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RESEARCH ARTICLE

Study of the Transcutaneous Auricular Vagus Nerve Stimulation May Advance Outcome in Chronic Pediatric Inflammatory Diseases

Ulf Andersson[1]

[1] Department of Women's and Children's Health, Karolinska Institutet at Karolinska University Hospital, 17176 Stockholm, Sweden

*ulf.andersson@ki.se

Abstract

The recent insight that the immune system is innervated has initiated a search for neural reflex circuits suitable for therapeutic targeting in human inflammatory diseases. The inflammatory reflex, signaling along the vagus system to maintain immune system homeostasis, is the best characterized such circuit. Proinflammatory molecules, extracellularly released during infectious or sterile injury, are sensed by afferent vagal nerves that transmit this information to the nucleus of tractus solitarius in the brainstem. The afferent signals generate efferent action potentials that travel from the brainstem via efferent vagal nerves to the spleen and other organs. This culminates in T cell release of acetylcholine, which interacts with α7 nicotinic acetylcholine receptors on immunocompetent cells to inhibit proinflammatory cytokine release. These mobile anti-inflammatory T lymphocytes thus operate both inside and outside compartments innervated by the vagus system. Therapeutic proof-of-concept anti-inflammatory studies following surgical implantation of electrical vagus nerve stimulators were first conducted in rheumatoid arthritis and Crohn´s disease. Long term use of these devices was uneventful, while the initial surgical procedure caused adverse effects in some patients. The auricular branch of the vagus nerve reaches superficial parts in the concha and tragus in both ears, enabling transcutaneous electrical auricular vagus nerve stimulation (taVNS) as a safer therapeutic alternative. Invasive VNS and taVNS activate similar parts of the central nervous system indicated by functional imaging methods. Pilot taVNS studies in patients with inflammatory diseases have so far been conducted to treat rheumatoid arthritis, osteoarthritis, lupus, pediatric inflammatory bowel diseases, and pediatric nephrotic syndromes.

Keywords: Inflammatory reflex; taVNS; Acetylcholine; HMGB1; Inflammation; Pain

Introduction

Acute inflammation at adequate intensity is a beneficial healing process, while chronic inflammation reflects adaptive failure. Unresolved or excessive inflammation can be deleterious and generate severe tissue injury and cause chronic organ malfunctions including metabolic syndrome, obesity, and type 2 diabetes[1-3]. Therapeutic effects provided by existing biological and pharmaceutical agents are insufficient for many patients with inflammatory diseases[4]. Furthermore, these treatments often come with high costs and risks of serious adverse side-effects including increased susceptibility to infections[5, 6]. Growth retardation caused by long-term systemic corticosteroid treatment is one of many side-effects afflicting young patients severely. There is thus an unmet clinical need for alternative therapies that can ameliorate inflammatory conditions, in particular pain and fatigue. Novel insights into two research fields bring important information that when combined offer encouraging strategies to counteract dysregulated inflammation. One recent breakthrough concerns the revelation of the ability of the nervous system to regulate inflammation and that these neuronal pathways can be successfully therapeutically targeted[7-9]. The other important discovery concerns innovations in bioelectronic technology empowering therapeutic management of the endogenous nervous control of the immune system[10-14]. The aim of this review is to outline mechanisms enabling neuronally-mediated regulation of inflammation that provide therapeutic pediatric opportunities. The focus is on transcutaneous auricular vagus nerve stimulation (taVNS), acetylcholine, and high mobility group box 1 protein (HMGB1) biology.

The purpose of taVNS is to treat dysregulated inflammation and pain non-invasively by applying electrical current to the cutaneous zone innervated by the auricular branch of the vagus nerve (ABVN) in the left auricle.

The cholinergic anti-inflammatory pathway inhibits inflammation

Immune functions, including the generation of inflammation, were until lately presumed to be independent of neuronal control. The nervous system is hardwired, while cells in the immune system are mobile or at least have the potential to move. These facts were for long interpreted to mean that the immune and the nervous systems operate independently of each other. However, results derived from preclinical and clinical studies during the last two decades have gradually demonstrated that the immune system, like all other organs, is under nervous control[3, 15-18]. The inflammatory reflex is a brain-integrated physiological mechanism based on afferent and efferent vagus nerve circuitry that detects and controls inflammation[16]. The vagus nerve-mediated inflammatory reflex with its efferent arm the cholinergic anti-inflammatory pathway is so far the most extensively studied example of an immunoregulatory neural circuit (Fig.1)[3]. The vagus nerve is a mixed nerve composed of 80% afferent and 20% efferent fibers. The efferent fibers originate in two brainstem nuclei, the dorsal motor nucleus and the nucleus ambiguus. The afferent arm of the inflammatory reflex that projects to the nucleus of the tractus solitarius (NTS) in the brainstem is mediated by sensory vagal fibers that monitor both extracellular proinflammatory endogenous and exogenous molecules (Fig. 1)[19]. This information is neuronally conveyed to activate an opposing motor response in efferent vagus nerves which thru acetylcholine release counteract inflammation via inhibited cytokine release and a reduced accumulation of inflammatory cells at sites with ongoing inflammation[20]. Recent studies demonstrate that afferent vagus nerve signaling from the NTS also reaches forebrain regions including hippocampus, cortex, and identified basal forebrain cholinergic nuclei[21-23]. This interaction results in M1 muscarinic acetylcholine receptor (M1mAChR)-dependent forebrain cholinergic signaling that down-regulates inflammation via vagus nerve-mediated signaling[23]. The inflammatory reflex can thus also be activated via brain mAChR-mediated mechanisms by centrally acting M1 mAChR agonists and acetylcholinesterase inhibitors like galantamine[23, 24].

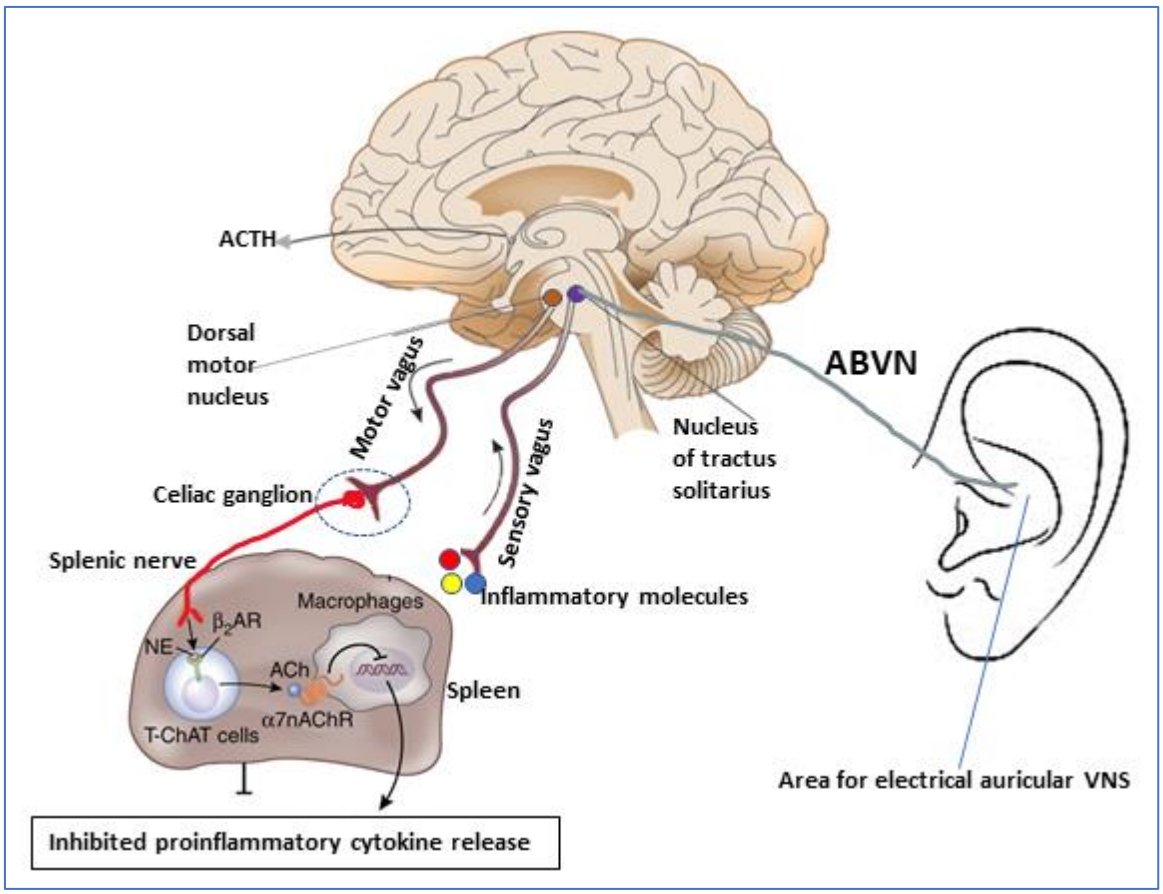

Fig 1. The inflammatory reflex and how transcutaneous auricular vagus nerve stimulation may activate the pathway. Afferent vagus nerve fibers residing in the nodose ganglion are stimulated in the periphery by damage-associated molecular pattern molecules (DAMPs), including cytokines, and pathogen-associated molecular pattern molecules (PAMPs). The signals are transmitted to the nucleus of tractus solitarius (NTS). Reciprocal connections between the NTS and the dorsal motor nucleus (DMN) of the vagus mediate communication with and activation of efferent vagus fibers (motor vagus) from the DMN. The signal is propagated to the celiac ganglia, where the splenic nerve originates. Norepinephrine (NE) released from the splenic nerve interacts with beta2-adrenergic receptors (beta2-AR) on certain splenic T lymphocytes and causes the release of acetylcholine (ACh) from T cells containing choline acetyltransferase (T-ChAT cells). ACh interacts with α7-subunit-nicotinic acetylcholine receptors (α7nAChR) on macrophages and inhibits proinflammatory cytokine production and inflammation. Transcutaneous electrical stimulation of the auricular branch of the vagus nerve (ABVN) in the left ear also signals to the NTS and thus activates efferent vagus signals to the splenic T-ChAT cells. Abbreviations not explained in the text: ACTH= adrenocorticotropic hormone; VNS=vagus nerve stimulation.

Acetylcholine impedes inflammation via alpha 7 subunit nicotinic acetylcholine receptors (α7nAChR) expressed on both professional immunocompetent cells and additional cell types[25, 26]. Suppression of NF-kB nuclear translocation is one mechanism activated by acetylcholine-α7nAChR signaling that leads to reduced proinflammatory cytokine synthesis[27, 28], but additional means will be outlined further on in this review. Acetylcholine is released in all vagally innervated organs including the celiac ganglion where it activates the catecholaminergic splenic nerve to release norepinephrine in the spleen to stimulate a subset of splenic T lymphocytes capable of acetylcholine synthesis (Fig.1)[29, 30]. These activated T cells may then leave the spleen and function like mobile neurons to release acetylcholine that will down-regulate inflammation also in body compartments that lack vagal innervation.

Acetylcholine inhibits neuronal HMGB1-induced inflammation

Homeostasis depends on reflexive neural circuits that are counteractive. The cholinergic anti-inflammatory pathway inhibits inflammation, while separate neural circuits in contrast promote inflammation by releasing molecules such as

histamine and neuropeptides[31]. High mobility group box 1 protein (HMGB1) has recently been discovered by us as a proinflammatory molecule actively released by stimulated sensory neurons (nociceptors) in a retrograde manner (antidromically)[32, 33]. HMGB1 was originally discovered as a nuclear protein present in all cells to provide a structural chromatin function[34]. The protein was later established by us also to act as a potent proinflammatory molecule when actively released extracellularly from stimulated innate immunity cells or passively discharged from necrotic or pyroptotic cells as a damage-associated molecular pattern molecule (DAMP)[35, 36]. Extracellular HMGB1 induces proinflammatory cytokine production, dendritic cell maturation, neutrophil and monocyte recruitment via receptor systems including TLR4, RAGE, and CXCR4[37-39]. Extensive preclinical studies of models of sterile injury as well as infections have established that HMGB1 plays an important role in the pathogenesis of inflammation since preclinical treatments with HMGB1 antagonists are very successful[40-42]. Recent studies of mice with neuronal-specific HMGB1 gene ablation demonstrated that the animal expressed much milder inflammation and much less pain in several experimental inflammatory models[33]. HMGB1 is actively released during nociceptor depolarization and plays a key etiologic role in the initiation of neuroinflammation and pain.

It is thus of great clinical interest that acetylcholine via α7nAChR signaling operates as a potent HMGB1 antagonist capable of downregulating HMGB1-induced inflammation via several mechanisms. α7nAChR-mediated signaling suppresses HMGB1 release by inhibiting NF-kB nuclear translocation as well as by deacetylating nuclear HMGB1 (Fig.2)[27, 43, 44]. Nuclear HMGB1 must be hyperacetylated to be exported from the nucleus to the cytosol as a first step required for active extracellular release[45]. Acetylcholine-α7nAChR activation upregulates the intranuclear function of the histone deacetylase system NAD^+-SIRT1 that enzymatically detaches acetyl groups from multiple lysine residues in nuclear HMGB1 and thus prevents HMGB1 from being translocated to the cytosol[44]. SIRT activity declines with aging and causes defects in nuclear and mitochondrial functions resulting in many age-associated pathologies[46]. Stimulating SIRT1 activity may thus serve mutiple beneficial purposes.

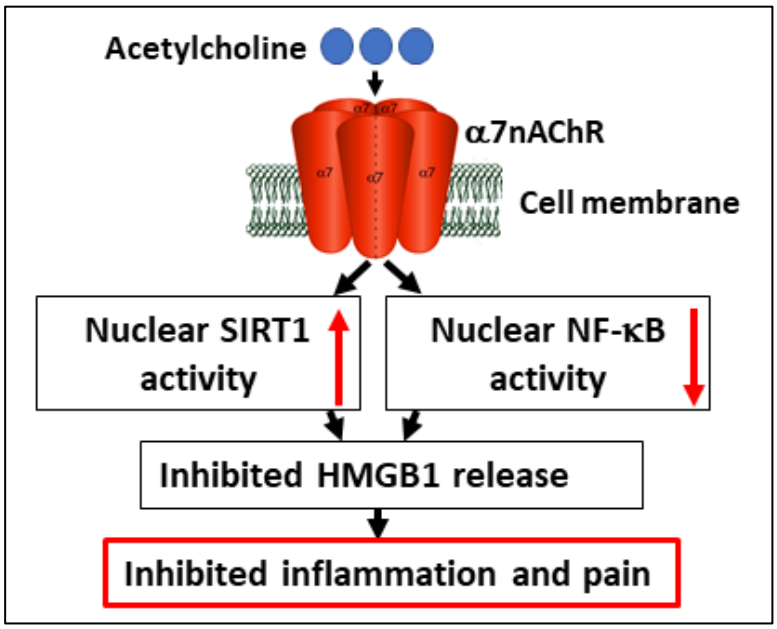

Fig 2. Acetylcholine inhibits cellular HMGB1 release via α7nAChR signaling. Acetylcholine-α7nAChR interaction inhibits the nuclear translocation of NF-kB and upregulates nuclear SIRT1 function. These combined events suppress extracellular HMGB1 release and subsequent induction of inflammation and pain. Nuclear HMGB1 needs to be hyperacetylated to leave the nucleus in order to be extracellularly released and SIRT1, also known as NAD-dependent deacetylase sirtuin-1, deacetylates HMGB1 and thus prevents the HMGB1 export. HMGB1 is also dependent on NF-kB assistance for this translocation and SIRT1 inhibits NF-κB activity by deacetylating the RelA/p65 subunit[47].

Furthermore, extracellular neuronally-derived HMGB1 preferentially induces inflammation and pain via the TLR4/MyD88/NF-kB-pathway[33, 48, 49], a route that is specifically inhibited after acetylcholine-α7nAChR stimulation[50]. Finally, α7nAchR-signaling also inhibits inflammasome activation by preventing release of mitochondrial DNA[51]. Increased extracellular ATP levels enable acetylcholine to translocate into the cytoplasm of innate immunity cells to bind and activate α7nAChR abundantly expressed on the surface of mitochondria. Inhibited inflammasome activities reduce the release of HMGB1, IL-1α, IL-1β, and IL-18, bringing potent anti-inflammatory consequences [52]. In a simplified interpretation of the neuronal control of the immune system it can be summarized that inflammation can be either enhanced or inhibited. Neuronally released HMGB1 activates inflammation and pain, while the neurotransmitter acetylcholine mediates the opposite effects. Furthermore, acetylcholine inhibits HMGB1 release.

Clinical experiences from implanted electrical nerve stimulators

Historically there is a wide clinical experience of implanted electric stimulators that began in 1958 with surgically inserted pacemakers to regulate the electrical conduction system of the heart[53], followed by the creation of vagus nerve stimulators to control drug-resistant epilepsy[54] and treatment-resistant depression[55]. In 2011 vagus nerve stimulators started to be implanted in patients to activate the cholinergic anti-inflammatory pathway to treat inflammatory diseases including rheumatoid arthritis and Crohn´s disease[10, 11, 56]. VNS implantation requires a surgical procedure that positions the lead wire at the cervical portion of the trunk of the left vagus nerve while the pulse generator is inserted subcutaneously in a pocket created in the upper chest. Stimulating parameters regarding duration and timing are quite different in the treatment of epilepsy and depression versus inflammatory diseases[57, 58]. Epilepsy treatment typically uses a stimulation on-time of 30− 90 seconds alternating with off-time of five minutes continuously round the clock, while the stimulation time for treatment of inflammatory diseases is generally limited to minutes rather than hours every 24 hrs. The most common side effects are related to the surgical intervention. Laryngo-tracheal dysfunction, which is related to the stimulation of the inferior recurrent laryngeal nerve, occurs in approximately two thirds of cohorts treated for epilepsy and is usually transient[58]. No age-dependent differences between patients regarding side-effects have been reported.

The anti-inflammatory and disease-alleviating efficacy of VNS has been demonstrated in many animal models, including endotoxemia, sepsis, arthritis, and inflammatory bowel disease. In addition to VNS, pharmacological cholinergic modalities including α7nAChR agonists and acetylcholinesterase inhibitors have been shown to suppress aberrant inflammation and alleviate disease severity in preclinical models of a substantial number of diseases[3, 24, 59].

Encouraging results were reported in 2016 from the initial study of adult patients with rheumatoid arthritis treated with an implantable VNS device[11]. The capacity for TNF synthesis was reduced by VNS, which is relevant since overproduction of TNF is part of the pathogenesis in chronic arthritis. Several, but not all, patients improved significantly despite that some of them had failed multiple biological disease-modifying antirheumatic agents prior to enrolment in the VNS study. When the device was turned off for two weeks in the middle of the treatment period, the disease got worse and improved again after restart of the pulse generator. VNS with an implanted miniaturised neurostimulator in another study likewise reduced signs and symptoms of rheumatoid arthritis in patients with multidrug-refractory disease[60].

An additional proof-of-concept study supporting the strategy of stimulating the cholinergic anti-inflammatory pathway in a clinical setting has been accomplished in patients with Crohn´s disease[61]. After 12 months of treatment with VNS, 5 out of 9 treated patients with moderate Crohn´s disease reached clinical remission and 6 patients were in endoscopic remission. C-reactive protein and fecal calprotectin decreased in 6 and 5 patients, respectively. Seven patients restored their vagal tone and decreased their digestive pain score. Substantial pain relief has been a prominent achievement also reported in VNS treatment of arthritis[10, 11].

Transcutaneous auricular VNS (taVNS) for inflammation therapy

A promising approach to circumvent the caveats of pharmacological or invasive stimulation in humans is taVNS, applied mainly to the cymba conchae or, in some studies to the tragus of the left external ear.

An increasing number of studies have shown that several therapeutic effects induced by invasive VNS, can be reproduced by noninvasive auricular-nerve stimulation. A sensory branch of the vagus nerve innervates the cymba conchae exclusively, and parts of the antihelix, the cava conchae, and the tragus of human auricles (Fig.3)[13, 62].

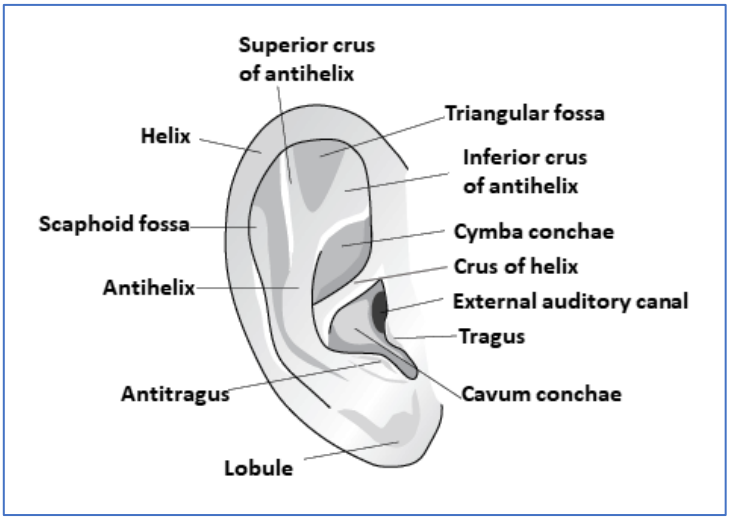

Fig 3. Auricular anatomy and remarks on the distribution of the auricular branch of the vagus nerve (ABVN). The nerve fibers in the auricle, including the ABVN, are located in the dermis and the perichondrium in the 1-2 mm space between the auricular cartilage and the skin[63]. The cymba conchae innervation is to 100% dominated by the ABVN. Additional auricular areas partly innervated by ABVN include the antihelix (73%), cavum conchae (45%), tragus (45%), crus of helix (20%), and crura of antihelix (9%)[62]. These numbers are based on studies of auricular nerve distribution in seven cadavers.

The auricular branch of the vagus nerve projects sensory input to the brain stem nucleus tractus solitarius (NTS) which receives approximately 95% of all vagal afferents[64, 65]. The NTS projects to numerous areas in forebrain, amygdala, hippocampal, limbic, and brainstem structures including the nucleus ambiguus, the locus coeruleus, and the dorsal motor nucleus, which promotes motor outflow of the vagus nerve[66]. Functional magnetic resonance imaging (fMRI) studies in humans confirm that the central projections of the auricular branch of the vagus nerve are consistent with the vagal projections activated after invasive VNS and can be accessed non-invasively via the external ear[13, 67].

Lessons learnt from taVNS treatment in animal disease models

The efficacy of taVNS treatment to inhibit inflammation was first demonstrated in endotoxemic rats[68]. This therapeutic intervention suppressed LPS-induced proinflammatory cytokine production via a7nAChR-mediated activation of the cholinergic anti-inflammatory pathway. The results were later recapitulated in a mouse model of endotoxemia, where it was clearly demonstrated that selecting optimal pulse frequency parameters for the taVNS is crucial to optimize the beneficial therapeutic results[69]. Furthermore, VNS has also been demonstrated to attenuate organ dysfunction in porcine progressive sepsis[70]. The importance of acetylcholine-releasing T lymphocytes for the function of the inflammatory reflex was further highlighted in a separate study of VNS-treated endotoxemic nude mice. Nude mice, that are devoid of functional T cells, did not reduce LPS-induced proinflammatory cytokine release after VNS intervention[29]. Furthermore, treatment with taVNS has demonstrated beneficial inhibitory results also in disease models of non-infectious conditions such as depressive-like behavior in rats[71, 72], diabetic neuropathy[73], seizures[74], several anti-diabetic effects[75], and in cerebral ischemia–reperfusion injury[76].

The somewhat remarkable result that VNS performed for only a few minutes once a day generates a significant inhibition of proinflammatory functions in macrophages for more than 24 hrs was addressed by Tarnawski *et al* using

a7nAChR-specific agonists or VNS in *in vitro* experiments and in *in vivo* studies of animal models of inflammation[77]. Their results indicate that action potentials in the inflammatory reflex trigger an inhibitory change in macrophage behavior that depends on a7nAChR, adenyl cyclase and subsequent phosphorylation of the cAMP response element binding protein (CREB), a transcription factor involved in memory formation. This pathway has been demonstrated to inhibit NF-kB-induced transcription of proinflammatory cytokines[78]. Memory is thus a feature of both adaptive and innate immunity.

Clinical results from taVNS treatment studies in inflammatory diseases

The auricular vagus nerve stimulation performed in at home environment is generally accomplished using a small battery-powered hand-held stimulating device which generates pulsed electrical currents which are delivered via skin electrodes in the auricle. Users can adjust the pulse amplitude (mA), frequency (Hz), width or duration (μs) and pattern of the currents. The initial, and so far, most extensive experience from clinical taVNS-based therapy comes from treatment of patients with epilepsy[79-81] and depression[82, 83]. The original reason for starting taVNS treatment in depression was that many patients with epilepsy experienced mood improvement during taVNS therapy. Since the mechanisms for beneficial therapeutic taVNS results seen in many patients with these diseases are not fully elucidated, this clinical area is not further covered in the present review focusing on universally accepted signs and symptoms of inflammation. One important piece of information from the studies in epilepsy and depression is that the treatment is safe. Transcutaneous auricular VNS treatment for drug-resistant epilepsy carried out in 10 separate trials with 350 patients has resulted in only minor adverse effects with skin irritation related to electrode placement in the ear.

The pioneering taVNS intervention, reported in 2019, to treat a systemic inflammatory disease was accomplished with a vibrotactile device stimulating the left cymba conchae of patients with rheumatoid arthritis[84]. The adherence of the skin to the perichondrium in the concha region makes it especially susceptible to the cartilage movements including vibrations and therefore mechanical forces moving the cartilage may also stimulate the auricular nerves[63]. The vibration treatment attenuated systemic inflammatory signs and symptoms including pain in the patients. Furthermore, the vibrotactile stimulation also inhibited peripheral blood production of TNF, IL-1β, and IL-6 in healthy subjects. A 12-week open-label, proof-of-concept pilot study of electrical taVNS treatment in moderately to severely active rheumatoid arthritis generated significant reductions in the disease severity[85]. Out of 30 RA patients, 11 attained low disease activity and 7 achieved remission. 15 of the patients continued the treatment with a daily stimulation time up to 30 min for another 9 months with sustained or further improved disease course. No serious adverse events were reported during the study extension phase[86]. Another taVNS pilot study performed for 4 weeks in patients with erosive hand osteoarthritis demonstrated considerable analgesic outcomes and objective effects on joint inflammation[87]. The number of swollen joints decreased in 15 of the studied 18 patients. A recent, randomised, double-blind, sham-controlled pilot taVNS trial in patients with active systemic lupus erythematosus (SLE) resulted in significantly reduced pain, fatigue, and inflammatory joint scores[88]. This is a very encouraging outcome since both pain and fatigue are common symptoms severely reducing the quality of life in active SLE.

The pediatric experience of taVNS treatment in inflammatory diseases is so far restricted to two studies. Transcutaneous auricular VNS performed for 5 min twice daily at home during 4 months in 22 teenage patients with mild/moderate inflammatory bowel disease (12 patients with ulcerative colitis and 10 with Crohn´s disease) reduced fecal calprotectin and improved symptoms significantly[89]. Eleven of the 17 patients with pathological baseline fecal calprotectin scores experienced a reduction of more than 50% by week 16. There were no safety concerns. An open-label, pilot study of taVNS for five minutes daily for 26 weeks in children with nephrotic syndromes also demonstrated promising results[90]. Three out of 3 children with frequently relapsing nephrotic syndrome remained relapse-free during the study period. Two patients continued the treatment and remained in remission for 15 and 21 months, respectively. Three out of 4 children with steroid-resistant nephrotic syndrome demonstrated moderately reduced proteinuria. A clinical trial investigating the effects of taVNS in juvenile inflammatory arthritis is ongoing (ClinicalTrials.gov Identifier: NCT01924780).

Presently approved indications for taVNS treatment and description of taVNS devices

VNS via a surgically implanted device is currently FDA-approved for the treatment of epilepsy, refractory depression, and chronic obesity. Clinical studies are presently ongoing to evaluate the effects provided by implanted VNS devices in multiple chronic inflammatory diseases. The GammaCore® transcutaneous cervical VNS device has been approved by the FDA for acute treatment of migraine and acute or prophylactic treatment of cluster headaches.

There is presently only a very restricted number of companies distributing devices for taVNS studies (reviewed in [91]). One device for transcutaneous auricular VNS named NEMOS (distributed by tVNS Technologies GmbH, Germany) has been granted the CE mark for the treatment of resistant epilepsy. Other taVNS devices, NET-1000 and NET-2000, developed by Auri-Stim, have been approved by the FDA for the treatment of depression, anxiety and insomnia. A typical taVNS device is comprised of two main components: the stimulation unit, which houses the battery and pulse generator (approximately the size of a mobile phone), and a dedicated pair of ear electrodes, which are connected to the stimulator via a cable.

Does VNS- or taVNS-based therapy offer exceptional prospects for pain relief in chronic inflammation?

I am particularly impressed by observations I have made while meeting patients with rheumatoid arthritis and Crohn´s disease regarding the beneficial effects seen on pain alleviation mediated by vagus stimulation. These soft data are supported by published results witnessed during VNS- as well as taVNS-therapy in chronic inflammation[11, 88]. When Koopman *et al* treated rheumatoid arthritis patients (n=17) with an implanted vagus nerve stimulator the pain score declined after 6 weeks of treatment from a mean value of 71 to 34 mm measured on a visual analogue scale (VAS) ranging from 0-100 mm with 100 mm representing maximal pain (Table S2)[11]. At the same time mean serum C-reactive protein levels, reflecting inflammation, declined only quite modestly from 17 to 13 mg/L. Likewise, in a sham-controlled taVNS treatment of active SLE (n=18 patients) Aranow and colleagues observed correspondingly favorable outcome regarding pain relief [88]. Acetylcholine discharged after vagus stimulation inhibiting nociceptor-released HMGB1 might conceivably mediate the analgesic effects.

Concluding remarks

The aim to harness the body´s own protective neural circuits to treat disease is a very appealing strategy. Controlled clinical studies are what is urgently required. There is a need to design and produce commercially available devices enabling controlled electrical auricular stimulation since these are presently a scarce commodity. Other important tasks will be to define the most favorable anatomical positions for the ear electrodes and to optimize the specific stimulation parameters for treatment regarding pulse frequency, pulse width, waveform, amplitude, timing, and duration. The success of this work will depend on well-organized, multidisciplinary, collaborative research efforts.

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Conflicts of Interest

I declare that I have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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