The Effects of Neurotrophins and the Neuropeptides VIP and PACAP on HIV-1 Infection: Histories with Opposite Ends

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Key Words

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Abstract

The nerve growth factor (NGF) and other neurotrophins, and the neuropeptides vasoactive intestinal peptide (VIP) and pituitary adenylate cyclase-activating peptide (PACAP) are largely present in human tissue and can exert modulatory activities on nervous, endocrine and immune system functions. NGF, VIP and PACAP receptors are expressed systemically in organisms, and thus these mediators exhibit pleiotropic natures. The human immunodeficiency virus type 1 (HIV-1), the causal agent of the acquired immunodeficiency syndrome (AIDS), infects immune cells, and its replication is modulated by a number of endogenous factors that interact with HIV-1-infected cells. NGF, VIP and PACAP can also affect HIV-1 virus particle production upon binding to their receptors on the membranes of infected cells, which triggers cell signaling pathways that modify the HIV-1 replicative cycle. These molecules exert opposite effects on HIV-1 replication, as NGF and other neurotrophins enhance and VIP and PACAP reduce viral production in HIV-1-infected human primary

macrophages. The understanding of AIDS pathogenesis should consider the mechanisms by which the replication of HIV-1, a pathogen that causes chronic morbidity, is influenced by neurotrophins, VIP and PACAP, i.e. molecules that exert a broad spectrum of physiological activities on the neuroimmunoendocrine axis. In this review, we will present the main effects of these two groups of mediators on the HIV-1 replicative cycle, as well as the mechanisms that underlie their abilities to modulate HIV-1 production in infected immune cells, and discuss the possible repercussion of the cross talk between NGF and both neuropeptides on the pathogenesis of HIV-1 infection.

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Introduction

Infection by human immunodeficiency virus type 1 (HIV-1), the etiological agent of the acquired immunodeficiency syndrome (AIDS), is characterized by persistent viral replication in lymphoid tissue and a continuous

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decline in CD4⁺ T cell numbers. In the absence of anti-retroviral treatment, HIV-1 infection induces progressive deterioration in immune system function, promotes central nervous system (CNS) degeneration, increases host susceptibility to opportunistic infections and leads to severe immunodeficiency [1].

HIV-1 infects and replicates in CD4⁺ T lymphocytes, macrophages, dendritic cells and in CNS microglial cells. HIV-1 uses the CD4 molecule as the main cellular receptor to penetrate into the target cells; HIV-1 also requires a coreceptor, either CCR5 or CXCR4 (receptors for β - or α -chemokines, respectively) [2]. Several conditions or endogenous factors are well known to modulate viral replication in infected cells, and these factors can affect the viral load in HIV-1-infected individuals. For example, chronic immune system activation favors HIV-1 replication and is associated with accelerated infection progression to the clinical symptoms of immune deficiency that are typical of AIDS [3]. Likewise, many natural molecules can promote enhanced HIV-1 production or induce the opposite effect by diminishing HIV-1 growth in infected cells [4].

Neurotrophic factors are critical molecules for nervous system development and neuronal cell survival, and play important roles during the adult life. In mammals, the neurotrophin family includes nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin (NT)-3 and NT-4 and NT-5 [5]. NGF was the first identified neurotrophic factor, following the pioneering and seminal studies of Levi-Montalcini and Hamburger, who described in the early 1950s that NGF was a trophic and neuritogenic factor for dorsal root ganglion and sympathetic neurons [6]. The effects of NTs are not restricted to the nervous system, as they also regulate immune cell functions, modulate inflammatory responses and are associated with many other peripheral physiological effects [7, 8].

The neuropeptides vasoactive intestinal peptide (VIP) and pituitary adenylate cyclase-activating peptide (PACAP) are expressed in both the central and peripheral nervous system and are widely distributed throughout the organism [9]. VIP and PACAP share structural similarities with each other and with peptides from the secretin/glucagon peptide family, and both exert a large range of biological effects that target the nervous, endocrine, gastrointestinal, circulatory and immune systems [9]. Both neuropeptides can act as natural anti-inflammatory agents due to their ability to inhibit the activity or production of several proinflammatory molecules and to stimulate the production of anti-inflammatory mediators [9, 10].

Considering that HIV-1/host cell interactions and viral replication are influenced by numerous endogenous molecules with diverse biochemical natures and physiological roles, it is relevant to understand how HIV-1 infection is affected by NTs and the neuropeptides VIP and PACAP, which are endowed with a wide range of physiological activities. In this review, we will describe the main effects of these molecules on HIV-1 replication and discuss the mechanisms that underlie their abilities to modulate HIV-1 growth in infected immune cells, as well as the possible repercussion of the cross talk between NGF and both neuropeptides on the pathogenesis of HIV-1 infection.

HIV-1 Infection

According to the United Nations Program on HIV/AIDS, approximately 34 million people worldwide are infected with HIV [11]. HIV belongs to the Retroviridae family, in which genomic RNA is reverse transcribed to DNA by the retrovirus-typical enzyme reverse transcriptase, and to the Lentivirinae subfamily, which is associated with persistent infections and long clinical latency periods [12]. Regarding viral genetic diversity, HIV isolates have been classified into two types, HIV-1 and HIV-2, which have been further subclassified into groups and subtypes [see ref. 12 for more information on this subject]. Thereafter, in this review, we will refer to HIV-1, which is responsible for the worldwide pandemic and induces a more profound immunosuppression than HIV-2 [12].

The HIV-1 replicative cycle begins when the virus envelope glycoprotein gp120 interacts with its receptors on the cell membrane, followed by the fusion of the cell membrane with the viral envelope and the release of the viral nucleocapsid into the cell cytoplasm. Next, the viral RNA genome is transcribed by reverse transcriptase into double-stranded proviral DNA, which is then integrated into the host cell genome by the viral enzyme integrase. In response to a number of stimuli, the viral DNA is transcribed to produce new virus particles that are subsequently released into the extracellular environment via budding from the cell membrane [for a review of the HIV-1 replicative cycle, see ref. 13–16]. HIV-1 isolates are phenotypically classified according to the chemokine receptor to which they preferentially bind on the cell membrane during the infection process. Thus, the isolates are designated as R5-tropic, X4-tropic or R5X4 dual-tropic viruses, according to their interactions with CCR5,

CXCR4 or with either receptor, respectively. Individuals who carry a homozygous mutant allele with a 32-base pair deletion in the CCR5 gene (*ccr5Δ32*), which encodes a truncated protein that is not expressed in the cell membrane, are refractory to infection by R5-tropic HIV-1 isolates [17].

During the acute HIV-1 infection phase, due to viral propagation throughout the tissues, many viral reservoirs are established that consist mainly of memory CD4⁺ T cells and resident macrophages from the gastrointestinal lymphoid tissues (GALT) [18, 19]. These reservoirs of HIV-1-infected cells can sustain continuous viral replication for long periods of time, thus affecting HIV-1 evolution and genetic diversity in different compartments of the human body [20]. Today, identification of the mechanisms that underlie HIV-1 reservoir survival and maintenance is generally believed to be critical to the search for new and effective anti-HIV-1 therapeutic strategies [21–23].

One particular HIV-1 reservoir established early after seroconversion is found in the CNS and represents a constant threat to neuronal cells [24, 25]. In fact, studies of postmortem nervous tissues or animal models have shown that brain macrophages and microglia are permissive to productive HIV-1 and simian immunodeficiency virus (SIV) infections and can become viral reservoirs [26–28]. Persistent HIV-1 replication may occur even in the CNS of individuals with undetectable viral loads in the peripheral blood due to antiretroviral therapy [29]. Although HIV-1 does not replicate in neurons, its productive infection in other cell types leads to the release of neurotoxic viral proteins, such as Tat, Nef, Vpr and gp120 [30–33]. These viral proteins induce neuronal apoptosis and affect axonal projections to their targets, impairing neuronal plasticity and function [34–41]. Moreover, the chronic immune activation due to HIV-1 proteins and other antigens (as discussed below) results in a persistent proinflammatory condition in the CNS with concomitant homing of anti-HIV-1-specific CD8⁺ T lymphocytes to this site, which may accentuate the cell death [42]. The nervous tissue damage during the course of HIV-1 infection is also aggravated by the systemic increase in proinflammatory cytokines, such as tumor necrosis factor (TNF)- α , interleukin (IL)-1 β and IL-6, which induce oxidative stress in neuronal cells and modify the permeability of the blood-brain barrier [43–46].

Before the introduction of highly active antiretroviral therapy, an elevated number of HIV-1-infected patients presented a panel of severe neurocognitive symptoms that were designated as HIV-1-associated dementia, the onset of which correlated with the viral load and infection

time course [47]. Today, despite better HIV-1 infection management, neurocognitive disorders continue to be diagnosed, although these conditions may be dissociated from viral load levels and CD4⁺ T cell counts. Since highly active antiretroviral therapy allows HIV-1-infected individuals to live longer, age-associated neurocognitive disorders converge with HIV-1-persistent replication in the CNS reservoirs, resulting in an increased risk factor for injuries in this compartment [48]. In contrast to the era before highly active antiretroviral therapy, the newly recognized CNS impairments present as asymptomatic forms or with only mild symptoms [49]. These dysfunctions are now grouped as asymptomatic neurocognitive impairments or mild neurocognitive disorders that, together with HIV-1-associated dementia, comprise the complex designated as HIV-1-associated neurocognitive disorders [50]. Neurocognitive injuries can be recognized by a collection of signs, such as behavioral alterations, forgetfulness, nerve pain, motor control impairment, headache and depression, which are associated with anatomical alterations in the brain [50].

Several conditions contribute to maintain or increase viral loads in HIV-1-infected individuals. For example, persistent immune system activation due to HIV-1 proteins, co-infecting agents or microbial products translocated from the intestinal lumen favors HIV-1 replication and can accelerate infection progression from an asymptomatic state to the clinical symptoms of immunosuppression typical of AIDS [51]. Chronic immune system stimulation elevates the number of HIV-1-permissive cells, promotes a more rapid turnover of T cells and eventually provokes a severe loss of CD4⁺ T lymphocytes, which is the hallmark of HIV-1 infection and AIDS [51]. The degree of immune system activation has now been considered the best predictor of HIV-1 disease progression, and decreased viral loads in HIV-1-infected patients who receive antiretroviral drugs are associated with steady reductions in systemic immune activation levels [52]. Many experimental studies have also consistently shown that endogenous molecules can promote enhanced HIV-1 production, including the cytokines TNF- α , IL-1 β , IL-6, macrophage migration inhibitory factor and NGF, whereas other natural soluble mediators can induce the opposite effect by diminishing HIV-1 growth, including the cytokines IL-10, IL-27 and interferon (IFN) α/β , the β -chemokines CCL3, CCL4 and CCL5, and the neuropeptides VIP and PACAP [53–61].

The final outcome of an HIV-1-productive infection can also depend on the balance between innate natural factors that can limit viral replication or release and

HIV-1 accessory genes that neutralize the antiretroviral effects of these natural restriction factors [62]. Lately, some IFN-stimulated products have been described as intrinsic restriction factors against HIV-1 replication. For example, the cytidine-deaminase enzymes apolipoprotein B mRNA-editing enzyme catalytic polypeptide-like (APOBEC) 3G/3F/3A are incorporated into virus particles, where they induce guanosine → adenosine mutations in the nascent viral DNA during RNA reverse transcription [63]. These hypermutations generate defective virions that are incapable of producing new rounds of successful infections [63]. APOBEC proteins can also inhibit HIV-1 independently of their catalytic activity, as they bind to viral DNA and prevent its processing by the HIV-1 enzyme integrase, thus reducing the integration of HIV-1 DNA to the host cell genome [64, 65]. The APOBEC anti-HIV-1 effects are antagonized by the viral protein Vif through the induction of APOBEC degradation [63]. The bone marrow stromal cell antigen 2 protein (BST2/CD317; also known as tetherin) restricts the budding of mature HIV-1 viral particles by retaining the virions in the membrane of the infected cell [66]. This anti-HIV-1 activity is counterbalanced by the virus proteins Vpu and Nef [67, 68]. Three members of the IFN-induced transmembrane family of proteins (IFITMs) also naturally hinder HIV-1 replication by mechanisms not yet clearly defined, although recent findings suggest that these factors interfere with the production of viral proteins [69, 70]. It is not known how HIV-1 counterbalances the antiviral effects of IFITMs. In dendritic cells and other myeloid-derived cells, the sterile α motif domain and HD domain-containing protein 1 (SAMHD1) restricts HIV-1 and HIV-2 replication through the hydrolysis of deoxynucleoside triphosphates into deoxynucleosides and triphosphates [71], therefore depleting the deoxynucleoside triphosphate pool and diminishing the efficiency of both, HIV-1 RNA reverse transcription and provirus transcription. SAMHD1 has also a 3'-5' exonuclease activity [71–73], which possibly contributes for its anti-HIV-1 effects, which are counteracted by the HIV-2 protein Vpx through promoting SAMHD1 degradation. Macrophages and dendritic cells release much less HIV-1 particles than lymphocytes, which may be partially explained by the activity of viperin (also known as RSAD2), a cell membrane-interacting protein induced by viral infection and IFNs [74–76]. Viperin interferes with the virus budding process of various viruses, including HIV-1 [77–81], through inhibition of FPPS, an enzyme involved in the maintenance of lipid-raft and lipid-protein interaction/modification

[79, 82]. It is not yet known how HIV-1 escapes from the viperin effects. Recently, a new IFN-stimulated HIV-1 restriction factor was identified, the myxovirus resistance 2 protein (MX2), which restricts both HIV-1 and simian immunodeficiency virus replication by blocking provirus integration in the cell genome, possibly by its interaction with the capsid region of the viral protein Gag [83].

NGF, Other NTs and HIV-1 Infection

NGF is the most studied NT and the one from which the most findings related to the role of neurotrophic factors in HIV-1 infection have been obtained. This molecule is synthesized as a 305-amino acid precursor protein and is cleaved to derive the mature 118-amino acid structure; the biologically active NGF molecule is formed as a dimer with subunits of approximately 13 kDa [84, 85]. The signaling pathways activated by NGF depend on two distinct receptor types, tropomyosin-related kinase (Trk) and p75^{NTR} [84, 85]. Trk receptors belong to the tyrosine kinase group of receptors, and their activation promotes cell survival and differentiation [84, 85]. NTs preferentially bind three Trk isoforms, such that NGF activates TrkA, BDNF and NT-4/NT-5 engage TrkB, and NT-3 binds TrkC [84, 85]. Due to the existing homology between NGF and the other NTs and between the TrkA receptor and other receptor family members, a certain degree of promiscuity exists between the ligands and receptors; for example, NT-3 can also activate the TrkA and TrkB receptors [85]. The NGF-TrkA interaction promotes the dimerization and transphosphorylation of the TrkA tyrosine residues and triggers a signaling cascade that culminates in the differentiation and survival of several cell types [84]. Once phosphorylated, TrkA can activate other intracellular proteins such as nuclear factor (NF)- κ B, phosphoinositide 3-kinase, protein kinases PKC and PKA and the mitogen-activated protein kinases (MAPKs) and modulate different signaling pathways [84–86].

The Trks and p75^{NTR} receptors can trigger either synergistic or antagonistic actions or function independently according to the target cell [87, 88]. Similar to the TNF receptor superfamily, p75^{NTR} contains a death domain in its intracellular region [89]. The death domain mediates interactions with other cellular components that allow p75^{NTR} to regulate intracellular signaling events, such as NF- κ B, JNK and caspase activation, in response to NT ligation [90].

NTs play an important role in the establishment of the neuronal population throughout development [91, 92]. They are produced by nerve target cells and can determine axonal migration; neurons that assemble efficient synapses and retain NTs are more likely to survive [92]. In adult animals, cells continue to produce NTs and express NT receptors, suggesting that these neuromodulators contribute to internal homeostasis throughout the life of an organism [93, 94]. Today, it is well known that the effects of NTs are not restricted to the central and peripheral nervous systems. In fact, these molecules affect neuroimmunoendocrine interactions by activating the hypothalamic-pituitary-adrenal axis and thus regulate inflammatory responses [7, 8, 95]. Increased serum NGF and corticosteroid levels are associated with chronic stress events, and NGF synthesis is induced by the cytokines IL-1, IL-6, TNF- α and transforming growth factor- β during the course of an inflammatory reaction [8, 96]. NGF regulates the immune system by promoting myeloid precursor growth and maturation, inducing B and T lymphocyte proliferation and B cell differentiation into plasma cells, and stimulating immunoglobulin and eicosanoid production [97–101]. Following NGF treatment, CD4+ T cells produce and release this NT, suggesting that NGF participates in lymphocyte survival and functioning [102, 103]. NGF is also associated with neutrophil chemotaxis, mast cell degranulation and differentiation, and the induction of monocyte cytotoxic activity [104–107].

Some pathogens have established curious interactions with NTs. For example, human syncytial respiratory virus, the main causal agent of bronchiolitis in children up to 6 months old, stimulates the expression of NGF and its receptor [108, 109]; herpes simplex virus (HSV-1), which can provoke mucocutaneous lesions, keratoconjunctivitis and encephalitis, establishes long latent periods in the trigeminal ganglia in an NGF-TrkA interaction-dependent fashion [110, 111]; the protozoan parasite *Trypanosoma cruzi*, the etiological agent of Chagas disease, expresses a transsialidase that mimics mammalian NTs and is named parasite-derived neurotrophic factor due to its ability to engage Trk receptors and promote host cell survival [112, 113], and the NTs NGF, proNGF and BDNF facilitate HIV-1 replication in human monocyte-derived macrophages, as we will discuss further in this review.

The initial suggestions that NTs could interfere in the HIV-1 replicative cycle came from independent studies performed in the mid-1990s. Ensoli et al. [114], using a model that might be representative of pediatric infection as a consequence of vertical transmission, observed that NGF can stimulate HIV-1 replication in neuroblastic and

embryonic glial cell lines, which are immature cells of the CNS. Recio and Aranda [115], by means of transfecting HIV-1 long-terminal repeats in NGF-induced neuronal-differentiated PC12 pheochromocytoma cells, showed that this NT promoted a strong activation of HIV-1 gene transcription in a Ras/Raf-dependent fashion. Although defining studies, the actual significance of their findings could be limited due to the evidence that neurons are refractory to HIV-1 productive infection. In the late 1990s, Levi-Montalcini and her group performed their investigation using human primary macrophages, providing a more comprehensible understanding of how NGF affects the HIV-1 interaction with a naturally virus-producing cell. They demonstrated that HIV-1-infected macrophages expressed elevated levels of NGF and the TrkA receptor, and that anti-NGF neutralizing antibodies reduced HIV-1 production and induced macrophage apoptosis [116]. In summary, this study unequivocally showed that NGF could act as an autocrine survival factor for HIV-1-infected macrophages, but left open the hypothesis that NGF could also directly facilitate HIV-1 replication in primary macrophages.

In a more recent investigation, we reported that NGF (as well as BDNF and proNGF) enhanced HIV-1 replication in human primary monocyte-derived macrophages via PKC, NF- κ B, extracellular signal-regulated kinase and p38 kinase-dependent signaling pathways that were triggered by the NGF-TrkA interaction [57]. We also reported that NGF increased HIV-1 proviral DNA transcription, eventually leading to increased HIV-1 production in macrophages. NGF also inhibited the synthesis of the endogenous HIV-1 restriction factor APOBEC3G (A3G), thus reducing G $\rightarrow$ A hypermutation events in the viral genome and retaining viral fitness.

Taken together, these studies revealed that NGF is not only an essential factor for HIV-1-infected macrophage survival and an enhancer of viral transcription and replication, but is also a critical mediator that permits cellular HIV-1 reservoirs to produce virus particles with a preserved replicative capacity and infectivity. These findings are summarized in figure 1. Samah et al. [117, 118], however, described opposite results by observing not only that NGF failed to facilitate HIV-1 replication in macrophages, but also that HIV-1 production decreased in a NGF-treated monocyte population that migrated in response to the chemokine CXCL12. NGF, which is produced by perivascular macrophages and multinucleated giant cells during HIV-1-induced encephalitis, stimulates monocytes to express CXCR4, the receptor for CXCL12. Thus, although their and our studies differ in the culture

Fig. 1. NTs and the neuropeptides VIP and PACAP present opposing effects on HIV-1 replication. The center of the figure shows a simplified model of the HIV-1 replicative cycle (as described in main text). NTs (left side) enhance HIV-1 replication in macrophages by means of Trk receptor activation and by triggering multiple signaling pathways that culminate with concomitant increases in viral transcription and production. The NGF-mediated downregulation of APOBEC3G synthesis and activity allows the production of HIV-1 virions with preserved replicative capacity. The neuropeptides VIP and PACAP (right side) inhibit HIV-1 production in macrophages through upregulation of cell produc-

tion of β -chemokines and IL-10, after engaging VPAC1/2 receptors by VIP, and VPAC1/2 and PAC1 by PACAP. In macrophages and CD4+ T cells, the sole activation of VPAC2 (Bay 55-9837 [200], helodermin [201], R3P55 [202] and RO 25-1553 [203]) and PAC1 (maxadilan [182]) by specific agonists also decreases HIV-1 replication, whereas ligation of VPAC1 only (secretin [180] and ALA-VIP [181]) results in an opposite effect. PIP2 = Phosphatidylinositol 4,5-bisphosphate; DAG = diacylglycerol; IP3 = inositol 1,4,5-trisphosphate; Ras/ERK = rat sarcoma/extracellular signal-regulated kinase).

conditions, activating factors added to cells and the time point at which NGF was added to monocytes or macrophages with respect to HIV-1 infection, they are to some extent complementary, as HIV-1-infected macrophages are constantly exposed to numerous stimuli and respond according to the mediators present in the cell microenvironment.

The HIV-1 proteins Tat, Nef, gp120 and Vpr can cause neuronal loss, either due to proapoptotic stimuli or by impairing axonal projections and neuronal plasticity/function, especially in sympathetic neurons [34–41]. NT signaling through Trk receptors has been associated with anti-apoptotic activity and with enhancement of synaptic connections, and many works, using different cellular

models and treatment strategies, have shown that NGF and BDNF are able to recover neuronal plasticity impaired by HIV-1 proteins and to rescue neuronal cells from HIV-1-induced apoptosis [119–122]. Clinical trials in which NGF was used as a treatment for HIV-associated distal sensory polyneuropathy showed that this therapy was safe and well tolerated, and significantly relieved the pain symptoms. Nevertheless, no amelioration of neuropathy severity was recorded [123, 124].

Although the results of such studies point to neuroprotective effects of both NTs with obvious benefits for patients with AIDS-associated dementia, the therapeutic use of NGF or BDNF should be examined carefully due to the enhancer activities of NGF and other NTs on HIV-1 replication [57, 125]. Thus, it is critical to identify the ideal balance between the neuroprotective and the HIV-1-productive infection-promoting effects of NTs.

The Neuropeptides VIP and PACAP and HIV-1 Infection

VIP, a 28-amino acid molecule that was initially identified in the swine small intestine, is endowed with numerous biological effects and can cause systemic vasodilation, increased cardiac output, hypotension and hyperglycemia [126]. VIP also modulates the functions of the immune system and the central and peripheral nervous systems, and acts as an endogenous anti-inflammatory agent [127, 128]. PACAP was first isolated from the ovine hypothalamus and was named for its ability to activate the enzyme adenylate cyclase and induce the production of cyclic adenosine monophosphate in anterior pituitary cells [129]. PACAP shares 68% amino acid sequence homology with VIP and exists in two isoforms, which are designated PACAP-27 and PACAP-38 according to their amino acid numbers [130–136]. PACAP (predominantly PACAP-38) is widely expressed in the CNS and peripheral tissues, and can play a large number of modulatory roles in the nervous tissue and the immune system [10, 137].

VIP and PACAP share cellular receptors, which are named VPAC1, VPAC2 and PAC1. PAC1 recognizes both PACAP isoforms with high affinity and VIP with low affinity, whereas VPAC1 and VPAC2 are engaged with similarly high affinities by both neuropeptides [138–141]. Thus, VPAC1 and VPAC2 can mediate the effects of VIP and PACAP with the same efficiency, whereas PAC1 is activated primarily by PACAP. These three receptors belong to the G protein-coupled receptor com-

plex and thus present the typical structure of seven transmembrane domains joined by intra- and extracellular loops [140, 142–144]. VPAC1 and VPAC2 can also present an uncommon five transmembrane structure derived from alternative splicing, and PAC1 can also express a high number of spliced isoforms; all these isoforms are related to tissue/cell expression and differential signaling activation [145, 146]. VPAC1, VPAC2 and PAC1 are widely distributed in the tissues and, within the immune system, VPAC1 and VPAC2 are expressed in lymphocytes, macrophages and dendritic cells, whereas PAC1 expression is limited to macrophages [147–152]. No systematic studies have explored the expression of these receptors in other immune cells. The binding of VIP and PACAP to their receptors activates signaling pathways that involve cyclic adenosine monophosphate/PKA and diacylglycerol/inositol 1,4,5-triphosphate/PKC that, in turn, can trigger the activation of other signaling cascade proteins, such as phosphoinositide 3-kinase, Src, MAPKs, Jak/STAT and NF- κ B [153].

VIP and PACAP are produced and expressed in the central and peripheral nervous systems and in many other sites, including the gastrointestinal and urinary tracts and glandular tissues [154, 155]. Both peptides are found in lymphoid tissues (e.g. thymus and spleen), in which thymocytes and CD4⁺ T helper (Th) 2 and CD8⁺ T lymphocytes are their main sources [132, 149, 156, 157]. VIP and PACAP regulate metabolic and endocrine activities and gastrointestinal tract functioning, and act as immunomodulatory and anti-inflammatory agents [127, 128, 137]. These peptides participate in neural development and exert neuroprotective effects due to their ability to protect nervous tissue from injuries caused by a number of neurotoxic agents, such as ethanol, hydrogen peroxide and the HIV-1 gp120 [158–161].

One of the most prominent VIP and PACAP features concerns their role as natural anti-inflammatory agents, a property that has been experimentally well demonstrated in a variety of pathological conditions, including autoimmune and infectious diseases and the prevention of graft-versus-host disease [162, 163]. The anti-inflammatory effects of VIP and PACAP derive from the ability of both neuropeptides to inhibit the activity or production of several proinflammatory molecules (such as chemokines, reactive oxygen and nitrogen species, IL-12, IL-6, IL-1 β , TNF- α and macrophage migration inhibitory factor), in addition to their capacity to stimulate the production of anti-inflammatory factors (e.g. IL-4 and IL-10) [127, 128, 137]. VIP and PACAP also participate in Th2 cell differentiation by inhibiting IL-12 production and in-

ducing IL-4 and IL-5 synthesis in macrophages and dendritic cells [127, 128, 137]. Moreover, VIP- or PACAP-treated macrophages stimulate CD4⁺ T cells to produce the Th2 cytokines IL-4 and IL-5 and inhibit the ability of the same cells to synthesize the Th1 cytokines IFN- γ and IL-2 [127, 128, 137]. Additionally, dendritic cells differentiated in vitro in the presence of VIP exhibit tolerogenic properties, such as producing high amounts of IL-10 and inducing the differentiation of lymphocytes to T regulatory cells [127, 164].

VIP and PACAP also exert their anti-inflammatory effects by reducing cellular expression of the Toll-like receptors (TLRs), components of the innate immune system whose activation by pathogen molecules or endogenous ligands results in potent proinflammatory reactions [165, 166]. VIP reduces TLR2 and TLR4 expression on CD4⁺ T cells in mice with experimental colitis and on the synovial cells of rheumatoid arthritis patients, and also downregulates TLR expression on macrophages activated by cognate TLR agonists [167–169]. PACAP reduces TLR2, TLR3, TLR4 and TLR6 expression, and also regulates MyD88 and NF- κ B activation induced upon TLR engagement in models of renal ischemia and traumatic brain injury [170–172].

The small homology (five amino acids) between the peptide sequences of VIP and the variable region V2 of HIV-1 gp120 suggested that VIP and PACAP or their receptors might somehow participate in the pathogenesis of AIDS, based on the possibility that VIP could block HIV-1 entry into target cells through direct interaction with the CD4 molecule [173, 174] or that gp120 binding to VIP receptors could modify cellular processes related to the clinical course of HIV-1 infection [175, 176]. For example, some authors proposed that HIV-1 gp120 interaction with VIP receptors could be associated with HIV-1-induced neuron damage, through disrupting VIPergic networks and glial function [176]. This hypothesis was soon disregarded on the basis of the VIP inability to bind liver, intestinal and colonic tissues [177]. However, because of the current knowledge that VIP receptors present a plethora of isoforms, including those expressed in the CNS, the initial suggestion needs to be reevaluated.

Some experimental studies have addressed the role of VIP and PACAP in HIV-1 biology and viral production in target cells by using specific agonists of VPAC1, VPAC2 and PAC1 or native VIP and PACAP molecules. These investigations showed that, whereas productive HIV-1 infection can be facilitated by the sole activation of VPAC1, viral production is reduced in HIV-1-infected cells either following the engagement of VPAC2 or PAC1

by specific ligands or when infected cells are treated with VIP and PACAP [61, 178, 179]. These effects are summarized in figure 1.

Initial studies reported that VPAC1 activation by secretin, a hormone peptide with affinity for VPAC1 [180], resulted in increased HIV-1 replication in CD4⁺ T cell lines and peripheral blood mononuclear cells [179], a phenomenon that was more recently found in primary monocyte-derived macrophages using ALA-VIP [61], a synthetic ligand of VPAC1 [181]. The sole VPAC1-specific engagement could occur by secretin or by the putative interaction of VPAC1 with the HIV-1 gp120 [173], but this last possibility needs to be tested. Together, these findings suggest that, in the absence of simultaneous VPAC2 and PAC1 activation, VPAC1 engagement sustains HIV-1 growth in immune cells. On the other hand, similar studies that used several specific ligands of the VPAC2 receptor (as shown in fig. 1) demonstrated that the isolated activation of this pathway reduced HIV-1 proviral DNA integration into the lymphocyte genome and also diminished viral growth in peripheral blood mononuclear cells, CD4⁺ T cell lines and in monocyte-derived macrophages [61, 178]. Likewise, reduced HIV-1 production was also found in HIV-1-infected macrophages after stimulation of PAC1 with maxadilan [61], a *Lutzomyia longipalpis*-derived specific ligand of this receptor [182]. We should note that, up to now, there is no evidence of natural sole activation of VPAC2 and PAC1 receptors in cells targeted by HIV-1.

The opposing effects on HIV-1 replication that are elicited by the isolated activation of these three receptors converge only to inhibit viral production when HIV-1-infected cells are treated with VIP or PACAP, or with both neuropeptides simultaneously, meaning that under natural conditions, the engagement of VPAC1 and VPAC2 by VIP, or of both receptors plus PAC1 by PACAP, might ultimately reduce HIV-1 production in the target infected cells [61]. Interestingly, the combined use of specific agonists to mimic receptor ligation by natural neuropeptides resulted in reduced HIV-1 replication to a similar degree as the VIP- and PACAP-induced HIV-1 inhibition [61].

In addition to their ability to reduce HIV-1 replication, VIP and PACAP might also counteract other harmful effects caused by HIV-1 infection. In experimental studies, VIP restored the impaired cytotoxic function of natural killer cells that were exposed to the HIV-1 gp120, and both neuropeptides protected nervous tissue from gp120-induced toxic effects [160, 161, 183]. Interestingly, the neuroprotection offered by VIP and PACAP is derived

from their capacity to stimulate the synthesis and release of the β -chemokines CCL3 and CCL5, which, together with the cytokine IL-10, are associated with the ability of VIP and PACAP to inhibit HIV-1 replication [61, 160, 161].

An understanding of the effects resulting either from the individual activation of VPAC1, VPAC2 and PAC1, or from their engagement by the natural ligands VIP and PACAP on HIV-1 replication, is relevant to the development of anti-HIV-1 therapies based on VIP and PACAP, as well as alternative molecules that can block or activate the receptors to control HIV-1 propagation in tissues. Finally, it is important to indicate that additional studies should be conducted to evaluate the effects of VIP and PACAP, as well as those of receptor agonists, on viral production in primary CD4⁺ T lymphocytes, which are the most significantly affected cells in HIV-1-infected individuals.

Cross Talk between NGF and VIP/PACAP and Possible Repercussions on HIV-1 Pathogenesis

Several studies have demonstrated a cross-modulatory relationship between NGF and the neuropeptides VIP and PACAP at the levels of receptor gene transcription, release and transactivation. Additionally, the interdependent actions of NGF, VIP and PACAP have been described as responsible for the proliferation, differentiation and survival of nervous system cells, as well as the regulation of neurotransmitter release from the same cells.

In the scenario of a cross relationship between these mediators, many other effects can be quoted. For example, VIP promotes the survival of sympathetic neuroblasts during early developmental stages, in which it acts as a trophic agent for the cell progenitors before NGF production is initiated [184]; PACAP and NGF synergize to induce the postnatal differentiation and maturation of cholinergic neurons from the basal forebrain and to regulate the activity of these neurons through the induction of choline acetyl-transferase mRNA isoforms [185]; together, PACAP and NGF also promote the differentiation of neuronal classes and recruit transcriptional factors involved in the sympathetic-adrenal differentiation process [186, 187].

NGF, VIP and PACAP regulate each other, and thus form a complex functional network. Studies in animal models and tumor cell lines showed that NGF activates the PACAP and PAC1 promoters, indicating that NGF regulates PACAP-stimulatory activity towards its target

cells [188, 189]. NGF is also a potential regulator of VIP, as it induces VIP expression in the rat suprachiasmatic nucleus during senescence and after chronic ethanol treatment (conditions in which the VIP levels are reduced in this site), thus restoring VIP production to initial levels [190, 191].

In addition, VIP and PACAP also regulate the expression of NGF and its receptors. For example, in a rat model of Parkinson's disease, VIP protects motor functions by inducing, among other mechanisms, NGF release from brain mast cells [192]; likewise, VIP enhances NGF gene expression and the release of both precursor and mature NGFs in human keratinocytes [193]. PACAP induces the expression of the NGF TrkA receptor via PAC1 activation [194] and can also transactivate the TrkA receptor in both neurons and monocytes [195–197], thus triggering TrkA receptor-related signaling pathways.

The ability to transactivate TrkA confers to PACAP the ability to mimic the trophic actions of NGF and thus maintain the integrity of the nervous tissue in the event of loss of neurotrophic primary factors. Studies of neurons also showed that PACAP-transactivated receptors are located mainly within the cell [195], thus highlighting the neurotrophic role of PACAP, which can act continuously, even during NT receptor turnover. Finally, both PACAP-38 and PACAP-27 have been described as VIP gene expression regulators [198, 199], suggesting that a reciprocal regulation might exist between both neuropeptides, although, to date, no reports have shown that VIP can modulate PACAP expression.

Thus, based on the neuromodulatory activities of NGF, VIP and PACAP, and their effects on the HIV-1 replicative cycle, it is possible to predict that the associated actions of these molecules could influence the pathogenesis of HIV-1 infection in nervous tissue. HIV-1 infects microglial cells and brain-resident macrophages, leading to the local release of neurotoxic HIV-1 proteins, such as gp120, Nef and Tat. Thus, considering the apoptotic potential of HIV-1 gp120 on nervous cells, the ability of VIP and PACAP to induce β -chemokine production (which inhibits gp120 binding to cell receptors) by astrocytes and microglial cells, along with the neuroprotection offered by NGF, suggests that these molecules can cooperate to counterbalance the harmful effects of HIV-1 gp120 on nervous cells. The abilities of VIP and PACAP to act as endogenous anti-inflammatory agents and of PACAP to transactivate the TrkA receptor, thus mimicking NGF and offering neuronal cell survival, could eventually protect nervous tissue from the local inflammatory responses triggered by the toxic HIV-1 proteins Nef and Tat.

Limitations and Perspectives

Considering the systemic aspects of HIV-1 infection, the better understanding of the ability of the NT-neuropeptide network to modulate viral replication in HIV-1 cell reservoirs might bring new challenges for researchers devoted to develop new strategies to control HIV-1 tissue propagation and the infection progress, due to the knowledge pointing to the relevance of these mediators on the physiopathology of HIV-1 infection. The systemic physiological effects resulting from the cross talk between NGF and VIP/PACAP should be considered as potential targets for therapeutic interventions based on the ability of endogenous molecules to restrain HIV-1 replication and propagation in the peripheral tissues and CNS.

Based on the anti-apoptotic activity of NTs via Trk signaling, models intended to rescue neuronal cells from apoptosis induced by HIV-1 proteins have been developed with the aim to provide therapeutic benefits to patients with HIV-1-induced neurocognitive disorders. However, an ideal balance between the neuroprotective effects of NTs and their abilities to promote HIV-1-productive infections should be pursued to provide safe therapies for HIV-1-infected individuals.

The current paradigms of HIV-1-induced cell dysfunction and tissue damage are largely based on the use

of ex vivo cell infections, mechanistic insights derived from mouse and nonhuman primate models, and post-mortem analyses. The difficulties to access the CNS and to collect samples from living HIV-1-infected individuals and the absence of robust animal models to study HIV-1-induced neuropathogenesis represent critical obstacles to significantly improve the knowledge in this field. Nonetheless, we wonder whether, prospectively, an association between the neuroprotective effects of NGF and VIP/PACAP and the anti-inflammatory and anti-HIV-1 activities of both neuropeptides might offer an alternative approach to control HIV-1 infection progression and to prevent the development of cognitive dysfunction secondary to HIV-1 propagation to nervous tissue.

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