholinergic postganglionic sympathetic neurons located in the para- and/or prevertebral chains. VNS induces ACh release in the coeliac mesenteric ganglia which binds with postsynaptic α7nAChRs of the splenic nerve, releasing norepinephrine in the spleen (Rosas-Ballina *et al.* 2011). The anti-inflammatory effectiveness of central cholinergic activation following intracerebroventricular infusion of the M1 muscarinic acetylcholine receptor agonist, to activate the CAP, is suppressed in mice after vagotomy or splenic neurectomy (Munyaka *et al.* 2014). However, Cailotto *et al.* (2014) using anterograde tracing experiments did not reveal dextran-labelled vagal fibres or terminals in the mesenteric ganglion or spleen. Martelli

et al. (2014) proposed a model with a non-neural link from the VN to the spleen. In this model, the α7nAChRs are located on the peripheral terminals of the splenic sympathetic nerves. When stimulated by ACh from incoming T-cells, these terminals release norepinephrine which then acts on β adrenergic receptors on splenic macrophages to suppress their release of TNF-α.

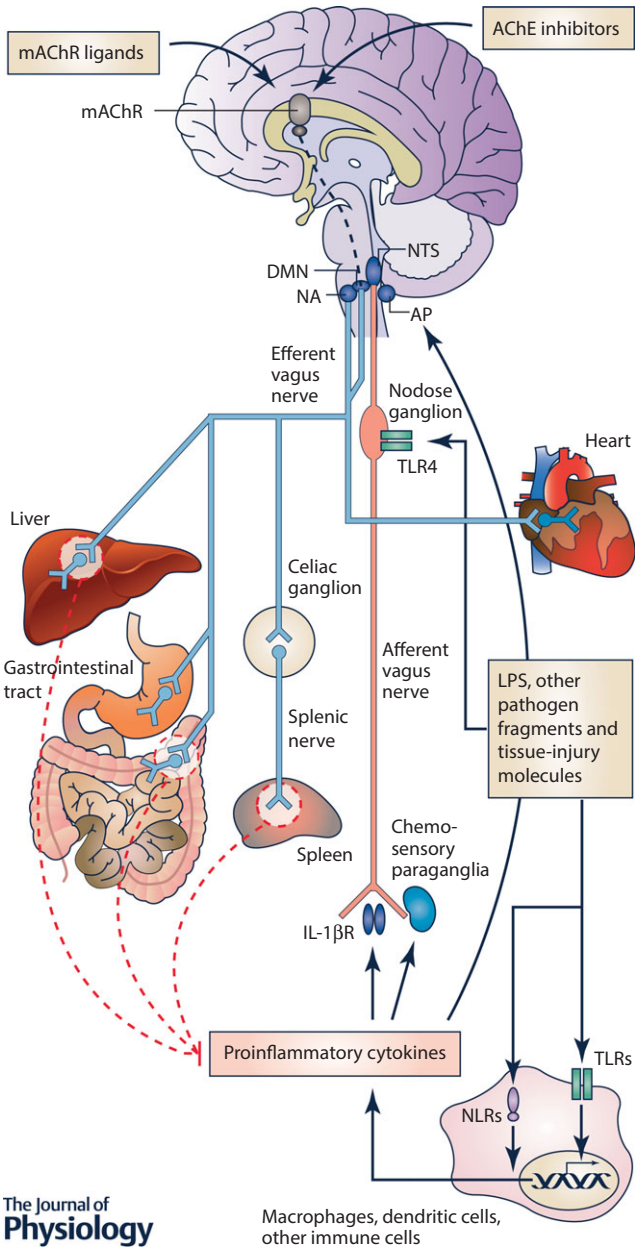

Figure 2. The functional anatomy of the inflammatory reflex (according to Pavlov & Tracey, 2015)
AChE, acetylcholinesterase; AP, area postrema; DMN, dorsal motor nucleus of the vagus nerve; LPS, lipopolysaccharide (endotoxin); mAChR, muscarinic acetylcholine receptor; NA, nucleus ambiguus; NLRs, nucleotide-binding oligomerization domain-like receptors; NTS, nucleus tractus solitarii; TLR4, Toll-like receptor 4.

Another pathway could be the activation of the coeliac ganglion, at the origin of the innervation of the spleen, by brain nuclei that are part of the CAN and that generate patterns of autonomic responses via projections to preganglionic sympathetic neurons in the spinal cord. Indeed, five cell groups in the brain regulate the entire sympathetic outflow (Strack *et al.* 1989*a*,*b*): the PVN, the A5 noradrenergic cell group, the caudal raphe region, the rostral ventrolateral medulla, and the ventromedial medulla. The activation of the afferent arm (i.e. vagal afferents) of the inflammatory reflex could activate the CAN, through projections from the NTS, to modulate the sympathetic nervous system through these five cell groups. In this case, the VN would induce an indirect anti-inflammatory reflex by activating the sympathetic nervous system.

Therapeutic implications of vagus nerve reinforcement

The inflammatory reflex, i.e. the CAP, opens new therapeutic alternatives based on its anti-TNFα effect. Indeed, inflammatory diseases in which TNFα is a key cytokine are good candidates for treatment targeting the CAP. Rheumatoid arthritis (RA) and inflammatory bowel diseases (IBD) are potential therapeutic targets for such an approach, particularly as an alternative to anti-TNFα therapy, which is the gold standard treatment. Such treatments act downstream of the release of TNFα by macrophages and other cytokine-producing cells. Consequently, treatments acting upstream would be of interest, as summarized in Table 1. Among these treatments, VNS seems particularly interesting (Bonaz & Bernstein, 2013; Bonaz *et al.* 2013).

Vagus nerve stimulation. VNS was approved by the FDA for the treatment of drug-resistant epilepsy and depression in 1997 and 2005, respectively. Today ~80,000 patients have been implanted for epilepsy and ~4000 for depression (data from Cyberonics, Houston, TX, USA). Approximately fifty per cent of patients reached a clinically significant reduction in seizure frequency ($\geq$ 50%), with about 12% experiencing a 90% decrease in seizures (Englot *et al.* 2011*a*,*b*). VNS exerted its effect with some latency in the treatment of epilepsy and its effectiveness improved over time in a 3 year follow-up study (Morris & Mueller, 1999). The anti-epileptic effect of VNS was suggested to be related to vagal C-fibres, but their destruction did not alter subsequent VNS-induced seizure suppression in rats thus suggesting that seizure suppression resulted from the activation of vagal A- and B-fibres (Krahl *et al.* 2001). The most common post-implantation adverse events were hoarseness (20–28%), paraesthesia (12%), headache (4.5%), and shortness of breath (3.2%) (Morris & Mueller, 1999). No significant impact on heart rate has

Table 1. Main potential therapeutic treatments currently considered as acting upstream to decrease TNFα release

Therapy	Target/therapy sub-type	Mechanism	References
Pharmacological therapy	GTS-21 AR-R17779	α7nAChR agonists	van Westerloo *et al*. (2006) The *et al*. (2007)
	Galantamine	Central cholinergic pathway stimulation	Pavlov *et al*. (2009); Ji *et al*. (2014)
	Semapimod (CNI 1493)	p38 mitogen-activated protein kinase inhibitor	Borovikova *et al*. (2000*a*); Bernik *et al*. (2002*b*)
Nutritional therapy	Fat nutrition	Stimulation of vagal afferent fibres through fat-induced CCK release and, in return, of the CAP	Luyer *et al*. (2005)
	Choline	Precursor in the biosynthesis of ACh and selective natural α7nAChR agonist	Parrish *et al*. (2008)
	Ghrelin	Activation of the CAP	Mao *et al*. (2015*a*,*b*,*c*)
Complementary therapy	Acupuncture Hypnosis Meditation Tai chi	Stimulation of vagal efferent fibres and CAP	Gamus (2011)
Physical activity and exercise		Stimulation of vagal efferent fibres and CAP	Heffernan *et al*. (2009); Jae *et al*. (2009*a*,*b*)

ever been reported. Adverse events are typically mild to moderate, and usually occur during stimulation and often decrease over time. The commonly used VNS parameters that activate vagal afferents in epilepsy and depression are: frequency, 20–30 Hz; intensity, 0.5–1.5 mA; pulse width, 500 μs; on-time, 30 s; off-time, 5 min. VNS parameters may easily be adjusted with a programming wand. The implantation of a VNS device is performed under general anaesthesia usually by a neurosurgeon familiar with this technique. Surgery lasts ∼1 h. An electrode (Model 302, Cyberonics) is wrapped around the left VN in the neck, near the carotid artery, tunnelled under the skin and connected to a bipolar pulse generator (Model 102) implanted subcutaneously in the left chest wall or in the axilla. VNS is generally performed on the left cervical VN since the right VN innervates the sinoatrial node (involved in the pace-making 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). The device is switched on at 0.25 mA at surgery and progressively increased up to 1.25 mA, patient tolerance permitting. VNS is performed continuously with alternating ON–OFF phases.

VNS should be of interest in the treatment of chronic inflammatory diseases to decrease inflammation in the long run and thus to maintain the remission status of the disease as long as possible. The effect of VNS in epilepsy and depression is mediated through the activation of vagal afferent fibres, performed at high frequency of stimulation (20–30 Hz), but the activation of the CAP is mediated through vagal efferent fibres and involves a low-frequency (1–10 Hz) stimulation of the VN. Indeed, in Borovikova's reference study (Borovikova *et al.* 2000*b*) a 1 Hz frequency stimulation for 20 min (10 min before LPS administration and 10 min after) was performed and was effective for the preferential recruitment of efferent parasympathetic fibres. Other reports have also shown that low frequency (5 Hz) VNS is able to activate vagal efferents and thus the CAP (Bernik *et al.* 2002*a*).

Based on the CAP and on the initial VNS data, we studied VNS in an experimental model of TNBS (2,4,6-trinitrobenzenesulfonic acid)-induced colitis (Th1-induced inflammation) in rats, resembling Crohn's disease (CD) (Meregnani *et al.* 2011). The aim of this study was to perform chronic VNS in freely moving animals 3 h day^{-1} for 5 days, starting 1 h before colitis, with stimulation parameters (1 mA, 5 Hz, pulse width of 500 μs; 10 s ON, 90 s OFF; continuous cycle) adapted from previous studies (Bernik *et al.* 2002*a*) with an external stimulator. Control rats implanted according to the same procedure were not stimulated. The assessment of colonic inflammation was performed using physiological (e.g. body weight, temperature, and locomotor activity), macroscopic (area of lesions), histological, and biological parameters (e.g. myeloperoxidase activity, cytokine and cytokine-related mRNAs), both at the level of the damaged colon and immediately above (a 1-cm-long piece proximal to the most anterior aspect of the macroscopically observed damage). A global multivariate index of colitis was then generated for a better characterization of colonic

inflammation. VNS reduced the extent of body weight loss 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 suggest an anti-inflammatory role of VNS chronically performed in freely moving rats with colitis and provide potential therapeutic applications for patients with IBD.

To assess if, in our experimental conditions of low (5 Hz) frequency stimulation, VNS was limited to vagal efferent fibres, we performed a dynamic causal modelling study to estimate neuronal connectivity from functional magnetic resonance imaging (fMRI) of VNS-treated rats, in a small brain network including the NTS known to receive vagal afferents (Reyt *et al.* 2010). Indeed, the central effects of VNS at a low frequency stimulation have rarely been explored (George *et al.* 2002; Lomarev *et al.* 2002) and we have provided the first fMRI study of acute low frequency (5 Hz) VNS performed in rodents. Highly significant VNS-related deactivation was found in large portions of the brain, and particularly in the NTS and closely connected structures, such as the parabrachial nucleus, the locus coeruleus and the hippocampus. The VNS-induced fMRI deactivation of the cerebellum correlated with the known anatomical projections of the NTS to the cerebellum. Thus, even low-frequency stimulation at 5 Hz, which theoretically activates vagal efferent fibres (Borovikova *et al.* 2000*b*; Bernik *et al.* 2002*a*; Lomarev *et al.* 2002), also activates vagal afferents to the brain and suggests that the anti-inflammatory effect of low frequency VNS of the intact VN involves both a peripheral (i.e. the CAP) and central effect (through vagovagal inflammatory reflex, and/or a stimulation of the HPA axis, and/or a modification of the CAN). These results were corroborated by an electroencephalographic study performed in a CD patient treated by chronic low frequency (10 Hz) VNS; electroencephalographic (EEG) and electrocardiographic recordings were performed 1 week before, then at week 6, and months 6, 9, and 12 after VNS implantation. VNS induced significant changes in resting EEG in all frequency bands. In particular, activation was observed on the mediofrontal electrodes for both low and high frequency bands, with the most important activation for the theta band. An additional activation was found in the occipital electrodes for the gamma band. We observed significant correlations between EEG and the high frequency component of the heart rate variability (HRV) marker of vagal tone for the delta, theta, beta, and gamma frequency bands. We suggested that the increase in the mediofrontal theta band could reflect an activation of the anterior cingulate cortex, part of the CAN that modulates the parasympathetic nervous system. The changes in theta and gamma bands observed in this study provide evidence that forebrain areas could be involved in the mediation of the VNS effect on HRV. In parallel, the hypotonicity of vagal tone observed in this patient before VNS was regularly progressively corrected during the 1 year of VNS and the patient was in deep (clinical and endoscopical) remission (Clarencon *et al.* 2014). We are currently performing a pilot study of VNS in patients with CD (ClinicalTrials.gov Identifier: NCT01569503) where VNS is positioned as an alternative to the usual anti-TNFα treatment. We have currently implanted seven patients (with moderate to severe CD) with a neurostimulator (Model 102) and an electrode (Model 302) from Cyberonics, using the following stimulation parameters: intensity, 0.5–1.5 mA; frequency, 10 Hz; pulse width, 500 μs; and stimulation on-time of 30 s followed by off-time of 5 min. The first patient was implanted in April 2012 and the last patient in December 2014. At the 6 month follow-up, 5/7 patients had responded to VNS with clinical, biological and endoscopic improvement/healing. Two patients were withdrawn from the study after 3 months, due to worsening of their disease. One patient underwent surgery (ileocaecal resection), and the other patient received a combo therapy with azathioprine and infliximab and is presently in remission while the VNS intensity has been turned down to 0.25 mA. Only one of the six patients, still being treated with VNS at 6 months was also still treated with immunosuppressant (azathioprine) (Bonaz *et al.* 2016). TNF or other pro-inflammatory mediators, in tissues or in the blood, have not yet been assessed in our patients with VNS but these assays are underway. Our preliminary results show that VNS is feasible and could be an interesting tool in the treatment of active CD; nevertheless further investigation in a larger longitudinal cohort of CD patients is required. Another clinical trial study on VNS for CD was recently launched and is ongoing (ClinicalTrials.gov Identifier: NCT02311660; SetPoint Medical Corporation).

VNS should be of interest in other inflammatory conditions such as RA. Indeed, knockdown of the α7nAChR in RA fibroblast-like synoviocytes increased the production of mediators of inflammation, and degradation and activation of α7nAChRs in an animal model of RA resulted in reduced arthritis activity (Koopman *et al.* 2014). Accordingly, stimulation of the CAP by VNS improved an experimental model of arthritis while aggravation of arthritis activity was observed after unilateral cervical vagotomy, as well as in α7nAChR-knockout mice. Based on these data, the authors performed VNS in RA patients as a novel anti-inflammatory approach (ClinicalTrials.gov Identifier: NCT01552941; SetPoint Medical Corporation). This study was completed in May 2014 and the results are pending. VNS was also studied in postoperative ileus (ClinicalTrials.gov Identifier: NCT01572155; Katholieke Universiteit Leuven) since experimental studies showed that CAP activation improved postoperative ileus (The *et al.* 2007).

The development of new non-invasive VNS techniques, i.e. that do not require surgical implantation of the electrode and neurostimulator, is of interest, as expected. This involves transcutaneous VNS (tVNS) of the auricular concha which is innervated by the VN (Peuker & Filler, 2002); stimulation of this anatomical part of the ear should be of interest. The Cerbomed Nemos device (Erlangen, Germany) is an external device that provides tVNS by using a dedicated intra-auricular electrode (like an earphone) which stimulates the auricular branch of the VN (Stefan *et al.* 2012). This device received the European clearance (CE mark) in 2010 for epilepsy and is currently available in Germany, Austria, Switzerland, and Italy. Likewise, the Electrocore LLC Gammacore device (Basking Ridge, NJ, USA) is a non-invasive VN stimulator that uses proprietary electrical signals to treat primary headache. Such a device could be used, like the NEMOS, for inflammatory digestive disorders.

In conclusion, the VN has anti-inflammatory properties both through its afferent (activation of the HPA axis) and efferent (activation of the CAP) fibres. Given its position as a key element of the ANS in the brain–gut interactions in IBD (Bonaz & Bernstein, 2013), the VN seems to be a good therapeutic target in inflammatory conditions of the digestive tract (e.g. IBD) but also other inflammatory conditions such as RA, and others. We reported an abnormal ANS in IBD patients (Pellissier *et al.* 2010) negatively correlated with TNFα levels (Pellissier *et al.* 2014). VNS, by restoring the ANS balance in such patients through the activation of the VN, is a novel therapeutic treatment. Furthermore, such a treatment should be devoid of the usual adverse events of anti-TNFα drugs feared by patients, one of the reasons for non-adherence to treatment. Finally, VNS would be cheaper than anti-TNF drugs. The use of neuromodulation by bioelectronics devices as a treatment is an emerging field in the domain of bioelectronic medicine. It could be an alternative non-drug therapy to conventional treatment or could be combined with such treatments, but further investigation in a large longitudinal cohort of patients is required.

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Additional information

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Author contributions

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