



Are vasoactive neuropeptide autoimmune fatigue-related disorders mediated via G protein-coupled receptors?

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Summary Vasoactive neuropeptides such as pituitary adenylate cyclase activating polypeptide (PACAP), calcitonin gene related peptide (CGRP) and vasoactive intestinal peptide (VIP) have been implicated in a number of fatigue-related conditions. Associations of these vasoactive neuropeptides with heat shock proteins (hsps) and cytosine–guanosine dinucleotide (CpG) DNA fragments in autoimmune phenomena have been postulated to interfere with receptor signal activation for adenylate cyclase and other vital cellular processes. However, a specific mechanism for receptor dysfunction has not been explored to date.

G protein-coupled receptors (GPCRs) constitute a high proportion of biological receptor mechanisms and serve a wide range of substances including nucleosides, nucleotides, catecholamines, calcium, histamine, serotonin and prostaglandins. They are complex transmembrane hepta-helical serpentine structures with specific binding capabilities resulting in conformational changes that activate cognate cyclic GMP (G proteins). GPCRs adapt to certain stimuli through desensitisation and changes in phosphorylation and are subject to distortions of signalling processes. Hence, these vital signalling structures are susceptible to impairment of function through a range of mechanisms.

One of their vital functions is signalling through adenylate cyclase, a vital step in cyclic AMP metabolism. This step involves ATP metabolism and therefore is a crucial mediator of cellular energy pathways. Some GPCRs act to inhibit adenylate cyclase (Gi proteins). Also vasoactive neuropeptides, such as PACAP display a number of receptor isotypes including null variants. Overexpression of Gi proteins and null variant receptors may account for major disruptions of signal transduction and ATP/cAMP metabolism.

This paper examines the possible role of GPCR dysfunction in contributing to fatigue-related vasoactive neuropeptide autoimmune disorders which may include chronic fatigue syndrome (CFS), Gulf War syndrome (GWS) and even sudden infant death syndrome (SIDS).

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Introduction

Vasoactive neuropeptides, such as pituitary adenylate cyclase-activating polypeptide (PACAP), calcitonin gene-related peptide (CGRP) and

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vasoactive intestinal peptide (VIP) are powerful effectors of neuroregulatory, immunomodulatory and hormonal functions. These neuropeptides and their receptors are present in brain and a wide range of other tissues. They have significant co-transmission roles with adrenergic and cholinergic neurotransmitters [1,2].

Vasoactive neuropeptide (VN) dysfunction has been implicated in a number of fatigue-related conditions [3]. While a valid patho-mechanism to explain these fatigue-related conditions remains unproven, associations of certain vasoactive neuropeptides with heat shock proteins and cytosine–guanosine dinucleotide (CpG) DNA fragments in causing autoimmune phenomena have also been postulated [4,5]. However, a specific mechanism, such as receptor dysfunction has not been carefully explored to date. This paper explores the possible role of G protein-coupled receptor abnormalities in producing fatigue-related VN autoimmune disorders.

GPCR receptor structure and function

Receptors for VNs of the VIP/PACAP family are G-protein coupled heptahelical transmembrane receptor structures (GPCRs) with intracellular and extracellular components. Alterations to their structure have significant effects on their functioning. GPCRs have significant sequence homology, demonstrate overlap of distribution patterns and may interact to promote synergy or complementarity of function [6,7]. Limited redundancy exists between receptors, for example PACAP may activate all three known receptor types (PAC1, VPAC1 and VPAC2) although PAC1 is dominantly activated by PACAP. However, a degree of interdependency also exists with co-transmission known to occur. Hence, susceptibility to dysfunction of one of these substances may impact on the effective functioning of another. Other naturally occurring ligands also exist such as maxadilan, a sand fly salivary vasodilatory peptide [8], which activates the PAC1 receptor while not having a high degree of homology with natural ligands [9].

Multiple signalling pathways are implicated in VIP/PACAP systems [12]. These receptors mostly activate adenylate cyclase, an essential step in cyclic AMP metabolism, with effects also on calcium, protein kinase (PKA, PKC) and inositol phosphate pathways. While these effects are complex and specialised sources should be consulted, the

predominant role of cyclic AMP activation is the core function postulated to be compromised in VN autoimmune disorders.

Numerous isoforms and splice variants exist for some of these receptors (e.g., PAC1). They have variable lengths and shapes including so-called 'hip' and 'hop' cassette variants. Interestingly the 'hip' variant is believed to be unable to transduce an activating signal despite being able to bind ligands satisfactorily. Mutation/conversion to 'hip' variants of the PAC1 receptor would have significant implications for loss of function of the PAC1 receptor and hence PACAP function.

Receptors may be altered by receptor activity modifying proteins (RAMPS) and are also affected by radiological, chemical and biological intervention with consequent alteration of receptor expression and function. For example, RAMPS interact with certain VN receptors to promote receptor functional specificity, glycosylation, transport and activity [10].

Alterations in vasoactive neuropeptide GPCR receptor structure and function are documented. Mutations in the third intracellular loop of the human recombinant VPAC1 receptor differentially affect adenylate cyclase and calcium activities [11]. As noted above the 'hip' cassette is known to impair adenylate cyclase function and abolishes phospholipase C (PLC) stimulation [12]. Hence aberrations of receptor structure involving 'hip' cassette expression arguably will have a significant impact on functional capacity of this GPCR and downstream activation of energy and metabolic pathways and upstream genomic expression pathways.

Deletion variants of VPAC receptors occur. A deletion variant of mouse VPAC2 lacking amino acids 367–380 at the carboxyl-terminal end of the seventh transmembrane domain has been identified in immune cells. This variant does not transduce VIP-elicited increases in intracellular concentrations of cyclic AMP. Hence natural deletion of the last transmembrane domain of VPAC2 abrogates signalling functions without apparent alterations of expression or ligand binding [13].

Overexpression of VIP/PACAP receptors is noted in certain pathological conditions such as bronchitis and cancer [14,15]. However, their role in mediating possible autoimmune reactivity in fatigue-related conditions is not clear and little information appears available on whether these VN receptors instigate autoimmune responses.

Do autoimmune aberrations influence VIP/PACAP receptor expression, structure and function?

The question remaining to be answered is whether autoimmune complexes (e.g., VN–hsp–CpG complexes) could trigger autoimmune dysfunction of VIP/PACAP GPCR receptors. Evidence for such a mechanism so far appears thin. Lombardi et al demonstrated that inflammatory processes in vivo induced a tissue-specific down-regulation of GPCR kinases (GRKs) in adjuvant arthritis. Responsiveness of GPCRs is modulated by the family of GPCR kinases (GRK1-6) and these act by phosphorylating GPCRs in an agonist-dependent manner, resulting in homologous desensitisation of the receptor. GRKs and arrestins also play a key role in GPCR internalisation, dephosphorylation and recycling, thus contributing to the extent of both desensitisation and resensitisation of the receptors. Additionally oxygen radicals may be responsible for degradation of GRK2 protein in activated immune organs [16].

A self-defeating feedback loop of inflammatory mediators, such as cytokines/chemokines could conceivably result in prolonged down-regulation of vital neuropeptide receptors should a perverse autoimmune stimulus be instigated and continue unabated. The role of heat shock proteins and CpG DNA fragments in this context has already been suggested above. Of particular interest is the question of whether ineffective transduction isotypes such as the 'hip' cassette variant of the PAC1 receptor are preferentially upregulated hence effectively 'silencing' the PAC1 receptor and causing a disproportionately magnifying effect on VN receptor dysfunction.

Clearly if long-term disorders such as the chronic fatigue syndromes are associated with vasoactive neuropeptide GPCR abnormalities these disorders would have to manifest dysfunction of expression, ligation/binding capacity or signal transduction of their receptors. Hence, disorders of genomic expression, receptor structure, cellular migration or immune tolerance could occur as a result of autoimmune effects on VN GPCRs and be sustained over a prolonged time period resulting in phenotypic fatigue-related conditions.

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