Review

Targeting the neurological comorbidities of multiple sclerosis: the beneficial effects of VIP and PACAP neuropeptides

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Vasoactive intestinal peptide (VIP) and pituitary adenylate cyclase-activating polypeptide (PACAP) are two widely expressed neuropeptides with important immunomodulatory and neuroprotective properties in the central nervous system (CNS). Both VIP and PACAP have been implicated in several neurological diseases and have shown favourable effects in different animal models of multiple sclerosis (MS). MS is a chronic inflammatory and neurodegenerative disease of the CNS affecting over 2.5 million people worldwide. The disease is characterised by extensive neuroinflammation, demyelination and axonal loss. Currently, there is no cure for MS, with treatment options only displaying partial efficacy. Importantly, epidemiological studies in the MS population have demonstrated that there is a high incidence of neurological and psychological comorbidities such as depression, anxiety, epilepsy and stroke among afflicted people. Hence, given the widespread protective effects of the VIP/PACAP system in the CNS, this review will aim at exploring the beneficial roles of VIP and PACAP in ameliorating some of the most common neurological comorbidities associated with MS. The final scope of the review is to put more emphasis on how targeting the VIP/PACAP system may be an effective therapeutic strategy to modify MS disease course and its associated comorbidities.

Keywords

Multiple sclerosis; Vasoactive intestinal peptide; Pituitary adenylate cyclase-activating peptide; Comorbidities; Stroke; Epilepsy; Depression; Anxiety; Schizophrenia; Migraine

1. Introduction

1.1 Neuropeptides

Over the last couple of decades, general knowledge on the biological role of neuropeptides in the central nervous system (CNS) has increased substantially. Currently, more than a hundred different neuropeptides have been described in the CNS, most of which are involved in the modulation of different brain functions [1–5]. Neuropeptides are small, amino acid-based molecules that can influence neuronal activity, neuro-immune responses and whose dysregulations have been implicated in the pathogenesis of several mental illnesses such as Alzheimer's or Parkinson's disease, depression, anxiety, stroke, migraines, epilepsy and multiple sclerosis [6–15].

It has become increasingly clear that certain neuropeptides such as neuropeptide Y (NPY), somatostatin, calcitonin gene-related peptide (CGRP), vasoactive intestinal peptide (VIP) and pituitary adenylate cyclase-activating polypeptide (PACAP) exert anti-inflammatory effects in the CNS [16]. This has resulted in research focusing on these neuropeptides as potential therapeutic targets for the treatment of neuroinflammatory diseases [17–21].

1.2 PACAP and VIP

The neuropeptides pituitary adenylate cyclase-activating polypeptide (PACAP) and vasoactive intestinal peptide (VIP) are widely distributed throughout the CNS and the peripheral nervous system (PNS) and are involved in neuroprotection and immunomodulation [22–27]. The activities of PACAP and VIP are mediated by three G protein-coupled receptors (GPCRs), namely PAC1, VPAC1 and VPAC2 [28] (Fig. 1).

PACAP binds with high affinity to both PAC1, VPAC1 and VPAC2 receptors and its activity is believed to be predominantly neuroprotective [29–31]. For example, PACAP can prevent neuronal cell death after ischemia [32] and can promote axonal regeneration after spinal cord injury [33]. In contrast, VIP binds less efficiently to PAC1 receptors, whereas its exhibits similar high affinities for VPAC1 and VPAC2 receptors as PACAP [34]. The latter two receptors' activities are thought to be mainly associated with immune modulatory roles in the CNS as well as in peripheral organs [35–37]. Given VIP binding preference towards VPAC receptor subtypes, this peptide has emerged as a potential anti-inflammatory target to treat multiple sclerosis (MS) and perhaps, other inflammatory diseases [38, 39]. For example, in human rheumatoid arthritis (RA), VIP treatment downregulated chemokines production and interleukin-6 (IL-6) and decreased the levels of other pro-inflammatory mediators in RA patients [40]. Additionally, a single intracerebroventricular injection of VIP was able to attenuate microglial activation and prevented neurodegeneration in animal models of Parkinson's disease [41].

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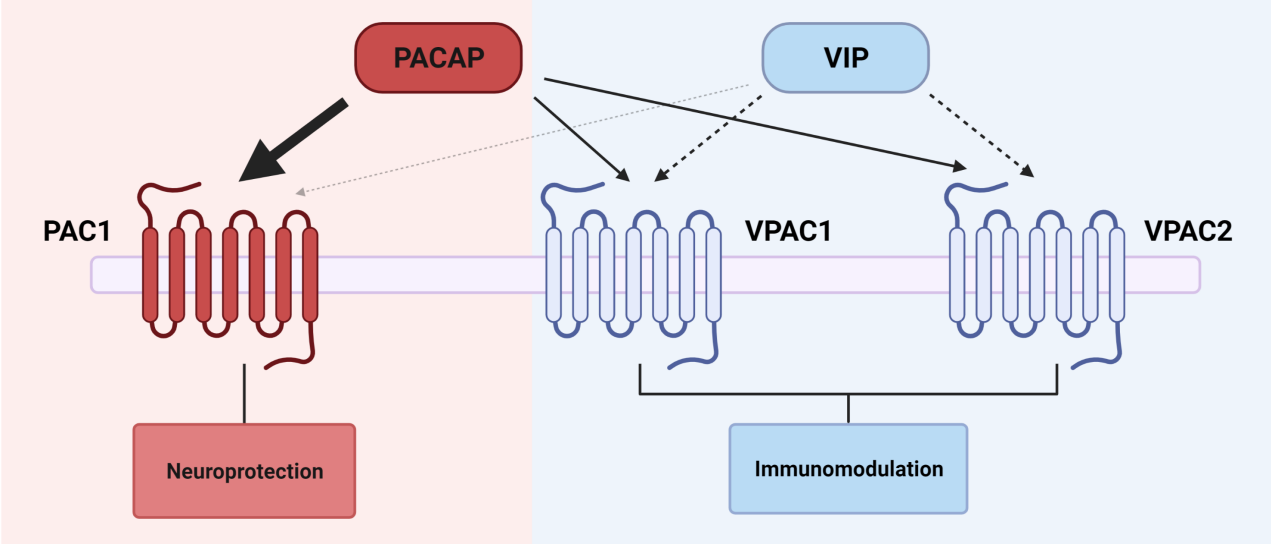

Fig. 1. PACAP and VIP and their downstream effects. A schematic overview of the binding of VIP and PACAP to the PAC1, VPAC1 and VPAC2 receptor with its main down-stream effects.

1.3 VIP/PACAP system in multiple sclerosis

1.3.1 Multiple sclerosis

MS is a chronic neuroinflammatory disease of the CNS that is characterised by episodes of demyelination within the CNS, with consequent axonal loss and gliosis. Both genetic vulnerability and/or exposure to certain environmental pollutants or unhealthy life styles are considered risk factors for disease development [42, 43]. The prevalence of MS has been increasing over the last decade with currently approximately 2.8 million people suffering from the disease worldwide [44].

There is a clear involvement of the immune system in MS, with the infiltration of auto-reactive T cells into the CNS believed to be a major pathophysiological event in disease aetiology [45]. Moreover, B cells, natural killer (NK) cells, astrocytes and microglia have also been shown to exacerbate CNS inflammation, thus playing a role in both disease onset and progression [46–48]. In fact, the heightened inflammatory state of the CNS observed in MS is believed to exacerbate the ongoing loss of myelin and axonal degeneration caused by a myelin-targeted autoimmunity [49]. A brief overview of current knowledge on the role of the VIP/PACAP system in neuroinflammation and myelination in MS is highlighted below.

1.3.2 VIP/PACAP and neuroinflammation in multiple sclerosis

Given the known neuroprotective and immunomodulatory roles of the VIP/PACAP system in the body, these endogenous neuropeptides have been thoroughly investigated in MS. In MS patients, it was found that both PACAP and VIP levels are reduced in the cerebrospinal fluid [50]. Moreover, using global and conditional knockouts for VIP, PACAP and their receptors, Waschek and colleagues have been able to dissect many of the neuroprotective and immunomodulatory actions elicited by these peptides in acute monophasic MOG_{33-35} experimental autoimmune encephalomyelitis (EAE) models, a well-established mouse model of MS [51–54]. These authors revealed that mice lacking PACAP or VPAC2 displayed more severe and prolonged disease than wild type controls while VIP or VPAC1 knockout mice showed EAE resistance [53–55].

When examining the effects of VIP and PACAP at a cellular level in MS models, a clear immunomodulatory influence can be observed. Extensive research has focused on the effect of PACAP and VIP on T-cell function, but additional effects have been reported on other immune cell populations. VIP and PACAP have been shown to regulate Th1 and Th17 profiles, triggering the shift towards anti-inflammatory phenotypes, whilst also assisting in the recruitment of anti-inflammatory Th2 and Treg cells [17, 39, 51–53, 56, 57]. Additionally, $PACAP^{-/-}$ and $VPAC2^{-/-}$ mice subjected to MOG_{33-35} induced EAE showed increased immune cell infiltration in the CNS, whereas reduced infiltration was seen in $VIP^{-/-}$ and $VPAC1^{-/-}$ mice [51, 53–55]. *In vitro* analyses also determined that PACAP or VIP treatment in cultured cells and in EAE models resulted in reduced levels of pro-inflammatory cytokines, chemokines, chemokine receptors and inducible nitric oxide synthase (iNOS) produced by T-cells, macrophages and microglia [39, 58–63]. This could potentially contribute to the observed neuroprotective effect, as a reduced inflammatory microenvironment is likely to promote the upregulation of cell survival genes by neurons and glia [64]. Moreover, PACAP treatment was also shown to suppress the function of antigen presenting cells in the EAE model, which is an important mediator of T-cell differentiation [56, 65].

Taken together, these findings corroborate the idea that VIP and PACAP play a critical role in the modulation of the inflammatory response of the CNS both *in vivo* and *in vitro*. However, given the diversified activities of either peptides in

several pathological domains of the disease, there is the need for additional research to further breakdown the differential effects of these peptides and their receptors on the immune system and the CNS.

1.3.3 VIP/PACAP and myelination

In addition to the established immunomodulatory and neuroprotective effects of VIP and PACAP in the nervous system, there is evidence to indicate that both neuropeptides may be critically involved in regulating certain aspects of oligodendrocyte and Schwann cell proliferation and maturation [66, 67].

Oligodendrocytes are the myelin producing cells of the CNS and the main cell-type affected in MS. PACAP is a known stimulator of oligodendrocyte progenitor cell proliferation, although it delays oligodendrocytes maturation *in vitro* [68, 69]. These findings have been substantiated using knockout mice, where PACAP-deficiency anticipated CNS myelination, although within a limited time window [70]. As far as these data may appear counterintuitive, the authors suggested that the physiological and transient inhibitory role of endogenous PACAP on myelination may serve to reduce the secretion of factors that impede axonal development and synapse formation by myelinating glia, and therefore, promote neuronal outgrowth over myelination, at least during the earliest stages of CNS development, when the former process should be prioritised. In this scenario, endogenous PACAP acts as a master regulator of CNS maturation. Nonetheless, additional investigations are warranted to clarify the exact involvement of PACAP/VIP receptors in relationship to CNS myelination at different developmental ages and in adulthood, as it is possible that a developmentally regulated expression of PACAP/VIP receptors or specific receptor isoforms may be at the basis of a diversified activity of the peptides, a mechanism already proposed for cortical development [71].

In Schwann cells, the myelin-producing cells of the PNS, PACAP and VIP activities are more obvious. In fact, as shown in PACAP knockout mice, genetic ablation of the *PACAP* gene results in impaired axon regeneration upon facial nerve injury [72]. *In vitro*, PACAP or VIP treatment of cultured Schwann cells induces the up-regulation of myelin-related proteins, suggesting that PACAP may enhance PNS myelination [73–75]. VIP, on the other hand, is believed to play a more differentiating role in oligodendrocyte progenitor cells in the CNS [67]. VIP/VPAC1 signaling was shown to reduce the severity of ibotenate-induced white matter lesions under inflammatory conditions [76]. Moreover, there are some indications of a myelin deficit in the CNS of VIP-deficient mice, although more in-depth studies are needed before this can be confirmed [67]. In the PNS, VIP administration in the proximity of transected nerves promoted early myelination and re-myelination of damaged nerves, and it induced the expression of myelin-related proteins by Schwann cells [77].

Thus, there are indications of a differential effect of VIP and PACAP in regulating CNS and PNS myelination. Although it is beyond the scope of this review to further dive into the details of the role of VIP and PACAP in myelin development, there are excellent reviews [67, 73] summarizing some of the current knowledge in the field.

1.4 Multiple sclerosis and associated comorbidities

Recent studies have demonstrated both a higher incidence and prevalence of comorbidities in MS patients compared with the healthy population [78, 79]. MS comorbidities often appear in people suffering from concurrent vascular and/or metabolic diseases, as well as certain neurological and psychiatric disorders [80–84]. Epidemiological studies in MS patients have demonstrated that afflicted people also have a higher chance of developing epilepsy [83], migraines [84], as well as affective/emotional disturbances such as depression or anxiety [79]. In view of the comorbidities often seen in MS patients and the critical role of neuropeptides in many pathological domains of these comorbidities, exploring the mechanisms and the extent at which both PACAP and VIP peptides can contribute to ameliorate the comorbidities of MS is becoming a hot topic. For this purpose, this review will summarise literature on the role of neuropeptides, focussing on the role of VIP and PACAP in the neurological comorbidities of MS, and how these neuropeptides could contribute to improve the clinical presentation of MS and disease course.

2. The role of PACAP and VIP neuropeptides in the comorbidities of MS

2.1 Depression, anxiety and bipolar disorder

MS is a devastating disease that comes with physical as well as psychological hardship, which may ultimately lead to the development of mood disorders or facilitate its onset in vulnerable people. One of the most prevalent comorbidities seen in MS patients is depression (23.7%), followed by anxiety (21.9%) and bipolar disorder (BD; 5.83%) [79]. Interestingly, these disorders are all associated with neurochemical evidence of CNS inflammation, supporting a pathological link with MS [12, 85–88]. Moreover, depression, anxiety and BD are all known to be influenced and triggered by stress, a risk factor able to also affect oligodendrocyte's health [89–93]. Here, the role of PACAP and VIP in depression, anxiety and BD is highlighted.

Several neuropeptides such as NPY, somatostatin, galanin and orexin, at different extents, have been implicated in the pathology of affective disorders and have been suggested as potential therapeutic targets [21, 94–101]. The VIP/PACAP system has also been implicated with depression and anxiety, although there are some conflicting findings. In some studies using PACAP-deficient mice, *PACAP* gene ablation increased depressive-like and anxiety-like behavior under stress conditions [102–104], whereas in a study by Lehmann and colleagues it was found that PACAP$^{-/-}$ mice had reduced anxiety and did not develop depressive-like behaviors [105]. However, this study used social defeat to trigger chronic

stress whereas the other studies either used naïve mice or mice exposed to a milder form of stress. Thus, PACAP$^{-/-}$ mice might exhibit a whole spectrum of behavioral disorders, depending on the initial source of stress, type and duration. In a clinical study, VIP serum levels negatively correlated with depression and anxiety state and positively correlated with brain volume of the left amygdala [106]. Similarly, decreased serum levels of VIP were detected in a rat depression model [107]. Moreover, single nucleotide polymorphisms (SNPs) in the *VIPR2* gene and *VIP* gene were found to be associated with unipolar major depression and BD, respectively [108]. In addition, a VIP injection into the CA1 region of the hippocampus of rats showing anxiety-like behaviors attenuated the symptoms [109], although, for unexplained reasons, VIP antagonist was not able to abolish VIP-mediated behavioral improvements.

Corticosterone, one of the hormones produced by the adrenal gland as part of the hypothalamic-pituitary-adrenal axis (HPA-axis), is known to play a critical role in the development of depression, anxiety and bipolar disorder [92, 110, 111]. Under stress conditions, PACAP-deficient mice exhibited an attenuated corticosterone response, which occurred irrespectively of the development of depressive-like behaviors [105, 112]. Moreover, PACAP injections into the amygdala and bed nucleus of the stria terminalis (BNST) have been found to reliably increase corticosterone levels and anxiety-like behaviors in rodents [113–115]. Interestingly, under chronic stress conditions there was a region-specific increase of PACAP and PAC1, but not VIP and VPAC1-2 levels in the BNST [116]. VIP has also been implicated in the control of glucocorticoid hormones release, although in a work focused on studying its effects in relationship to circadian rhythmicity [117]. Thus, there appears to be a link between PACAP, stress and the HPA axis, which could be relevant for the development of certain affective disorders.

In summary, the VIP/PACAP system appears to be a key player in the modulation of mood and other affective disturbances. Each peptide exerts intrinsic regulatory functions in brain homeostasis and it is not surprising that dysfunctional regulation of PACAP or VIP in specific brain regions or cell populations may be critical for the development of conditions such as depression, anxiety and bipolar disorder. Future research is warranted to explore if targeting this neuropeptide system can be used as an effective therapeutic strategy to treat mood disorders.

2.2 Psychotic disorders-focus on schizophrenia

Psychotic disorders encompass a broad range of mental illnesses such as schizophrenia, affective psychosis, delirium and drug-induced psychosis. Approximately 2–4% of MS patients have reported to experience psychotic episodes at some point during the course of the disease, which is a considerably higher rate than in the general population [118]. In line with MS pathology, neuroinflammation and increased oxidative stress events in the CNS are pathogenic events that are associated with the occurrence of certain psychotic disorders [119, 120].

Several neuropeptides have shown to activate signalling pathways that are implicated in the genesis of psychosis. Clear associations have been found between schizophrenia and neuropeptide Y, neurotensin, somatostatin and oxytocin and the number of psychotic episodes (reviewed in [121, 122]). Moreover, a genetic link between neuregulin-1 (NRG1), cholecystokinin A and schizophrenia has been described [123–127]. Similarly, genetic polymorphisms of genes encoding PACAP peptide or its receptors have also been correlated with schizophrenia [128–132], although for the former gene target (*PACAP* gene, aka *Adcyap1*), a replication study failed to reproduce the same findings [133].

There are certain indications that neuroinflammation can lead to the development of schizophrenia and psychotic episodes. In schizophrenia and first-time psychosis patients, studies have reported increased levels of pro-inflammatory cytokines and decreased levels of the anti-inflammatory cytokine IL-2 in the CNS [134, 135]. Additionally, increased microglia activation has been observed in recent-onset schizophrenic patients [136, 137]. However, since no comprehensive animal model exists for psychiatric diseases, reliable strategies to study neuroinflammation at a molecular level in psychiatric illnesses remains a daunting task. Furthermore, since neuroinflammation in schizophrenia is a relatively novel concept, the exact mechanisms through which it could contribute to disease aetiology remain uncertain. However, it would be interesting to explore if the anti-inflammatory activities of the VIP/PACAP system could potentially attenuate the CNS inflammation seen in people with psychotic disorders.

There is a leading hypothesis featuring schizophrenia as a neurodevelopmental disorder [138, 139]. In *post mortem* brain tissue from schizophrenic patients, the pool of neural stem cells (NSC) is reduced, suggesting decreased NSC proliferative activity [140]. Moreover, schizophrenia is characterised by abnormal connectivity among brain regions and axonal abnormalities [141–143]. A noteworthy link between brain development, schizophrenia and white matter can be seen for NRG1. NRG1 signals through disrupted-in-schizophrenia 1 (DISC1) and interestingly, PACAP has been found to affect DISC1 signalling as well [144]. In addition, both NRG1 and DISC1 are involved in neuronal migration, axon ensheathment and oligodendrocyte maturation [145, 146], suggesting a role for PACAP in modulating NRG1-mediated activities. Moreover, PACAP, PAC1 and DISC1 are known to be essential components of the cellular machinery that regulates neurite outgrowth [144, 147, 148]. PACAP stimulated NSC proliferation in mice and prevented the reduction of NSCs in a ketamine-induced schizophrenia-like mouse model via PAC1 receptor activation [149, 150].

Taken together, these data suggest that PACAP (and perhaps other PACAP/VIP receptor agonists) could aid in the treatment of schizophrenia. Based on the reported pro-mitotic activities of PACAP in NSCs, it is reasonable to hy-

pothesise that PACAP treatment could aid in replenishing the depleted pool of NSCs in the brain of schizophrenic patients, hence promoting neurogenesis. Additionally, it could reduce the chronic CNS inflammation that seems to also contribute to the development of psychotic disorders. Whilst additional investigations into this topic are still needed, there is already some indication that the beneficial actions of certain neuroleptic drugs can occur by mechanisms that involve restoring the dysfunctional VIP/PACAP signalling in the brain [151].

2.3 Epilepsy

Epilepsy is a chronic neurological disorder that is characterised by recurring and unprovoked seizures [152]. Seizures occur when the balance between excitatory and inhibitory signals in the brain is disrupted [152]. Epilepsy is one of the most common neurological disorders that is disproportionately prevalent in MS patients compared to the general population [153]. It has been reported that the prevalence of epilepsy is six times higher in MS patients than in the healthy population [154]. This is not surprising, as both lesions, inflammation and neurotransmitter imbalances within the CNS of people with MS may trigger such disabling ailment, with evidence also suggesting that the frequency of seizures tends to increase as the disease progresses [155].

As mentioned, an imbalance in neuronal activity is a critical neurochemical feature of seizure episodes [152]. It is well-documented that neuropeptides can contribute to reset this imbalance, including PACAP and VIP [156, 157]. Despite the emerging evidence, to date only one neuropeptide-based hormone, adrenocorticotropic hormone (ACTH), is currently being tested in clinical trials for the treatment of seizures in a rare disease that affects infants (West syndrome), whereas investigations on the efficacy of other neuropeptides in epilepsy has been limited to preclinical studies [152, 158].

PACAP and VIP are released during high neuronal firing activity [159, 160]. VIP exerts an overall excitatory effect on synaptic transmission, which is mediated by VPAC1 and VPAC2 receptors [161, 162]. In hippocampal surgical samples of patients suffering from human temporal lobe epilepsy, both VPAC1 and VPAC2 receptors were shown to be up-regulated [15] and up-regulation of VPAC receptor subtypes has been associated with increased neuronal survival [161]. These data support the idea that hippocampal VPAC receptors are increased as a homeostatic mechanism to prevent excessive neuronal damage/death caused by epileptic episodes.

Research into the role of PACAP in epilepsy has been centred on its ability to modulate microglia and glutamate transmission [163–165]. The expression of PACAP increases after kainic acid-induced seizures in rats, with many suggesting that this seizure-induced increase in PACAP may help to reduce excitotoxicity and promote overall neuroprotection to protect the CNS from damage [163]. Specifically, PACAP acts on microglia to promote the release of anti-inflammatory factors that polarise microglia towards an anti-inflammatory phenotype, whilst concurrently increasing the expression of glutamate transporters that promote glutamate re-uptake, resulting in two parallel protective mechanisms [166, 167].

Magnetic resonance imaging and pathological studies have proposed that cortical inflammation, demyelination and grey matter damage in MS patients may be responsible for the onset and development of epileptic seizures [155]. However, epilepsy is an active process, so it is difficult to determine if any aspect of MS pathology may specifically promote or trigger seizure episodes, especially given that MS patients often present with unique pathogenic profiles and distributions of lesion within the CNS. This should be considered as a further incentive to invest more in researching the efficacy of PACAP and VIP as therapeutic targets for epilepsy and MS, as both conditions are often comorbid and targeting these peptides or their receptors may prove to be effective in ameliorating epilepsy associated to MS.

2.4 Stroke

Stroke is the leading cause of adult disability, with one in four people globally experiencing a stroke event in their lifetime [168]. Stroke occurs when there is a long-lasting interruption or severe reduction of cerebral blood flow, which triggers a cascade of pathological events including excitotoxicity, oxidative stress, blood brain barrier (BBB) leakage and neuronal cell death [169]. Inflammatory processes, including the autoimmune activities of MS are thought to contribute to endothelial dysfunction and atherosclerosis, which may promote the development of micro- and macro-vascular alterations that culminate in ischaemic or hemorrhagic stroke. As such, it should not be surprising that compared with the general population, people with MS are at increased risk of experiencing a stroke, and if they do, they tend to suffer more severe symptoms [170].

It has been shown that administration of PACAP in animal models of stroke is neuroprotective and causes a reduction of both neurological deficits and the degree of pathological change of the CNS tissue of the ischaemic brain area [26]. There is an increase in inflammation post-stroke [13, 171], and PACAP and VIP are well-known anti-inflammatory agents in the CNS [10]. Both peptides are expressed in different types of immune cells, including microglia and astrocytes [37, 172]. Masmoudi-Kouki *et al.* [173] suggested that the neurotrophic and neuroprotective effects of PACAP and VIP can be partly accounted for by their activities on astrocytes. This idea is supported by the increase in astrocytic PACAP and PAC1 expression immediately following cerebral ischemia [174]. Additionally, exposure of cultured astrocytes to PACAP was found to be capable of up-regulating glutamate uptake via PAC1-mediated signalling, suggesting that the PACAP-PAC1 axis can reduce post-stroke excitotoxicity caused by excessive glutamate release [167].

In a study, treatment with VIP has shown to reduce brain damage and to promote neurogenesis following ischemic injury in the rat brain [175], although it should be highlighted that most of the neuroprotective effects reported for experimental stroke are related to PACAP and not VIP. Indeed,

studies in PACAP knockout mice suggest that the peptide prevents post-ischemic neuronal cell death [176, 177]. In animal models of stroke, intranasal administration of PACAP reduced infarct volume and improved functional recovery [178]. Additionally, PACAP-dependent polarisation of microglia towards an anti-inflammatory phenotype resulted in improved functional recovery in mice post-ischemic mice [179].

These studies provide evidence that both PACAP and VIP have potential therapeutic validity for the treatment of stroke, and may find application to aid in the recovery of neuronal injury post-stroke due to their neuroprotective and anti-inflammatory functions.

2.5 Neuropathic pain

Neuropathic pain is defined as pain caused by a damage or disease to the somatosensory nervous system [180]. WHO defines MS as one of the main CNS diseases responsible for the development of central neuropathic pain, followed by traumatic causes and other conditions such as spinal cord injury, traumatic brain injury and stroke, with neuropathic pain reported by about 86% of MS patients [181]. Neuropeptides are key regulators of peripheral nociception and contribute to the mechanisms regulating central sensitisation to pain. In many cases, chronic inflammation, ion channel imbalance and a lack of inhibition in the dorsal horn of the spinal cord partake in maintaining pain sensation [182]. PACAP and VIP are expressed across key anatomical regions/structures that are important in somatosensory processing and the transmission of pain signals [183]. In the PNS, these includes the dorsal root ganglia and peripheral nerves, whereas in the CNS, these peptides are detected in regions that process pain sensation and the associated emotional load, including the thalamus, periaqueductal grey (PAG), parabrachial nucleus and amygdala [22, 184, 185]. Despite the anatomical relevance of PACAP and VIP in neuropathic pain pathways, their distinct roles in the pathophysiology of neuropathic pain remains to be clarified. Studies using PACAP knockout mice have indicated that the peptide plays an excitatory role in pain transmission, as knockout animals do not develop symptoms of neuropathy after a spinal nerve transection, although they retain normal nociceptive responses [186]. These findings have been supported by subsequent studies showing that PACAP up-regulation in the dorsal horn is necessary for spinal sensitation and the development of neuropathic pain [187].

There is evidence to indicate that intrathecal injections of PACAP in mice cause both hyperalgesia and allodynia, two common symptoms of neuropathy [188]. In another study, intrathecal PACAP caused prolonged allodynia that was associated with sustained astrocytic activation [189]. Administration of a PAC1 receptor antagonist inhibited PACAP- and nerve injury-induced allodynia, suggesting a crucial role of PACAP-PAC1 interaction in the induction of neuropathic pain [190].

The overlap between pain and inflammatory signalling has been extensively studied and reviewed [189]. Additionally, an association between axonal regeneration and pain has been hypothesised, with evidence suggesting that accelerated regeneration may ameliorate pain of nerve origin [182]. PACAP and VIP have well-described roles in promoting axonal regeneration after peripheral nerve injury [34, 191]. For example, RNA sequencing data revealed the *ADCYAP1*, the gene encoding for the PACAP peptide, was the most differentially expressed gene associated with post-surgical nerve regeneration in patients with carpal tunnel syndrome [192].

Most studies have suggested PACAP has a dominant role in pain pathophysiology compared to VIP. However, Dickinson and collaborators identified distinct regulatory roles of spinal PAC1 and VPAC receptor subtypes in animals with experimentally-induced neuropathy [193], also implicating the VIP-VPAC axis as a further contributor to pain-related regulatory responses. Centrally, PACAP activity in the amygdala has been linked with the emotional expression of pain [185], whereas VIP expression in the PAG has been correlated with the development of co-morbid behaviors in nerve-injured rats [22]. Nonetheless, taken together these data pinpoint the critical role of the PACAP/VIP system in perpetuating both spinal and supraspinal pathways that are involved in the transmission of pain originating from peripheral nerve damage as well as its emotional components. With this in mind, it is conceivable that strategies aimed at blocking PACAP/VIP signaling may be beneficial in mitigating neuropathic pain, a disabling comorbid event associated with MS.

2.6 Migraine

Migraine is a complex and debilitating headache disorder that affects one in seven people worldwide [194]. Despite clinically manifesting as recurrent attacks of headache, migraines are associated with a range of symptoms (i.e., nausea, vomiting, and extreme sensitivity to light and sound) and are linked to other conditions like depression, anxiety, sleep disorders, chronic fatigue and cognitive dysfunction [195]. The role of neuropeptides in migraine in an active area of research. The most studied neuropeptide in relation to migraine is CGRP [196]. CGRP administration has been shown to induce migraine-like headaches that are indistinguishable from spontaneous migraine attacks [197, 198]. This has led to the discovery and use of CGRP antagonists that are effective in treating migraine symptoms [198].

PACAP has been shown to act in a similar way to CGRP, with infusion of PACAP inducing headaches in healthy volunteers [199]. Similarly, plasma levels of CGRP and PACAP are elevated following a migraine attack [200]. Interest in PACAP has a therapeutic target for migraine, stems from the discovery that PACAP-induced migraine did not results in an increase in CGRP, suggesting an alternate route of migraine initiation [201]. Human provocation studies have provided the most robust evidence describing the involvement of PACAP in migraine [201]. Intravenous administration of PACAP produced immediate headache in healthy participants, and delayed migraine in migraine sufferers [203]. Additionally, PACAP induced pronounced dilations of extra-

cranial arteries [202]. This dilation was further confirmed using magnetic resonance angiography that caused dilation of the middle meningeal artery in participants who reported migraine after PACAP infusion [203].

Despite sharing similar anatomical distribution and vasodilation properties to PACAP, the role of VIP in migraine remains controversial. Previously, it was suggested that VIP was unable to induce migraine [188]. Additionally, Bertels and colleagues, investigated the impact of PACAP and VIP on functional brain connectivity using functional magnetic resonance imaging, and revealed that PACAP, but not VIP, altered connectivity that coincided with the development of migraine in migraine patients [201]. However, recent studies have reported contrasting findings. Pellesi and colleagues, demonstrated that a 2-hour infusion of VIP could induce migraine attacks in 71% of patients with migraines without aura, similarly to PACAP [204]. They revealed that this induction was caused by dilation of cranial arteries that was mediated by VPAC1 and VPAC2 receptors, contradicting previous notions that migraine was induced via PAC1 receptor activation [204]. This could explain why novel therapeutics targeting PAC1 have had low efficacy in preventing migraine attacks in clinical trials [201, 205, 206]. Therefore, more research is needed to elucidate the specific role of each receptor in migraine induction and maintenance. For example, recent studies have proposed a link between mast cell degranulation and migraine pathology in PACAP-induced attacks, however the receptor mediating this effect has not been identified [207]. Chronic inflammation has been shown to promote the maintenance of pain states. As such, both migraine and neuropathic pain demonstrate the delicate balance of targeting PACAP and VIP in neurological disease due to their pleiotropic functions and global expression.

3. PACAP and VIP as a broad-spectrum therapy for MS and its associated comorbidities

The broad beneficial functions of PACAP and VIP in the CNS make them ideal neuroprotective agents, capable of promoting neuronal survival, function and protect neurons against inflammation. These peptides can be useful in a myriad of neurological diseases. In addition, as highlighted in this review, the VIP/PACAP system is clearly implicated in ameliorating several neurological comorbidities associated with MS (Fig. 2).

As highlighted in this review, in MS the VIP/PACAP system has shown to partly counteract autoimmunity. The neuropeptide system causes a shift from autoreactive T cells to anti-inflammatory state of T-cells by promoting the recruitment of anti-inflammatory Th2 and Treg cells as well as several other immunomodulatory effects (see 1.3.2; reviewed in [57, 208]). During the active stages of the disease, the MS brain shows signs of white matter inflammation in lesioned areas, which is believed to cause not only myelin damage, but also oligodendrocyte cell loss [49]. As discussed above, an increased inflammatory *milieu* in the CNS is also a major trigger of the comorbidities associated with MS. Strikingly, CNS white matter pathology has recently been suggested as a contributing factor to disease pathology in schizophrenia, epilepsy and stroke [89, 137, 209–215]. Thus, despite the different clinical presentations and domains of some of the pathologies discussed in this review, the underlying similarities at a molecular level highlight a potential link with a dysfunctional VIP/PACAP system and identify it as a valid target for therapeutic intervention that could span these disorders.

This idea is further substantiated by the overlapping positive effects of the VIP/PACAP system in MS and its comorbidities. The multiple actions of this neuropeptide system could mean that various downstream pathways are likely to be targeted. We predict that targeting this system could potentially ameliorate MS symptoms both by protecting oligodendrocytes, likely through a reduction of the pro-inflammatory CNS microenvironment, while also positively affecting comorbid conditions arising as the disease progresses. For example, for those MS patients who also experience strokes, targeting the VIP/PACAP system could stimulate neuroprotection and neurogenesis, inhibit apoptosis and promote axon regeneration, while also triggering an anti-inflammatory phenotype of activated glial cells, which could help preventing oligodendrocyte cell death [10, 216, 217].

PACAP and VIP have been tested in clinical trials as therapeutics for peripheral disorders such as arthritis [218], in which when injected intraperitoneally, the peptides reduced the incidence and severity of the disease, even when administered in the late stages [40]. Moreover, since data from the aforementioned migraine studies show that stimulating the VIP/PACAP system can lead to an increased incidence of migraines, several clinical trials are currently exploring the option of blocking VIP/PACAP activity in the brain to help migraine patients. Outside of the brain, there has been some success in using a VPAC2 agonist in asthma patients because of its bronchodilatory effects [219]. Of note, a recent study described the prospect of two well-known broad-spectrum antibiotics as potential compounds to target the PAC1 receptor. Doxycycline and Minocycline were shown to act as positive allosteric modulators of PAC1, stimulating axonal regeneration activities in cultured Schwann cells [191, 220]. These findings highlight that targeting the VIP/PACAP system is a feasible therapeutic approach for treating a spectrum of pathological conditions.

To achieve potential beneficial effects of PACAP and VIP in the CNS, passage through the blood brain barrier (BBB) must be improved. To overcome this issue, recently Yu and colleagues generated a VIP-TAT construct with enhanced efficiency to cross the BBB, which showed increased neuroprotection compared with VIP alone in an 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) mouse model of Parkinson's disease [221, 222]. Therefore, in view of the current advances in biotechnology, it is reasonable to anticipate more research into PACAP and VIP and more opportunities for

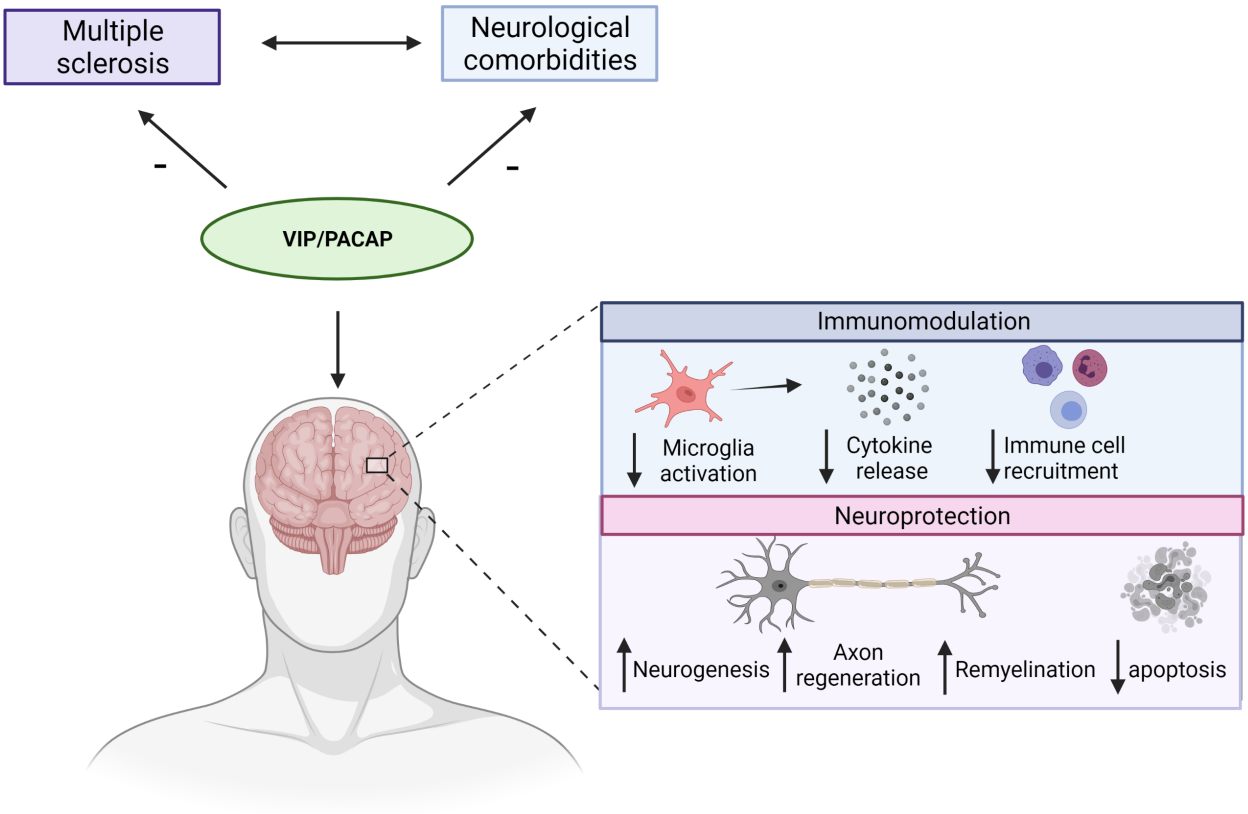

Fig. 2. Immunomodulation and neuroprotection functions of PACAP and VIP in multiple sclerosis and neurological comorbidities. Both PACAP and VIP are promising neuropeptides that can aid in MS and the associated neurological comorbidities of MS through the potent immunomodulatory actions and neuroprotective effects of both these peptides.

these peptides to be used as therapeutics for neurological and cognitive diseases.

However, it is important to recognize that targeting the VIP/PACAP system can come with its downsides. Since PACAP and VIP are pleiotropic molecules and can target different cell types within the CNS and periphery, it would require specialised administration strategies to deliver the peptides so that they can target the desired cell population or CNS region. Adeno-associated viruses and/or other vectors to deliver personalised gene therapy approaches are becoming closer to achieving the targeted administration of therapeutics to the brain via systemic route [223]. This could limit potential off-target effects by limiting the availability of peptides to, for example, the PAC1 receptor in cardiac cells [223].

Altogether, despite some yet to control side effects, the plethora of positive effects of both VIP and PACAP in the CNS justify the ongoing efforts to target this neuropeptide system using synthetic analogues or other technologies, with the aim to identify drug candidates with tropism towards selected CNS regions/tissues. This could result in an effective disease-modifying therapy able to improve MS disease course and prevent the development of most of the associated comorbidities.

4. Conclusion & future directions

The VIP/PACAP system appears to have protective benefits in MS and most of its associated neurological comorbidities, making it an attractive therapeutic target to pursue (Fig. 2). With the recent advancements in CNS-targeted drug administration, there is more flexibility in treatment options as new ways of overcoming the challenge of delivering drugs that pass through the BBB or that impede rapid degradation are being developed. As such, future research can now explore the potential of PACAP and/or VIP as targets for the treatment of a range of neurological diseases that involve inflammation and consequently, neurodegeneration.

Abbreviations

ACTH, adrenocorticotrophic hormone; BBB, blood brain barrier; BD, bipolar disorder; BNST, bed nucleus of the stria terminalis; CNS, central nervous system; PNS, peripheral nervous system; CGRP, calcitonin gene-related peptide; DISC1, disrupted-in-schizophrenia 1; EAE, experimental autoimmune encephalomyelitis; GPCR, G protein-coupled receptors; HPA, hypothalamic-pituitary-adrenal; IL, interleukin; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; MRI, magnetic resonance imaging; MS, multiple sclerosis; NK, natural killer cells; NPY, neu-

ropeptide Y; NSC, neural stem cells; NRG1, neuregulin-1; PACAP, pituitary adenylate cyclase-activating polypeptide; PNS, peripheral nervous system; SNPs, single nucleotide polymorphisms; RA, rheumatoid arthritis; VIP, vasoactive intestinal peptide; iNOS, inducible nitric oxide synthase; NO, nitric oxide.

Author contributions

MIJ and STB wrote the paper; AC conceived the study and revised the manuscript.

Ethics approval and consent to participate

Not applicable.

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Conflict of interest

The authors declare no conflict of interest.

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