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The vagus nerve and the inflammatory reflex—linking immunity and metabolism

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Abstract

The vagus nerve has an important role in regulation of metabolic homeostasis, and efferent vagus nerve-mediated cholinergic signalling controls immune function and proinflammatory responses via the inflammatory reflex. Dysregulation of metabolism and immune function in obesity are associated with chronic inflammation, a critical step in the pathogenesis of insulin resistance and type 2 diabetes mellitus. Cholinergic mechanisms within the inflammatory reflex have, in the past 2 years, been implicated in attenuating obesity-related inflammation and metabolic complications. This knowledge has led to the exploration of novel therapeutic approaches in the treatment of obesity-related disorders.

Introduction

The vagus nerve (cranial nerve X) is the main nerve of the parasympathetic division of the autonomic nervous system. The vagus nerve regulates metabolic homeostasis by controlling heart rate, gastrointestinal motility and secretion, pancreatic endocrine and exocrine secretion, hepatic glucose production, and other visceral functions. In addition, the vagus nerve is a major constituent of a neural reflex mechanism—the inflammatory reflex—that controls innate immune responses and inflammation during pathogen invasion and tissue injury.[1–3]

Innate immune responses are activated by pathogen-associated and danger-associated molecular patterns that are recognized by sensors on the immune cell surface or in intracellular compartments. These cellular sensors include Toll-like receptors (TLRs), nucleotide-binding oligomerization domain-like receptors (NLRs) and other pattern-recognition receptors (Figure 1).[4–6] Activation of signalling cascades downstream of TLRs results in increased production and release of tumour necrosis factor (TNF), IL-6 and other

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proinflammatory cytokines.[1,7] In addition, activation of NLRs is associated with the formation of multimeric protein complexes, termed inflammasomes, which regulate maturation and release of the proinflammatory cytokines IL-1β and IL-18.[5] Proinflammatory cytokines, along with chemo-kines, reactive oxygen species, nitrogen inter mediates and other inflammatory molecules, are critically implicated in extracellular pathogen clearance, vasodilatation, neutrophil recruitment, increased vascular permeability and induction of acute-phase proteins, such as C-reactive protein (CRP), and coagulation molecules.[1,5,7] Proinflammatory progression is balanced by the release of IL-10, TGF-β, soluble cytokine receptors and other anti-inflammatory molecules.

Inflammation is normally a local and temporary event and, upon its resolution, immune and physiological homeostasis is restored. However, disrupted innate immune regulation can result in continual pro-inflammatory cytokine activity and excessive or chronic inflammation. This state underlies the pathogenesis of a range of disease syndromes, including sepsis, rheumatoid arthritis, inflammatory bowel disease and other inflammatory and autoimmune disorders.[8–10] Understanding endogenous mechanisms that prevent or neutralize excessive proinflammatory responses could lead to novel therapeutic options for diseases associated with an excessive or chronic inflammatory state.

Chronic inflammation as a result of immune and metabolic dysregulation is a characteristic feature in patients with obesity and is causally linked with insulin resistance and other metabolic complications.[11–13] Decreased vagus nerve activity in the context of obesity has been reported.[14–17] Selective cholinergic activation within the efferent vagus nerve-mediated arm of the inflammatory reflex can suppress obesity-associated inflammation and reverse metabolic complications.[18–20] These findings raise the intriguing possibility that dysregulation of vagus nerve-mediated signalling might contribute to the pathogenes is of obesity and its related comorbidities.

In this Review, we provide a conceptual view of the inflammatory reflex as a physiological mechanism that functions on the path between immunity and metabolism and could be exploited in the treatment of obesity-associated inflammation and obesity-related disorders.

The inflammatory reflex

Communication between the immune system and the brain is vital for controlling inflammation. The inflammatory reflex is a centrally integrated physiological mechanism in which afferent vagus nerve signalling, activated by cytokines or pathogen-derived products, is functionally associated with efferent vagus nerve-mediated output to regulate proinflammatory cytokine production and inflammation (Figure 1). The absence of this inflammatory reflex—resulting from neural lesions or genetic ablation of essential components—results in excessive innate immune responses and cytokine toxicity.[21,22] The inflammatory reflex has been comprehensively reviewed elsewhere.[1–3,9,23]

Afferent arm

Afferent vagus nerve fibres sense peripheral inflammatory molecules and convey signals to the brain and are, therefore, important for immune-to-brain communication (Figure 1).[1,24]

Afferent vagus neurons, residing in the nodose and jugular ganglia, terminate primarily in the nucleus tractus solitarius in the brainstem medulla oblongata (Figure 1).[24] Afferent signalling is further communicated through neural contacts between brainstem nuclei, the hypothalamus and forebrain regions associated with integration of visceral sensory information as well as coordination of autonomic function and behavioural responses.[23,24]

Peripheral administration of bacterial lipopolysaccharide (also known as endotoxin) or IL-1β causes afferent vagus nerve activation, as determined by increased c-Fos expression and electrical activity.[24,25] IL-1β receptors expressed on vagus nerve afferents and chemosensory (glomus) cells in paraganglia surrounding afferent vagus nerve endings have been implicated in the recognition of immune activation (Figure 1).[24,26,27] Prostaglandin-dependent mechanisms have also been implicated in activating vagus nerve afferents by increasing levels of circulating IL-1β.[26] Intraportal administration of IL-1β to rats increases afferent and, subsequently, efferent vagus nerve and splenic nerveactivity.[25] Peripheral immune stimulation causes sickness behaviour, which is attenuated in rodents who have undergone surgical transection of the vagus nerve to prevent immune signalling at peripheral nerve endings.[24] This attenuation is dependent on the magnitude of immune activation, because fairly large quantities of circulating IL-1β produce fever and sickness behaviour by bypassing the neural circuits, acting through brain circumventricular organs (including the area postrema) and via other humoral mechanisms (Figure 1).[28,29] Accordingly, afferent vagus nerve endings appear to be important for relaying information about immune status to the brain when proinflamma tory cytokines are present at fairly low levels.[24,28] However, TLR4 (which can be activated by lipopolysaccharide) is expressed in the nodose ganglion,[30,31] which suggests a mechanism by which lipopolysaccharide and other inflammatory molecules can activate vagus nerve afferents above their visceral endings (Figure 1).

Efferent arm

A little more than a decade ago, an important role of efferent vagus nerve cholinergic signalling in brain-to-immune communication was revealed by observations that vagus nerve stimulation suppresses local and serum proinflammatory cytokine levels in rodents with endo-toxaemia, and that acetylcholine inhibits the release of TNF, IL-1β and IL-18 from lipopolysaccharide-stimulated macrophages.[21] These findings led to the definition of the cholinergic anti-inflammatory pathway as the efferent vagus nerve-based arm of the inflammatory reflex (Figure 1).[21] This efferent arm can be centrally regulated, and muscarinic acetylcholine receptors in the brain have been implicated in this regulation (Figure 1).[32–35] Galantamine is a centrally acting acetylcholinesterase inhibitor and activates the efferent cholinergic arm of the inflammatory reflex by through a muscarinic receptor-dependent mechanism in the brain.[34] Defining a brain network that can be used to explore central mechanisms of inflammatory reflex control is the aim of ongoing studies.

In peripheral tissues, the α7 nicotinic acetylcholine receptor (α7nAChR) is important for mediating anti-inflammatory signalling within the efferent arm of the inflammatory reflex (Figure 2).[22] Accordingly, several α7nAChR agonists have been identified as experimental anti-inflammatory therapeutics with potential for clinical development.[2,36–38] The α7

subunit of the receptor is expressed in macrophages, monocytes, dendritic cells, T cells, endothelial and other non-neuronal cells, and the structural and functional characterization of non-neuronal α7nAChRs is an area of ongoing study.[2,38] The presence of α7nAChRs on bone marrow-derived cells is required for the functional integrity of the inflammatory reflex, but their presence on T cells and neuronal cells is not essential.[39] The anti-inflammatory action of α7nAChR agonists or vagus nerve stimulation is associated with downregulation of CD14 and TLR4 expression in immune cells.[40] Cholinergic inhibition of proinflammatory cytokine production is mediated through intracellular signal pathways downstream of α7nAChR that culminate in suppression of NF-κB nuclear translocation (Figure 2).[36–38,40–42] The tyrosine protein kinase JAK2 might also be recruited and activated by α7nAChR upon cholinergic stimulation.[43] Subsequent phosphorylation of the transcription factor STAT3 results in suppression of proinflammatory cytokine production in intestinal macrophages (Figure 2).[43]

The anti-inflammatory effects of vagus nerve stimulation in animals with endotoxaemia require neural signals along the adrenergic splenic nerve,[44] which originates in the celiac ganglion that is innervated by the efferent vagus nerve (Figure 1, Figure 2). Somewhat unexpectedly, a specific subset of T cells (memory $CD4^+$ T cells, which express adrenoreceptors), instead of neurons, provide an endogenous source of acetylcholine in the spleen (Figure 2).[45] T cell production of acetylcholine in the spleen is critical for the inflammatory reflex. In nude mice, which lack T cells, vagus nerve stimulation fails to suppress TNF levels during endotoxaemia. However, transfer of acetylcholine-producing T cells, which repopulate the spleen in nude mice, restores the integrity of the neural circuit.[45] In addition to this spleen-mediated mechanism in endotoxaemia, efferent vagus nerve endings might directly regulate the immune function by releasing acetylcholine, without the requirement for either signalling along the splenic nerve or T cells (Figure 1, Figure 2). Such a direct mechanism has been implicated in the suppression of inflammation in experimental settings of haemorrhagic shock, ileus, inflammatory bowel disease, autoimmune myocarditis and other inflammatory and autoimmune conditions.[2,10,41,43,46–49] Importantly, many of these disorders are associated with autonomic dysfunction and decreased vagus nerve tone. Enhancement of vagus nerve output could, therefore, have therapeutic potential in these settings.[50,51]

Vagus nerve in metabolic regulation

Vagus nerve afferent and efferent signalling has an important role in the regulation of feeding behaviour and metabolic homeostasis. This finely tuned regulation is aimed at preserving energy balance and preventing fluctuations in body weight and metabolism that can be detrimental to the individual.

Dietary intake and metabolism

Vagus nerve afferents innervating the gastrointestinal tract and liver are major constituents of a sensory system that detects changes in micronutrient and metabolic molecules. These nerve fibres transmit information detected by associated mechanoreceptors, chemoreceptors and specific metabolite receptors in the gut and hepatic portal system concerning levels of lipids, cholecystokinin, leptin, peptide YY, insulin and glucose to the brain (Figure 3).[52,53]

Vagus nerve efferents, on the other hand, provide brain-derived output to the gastrointestinal tract, liver and pancreas (Figure 3).[52] Vagus nerve innerva-tion of white adipose tissue has also been demonstrated using retrograde neuronal tracing.[54] Other researchers, however, found limited retrograde labelling in the dorsal motor nucleus following injection of the tracer into white adipose tissue and a lack of some parasympathetic markers in adipose tissue.[55] Therefore, the anatomy and a functional role of the vagus nerve in relation to adipose tissue is an area of active study.

Hepatic and gastrointestinal vagus nerve afferents are involved in the regulation of short-term feeding behaviour.[52,53] Afferent vagus nerve signalling mediates gastric cholecystokinin-induced satiety and meal termination.[52] Synergistic activation of vagus nerve afferents by both cholecystokinin and leptin mediates their short-term inhibitory effects on food intake.[53] Lipid accumulation in the upper intestine, and consequent intestinal cholecystokinin release, also triggers vagus nerve-mediated and brain-integrated suppression of hepatic glucose production.[56,57] Additionally, the efferent arm of this reflex mechanism can be stimulated by direct activation of neurons in the dorsal vagal complex.[58]

Glucose-induced pancreatic insulin secretion can be stimulated by treatment with a truncated form of glucagon-like peptide-1, which activates hepatic vagus nerve afferents and, subsequently, vagus nerve efferents innervating the pancreas.[59,60] In addition to its intrinsic effects on the enteric nervous system, glucose in the lumen of the gastrointestinal tract causes neuronal activation in vagus nerve afferents, the nucleus tractus solitarius, the arcuate hypothalamic nucleus and the dorsal motor nucleus, thus highlighting the role of brain circuitry in vagus nerve regulation of gastrointestinal function and pancreatic secretion.[61]

Additionally, metabolic molecules can act directly in the brain to trigger efferent output that regulates metabolic homeostasis (Figure 3). Insulin signalling in the mediobasal hypothalamus is mediated through modulation of ATP-sensitive potassium channels and has been implicated in the suppression of hepatic glucose production.[62,63] Efferent vagus nerve signalling to the liver is essential for this regulation.[63] Hypothalamic sensing of fluctuations in circulatory lipid levels triggers efferent vagus nerve-mediated output that regulates hepatic glucose production and glucose homeostasis.[64] In the hypothalamus, cholinergic activation dependent on muscarinic receptors increases hepatic glycogen synthesis through a mechanism mediated by the vagus nerve.[65,66] Vagus nerve-derived cholinergic signalling through a mechanism mediated by M3 muscarinic receptors has also been implicated in stimulating insulin release in the pancreas.[67]

The dorsal vagal complex and different hypothalamic regions are important constituents of a brain network associated with vagus nerve-mediated regulation of peripheral metabolic functions (Figure 3). Interconnections between these and other forebrain regions also have a role in the integration of afferent and efferent signalling in terms of the control of metabolic homeostasis and the regulation of hedonic and motivational aspects of feeding behaviour (Figure 3).[68–70]

Postprandial inflammation

Serum lipopolysaccharide levels in healthy individuals are increased after consuming high-fat, or high-fat and high-carbohydrate meals.[71,72] Endotoxaemia following high-fat diet ingestion or lipid intake has been implicated in postprandial inflammation as plasma levels of IL-6 and the expression of TLR4, TLR2 and SOCS3 in mononuclear cells increases.[72–74] Postprandial endotoxaemia and inflammation are transient, however, and data from several studies suggest that the vagus nerve and the inflammatory reflex might have a role in suppressing postprandial inflammation. Afferent vagus nerve signalling has a primary role in informing the brain about the presence of peripheral inflammation during exposure to low levels of proinflammatory stimuli.[28] TLR4, which is expressed by vagus nerve afferents,[30,31] provides a molecular sensory component for neural detection of lipopolysaccharide in postprandial inflammation.

Dietary lipid infusion causes the release of chole-cystokinin, which acts both via vagus nerve afferents and directly in the brain to trigger activation of efferent vagus nerve signalling, which in turn suppresses the release of proinflammatory cytokines.[75] Activation of vagus nerve afferents by cholecystokinin or leptin is potentiated by IL-1β.[76,77] Furthermore, in rats, truncal vagotomy is associated with increased bacterial trans location across the intestinal mucosa,[78] which suggests a tonic vagus nerve control of intestinal permeability and postprandial endotoxaemia.

The inflammatory reflex and obesity

Disruption in metabolic and immune homeostasis in obesity is associated with hyperglycaemia, insulin resistance, dyslipidaemia and hypertension. This cluster of conditions characterizes the metabolic syndrome.[79] Moreover, levels of proinflammatory cytokines and acute-phase proteins such as CRP are increased in individuals with obesity, indicating chronic inflammation.[11,80] This inflammatory state is considered to be an essential pathophysiological constituent in obesity, underlying its adverse consequences and linking it to the other components of the metabolic syndrome.[80,81] Several lines of evidence indicate that vagus nerve activity could be impaired in obesity, and enhancing cholinergic signalling within the inflammatory reflex can suppress obesity-associated inflammation and its adverse implications.

Inflammation and obesity pathogenesis

The characteristic inflammatory state in obesity has been extensively discussed elsewhere.[11,13,80,82,83] White adipose tissue in individuals with obesity is expanded and infiltrated with T cells, bone-marrow-derived macrophages, mast cells and B cells.[83] As such, this tissue becomes a major source of proinflammatory factors.[80,83–86] Adipocyte enlargement as a result of increased lipid deposition leads to metabolic alterations associated with dysregulated secretion of adipokines, including leptin, resistin, adiponectin and visfatin.[80,84] Additionally, cell death and hypoxia occur in adipose tissue, leading to increased macrophage recruitment and generation of reactive oxygen species, which promote inflammation.[85,87] Increased production and release of TNF, IL-6, CCL2 and other proinflammatory mediators from adipocytes, as well as from activated M1 macrophages and

other immune cells, also drives inflammation.[80,82,84,85] In addition to adipose tissue, activation of inflammatory pathways also occurs in the liver, skeletal muscle and brain.[12,82]

Adipocyte and macrophage expression of TLR4 and TLR2 provides a mechanism for transforming metabolic overload (high levels of saturated free fatty acids and glucose) into proinflammatory responses, as free fatty acids and glucose can be ligands for TLRs. Subsequent intracellular signalling mediated through JNK, IKK and PKR leads to stimulation of the transcription factors NF-κB and AP-1, and increased production of pro-inflammatory mediators.[82,84,85,88] Metabolic overload also results in endoplasmic reticulum stress, which is associated with JNK and IKK signalling and generation of reactive oxygen species, triggering inflammation.[13,82] In addition, activation of macrophages and T cells in the adipose tissue of patients with obesity can be mediated by inflammasomes.[89] Pancreatic islet inflammation, as occurs in patients with type 2 diabetes mellitus, can also be mediated by these protein complexes.[90]

The intestinal microflora also contribute to the development of inflammation in individuals with obesity.[91,92] Chronic ingestion of a high-fat diet increases the proportion of lipopolysaccharide-containing microbiota in the gut and this increase is associated with low-grade metabolic endotoxaemia in both mice and humans.[92,93] In mice, mimicking metabolic endotoxaemia by chronic administration of a low dose of lipopoly-saccharide results in inflammation, increased adiposity, weight gain and metabolic complications.[93] These changes result from activation of CD14–TLR4 signalling, which triggers increased production of proinflammatory cytokines by adipocytes and macrophages.[85,93]

The role of specific cytokines and adipokines in mediating obesity-associated complications (Table 1) has been the subject of several reviews.[83,84,88,94–96] Inflammation is causally linked with impaired insulin signalling in peripheral target tissues and insulin resistance, a major complication in obesity and type 2 diabetes mellitus.[12,84,85,95] Activation of inflammatory pathways in the brain interferes with insulin and leptin signalling in the brain and also contributes to insulin resistance.[82,97] Increased lipolysis in insulin-resistant adipose tissue leads to enhanced release of free fatty acids, which stimulate inflammatory responses and reinforce insulin resistance. Proinflammatory cytokines and adipokines can inhibit insulin signalling by targeting IRS proteins or the insulin receptor. Specifically, TNF induces inhibition of insulin-activated tyrosine phosphorylation of the insulin receptor and serine phosphorylation of IRS-1, which leads to inactivation of insulin signalling.[85,95] TNF and other proinflammatory cytokines act in a paracrine manner to stimulate JNK, IKK and possibly other kinases, which mediate serine phosphorylation of IRS proteins.[82] Additionally, proinflammatory cytokines such as TNF, IL-6 and the adipokine resistin induce the expression of SOCS proteins, which block insulin signalling through inhibition of insulin receptor tyrosine kinase activity or IRS-1 ubiquitination and degradation.[95,98] The net result is a negative influence of proinflammatory cytokines on insulin signalling.

Vagus nerve signalling in obesity

Obesity and obesity-related type 2 diabetes mellitus are associated with attenuation of the afferent and efferent vagus nerve signalling that is implicated in metabolic regulation. Obesity in rodents fed a high-fat diet results in leptin resistance (indicated by decreased

leptin-mediated STAT3 phosphorylation) in afferent vagus neurons.[31] This decrease occurs in parallel with an increase in expression of SOCS3 (an inhibitor of STAT3 phosphorylation) in nodose ganglia.[31] SOCS3 expression is also induced by lipopolysaccharide. Consequently, endotoxaemia, resulting from impaired gut permeability,[99] could also contribute to leptin resistance in vagus nerve afferents through activation of TLR4.[31] In rodents, the progression of obesity-related type 2 diabetes mellitus is associated with attenuation of glucose-induced afferent vagus nerve signalling.[100]

Experimental evidence indicates that vagus nerve signalling regulating hepatic glucose production, a major determinant of blood glucose levels, is decreased in obesity. For instance, a high-fat diet causes impairment of a cholecystokinin-triggered vagus nerve reflex circuit that suppresses hepatic glucose production.[57] Additionally, the fatty acid-triggered hypothalamic suppression of hepaticglucose production, which is vagus nerve-mediated,[101] is attenuated by voluntary overeating.[102] This attenuation has been suggested to contribute to the rapid onset of weight gain and hepatic insulin resistance,[102] and has been postulated as a feature of obesity-related type 2 diabetes mellitus.[101]

Insulin resistance in sucrose-fed rats is associated with impaired muscarinic receptor-mediated hepatic vagus nerve signalling,[103] as well as impaired baroreflex sensitivity resulting from decreased vagus nerve activity[104] Vagus nerve cholinergic and muscarinic receptor-dependent signalling controls glucose-stimulated insulin secretion by pancreatic β cells, and mice with selective deficiency of the M3 muscarinic acetylcholine receptor in pancreatic β cells have suppressed insulin secretion and impaired glucose tolerance.[105] Furthermore, a high-fat diet fails to induce glucose intolerance and hyperglycaemia in transgenic mice with selective overexpression of the M3 muscarinic receptor on pancreatic β cells.[105]

Autonomic dysfunction and diminished vagus nerve activity occur frequently in individuals with obesity and type 2 diabetes mellitus. [14–16] A 15-year follow-up study has revealed a strong relationship between autonomic dysfunction and insufficient vagus nerve activity (revealed by impaired heart rate recovery following exercise cessation), impaired glucose homeostasis and development of type 2 diabetes mellitus.[17] Together, these preclinical and clinical findings support the hypothesis that diminished vagus nerve signalling in obesity could lead to enhanced inflammation and metabolic complications.

Cholinergic alleviation of obesity

Targeting cholinergic mechanisms in the inflammatory reflex using α7nAChR agonists or a centrally-acting acetylcholinesterase inhibitor could alleviate in flammation and metabolic complications in obesity.

Attenuation of inflammation—Murine adipocytes express α7nAChR, and treatment with the receptor agonist nicotine suppresses adipocyte TNF levels.[19,106] This receptor is also expressed in human adipocytes and has a role in suppressing proinflammatory gene expression.[107] Oral administration of the selective α7nAChR agonist TC-7020 to genetically obese Lepr$^{db/db}$ mice (which lack functional leptin receptors) significantly suppresses serum TNF levels. This effect is abolished by co-administration of the selective α7nAChR

antagonist methyllycaconitine.[18] Administration of a selective α4/α2nAChR agonist failed to suppress serum TNF levels in Lepr$^{db/db}$mice, which indicates the α7nAChR-specific nature of this effect[18] Administration of nicotine to Lepr$^{db/db}$ mice and mice with high-fat-diet-induced obesity—in doses that did not affect food intake and body weight—significantly ameliorated inflammation in adipose tissue and the systemic proinflammatory state that is characteristic of both groups of mice.[19] Nicotine administration significantly reduced levels of TNF in serum and abdominal adipose tissue, and decreased adipose tissue levels of *TNF*, *IL-6* and *IL-1*β mRNAs. Nicotine also inhibited adipose tissue expression of *CCL2* and the macrophage marker *F4/80*, indicating that macrophage infiltration and inflammation were both suppressed.[19]

Mice lacking α7nAChR that were fed a high-fat diet had greater M1 macrophage infiltration and higher expression of TNF and CCL2 in adipose tissue than was observed in wild-type mice.[19] Additionally, expression of proinflammatory genes following exposure to free fatty acids or TNF was higher in macrophages from mice lacking α7nAChR than in macrophages from wild-type mice.[19] Nicotine treatment failed to suppress this increased expression of proinflammatory genes in macro-phages from mice lacking the receptor.[19] These results provide a mechanistic link between the inflammatory reflex and signalling through α7nAChR in the inhibition of obesity-associated inflammation.

One pharmacological strategy to activate the efferent cholinergic arm of the inflammatory reflex is administration of the centrally-acting acetylcholinesterase inhibitor galantamine.[34] This compound enhances cholinergic muscarinic receptor-mediated signalling in the brain and stimulates efferent vagus nerve activity.[34,108] Galantamine treatment of mice with high-fat-diet-induced obesity and hyperglycaemia suppressed plasma IL-6, CCL2, leptin and resistin levels.[20] Importantly, galantamine treatment of obese mice lowered plasma IL-6 and CCL2 expression to levels detected in lean control mice.[20] Collectively, these findings highlight that activation of components of the inflammatory reflex results in inhibition of obesity-associated inflammation.

Alleviation of metabolic complications—Nicotine administration to mice fed a high-fat diet significantly improves glucose homeostasis and insulin signalling by restoring tyrosine phosphorylation of the insulin receptor and IRS-1 in skeletal muscle, adipose tissue and liver. [19] Administration of the α7nAChR agonist TC-7020 to Lepr$^{db/db}$mice reduces weight gain, food intake and levels of blood glucose, HbA_{1c} and triglycerides.[18] When this treatment is combined with administration of the selective α7nAChR antagonist methyllycaconitine, these effects are abolished.[18] In contrast to the effects of TC-7020 administration, treatment with a selective agonist targeting α4/β2nAChR, which is predominantly expressed on brain neurons, suppresses only weight gain and food intake.[18] Administration of an inhibitor of JAK2 counters the effects of TC-7020 treatment on body weight, food intake and blood glucose levels, thus indicating a mechanistic link between α7nAChR and JAK2-mediated signalling. Mice lacking α7nAChR that are fed a high-fat diet have greater impairment in glucose homeostasis, insulin sensitivity, and insulin signalling in muscle and liver than do wild-type mice fed the same diet.[19] In addition, the expression of human adipocyte α7nAChR (which suppresses proinflammatory responses) is significantly decreased in individuals with obesity; however, weight loss partially restores

expression of α7nAChR.[107] These findings further suggest a critical role for α7nAChR in maintaining metabolic homeostasis.

Galantamine treatment of mice with high-fat-diet-induced obesity reduces body weight and food intake, and selectively suppresses abdominal adiposity.[20] Galantamine also lowers fasting blood glucose, plasma insulin and cholesterol levels, improves glucose homeostasis and insulin resistance, and decreases serum alanine transaminase levels, liver weight and hepatosteatosis in these mice.[20] The role of brain and peripheral cholinergic activation by galantamine (acting as an acetylcholinesterase inhibitor and a positive allosteric modulator of nicotinic receptors, including α7nAChRs)[109] in mediating these beneficial metabolic alterations remains to be further elucidated.

Cholinergic signalling, including via α7nAChR-mediated mechanisms in brain regions such as the lateral hypothalamus, is implicated in the complex regulation of appetite and feeding behaviour.[110] Suppression of food intake following nicotine administration is thought to involve activation of α3/β4nAChR in pro-opiomelanocortin neurons of the hypothalamic arcuate nucleus, which induces activation of melanocortin 4 receptors in the hypothalamic paraventricular nucleus.[111] Thus, several cholinergic receptors, including α7nAChR, α4/β2nAChR and α3/β4nAChR, have been implicated in suppressing food intake and weight gain. However, α7nAChR is unique in having been specifically linked to controlling obesity-associated metabolic complications through suppression of inflammation.

Therapeutic implications

Obesity is directly linked with the epidemic of type 2 diabetes mellitus and cardiovascular disease.[112,113] Although weight loss through increased physical activity and dietary alterations can be beneficial in counteracting obesity and its adverse consequences,[81] these lifestyle modifications are difficult to implement and can be problematic to sustain. Permanent weight loss and attenuation of dyslipidaemia and type 2 diabetes mellitus can be achieved through bariatric surgery, but these operations are associated with considerable risks and are currently recommended only for individuals with morbid obesity. Current pharmacological options for treatment of the metabolic syndrome are limited to targeting its individual components separately and, therefore, multiple medications are required.[81,114] Accordingly, an unmet need remains for new treatments for the metabolic syndrome and type 2 diabetes mellitus, especially for drugs that target common steps in their pathogenesis.

The chronic inflammatory state associated with obesity is one such common step that could be targeted. Some anti-inflammatory approaches have already been explored in the treatment of obesity-linked disorders in preclinical and clinical scenarios.[82,95] For example, patients with type 2 diabetes mellitus who were treated with a recombinant human IL-1 receptor antagonist (anakinra) experienced reductions in levels of IL-6 and CRP. Additionally, HbA_{1c} levels in these patients were reduced and their pancreatic β-cell secretory function improved.[115] Administration of salicylate—a known IKK inhibitor in rodents, which propagates proinflammatory signals—significantly improved glucose homeostasis, reduced free fatty acid levels and increased adiponectin levels in patients with type 2 diabetes mellitus.[116]

The importance of the vagus nerve in the regulation of metabolic and immune homeostasis and the efficacy of selective cholinergic modalities in alleviation of inflammation and metabolic complications indicate that specific drugs or devices that stimulate neural circuits directly to activate the inflammatory reflex could be used in the treatment of obesity-related complications (Figure 4).

Pharmacological approaches

The efficacy of galantamine in suppressing inflammation, insulin resistance and development of fatty liver in preclinical settings provides a rationale for further clinical studies.[20] Galantamine is approved by the FDA as a treatment for Alzheimer disease, and this drug has also been used in Europe for decades in the clinical management of myasthenia gravis as well as other diseases. The availability of data related to the previous clinical use of galantamine might facilitate its utilization in obesity-driven disorders, particularly in subgroups of individuals who have limited capacity to benefit from lifestyle interventions, such as elderly people. The efficacy of galantamine in alleviating liver damage and inhibiting hepatosteatosis in rodents with obesity is a rationale for clinical studies to investigate the utility of this drug in the treatment of fatty liver diseases (Figure 4), such as nonalcoholic steato-hepatitis, which is considered to be the hepatic manifestation of the metabolic syndrome. Current treatments for nonalcoholic steatohepatitis are not efficient and the disease can progress to cirrhosis and liver cancer—new therapies are clearly needed.[117]

The preclinical efficacy of nAChR agonists in obesity models identify nAChRs, including α7nAChR, as molecular targets for drug development for the treatment of the metabolic syndrome and type 2 diabetes mellitus (Figure 4). Several α7nAChR agonists, including TC-7020 and GTS-21, have proven anti-inflammatory properties and the latter had good tolerability in human volunteers.[118] Currently, a phase II clinical trial of the selective α7nAChR agonist TC-6987 is ongoing in patients with type 2 diabetes mellitus.[119]

Obesity and the metabolic syndrome are closely associated with atherosclerosis and impaired cardiovascular regulation. Donepezil, another centrally acting and FDA-approved acetylcholinesterase inhibitor, suppresses CCL2 and TNF expression, attenuates atherosclerosis and improves long-term survival following chronic heart failure in rodents.[120,121] These findings suggest that centrally acting acetylcholinesterase inhibitors could potentially be developed for the treatment of atherosclerosis.

Efferent vagus nerve-derived cholinergic activation of pancreatic insulin secretion is well documented. In addition, human pancreatic α cells secrete acetylcholine, which might act through a M3 muscarinic receptor-mediated mechanism as a paracrine stimulator of glucose-induced β-cell insulin release.[122] An interesting consideration is the hypothesis that this local acetyl-choline release in the pancreas could also exert a regulatory influence on immune cells. Future studies might test the capacity of M3 muscarinic receptor agonists to promote pancreatic insulin secretion and of α7nAChR agonists to suppress pancreatic inflammation in patients with type 2 diabetes mellitus (Figure 4).

Patients taking antipsychotic medication, particularly clozapine or olanzapine, frequently develop obesity, insulin resistance and other metabolic complications. These adverse effects

represent a challenging clinical problem.[123] Adipose tissue inflammation could contribute to the metabolic derangements associated with antipsychotic treatment, as chronic administration of olanzapine to rodents even at a dose that does not induce significant body weight gain results in enhanced adiposity and increased adipose tissue macrophage infiltration and TNF expression.[124] Furthermore, the adverse metabolic effects of antipsychotic treatments are postulated to be related to their antagonism of peripheral and central M3 muscarinic receptors and their consequent suppression of positive cholinergic effects on insulin signalling and glucose homeostasis.[125,126] Using galantamine treatment to alleviate the metabolic syndrome in patients taking antipsychotics is an interesting possibility to explore. Galantamine and other centrally-acting acetylcholinesterase inhibitors have already been tested in the context of schizophrenia and shown to ameliorate selective cognitive deficits in patients with the disease.[127] Intriguingly, progression of type 2 diabetes mellitus often correlates with cognitive deterioration, and new therapeutic agents for type 2 diabetes mellitus that prevent or reduce this decline in cognitive function would be advantageous.[128] Treatment of patients with type 2 diabetes mellitus with galantamine, which has proven efficacy in ameliorating cognitive deficiency, could represent a promising intervention targeting this pathophysiological relationship.[127]

Devices for vagus nerve stimulation

Vagus nerve activity is decreased in chronic inflammatory conditions, including obesity. Weight loss resulting from increased physical activity, dieting or bariatric surgery is accompanied by reductions in levels of inflammatory markers, amelioration of metabolic complications and an increase in the vagus nerve-activity component of heart rate variability.[15,129] A role for vagus nerve signalling in achieving and maintaining weight loss after bariatric surgery has also been reported.[130] In view of these findings and the importance of the vagus nerve in immune and metabolic regulation, restoring or augmenting vagus nerve activity could attenuate inflammation and other conditions associated with obesity (Figure 4).

Stimulation of the vagus nerve using surgically implanted devices is in clinical use for epilepsy and depression and, as a result, the safety profile for this approach is well described.[131] In patients with obesity and treatment-resistant depression, vagus nerve stimulation is associated with significant weight loss, which was positively correlated with the degree of obesity.[132] These clinical findings are in agreement with those of several studies in obese rodents and minipigs, in which vagus nerve stimulation suppressed food intake and weight gain.[133,134] The devices used in these studies predominantly stimulated afferent vagus nerve fibres; however, afferent and efferent vagus nerve signals converge within the dorsal vagal complex and higher brain regions, which also provide neurophysiological circuitry to activate efferent vagus nerve activity that regulates metabolic homeostasis and immune function. Thus, stimulation of afferent and efferent vagus nerve signalling has the potential for controlling the inflammatory state in obesity and re storing metabolic regulation and insulin signalling (Figure 4).

Unpublished data from our group indicate that vagus nerve stimulation suppresses insulin resistance in rodents. Vagus nerve activities regulating hepatic glucose metabolism and

cardiac function are both impaired in obesity, which suggests that augmentation of efferent vagus nerve activity could be beneficial in patients with hyper glycaemia, insulin resistance and cardiovascular diseases (Figure 4). Vagus nerve stimulation is also being studied as a method to suppress cardiac inflammation in patients with heart failure.[135] Additionally, this approach could be studied in patients with obesity-associated disorders, such as the metabolic syndrome and type 2 diabetes mellitus. Carefully designed clinical studies are needed to assess the potential for treatment with selective cholinergic agonists or neurostimulating devices in such patients.

Conclusions

The inflammatory reflex mediated by the vagus nerve has been successfully exploited therapeutically in preclinical models of diseases with aetiologies characterized by excessive inflammatory responses. Insufficient efferent vagus nerve cholinergic output might have a causative role in the dysfunctional immune and metabolic regulation observed in obesity, as selective activation of the efferent cholinergic arm of the inflammatory reflex attenuates both inflammation and metabolic derangements. Although cholinergic suppression of inflammation can contribute specifically to alleviating metabolic complications, direct cholinergic effects on metabolic pathways could also have a role in alleviating symptoms associated with the metabolic syndrome and type 2 diabetes mellitus. These complex interactions and the contribution of central and peripheral mechanisms in this regulation are topics of ongoing study. Additionally, intracellular mechanisms by which cholinergic signals control obesity-associated inflammation and modulate insulin signalling are under investigation. α7nAChR agonists, centrally acting acetylcholinesterase inhibitors and direct electrical stimulation of the vagus nerve offer potential therapeutic strategies for treating obesity, the metabolic syndrome, type 2 diabetes mellitus and other disorders associated with obesity. The use of cholinergic modalities in combination with existing or new therapeutic approaches to target neural, endocrine and immune functions for therapeutic benefit in patients with obesity-related disorders should also be considered.

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Key points

- The inflammatory reflex is a physiological mechanism through which the vagus nerve regulates immune function and inhibits excessive proinflammatory cytokine production
- Vagus nerve signalling has an important role in the regulation of feeding behaviour and metabolic homeostasis
- Disruption of metabolic and immune regulation in obesity results in inflammation, which mediates insulin resistance and the development of type 2 diabetes mellitus as well as other debilitating and life-threatening conditions
- Activation of cholinergic signalling in the efferent arm of the inflammatory reflex alleviates obesity-associated inflammation and metabolic derangements
- The inflammatory reflex can potentially be exploited for treatment of the metabolic syndrome, type 2 diabetes mellitus and other obesity-driven disorders

Review criteria

Original research papers and reviews (in English) were considered for inclusion in this manuscript on the basis of the authors' knowledge of the field and the results of PubMed and Google Scholar searches using the following keywords: "vagus", "feeding behaviour", "metabolism", "obesity", "inflammation", "metabolic syndrome", "type 2 diabetes mellitus", and "cholinergic", alone and in combination. The authors have also reviewed the reference lists of key manuscripts to identify additional relevant papers. Paper selection was limited to the past 35 years (1967–2012).

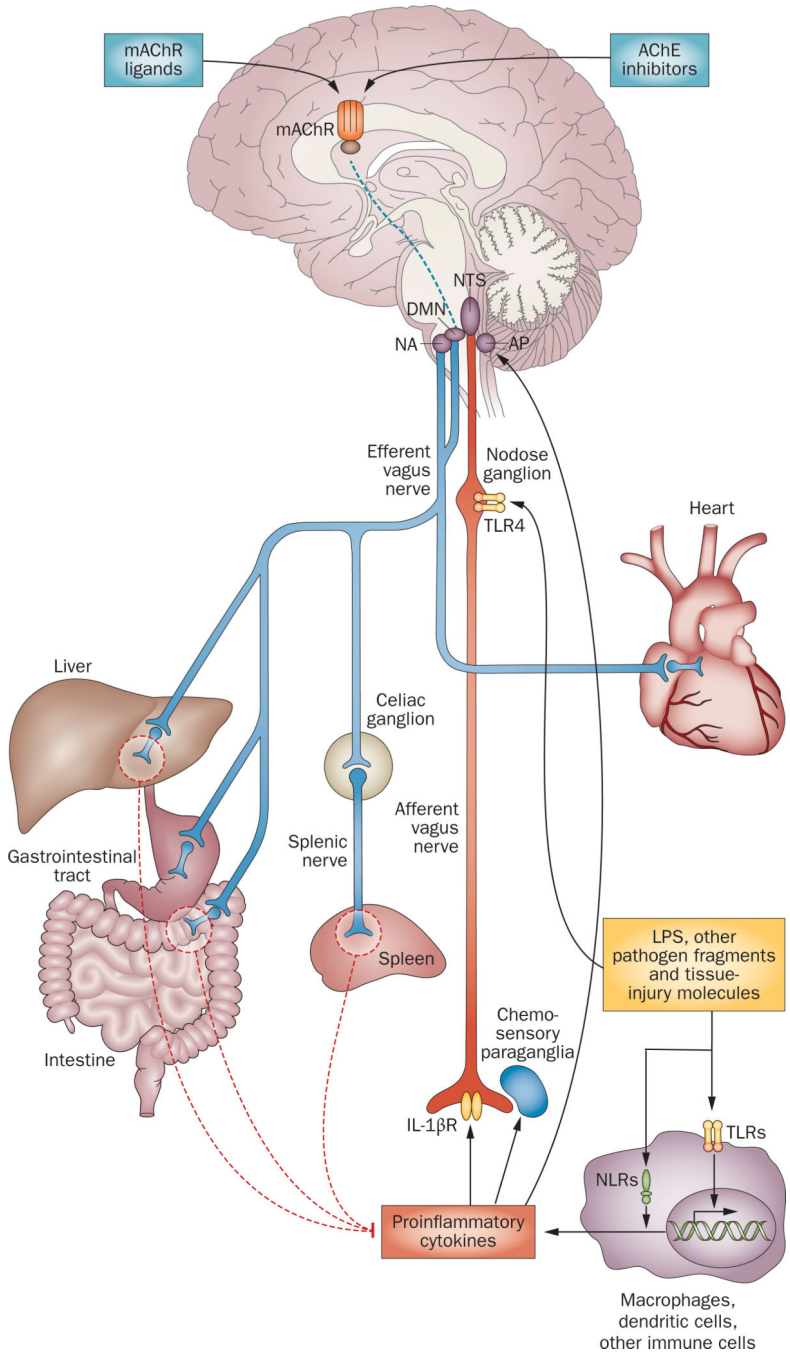

Figure 1.

The functional anatomy of the inflammatory reflex. Inflammatory mediators, such as cytokines, are released by activated macrophages and other immune cells when TLRs and NLRs are activated upon immune challenge. These mediators are detected by sensory components of the afferent arm of the inflammatory reflex (red). Neuronal interconnections between the NTS, A P, DMN, NA, and higher forebrain regions (not shown) integrate afferent signalling and efferent vagus nerve-mediated immunoregulatory output. Efferent vagus nerve cholinergic output to the spleen, liver and gastrointestinal tract (blue) regulates

immune activation and suppresses proinflammatory cytokine release (dotted red lines). This efferent cholinergic arm of the inflammatory reflex can be activated in the brain through mAChR-mediated mechanisms triggered by mAChR ligands and AChE inhibitors, such as galantamine. Abbreviations: AChE, acetylcholinesterase; A P, 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 solitarius; TLR4, toll-like receptor 4.

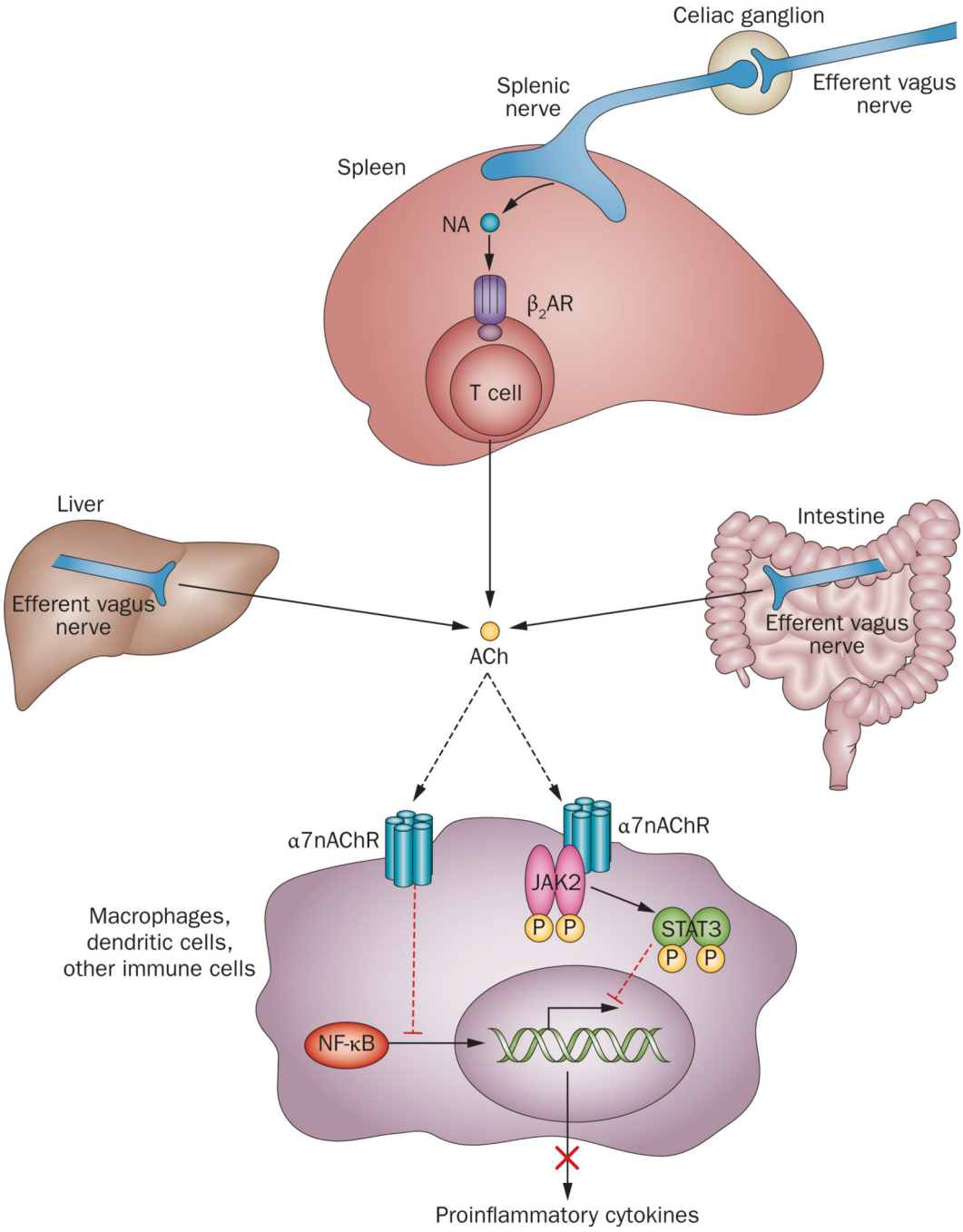

Figure 2.

Molecular mechanisms of cholinergic control of inflammation. Efferent vagus nerve activity is translated into catecholamine-mediated activation of T-cell-derived acetylcholine release in the spleen and into direct acetylcholine release from efferent vagus nerve endings in other organs. Inhibition of NF-κB nuclear translocation and activation of a JAK2-STAT3-mediated signalling cascade in macrophages and other immune cells are implicated in cholinergic α7nAChR-mediated control of proinflammatory cytokine production. Abbreviations: ACh, acetylcholine; β_2AR, β_2 adrenergic receptor; JAK2, Janus kinase 2;

α7nAChR, α 7 nicotinic acetylcholine receptor; NA, noradrenaline; NF-κB, nuclear factor κB; STAT3, signal transducer and activator of transcription 3.

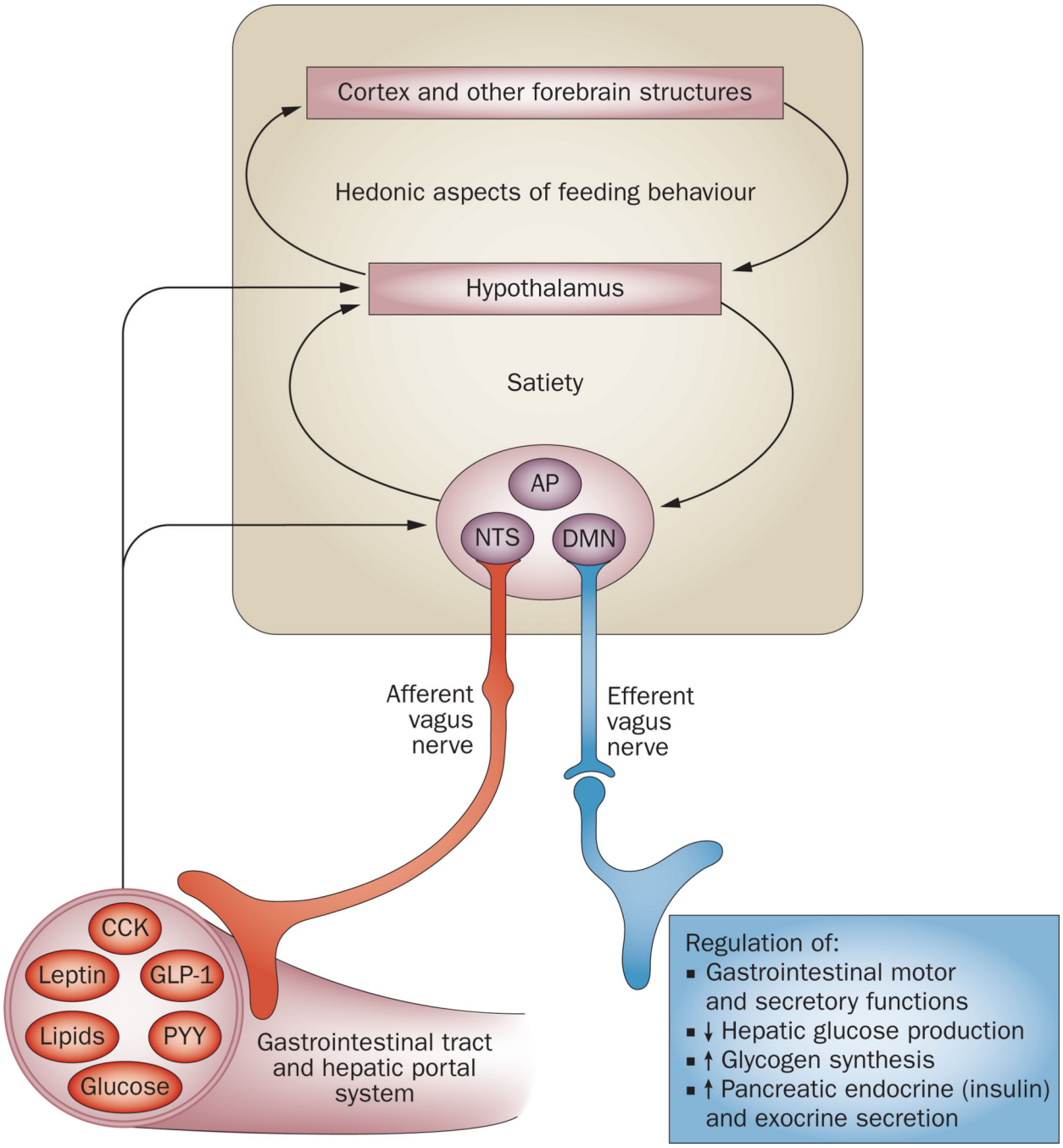

Figure 3.

The role of the vagus nerve in metabolic regulation. Gastrointestinal and hepatic vagus nerve afferents (red) communicate alterations in peripheral levels of micronutrients and metabolic molecules to the brain. Neural interaction between the interconnected NTS, DMN and A P, within the dorsal vagal complex, and reciprocal projections between this brainstem region and several hypothalamic areas (arcuate and paraventricular nuclei and mediobasal and lateral areas), underlie brain integration of visceral information and the modulation of efferent motor vagus nerve output, leading to regulation of metabolic homeostasis. Efferent

vagus nerve signalling (blue) can be triggered by sensing metabolic alterations in the brainstem and the hypothalamus. Complex communication between hypothalamic nuclei and other forebrain structures (such as the insula and premotor cortex, amygdala, nucleus accumbens, parabrachial nucleus and thalamus) mediate hedonic, motivational and rewarding aspects of feeding behaviour and their interaction with vagus nerve-mediated homeostatic mechanisms. Abbreviations: A P, area postrema; CCK, cholecystokinin; DMN, dorsal motor nucleus of the vagus nerve; GLP-1, glucagon-like peptide-1; NA, nucleus ambiguus; NTS, nucleus tractus solitarius; PYY, peptide YY.

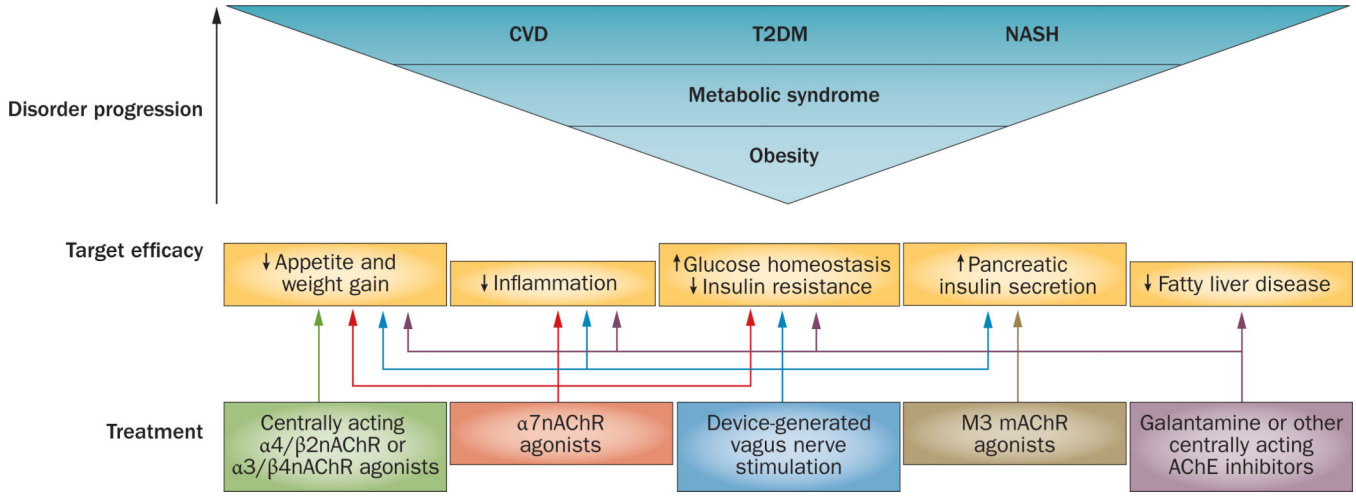

Figure 4.

Possible therapies based on cholinergic-based approaches for the treatment of obesity-driven disorders. Obesity progression and the metabolic syndrome are closely associated with debilitating diseases, including T2DM, CVD and NASH. Dysregulated immune and metabolic homeostasis is associated with inflammation underlying obesity-related disease pathogenesis, which mediates insulin resistance and other complications. Efficacy of cholinergic agents in activating regulatory mechanisms in the inflammatory reflex in animals indicates a rationale for developing novel treatments for humans. Cholinergic therapeutics and devices for vagus nerve stimulation could selectively control the complex interplay between immune and metabolic pathways. Abbreviations: CVD, cardiovascular disease; M3 mAChR, M3 muscarinic acetylcholine receptor; α7nAChR, α7 nicotinic acetylcholine receptor; α4/β2nAChR, α4/β2 nicotinic acetylcholine receptor; α3/β4nAChR, α3/β4 nicotinic acetylcholine receptor; NASH, nonalcoholic steatohepatitis; T2DM, type 2 diabetes mellitus; VNS, vagus nerve stimulation.

Table 1

Contributions of key adipokines and cytokines to obesity-related disease

Cytokines and adipokines	Cell source	Role in obesity pathogenesis
Leptin (adipokine)	Adipocytes Gastric mucosa cells	Regulates food intake and energy balance[136]* Increased release in parallel with weight gain* Activates anorexigenic pathways in the hypothalamic arcuate nucleus and other brain regions[69,136]* Acts through leptin receptors on vagus nerve afferents and on brain neurons and via intracellular JAK2–STAT3-mediated signalling[31,69,136]* Positive regulator of glucose homeostasis and insulin sensitivity in muscle and liver[136]* Leptin resistance in obesity associated with intracellular SOCS3-mediated suppression of STAT3 phosphorylation[98,136] Hyperleptinaemia has proinfammatory effects in obesity[84,137,138]
TNF (proinfammatory cytokine)	Macrophages Other immune cells Adipocytes	Major contributor to local and systemic insulin resistance[80,82,85,139] Increased expression in adipose tissue[139] Stimulates resistin expression in human macrophages and suppresses adiponectin expression in human adipocytes[83,88] Implicated in pathogenesis of fatty liver disease[94]
IL-6 (proinfammatory cytokine)	Macrophages Other immune cells Adipocytes	Mediates local and systemic insulin resistance[80,82,85] Suppresses adiponectin expression in human adipocytes Implicated in pathogenesis of fatty liver disease[94] Inducer of acute-phase proteins, including C-reactive protein in liver, which is linked to an increased risk of cardiovascular disease[80,96,140] Increased expression in adipose tissue and increased circulating levels in obesity[13,80]
CCL2 (chemokine)	Adipocytes Endothelial cells Macrophages	Mediates macrophage and other immune cell infltration into adipose tissue[141] Linked to insulin resistance and hepatosteatosis in rodents[141] Increased levels in obesity[85]
Resistin (adipokine)	Adipocytes (mice) Macrophages (humans)	Induces insulin resistance in mice, and might have a similar role in humans[85,142] Stimulates TNF and IL-6 production[84] Increased circulating and adipose tissue levels in rodent models of obesity[11]
Adiponectin (adipokine)	Adipocytes	Anti-infammatory and insulin-sensitizing functions[95] Suppresses macrophage activation and proinfammatory cytokine release[84] Inversely correlated with insulin resistance, type 2 diabetes mellitus, fatty liver disease and atherosclerosis[84,94] Decreased circulating levels in obesity[84]

* Physiological functions under normal conditions.

Abbreviations: CCL2, CC-motif chemokine 2 (also known as monocyte chemotactic protein 1 or MCP-1); TNF, tumour necrosis factor.