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Novel pathomechanisms in Chronic Fatigue Syndrome/Myalgic Encephalomyelitis: Do purinergic signalling perturbations and gliosis play a role?

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ABSTRACT

CFS/ME is, in some cases, a serious fatigue-related condition exhibiting a range of neurological, immunological and metabolic dysfunctions in symptom presentation. The present paper explores the possibility of perturbations of purinergic signalling (PS) as a pathomechanism of CFS/ME involving glial cell dysfunction, disruption of neuronal transmission, neuroinflammation and possible disturbances in the functioning of the blood-brain and blood-spinal barriers (BBB/BSB). This paper discusses the possibility that the putative neuroinflammatory processes may occur through perturbations of PS involving vasoactive neuropeptide (VN) dysfunction (e.g. through autoimmune mechanisms).

Pituitary adenylate cyclase-activating polypeptide (PACAP) and vasoactive intestinal peptide (VIP) function as neurotransmitters, vasodilators and regulators of immunity, nociception and hypoxic injury. They are important in the central nervous system (CNS) by activating adenylate cyclase (AC) to produce cAMP from ATP. Compromise of ATP metabolism may promote neuronal and glial toxicity through impaired cAMP production or impaired ATP metabolism and these may alter BBB/BSB function.

Although speculative, diagnostic and therapeutic implications may exist for CFS/ME if VN compromise, along with perturbations of PS, do indeed disrupt neurological and glial cell functioning. Treatment opportunities involving phosphodiesterase inhibitors (PDEIs) and purinergic modulators may plausibly exist.

INTRODUCTION

Attempts have been made over many years to develop a viable hypothesis for the aetiology of chronic fatigue syndrome/ myalgic encephalomyelitis (CFS/ME). Numerous studies continue to contribute new data, but a coherent hypothesis to explain the clinical picture of this debilitating condition in the light of research data remains elusive. This paper attempts to develop further a hypothesis previously expounded involving vasoactive neuropeptide (VN) dysfunction with a more focused attention on disturbance of a neurotransmitter system recently attaining prominence. This system is termed purinergic signalling (PS) and derives its name from purine molecules forming part of the major non-neuronal neurotransmission system. It is based on adenosine triphosphate (ATP) and its derivatives, ADP and adenosine as well as the purine nucleotide UTP. Perturbations of PS have serious consequences for neuronal and glial cells and may lead to inflammatory conditions such as gliosis. Disruption of the blood-brain and blood-spinal barriers (BBB/BSB) may also occur as a consequence of PS disturbances. This paper examines a number of pathophysiological processes associated with PS and VNs and how these may theoretically be associated with the pathophysiology of CFS/ME.

VNs, including pituitary adenylate cyclase-activating polypeptide (PACAP) and vasoactive intestinal peptide (VIP) are distributed widely throughout the body and their loss of function may have systemic impacts, particularly in heart, lung, gut, urogenital, endocrine and skeleto-muscular systems. Similarly perturbations of PS may be associated with pathomechanisms in these systems. However, the present paper is largely confined to CNS effects and the potential role of gliosis in contributing to CFS/ME symptoms.

PURINERGIC SIGNALLING IN HEALTH AND DISEASE

ATP and PS form a major neurotransmitter system involving glia i.e. oligodendrocytes, microglia and astrocytes. Glial cells comprise a vital support system for effective neuronal signalling including synaptic transmission. The concept of the tripartite synapse is gaining prominence due to the extensive influence glial cells have in anatomical contact with, and functional activity of neuronal synapses (1). Astrocytes play an extraordinarily important role in higher animals, with one astrocyte potentially influencing up to several hundred thousand neuronal synapses. Rather than the traditional view of neurons being the only significant player in central

nervous system (CNS) homeostasis, it is now suggested that glial cells also exert significant control over neuronal signalling and CNS function.

ATP is released during normal signalling as well as under inflammatory and traumatic conditions. It is rapidly metabolised to adenosine by ectonucleotidase enzymes, but these have physiological limits and excess ATP can contribute to further pathogenic ATP release by cells (2). In the nervous system the concentration of extracellular purines is dependent on enzyme-associated stabilizing processes (3). Thus, it is conceivable that any alteration in this fine balance, for example decreased conversion of ATP to cyclic AMP (cAMP) by adenylate cyclase (AC) may induce perturbations of PS which invoke ATP toxicity with potentially serious consequences. ATP released by astrocytes modulates astrocyte-neuron synaptic transmission in different brain regions (4) and distortions in these signalling pathways may also affect neuronal calcium necessary for synaptic transmission. Further, ATP effects on purinergic receptors may contribute to neuropathic pain (5). Neuropathic pain as observed in CFS/ME may theoretically have an association with microglial activation and gliosis, although there is no direct evidence for this notion which has been published to date. Neuropathic pain syndromes, potentially including CFS/ME are associated with the production of inflammatory cytokines and chemokines (6). Similarly adenosine has primarily an anti-inflammatory action, but it can become pathogenic if produced in excess. Its accumulation is associated with wakefulness and cognitive consequences of sleep loss and involves astroglia (7).

PS disruption will impact on CNS homeostasis (8). Neurons and glia have bi-directional communication which sustains delicate neuronal function and ensures that inflammatory modulation is protective rather than pathological (9). PS must be tightly controlled to permit flexible inflammatory responses without unregulated deterioration in the neuroinflammatory environment (10). These concepts may be of importance in understanding the pathomechanisms and disease profiles of CFS/ME.

VASOACTIVE NEUROPEPTIDES IN NEUROLOGICAL HOMEOSTASIS

PS disruption may follow from compromise of VNs in particular, pituitary adenylate cyclase-activating polypeptide (PACAP) and vasoactive intestinal peptide (VIP) which have an emerging significant role in CNS function. These VNs activate AC and are important in cAMP production from ATP.

PACAP and VIP function in the CNS and periphery as neurotransmitters, vasodilators, regulators of perfusion and hypoxia, and modulators of nociception and immune responses. PACAP and VIP are also present in the heart, lung, pancreas, adrenal gland, gonads, gastrointestinal tract, immune and lymphatic systems (11, 12). They are involved in protective activities in the CNS, exerting an influence on both neuronal and glial function (13, 14). VNs and their receptors have been identified in a range of cells which may include perivascular macrophages, pericytes and endothelial cells as well as microglia, astrocytes and oligodendrocytes (13, 15). PACAP27 and PACAP38 have been recognized in oligodendrocytes in normal neocortex and hippocampus of human brain (16). These VNs influence other system functions including cardiovascular, respiratory, metabolic, immune and nervous system functioning (17). They help maintain BBB/BSB perivascular structures (18) in the CNS. Their pre-eminent role in CNS homeostasis likely places them at the centre of neuroinflammatory and immunoregulatory modulation with implications for neurodegeneration.

PACAP and VIP assist in controlling inflammatory processes, such as regulating Th1/Th2/Th17 shift and inflammatory activity (19). Pro-inflammatory cytokines such as TNF alpha increase the vascular permeability of endothelial layers when intracellular cAMP is low (20). Cytokines such as IL-10 and IL-4 are Th2 directed cytokines which influence the regulatory and anti-inflammatory functions of PACAP and VIP (17). As PACAP and VIP act as modulators of anti-inflammatory responses (21, 22), protective mechanisms within the CNS potentially may be disrupted. VPAC2R, a structurally related G protein-coupled receptor (GPCR) to PAC1R, has also been detected in reactive astrocytes in vivo (23).

As well as mediating inflammatory processes in microglial activation (19)), signalling pathways involving PACAP and VIP contribute to the maintenance of high level neurological functioning such as memory and learning (24) and neuroprotection (18). VIP tightly regulates glycogen metabolism in astrocytes and regulates the expression of a number of genes related to energy metabolism including glycogen (25). Thus neuroinflammatory, neurodegenerative and other disease processes in the CNS might develop where PACAP and VIP become impaired.

PACAP and VIP in autoimmunity

Autoimmunity of VNs or their receptors, may conceivably compromise AC function, resulting in impaired production of cAMP and failure to maintain metabolism of ATP at appropriate rates. This may have an important role in promoting neuronal and glial toxicity. Moreover cAMP has a vital role in activating gene translation through the transcription factor cAMP response element binding protein (CREBP). Changes in mRNA expression of genes responsible for neuronal-glial cell signalling pathways and neuroimmume function may be linked to CREBP dysfunction (26). Additionally, cAMP is essential in regulating pulsatile insulin secretion in response to glucose signals (27) and this may influence neurological function.

While not proven, impairment of PACAP/VIP or their receptors could also affect the permeability and function of the BBB/BSB, thus influencing CNS and immunological homeostasis. Similarly, direct effects on neuronal and glial cell activity are likely to impact on CNS functioning. As potent activators of AC and hence cAMP production, VNs may be important in influencing regulatory T cell (Treg) functio,n which is dependent on cAMP activity (28). Other immune functions involving glial cells may be affected during VN compromise such as endogenous triggering of microglial activation and reactive gliosis. Microglial cells are influenced by PACAP and VIP in immunoregulation (29).

PS in neuron-glia interactions is emerging as a vital component of CNS homeostasis (9) and this signalling is intimately connected to VN function. ATP released from astrocytes activates P2 receptors on astrocytes and other brain cells, allowing a form of homotypic and heterotypic signalling, which also involves microglia, neurons and oligodendrocytes (30). It is possible that underutilisation of intracellular ATP may lead to its extravasation to the extracellular compartment and coupled with a range of insults including trauma and hypoxia result in microglial activation (31, 32). However, nucleotide-induced activation of astrocytes and microglia may originally start as an acute defence mechanism aimed at protecting neurons from cytotoxic and ischaemic insults (30). Defects in AC activation may cause levels of ATP to accelerate, thus activating microglia and adjacent cells in pathophysiological conditions.

PACAP deficient mice have been shown to have diminished $CD4^{+}CD25^{+}$Foxp3Treg in lymph nodes and Foxp3 mRNA expression in spinal cord (13) and Treg function is important in maintaining CNS homeostasis. Also as

macrophage and perivascular infiltration of CNS is regulated by Virchow Robin spaces (VRS), VNs also becomes of considerable interest in other neurological conditions (33-35). Moreover, synchronization of Treg and dendritic cell immunoregulatory functions entail the activity of CD39 in conjunction with regulated PS (11). The ectoenzyme CD39 is a necessary component of the degradation process of ATP to AMP and is expressed mainly by Foxp3 (+) Treg cells. Foxp3 stimulates the expression of CD39 while ligation of the T-cell receptor has an added advantage of increasing CD39 catalytic activity. Thus, in the presence of activated Tregs, P2 receptor-related cell toxicity and maturation of dendritic cells from ATP induction may be prevented. Interestingly, a decline in CD39 (+) Treg cells in the circulation has been noted in some diseases. This implicates CD39 as a marker for a subset of Tregs that are important in the suppression of inflammation in some autoimmune diseases (12). As Foxp3 expression is likely to be under VN influence (13), the capacity for CD39 activity may be compromised following VN impairment resulting in ATP toxicity effects. Perturbations of PS thus may link VN functional abnormalities, neuroimmunological disruption, neuroinflammation and even neurodegeneration in some neurological conditions.

Autoimmunity of VN GPCRs is currently unverified, with little information available on loss-of-function autoimmunity to GPCRs generally (36). By comparison, Sjogren's syndrome has known T cell and/or B cell antibody targeting of acetylcholine GPCRs (37). Interestingly, autoimmunity involving gain of function to inhibitory VN GPCRs (Gi) may theoretically emulate loss of function autoimmunity to stimulatory (Gs) VN GPCRs although whether this assertion applies in glial VN-related immunopathology requires further research. Alternatively, receptor destruction or cell cycling of receptors may occur resulting in compromise of function. However, for GPCRs to become immunogenic they would normally require fragmentation and expression to the immune system by appropriate major histocompatibility complex (MHC) molecules. The expression of MHC Class II molecules within the BBB/BSB may stimulate T helper cell (Th) immunological responses (38). PACAP and VIP may protect against BBB pathology by exerting inhibitory effects on MHC expression (39). PACAP and VIP also provide hypoxia protection in the BBB (40, 41). However it would not be surprising to find novel pathomechanisms of autoimmunity in the CNS noting that neuroinflammation and

neurodegeneration may occur through autoimmunity to a range of possible antigenic epitopes (42).

Gliosis and VN Compromise

PACAP and VIP have a major influence in the astrocytic compartment (43). Glial cells have a crucial role in multiple CNS activities, including neuroprotection and neurotoxicity (44), as well as functioning of the neurovascular unit and central pain mediation involving the BBB/BSB (6). Gliosis usually comprises activation of glial cells accompanied by swelling, hypertrophy and an increase in glial fibrillary acidic protein (GFAP). Gliosis accompanies many neurological conditions and may be the result of homeostasis disturbance. Gliosis is known to follow hypoxia and trauma and VN failure could be a mechanism for failure of neuroprotection, although the involvement of pro-inflammatory cytokines remains controversial (45). Gliosis from any cause may result in neuronal damage and loss through unregulated neuroinflammatory mechanisms. Humans have a different proportion of glial cells to neurons compared with experimental animals hence there may be difficulties in extrapolating findings from animals to humans. Thus finding a suitable animal model for CFS/ME may be problematic. Nevertheless dysfunction of VNs and PS may be expected to have more serious consequences in humans

Markers of gliosis have been found in some neuro-inflammatory diseases. For instance, an increase in cerebrospinal fluid (CSF) levels of GFAP, a marker of astrogliosis has been observed (46). PACAP and the PAC1R receptor play important roles in glial cells as well as in neurons (47) and cAMP-dependent PACAP-induced GFAP expression is associated with astrocytogenesis (48). GFAP also occurs in reactive astrocytes associated with PAC1 binding (49) suggesting a possible modulating role of PACAP in gliosis. VIP-like immunoreactive (VIP-LIR) astrocytes have been found in the subcortical white matter of the human forebrain parietal lobe although comprising a minority of the GFAP-stained astrocyte population. The close anatomical relationship between the VIP-LIR astrocyte bodies and processes and the brain vasculature strongly suggests that they play a role in the local control of blood flow and of the barrier properties of the vessel walls (50). As VNs regulate hypoxic consequences it is interesting to ask whether their failure results in ‘virtual’ hypoxia thus triggering a range of pathogenic mechanisms through ATP and P2 receptors (51). Reduced perfusion of normal appearing white matter (NAWM) might

be caused by a widespread astrocyte dysfunction, possibly related to a deficiency in astrocytic beta (2)-adrenergic receptors and a reduced formation of cAMP (52). Hypoperfusion and possible loss of cAMP production may also occur in connection with PACAP/VIP compromise.

Other CNS receptor proteins are influenced by VNs. PACAP/VIP influence water channels such as the astroglial water channel aquaporin (AQP4), which are required for BBB maintenance (53). Neuromyelitis optica (NMO) in the CNS is associated with the binding of highly specific serum NMO- immunoglobulin (Ig)G antibodies to AQP4 on cerebral microvessels, pia mater and VRS (54). The binding of NMO-IgG to astrocytes and brain endothelium cells induces changes in the expression AQP4 thus compromising BBB permeability, granulocyte recruitment and degranulation of specific lymphocytes (55). Furthermore, secretin, a hormone related to PACAP/VIP, is implicated in electrolyte transport in cells and is a necessary component of aquaporin function via vasopressin. Secretin receptor-null mice have reduced expression of AQP2 and AQP4 in the kidney (56). Impaired VIP function may have serious consequences for water channel function especially those involving AQP4. Should VIP function become impaired, increased levels of ATP could result in activation of P2X7R in astrocytes and down-regulation of the expression of AQP4 (57). Hence ATP increase from dysfunctional AC mechanisms may affect AQP4 function along with inducing other possible ATP toxicity effects.

Potential role of VN compromise in CFS/ME

In addition to protective effects noted above, PACAP and VIP have neuroprotective effects, which involve hypoxia prevention in the BBB (40) via a transport mechanism which allows peptides to penetrate the brain parenchymal space (58). Furthermore, PACAP27 and PACAP38 have been recognized in oligodendrocytes in normal neocortex and hippocampus of human brain (16) and PACAP and VIP protect neurons and glial cells (14). These findings may support the view that CFS/ME patients have a neurological abnormality that may contribute to the clinical picture of the illness and that immune dysregulation within the central nervous system may be involved in this process although evidence remains modest (59). Macrophage-related toxic molecules, nitric oxide, glutamate, PACAP and VIP may theoretically have an important influence in these inflammatory events (60). Disruption of the balance between Th1 and Th2 cytokines has been suggested in CFS/ME

development (61, 62). CFS/ME has been observed by some, but largely unconfirmed by many others, to have an inflammatory component, exhibiting a pro-inflammatory cytokine shift (63, 64) and significant changes in Treg profile (65).

In perivascular spaces such as VRS immunoreactive lymphocytes permeate neuronal parenchyma and normally protect the BBB/BSB (66). Trauma may activate macrophage proliferation and migration into the VRS initiating pro-inflammatory immune responses (67), in particular, perivascular macrophages (PVM) expressing VN receptors possibly influence BBB/BSB integrity and modulate immune responses (68). Pathological dilatation of VRS may occur from a variety of causes including ischaemia (69). As VRS are localised in specific brain regions, pathological dilation of the VRS and other abnormal changes in VRS may predispose these anatomical locations to impairment or loss of their functions. Compromise of VRS in areas such as the nucleus tractus solitarius and dorsal medulla oblongata may damage viscerosensory and autonomic functions (70). Similarly, capillary diversity within the subfornical organ (SFO) and area postrema (AP) may promote low-resistance pathways for rapid dispersion of blood-borne hormones inside their organ boundaries and this may have a role in regulation of blood pressure and body fluids (71). Circumventricular organs (CVO) are regions in the CNS devoid of an efficient BBB, therefore, they may be more liable to macromolecule contamination of CSF including autoimmune dysfunction in the CNS (72). These anatomical locations and their functions rely on PACAP/VIP for protection from adverse events.

As noted above, macrophages and microglia associated with VRS usually express low levels of MHC class II molecules and in conjunction with lymphocytes, initiate and promote immune reactions against foreign antigens in the brain. However in a disorder like CFS/ME this mechanism may be stimulated and encourage neuroinflammation. Impaired NK cell activity noted in most CFS/ME cases and loss of anti-inflammatory functions could be associated with downregulation of IL-10RA gene found in some patients in some gene expression studies in CFS/ME (73-81). Importantly, atypical NK cell activity occurs in a number of autoimmune diseases (82) and their revival that is restoration of both NK activity and phenotypes (in particular, CD56bright NK cells) reduces inflammation (83).

As these VNs control catecholaminergic and cholinergic synthesis their failure would have significant impact on dopamine, adrenaline, noradrenaline and acetylcholine synthesis. PACAP stimulates the sustained phosphorylation of tyrosine

hydroxylase at serine 40 in bovine chromaffin cells thus controlling a rate limiting step in catecholamine synthesis (84) as well as acting as a primary secretagogue for catecholamine release at the sympatho-adrenal synapse under the stress response (85). PACAP also controls synthesis of enzymes producing adrenaline in the mouse adrenal medulla (86). PACAP has a prominent role in promoting survival of basal forebrain cholinergic neurons in the rat brain (87). Because of the important distribution of PACAP in the brain it is conceivable gliosis from any cause would not be properly controlled following VN impairment. Hence multiple CNS effects including impairment of memory and concentration might result. Fatigue as well as behavioural and emotional function would also likely be affected along with profound experience of unwellness. Compromise of these functions of catecholaminergic and cholinergic synthesis would be quite disabling and could manifest as the symptom profile of CFS/ME.

Decreased white matter perfusion has found in some but not all CFS studies of cases using MRI techniques (88, 89). Microvessel abnormalities with respect to cerebrovascular compromise may occur during PACAP/VIP compromise, and hence may be associated with CFS/ME thus manifesting itself in the form of cognitive impairments related to memory and concentration. Interestingly, MRI and SPECT studies in some CFS patients have observed CNS lesions and atypical hypoperfusion in varying brain regions in some CFS patients (90-93).

FUTURE DIRECTIONS AND IMPLICATIONS FOR TREATMENT

Recent developments in understanding PS, neurological models of autoimmunity such as experimental autoimmune encephalomyelitis (EAE), reactive gliosis, and pathomechanisms involving VNs have the potential to contribute to a better understanding of CFS/ME. Now recognised as non-adrenergic non-cholinergic (NANC) transmission, this neurotransmitter system may be vulnerable to compromise and relevant to CFS/ME. ATP, NO and VIP for example are now recognised as co-transmitters and are likely to be inter-dependent (94). In addition, gliosis and its association with brain insult (95) and the possibility of ‘virtual’ oxygen glucose deprivation (OGD) likely to occur from VN failure may be relevant. As astrocytes have a vital role in cerebral vasculature function (96) further research should consider how this function might be lost in VN impairment.

Disruption of PS as a result of VN malfunction would be expected to be more severe in the brain where BBB function could be compromised by cAMP failure to protect tight junctions and excess ATP could activate purinergic receptors and initiate inflammatory mechanisms. These pathomechanisms have self-perpetuating and exacerbating mechanisms which may involve upregulation of P receptors (e.g. P2XR) and prolongation of the disease response. The BBB is considered an integral part of the neuroimmune axis (41) and its disruption is implicated in range of neuropathological disorders (97). New techniques for investigating BBB function are being developed (98, 99) such as photon microscopy and may theoretically have applications in the diagnosis of CFS/ME but their application in living subjects is some way off. Rather than being an isolated and hitherto obscure condition, CFS/ME may turn out to be an exemplar disease model of aberrant PS.

Therapeutic opportunities for CFS/ME may arise through renewed understanding of neuroinflammation involving the BBB (9) and have applications in developing treatments for several neurological conditions. PACAP stimulates PAC1R, VPAC1R and VPAC2R receptor families in the substantia nigra (SN) (100), hence PACAP and VIP have a key involvement in neuronal survival and their defect may also affect motor neuron and other neuronal function (101). Changes in gene regulation at the promoter regions of the VPAC2R can activate abnormal expression of VPAC2, causing failure of VIP receptor function which disrupts the Th1 and Th2 response in other conditions (102). As PACAP and VIP in normal healthy conditions modulate their metabolic actions on ATP through the regulation of cAMP production, in pathological conditions that impair AC production and alter cAMP levels, phosphodiesterase (PDE) enzymes arguably could reverse this defect. PDE inhibitors (PDEIs) are relatively novel therapeutic substances that enhance cAMP production (103) and therefore may have applications in VN autoimmune conditions. The therapeutic advantages of PDEIs are associated with neuronal survival, depression and learning and long term memory formation, processes that have been shown to decline in neuropsychiatric and neurodegenerative disorders (104).

Neurological damage can be reduced by an increase in intracellular cAMP as a result of rolipram treatment, decreasing permeability of inflammatory and humoral cells in the cerebrovascular endothelial layer (105). In inflammatory responses in experimental autoimmune neuritis, rolipram effectively reduces pro-inflammatory cytokine and the chemokines macrophage inflammatory protein-1 alpha (MIP-1

alpha), MIP-2 and monocyte chemotactic protein-1(MCP-1) production and promotes an increase in anti-inflammatory mediators such as IL-4 in the peripheral nervous system (106). Thus PDEIs have a number of potentially therapeutic functions. However, molecular mechanisms of pathological pain are being elucidated through improved understanding of purinergic signalling and this may lead to purinergic treatment opportunities (107-109). The question of ATP toxicity might be addressed through purinergic receptor modifiers.

CONCLUSION

Patients with CFS/ME often exhibit a variety of sensory and motor disturbances, which may be consistent with neuronal and glial cell dysfunction. Whether this condition is linked to neuroinflammatory and neurodegenerative molecular pathology associated with perturbations of PS is yet to be established. Gliosis and other CNS pathology associated with PS perturbations may result from disruption of VN function, possibly associated with autoimmunity affecting VN GPCRs.

It is important to note that the concept that perturbations of PS may induce neuro-inflammation and possibly neurodegeneration is relatively novel and yet to find wider acceptance in the scientific and clinical community. However, treatment implications follow from VN involvement in glial cell functioning and pathologies such as gliosis. As PDEs catalyse cAMP, PDEIs maintain cAMP levels and have proven and well known therapeutic benefit in animal models such as EAE. PDEIs thus may have a role in therapy for neurological conditions in which these mediators can be reliably implicated. Should these conditions be proven to be associated with perturbations in PS associated with VN dysfunction there may also be implications for purinergic receptor modulators in treatment.

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