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MINI REVIEW

Role of mu-opioids as cofactors in human immunodeficiency virus type 1 disease progression and neuropathogenesis

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Abstract About one third of acquired immunodeficiency syndrome cases in the USA have been attributed to the use of injected addictive drugs, frequently involving opioids like heroin and morphine, establishing them as significant predisposing risk factors for contracting human immunodeficiency virus type 1 (HIV-1). Accumulating evidence from in vitro and in vivo experimental systems indicates that opioids act in concert with HIV-1 proteins to exacerbate dysregulation of neural and immune cell function and survival through diverse molecular mechanisms. In contrast, the impact of opioid exposure and withdrawal on the viral life cycle and HIV-1 disease progression itself is unclear, with conflicting reports emerging from the simian immunodeficiency virus and simian–human immunodeficiency virus infection models. However, these studies suggest a potential role of opioids in elevated viral production. Because human microglia, astrocytes, CD4+ T lymphocytes, and monocyte-derived macrophages express opioid receptors, it is likely that intracellular signaling events triggered by morphine facilitate enhancement of HIV-1 infection in these target cell populations. This review highlights the biochemical changes that accompany prolonged exposure to and withdrawal from morphine that synergize with HIV-1 proteins to disrupt normal cellular physiological functions especially within the central nervous system. More importantly, it collates evidence from epidemiological studies, animal models, and heterologous cell systems to propose a mechanistic link between such physiological adaptations and direct modulation of HIV-1 production. Understanding the opioid–HIV-1 interface at the molecular level is vitally important in designing better treatment strategies for HIV-1-infected patients who abuse opioids.

Keywords Opioids · HIV-1 · Cyclic AMP (cAMP)

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Introduction

Opioids are one of the major classes of addictive drugs, heroin being the most abused opioid. Injection of both heroin and its most active metabolite, morphine, puts abusers at a high risk for contracting human immunodeficiency virus type 1 (HIV-1). In fact, more than one third of acquired immunodeficiency syndrome (AIDS) cases reported in the USA have been attributed to injection drug

use (Donahoe and Vlahov 1998; Hauser et al. 2005). During addiction, heroin abusers may inject up to four times a day (Clinic 2010), exposing themselves to doses as high as 2 g per day (Jessop and Taplits 1991). Chronic opioid administration leads to the establishment of dependence in vivo, and drug cessation in abusers gives rise to withdrawal symptoms (Rahim et al. 2004). Evidence is accumulating that in addition to increasing the risk of exposure to HIV-1, high levels of opioids in the circulation can accelerate the progression to AIDS (Bell et al. 2002; Donahoe and Vlahov 1998). Furthermore, opiate-addicted individuals have a higher incidence of HIV-associated dementia and have more severe neurocognitive and pathological abnormalities (Nath et al. 2002).

Opioids such as heroin and morphine stimulate cells through the interaction of the drug with the μ-opioid receptor present on the cell surface. Stimulation of the μ-opioid receptor triggers a signaling cascade that alters viral gene expression and hence HIV-1 infection and replication. This review discusses the connections between the μ-opioid signaling pathway and HIV-1 replication and proposes implications of this link in overall HIV-1 disease pathogenesis. In addition, opioids have been shown to act synergistically with HIV-1 neurotoxic proteins such as Tat and gp120, enhancing their neurotoxic effects. It has also been observed that HIV-1 patients abusing opioids have an increased occurrence of neurocognitive disorders. Therefore, this review also discusses the impact of μ-opioids on HIV-1 central nervous system (CNS) disease and neurotoxicity.

Opioids and opioid receptors

Opioids are classified pharmacologically as analgesics and neurotransmitters (Pleuvry 1991). Endogenous opioids are peptides derived from precursor molecules and were discovered initially in nervous system tissue (Pleuvry 1991). Endogenous opioids can be divided into four families of peptides based on the precursor gene: (1) enkephalins, including Met-enkephalin (Tyr-Gy-Gly-Phe-Met) and Leu-enkephalin (Tyr-Gy-Gly-Phe-Leu), which are pentapeptides derived from proenkephalin (Dhawan et al. 1996); (2) dynorphins, including big dynorphin, dynorphin A and B, and α/β-neoendorphin, which are all derived from prodynorphin (Khalap et al. 2005; Merg et al. 2006); (3) β-endorphin, which is derived from the precursor molecule proopiomelanocortin (Pleuvry 1991); and (4) endomorphin-1 (Tyr-Pro-Trp-Phe-NH_2) and endomorphin-2 (Tyr-Pro-Phe-Phe-NH_2), which are tetrapeptides presumed to be derived from a larger precursor molecule that has not yet been identified (Zadina et al. 1997). Because endogenous opioids are prone to peptidase-induced degradation, experimental studies use exogenous synthetic peptides, such as D-Ala^2,*N*-Me-Phe^4,Gly^5-ol-enkephalin (DAMGO), that are resistant to the action of peptidase (Dhawan et al. 1996) and therefore retain their biological activity.

Opioid receptors are members of a superfamily of seven transmembrane domain receptors that are coupled with G_i/G_o pertussis toxin-sensitive G proteins (hence, G-protein-coupled receptors). This association was established during their initial identification by radioactive ligand binding assays (Pert and Snyder 1973). Based on the chromosomal position and "signature motifs" in their genes, opioid receptors have been categorized in the rhodopsin family within the G-protein-coupled receptor superfamily (Fredriksson et al. 2003; Perez 2003). The four families of endogenous opioids have distinct selectivity profiles for the opioid receptors (μ, δ, or κ). Enkephalins have a higher affinity for the δ-opioid receptor (DOR), dynorphins bind with higher affinity to the κ-opioid receptor (KOR), and β-endorphin and endomorphins bind to the μ-opioid receptor (MOR) with the highest affinity (Dhawan et al. 1996; Merg et al. 2006). Morphine, DAMGO, and the most commonly used opioid receptor antagonist naloxone bind to all three classes of opioid receptors but have the greatest affinity for the MOR (Martin-Kleiner et al. 2004). MORs, DORs, and KORs are encoded by three different genes, *mor*, *dor*, and *kor*, respectively, each characterized by alternate splice variants (Pan 2003; Pan et al. 2000; Zimprich et al. 1995). These three genes have a similar genomic structure which is characterized by three highly conserved exons. Exon I encodes transmembrane domain 1, exon II encodes transmembrane domains 2 to 4, and exon III encodes transmembrane domains 5 to 7. MOR contains an additional exon, IV, coding for the last 18 amino acids of the protein, and KOR contains an additional exon prior to the conserved exon I, which encodes for a portion of its 5′-UTR (Law et al. 2004; Wei and Loh 2002).

The MOR signaling mechanism

Binding of ligand to the G-protein-coupled receptor results in a signaling cascade whose result depends on whether the interaction is by acute or chronic stimulation. Acute activation of the $G\alpha_i$-coupled MOR elicits multiple downstream signaling cascades (Law et al. 2000), resulting in a decline in the levels of intracellular cyclic adenosine monophosphate (cAMP) via adenyl cyclase (AC) inhibition (Figs. 1 and 2a) (Ozawa et al. 1999), an increase in phospholipase C activity resulting in transient enhancement in cytosolic Ca^{2+} levels (Spencer et al. 1997), inhibition of N-type (Tallent et al. 1994) and L-type voltage-gated (Piros

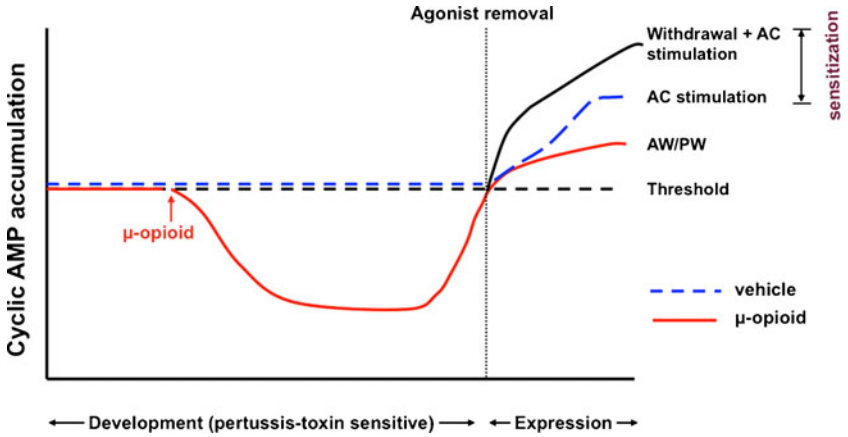


Fig. 1 Hypothetical model for $G\alpha_{i/o}$-coupled receptor-induced heterologous sensitization. Whereas acute activation of $G\alpha_{i/o}$-coupled opioid receptors leads to inhibition of cAMP accumulation, prolonged (chronic) activation enhances adenyl cyclase-mediated cAMP response when the opioid agonist is removed. The supersensitivity of adenyl cyclase, also termed heterologous sensitization, manifests itself as cAMP overproduction. *AC* adenyl cyclase, *AW* abrupt withdrawal, *PW* precipitated withdrawal

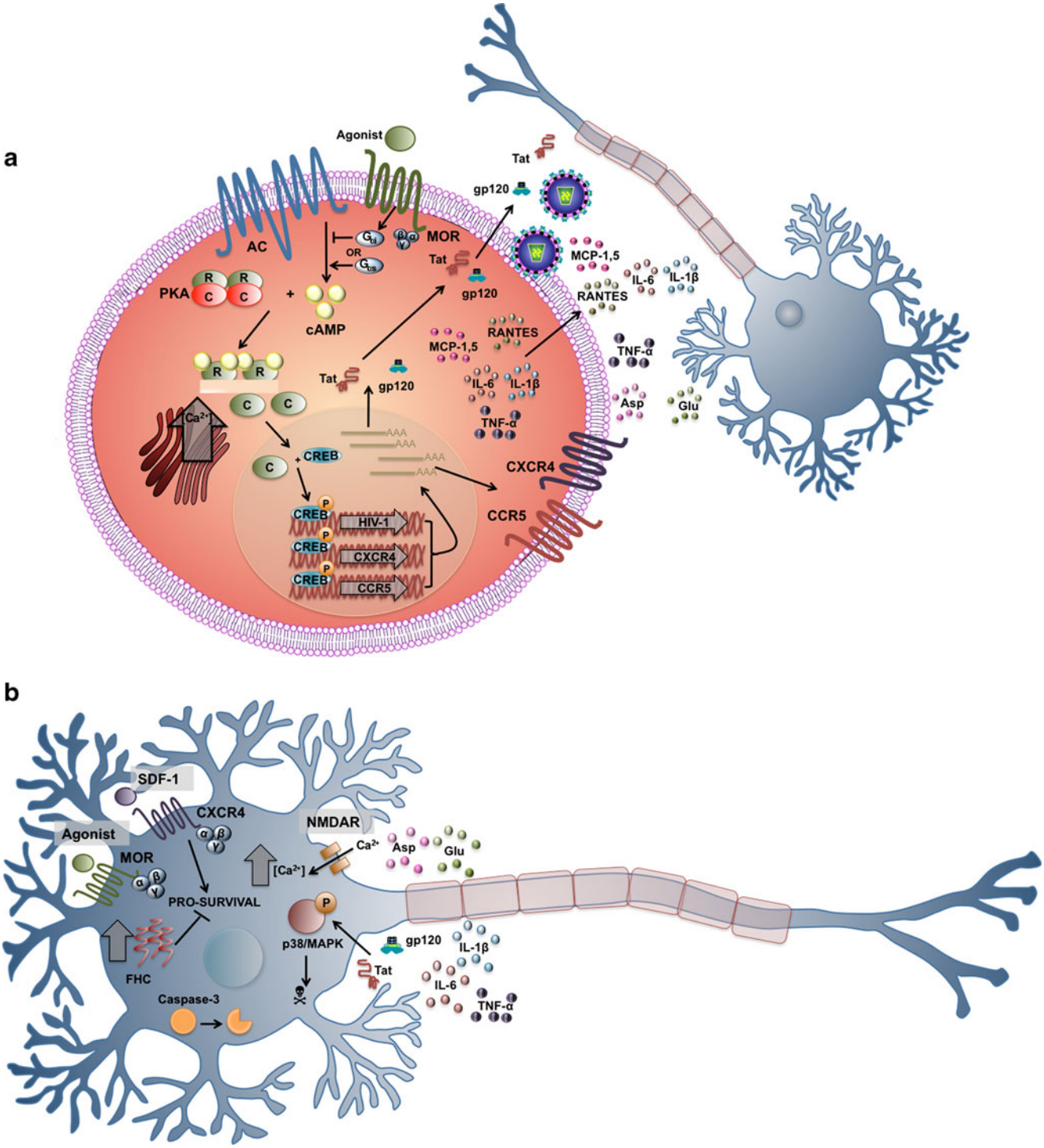
et al. 1995) Ca^{2+} channels (preventing Ca^{2+} influx), stimulation of inward-rectifying K^+ channels (Henry et al. 1995), and activation of the mitogen-activated protein kinase (MAPK), ERK1/2 (Fukuda et al. 1996).

In contrast to acute activation, sustained stimulation of MOR through chronic morphine exposure increases AC activity (Belcheva et al. 2001; Chao and Nestler 2004), and the activity is further enhanced on agonist withdrawal (Liu and Anand 2001) (Figs. 1 and 2a). Agonist cessation or withdrawal can be classified as either abrupt withdrawal, which is agonist removal, or precipitated withdrawal, which is agonist removal coupled with treatment with an opioid antagonist such as naloxone. The phenomenon of upregulation of the cAMP signal transduction system during agonist cessation or withdrawal is referred to as supersensitization or heterologous sensitization of AC and is thought to represent a cellular adaptive mechanism following sustained $G\alpha_i$-receptor activation (Watts 2002). AC stimulation and the subsequent increase in cAMP trigger the PKA signaling cascade. Binding of cAMP to the paired regulatory "R" subunits of the inactive PKA holoenzyme releases the catalytic "C" subunits. The functionally active C subunits passively diffuse into the nucleus and phosphorylate the Ser133 residue (Gonzalez and Montminy 1989) in the P-box region of the cAMP response element binding (CREB) protein family of transcription factors. The CREB family comprises transcription factors that contain a carboxy terminus basic domain/leucine zipper motif and binds to a specific DNA *cis* element designated the cAMP response element (Montagne et al. 1990). Once it is phosphorylated, CREB is able to associate with CREB binding protein (CBP) and its paralog p300 (Kwok et al. 1994; Parker et al. 1996). In addition to interacting with the RNA polymerase II complex (Kee et al. 1996; Nakajima et al. 1997), CBP acetylates nucleosomal histone tails due to its intrinsic histone acetyltransferase activity, thereby leading to derepression or enhancement of target gene promoters (Korzus et al. 1998), inducing gene expression.

Given this pathway, the following factors have been proposed to contribute to the development of heterologous sensitization: specificity of a G protein α subunit coupling ($G\alpha_{i1}/G\alpha_{i2}/G\alpha_{i3}/G\alpha_0$) to the MOR; change in expression, localization, or interaction of $G\alpha_i$ versus $G\alpha_s$ relative to AC; G protein βγ subunit interaction with AC; and protein kinase A (PKA) expression, increased expression of AC, and AC isoform specificity. Following chronic morphine exposure, specific increases in AC1, AC8, and PKA expression have been observed (Lane-Ladd et al. 1997). Therefore, with respect to HIV-1 pathogenesis, chronic drug users potentially have an increase in HIV-1 transcription leading to an increase in toxic viral protein production, viral replication, and accelerated pathogenesis, discussed in detail below.

The role of μ-opioids in HIV-1/AIDS disease pathogenesis

High levels of illicit drugs including opioids in the circulation of an HIV-1-infected patient can impact disease progression. In fact, slower disease progression was noted in an HIV-1-infected cohort when drug use was disrupted (Ronald et al. 1994). However, epidemiological studies involving opiate abusers have been plagued with complicated design strategies, including interpretation of data based on improperly defined variables, such as polydrug abuse (instead of examining each drug-abuse group in isolation); dose regimens (stability of dependency and intensity of withdrawal alter levels of stress, which in turn modulate progression of HIV-1/AIDS); parameters of



measuring progression of HIV-1/AIDS (most studies use only CD4 count without additional clinical parameters such as viral load) (Donahoe 2004); and associated neuropathological manifestations (neurocognitive defects and behavioral alterations). Another confounding factor in such studies is the potential interaction between opiates and highly active antiretroviral therapy (HAART), wherein one drug could possibly alter the metabolic disposition or clearance of the other (Faragon and Piliero 2003).

μ-Opioids alter viral replication in immune cell populations

To work around these difficulties, many studies of μ-opioids and HIV-1 pathogenesis have been done using

◀ **Fig. 2** Morphine signaling in an HIV-1-infected CNS resident cell alters the transcriptional profile of the cell and enhances neurotoxicity. **a** Acute exposure of an infected perivascular macrophage or microglial cell within the CNS to morphine will activate the receptor-coupled $G\alpha_i$ subunit leading to inhibition of adenyl cyclase (*AC*) and decreased levels of cAMP. However, under prolonged exposure, a $G\alpha_s$ subunit associates with the μ-opioid receptor (*MOR*) and becomes activated, activates AC, and increases levels of cAMP. Protein kinase A (*PKA*) exists in the cytoplasm in an inactive form as two regulatory (*R*) subunits associated with two catalytic (*C*) subunits. Binding of cAMP to the R subunits causes activation and dissociation of the C subunits, which can then enter the nucleus where they can phosphorylate the transcription factor cAMP response element binding (*CREB*) protein, and the now active CREB binds cAMP response element (*CRE*) sequences within the promoter region of the target genes. These target genes include the HIV-1 LTR, leading to increased viral transcription, as well as the HIV-1 coreceptors CXCR4 and CCR5, leading to increased expression of the receptors on the cell surface, potentially enhancing infection by HIV-1. Cytoplasmic Ca^{2+} levels also increase. Increased viral synthesis; increased production and secretion of the viral proteins gp120 and Tat along with host proteins RANTES, MCP-1,5, IL-1β, IL-6, and TNF-α; and increased levels of glutamate and aspartate in the CSF impact surrounding neurons. In addition, morphine can act synergistically with these viral proteins to further increase these signaling cascades. **b** High levels of Tat, gp120, IL-1β, IL-6, and TNF-α lead to the phosphorylation and activation of p38/MAPK, triggering a signaling cascade resulting in apoptosis of the neuron. The increased presence of aspartate and glutamate can lead to excitotoxicity through increased binding of either amino acid to the *N*-methyl-D-aspartic acid receptor (*NMDAR*), causing an influx of Ca^{2+}, which can either trigger an independent apoptotic cascade or enhance activation of p38/MAPK, also resulting in neuronal apoptosis. Direct exposure of neurons to morphine causes an increase in cleaved, active caspase-3 and an increase in ferritin heavy chain protein (*FHC*), which can inhibit the prosurvival signaling cascade triggered by SDF-1 binding CXCR4, both of which may contribute to neuronal cell death

both in vitro culture and in vivo nonhuman primate systems (Table 1). Apart from several studies linking persistent or chronic opioid use to immunomodulation (McCarthy et al. 2001) and increased susceptibility to bacterial infections (Wang et al. 2005), evidence from morphine-pretreated peripheral blood monocyte cocultures showed increased HIV-1 replication, which suggests that they have a role as potential cofactors in the pathogenesis associated with HIV-1 infection (Peterson et al. 1990). Further studies demonstrated that the expression of HIV-1 in cocultures of the U1 promonocytic cells and human brain cells increased after exposure to morphine (Peterson et al. 1994). In fact, prolonged treatment with morphine or the selective μ-opioid agonist DAMGO enhanced the percent of T cells and monocytes expressing HIV-1 coreceptors CXCR4 and CCR5, respectively, likely mediating the observed increase in viral replication in vitro (Steele et al. 2003). Related studies have demonstrated that morphine enhances HIV-1 R5 strain infection of macrophages through the down-regulation of β-chemokine production and upregulation of CCR5 receptor expression (Guo et al. 2002; Li et al. 2003). However, the molecular basis for this increase in coreceptor expression and concomitant enhancement in susceptibility and/or replication is not known. In addition, morphine treatment of T cells during infection with simian immunodeficiency virus (SIV) also demonstrated an increase in SIV replication and syncytia formation (Chuang et al. 1993). In vitro studies involving cells of the CNS, like those involving cells of the immune system, have shown increased HIV-1 replication when treated with opioids (Chao et al. 1995; Peterson et al. 1999). Specifically with respect to μ-opioids, endomorphin-1 potentiated viral expression in a bell-shaped dose–response manner in both mixed glial/neuronal cell and purified microglial cell cultures (Peterson et al. 1999). Interestingly, few in vitro studies have been published to date that directly test the effect of morphine on HIV-1-infected cells of the CNS. One such study has demonstrated in mouse microglial cells that morphine treatment could enhance CCR5 expression as well as induce an activated cell phenotype (Bokhari et al. 2009). In another study, heroin was shown to potentiate HIV-1 replication in normal human astrocytes (Reynolds et al. 2006).

To further clarify the role of opiate exposure in the progression of HIV-1/AIDS, researchers have used nonhuman primate models of morphine and SIV/simian–human immunodeficiency virus (SHIV) infection. Studies performed in the rhesus macaque model with SIV_{mac239} demonstrated that long-term treatment with morphine adversely affected T cell and polymorphonuclear-mediated immune functions while exacerbating the rate of viral replication over that of saline controls (Chuang et al. 2005). In a separate study with SIV_{smm9}, although morphine exposure followed by agonist-precipitated withdrawal with naloxone transiently increased circulating numbers of SIV-infected T cells (Donahoe et al. 1993), an overall decrease in the severity and rate of simian AIDS progression was observed (Donahoe et al. 1993). Although the outcomes of these studies are inconsistent because of small sample sizes, variability in the virulence of the viral strain used and variability in the amount of opiate per injection cycle in both studies prevented the researchers from drawing any firm conclusions. More recently, infection with a triple combination of SIV/17E-Fr, $SHIV_{89.6}P$, and $SHIV_{KU}$ strains reported rapid disease progression with high plasma viremia, a large decline in the numbers of circulating $CD4^+$ T cells, and rapid ablation of the adaptive immune response in 50% of morphine-dependent macaques as well as high cerebrospinal fluid (CSF) viral loads and marked neuropathogenesis, culminating in mortality by 20 weeks post-infection not observed in the nonaddicted, infected macaques (Kumar et al. 2006, 2004). The most recent study to address the impact of morphine in a nonhuman primate model (Bokhari et al. 2011) infected Indian rhesus macaques with SIVmacR71/17E and demonstrated the

Table 1 μ-Opioids impact HIV-1 infection, replication, and pathogenesis

Observation	Model system	Reference(s)
In vitro		
Increased HIV-1 viral replication	In vitro monocyte cocultures pretreated with morphine before infection with HIV-1	Peterson et al. 1990
Increased SIV_{mac239} viral replication and syncytia formation	In vitro CEM×174 T cells treated with morphine during SIV-1 infection	Chuang et al. 1993
Increased HIV-1 expression	U1 promonocytic cells cocultured with brain cells treated with morphine	Peterson et al. 1994
Increased HIV-1 expression	Mixed glial/neuronal cell and purified microglial cell cultures treated with endomorphin-1	Peterson et al. 1999
Enhanced R5 strain infection of macrophages through the downregulation of β-chemokine production and upregulation of CCR5 receptor expression	In vitro MDM culture pretreated with morphine for 12 h before and throughout infection with HIV-1; RT activity measured on day 8; β-chemokine production, on day 4; and CCR5 expression, at 24 h	Guo et al. 2002; Li et al. 2003
Increased CXCR4 and CCR5 expression levels on T cells and monocytes; increased viral replication	In vitro T cell and monocyte cultures pretreated with morphine or DAMGO before infection with HIV-1	Steele et al. 2003
In vivo		
Increased circulating SIV-infected T cells; decreased severity and rate of simian AIDS progression	Macaques infected with SIV_{smm9}, morphine exposure and agonist-precipitated withdrawal	Donahoe et al. 1993
Slower overall disease progression when drug use ceased	In vivo using an HIV-1-infected cohort	Bell et al. 2002
Increased incidence and severity of HIV-1 encephalitis	Human autopsy samples from opiate-abusing, HIV-1-infected patients	Bell et al. 2002
Rapid disease progression, high plasma viremia, decreased circulating $CD4^+$ T cells, high cerebrospinal fluid viral load, increased neuropathogenesis, mortality by 20 weeks	Morphine-dependent macaques infected with a triple combination of SIV/17E-Fr, $SHIV_{89.6}P$, and $SHIV_{KU}$ strains	Kumar et al. 2004, 2006
Increased viral replication	Macaques infected with SIV_{mac239}, treated with long-term morphine	Chuang et al. 2005
Exhibited a trend towards higher mortality rates, retardation in weight gain, higher plasma and CSF viral loads, higher numbers of circulating CD4+ and CD8+ T cells but CD4/CD8 ratios remained unchanged, increased virus build-up in the brains along with an increased influx of infected monocyte/macrophages in the brain	Macaques infected with SIVmacR71/17E and treated four times daily, at 6-h intervals, at a dose of 3 mg/kg	Bokhari et al. 2011

macaques, which received morphine and virus (M+V), exhibited a trend towards higher mortality rates, retardation in weight gain, and higher plasma and CSF viral loads. Interestingly, the M+V group demonstrated higher numbers of circulating CD4+ and CD8+ T cells; however, the CD4/CD8 ratios between the groups remained unchanged. In addition, a subset of M+V animals succumbed to disease within weeks postinfection, had higher incidence of other endorgan pathologies and therefore were classified as rapid progressors. The M+V animals but especially the rapid progressors also exhibited a trend toward increased virus build-up in the brains along with an increased influx of CD68+ infected monocyte/macrophages in the brain. Evidence linking opioid abuse to HIV-1 neuropathogenesis in humans includes autopsy samples from the Edinburgh HIV-1/AIDS cohort, which have shown that in AIDS patients, HIV-1 encephalitis was more likely to be found in opiate abusers than in non-drug abusing homosexual men (Bell et al. 2002), further implicating opioid use and/or withdrawal in amplifying neurological dysfunction. Collective insight gained as a result of these studies suggests that chronic morphine exposure and withdrawal play a role in dysregulating the immune system, thereby acting as cofactors to hasten viral production and peripheral and neurological disease progression.

μ-Opioids impact viral replication through cAMP–PKA signaling

As stated previously, the cell signaling pathway most affected by the stimulation of the MOR is the cAMP–PKA pathway. However, the mechanism through which viral production is impacted has not yet been elucidated. Interestingly, the HIV-1 long terminal repeat (LTR), which serves as the viral promoter, contains a cAMP response element (CRE) sequence upstream of the transcription start site, immediately 5′ of the CCAAT-enhancer-binding protein site I (Krebs et al. 1997). The

CRE sequence is termed the activating transcription factor (ATF)/CREB site (TGC***TGACATCG***AGC). This site has been shown to bind members of the ATF/CREB family of proteins differentially, depending on cellular phenotype and binding site sequence (Krebs et al. 1998; Ross et al. 2001). Given this result, one hypothesis is that morphine directly induces HIV-1 transcription through upregulation of ATF/CREB factors that bind the CRE element, resulting in increased viral replication (Fig. 2a). We recently demonstrated that the phosphoactivating transcription factor/CREB protein binds to this site in response to specific cAMP activators like forskolin (Banerjee and Wigdahl, unpublished observations).

As stated previously, studies have also shown that morphine induces expression of CXCR4 and CCR5 on T cells, monocyte–macrophages, and microglial cells (Bokhari et al. 2009; Guo et al. 2002; Li et al. 2003; Steele et al. 2003). More recently, studies have identified a functional cAMP response element within the CCR5 promoter (Kuipers et al. 2008). They show specifically that the contribution of IRF-1, NF-κB, and CREB-1 to the transcriptional regulation of the chemokine receptor CCR5 involves only CREB-1 and is neither induced nor modulated by IRF-1 and NF-κB in T lymphocytes. Experiments performed in our laboratory using the TF-1 hematopoietic progenitor cell line have demonstrated that CCR5 transcription follows an asymmetrical sinusoidal pattern in response to an increase in the concentration of intracellular cAMP (Banerjee et al. 2011). Taken together, these results indicate the potential for the cAMP/CREB pathway to also regulate expression of CCR5 on the cell surface. With respect to this hypothesis, studies have shown that a CRE element within the CXCR4 promoter partially regulates CXCR4 transcription in response to changes in cAMP signaling (Cristillo et al. 2002a, b). Overall, these studies would then allow for a two-tiered impact on HIV-1 infection and replication through MOR stimulation, the first involving increased coreceptor expression and the second centered on increased HIV-1 LTR activity/replication.

In support of this observation, following chronic exposure to morphine, increases in cAMP levels have been observed in the nucleus accumbens (Nestler 1997, 2001; Nestler and Aghajanian 1997), part of functionally related nuclei comprising the basal ganglia and is therefore anatomically located in the region of greatest HIV-1 concentration in the brain (Wiley et al. 1998). This topographical overlap between cAMP accumulation and HIV-1 aggregation supports the possibility that opioid exposure/withdrawal might also serve to modulate HIV-1 gene expression and/or replication in susceptible CNS cell populations, thereby potentiating the direct neurotoxic effects of the virus.

Impact of opioids on HIV-1 neurotoxicity

Within the CNS, perivascular macrophages and microglia are the major sites of viral RNA, protein, and virion production in the brain (Nath 1999). However, many of the observed neurocognitive deficits result from neuronal death caused by cytotoxic factors secreted from infected cells rather than from the direct viral infection of neurons, which is not likely to occur. Beyond the discussed impact of μ-opioids on the viral load within the CNS, μ-opioids can directly enhance neurotoxicity either by acting on the neuron directly or by enhancing HIV-1 replication in infected cells of the CNS, thereby inducing secretion of known HIV-1 neurotoxic proteins (Tat, Nef, gp120, and Vpr) or induction of other potentially toxic products such as proinflammatory cytokines, glutamate, arachidonic acid, reactive oxygen species, and nitric oxide. Although neurons display the greatest abundance of MORs (Patel et al. 2006), it is widely believed that they are not direct targets of HIV-1 infection due to the paucity of CD4 cellular receptors. MORs have been observed on perivascular macrophages and resident glial cells including astrocytes and microglia (Chao et al. 1997; Hauser et al. 2006; Ruzicka et al. 1995; Stiene-Martin et al. 1998), the major sites of virion and viral protein production in the brain (Nath 1999) as well as on circulating monocytes (Chuang et al. 1995), which likely play an important role in the continuous viral seeding of the CNS.

With respect to the direct impact of opioids on the neuron in the context of the potential impact on HIV-1 neurotoxicity, it has been shown that morphine or the MOR agonist DAMGO can interact directly with neurons. Specifically, DAMGO treatment of neurons has been shown to inhibit intracellular signaling pathways (ERK1/2 and Akt) activated by CXCL12 abolishing the neuroprotective effect of CXCL12 (Patel et al. 2006). In addition, chronic exposure to morphine caused an upregulation of ferritin heavy chain protein (FHC), a negative intracellular regulator of CXCR4, thereby inhibiting prosurvival signals through CXCR4 (Sengupta et al. 2009). Initial studies in postmortem human tissue suggest that opiate abuse increases FHC levels approximately tenfold within the frontal cortex and that FHC is found within neurons (Pitcher et al. 2010). Furthermore, an increase in FHC-positive cells was found in the frontal cortex of HIV-1-infected patients affected by neurological deficits (HIV-1/HAD) along with a reduction in phospho-CXCR4 (pCXCR4), suggesting that FHC is induced in HIV-1 neuropathology and associated with a disruption in CXCR4 signaling (Pitcher et al. 2010).

Opioids in the CNS also interact with infected cells, causing increases in viral replication and viral protein production and secretion, and act synergistically or

additively with the viral proteins, which also have neurotoxic effects (Fig. 2). Morphine synergizes with Tat and gp120 to dysregulate intracellular Ca^{2+} homeostasis in astrocytes (El-Hage et al. 2008; Mahajan et al. 2005). In combination with Tat, morphine induced high levels of active caspase-3 in neurons (Bruce-Keller et al. 2008); however, this increase did not directly correlate with increased neuronal apoptosis (Bruce-Keller et al. 2008) despite the observed synergistic increase in neuronal apoptosis (Gurwell et al. 2001); therefore, this mechanism should be studied further to gain a full understanding of this cascade. In a more recent study, exposure to both morphine and Tat in combination induced morphological changes in microglia from a quiescent to an activated morphology, with a dramatic increase in the expression of the microglial activation marker CD11b, inducible nitric oxide synthase (iNOS), CD40 ligand, interferon-gamma-inducible protein 10 (IP-10), and the proinflammatory cytokines TNFα, IL-1β, and IL-6 (Bokhari et al. 2009). Morphine also has been shown to promote gp120 neurotoxicity through activation of p38 MAPK (Hu et al. 2005), which is necessary for gp120-mediated neuronal death (Kaul et al. 2001). The p38 MAPK signaling pathway is also responsible for increased secretion of arachidonic acid and TNF-α from macrophages (Kaul et al. 2001). It has been suggested that morphine in combination with gp120BaL, a CCR5-utilizing protein, increased secretion of CCL5 from astrocytes leading to neuroprotection (Avdoshina et al. 2010); however, a strong decrease in neuronal viability was not observed following exposure to gp120BaL alone, and therefore, the neuroprotection factor was low.

In addition to viral proteins, the levels of other potentially toxic products such as proinflammatory cytokines, glutamate, arachidonic acid, reactive oxygen species, and nitric oxide (Persidsky and Gendelman 2003) that are elevated during HIV-1 infection of the brain can also be modulated by opioids (Fig. 2a). High levels of arachidonic acid impair glutamate uptake in astrocytes, enhancing already increased levels of glutamate (Kaul and Lipton 2006). Glutamate and aspartate levels in the CSF have been shown to be increased following morphine exposure (Tai et al. 2006), and high levels of the two stimulated the *N*-methyl-D-aspartic acid receptor (NMDAR) on neurons at an enhanced level, leading to excitotoxicity involving an influx of Ca^{2+}, activation of p38 MAPK, and release of cytochrome c (Kaul and Lipton 2004). Patients receiving long-term opioid treatment have significantly increased levels of IL-6 in their CSF compared with patients on short-term treatment (Zin et al. 2010), and increased secretion may be from glial cells. Glial cells are highly activated following combined exposure to morphine and Tat (Bokhari et al. 2009; Bruce-Keller et al. 2008) and secrete increased levels of TNF-α, IL-1β, and IL-6 (Sawaya et al. 2009; Tai et al. 2006). Increased TNF-α has been shown to be induced by morphine through an NF-κB pathway (Sawaya et al. 2009). Increased IL-6 secretion within the CNS may act to enhance an inflammatory state; however, due to the pleiotropic nature of IL-6, further studies should be done on the exact role played. Exposure of astrocytes to morphine and Tat increased production of proinflammatory chemokines, including monocyte chemoattractant protein-1 (MCP-1), MCP-5, and RANTES (El-Hage et al. 2005), which will recruit additional monocytes and lymphocytes to the site of infection. These newly recruited cells may either already be infected, trafficking in more virus, or may become infected once in the CNS. Despite this, there is evidence that MCP-1 has neuroprotective functions against Tat-mediated neuronal apoptosis (Eugenin et al. 2003).

Concluding remarks

This review provides the current evidence that demonstrates a strong relationship between the use of μ-opioids, especially morphine, and HIV-1 infection/replication, disease progression, and HIV-1 neuropathogenesis. Although some nonhuman primate work suggests adding a note of caution in this regard, overall, the research results in the nonhuman primate and humans point to this conclusion. With respect to the impact of μ-opioids on neuropathogenesis, the studies reviewed here depict an increase in opioid-mediated virus production that can further enhance the neurotoxic effects of HIV-1 by exacerbating the secretion of by-products and viral proteins from microglia and astrocytes that leads to secondary destabilization of neurons, ultimately leading to neuronal injury or death. The known HIV-1 neurotoxic proteins Vpr and Nef have not been studied in combination with μ-opioids, leaving unanswered the question of whether the neurotoxicity of these proteins is also enhanced like that of Tat and gp120.

Another understudied area with respect to the effects of μ-opioids on HIV-1 infection/replication that impacts not only peripheral disease but also CNS disease is the cellular developmental pathways in the bone marrow. It has been suggested that bone marrow is an important anatomical sanctuary for HIV-1 (Alexaki et al. 2008; Alexaki and Wigdahl 2008; Carter et al. 2010; Clements et al. 2011; McNamara and Collins 2011). This supposition is corroborated by the observation that levels of both cell-free (Regez et al. 2005) and cell-associated virus (McElrath et al. 1989) in bone marrow are similar to those in the blood. In fact, studies have shown that HIV-1-specific sequences from bone marrow resemble sequences from the deep white matter of the brain, more so than any other tissue, suggesting direct HIV-1 trafficking from the bone marrow

followed by rapid movement into the CNS (Liu et al. 2000), most likely due to the continuous bone marrow-derived monocyte–CNS macrophage turnover. In fact, a recent study in the SIV rhesus macaque system demonstrated that an increased rate of monocyte recruitment from the bone marrow into the blood correlates with rapid progression to AIDS and that the magnitude of the $BrdU^+$ monocytes in the blood correlated with the severity of encephalitis (Burdo et al. 2010). Because MORs have been identified on $CD34^+$ hematopoietic progenitor cells in the bone marrow (Steidl et al. 2004), it is plausible that μ-opioids might orchestrate enhanced generation of viral particles from these $CD4^+CXCR4/CCR5^+$ hematopoietic precursors or enhance the rate at which these cells traffic to the peripheral blood and end organs. Therefore, a μ-opioid-induced increase in viral replication in hematopoietic progenitor cells in the bone marrow could allow for enhanced viral loads in the CNS.

This review also points to the hypothesis that the cAMP–PKA–CREB signaling cascade might serve as at least one of the missing molecular link(s) between chronic opioid exposure/withdrawal and HIV-1 production in opioid receptor-positive HIV-1-infected cellular populations. It would be interesting to elucidate whether the PKA–CREB cascade triggered by μ-opioids in the CNS is involved in regulating the expression of these intracellular signaling mediators through putative CREB sites in their promoters. Exploring the PKA–CREB nexus may provide the elusive molecular basis of μ-opioid-mediated exacerbation of HIV-1 production in the periphery and of the secretion of by-products from microglia and astrocytes that leads to secondary destabilization, neuronal injury, and apoptosis in the CNS. This connection would then trigger a feedback loop progressively involving other neighboring cells either within or outside the CNS. Because opioid receptors have been identified on macrophages, microglia, and astrocytes (Hauser et al. 2006) as well as on terminally differentiated mature immune cell populations, including human $CD4^+$ T cells, monocyte–macrophages, polymorphonuclear cells (Chuang et al. 1995), and cells of bone marrow origin, this hypothesis underlines a plausible, novel mechanism to explain the role of opioids as cofactors in HIV-1 pathogenesis, both in the CNS and in the periphery.

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