Published in final edited form as:

J Cell Physiol. 2012 July ; 227(7): 2832–2841. doi:10.1002/jcp.24004.

Epigenetics of μ -Opioid receptors: Intersection with HIV-1 infection of the Central Nervous System

Patrick M. Regan¹, Rajnish S. Dave¹, Prasun K. Datta¹, and Kamel Khalili^{1,*}

Department of Neuroscience, Center for Neurovirology, Temple University School of Medicine, 3500 North Broad Street, 7th Floor, Philadelphia, PA 19140

Abstract

The abuse of intravenous drugs, such as heroin, has become a major public health concern due to the increased risk of HIV-1 infection. Opioids such as heroin were originally identified and subsequently abused for their analgesic effects. However, many investigations have found additional effects of opioids, including regulation of the immune system. As such, chronic opioid abuse has been shown to promote HIV-1 pathogenesis and facilitate HIV-1-associated neurocognitive dysfunction. Clinical opioids, such as morphine and methadone, as well as illicit opioids, such as heroin, exert their effects primarily through interactions with the μ -opioid receptor (MOR). However, the mechanisms by which opioids enhance neurocognitive dysfunction through MOR-mediated signaling pathways are not completely understood. New findings in the regulation of MOR expression, particularly epigenetic and transcriptional regulation as well as alternative splicing, sheds new insights into possible mechanisms of HIV-1 and opiate synergy. In this review, we identify mechanisms regulating MOR expression and propose novel mechanisms by which opioids and HIV-1 may modulate this regulation. Additionally, we suggest that differential regulation of newly identified MOR isoforms by opioids and HIV-1 has functional consequence in enhancing HIV-1 neurocognitive dysfunction.

Keywords

CNS; neurotoxicity; HIV-1; epigenetics; morphine; alternative splicing; neurons

Introduction

The abuse of intravenous drugs, such as heroin, contributes substantially to the global HIV-1 epidemic, with an estimated 20% of injection drug users (IDUs) infected with HIV-1. The direct contribution of intravenous (IV) drug abuse to HIV-1 infection is about 30% worldwide, excluding sub-Saharan Africa, and is the third most frequently reported risk factor for HIV-1 infection in the United States (CDC, 2009; Vlahov et al., 2010). Risk behaviors associated with IV drug abuse, primarily sharing of syringes, has driven the HIV-1 epidemic in Eastern Europe, Asia, North Africa, and North & South America. The largest reports of HIV-1 infection among IDUs are found in China, the United States, and Russia (Vlahov et al., 2010). Although the incidence of HIV-1 infection among IDUs has decreased nearly 80% since the late 1980's, IDUs still represent a large proportion of the population with new HIV-1 diagnoses (CDC, 2009).

In addition to the devastating effects it has on the immune system, HIV-1 infection is a major cause of several neurocognitive dysfunctions, collectively known as HIV-1-associated

*Corresponding Author: Dr. Kamel Khalili, Phone: 215-707-4500; Fax: 215-707-4888, kamel.khalili@temple.edu.

neurocognitive disorders or HAND. Symptoms of HAND are associated with neuronal damage. Since HIV-1 does not produce active infection in neurons, neuronal damage in HIV-1 infection has been proposed to be mediated either by direct interactions of secreted viral proteins with neurons or indirectly by inflammatory molecules secreted by infected glia (Lindl et al., 2010) and/or astrocytes. Introduction of highly active antiviral therapy (HAART), now called combination antiretroviral therapy (cART), has significantly reduced the progression of HIV-1 as well as HAND (Ances and Ellis, 2007; Gray et al., 2003; Lindl et al., 2010; Sacktor, 2002). However, as a result of therapy, HIV-1-infected individuals are living longer, leading to an increased prevalence of HAND (Anthony and Bell, 2008; González-Scarano and Martín-García, 2005; McArthur, 2004; Wang et al., 2006).

Aside from providing a method of viral transmission, injection drug abuse also intrinsically affects HIV-1 pathogenesis, particularly in the central nervous system (CNS). The immunosuppressive effects of opioids enhance HIV-1 pathogenesis (Cabral, 2006) while synergistic HIV-1-opioid interactions mediate inflammation and glial dysfunction, leading to neurological complications (Hauser et al., 2006; Hauser et al., 2007). Neurotoxicity during HIV-1 infection may occur indirectly, with toxic cellular products released from infected glial cells causing dysfunction in nearby neurons (Hauser et al., 2007), or directly by neurotoxic HIV-1 viral proteins such as gp120, Tat, and Vpr (Crews et al., 2008; Lindl et al., 2010). It is suggested that opioid abuse reduces the threshold for HIV-1 neurotoxicity due to overlapping apoptotic pathways activated by opioids through the μ -opioid receptor (MOR) (Carmody, 1987) and by HIV-1 viral proteins through chemokine receptors, primarily CXCR4 and CCR5 (Hauser et al., 2006). However, specific mechanisms and common cellular pathways have yet to be fully established.

In this review, we will examine the role of opioid abuse in directly enhancing HIV-1 neurocognitive dysfunction and how newly identified regulatory mechanisms of MOR expression may affect this process. We postulate that epigenetic activation and transcriptional modulation of *OPRM1* by signaling cascades mediated by HIV-1 infection and opioid abuse may alter MOR expression, thus modifying HIV-1-opioid crosstalk. Additionally, we examine how alternative splicing may alter MOR function. Common epigenetic, transcriptional, translational, and signal transduction mechanisms that regulate alternative splicing and how these mechanisms may be altered by both opioids and HIV-1 infection to differentially regulate MOR isoform expression are discussed. We examine how differential expression of MOR isoforms may result in the activation of different biochemical pathways, altering HIV-1-opioid crosstalk and neurotoxic signaling. Lastly, we identify similar biochemical pathways activated separately by opioids and HIV-1 infection that potentially overlap, resulting in a decreased threshold for HIV-1 proteins or opioid signaling and increased neurotoxicity.

Exceptional diversity of opioids and their receptors: Biological significance

Opioid receptors are members of the G protein-coupled receptor (GPCR) family, which are characterized by having seven transmembrane domains and associating with G protein subunits. GPCRs bind a variety of physiological and environmental molecules, including hormones and neurotransmitters (Devi, 2010). Natural alkaloids derived from the resin of the opium poppy, called opiates, as well as endogenous opioids, such as enkephalins, endorphins, and dynorphins, and synthetic opioids, such as morphine, methadone, and heroin, all bind opioid receptors with various affinities (Carmody, 1987; Drake et al., 2007). This variation in binding affinity was originally used to classify three different receptor subtypes, designated as μ (MOR), κ (KOR), and δ (DOR) opioid receptors (Reisine, 1995). Later, genetic screening verified the existence of these three distinct subtypes (Smith and Lee, 2003). The existence of three related, yet distinct, genes coding for μ , κ , and δ

receptors, and the physical separation of these genes, has allowed for the independent evolution of protein coding sequences and promoter elements, generating great diversity in receptor expression and function (Gray et al., 2006; Mrkusich et al., 2004; Peckys and Landwehrmeyer, 1999; Singh et al., 1997; Wang and Wessendorf, 2002). Of the three opioid receptor subtypes, functional diversity among the MOR is of particular interest because of its high affinity for clinically used opioids as well as illicit opioids of abuse.

Opioid compounds were originally identified for their analgesic properties and, likewise, investigations of MOR regulation and diversity have primarily focused on its role in the sensation and modulation of pain. However, the expression of MORs in multiple cell types indicates that opioids and their receptors may modulate additional physiological systems, including the immune system (Groneberg and Fischer, 2001; Reisine, 1995; Toda et al., 2009). In this regard, the role of opioids in HIV-1 pathogenesis has become an increasingly important topic. While the increased risk of HIV-1 contraction due to IDU behavior can account for most the HIV-1 pathology (CDC, 2009), the rapid progression to AIDS and other HIV-1-associated diseases, such as HAND, in IDUs suggests that opioids actively play a role in HIV-1 pathogenesis (Hauser et al., 2005; Hauser et al., 2006; Hauser et al., 2007; Nath, 2010). This may, in part, be due to the immunosuppressive effects of opioids. However, direct interactions between neurons, opioids, and HIV-1 viral proteins are now believed to also participate in increased neuronal dysfunction (Rogers, 2011). Therefore, regulation and functional variation of MOR expression has major significance not only in the modulation of pain but also in the modulation of disease pathogenesis, particularly in HIV-1 (CDC, 2009; Guo et al., 2002; Li et al., 2003; Li et al., 2002; Peterson et al., 1994; Peterson et al., 1999; Peterson et al., 1990; Suzuki et al., 2002).

Structural organization and regulation of *OPRM1* gene

The *OPRM1* gene, localized on chromosome 6q24-q25 spans a region of 243366 base pairs (Gene ID 4988) and comprises over 13 identified exons (Figure 1). Pre-mRNA transcripts from the *OPRM1* gene undergo extensive splicing to generate 21 known isoforms of the human μ -opioid receptor (Table 1).

MOR-1 like other G-protein coupled receptor contains seven transmembrane (TM) domains with an extracellular N-terminus and an intracellular C-terminus (Figure 2). The first three exons encode for the N-terminus and the TM domains.

Originally, the *OPRM1* promoter was thought to contain both distal and proximal promoter, however it now appears that this is not the case. The *OPRM1* gene is unique in that there is no distinct singular promoter region, as in the mouse homologue (Choi et al., 2008). Instead, it has been found that the *OPRM1* promoter region is TATA-less and therefore has multiple transcriptional initiation sites (Bedini, 2008; Xu and Carr, 2001b). Transcription of *OPRM1* is controlled by a complex interplay of positive and negative regulatory elements (Xu and Carr, 2001b). These regulatory elements include NF κ B (Kraus et al., 2003), SP1 & SP3 (Xu and Carr), AP-1, and STAT6 (Börner et al., 2004; Hwang et al., 2010; Kraus, 2009; Kraus et al., 2001; Kraus et al., 2003). Cytokines, such as interleukin-4 (IL-4) (Kraus et al., 2001), interferon- γ (IFN- γ) (Kraus et al., 2006), insulin-like growth factor-1 (Bedini et al., 2008), and phorbol esters (Wei and Loh, 2011), among many others, also modulate *OPRM1* transcription (Figure 2). As we will discuss in detail, epigenetic mechanisms may regulate *OPRM1* expression by modulating these transcriptional initiation sites.

MOR-mediated signal transduction

The classical MOR is a prototypical G_i/G_o coupled G-protein coupled receptor. As such, activation of the typical MOR stimulates inhibitory pathways mediated by heterotrimeric G_i

and/or G_o proteins. These pathways include inhibition of adenylyl cyclases and N-, P/Q-, and L-type Ca^{2+} channels, altering intracellular Ca^{2+} levels. Activation of the MOR is also known to stimulate K^+ channels and regulate the mitogen-activated protein (MAP) kinase cascade (Law et al., 2000). However, GPCRs have been shown to activate additional pathways, including the anti-apoptotic phosphoinositide 3 kinase (PI3K)/Akt pathway involved in neuronal survival (Polakiewicz et al., 1998; Yin et al., 2006). This has been shown to occur through the association of the $p110\gamma$ PI3K isoform with either the G_α or $G_{\beta\gamma}$ subunit of the heterotrimeric G protein (Duronio, 2008; Tegeder and Geisslinger, 2004).

If β -arrestins compete with G proteins for the receptor before G protein activation, G protein-independent β -arrestin-related pathways will be activated. If β -arrestins compete with G proteins for the receptor after G protein activation, then G protein-dependent β -arrestin-related pathways will be activated. Regardless of this competition, translocation of the β -arrestins to the receptor disrupts G protein coupling, dampens the signal transduction processes, and promotes receptor internalization by targeting the receptor to clathrin-coated pits (Chu et al., 2010; Connor et al., 2004; Zhang et al., 2009). Intracellular signaling stimulated by β -arrestins then designate whether the receptor is recycled back to the membrane or is targeted for degradation by lysosomes. As such, β -arrestins can act as negative regulators of GPCR-mediated signaling.

In addition to desensitizing GPCR signaling, β -arrestins can also function as a scaffolding molecule for the recruitment of signaling molecules. β -arrestins recruit a variety of endocytic proteins and signaling molecules to the receptor, thereby linking GPCRs to additional signaling pathways. Signaling components that are recruited via β -arrestin include components of the JNK3 and ERK1/2 MAP kinase cascades, p53 and NF κ B pathways, Akt, and the phosphodiesterase 4D3 and 4D5 isoforms of cAMP (Gintzler and Chakrabarti, 2006; Ma and Pei, 2007; Moorman et al., 2009). Cytoplasmic β -arrestins may also interact with regulators of transcription factors, such as the E_3 ubiquitin ligase MDM2 and the NF κ B-inhibitor I κ B α , indirectly regulating transcription. Indeed, previous studies have shown that opioid receptor activation stimulates the cytoplasmic retention of MDM2 in a β -arrestin/MDM2/receptor complex, reducing nuclear expression of MDM2 and resulting in increased p53-dependent transcription and apoptosis. Similarly, interaction with β -arrestin 2 prevents phosphorylation and degradation of I κ B α , thereby attenuating activation of NF κ B and transcription of NF κ B target genes. Direct epigenetic regulation by β -arrestins has also been shown by opioid receptor activation. Following activation of DOR and KOR, but not MOR, β -arrestin 1 translocates into the nucleus where it accumulates at the *p27* and *FOS* promoters. It then recruits the histone acetyltransferase p300, promoting histone H4 hyperacetylation at the promoter sites, activating their transcription (Ma and Pei, 2007). It is thought that the distinction between DOR, KOR, and MOR activation is due to the high affinity of DOR and KOR, but not MOR, for β -arrestin 1. This is due to the fact that while β -arrestin 1 and β -arrestin 2 are highly similar, β -arrestin 2 possesses a strong nuclear export signal in its C terminus, which hinders its retention in the nucleus. Given this fact, β -arrestin 1 may play a more important role in GPCR-mediated nuclear signaling and preferential activation of particular β -arrestin-dependent signaling and epigenetic pathways is dependent on which member of the β -arrestin family is recruited by a given GPCR (Kang et al., 2005; Ma and Pei, 2007). Overall, an important distinction to be made in β -arrestin signaling is that while most G protein-dependent pathways are rapid and transient, the majority of β -arrestin-dependent pathways are slower in onset and prolonged. Therefore, β -arrestin binding of GPCRs is not only a regulatory mechanism of G protein signal transduction but also serves as a critical initiator of a second-wave of prolonged signal transduction as well as epigenetic and transcriptional regulation (Ma and Pei, 2007).

The competition between G proteins and β -arrestins determines the selection of the downstream pathways. Receptor phosphorylation can increase the affinity of the receptor for β -arrestins, promoting the activation of β -arrestin dependent pathways over G protein pathways (Zheng et al., 2010). Specific residues of the intracellular loops and C-terminal tail of GPCRs, including the MOR, are phosphorylated in response to agonist-binding by one of two types of serine/threonine protein kinases, G protein-coupled receptor kinases (GRKs) or second messenger-activated kinases, which include PKA, PKC, Src, MAPK, and Ca^{2+} /calmodulin-dependent protein kinase II (Chu et al., 2010; Clayton et al., 2010; Feng et al., 2011). GRK-mediated phosphorylation of the agonist-occupied receptor subsequently increases the affinity of the agonist-receptor complex for β -arrestins. The functions of second-messenger-mediated phosphorylation are still unclear (Feng et al., 2011). Therefore, C-terminal phosphorylation site specificity contributes to the specificity of β -arrestin binding. As such, MOR signaling pathways are dependent on the extent to which the agonist-receptor complex is phosphorylated and the subsequent recruitment of β -arrestins or other secondary messengers. Interestingly, morphine exposure generally results in less phosphorylation of the MOR than other, more efficacious agonists, such as DAMGO. This may be due to specific agonist-induced phosphorylation at different residues (Johnson et al., 2005). Therefore, weak phosphorylation-inducing agonists, such as morphine, might desensitize the receptor via pathways other than those involving GRK and β -arrestins (Chu et al., 2010; Zheng et al., 2010). Likewise, these agonists will not induce β -arrestin specific signaling components.

Alternative splicing of the μ -opioid receptor: Functional consequences

Multiple studies in mice, rats, and humans have shown that the MOR pre-mRNA undergoes extensive splicing to give multiple isoforms (Bare et al., 1994; Kvam et al., 2004). Both C-terminal (Bare et al., 1994; Doyle et al., 2007; Pan et al., 1999; Pan et al., 2005b; Pan et al., 2003; Pan et al., 2000) and N-terminal (Pan, 2002; Pan et al., 2001; Xu et al., 2009) variants have been identified, all shown to have distinct regional, cellular, and subcellular distributions (Abbadie et al., 2000a; Abbadie et al., 2000b; Abbadie et al., 2000c; Abbadie et al., 2004; Abbadie et al., 2001; Abbadie et al., 2002; Pasternak, 2010; Ständer et al., 2002; Zhang et al., 2006). Some general studies have shown differences in ligand binding affinity between these receptors (Bolan et al., 2004; Oldfield et al., 2008; Pan et al., 2005a; Pasternak et al., 2004; Pasternak, 2004; Ravindranathan et al., 2009). Furthermore, these receptors seem to have different rates of [^{35}S]GTP γ S binding, internalization, and resensitization in response to different μ -selective ligands (Abbadie and Pasternak, 2001; Koch et al., 2001; Koch et al., 1998; Mizoguchi et al., 2003; Tanowitz et al., 2008). Studies in mice suggest that these isoforms have functional significance. Some studies on exon-specific KO studies in mice have found that sequences encoding for particular exons are an important molecular target for the activation of G-proteins by MOR agonists and are required for morphine-induced nociception in the spinal cord (Mizoguchi et al., 2002a; Mizoguchi et al., 2002b; Mizoguchi et al., 2003). However, there are discrepancies between studies, with some studies showing retention of nociception with heroin and M6G (a morphine metabolite) in exon KO mice (Schuller et al., 1999). These differences arise from different knockout methods and it is suggested that other exons near the KO exon were also disrupted (Mizoguchi et al., 2003). As such, functional differences observed among different exon KO mice strains are attributed to alterations of additional exons, suggesting functional roles for individual isoforms incorporating these exons. Therefore, closer examinations of the functional significance of individual exons of *OPRM1*, as well as the isoforms that incorporate them, are needed.

Despite the functional significance of individual exons, examination of the downstream functional consequences of individual MOR isoforms is scarce. However, one functional

study of the truncated variant MOR-1K showed interesting results. As previously described, the classical MOR, or MOR-1, is a G_i/G_o coupled 7-transmembrane receptor that is expressed on the cell membrane, activation of MOR-1 resulted in inhibition of adenylyl cyclase and Ca^{2+} channels, among other results (Law et al., 2000). The alternatively spliced MOR-1K receptor does not fit this model in that it is a 6-transmembrane receptor sequestered in the intracellular compartment. Despite this lack of membrane expression, MOR-1K is still functional and is primarily coupled to G_s proteins, in contrast to MOR-1. As such, activation of MOR-1K was shown to increase adenylyl cyclase and Ca^{2+} channel activity (Gris et al., 2010). Unfortunately, no investigations on the downstream signal transduction pathway stimulated by MOR-1K activation has been conducted.

Although MOR-1K is only a single example of an MOR isoform with alternative functions, it has a major consequence on our understanding of opioid signaling since opioids may now have an excitatory or inhibitory effect based on which isoform is stimulated. Given that each region of a given GPCR has important functional roles, it is likely that alternatively spliced isoforms are functionally different from each other in numerous ways. Furthermore, the differential expression of MOR isoforms may have functional consequences, given that agonist selectivity and C-terminal phosphorylation site availability contributes to the specificity of receptor phosphorylation and the recruitment of β -arrestins or other secondary messenger proteins. Therefore, alternative splicing resulting in an isoform with altered agonist selectivity or an alteration of the C-terminal phosphorylation sites will affect how the isoform is phosphorylated, the competition between G proteins and β -arrestins, and ultimately which signaling pathways are stimulated. Currently, however, there is little to no data on the signal transduction mediated by individual MOR isoforms or how differential regulation of their expression alters the overall signal transduction of opioids (Table I).

Regulation of MOR alternative splicing by signal transduction

Normally, the regulation of splicing is achieved by SR proteins, which bind to areas within and flanking alternative exons of pre-mRNA, promoting or inhibiting assembly at nearby splice sites. Changing the activity of these proteins by posttranslational modifications alters splicing regulation. Interestingly, a study in human blood lymphocytes found that the MOR isoform MOR-1A is specifically and significantly up regulated in methadone maintenance treated individuals while MOR-1O is specifically and significantly down regulated (Vousooghi et al., 2009). Regulation of *OPRM1* promoter activity alone does not account for this differential regulation of MOR isoform expression. Therefore, in addition to regulating epigenomic and translational activity, opioids may also regulate alternative splicing mechanisms.

To date, there have been no studies investigating the mechanisms involved in the regulation of MOR alternative splicing and, as such, the effects of opioids on this process are completely unknown. Interestingly, however, one mechanism of post-translational modification of SR proteins is phosphorylation by Akt, which alters the role of SR proteins in both splicing and translation (Blencowe and Graveley, 2007). Since the activation of the MOR, as well as other GPCRs, has been shown to modulate Akt, it is possible that the MOR may self-regulate its own splicing via modulation of Akt-dependent phosphorylation of regulatory SR proteins. Many additional cellular signaling pathways stimulated by MOR activation, such as Ras/MEK/ERK, MAPK, Ca^{2+} /calmodulin/CaMK IV, and Rac/JNK/p38 have also been shown to regulate alternative splicing (Tarn, 2007). However, none of these mechanisms have been implicated specifically in regulating alternative splicing of the MOR pre-mRNA (Figure 2).

Epigenetic regulation of *OPRM1* expression

Epigenetic regulation involves reversible, heritable changes and chromatin modifications without DNA alterations and results in altered gene activity. Differential expression of the opioid receptors during development and adulthood requires epigenetic regulation. DNA methylation and histone modifications have been found in the regulatory regions of all three genes encoding for the different opioid receptor subtypes (Figure 2). During embryonic development, the *OPRM1* gene is completely silenced while expression of genes encoding for the KOR and the DOR are gradually increased. During neuronal differentiation there is a shift in this expression, with the KOR and DOR encoding genes being silenced and a robust activation of *OPRM1*. This is followed by a reactivation of the KOR and DOR genes (Wei, 2008).

Role of DNA methylation

Epigenetic regulation of *OPRM1* expression in mice has been shown to be mediated by MeCP2, through its association with the chromatin-remodeling factor Brg1 and the DNA methyltransferase Dnmt1. This regulation occurs through DNA methylation, demethylation, and subsequent chromatin remodeling of the *OPRM1* promoter in various CNS regions. When methylated, CpG regions of the promoter facilitate MeCP2 binding. MeCP2 then recruits repressor proteins that deacetylate associated histones, forming a compact structure around the promoter region and silencing *OPRM1* transcription. Demethylation of these CpG regions causes MeCP2 and associated repressor proteins to dissociate from the promoter region, facilitating *OPRM1* transcription (Hwang et al., 2010; Hwang et al., 2007; Hwang et al., 2009; Meaney and Ferguson-Smith, 2010).

Promoter methylation of the human *OPRM1* gene has also been demonstrated to play a role in its regulation. It was demonstrated that the *OPRM1* promoter region is CpG methylated in non-expressing neuronal cells while it is unmethylated in expressing neuronal cells (Andria and Simon, 1999). Hypermethylation of two CpG sites in the *OPRM1* gene promoter has been demonstrated in peripheral lymphocytes of Caucasian methadone maintained former heroin addicts (Nielsen et al., 2009). The same group has also demonstrated ethnic diversity in the DNA methylation of the *OPRM1* gene promoter. In African-Americans, the degree of methylation was significantly decreased, while in Hispanics, the degree of methylation was increased in former heroin addicts (Nielsen et al., 2010). Although these findings do not definitively prove that opioids alter the epigenetic regulation of the *OPRM1* gene, they are interesting given the known roles of KORs and DORs in β -arrestin dependent epigenetic regulation (Kang et al., 2005; Ma and Pei, 2007).

Role of Histone modification

The other major form of epigenetic regulation is mediated by posttranslational modification of DNA-associated histone proteins in chromatin. Notably, histone acetylation by histone acetyltransferase (HAT) and deacetylation by histone deacetylases (HDACs) play a crucial role in the regulation of gene expression. Indeed, it has been demonstrated that HDAC1 and HDAC2 are recruited to the proximal region of the *OPRM1* promoter in human neuronal NMB cells. Additionally, the HDAC inhibitor trichostatin A (TSA) affected the *OPRM1* gene expression both at the transcriptional and post-transcriptional levels (Lin et al., 2008). A recent study demonstrated that cycloheximide induced the murine *OPRM1* gene in an epigenetic fashion with increased recruitment of acetylated histone H3 and methylated H3-K4 as well as a concomitant decrease in HDAC2 binding and H3-K9 methylation on the promoter (Kim et al., 2011).

Role of MicroRNA

MicroRNAs (miRNAs) are noncoding RNAs, generally 20–30 nucleotides in length, that are processed and assembled into ribonucleoprotein (RNP) complexes called micro-RNPs (miRNPs) or into miRNA-induced silencing complexes (miRISCs) (Carthew and Sontheimer, 2009; Filipowicz et al., 2008; Zhang and Su, 2009). miRNA complexes primarily reside in cytoplasmic RNA processing bodies (P-bodies) where they inhibit gene expression at the post-transcriptional level (Zhang and Zeng, 2010). This is mediated by the binding of miRNA complexes to miRNA binding sites generally located within the 3'-untranslated region (3'-UTR) region of the mRNA transcript. The mechanism of regulation is determined by the degree of miRNA-mRNA complementarity, with perfect complementarity promoting cleavage and incongruity promoting repression of the mRNA. Whether this cleavage or repression occurs at translation initiation or post-initiation is still subject to debate. Alternatively, miRNAs complexes may also regulate gene expression at the epigenetic level. Epigenetic regulating miRNAs, dubbed epi-miRNAs, modulate gene expression indirectly by targeting components of epigenetic machinery, including DNA methyltransferases and enzymes regulating histone acetylation (Carthew and Sontheimer, 2009; Iorio et al., 2010).

The presence of a long 3'-UTR in *OPRM1* mRNA (Han et al., 2006; Ide et al., 2005) suggests that it may contain regions that could play a role in the regulation of receptor expression. The role of miRNA in the regulation of murine MOR is seen in the fact that miRNA23b blocks the association of MOR1 mRNA with polysomes, leading to inhibition of MOR1 protein translation (Wu et al., 2008). Additionally, the miRNA let-7 promotes translocation and sequestration of MOR mRNA to P-bodies, leading to translation repression. As such, anti-let-7 treatment was shown to decrease brain let-7 levels and partially attenuate opioid antinociceptive tolerance in mice, suggesting a direct role of miRNA regulation in MOR function (He et al., 2010).

Regulation of *OPRM1* and MOR expression by Opioids and HIV-1

Given that the activity of the MOR is tightly regulated by the activation and transcription of *OPRM1* (Xu and Carr, 2001b), a particularly important question is what environmental factors alter the epigenome and transcription of *OPRM1*. The up-regulation of MORs by pro-inflammatory cytokines like IL-1, IL-6 and TNF α , as well as anti-inflammatory cytokines, like IL-4, in neurons suggests that these factors mediate epigenetic and transcriptional regulation of the *OPRM1* gene (Kraus, 2009). A study in Jurkat T cells showed that IL-4 stimulation leads to phosphorylation and activation of STAT6. Remodeling of the chromatin structure, including histone modifications, is then signaled by IL-4 activated STAT6. This remodeling is most likely necessary to switch the *OPRM1* gene from a heterochromatic, or inactive, state to a euchromatic, or active, state. Additionally, IL-4 induces the disassociation of MeCP2 from the *OPRM1* promoter, demethylating the promoter. Following these events, STAT6 binds to a regulatory DNA element and facilitates its transcription (Kraus et al., 2010). Additional regulatory mechanisms involve IL-1 and TNF α , mediated by AP-1 and/or NF- κ B, and IL-6, mediated by STAT1 and/or STAT3 (Kraus, 2009).

These mechanisms of *OPRM1* regulation are interesting since both morphine and HIV-1 have been shown to alter cytokine release (Bonnet et al., 2008; Turchan-Cholewo et al., 2009). It has previously been shown that HIV-1 infection increases MOR mRNA expression (Beltran et al., 2006). However, regulation of MOR expression through an IL-4/STAT6-dependent pathway has not been shown in HIV-1 infection. Rather, HIV-1 infection has been shown to regulate *OPRM1* expression in an SP1-dependent manner, although the

mechanism of this regulation is not completely understood (Liu et al., 2009). Likewise, MOR ligands have been shown to alter MOR expression (Zadina et al., 1993). It was previously shown that DAMGO increases binding of SP1 and SP3 to the *OPRM1* promoter region. It is likely that this is mediated through the cAMP-PKA pathway, however an exact mechanism has not been determined. Regardless, this DAMGO-mediated increase in SP1/SP3 binding may modulate transcription (Xu and Carr, 2001a). In addition to this mechanism, a study in CEM $\times$ 174 cells suggests a role for PI3K and Akt in morphine-induced initiation of *OPRM1* transcription. Here the authors suggest that the transcription factor E2F1, a downstream effector of Akt, along with additional transcription factors Sp1 and YY1, interact with the promoter region of the *OPRM1*. In short, morphine treatment was shown to promote nuclear translocation of E2F1 and enhance E2F1 gene expression through a PI3K/Akt dependent pathway. This enhanced E2F1 interacted directly and indirectly with Sp1 and YY1, respectively, to bind to the *OPRM1* promoter and facilitate transcription. Therefore, in addition to epigenetic regulation by cytokines and interleukins during HIV-1 infection, morphine may directly regulate transcription of the *OPRM1* gene through either a cAMP/PKA/SP1/SP3 or PI3K/Akt/E2F1 pathway (Li et al., 2008; Liu et al., 2010).

Opioid abuse may also modulate MOR mRNA transcript levels specifically and directly by miRNA mechanisms. A recent study demonstrated that morphine significantly up regulates let-7 miRNA expression in SH-SY5Y neuronal cells (He et al., 2010). Additionally, morphine treatment of primary human monocyte-derived macrophages leads to differential regulation of miRNA expression. Among the miRNAs differentially regulated were hsa-miR-15b and hsa-miR-181b, both of which have several targets in pro-inflammatory pathways (Dave and Khalili, 2010). Therefore, opioid abuse may regulate MOR expression through both direct miRNA-dependent inhibition of MOR mRNA or indirectly by miRNA modulation of pro-inflammatory or other signal transduction pathways (Figure 2).

Viruses, such as HIV-1, are dependent on intracellular machinery to facilitate their own replication. As such, many viruses, including HIV-1, have developed mechanisms to hijack cellular processes to facilitate replication while evading immune responses. Those viruses that cause latent infection benefit from heritable, epigenetic changes in host transcription and, therefore, have evolved mechanisms of modulating host epigenetic, transcriptional, and translation regulation. HIV-1 has been shown to increase cellular DNA methyltransferase activity to produce a general increase in cellular DNA methylation (Paschos and Allday, 2010). Furthermore, a miRNA generated by the HIV-1 TAR element specifically recruits HDAC-1 in order to silence its own transcription (Klase et al., 2007; Ouellet et al., 2009). While this HIV-1-generated miRNA may silence transcription for the purpose of driving latency, HIV-1 replication may be actively suppressed by cellular miRNAs. HIV-1 mRNA expression is modulated by RISCs and P bodies, and a depletion of P bodies, as well as cellular anti-HIV-1-miRNAs, enhances HIV-1 production and infection both *in vitro* and *in vivo* (Nathans et al., 2009; Wang et al., 2009). Interestingly, of the five known anti-HIV-1-miRNAs highly expressed in human monocytes, morphine treatment reduces the expression of four. This effect was specific to anti-HIV-1-miRNAs, as no change was seen in the expression of known anti-HCV-miRNA (Wang et al., 2011). Therefore, in addition to SP1-dependent modulation of *OPRM1* expression, the existence of HIV-1-generated miRNAs, and their interaction with the epigenetic, transcriptional, and translational machinery, leave open the possibility of direct or indirect modulation of *OPRM1* and MOR expression by HIV-1. Furthermore, there is clear evidence that opioids modulate HIV-1 replication by inhibiting anti-HIV-1-miRNAs. As such, alterations in *OPRM1* and MOR expression may have functional consequences in HIV-1 pathogenesis due to changes in anti-HIV-1-miRNA regulation.

Opioid abuse enhances HIV-1-associated neurocognitive dysfunction by stimulating overlapping biochemical responses in the CNS

Nervous system disorders caused by HIV-1 infection, such as HAND, remain a significant problem among HIV-1 infected individuals, occurring in approximately 30–50% of HIV-1 infected persons in the United States (Power et al., 2009; Rappaport and Berger, 2010). While any cognitive ability can be compromised in HAND, disturbance in memory, particularly in learning and retrieval of new information, tends to be prominent. Many individuals also exhibit psychomotor slowing, problems in attention, and disturbance in executive functions (Grant, 2008). While it is well accepted that HAND results from HIV-1-mediated neuronal damage, exact mechanisms have yet to be fully established.

Currently, there are two models for the development of neurocognitive dysfunction in HIV-1. The indirect model proposes that neuronal dysfunction is mediated by the inflammatory and excitotoxic responses mounted by infected and uninfected glial cells in response to HIV-1 infection or HIV-1 viral proteins. Microglia, the resident macrophage of the CNS, are productively infected with HIV-1 and are the major source of toxic cellular proteins, including TNF- α , interleukins, and nitric oxide, in addition to serving as viral reservoirs (Fischer-Smith and Rappaport, 2005; Garden, 2002; Lindl et al., 2010; Louboutin et al., 2010; Yadav and Collman, 2009). Additionally, HIV-1 infection increases cytokine and reactive oxygen species (ROS) release by astrocytes, despite the absence of infection in these cells (Kaul et al., 2001; Turchan-Cholewo et al., 2009). As such, HIV-1-mediated neurotoxicity may be secondary to the inflammatory response mounted by glial cells, resulting in a simultaneous loss of trophic support and an increasingly neurotoxic environment (Hauser et al., 2006). Alternatively, the direct model proposes that HIV-1-mediated neuronal dysfunction is mediated by viral proteins, such as gp120, Tat, and Vpr, secreted by actively infected glial cells, such as macrophages and microglia. These secreted viral proteins directly interact with uninfected neurons to promote neuronal apoptosis (Lindl et al., 2010). Although these models present two separate and distinct pathways of HIV-1-mediated neurotoxicity, they are not mutually exclusive. Therefore, neuronal dysfunction present in HIV-1 infection is most likely due to a combination of both direct and indirect insults.

It is thought that opioid abuse enhances HIV-1-mediated neurotoxicity by sensitizing neurons to both direct and indirect insults mediated by HIV-1 infection. Opioids modify multiple aspects of microglial function, including phagocytosis, chemotaxis, and chemokine production (El-Hage et al., 2006). Likewise, opioids increase cytokine and chemokine production in astrocytes (El-Hage et al., 2008). In this manner, opioid abuse may exacerbate the inflammatory environment common in HIV-1 infection by further increasing chemokine, cytokine, and ROS production by glial cells. However, opioid enhancement of HIV-1-mediated neurotoxicity is more commonly attributed to direct interactions between HIV-1 viral proteins, opioids, and neurons. Multiple pathways activated by opioids and HIV-1 viral proteins intersect at various levels, allowing for HIV-1-opioid crosstalk. This includes the recruitment of β -arrestin, which augments both MOR and CXCR4 activation of p38 and ERK and regulates downstream MAPK signaling (Hauser et al., 2006). Another point of HIV-1 and opioid intersection is the PI3K/Akt/GSK3 β pathway, which, in addition to modulating transcriptional and alternative splicing mechanisms, facilitates apoptotic signaling. PI3K-activated Akt enhances neuronal stability and survival during stress, particularly in inflammation, by diminishing neuronal damage caused by interleukins, nitric oxide, TNF- α , and other inflammatory molecules. Although Akt mediates this process through multiple mechanisms, it has most notably been shown to inactivate the pro-apoptotic proteins glycogen synthase kinase-3 (GSK3) and Bad by phosphorylating them. Activation of Akt also removes IKK inhibition of NF- κ B, activating anti-apoptotic genes.

Additionally, Akt can block caspase activation within the CNS both directly, by inhibition of caspase 9, or indirectly through the modulation of caspase substrates (Chong et al., 2005; Song et al., 2005). Morphine has been shown to dephosphorylate Akt, inactivating it and resulting in microglial apoptosis (Xie et al., 2010). Interestingly, it has been shown that both gp120 and Tat activate GSK3 β in neurons, promoting apoptosis (Hauser et al., 2006). Therefore, in addition to MAPK signaling, opioid enhancement of HIV-1-mediated neurotoxicity may, in part, be due to the combination of Akt inhibition by morphine and GSK3 β activation by HIV-1 viral proteins, resulting in increased neuronal apoptosis.

Conclusion

It is generally accepted that neurological complications caused by HIV-1 infection, such as HAND, are enhanced by opioid abuse. Although this may occur indirectly by HIV-1 and opioid mediated glial dysfunction, opioid enhancement of HAND is regularly attributed to the direct, combinational effect of HIV-1 viral proteins and opioids stimulating overlapping apoptotic pathways in neurons. While exact mechanisms of this interaction are the subject of intense investigation, an equally important mechanism in this interaction, the regulation of MOR expression by HIV-1 and opioids, is severely lacking.

The activity of the MOR is tightly regulated by the transcription of *OPRM1*, of which the epigenetic and transcriptional regulatory mechanisms have been partially characterized. Many of these mechanisms overlap with known opioid and HIV-1 viral protein signaling cascades as studies have shown that both modulate MOR expression. Exact mechanisms of *OPRM1* regulation are not fully characterized but may include miRNA regulation as well as transcriptional, translational, and epigenetic modulation.

Extensive alternative splicing of the MOR pre-mRNA has been shown to give multiple isoforms of the MOR. Given the importance of multiple GPCR regions in receptor function, alterations of these regions by alternative splicing may generate functionally distinct isoforms. This is particularly true for C-terminal variants, since site-specific phosphorylation at conserved residues regulates the recruitment of second messenger proteins and the signal transduction mechanism. Some differences have been observed between MOR isoforms, including both excitatory and inhibitory functions, suggesting that MOR isoforms individually contribute to opioid signaling, including opioid enhancement of HIV-1 neuropathogenesis. Unfortunately, the signal transduction mediated by individual isoforms is not understood.

In conclusion, opioid abuse has become a major public health concern due to the increased risk of HIV-1 infection as well as the exacerbation of HIV-1 neuropathogenesis. Complicating the understanding of HIV-1 and opioid interactions is the dynamic interplay between opioid abuse, HIV-1 infection, *OPRM1* regulation, and the generation and differential expression of functionally distinct MOR isoforms by alternative splicing. Modulation of the mechanisms regulating the epigenetic and transcriptional expression of *OPRM1*, and/or splicing of MOR pre-mRNA, will alter the MOR isoforms expressed. This may, in turn, alter the overall physiological affect of opioids. Although mechanisms regulating all of these processes have been partially characterized, our understanding of how opioids or HIV-1 modulate these mechanisms is incomplete. Furthermore, there has been no investigation to date examining the signal transduction mechanisms mediated by individual MOR isoforms or their individual role in opioid driven pathogenesis, such as HAND. As such, extensive studies are needed first to understand how opioid abuse and pathological conditions, such as HIV-1 infection, modulate the epigenetic, transcriptional, and post-transcriptional regulation of MOR expression. Second, how MOR isoforms individually contribute to the signal transduction of different opioids needs to be investigated. Finally,

studies are needed to determine how the differential regulation of MOR isoforms alters the physiological response to opioids in both homeostatic and pathological conditions.

Acknowledgments

The authors wish to thank members of the Department of Neuroscience and the Center for Neurovirology for their support, and sharing of ideas and reagents. We also wish to thank Dr. Martyn White for critical reading of this manuscript and C. Papaleo for editorial assistance. This work was made possible by grants awarded by NIH to KK.

Literature Cited

- Abbadie C, Gultekin SH, Pasternak GW. Immunohistochemical localization of the carboxy terminus of the novel mu opioid receptor splice variant MOR-1C within the human spinal cord. *NeuroReport*. 2000a; 11(9):1953–1957. [PubMed: 10884050]
- Abbadie C, Pan Y-X, Drake CT, Pasternak GW. Comparative immunohistochemical distributions of carboxy terminus epitopes from the mu-opioid receptor splice variants MOR-1D, MOR-1 and MOR-1C in the mouse and rat CNS. *Neuroscience*. 2000b; 100(1):141–153. [PubMed: 10996465]
- Abbadie C, Pan Y-X, Pasternak GW. Differential distribution in rat brain of mu opioid receptor carboxy terminal splice variants MOR-1C-like and MOR-1-like immunoreactivity: evidence for region-specific processing. *The Journal of comparative neurology*. 2000c; 419(2):244–256. [PubMed: 10723002]
- Abbadie C, Pan Y-X, Pasternak GW. Immunohistochemical study of the expression of exon11-containing μ opioid receptor variants in mouse brain. *Neuroscience*. 2004; 127(2):419–430. [PubMed: 15262332]
- Abbadie C, Pasternak GW. Differential in vivo internalization of MOR-1 and MOR-1C by morphine. *NeuroReport*. 2001; 12(14):3069–3072. [PubMed: 11568638]
- Abbadie C, Pasternak GW, Aicher SA. Presynaptic localization of the carboxy-terminus epitopes of the μ opioid receptor splice variants MOR-1C and MOR-1D in the superficial laminae of the rat spinal cord. *Neuroscience*. 2001; 106(4):833–842. [PubMed: 11682168]
- Abbadie C, Rossi GC, Orciuolo A, Zadina JE, Pasternak GW. Anatomical and functional correlation of the endomorphins with mu opioid receptor splice variants. *The European journal of neuroscience*. 2002; 16(6):1075–1082. [PubMed: 12383236]
- Ances BM, Ellis RJ. Dementia and neurocognitive disorders due to HIV-1 infection. *Seminars in neurology*. 2007; 27(1):86–92. [PubMed: 17226745]
- Andria ML, Simon EJ. Localization of promoter elements in the human mu-opioid receptor gene and regulation by DNA methylation. *Molecular brain research*. 1999; 70(1):54–65. [PubMed: 10381543]
- Anthony IC, Bell JE. The Neuropathology of HIV/AIDS. *International review of psychiatry*. 2008; 20(1):15–24. [PubMed: 18240059]
- Bare LA, Mansson E, Yang D. Expression of two variants of the human μ opioid receptor mRNA in SK-N-SH cells and human brain. *FEBS letters*. 1994; 354(2):213–216. [PubMed: 7957926]
- Bedini, A. Transcriptional regulation of human mu-opioid receptor gene: Functional characterization of activating and inhibitory transcription factors [Doctoral Thesis]. Bologna: Alma Mater Studiorum Universita Degli Studi di Bologna; 2008. p. 147
- Bedini A, Baiula M, Spampinato S. Transcriptional activation of human mu-opioid receptor gene by insulin-like growth factor-I in neuronal cells is modulated by the transcription factor REST. *Journal of neurochemistry*. 2008; 105(6):2166–2178. [PubMed: 18284609]
- Beltran JA, Pallur A, Chang SL. HIV-1 gp120 up-regulation of the mu opioid receptor in TPA-differentiated HL-60 cells. *International immunopharmacology*. 2006; 6(9):1459–1467. [PubMed: 16846840]
- Blencowe, B.; Graveley, B., editors. *Alternative splicing in the postgenomic era*. New York: Landes Bioscience and Springer Science+Business Media, LLC; 2007.
- Bolan EA, Pan Y-X, Pasternak GW. Functional analysis of MOR-1 splice variants of the mouse mu opioid receptor gene Oprm. *Synapse*. 2004; 51(1):11–18. [PubMed: 14579421]

- Bonnet M-P, Beloeil H, Benhamou D, Mazoit J-X, Asehnoune K. The μ opioid receptor mediates morphine-induced tumor necrosis factor and interleukin-6 inhibition in toll-like receptor 2-stimulated monocytes. *Anesthesia and analgesia*. 2008; 106(4):1142–1149. [PubMed: 18349186]
- Börner C, Kraus J, Schröder H, Ammer H, Höllt V. Transcriptional regulation of the human μ -opioid receptor gene by interleukin-6. *Molecular pharmacology*. 2004; 66(6):1719–1726. [PubMed: 15448191]
- Cabral GA. Drugs of abuse, immune modulation, and AIDS. *Journal of neuroimmune pharmacology*. 2006; 1(3):280–295. [PubMed: 18040805]
- Cadet P. Mu opiate receptor subtypes. *Medical science monitor*. 2004; 10(6):28–32.
- Carmody JJ. Opiate receptors: an introduction. *Anaesthesia and intensive care*. 1987; 15(1):27–37. [PubMed: 3032015]
- Carthew RW, Sontheimer EJ. Origins and Mechanisms of miRNAs and siRNAs. *Cell*. 2009; 136(4):642–655. [PubMed: 19239886]
- CDC. HIV infection among injection-drug users - 34 states, 2004–2007. *MMWR Morbidity and mortality weekly report*. 2009; 58(46):1291–1295. [PubMed: 19940834]
- Choi HS, Song KY, Hwang CK, Kim CS, Law P-Y, Wei L-N, Loh HH. A proteomics approach for identification of single strand DNA-binding proteins involved in transcriptional regulation of mouse μ opioid receptor gene. *Molecular & cellular proteomics*. 2008; 7(8):1517–1529. [PubMed: 18453338]
- Chong ZZ, Li F, Maiese K. Activating Akt and the brain's resources to drive cellular survival and prevent inflammatory injury. *Histology and histopathology*. 2005; 20(1):299–315. [PubMed: 15578447]
- Chu J, Zheng H, Zhang Y, Loh HH, Law P-Y. Agonist-dependent μ -opioid receptor signaling can lead to heterologous desensitization. *Cellular signalling*. 2010; 22(4):684–696. [PubMed: 20043990]
- Clayton CC, Bruchas MR, Lee ML, Chavkin C. Phosphorylation of the μ -opioid receptor at tyrosine 166 (Tyr3.51) in the DRY motif reduces agonist efficacy. *Molecular pharmacology*. 2010; 77(3):339–347. [PubMed: 19959593]
- Connor M, Osborne PB, Christie MJ. μ -opioid receptor desensitization: is morphine different? *British journal of pharmacology*. 2004; 143(6):685–696. [PubMed: 15504746]
- Crews L, Lentz MR, Gonzalez RG, Fox HS, Masliah E. Neuronal injury in simian immunodeficiency virus and other animal models of neuroAIDS. *Journal of neurovirology*. 2008; 14(4):327–339. [PubMed: 18780234]
- Dave RS, Khalili K. Morphine treatment of human monocyte-derived macrophages induces differential miRNA and protein expression: impact on inflammation and oxidative stress in the central nervous system. *Journal of cellular biochemistry*. 2010; 110(4):834–845. [PubMed: 20564181]
- Devi, LA., editor. Softcover reprint of hardcover 1st ed. 2005 ed. Totowa: Humana Press; 2010. *The G Protein-Coupled Receptors Handbook (Contemporary Clinical Neuroscience)*.
- Doyle GA, Sheng XR, Lin SSJ, Press DM, Grice DE, Buono RJ, Ferraro TN, Berrettini WH. Identification of five mouse μ -opioid receptor (MOR) gene (Oprm1) splice variants containing a newly identified alternatively spliced exon. *Gene*. 2007; 395:98–107. [PubMed: 17398041]
- Drake CT, Chavkin C, Milner TA. Opioid systems in the dentate gyrus. *Progress in brain research*. 2007; 163:245–263. [PubMed: 17765723]
- Duronio V. The life of a cell: apoptosis regulation by the PI3K/PKB pathway. *The Biochemical journal*. 2008; 415(3):333–344. [PubMed: 18842113]
- El-Hage N, Bruce-Keller AJ, Yakovleva T, Bazov I, Bakalkin G, Knapp PE, Hauser KF. Morphine exacerbates HIV-1 Tat-induced cytokine production in astrocytes through convergent effects on $[Ca^{2+}]_i$, NF-kappaB trafficking and transcription. *PloS one*. 2008; 3(12):1–14.
- El-Hage N, Wu G, Wang J, Ambati J, Knapp PE, Reed JL, Bruce-Keller AJ, Hauser KF. HIV-1 Tat and opiate-induced changes in astrocytes promote chemotaxis of microglia through the expression of MCP-1 and alternative chemokines. *Glia*. 2006; 53(2):132–146. [PubMed: 16206161]
- Feng B, Li Z, Wang JB. Protein kinase C-mediated phosphorylation of the μ -opioid receptor and its effects on receptor signaling. *Molecular pharmacology*. 2011; 79(4):768–775. [PubMed: 21212139]

- Filipowicz W, Bhattacharyya SN, Sonenberg N. Mechanisms of post-transcriptional regulation by microRNAs: are the answers in sight? *Nature reviews genetics*. 2008; 9(2):102–114.
- Fischer-Smith T, Rappaport J. Evolving paradigms in the pathogenesis of HIV-1-associated dementia. *Expert reviews in molecular medicine*. 2005; 7(27):1–26. [PubMed: 16321172]
- Garden GA. Microglia in human immunodeficiency virus-associated neurodegeneration. *Glia*. 2002; 40(2):240–251. [PubMed: 12379911]
- Gintzler AR, Chakrabarti S. Post-opioid receptor adaptations to chronic morphine; altered functionality and associations of signaling molecules. *Life sciences*. 2006; 79(8):717–722. [PubMed: 16581089]
- González-Scarano F, Martín-García J. The neuropathogenesis of AIDS. *Nature reviews immunology*. 2005; 5(1):69–81.
- Grant I. Neurocognitive disturbances in HIV. *International review of psychiatry*. 2008; 20(1):33–47. [PubMed: 18240061]
- Gray AC, Coupar IM, White PJ. Comparison of opioid receptor distributions in the rat central nervous system. *Life sciences*. 2006; 79(7):674–685. [PubMed: 16546223]
- Gray F, Chrétien F, Vallat-Decouvelaere AV, Scaravilli F. The changing pattern of HIV neuropathology in the HAART era. *Journal of neuropathology and experimental neurology*. 2003; 62(5):429–440. [PubMed: 12769183]
- Gris P, Gauthier J, Cheng P, Gibson DG, Gris D, Laur O, Pierson J, Wentworth S, Nackley AG, Maixner W, Diatchenko L. A novel alternatively spliced isoform of the mu-opioid receptor: functional antagonism. *Molecular pain*. 2010; 6(33):1–10. [PubMed: 20089138]
- Groneberg DA, Fischer A. Endogenous opioids as mediators of asthma. *Pulmonary pharmacology & therapeutics*. 2001; 14(5):383–389. [PubMed: 11603951]
- Guo C-J, Li Y, Tian S, Wang X, Douglas SD, Ho W-Z. Morphine enhances HIV infection of human blood mononuclear phagocytes through modulation of β -chemokines and CCR5 receptor. *Journal of investigative medicine*. 2002; 50(6):435–442. [PubMed: 12425430]
- Han W, Kasai S, Hata H, Takahashi T, Takamatsu Y, Yamamoto H, Uhl GR, Sora I, Ikeda K. Intracisternal A-particle element in the 3' noncoding region of the mu-opioid receptor gene in CXBK mice: a new genetic mechanism underlying differences in opioid sensitivity. *Pharmacogenetics and genomics*. 2006; 16(6):451–460. [PubMed: 16708053]
- Hauser KF, El-Hage N, Buch S, Berger JR, Tyor WR, Nath A, Bruce-Keller AJ, Knapp PE. Molecular targets of opiate drug abuse in neuroAIDS. *Neurotoxicity research*. 2005; 8(1–2):63–80. [PubMed: 16260386]
- Hauser KF, El-Hage N, Buch S, Nath A, Tyor WR, Bruce-Keller AJ, Knapp PE. Impact of opiate-HIV-1 interactions on neurotoxic signaling. *Journal of neuroimmune pharmacology*. 2006; 1(1):98–105. [PubMed: 18040795]
- Hauser KF, El-Hage N, Stiene-Martin A, Maragos WF, Nath A, Persidsky Y, Volsky DJ, Knapp PE. HIV-1 neuropathogenesis: glial mechanisms revealed through substance abuse. *Journal of neurochemistry*. 2007; 100(3):567–586. [PubMed: 17173547]
- He Y, Yang C, Kirkmire CM, Wang ZJ. Regulation of opioid tolerance by let-7 family microRNA targeting the μ opioid receptor. *The Journal of neuroscience*. 2010; 30(30):10251–10258. [PubMed: 20668208]
- Hwang CK, Kim CS, Kim DK, Law P-Y, Wei L-N, Loh HH. Up-regulation of the μ -opioid receptor gene is mediated through chromatin remodeling and transcriptional factors in differentiated neuronal cells. *Molecular pharmacology*. 2010; 78(1):58–68. [PubMed: 20385708]
- Hwang CK, Song KY, Kim CS, Choi HS, Guo X-H, Law P-Y, Wei L-N, Loh HH. Evidence of endogenous mu opioid receptor regulation by epigenetic control of the promoters. *Molecular and cellular biology*. 2007; 27(13):4720–4736. [PubMed: 17452465]
- Hwang CK, Song KY, Kim CS, Choi HS, Guo X-H, Law P-Y, Wei L-N, Loh HH. Epigenetic programming of μ -opioid receptor gene in mouse brain is regulated by MeCP2 and Brg1 chromatin remodelling factor. *Journal of cellular and molecular medicine*. 2009; 2009; 13(9B):3591–3615. [PubMed: 19602036]
- Ide S, Han W, Kasai S, Hata H, Sora I, Ikeda K. Characterization of the 3' untranslated region of the human mu-opioid receptor (MOR-1) mRNA. *Gene*. 2005; 364:139–145. [PubMed: 16122888]

- Iorio MV, Piovan C, Croce CM. Interplay between microRNAs and the epigenetic machinery: an intricate network. *Biochimica et biophysica acta*. 2010; 1799(10–12):694–701. [PubMed: 20493980]
- Johnson EE, Christie MJ, Connor M. The role of opioid receptor phosphorylation and trafficking in adaptations to persistent opioid treatment. *Neuro-Signals*. 2005; 14(6):290–302. [PubMed: 16772732]
- Kang J, Shi Y, Xiang B, Qu B, Su W, Zhu M, Zhang M, Bao G, Wang F, Zhang X, Yang R, Fan F, Chen X, Pei G, Ma L. A nuclear function of β -arrestin1 in GPCR signaling: regulation of histone acetylation and gene transcription. *Cell*. 2005; 123(5):833–847. [PubMed: 16325578]
- Kaul M, Garden GA, Lipton SA. Pathways to neuronal injury and apoptosis in HIV-associated dementia. *Nature*. 2001; 410(6831):988–994. [PubMed: 11309629]
- Kim DK, Hwang CK, Wagley Y, Law P-Y, Wei L-N, Loh HH. p38 Mitogen-activated protein kinase and PI3-kinase are involved in up-regulation of mu opioid receptor transcription induced by cycloheximide. *Journal of neurochemistry*. 2011; 116(6):1077–1087. [PubMed: 21198637]
- Klase Z, Kale P, Winograd R, Gupta MV, Heydarian M, Berro R, McCaffrey T, Kashanchi F. HIV-1 TAR element is processed by Dicer to yield a viral micro-RNA involved in chromatin remodeling of the viral LTR. *BMC Molecular Biology*. 2007; 8(63):1–19. [PubMed: 17204145]
- Koch T, Schulz S, Pfeiffer M, Klutzy M, Schröder H, Kahl E, Höllt V. C-terminal splice variants of the mouse μ -opioid receptor differ in morphine-induced internalization and receptor resensitization. *The Journal of biological chemistry*. 2001; 276(33):31408–31414. [PubMed: 11359768]
- Koch T, Schulz S, Schröder H, Wolf R, Raulf E, Höllt V. Carboxyl-terminal splicing of the rat μ opioid receptor modulates agonist-mediated internalization and receptor resensitization. *The Journal of biological chemistry*. 1998; 273(22):13652–13657. [PubMed: 9593704]
- Kraus J. Regulation of mu-opioid receptors by cytokines. *Frontiers in bioscience (Scholar edition)*. 2009; 2009; S1:164–170. [PubMed: 19482692]
- Kraus J, Borner C, Giannini E, Hickfang K, Braun H, Mayer P, Hoehe MR, Ambrosch A, König W, Höllt V. Regulation of μ -opioid receptor gene transcription by interleukin-4 and influence of an allelic variation within a STAT6 transcription factor binding site. *The Journal of biological chemistry*. 2001; 276(47):43901–43908. [PubMed: 11572871]
- Kraus J, Börner C, Giannini E, Höllt V. The role of nuclear factor in tumor necrosis factor-regulated transcription of the human μ -opioid receptor gene. *Molecular pharmacology*. 2003; 64(4):876–884. [PubMed: 14500744]
- Kraus J, Börner C, Lendeckel U, Höllt V. Interferon- γ down-regulates transcription of the μ -opioid receptor gene in neuronal and immune cells. *Journal of neuroimmunology*. 2006; 181(1–2):13–18. [PubMed: 16914208]
- Kraus J, Lehmann L, Börner C, Höllt V. Epigenetic mechanisms involved in the induction of the mu opioid receptor gene in Jurkat T cells in response to interleukin-4. *Molecular immunology*. 2010; 48(1–3):257–263. [PubMed: 20828825]
- Kvam T-M, Baar C, Rakvåg TT, Kaasa S, Krokan HE, Skorpen F. Genetic analysis of the murine μ opioid receptor: increased complexity of Oprm gene splicing. *Journal of molecular medicine*. 2004; 82(4):250–255. [PubMed: 14991152]
- Law PY, Wong YH, Loh HH. Molecular mechanisms and regulation of opioid receptor signaling. *Annual review of pharmacology and toxicology*. 2000; 40:389–430.
- Li H, Liu H, Wang Z, Liu X, Guo L, Huang L, Gao L, McNutt MA, Li G. The role of transcription factors Sp1 and YY1 in proximal promoter region in initiation of transcription of the mu opioid receptor gene in human lymphocytes. *Journal of cellular biochemistry*. 2008; 104(1):237–250. [PubMed: 17990281]
- Li Y, Merrill JD, Mooney K, Song L, Wang X, Guo C-J, Savani RC, Metzger DS, Douglas SD, Ho W-Z. Morphine enhances HIV infection of neonatal macrophages. *Pediatric research*. 2003; 54(2):282–288. [PubMed: 12736382]
- Li Y, Wang X, Tian S, Guo C-J, Douglas SD, Ho W-Z. Methadone enhances human immunodeficiency virus infection of human immune cells. *The Journal of infectious diseases*. 2002; 185(1):118–122. [PubMed: 11756991]

- Lin Y-C, Flock KE, Cook RJ, Hunkele AJ, Loh HH, Ko JL. Effects of trichostatin A on neuronal mu-opioid receptor gene expression. *Brain research*. 2008; 1246:1–10. [PubMed: 18950606]
- Lindl KA, Marks DR, Kolson DL, Jordan-Sciutto KL. HIV-associated neurocognitive disorder: pathogenesis and therapeutic opportunities. *Journal of neuroimmune pharmacology*. 2010; 5(3): 294–309. [PubMed: 20396973]
- Liu H, Li H, Guo L, Li C, Li M, Jiang W, Liu X, McNutt MA, Li G. The mechanism involved in the repression of the μ opioid receptor gene expression in CEM $\times$ 174 cells infected by simian immunodeficiency virus. *Journal of leukocyte biology*. 2009; 85(4):684–691. [PubMed: 19164126]
- Liu H, Li H, Guo L, Li M, Li C, Wang S, Jiang W, Liu X, McNutt MA, Li G. Mechanisms involved in phosphatidylinositol 3-kinase pathway mediated up-regulation of the mu opioid receptor in lymphocytes. *Biochemical pharmacology*. 2010; 79(3):516–523. [PubMed: 19765550]
- Louboutin J-P, Reyes BAS, Agrawal L, van Bockstaele EJ, Strayer DS. HIV-1 gp120-induced neuroinflammation: relationship to neuron loss and protection by rSV40-delivered antioxidant enzymes. *Experimental neurology*. 2010; 221(1):231–245. [PubMed: 19913017]
- Ma L, Pei G. β -arrestin signaling and regulation of transcription. *Journal of cell science*. 2007; 120(2): 213–218. [PubMed: 17215450]
- McArthur JC. HIV dementia: an evolving disease. *Journal of neuroimmunology*. 2004; 2004; 157(1–2):3–10. [PubMed: 15579274]
- Meaney MJ, Ferguson-Smith AC. Epigenetic regulation of the neural transcriptome: the meaning of the marks. *Nature neuroscience*. 2010; 13(11):1313–1318.
- Mizoguchi H, Tseng LF, Suzuki T, Sora I, Narita M. Differential mechanism of G-protein activation induced by endogenous μ -opioid peptides, endomorphin and β -endorphin. *Japanese journal of pharmacology*. 2002a; 89(3):229–234. [PubMed: 12184727]
- Mizoguchi H, Wu HE, Narita M, Loh HH, Nagase H, Tseng LF. Loss of μ -opioid receptor-mediated G-protein activation in the pons/medulla of mice lacking the exons 2 and 3 of μ -opioid receptor gene. *Neuroscience letters*. 2002b; 335(2):91–94. [PubMed: 12459506]
- Mizoguchi H, Wu HE, Narita M, Sora I, Hall SF, Uhl GR, Loh HH, Nagase H, Tseng LF. Lack of μ -opioid receptor-mediated G-protein activation in the spinal cord of mice lacking Exon 1 or Exons 2 and 3 of the MOR-1 gene. *J Pharmacol Sci*. 2003; 93(4):423–429. [PubMed: 14737012]
- Moorman J, Zhang Y, Liu B, Lesage G, Chen Y, Stuart C, Prayther D, Yin D. HIV-1 gp120 primes lymphocytes for opioid-induced, β -arrestin 2-dependent apoptosis. *Biochimica et biophysica acta*. 2009; 1793(8):1366–1371. [PubMed: 19477204]
- Mrkusich EM, Kivell BM, Miller JH, Day DJ. Abundant expression of mu and delta opioid receptor mRNA and protein in the cerebellum of the fetal, neonatal, and adult rat. *Developmental brain research*. 2004; 148(2):213–222. [PubMed: 14766199]
- Nath A. Human immunodeficiency virus-associated neurocognitive disorder: pathophysiology in relation to drug addiction. *Annals of the New York Academy of Sciences*. 2010; 1187:122–128. [PubMed: 20201849]
- Nathans R, Chu C-Y, Serquina AK, Lu C-C, Cao H, Rana TM. Cellular microRNA and P bodies modulate host-HIV-1 interactions. *Molecular cell*. 2009; 34(6):696–709. [PubMed: 19560422]
- Nielsen DA, Hamon S, Yuferov V, Jackson C, Ho A, Ott J, Kreek MJ. Ethnic diversity of DNA methylation in the OPRM1 promoter region in lymphocytes of heroin addicts. *Human genetics*. 2010; 127(6):639–649. [PubMed: 20237803]
- Nielsen DA, Yuferov V, Hamon S, Jackson C, Ho A, Ott J, Kreek MJ. Increased OPRM1 DNA methylation in lymphocytes of methadone-maintained former heroin addicts. *Neuropsychopharmacology*. 2009; 34(4):867–873. [PubMed: 18650805]
- Oldfield S, Braksator E, Rodriguez-Martin I, Bailey CP, Donaldson LF, Henderson G, Kelly E. C-terminal splice variants of the μ -opioid receptor: existence, distribution and functional characteristics. *Journal of neurochemistry*. 2008; 104(4):937–945. [PubMed: 18005002]
- Ouellet DL, Plante I, Barat C, Tremblay MJ, Provost P. Emergence of a complex relationship between HIV-1 and the microRNA pathway. *Methods in molecular biology*. 2009; 487:415–433. [PubMed: 19301659]

- Pan L, Xu J, Yu R, Xu M-M, Pan Y-X, Pasternak GW. Identification and characterization of six new alternatively spliced variants of the human μ opioid receptor gene, Oprm. *Neuroscience*. 2005a; 133(1):209–220. [PubMed: 15893644]
- Pan Y-X. Identification and characterization of a novel promoter of the mouse mu opioid receptor gene (Oprm) that generates eight splice variants. *Gene*. 2002; 295(1):97–108. [PubMed: 12242016]
- Pan Y-X, Xu J, Bolan E, Abbadie C, Chang A, Zuckerman A, Rossi G, Pasternak GW. Identification and characterization of three new alternatively spliced μ -opioid receptor isoforms. *Molecular pharmacology*. 1999; 56(2):396–403. [PubMed: 10419560]
- Pan Y-X, Xu J, Bolan E, Moskowitz HS, Xu M, Pasternak GW. Identification of four novel exon 5 splice variants of the mouse μ -opioid receptor gene: functional consequences of C-terminal splicing. *Molecular pharmacology*. 2005b; 68(3):866–875. [PubMed: 15939800]
- Pan Y-X, Xu J, Mahurter L, Bolan E, Xu M, Pasternak GW. Generation of the mu opioid receptor (MOR-1) protein by three new splice variants of the Oprm gene. *Proceedings of the National Academy of Sciences of the United States of America*. 2001; 98(24):14084–14089. [PubMed: 11717463]
- Pan Y-X, Xu J, Mahurter L, Xu M, Gilbert A-K, Pasternak GW. Identification and characterization of two new human mu opioid receptor splice variants, hMOR-1O and hMOR-1X. *Biochemical and biophysical research communications*. 2003; 301(4):1057–1061. [PubMed: 12589820]
- Pan YX, Bolan E, Chang A, Pasternak GW. Isolation and expression of a novel alternatively spliced mu opioid receptor isoform, MOR-1F. *FEBS letters*. 2000; 466:337–340. [PubMed: 10682855]
- Paschos K, Allday MJ. Epigenetic reprogramming of host genes in viral and microbial pathogenesis. *Trends in microbiology*. 2010; 18(10):439–447. [PubMed: 20724161]
- Pasternak DA, Pan L, Xu J, Yu R, Xu M-M, Pasternak GW, Pan Y-X. Identification of three new alternatively spliced variants of the rat mu opioid receptor gene: dissociation of affinity and efficacy. *Journal of neurochemistry*. 2004; 91(4):881–890. [PubMed: 15525342]
- Pasternak GW. Multiple opiate receptors: déjà vu all over again. *Neuropharmacology*. 2004; 47(Supplemental No.1):312–323. [PubMed: 15464147]
- Pasternak GW. Molecular insights into μ opioid pharmacology: From the clinic to the bench. *The Clinical journal of pain*. 2010; 26(1):S3–S9. [PubMed: 20026962]
- Peckys D, Landwehrmeyer GB. Expression of mu, kappa, and delta opioid receptor messenger RNA in the human CNS: a ^{33}P in situ hybridization study. *Neuroscience*. 1999; 88(4):1093–1135. [PubMed: 10336124]
- Peterson PK, Gekker G, Hu S, Anderson WR, Kravitz F, Portoghese PS, Balfour HH, Chao CC. Morphine amplifies HIV-1 expression in chronically infected promonocytes cocultured with human brain cells. *Journal of neuroimmunology*. 1994; 50(2):167–175. [PubMed: 8120138]
- Peterson PK, Gekker G, Hu S, Lokensgard J, Portoghese PS, Chao CC. Endomorphin-1 potentiates HIV-1 expression in human brain cell cultures: implication of an atypical μ -opioid receptor. *Neuropharmacology*. 1999; 38(2):273–278. [PubMed: 10218868]
- Peterson PK, Sharp BM, Gekker G, Portoghese PS, Sannerud K, Balfour HH. Morphine promotes the growth of HIV-1 in human peripheral blood mononuclear cell cocultures. *AIDS*. 1990; 4(9):869–873. [PubMed: 2174676]
- Polakiewicz RD, Schieferl SM, Gingras AC, Sonenberg N, Comb MJ. μ -Opioid receptor activates signaling pathways implicated in cell survival and translational control. *The Journal of biological chemistry*. 1998; 273(36):23534–23541. [PubMed: 9722592]
- Power C, Boissé L, Rourke S, Gill MJ. NeuroAIDS: an evolving epidemic. *The Canadian journal of neurological sciences*. 2009; 36(3):285–295. [PubMed: 19534327]
- Rappaport J, Berger JR. Genetic testing and HIV dementia: teasing out the molecular mechanisms of disease. *AIDS*. 2010; 24(10):1585–1587. [PubMed: 20523202]
- Ravindranathan A, Joslyn G, Robertson M, Schuckit MA, Whistler JL, White RL. Functional characterization of human variants of the mu-opioid receptor gene. *Proceedings of the National Academy of Sciences of the United States of America*. 2009; 106(26):10811–10816. [PubMed: 19528663]
- Reisine T. Opiate receptors. *Neuropharmacology*. 1995; 34(5):463–472. [PubMed: 7566479]

- Rogers TJ. Immunology as it pertains to drugs of abuse, AIDS and the neuroimmune axis: mediators and traffic. *Journal of neuroimmune pharmacology*. 2011; 6(1):20–27. [PubMed: 20957520]
- Sacktor N. The epidemiology of human immunodeficiency virus-associated neurological disease in the era of highly active antiretroviral therapy. *Journal of neurovirology*. 2002; 8:115–121. [PubMed: 12491162]
- Schuller AG, King MA, Zhang J, Bolan E, Pan Y-X, Morgan DJ, Chang A, Czick ME, Unterwald EM, Pasternak GW, Pintar JE. Retention of heroin and morphine-6 β -glucuronide analgesia in a new line of mice lacking exon 1 of MOR-1. *Nature neuroscience*. 1999; 2(2):151–156.
- Singh VK, Bajpai K, Biswas S, Haq W, Khan MY, Mathur KB. Molecular biology of opioid receptors: recent advances. *Neuroimmunomodulation*. 1997; 4(5–6):285–297. [PubMed: 9650823]
- Smith AP, Lee NM. Opioid receptor interactions: local and nonlocal, symmetric and asymmetric, physical and functional. *Life sciences*. 2003; 73(15):1873–1893. [PubMed: 12899914]
- Song G, Ouyang G, Bao S. The activation of Akt/PKB signaling pathway and cell survival. *Journal of cellular and molecular medicine*. 2005; 9(1):59–71. [PubMed: 15784165]
- Ständer S, Gunzer M, Metze D, Luger T, Steinhoff M. Localization of μ -opioid receptor 1A on sensory nerve fibers in human skin. *Regulatory peptides*. 2002; 110(1):75–83. [PubMed: 12468112]
- Suzuki S, Carlos MP, Chuang LF, Torres JV, Doi RH, Chuang RY. Methadone induces CCR5 and promotes AIDS virus infection. *FEBS letters*. 2002; 519(1–3):173–177. [PubMed: 12023039]
- Tanowitz M, Hislop JN, von Zastrow M. Alternative splicing determines the post-endocytic sorting fate of G-protein-coupled receptors. *The Journal of biological chemistry*. 2008; 283(51):35614–35621. [PubMed: 18936093]
- Tarn W-Y. Cellular signals modulate alternative splicing. *Journal of biomedical science*. 2007; 14(4):517–522. [PubMed: 17385059]
- Tegeder I, Geisslinger G. Opioids as modulators of cell death and survival-unraveling mechanisms and revealing new indications. *Pharmacological reviews*. 2004; 56(3):351–369. [PubMed: 15317908]
- Toda N, Kishioka S, Hatano Y, Toda H. Interactions between morphine and nitric oxide in various organs. *Journal of anesthesia*. 2009; 23(4):554–568. [PubMed: 19921366]
- Turchan-Cholewo J, Dimayuga FO, Gupta S, Keller JN, Knapp PE, Hauser KF, Bruce-Keller AJ. Morphine and HIV-Tat increase microglial-free radical production and oxidative stress: possible role in cytokine regulation. *Journal of neurochemistry*. 2009; 108(1):202–215. [PubMed: 19054280]
- Vlahov D, Robertson AM, Strathdee SA. Prevention of HIV infection among injection drug users in resource-limited settings. *Clinical infectious diseases*. 2010; 50(Suppl 3):S114–S121. [PubMed: 20397939]
- Voussooghi N, Goodarzi A, Roushanzamir F, Sedaghati T, Zarrindast M-R, Noori-Daloii M-R. Expression of mu opioid receptor splice variants mRNA in human blood lymphocytes: a peripheral marker for opioid addiction studies. *International immunopharmacology*. 2009; 9(7–8):1016–1020. [PubMed: 19272466]
- Wang H, Wessendorf MW. μ - and δ -opioid receptor mRNAs are expressed in periaqueductal gray neurons projecting to the rostral ventromedial medulla. *Neuroscience*. 2002; 109(3):619–634. [PubMed: 11823071]
- Wang T, Rumbaugh JA, Nath A. Viruses and the brain: from inflammation to dementia. *Clinical science*. 2006; 110(4):393–407. [PubMed: 16526945]
- Wang X, Ye L, Hou W, Zhou Y, Wang Y-J, Metzger DS, Ho W-Z. Cellular microRNA expression correlates with susceptibility of monocytes/macrophages to HIV-1 infection. *Blood*. 2009; 113(3):671–674. [PubMed: 19015395]
- Wang X, Ye L, Zhou Y, Liu M-Q, Zhou D-J, Ho W-Z. Inhibition of anti-HIV microRNA expression: a mechanism for opioid-mediated enhancement of HIV infection of monocytes. *The American journal of pathology*. 2011; 178(1):41–47. [PubMed: 21224041]
- Wei L-N. Epigenetic control of the expression of opioid receptor genes. *Epigenetics*. 2008; 3(3):119–121. [PubMed: 18497574]
- Wei L-N, Loh HH. Transcriptional and epigenetic regulation of opioid receptor genes: present and future. *Annual review of pharmacology and toxicology*. 2011; 51:75–97.

- Wu Q, Law P-Y, Wei L-N, Loh HH. Post-transcriptional regulation of mouse μ opioid receptor (MOR1) via its 3' untranslated region: a role for microRNA23b. *The FASEB journal*. 2008; 22(12):4085–4095.
- Xie N, Li H, Wei D, Lesage G, Chen L, Wang S, Zhang Y, Chi L, Ferslew K, He L, Chi Z, Yin D. Glycogen synthase kinase-3 and p38 MAPK are required for opioid-induced microglia apoptosis. *Neuropharmacology*. 2010; 59(6):444–451. [PubMed: 20600172]
- Xu J, Xu M, Hurd YL, Pasternak GW, Pan Y-X. Isolation and characterization of new exon 11-associated N-terminal splice variants of the human mu opioid receptor gene. *Journal of neurochemistry*. 2009; 108(4):962–972. [PubMed: 19077058]
- Xu Y, Carr LG. Binding of Sp1/Sp3 to the proximal promoter of the hMOR gene is enhanced by DAMGO. *Gene*. 2001a; 274(1–2):119–128. [PubMed: 11675004]
- Xu Y, Carr LG. Transcriptional regulation of the human μ opioid receptor (hMOR) gene: evidence of positive and negative cis-acting elements in the proximal promoter and presence of a distal promoter. *DNA and cell biology*. 2001b; 20(7):391–402. [PubMed: 11506703]
- Yadav A, Collman RG. CNS inflammation and macrophage/microglial biology associated with HIV-1 infection. *Journal of neuroimmune pharmacology*. 2009; 4(4):430–447. [PubMed: 19768553]
- Yin D, Woodruff M, Zhang Y, Whaley S, Miao J, Ferslew K, Zhao J, Stuart C. Morphine promotes Jurkat cell apoptosis through pro-apoptotic FADD/P53 and anti-apoptotic PI3K/Akt/NF- κ pathways. *Journal of neuroimmunology*. 2006; 174(1–):101–107. [PubMed: 16529824]
- Zadina JE, Chang SL, Ge LJ, Kastin AJ. Mu opiate receptor down-regulation by morphine and up-regulation by naloxone in SH-SY5Y human neuroblastoma cells. *The Journal of pharmacology and experimental therapeutics*. 1993; 265(1):254–262. [PubMed: 8097244]
- Zhang R, Su B. Small but influential: the role of microRNAs on gene regulatory network and 3'UTR evolution. *Journal of genetics and genomics*. 2009; 36(1):1–6. [PubMed: 19161940]
- Zhang X, Zeng Y. Regulation of mammalian microRNA expression. *Journal of cardiovascular translational research*. 2010; 3(3):197–203. [PubMed: 20560040]
- Zhang Y, Pan Y-X, Kolesnikov Y, Pasternak GW. Immunohistochemical labeling of the mu opioid receptor carboxy terminal splice variant mMOR-1B4 in the mouse central nervous system. *Brain research*. 2006; 1099(1):33–43. [PubMed: 16793025]
- Zhang Y, Xiong W, Lin X, Ma X, Yu L-C. Receptor trafficking induced by μ -opioid-receptor phosphorylation. *Neuroscience and biobehavioral reviews*. 2009; 33(8):1192–1197. [PubMed: 19747597]
- Zheng H, Loh HH, Law P-Y. Agonist-selective signaling of G protein-coupled receptor: mechanisms and implications. *IUBMB life*. 2010; 62(2):112–119. [PubMed: 20058265]

Figure 1. Exon mapping of the human OPRM1 gene

Pre-mRNA transcripts from the OPRM1 gene undergo extensive splicing to generate 21 known isoforms of the human μ -opioid receptor. Shown above are the known exon regions of the OPRM1 gene. Solid arrows indicate that the exon below is derived from a region of the above exon. For example, exon 3c is generated from a segment of exon 3a. This may occur either due to incomplete incorporation of the exon or the incorporation of flanking intronic sequences. Given the complexity of the OPRM1 exon expression, transcriptional and epigenetic regulation, particularly interacting histone modifications, chromatin-binding proteins, and splicing factors at the intron-exon junctions, is critical for regulating differential splicing of the MOR. Exact mechanisms regulating alternative splicing specificity of the MOR are not known. As such, the corresponding exon names and numbers may not match those cited in earlier literature.

Figure 2. Schematic representation of the mechanisms involved in OPRM1 gene regulation
The OPRM1 gene expression is tightly controlled through a variety of transcription factors, alternative splicing mechanism, miRNAs, and by epigenetic mechanisms. The expression of OPRM1 by cytokines (1) and opioid agonist (2) can be regulated by a group of nuclear receptors and transcription factors. Recent evidences suggest that OPRM1 expression can also be regulated by epigenetic mechanisms, namely histone modifications (3) DNA methylation (4), and microRNAs (5). Finally, the expression of OPRM1 can also be regulated by mechanisms involving spliceosome recruitment to generate alternatively spliced MOR isoforms (6).

Isoforms of the human μ -Opioid Receptor

Table 1

Currently there are 21 known isoforms of the human μ -opioid receptor generated by alternative splicing. Here we show both the exon map and protein sequence of each individual MOR isoform as well as known localization in human tissue and downstream signaling. The exon sequence encoding for each isoform is indicated in the table. The exon map of the OPRM1 gene is shown in Figure 1. The protein sequences of conserved regions are indicated in the table and conserved protein sequences are shown as textured boxes. Full protein sequences of conserved regions are indicated below. Textured boxes that contain a short amino acid sequence indicate that in that given isoform, the conserved region is truncated after the sequence shown. All exon and protein sequences were obtained from the NCBI database. As such, the exon names and numbers corresponding to a given isoform may not match those cited in earlier literature.

Variant	Exon Map	Variant	Tissue Expression	Signaling	Ref
Mor-1	3a-5a-6a-11		Immune Cells, Spinal cord, Pre-frontal Cortex, Temporal cortex, Piriform cortex, Nucleus accumbens, Pons, Cerebellum	<ul style="list-style-type: none"> - Gi/Go Coupled GPCR - Inhibition of adenylyl cyclase and cAMP - Inhibition of N-, P/Q-, and L-type Ca²⁺ channels, - Stimulate K⁺ channels - Activate (MAP) kinase cascade 	(Law et al., 2000; Xu et al., 2009)
MOR-1A	3a-5a-6b		Cutaneous Nerve Fibers, Be(2)C Cells, CNS	-Inhibition of adenylyl cyclase and cAMP	(Bare et al., 1994; Pan et al., 2005a; Ständer et al., 2002)
MOR-1B1	3a-5a-6a-10		Be(2)C Cells	-Inhibition of adenylyl cyclase and cAMP	(Pan et al., 2005a)
MOR-1B2	3a-5a-6a-9b		Be(2)C Cells	-Inhibition of adenylyl cyclase and cAMP	(Pan et al., 2005a)
MOR-1B3	3c-5a-6a-9a		Be(2)C Cells	-Inhibition of adenylyl cyclase and cAMP	(Pan et al., 2005a)
MOR-1B4	3a-5a-6a-8b		Be(2)C Cells	-Inhibition of adenylyl cyclase and cAMP	(Pan et al., 2005a)
MOR-1B5	3a-5a-6a-8a		Be(2)C Cells	-Inhibition of adenylyl cyclase and cAMP	(Pan et al., 2005a)
MOR-1C	3d-5c-6a-12		Unknown	Unknown	

Variant	Exon Map	Variant	Tissue Expression	Signaling	Ref
MOR-1G1	1b-5b-6a-11		Spinal cord, Pre-frontal Cortex, Temporal cortex, Piriform cortex, Nucleus accumbens, Pons, Cerebellum	Unknown	(Xu et al., 2009)
MOR-1G2	1a-5a-6a-11		Spinal cord, Pre-frontal Cortex, Temporal cortex, Piriform cortex, Nucleus accumbens, Pons, Cerebellum	Unknown	(xu et al., 2009)
MOR-1H	1c-2-3c-5a-6a-11		Unknown	Unknown	
MOR-1I	1d-3c-5a-6a-11b		Pre-frontal cortex, Piriform cortex, Pons	Unknown	(Xu et al., 2009)
MOR-1JL	J-21-3J-4		Unknown	Unknown	
MOR-1K1	13a-2K-3K-4		Frontal lobe, Medulla oblongata, Insula, Nucleus accumbens, Pons, Spinal cord, and Dorsal root ganglion	<ul style="list-style-type: none"> - GS Coupled GPCR - Activation of adenylyl cyclase and cAMP - Activation of Ca2+ channels - Increased NO production 	(Gris et al., 2010)
MOR-1K2	13b-2K-3K-4		Frontal lobe, Medulla oblongata, Insula, Nucleus accumbens, Pons, Spinal cord, and Dorsal root ganglion	<ul style="list-style-type: none"> - GS Coupled GPCR - Activation of adenylyl cyclase and cAMP - Activation of Ca2+ channels - Increased NO production 	(Gris et al., 2010)
MOR-1O	3b-5c-6a-12		Be(2)/C Cells, CNS	Unknown	(Cadet, 2004; Pan et al., 2003)
MOR-1R	3d-5a-6a-7		Unknown	Unknown	
MOR-1V	3f-5a-6a-9-11c		Unknown	Unknown	
MOR-1W	W-5a-6b		Unknown	Unknown	

Variant	Exon Map	Variant	Tissue Expression	Signaling	Ref
MOR-1X	3b-5c-6a-7		Be(2)C Cells, CNS	Unknown	(Pan et al., 2003)
MOR-1Y	3d-5a-6a-9c		Be(2)C Cells	-Inhibition of adenylyl cyclase and cAMP	(Pan et al., 2005a)

MCLHRRVPSEETYSLDRFAQNPPLFPPLPPSLPASESRMAHPLLQRCGAARTGFCKKQQELWQRRKEAALGTRKYSVLLATSHSGARPAVST

MDSSAAPTNASNCTDALAYSSCSPAPSPGSWWNLSHLDGNLSDPCGPNRTDLGGRDGLCPPTGSPSMITAIMALYSIVCVVGLFGNFLVMYVIVRYTK

MKTATNYIFNLALADALATSTLTPFQSVNYLMGTWPFGTILCKIVISIDYYNNMFTSIFTLCTMSVDRYIAVCHPVKALDFRTPRAKIINVCNWILSSAIGLPVMFMATTKYRQGSIDCTLTFSHPTWY
WENLLKICVFIFAFIMPVLITVCYGLMILRLKSVRMLSGSKEKDRNLRITRMVLVVAVFVVCWTPHIYVVIKALVTIPETTQTVSWHFCIALGYTNSCLNPVLYAFLDENFKRCFREFCIP'TSSNI
EQQNSTRIRQNRDHPSTANTVDRTNHQ