Controlling inflammation: the cholinergic anti-inflammatory pathway

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Abstract
Innate immune responses and inflammation are regulated in part by neural mechanisms. In the present paper, we summarize experimental evidence that reveals that innate immunity and inflammation are controlled by the vagus nerve, previously known as a regulator of other vital physiological functions. Activation of vagus nerve cholinergic signalling inhibits TNF (tumour necrosis factor) and other pro-inflammatory cytokine overproduction through ‘immune’ $\alpha_7$ nicotinic receptor-mediated mechanisms. This efferent vagus nerve-based ‘cholinergic anti-inflammatory pathway’ has been elucidated as a critical regulator of inflammation in several experimental models of diseases. Our recent observations have shown that activation of central (brain) cholinergic transmission by selective muscarinic receptor ligands results in lower systemic TNF levels in rodents and indicate that the efferent vagus nerve may provide a functional brain-to-immune connection. Thus central cholinergic signalling is implicated in the activation of the cholinergic anti-inflammatory pathway. Electrical vagus nerve stimulation is clinically approved for the treatment of epilepsy and depression and current knowledge suggests that it could be utilized to control inflammation. Advances in understanding the receptor and molecular mechanisms of cholinergic anti-inflammatory signalling indicate that selective $\alpha_7$ nicotinic receptor agonists and centrally acting cholinergic enhancers can be used in the treatment of pathological conditions characterized by cytokine overproduction.

Introduction
Inflammation is a localized and highly regulated host response to infection and injury, which is directed towards neutralizing the invading pathogen and wound healing [1]. TNF (tumour necrosis factor) and other pro-inflammatory cytokines are important mediators of inflammation, produced by activated macrophages and other immune cells [1]. However, excessive cytokine production and release into the bloodstream is characteristics of abnormal chronic or overactivated inflammatory responses, resulting in secondary tissue injury and primarily related to the pathology of numerous diseases, including rheumatoid arthritis, Crohn’s disease, autoimmunity diseases and sepsis [1–3].

Controlling cytokine production is critical for preventing pathological unrestrained inflammation. The release of glucocorticoids, anti-inflammatory cytokines and soluble cytokine receptors are well-documented mechanisms directed towards counteracting abnormal pro-inflammatory cytokine excess [4,5]. Research in our laboratory demonstrated the importance of neural, vagus nerve-mediated pathway in controlling cytokine production and inflammation.

The cholinergic anti-inflammatory pathway and the inflammatory reflex
Experimental evidence accumulated over the last 10 years has demonstrated that activation of the efferent vagus nerve decreases systemic TNF and other pro-inflammatory cytokines in experimental models of acute systemic inflammation. These findings revealed that apart from its classical functions, such as regulation of heart rate, bronchoconstriction and gastrointestinal motility, the efferent vagus nerve is a neural regulator of the production of TNF and other pro-inflammatory cytokines. Activation of the efferent vagus nerve stimulates the release of acetylcholine, which inhibits the production of the pro-inflammatory cytokines TNF, IL-1 (interleukin 1), IL-6 and IL-18 by LPS (lipopolysaccharide; endotoxin)-activated macrophages, without affecting the production of anti-inflammatory cytokines [6]. Therefore we termed this physiological, neural mechanism ‘the cholinergic anti-inflammatory pathway’ [6]. Current research indicates that the cholinergic anti-inflammatory pathway inhibits TNF production by resident macrophages in organs of the reticuloendothelial system and prevents abnormal elevation of systemic TNF [6–8] (Figure 1). Electrical vagus nerve stimulation decreases systemic TNF during endotoxaemia (induced by administration of LPS) [6,8–11], ischaemia reperfusion as a result of aortic occlusion [12] and lethal hypovolemic haemorrhagic shock [13]. These studies also demonstrate that vagus nerve stimulation attenuates the development of shock, an effect that can be attributed to

Key words: $\alpha_7$ nicotinic acetylcholine receptor, cholinergic anti-inflammatory pathway, inflammation, muscarinic receptor, tumour necrosis factor (TNF), vagus nerve.

Abbreviations used: $\alpha_7nAchR$, $\alpha_7$ nicotinic acetylcholine receptor; CCK, cholecystokinin; DMN, dorsal motor nucleus of the vagus; HMGB1, high mobility group box 1; IL, interleukin; LPS, lipopolysaccharide; NTS, nucleus tractus solitarius; TNF, tumour necrosis factor.

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Efferent vagus nerve-mediated regulation of inflammation (proposed mechanisms)

Excessive inflammatory responses are inhibited by activated efferent vagus nerve-derived anti-inflammatory output in organs of the reticuloendothelial system, including the heart, liver, gastrointestinal (GI) tract and spleen. Current knowledge indicates that vagus nerve cholinergic signalling interacts with α7nAchR on immune cells in the spleen and inhibits TNF production and release into the circulation. Systemic TNF is inhibited by activation of muscarinic cholinergic signalling in the brain and the efferent vagus nerve may provide a functional brain-to-immune connection (DMN; Ach, acetylcholine). See text for details. Part of this Figure is adapted by permission from Macmillan Publishers Ltd: Nature, [1], copyright 2002. http://www.nature.com

suppressed TNF overproduction, since TNF is a causative mediator of hypotension and shock.

Controlling inflammation is associated with a bi-directional communication between the innate immune system and the brain. Revealing the anti-inflammatory function of the efferent vagus nerve allowed us to postulate the concept of reflex regulation of inflammation [1]. Studies by Watkins et al. [14] identified a role for the afferent vagus nerve as a sensor of inflammation. Inflammatory products, including LPS and IL-1, activate afferent vagus nerve fibres (with perikarya in the nodose ganglion), relay information for occurring peripheral inflammation to the brain. The efferent vagus nerve-based cholinergic anti-inflammatory pathway, which inhibits excessive pro-inflammatory cytokine production and curbs overactivated inflammatory responses, represents the efferent arm of this inflammatory reflex [1]. These two components (afferent and efferent) of the inflammatory reflex are centrally integrated through neural interactions between the NTS (nucleus tractus solitarius), where vagal afferents terminate, and the DMN (dorsal motor nucleus of the vagus), where most of the efferent vagal preganglionic fibres originate [4]. Neural interactions between the NTS and the DMN and other brain stem regions, and higher forebrain structures, including the hypothalamus, provide a neural network for central modulation of the inflammatory reflex and its integration with other brain-derived inflammatory response pathways, including the HPA (hypothalamo–pituitary–adrenal) axis and the sympathetic part of the autonomic nervous system [1,4,16]. The inflammatory reflex represents a complex brain-integrated mechanism, with several regulatory points, rather then a simple vago-vagal neuronal circuit.

Our recent study demonstrated a pivotal role for a brain muscarinic cholinergic mechanism in controlling acute inflammation [11]. Activation of central muscarinic acetylcholine receptors by selective ligands inhibits systemic TNF during endotoxaemia in rats [11] (Figure 1). Central muscarinic cholinergic stimulation also is accompanied by activation of the high-frequency component of heart rate variability, a measure of efferent vagus nerve activity [11]. This finding indicates that the efferent vagus nerve-driven cholinergic anti-inflammatory pathway may provide a functional brain-to-immune communication channel (Figure 1). CNI-1493, a compound previously shown as a central activator of the cholinergic anti-inflammatory pathway [9], interacts with central muscarinic receptors, and this observation also supports a role for central muscarinic signalling in the regulation of this pathway [11]. Although the central regions and circuits playing a mediating role remain unknown, this study indicates the possibility of using centrally acting cholinergic drugs to control inflammatory responses [11].

Cholinergic mechanisms controlling inflammation and their therapeutic implications

Acetylcholine receptors are divided into two classes: muscarinic (G-protein-coupled) and nicotinic (ligand-gated ion channels). While these receptors are distributed in the central nervous system and the periphery, they have different synaptic locations and exhibit varied function in cholinergic transmission. Identifying the receptors transmitting the anti-inflammatory cholinergic signal represents an important advance in our understanding of the cholinergic anti-inflammatory pathway. Research in our laboratory utilizing antisense and knockout approaches identified an anti-inflammatory role for the α7nAchR (α7 nicotinic acetylcholine receptor) expressed on macrophages and other cytokine-producing cells [10]. The α7nAchR is required for the anti-inflammatory effects of the vagus nerve because, in contrast with the wild-type mice, vagus nerve stimulation is ineffective in suppressing systemic TNF in α7nAchR knockout mice subjected to endotoxaemia [10]. Our recent study has also
indicated that inhibition of TNF by electrical vagus nerve stimulation does not require activation of muscarinic receptors on immune cells, because blockade of these receptors does not interrupt the effect [11]. These findings identified the α7nAchR as a peripheral ‘immune’ component of the cholinergic anti-inflammatory pathway (Figure 1) and indicated that pharmacological activation of the α7nAchR might be utilized as an anti-inflammatory strategy in addition to electrical vagus nerve stimulation [2,17].

Electrical vagus nerve stimulation and/or α7nAchR agonists have been successfully applied in models of inflammatory diseases, including endotoxaemia, sepsis, ischaemia reperfusion, haemorrhagic shock, subcutaneous and gastrointestinal inflammation and pancreatitis [6–8,10–13,18–21]. Several studies contributed to current understanding of the cholinergic anti-inflammatory pathway and its functions in controlling inflammation. Guarini et al. [13] have reported that vagus nerve stimulation counteracts hypotension and increases the survival time during lethal haemorrhagic shock in rats. The mechanisms underlying these beneficial effects are nicotinic receptor-dependent and include inhibition of the activation of nuclear factor κB and TNF synthesis in the liver [13]. De Jonge et al. [7] have shown that vagus nerve stimulation attenuates inflammation and postoperative ileus in mice by activating transcription STAT3 (signal transducer and activator of transcription 3) in intestinal macrophages through an α7nAchR-mediated mechanism. Luyer et al. [19] have recently shown important functions for the efferent vagus nerve and nicotinic receptors in mediating anti-inflammatory and protective effects of CCK (cholecystokinin) in rats subjected to haemorrhagic shock. This study demonstrates that CCK (induced by ingestion of dietary fat) stimulates CCK receptors, leading to subsequent activation of efferent vagus nerve signalling, which suppresses the inflammatory response during haemorrhagic shock [19]. The authors propose the intriguing hypothesis that the immune hyporesponsiveness in the intestinal tract to dietary antigens is related to a tonic anti-inflammatory function of the efferent vagus nerve [19]. Our recent study identified the spleen as an important organ source of TNF during endotoxaemia and demonstrated that activation of the cholinergic anti-inflammatory pathway by electrical stimulation of the vagus nerve inhibits splenic and systemic TNF levels [8]. The α7nAchR in the spleen is required for the efferent vagus nerve-mediated inhibition of splenic TNF production (Figure 1), because vagus nerve stimulation failed to inhibit splenic TNF in α7nAchR knockout mice [8]. Therefore it is plausible to consider that the severity of inflammation might be dependent on the functional connection between the cholinergic anti-inflammatory pathway and the spleen. Research in progress in our laboratory is aimed at revealing the cellular and molecular mechanisms of this connection.

Cholinergic modalities based on activation of the α7nAchR inhibit TNF and other important pro-inflammatory cytokines, including HMGB1 (high mobility group box 1). Studies with rodents have demonstrated that HMGB1 is critically involved in the pathogenesis of severe sepsis [3]. Recently, Wang et al. [18] have shown that acetylcholine inhibits the release of HMGB1 by LPS-stimulated macrophages. Nicotine also suppresses the release of HMGB1 by LPS-stimulated RAW 264.7 macrophage cell line through an α7nAchR-dependent mechanism [18]. Nicotine inhibits systemic HMGB1 and significantly improves survival of mice with caecum ligation and puncture-induced peritonitis, a widely used preclinical model of severe sepsis [18]. However, nicotine is ineffective in splenectomized mice [8] and this finding indicates a role for the spleen in mediating nicotinic beneficial effect in experimental sepsis. Ongoing research in our and other laboratories focuses on exploring selective α7nAchR agonists as anti-inflammatory agents.

Knowledge related to the vagus nerve-driven cholinergic anti-inflammatory pathway and its regulatory mechanisms can be exploited in the development of new therapeutic approaches for the treatment of inflammatory diseases. Rheumatoid arthritis and other inflammatory diseases are accompanied by autonomic dysfunction, characterized by a diminished vagal component of heart rate variability [5,22]. It is possible that this physiological dysfunction results in decreased vagus nerve anti-inflammatory output, which might be associated with loss of tonic suppression of inflammatory processes. Vagus nerve stimulation by surgically implanted devices is clinically approved for the treatment of pharmacoresistant epilepsy and treatment-resistant depression, with minor side effects [23,24]. Similar devices may have clinical implications in improving autonomic dysfunction and the treatment of unrestrained inflammation in humans. Cholinergic modalities directed towards peripheral α7nAchR activation or central muscarinic cholinergic stimulation present other promising therapeutic approaches in the treatment of inflammatory disorders.

Conclusion

Studies in our laboratory and other research centres have identified the cholinergic anti-inflammatory pathway as a neural, efferent vagus nerve-based mechanism that controls inflammation. Vagus nerve cholinergic signalling interacts with α7nAchR on immune cells and inhibits the production of TNF and other pro-inflammatory cytokines and excessive inflammatory responses. This pathway can be exploited in the treatment of inflammatory disorders.

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References


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