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Spikeotherapeutics: the Cholinergic Anti-inflammatory Pathway, the Vagus Nerve and Dysautonomia: is Nicotine an answer?

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ABSTRACT

Severe COVID-19 is characterised by immune dysregulation and a highly inflammatory cytokine storm with symptoms of dysautonomia. It has been proposed that this is at least partially a result of dysregulation of the cholinergic anti-inflammatory pathway and that nicotine may be a useful therapeutic intervention. Post Acute COVID Syndrome (also known as 'Long COVID') and Post COVID Vaccination Syndrome are being increasingly recognised and are also characterised by prominent inflammatory markers and dysautonomia. A narrative review of the literature found the actions of the cholinergic system are profoundly anti-inflammatory and act via the α7nACh receptors. The spike protein has been demonstrated to contain an amino acid sequence near the Receptor Binding Domain that has homology with a-1 neurotoxins and is demonstrated in silico to interact with α7nACh, impairing the cholinergic anti-inflammatory system. Nicotine has been proposed as having a possible therapeutic role in mitigating this effect. However, its place is limited due to widespread nicotine actions and significant adverse effects in chronic dosing. Other potential therapeutic interventions addressing background chronic sympathetic nervous system activation and dysautonomia are therefore considered for modulation of the cholinergic system in the management of Post Acute COVID Syndrome/ Post Acute COVID Vaccination Syndrome in the stead of nicotine.

Keywords: Cholinergic Anti-inflammatory Pathway; nicotine ; sympathetic activation ; dysautonomia; spike protein ; a-1 neurotoxin; spikeopathy; anticholinesterases; vagal nerve stimulation

1. Introduction

The aims and scope of this review are to explore the pathogenic effects of the SARS-CoV-2 spike protein on the cholinergic system, effects that can be described by the term 'Spikeopathy'[1]. Nicotine, through its action on the cholinergic system has been proposed as a therapeutic but has significant adverse effects over time and alternative methods are therefore considered.

Severe COVID-19 is characterised by immune dysregulation, a highly inflammatory 'cytokine storm'[2] and dysautonomia[3]. Although smoking is a well-known risk factor for respiratory infections and thus COVID-19 illness, an unusually low prevalence of smoking has been observed among hospitalized COVID-19 patients. It has thus been hypothesised that nicotine may be protective against COVID-19 due to its anti-inflammatory properties and to a potential direct interaction between SARS-CoV-2 and nicotinic acetylcholine receptors[4].

The nicotinic acetyl choline receptors (nAChR) were first discovered in the context of action of nicotine[5], and the discovery of acetylcholine as the signaling molecule followed later[6]. Thereafter protein α-neurotoxins from snake venom (α-bungarotoxin, α-cobratoxin, etc) were identified as antagonists. The "cholinergic anti-inflammatory pathway" (CAP) was thus discovered and is an important regulator of the inflammatory response[7]. Its effects are mediated mainly by the vagus nerve and by α7 nicotinic acetylcholine receptors (α7nAChRs)[8]. The CAP has now been implicated in spikeopathy as a result of the findings that part of the SARS-CoV-2 spike protein's receptor binding domain (RBD) has homology with the same snake venom α-neurotoxins[9,10], with possible resultant impairment of the nicotinic cholinergic transmission which is necessary for regulation of inflammatory responses as detailed in Parry et al (2023)[11].

The CAP forms a multi-faceted network, with distribution in neuronal and non-neuronal cells, and diverse functions throughout the body[12]. nAChR receptors are ubiquitous in the body: in addition to the nervous system, α7 nAChRs are expressed in non-neuronal cells such as lymphocytes, monocytes, macrophages, dendritic cells, adipocytes, keratinocytes, endothelial cells, platelets and epithelial cells of the intestine and lung. With such widespread distribution, nAChRs appear to be implicated in the pathophysiology of severe COVID-19 via various mechanisms including impairment of the CAP[13]. This same mechanism can explain both the breadth and severity of symptoms experienced in long COVID and in COVID-19 vaccine injuries. The former shows failure to clear spike protein and virus, with uncontrolled immune activation and sequelae[14], and in vaccine injuries, where vaccine mRNA persists[15], recombinant spike protein has been shown to persist for at least 6 months post injection in some 50%, and there is increased load with each subsequent injection[16]. A recent preprint reports persistent circulating vaccine-produced spike proteins at over 700 days since the previous injection[17].

The cholinergic control has several properties favoring a central role in immune homeostasis as compared with the Hypothalamic-Pituitary-Adrenocortical (HPA) axis or the local production of anti-inflammatory cytokines. As neural conduction is rapid, it is capable of providing an instantaneous modulatory input to the region of inflammation and can also modulate its output based on sensory information obtained from various parts of the host. The influence of the CAP is thus fast and integrated with respect to the general status of the host[18].

A current conceptualization for the mechanism of the cytokine storm in the context of severe COVID-19, is that cholinergic dysfunction together with direct inhibition of both peripheral and central nAChR by SARS -CoV-2 spike protein induces hyperinflammation[19] and immunopathogenesis[20]. Proinflammatory cytokines cross the blood -brain-barrier thus inhibiting the central anti-inflammatory nAChR with subsequent neuroinflammation[21] amongst other mechanisms. The neuroinflammation and dysregulated central nAChRs then stimulate a sympathetic discharge and development of an unregulated sympathetic storm[22,23] with dysautonomia[24] which triggers oxidative stress and hyperinflammation by increasing the generation of reactive oxygen species (ROS) and release of pro-inflammatory cytokines[25], while dysregulated nAChRs on immune cells also allows release of proinflammatory cytokines and further the development of a cytokine storm.

As the nAChRs are implicated in spikeopathy, interest has turned to the use of nicotine directly as an agonist to these receptors and its use has been

popularized. Nicotine as a potent parasympathomimetic alkaloid is considered the most addictive and pharmacologically active substance among the over 8000 chemicals present in tobacco products[26,27]. Nicotine is a strong competitive agonist with acetylcholine for the nicotinic AChRs. Activation of the receptors in the central nervous system (CNS) activates dopamine, with reward-pleasure effects[28]. Chronic nicotine use however results in inactivation of the receptors and their downregulation with decreased dopamine release resulting in tolerance, dependance and addiction[29].

Nicotine is therefore not appropriate for high dose or recurrent usage and thus it is necessary to gain a deeper understanding of the CAP, the autonomic nervous system and dysautonomia, and to find alternative methods of intervention. This paper explores such interventions as lifestyle changes, vagus nerve stimulation, nutraceuticals and pharmaceuticals and the underlying biochemical rationale for each. Widely biodistributed, spike protein production leads to myriad pathophysiological disturbances and spikeotherapeutic strategies need to cover the diverse spikeopathy that is mediated via disruption of the CAP and autonomic nervous system.

2. Discussion

3.1 Cholinergic Anti-inflammatory Pathway and the Vagus Nerve

The CAP is innervated by the vagal nerve as part of the parasympathetic nervous system, as distinct from the sympathetic nervous system, and via the vagal nerve, attenuates the systemic inflammatory response[30]. The modulation of inflammatory and immune response is by the CNS through the vagus nerve via bi-directional communication between the immune and nervous systems. IL-1 receptors in afferent vagus nerve fibres into the nucleus tractus solitarius, provide sensory input to the CNS about the inflammatory status in peripheral tissues[31], while systemic inflammation is signaled by cytokines or toxins in the area prostrema, a site where the blood-brain barrier is 'leaky' and specialized neurons are activated[32]. Resultant transmission of efferent signals, predominantly from the dorsal motor nucleus to modulate the inflammatory response, is both direct to provide local responses and via the coeliac ganglion and coeliac nerve to the spleen to provide a systemic response. This neural response is rapid and localized, in comparison to the chemokine and cytokine diffusible anti-inflammatory network, which is slow, non-local, and dependent on concentration gradients[33]. ACh released by the vagal nerve in the celiac mesenteric ganglia activates postsynaptic α7nAChR on adrenergic neurons of the splenic nerve, leading to the release of norepinephrine in the spleen which activates adrenergic receptors on splenic T cells thus stimulating ACh synthesis by splenic T cells interacting with α7nAChR located on adjacent macrophages[34]. ACh released by vagal nerve activation significantly and rapidly inhibits the release of macrophage tumour necrosis factor (TNF), and other proinflammatory cytokines, but not the macrophage secretion of anti-inflammatory cytokine IL-10[35].

Activated via the vagal nerve release of ACh, nAChRs are found in the immune system on T-cells, B-cells, macrophages, monocytes, neutrophils and mast cells and act overall to reduce inflammation, including the reduction of proinflammatory cytokines, such as IL-6, while promoting anti-inflammatory cytokines such as IL-4[36]. The vagus nerve thus plays a critical role in the modulation of innate immune response but also blood pressure control: vagus nerve activity is reduced in response to hyperinflammation and cytokine storm, allowing sympathetic activation of heart rate and blood pressure whereas vagus nerve stimulation inhibits the release of pro-inflammatory cytokines in various inflammatory disorders[37].

Interestingly, loss of parasympathetic tone in general has been noted to predate activation of the sympathetic nervous system, which then promotes both a dysautonomia[38], and exacerbates the inflammatory effects of nAChR blockade, particularly in the nervous system, with microglial and mast cell activation. Psychological stress itself from any cause can activate mast cells[39] and increase microglial reactivity to other challenges and lead to cognitive decline and neuro-inflammatory response.[40]

3.2 Nicotine, n-Acetylcholine Receptors, Inflammation

The cholinergic anti-inflammatory pathway (CAP) and nicotinic receptors are widespread through organ systems and hence impairment of the CAP is complex, as are the effects of exogenous nicotine. A detailed description of this can help to explicate these complexities.

The nAChRs are divided into two different groups of neuronal and non-neuronal receptors. Structurally, this receptor family is a pentamer composed of a combination of α(1–10), β(1–5), ε/γ, and δ subunits[41]. There is a specific neuronal and non-neuronal pentamer combination of subunits distributing throughout the body. Non-neuronal nAChRs are expressed in numerous tissues including lung epithelial, endothelial, fibroblast cells, immune cells and in muscles[42].

Nicotinic acetylcholine receptors (nAChRs) are members of the Cys-loop superfamily of pentameric ligand-gated ion channels[43], which include GABA (A and C), serotonin, and glycine receptors. Currently, alpha (α2-α10) and beta (β2-β4) subunits have been identified in the CNS. Of note, the most commonly expressed nAChR subtype in the CNS (90%) is the α4β2* receptor, characterized by its high-affinity for ACh and nicotinic agonists whereas the α7* nAChR is low-affinity ACh binding [44]. There are significant differences between the two main subtypes: the heteromeric receptors, which are specialized for rapid electrochemical signal transduction, and the homomeric α7 receptors, which apparently are involved in both rapid ionotropic and slower metabotropic signaling[45].

The α7 homo-pentamer is the most studied of these receptors and are distinguished from other nAChRs by unique physiological and pharmacological properties. The α7 subunit is expressed at high levels in the hippocampus and hypothalamus[46] and are the key nAChR in non-neuronal tissues such as cells of the immune system[47] as distinct to synaptic transmission. α7 receptors are not strictly receptors for acetylcholine but respond also to choline[48], the ubiquitous precursor to acetylcholine. The propensity of neuronal α7 nAChRs to rapidly desensitize in the presence of agonists, notably nicotine, is well described, and α7 nAChR agonists frequently demonstrate inverted U-shaped curves in cognitive tasks demonstrating a dose dependent effect and issues with chronic or repeated dosing[49].

Nicotine alters the physiological processes of cells that express nAChRs. It thus has profound systemic effects on many organs including the lungs, kidneys, heart, liver, and gastrointestinal tract, mediated through effects on epithelial, endothelial, and fibroblast cells[50] and altered immune function[51]. Depending on the cell type and combination of the various subunits of nAChRs, nicotine also contributes to releasing growth factors, modification of extracellular matrix, dysregulated growth, and angiogenesis[52].

The effect of nicotine on the immune system is generally beneficial in situations of inflammation. Nicotine is known to decrease the level of pro-inflammatory cytokines, inhibit dendritic cells, and prevent cell apoptosis[53,54]. Nicotine has been well demonstrated to exert an immunosuppressive effect on human dendritic cells, the major 'professional' antigen presenting cells, by reducing phagocytosis and thus antigen uptake, reducing production of proinflammatory cytokines including IL1β, IL10, IL12, and TNF-α and an inhibition of Th1 polarization and IFN-γ production[55]. Dosage has been demonstrated to be important, with pro-apoptotic activity in dendritic cells in high doses of nicotine and anti-apoptotic effect in the lower doses[56].

Similarly, the effects of nicotine on macrophages are generally thought to be anti-inflammatory: however data suggests that nicotine exerts a dual role in macrophages. A study of mycobacterium – infected macrophages demonstrated an inflammatory response to nicotine and differentiation to M1 phenotype, whereas uninfected macrophages expressed anti-inflammatory responses and M2 polarization, in a dose -dependent effect, with increased dosing increasing the proinflammatory effect[57].

In addition, nicotine has immune alteration effects via α7 nAChRs on CD 4+ T cells with skewing of polarization from Th1 and Th17 cells to T reg (FOXP3) and Th2 cells. While both Th1 (IFN-γ and TNF-α) and Th17 (IL17, IL21, and IL22) cytokines are downregulated, the Th2 cytokines (IL4, IL-6, IL-10 and IL-13) are upregulated, partially a result of altered dendritic cell function[58,59]. Nicotine blocks the activation of T cells through the inhibition of cytotoxic T lymphocyte-associated protein 4 (CTLAP4)[60] and induces T cell anergy with impairment of antigen-mediated signal transduction. However, in addition to action via α7nAChRs, nicotine appears to increment Th2 cells via the activation of α4nAChRs in the circulation system, spleen, bone marrow, and thymus[61] which may partially explain the Th2 cell activation.

Nicotine also has effects on neutrophils, via nAChRs and peroxynitrite generation with increased production of IL-8 and stimulation of neutrophil extracellular traps (NETs)[62]. Neutrophils are the

most abundant leukocytes in human circulation and are the primary cells recruited to sites of inflammatory innate immune response to tissue damage and/or infection[63]. Activated neutrophils release NETs in response to a variety of stimuli, with the release of intracellular granule components. The initial positive effects of pathogen control are countered by pro-inflammatory effects and disease enhancement particularly if NET production continues[64].

Mast cells have also been implicated in the spikeopathy-related pathology[65]. Nicotine has been shown to suppress the delayed phase of activated mast cells via α7/α9/α10 nAChRs[66]. Nicotine has also been shown to inhibit the activation of mucosal mast cells via *a*7 nAChRs and induce the expression of cytokines (Th1 and Th2 types)[67]. nAChR agonists also demonstrate a dose dependent effect of either histamine liberation or suppression of IgG activation of mast cells[68].

The effects of nicotine on the immune system are thus widespread and initially anti-inflammatory, which may explain the observed protection of tobacco smokers to COVID-19. However, nicotine may become proinflammatory in higher doses and chronic dosing.

3.3 Nicotine and Neurotransmitters

Since the epidemiological studies during the early 1960s which provided the first evidence of an inverse correlation between smoking and incidence of Parkinson's disease[69], the critical role of nAChRs activation in modulating neuro-immune pathways has been recognized, and nicotine investigated as an effective therapeutic in neurodegeneration and neuroinflammatory diseases. Although nAChRs are widely distributed in different nervous system regions, nicotine neuroprotection in the brain and spinal cord is mainly mediated via α7nAChRs and α4β2[70]. Presynaptic and preterminal nicotinic receptors enhance neurotransmitter release for ACh, dopamine, norepinephrine, serotonin, glutamate, and *gamma*–aminobutyric acid (GABA), postsynaptic nAChRs contribute a small minority of fast excitatory transmission, and nonsynaptic nAChRs modulate many neurotransmitter systems by influencing neuronal excitability. Nicotinic receptors thus have roles in development and synaptic plasticity, and nicotinic mechanisms participate in learning, memory, and attention[71]. These crucial roles in modulating presynaptic, postsynaptic, and extrasynaptic signaling, undergird the involvement of nAChRs in a complex range of CNS disorders including Alzheimer's dementia, Parkinson's disease, schizophrenia, Tourette´s syndrome, anxiety, depression and epilepsy[72] and in the neurological symptomatology of spikeopathy[73].

However, the effects of nicotine in the CNS cannot be equated to the effects of ACh nor the effects of ACh peripherally. The nAChRs of the brain are primarily localized at presynaptic, perisynaptic, or somatic sites and ACh is released in a relatively diffuse manner, where it functions primarily as a modulator of neuronal excitability, subsequently modulating the release of various neurotransmitters, including glutamate, GABA, norepinephrine, ACh itself, and dopamine[74]. Nicotine crosses the blood-brain barrier and so the presentation of nicotine to the receptors is even more diffuse than for ACh, slower by several orders of magnitude, and not rapidly reversed by metabolism. Additionally, nicotine will affect all the many different nAChR subtypes in the brain to varying degrees[75] but has an up to 30-fold higher affinity to α-7nAChRs than ACh)[76].

The conformational dynamics of nicotine binding is also different to that of ACh, resulting in a smaller transient, but more sustained response than ACh[77], with a measurable 'smoldering'[78] steady-state current from α4β2 receptors[79]. When nicotine is delivered slowly, as via a patch or pill, there is relatively little synchronized receptor activation, and, instead, receptors equilibrate between multiple conformational states[80] which predominantly favors desensitization and therefore also decreases receptor responses to endogenous cholinergic stimuli. The α4β2* receptors have been shown to be directly affected by chronic exposure to nicotine and are the key receptors implicated in nicotine dependance[81], a very likely outcome in chronic nicotine usage for any reason.

3.4 Nicotine Adverse Effects

While the effect of nicotine on nAChR may be beneficial initially in terms of controlling inflammation, nicotine also has negative effects on insulin resistance and glucose metabolism. Cigarette smoke contains many other chemicals, but nicotine is the major active ingredient:

smoking increases the risk of Type 2 Diabetes (T2DM) by about 30–40% (women more so than men)[82], intensifies the complications of diabetes, and the combination of smoking and diabetes increases the mortality and mortality rates. Diabetic smokers also have higher levels of cholesterol and blood pressure[83]. Nicotine itself increases the blood sugar level by alteration of energy metabolism[84] and this hyperglycemia inhibits nAChR activity via a negative feedback loop involved in nicotine dependence[85].

Nicotine also carries significant adverse effects for the liver and is recognized as a risk factor and perpetuating factor for Non-Alcoholic Fatty Liver Disease (NAFLD)[86], further exacerbated by a high fat diet[87]. In addition, chronic nicotine exposure upregulates inflammatory cytokines, the development of secondary polycythemia, and blockage of proliferation and activation of apoptosis in lymphocytes[88].

The cardiovascular adverse effects of nicotine are well documented and include acutely raised heart rate and blood pressure and platelet aggregation, contributing to plaque growth and thrombosis[89] and dyslipidemia resulting in a proatherogenic profile[90]. Long term, nicotine also induces endothelial activation and angiogenesis[91], vascular smooth muscle cell proliferation and macrophage activation to pro-inflammatory cytokines[92] further increasing inflammatory plaque, intimal thickening and progression of atherosclerosis.

Of significance with regards to the endotheliitis induced by spike protein[93], nicotine itself can cause endothelial dysfunction, the result of differential gene expression of endothelial cells, growth of atherosclerotic plaques, and angiogenesis[94] leading to inflammation and vascular disorders such as atherosclerosis. Nicotine alters the expression of endothelial genes whose products play major roles in regulating the vascular tone and thrombogenicity, with increased mRNA levels of endothelial nitric oxide synthase, angiotensin-I converting enzyme, tissue-type plasminogen activator, plasminogen activator inhibitor-1, von Willebrand factor, and vascular cell adhesion molecule-1[95], which would increase calcification of plaque.

In addition to direct effects of the nAChR activation, nicotine also indirectly affects the renin-angiotensin system (RAS). It has been shown that nicotine affects RAS homeostasis through upregulation of the expression of angiotensin converting enzyme (ACE), angiotensin II (AngII), and angiotensin receptor type2 (AT2R) with downregulation of the expression of angiotensin converting enzyme-2 (ACE2) and angiotensin (1-7)[96]. ACE2 is the peptidase for the metabolism of the vasoconstrictor angiotensinogen II to the vasodilators angiotensin-(1-7) and angiotensin-(1-9). Thus, reduction of ACE2 resulting from direct spikeopathy and indirect nAChR blockade, in addition to indirect nicotine effects on the RAS, will favor vasoconstriction and the development of ED, oxidative stress, and inflammatory disorders[97].

3.5 Dysautonomia in COVID-19, 'Long COVID'/Post Acute Covid Syndrome and Post Covid Vaccination Syndrome

The theory of the spike protein interaction with n AChR is attractive and supported by amino acid sequence analysis[98] and in silico studies[99], however in vitro experimental competitive ligand-binding competition assays have demonstrated that the mutually exclusive binding of SARS-CoV-2 and cholinergic ligands to the human α-7nAChR is unlikely to be a relevant aspect of the inflammatory processes. In particular, it was found that components of the spike protein had minimal competitive action in displacing α-bungarotoxin from the receptor sites, and that the S1 domain had no measurable effect on α-7nAChR channel function. It would thus appear that the binding of SARS-Cov-2 spike protein to the human α-7nAChRs and thus competition with ACh, choline or nicotine is unlikely to be of significance[100].

The autonomic nervous system (ANS) innervates all organs of the body to maintain biological homeostasis at rest and in response to stress through an intricate network of central and peripheral neurons. The ANS has traditionally been viewed as consisting of the sympathetic nervous system, the parasympathetic nervous system, and the enteric nervous system. However, more recent research has elucidated the role of the neuroendocrine and neuroimmune systems, resulting in a change of nomenclature to "extended autonomic system (EAS)". The recognition of the roles of the sympathetic adrenergic system, with epinephrine as the key effector; the HPA axis; arginine vasopressin; the RAS, with angiotensin II and aldosterone as the main effectors; and the cholinergic anti-inflammatory and sympathetic inflammasome pathways adds to

the complexity. A central autonomic network first delineated as the Chrousos/Gold "stress system" regulates these systems[101].

The term 'Dysautonomia' covers a range of clinical conditions with different characteristics and prognoses, including Reflex Syndromes, Postural Orthostatic Tachycardia Syndrome, Myalgic Encephalitis/Chronic Fatigue Syndrome, Neurogenic Orthostatic Hypotension and Carotid Sinus Hypersensitivity Syndrome. Cardiovascular Autonomic Neuropathy is a currently commonly used term to define dysautonomia with impairment of the sympathetic and/or parasympathetic cardiovascular autonomic nervous system and if present, implies greater severity and worse prognosis in various clinical situations[102]. This cardiovascular autonomic dysfunction, also called CVAD, is a malfunction of the cardiovascular system caused by deranged autonomic control of circulatory homeostasis and might affect one-third of highly symptomatic patients with COVID-19[103].

The SARS-CoV-2 virus is prone to neuroinvading via the lung along the vagus nerve up to the brainstem autonomic nervous centers which are involved in the coupling of cardiovascular and respiratory rhythms. The brainstem autonomic network allows SARS-CoV-2 to trigger a neurogenic switch to hypertension and hypoventilation, which may act in synergy with preexisting dysautonomias and an inflammatory "storm"[104]. Dysautonomia is characteristic in acute severe COVID-19 as a result of several proposed mechanisms including: direct tissue damage, CAP and immune dysregulation, hormonal disturbances, cytokine storm, persistent low grade infection and iatrogenic from the medications and hospital/ICU admission. This acute dysautonomia itself increases mortality risk, due to intrinsic effects on the respiratory, cardiovascular and neurological systems[105]. Dysautonomia with a significant drop in vagal cardiac modulation has been well documented associated with acute SARS-Cov-2 infection using Heart Rate Variability (HRV)[106]. Many of these mechanisms are directly related to spikeopathy[107].

The possible protective effect of nicotine particularly on the nervous system in acute COVID-19 has been studied superficially. Nicotine dosing prior to inoculation with SARS-CoV-2 intranasally in mice was shown to reduce the likelihood of SARS-CoV-2 RNA neuro-invasion and associated pathology[108]. However, a DBPCCO multicenter trial in mechanically ventilated patients with Covid pneumonia concluded that nicotine patches, 14 mg daily for a maximum of 30 days, did not reduce mortality, days of ventilation or rates of anxiety, depression, PTSD or insomnia 8 weeks after nicotine tapering[109]. There was no apparent benefit of nicotine in acute COVID-19.

WHO has defined a Post-COVID-19 condition that occurs in individuals with a history of probable or confirmed SARS-CoV-2 infection, usually 3 months from the onset of COVID-19 with symptoms that last at least 2 months and cannot be explained by an alternative diagnosis[110], and the term Post Acute Covid Syndrome (PACS) has been coined. Dysautonomia and chronic inflammation are common symptoms of this 'long COVID'. A recent study of health care workers used HRV as a measure of autonomic nervous system cardiac modulation, found sustained sympathetic cardiac modulation and diminished vagal cardiac modulation during the first 30 days after COVID-19 and that the SARS-CoV-2 associated autonomic imbalance in the post-acute phase after recovery of mild COVID-19 resolved 6 months after the first negative SARS-CoV-2 nasopharyngeal swab. However, a significant proportion of HCWs reported long-term symptoms, not apparently related to cardiac autonomic balance[111]. Vaccination status was not reported so these cases may instead represent Post Covid Vaccination Syndrome (PCVS) rather than purely PACS.

Distinct from acute COVID-19 or PACS, PCVS is a chronic disease triggered by SARS-CoV-2 vaccination with or without a history of COVID-19, with an estimated prevalence of 0.02%[112]. PCVS presents a phenotype of acquired autonomous dysfunction that overlaps with various established multisystemic dysautonomia syndromes such as myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS), POTS, fibromyalgia/chronic pain syndrome, small fiber neuropathy (SFN) and mast cell activation syndrome (MCAS)[113]. Autoantibodies against G-protein coupled receptors are increasingly recognised in these and similar diseases: antibodies against β2, M3 and M4 receptors have been found significantly elevated in ME/CFS patients[114].; adrenergic alpha 1 receptor antibodies, β2-adrenergic antibodies, and muscarinic acetylcholine $M_{2\text{-}4}$ receptor antibodies have been demonstrated

commonly in POTS[115], for example. These and other altered G protein receptor auto-antibodies can discriminate PCVS from the normal post-vaccination state, in particular ang II type 1 and alpha-2B adrenergic receptor antibodies.[116]. Clinically PCVS presents predominantly as malaise and chronic fatigue in >80% of cases, with overlapping clusters of (i) peripheral nerve dysfunction, dysaesthesia, motor weakness, pain, and vasomotor dysfunction; (ii) cardiovascular impairment; with (iii) cognitive impairment, headache, and visual and acoustic dysfunctions frequently present. Abnormal standard serum markers include increased interleukins 6 and 8 (>80%). Many of these patients fit the criteria for ME/CFS and dysautonomia syndromes[117]. They similarly share symptomatology with PACS[118], which is thought to be due to the common pathogenic mechanisms of spike S1 protein persistence[119]. There is considerable overlap in self-reported symptoms between PACS and PCVS, as well as shared exposure to SARS-CoV-2 spike (S) protein in the context of inflammatory responses during infection or vaccination. In a recent preprint by Battarcharjee et al from the Yale School of Medicine it was found that participants with PCVS had significantly higher circulating S1 levels compared with the control group ($p = 0.01$), with elevated levels of spike (S1 and full length S) in circulation up to 709 days after vaccination among a subset with PVS, even in those with no evidence of detectable SARS-CoV-2 infection[120].

As dysautonomia and persistent inflammation are characteristic of PCVS and persistent spike protein has been demonstrated, involvement of the CAP can be postulated. Nicotine has been trialled in several neurodegenerative diseases with variable and inconsistent results[121]. Acute, low dosing of nicotine has been trialed in the context of PACS and PCVS and is being widely promulgated in social media. One published series of 4 case studies involved patients who presented with 'long COVID', were nicotine naïve and had no significant co-morbidities. The intervention consisted of the use of a 7.5mg nicotine patch in the morning, for 7 days. Only minor adverse effects were noted although 1 patient did apparently use a 15mg patch in error. All 4 patients were reported as having significant and rapid recovery which was sustained when reviewed 3 to 6 months later[122]. At the time of writing, no further published or planned studies using nicotine could be found, perhaps indicative of the complexity of the issue.

It is apparent that CAP dysregulation is a part of the pathogenicity of the spike protein and spike-related symptoms. Support for the CAP is thus indicated, but direct agonist stimulation by potent agents such as nicotine while giving an initial acute benefit, is inappropriate for chronic treatment and does not mimic physiologic effects of normal CAP function. Short term, acute dosing of nicotine may have a beneficial anti-inflammatory effect particularly for the CNS and mast cell activation, but enthusiasm must be counteracted by the known risk of dependance and addiction, cardiovascular adverse effects and effects for diabetics and those with NAFLD or liver dysfunction should recurrent dosing be considered.

3.6 Stress and the Sympathetic Nervous System.

It is not so much a question of simply blockade of the CAP as it is of imbalance. The function of the sympathetic nervous system – or 'flight, fright, fight' system – is commonly conceived as being for high arousal, active states whereas the parasympathetic nervous system is viewed as the 'rest, digest, repair' system, suggesting an association between parasympathetic nervous system activity and social, relational and restorative processes[123]. However, these two systems do not function in a continuum but independently and the effect of this coordinated activity is highly variable in both context and in individuals[124].

Autonomic nervous system activity is a core and central component of motivation, emotional and stress responses, and individual differences in autonomic responses to high arousal states are powerful indicators of vulnerability and resilience to mental and health problems as evidenced by different Heart Rate Variability (HRV) under stress. HRV has been used for decades as a surrogate marker for parasympathetic tone and hence vagal function in patients with cardiovascular and inflammatory diseases[125] and does inversely correlate to markers of inflammation in patients with coronary artery disease, cardiovascular risk factors, autoimmune disease and traumatic brain injury[126]. Over the longer-term, reduced HRV correlates with immune dysfunction and inflammation, cardiovascular disease and mortality, attributable to the downstream effects of a poorly functioning

cholinergic anti-inflammatory reflex as a result of chronically poor vagal tone[127]. .

Encountering and managing stressors such as occur in daily life[128] elicits autonomic responses, with consequences for emotion functioning and well-being[129]. It has been well documented that the 3 years of the COVID-19 pandemic have resulted in high levels of perceived stress throughout society and across countries, both as a result of the COVID messaging itself but also the effects of lockdowns, masking and economic effects[130]. This apparent psychological stress response is itself a marker of alteration of homeostatic biological responses[131].

Some aspects of this relationship between stress and biological responses are also mediated via the hypothalamic-pituitary-adrenal (HPA) axis control of glucocorticoid and pro-inflammatory cytokine release. HPA axis activation following stress exposure results in the release of cortisol by the cortex, a significant regulator of the flight, fright, fight response[132] and activation of the sympathetic nervous system (SNS) which then triggers the release of proinflammatory cytokines notably IL-6 via α2-adrenoreceptor activation on immune cells[133]. This is a feed-forward loop in which IL-6 potently activates the HPA axis, further increasing SNS activity[134]. Once the stressor is removed, cortisol binding to the glucocorticoid receptors (GR) in the hippocampus and hypothal- amus will inhibit further release of glucocorticoids and cytokines[135] and the symp- athetic drive will quieten.

In chronic stress however, persistent activation of the HPA axis results in down- regulation of GRs, and GR-mediated signalling in the hippocampus is blunted[136] with with subsequent glucocorticoid resistance and loss of cortisol suppression of inflammatory responses. Additionally, under chronic stress, the HPA axis has reduced hippocampal mediated regulation, further contributing to cortisol dysregulation and uncontrolled proinflammatory cytokine release[137]. Stress itself can thus trigger neuroinflammation: stress-related neuropsychiatric disorders are associated with immune system activation/inflammation, high sympathetic tone, and corticotrophin-releasing hormone hypersecretion by the hypothalamus, which are all consistent with insufficient glucocorticoid-mediated regulation of stress hyperresponsiveness[138].

3.7. Modulating the Autonomic Nervous System.

It is apparent therefore that not only is the CAP affected directly by the spike protein interaction with nAChRs, but that the counteracting sympathetic nervous system is strongly activated by the chronic ongoing stress as a result of the societal and health changes resulting from the COVID-19 pandemic and pandemic management.

Lifestyle changes to reduce this sympathetic overload and activate the parasympathetic system and thus cholinergic stimulation via the vagal nerve to the nAChR should thus be implemented. This can be achieved by such measures as positive emotions meditation[139], deep diaphragmatic breathing[140], laughing and massage. Tai Chi[141], and Qi Gong[142], may also be helpful here both traditional gentle forms of movement with controlled breathing which have been shown to increase vagal tone and balance sympathetic outflow. Vagal tone can also be increased by HRV-biofeedback monitoring of slow-paced breathing techniques[143]. HRV variability, indexed as standard deviation of normal-to -normal heartbeat intervals (SDNN) has been found predictive in all cause mortality and of prognosis post acute myocardial infarction and cancer[144]. Higher HRV predicts greater chances of survival in acute COVID-19, especially in patients aged 70 years and older, independent of major prognostic factors while low HRV predicts admission in the first week after hospitalization[145]. Therefore, the use of biofeedback devices to enhance HRV and thus vagal tone is likely to also positively impact on other spikeopathy-related conditions.

Something as simple as 'forest bathing' or increasing outdoor green exposure has also been demonstrated to increase vagal tone and reduce sympathetic tone, as evidenced by reduction of blood pressure, lowering pulse rate, increasing the power of HRV, improving cardiac-pulmonary parameters, and metabolic function, inducing a positive mood, reducing anxiety levels, and improving the quality of life[146].

Vagal nerve stimulation (VNS) devices may also be of benefit. Previously developed for intractable epilepsy, VNS at low frequencies by implantable devices has been demonstrated of some benefit in a number of inflammatory disorders, including irritable bowel disorder[147], rheumatoid arthritis, migraine and fibromyalgia[148]. Recent non-invasive

methods of transcutaneous vagal nerve stimulation (tVNS) at two different anatomical locations been developed: at the cymba conchae of the ear or at the neck containing the cervical vagal nerve. These have now been trialled in a growing number of pilot studies for various musculoskeletal and pain conditions[149]. Vagus nerve stimulation via auricular stimulation has been tested in acute COVID-19 patients, been shown to reduce proinflammatory markers and increase anti-inflammatory markers[150]. It may positively influence the condition of these patients by suppressing inflammatory cytokine levels, especially IL-6, as a result of activation of the cholinergic inflammatory pathway[151], although a difference in clinical outcomes does not seem apparent[152]. Further developments in the field of non-invasive VNS include pulsed electromagnetic frequency (PEMF) devices, which have a higher profile than tVNS and have been demonstrated to result in improved HRV as a marker of parasympathetic system activation[153]. A recently published double – blind- placebo controlled trial of cervical vagal stimulation by a portable PEMF device demonstrated improvements in sleep and anxiety consistent with effects on parasympathetic tone[154]. Such non-invasive devices are well tolerated and may thus prove useful in conjunction with other measures to improve vagal tone in PACS/PCVS.

Electroacupuncture (EAP) has been demonstrated to also stimulate the vagal nerve and may provide another mode of intervention: EAP at the acupuncture point ST36 on the hind limb of mice functions via the vagal adrenal axis[155], but has yet to be documented in spikeopathy patients.

3.8. nAChR Agonism.

Nicotine is a naturally occurring pyrrolidine alkaloid[156] and can be found in small amounts in foods. especially the Solanaceae family (tomatoes, potatoes, eggplant), the highest being eggplant and tomatoes[157]. The brassica family (Brassica oleracea), which includes cultivars of cabbage, broccoli, cauliflower, brussels sprouts, collards, and kale, also contain measurable amounts of nicotine. Indeed, nicotine extracted from Brassica oleracea natural food sources has been found to be an effective anti-inflammatory compound at a low dosage for rheumatoid arthritis[158]. Dietary supplementation with Solanaceaea fruit and vegetables has been demonstrated to reduce the risk of Parkinson's disease[159], and supplementation of an extract, anatidine with nicotine-like properties as regards inflammation, has demonstrated benefit in osteoarthritis pain and inflammation[160]. Thus the inclusion of these foods may prove beneficial in the provision of a background level of agonism for the a7 nAChRs.

It bears mention that nicotinic acid is not related or derived from nicotine biologically, nor does it share effects with nicotine. Nicotinic acid was first synthesised by oxidising nicotine, and then the name changed to niacin to avoid confusion with nicotine[161]. While nicotinic acid has been demonstrated to have an anti-inflammatory action by modulation of macrophages, dendritic cells, neutrophils, and lymphocytes[162], it is via G protein receptor GPR 109a, with reduction of IFN-γ/LPS-induced expression of pro-inflammatory markers TNF-α, CXCL10 and CD197, rather than via the CAP[163].

Direct agonist action on nAChRs is also available via nutraceuticals. Choline is involved in the biosynthesis of brain phospholipids sphingomyelin and phosphatidylcholine and the biosynthesis of ACh by the action of choline acetyl transferase. Choline binds to α7 nAChRs[164] but not α4β2 receptors, unlike nicotine. It also exhibits neuroprotective properties but with a lower potency of about 3 orders of magnitude and only about 40% the level of cytoprotection of nicotine[165]. Choline is an essential brain nutrient for cholinergic neurotransmission, being sourced from the diet and by de novo synthesis. Dietary choline intake has been demonstrated neuroprotective over time and promotes improved cognitive function: in an analysis of Framingham Offspring Cohort data, past choline intake was significantly associated with changes in white matter hyperintensity volume seen in MRI in the brain, whereas cognitive function was affected by concurrent choline intake[166].

Phosphatidylcholine has been shown to have potency and effects similar to cholline in nAChR binding[167]. Phosphatidylcholine, a choline precursor, is a mixture of neutral lipids and phospholipids, which are essential components of the central nervous system, especially of the cellular membranes. It is natural emulsifier synthesized by plants and animals and found as food predominantly in soy lecithin and egg yolk. Phosphatidylcholine appears

to enhance neuronal development[168], has demonstrated positive effects in patients with cerebrovascular disease[169], and might decrease the risk of developing APOE4-associated Alzheimer's dementia (AD)[170]. A recent small concept study of phosphatidylcholine and computer based cognitive training in PACS/PCVS patients with cognitive impairment, showed a trend to improvement in all groups but with no statistical significance for the intervention groups. However, the study only included 29 patients with 4 weeks intervention and 8 weeks post intervention, which is limited in statistical power[171]. Nonetheless, cholinergic precursors choline and phosphatidylcholine (lecithin) are probably limited in enhancing brain levels of Ach. Other phospholipids involved in choline biosynthetic pathways such as CDP-choline, choline alphoscerate and phosphatidylserine have been demonstrated to clearly enhance ACh availability or release and provided a modest improvement of cognitive dysfunction in AD, these effects being more pronounced with choline alphoscerate[172,173,174,175]. Citicholine is a choline donor, and as CDP- choline, an endogenous precursor for phosphatidylcholine synthesis. It has also been shown to improve memory performance in elderly subjects with minimal negative effects, and improve the cognitive and mental performance in AD and vascular dementia[176].

In addition to choline and other choline derivatives phosphatidylcholine and citicholine, other nutrients have been found to act to activate nACHR signalling, notably epigallocatchin-3-gallate (EGCG)[177] and genistein[178], exerting their known antioxidant effects via activation of nAChR signalling and subsequent cascades. There may therefore be a role for supportive nutrients to activate nAChR signalling in spikeopathy.

3.9 Anticholinesterases.

The cholinergic pathway can be further supported by the use of anticholinesterases to increase levels of acetylcholine in the neurosynapses. The acetylcholinesterase (AChE) enzyme is found particularly in the brain, nerve cells, and erythrocytes, and is involved in hydrolyzing the acetylcholine ester[179]. By reducing ACh hydrolysis, altering the AChE activity may help to restore the cholinergic balance.

Pharmaceutical anticholinesterases increase both the levels and actions of acetylcholine found in the central and peripheral nervous system. Most commonly their use is in treating chronic neurogenerative diseases such as Alzheimer's dementia, Parkinson's disease and Lewy body dementia, characterised by destruction of Ach producing cells[180]. Peripheral-acting cholinesterase inhibitors which raise the postsynaptic muscarinic Ach receptor activation eg pyridostigmine, are often used in conditions such as myasthenia gravis and have been trialled in chronic fatigue syndrome (CFS) with some success[181]. As the symptomatic and exercise tolerance profile of PCVS overlaps signifificantly with ME/CFS[182], it is possible that this drug may be of therapeutic benefit also in managing ongoing symptoms of spikeopathy.

Numerous herbs and nutrients are demonstrated to possess AChE activity. The traditional nootropic herb Bacopa monniera has anti AChE activity as part of its spectrum of neuroprotective effects, which includes up-regulating brain-derived neurotropic factor and muscarinic muscarinic-1 receptor expression thereby restoring the levels of antioxidant enzymes and lipid peroxidation[183]. The traditional Chinese herb Huperzia serrata is the source of Huperzine A, a novel alkaloid which has proven to be a highly specific and reversible AChE inhibitor[184]. Huperzine A (HupA), a novel alkaloid isolated from the Chinese herb Huperzia serrata, is a potent, highly specific and reversible inhibitor of acetylcholinesterase (AChE). Flavonoids have also been found to have strong biological activity and AChE inhibitory activity[185]. Ginger and Cyperus rotunda have been demonstrated to have strong inhibitory action on AChE[186], as has Moringa oleifera leaves extract[187]. Intriguingly, Moringa has also been shown in rats to antidote the venom of Naja nigricollis, (black spitting cobra) which was the first identified *a*-neurotoxin[188], the 3-finger snake venom with which the spike protein has been demonstrated to share sequence homology.

In a recent study of 90 extracts from 30 medicinal plants on AChE activity, 23 active compounds were identified, including most abundantly flavonoids and dihydroxycinnamic acids. The commonly known and used herbs lavendar, oregano and thyme all tested positively with high AChE inhibition[189].These results are concordant with many studies of the activity of polyphenols as AChE inhibitors, which, in addition to inhibiting

AChE activity, also have an antioxidant effect, including scavenging free radical forms of oxygen and the ability to chelate transition metals, which reduces the initiation of inflammation that can cause the destruction of neuronal structures. Flavonoids, such as quercetin, kaempferol and, to a lesser extent, luteolin have also been reported as efficient AChE inhibitors[190]. Some other traditional herbs such as *Salvia* L. species *Angelica officinalis* L., *Hypericum perforatum* L., etc also have been demonstrated to have AChE activity[191].

In a recent review, Shoaib et al have listed numerous other plants and their derivatives with AChE inhibitory activity[192]. Interestingly, some bacterial products, such as novel exopolysaccharide EPSR4, a compound from the bacteria Bacillus subtilis and AG6 exopolysaccharide from the marine -derived Bacillus velezensis also have demonstrated AChE activity amongst other beneficial actions[193]. There are thus numerous plant-based and dietary options for enhancing ACh via AChE action.

MODULATING AUTONOMIC NERVOUS SYSTEM	REFERENCES
1. Lifestyle measures	1. 139,140,141,142
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3. Forest bathing	3. 146
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TABLE 1: ALTERNATIVE METHODS OF MODULATING THE AUTONOMIC NERVOUS SYSTEM

4. Conclusion

The dysregulation of the cholinergic anti-inflammatory pathway and dysautonomia are key players in spikeopathy and in the disease processes following, including Long COVID and PCVS. Impaired cholinergic signalling is further exacerbated by chronic stress and activation of the sympathetic nervous system. Spike protein does not bind directly to nicotinic cholinergic anti-inflammatory pathway receptors however and so while it is tempting to think that stimulation of these receptors by nicotine may be therapeutic, this must be tempered by the recognition that nicotine binding is neither physiological nor selective and has significant adverse effects in chronic dosing. A more physiological approach would be to consider stress reduction and vagal nerve activation techniques, and nutraceutical and herbal substances with anticholinesterase or acetylcholine agonist actions together with dietary change and nutraceuticals addressing inflammation. Pharmaceutical agonists/anticholinesterases may also be of benefit. This approach could have significant benefit in many other related disease processes in addition, for example ME/CFS and dysautonomia syndromes.

A new field of 'spikeotherapeutics' would need to cast the net wide in dealing with the myriad 'spikeopathy' induced biological dysfunctions and diseases triggered by SARS-CoV-2 spike protein, whether from the virus or mRNA and DNA genetic code vaccines. This review has outlined lifestyle, dietary, nutraceutical and pharmaceutical interventions that can help address these wide range of disorders without the adverse effects of nicotine.

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ABRREVIATIONS:

AChE - acetylcholinesterases
AChR: acetylcholine receptors
α7nAChRs - α7 nicotinic acetylcholine receptors
CAP- cholinergic anti-inflammatory pathway
CNS - central nervous system
EAP- electroaupuncture
GABA -*gamma*–aminobutyric acid
HPA- Hypothalamic-Pituitary-Adrenocortical Axis
HRV- Heart Rate Variability
ME/CFS - myalgic encephalomyelitis/chronic fatigue syndrome
nAChR- nicotinic acetyl choline receptors
PEMF: pulsed electromagnetic frequency
RBD - receptor binding domain
RAS- renin-angiotensin system
ROS- reactive oxygen species
SNS- sympathetic nervous system
POTS - Postural Orthostatic Tachycardia Syndrome
VNS- vagal nerve stimulation
tVNS- transcutaneous vagal nerve stimulation

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