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Functions of the Chemokine Receptor CXCR4 in the Central Nervous System and Its Regulation by μ-Opioid Receptors

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Abstract

Activation of the G protein-coupled receptor CXCR4 by its chemokine ligand CXCL12 regulates a number of physiopathological functions in the central nervous system, during development as well as later in life. In addition to the more classical roles of the CXCL12/CXCR4 axis in the recruitment of immune cells or migration and proliferation of neural precursor cells, recent studies suggest that CXCR4 signaling also modulates synaptic function and neuronal survival in the mature brain, through direct and indirect effects on neurons and glia. These effects, which include regulation of glutamate receptors and uptake, and of dendritic spine density, can significantly alter the ability of neurons to face excitotoxic insults. Therefore, they are particularly relevant to neurodegenerative diseases featuring alterations of glutamate neurotransmission, such as HIV-associated neurocognitive disorders. Importantly, CXCR4 signaling can be dysregulated by HIV viral proteins, host HIV-induced factors, and opioids. Potential mechanisms of opioid regulation of CXCR4 include heterologous desensitization, transcriptional regulation and changes in receptor expression levels, opioid–chemokine receptor dimer or heteromer formation, and the newly described modulation by the protein ferritin heavy chain—all leading to inhibition of CXCR4 signaling. After reviewing major effects of chemokines and opioids in the CNS, this chapter discusses chemokine–opioid interactions in neuronal and immune cells, focusing on their potential contribution to HIV-associated neurocognitive disorders.

1. CHEMOKINE SYSTEM OVERVIEW

In order for cells to communicate they must employ a language of sorts that allows them to respond to threats and to routine duties. Chemokines act as a part of this natural language and their physiological effects are myriad. Chemokine ligands are mostly secreted small proteins, although two chemokines, CX3CL1 and CXCL16, also exist in a membrane-bound form that allows their signaling events to happen specifically in nearby cells (Clark, Staniland, & Malcangio, 2011; La Porta, 2012). The chemokine superfamily is divided into different classes based on the order of four conserved cysteine residues. In alpha

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chemokines, the first two conserved cysteines are separated by any amino acid. Therefore, this class is denoted as CXC. The receptor or ligand designation (L/R) follows, and then a numerical identifier (Zlotnik & Yoshie, 2000). Other chemokine classes include CC, which has adjacent conserved cysteines, (X)C, which has only two conserved cysteines, and CX3C, which has three amino acids separating the first two conserved cysteines. Typically, chemokines in a particular class may only stimulate receptors of the same class, but this does not eliminate natural redundancy from the system as many chemokine ligands display promiscuous binding to receptors within their family (Zlotnik & Yoshie, 2012). Chemokine receptors are seven-transmembrane G protein-coupled receptors (GPCRs) that mostly signal through Gαi proteins (Réaux-Le Goazigo, Van Steenwinckel, Rostène, & Mélik Parsadaniantz, 2013) and thus are subject to GPCR–GPCR interactions that can modulate intracellular signals after ligand binding. In some cases, chemokine receptors can regulate the strength of an external signal by forming dimeric complexes (Mellado et al., 2001). Both homo- and heterodimers seem to occur within the chemokine receptor family, and heterodimers composed of chemokine/opioid receptors are thought to play an important role in signaling modulation of immune and neural cells (Mellado et al., 2001). Chemokine and opioid interactions at the receptor level will be covered in an upcoming section.

Chemokines have also been characterized on the basis of their function as inflammatory or homeostatic (Moser, Wolf, Walz, & Loetscher, 2004). Inflammatory chemokines are upregulated in damaged tissues and activated immune cells and have the ability to recruit immune effector cells to an area of infection or inflammation. Although there are a large number of potentially inflammatory chemokines, these proteins are typically more promiscuous in their binding, and many of them are located on the same areas of chromosomes 4 and 17 (Nomiyama, Osada, & Yoshie, 2011). This redundancy ensures that a proper immune response can be mounted in tissues that may possess different chemokine secretion profiles. Inflammatory chemokines also promote angiogenesis and help to activate the blood vessel endothelium to become leakier and express anchor proteins that allow circulating immune cells to more easily enter an inflamed area (Strieter, Burdick, Gomperts, Belperio, & Keane, 2005). The second functional classification comes from the discovery that chemokines are necessary for normal homeostatic processes to occur. These chemokine receptor pairs are usually located on distinct chromosomal sites and have much less redundancy compared with the inflammatory variety of chemokines (Zlotnik & Yoshie, 2012). CXCR4, which is the focus of this review, exerts several homeostatic effects throughout the body, including the CNS (Lazarini, Tham, Casanova, Arenzana-Seisdedos, & Dubois-Dalcq, 2003; Réaux-Le Goazigo et al., 2013). CXCL12 is the only chemokine ligand known to bind CXCR4; downstream effects of the CXCL12/CXCR4 axis in the CNS include regulation of the retinoblastoma cell-cycle protein, ensuring cell survival for postmitotic cells (Khan et al., 2008), migration of neuronal precursor cells (Stumm et al., 2003), neurogenesis (Réaux-Le Goazigo et al., 2013), protection against neurotoxic insults (Khan et al., 2004; Meucci et al., 1998; Shepherd, Loo, & Mohapatra, 2013), and regulation of dendritic spine density (Pitcher et al., 2014). These effects depend on the receptor and ligand being constitutively expressed in these tissues, which is in stark contrast to the highly inducible expression of inflammatory chemokines.

The homeostatic and inflammatory characterization is not a firm boundary, as many chemokines have activity in both facets, and still other chemokine receptors serve more diverse functions. CXCR7 was originally defined as an orphan receptor until CXCL12 was reported to bind it (Balabanian et al., 2005). Upon further investigation, CXCR7 was shown to lack a DRY amino acid motif, implying that it was not capable of coupling to G proteins and activating the classical pathways associated with GPCRs (Graham, 2009). This receptor is important in development, as it acts as a chemokine sink for CXCL12, but it may have functionality that is cell-type specific and has yet to be uncovered through β-arrestin-mediated signals or other G protein-independent signals. Recent studies show that CXCR7's ability to act as a sink for CXCL12 prevents CXCR4 internalization in the presence of high-chemokine levels, thus preserving CXCR4 function and ensuring proper migration of interneurons in the developing CNS (Sanchez-Alcaniz et al., 2011). Involvement of CXCR7 in other physiological and developmental processes has yet to be fully characterized, although studies have highlighted its involvement in various cancers (Hattermann & Mentlein, 2013).

2. OPIOID SYSTEM OVERVIEW

The opioid system has been covered extensively in several past reviews (Corbett, Henderson, McKnight, & Paterson, 2006; Hutchinson et al., 2011; Waldhoer, Bartlett, & Whistler, 2004; Williams et al., 2013) and is discussed here only briefly. Opioids have a long history of usage as analgesics and pain relievers, and research today continues to reveal novel actions of these compounds. Opioid receptor ligands are typically referred to as opiates if they are obtained from natural substances such as the opium poppy and as opioids if they are either natural or made in a synthetic process. Opioid receptors (also GPCRs) exist in three major isoforms (μ, κ, and δ), which are expressed throughout the body (Wittert, Hope, & Pyle, 1996), and are sensitive to the general opioid receptor antagonist naloxone. Similarly to chemokine receptors, formation of receptor complexes with other opioid receptor subtypes, or other GPCRs can effect signal transduction to varying degrees. Opioid receptors have been reported to interact with chemokine receptors (described later) and cannabinoid receptors (Rios, Gomes, & Devi, 2006) resulting in decreased signal transduction for both opioid and cannabinoid ligands (Rios et al., 2006). Although opioid drugs are tightly regulated and are common drugs of abuse, natural opioid ligands produced by the body are important physiological regulators of many different processes, such as cell membrane homeostasis, cell proliferation, immune function, gastrointestinal function, and neuromodulation, among others (Feng et al., 2012). Importantly, endogenous opioids are peptide based, and are much larger than exogenous small-molecule opioids, although they are able to bind and activate the same receptors. Currently, 10 different endogenous opioid ligands have been discovered, which are all slightly different in their binding affinities and specificity for opioid receptor subtypes. Met and Leu enkephalin, the first endogenous opioid ligands discovered from purified brain extracts (Hughes et al., 1975), both derived from the precursor proenkephalin and have the greatest affinity for δ-opioid receptors. Dynorphins, the second group of endogenous opioids, are derived from the protein precursor prodynorphin and preferentially bind κ-opioid receptors (Goldstein, Tachibana, Lowney, Hunkapiller, & Hood, 1979). Endorphins are the third group, derived from pro-

opiomelanocortin, and possess the highest affinity for the μ subtype (Li, Chung, & Doneen, 1976). Endogenous opioids have very diverse roles in many different tissues. For example, opioids typically have an inhibitory effect on neuronal activity, but the μ-opioid receptor agonist DAMGO (Tyr-D-Ala-Gly-*N*-methyl-Phe-Gly-ol) is able to regulate resting membrane potential in Purkinje neurons by its ability to increase intracellular calcium levels via a G protein-independent pathway, which results in increased neurotransmitter release (Iegorova, Fisyunov, & Krishtal, 2010). Painful stimuli including injuries and lipopolysaccharide administration are known to cause an upregulation of the μ-opioid receptor agonist β endorphin, which serves as a natural form of analgesia (Molina, 2002), and opioids are able to control feeding depending on which receptor subtype is bound (Gosnell & Levine, 2009).

Exogenous opioids may be both beneficial and problematic depending on the compound and dose taken. Common opioid compounds such as morphine and heroin are highly addictive and likely to be abused to achieve a sensation of euphoria. Many cellular processes that depend on tight regulation of the opioid system can be affected under conditions of abuse (covered later in this chapter). As opioid drugs, in particular morphine, are still the standard of care for chronic pain and other disorders involving pain, and their potential for abuse makes their use more widespread, understanding their total physiological influence will be important for determining potential effects of opioids in other pathophysiological conditions, such as neurocognitive disorders.

3. CXCR4 AND OPIOIDS ACTIONS IN THE CENTRAL NERVOUS SYSTEM

Although both the chemokine and opioid families are expressed in many different tissues, these proteins have been intensively investigated in the nervous and immune system where they exert important physiological and pathological effects. With regard to the CNS, both chemokines and opioids have been shown to act as neuromodulators (Rostene, Kitabgi, & Parsadaniantz, 2007) and as mediators of cell-to-cell communication (Sheridan & Murphy, 2013). The following sections cover the CXCL12/ CXCR4 signaling axis and opioid family members in different CNS cell types, and their normal as well as pathological roles with a focus on HIV-associated neurocognitive disorders (HAND).

3.1. Physiological and pathological roles of CXCR4

CXCR4 is expressed in the brain and the spinal cord *in vitro* and *in vivo* in a vast variety of species (Meucci et al., 1998; Ohtani et al., 1998; Pitcher et al., 2014) and in all major CNS cell types, including neurons (Meucci et al., 1998; van der Meer, Ulrich, Gonzalez-Scarano, & Lavi, 2000), astroglia (Bajetto et al., 1999), microglia (Lipfert, Odemis, Wagner, Boltze, & Engele, 2013), and oligodendrocytes (Maysami et al., 2006). The receptor has the potential to activate several distinct signaling pathways and elicit various biological responses. The natural ligand CXCL12 binding results in inhibition of adenylate cyclase, via activation of Gαi proteins, and decreased protein kinase A activity (Zheng et al., 1999)—while also increasing intra-cellular Ca^{2+} levels and protein kinase C, via the phospholipase C pathway (Cali & Bezzi, 2010; Khan et al., 2004; Meucci et al., 1998). Additional downstream signals with direct effect on gene transcription include activation of the ERK and Akt cascade (Khan et al., 2004), the JAK/STAT, and the nuclear factor-κB pathways

(Ganju et al., 1998). As a homeostatic chemokine/receptor pair, these proteins have much more varied roles than contributing to immune responses compared to their inflammatory counterparts. The signaling outcomes of the receptor are indeed similar to the classical immune chemotactic response, but the same signals can also occur on nonimmune cells that express CXCR4. CXCR4 and CXCL12 are expressed in both the developing and mature CNS, where they serve multiple vital functions. For instance, during development, CXCL12 guides developing interneurons to their proper cortical layer via a CXCL12 gradient that is produced by resident cells (Sanchez-Alcaniz et al., 2011; Stumm et al., 2003). As explained earlier, CXCR7 was shown to act as a chemokine sink, removing excess CXCL12 from the extracellular space and, in the process, preserving the developing interneurons' responsiveness to CXCL12-mediated chemotaxis (Sanchez-Alcaniz et al., 2011). Although this function does not involve the immune system, the signaling outcome of chemotaxis is similar to outcomes observed with inflammatory chemokines, suggesting that this chemokine/receptor pair is more ancient and more important because of its presence in organisms that do not have a functional immune system (Huising, Stet, Kruiswijk, Savelkoul, & Lidy Verburg-van Kemenade, 2003). Additionally, CXCR4 knockout animals do not survive past birth, further indicating the importance of this receptor during development (Ma et al., 1998). In the mature CNS, CXCL12 may also have other functions that are more diverse than chemotaxis. In periods of neuronal stress or excitotoxicity, CXCL12 can protect neurons by several different mechanisms, as discussed later.

The importance of the CXCL12/CXCR4 signaling axis becomes much more apparent in pathological states where the signaling is dysregulated, including HIV infection. HIV can infect peripheral immune cells and use them as a liaison to enter the CNS (Gonzalez-Scarano & Martin-Garcia, 2005). Upon entering, the virus can establish a CNS reservoir and by extension cause activation of CNS immune cells and an inflammatory response (Kraft-Terry, Buch, Fox, & Gendelman, 2009). Both cells that are infected and uninfected can contribute to the inflammatory/excitotoxic state, and the HIV proteins themselves can have detrimental effects on many different CNS cell types (recently reviewed by Gonzalez-Scarano & Martin-Garcia, 2005; Lindl, Marks, Kolson, & Jordan-Sciutto, 2010). The HIV-envelope protein from X4 viruses uses CXCR4 for entry into cells, but it can also cause cell damage via CXCR4-dependent signaling events (Pandey & Bolsover, 2000). Depending on the cell type expressing CXCR4, this binding event can result in different outcomes. For example, gp120-induced activation of CXCR4 in glia can cause secretion of several inflammatory mediators, including tumor necrosis factor-α and interleukin-1β (Bezzi et al., 2001). These mediators can activate nearby uninfected immune cells and precipitate inflammatory responses in the CNS (Kraft-Terry et al., 2009). Astrocytes are the main support cells in the CNS, and their typical functions can be altered in HIV infection, even though these cells are not the primary target of HIV in the CNS. Inflammatory mediators can alter their ability to prevent excessive stimulation of glutamate receptors in the tripartite synapse, resulting in the formation of an excitotoxic environment (Okamoto, Wang, & Baba, 2005). Additionally, gp120 can directly bind CXCR4 on neurons, which contributes to neuronal demise and simplification (Bardi, Sengupta, Khan, Patel, & Meucci, 2006; Ellis, Langford, & Masliah, 2007; Hesselgesser et al., 1998; Meucci et al., 1998). In summary, while activation of CXCR4 by its natural chemokine ligand, CXCL12, is generally

neuroprotective, abnormal stimulation of this receptor during HIV infection can have opposite effects in the CNS.

Patients infected by HIV who also abuse opioid drugs often show enhanced disease progression at the periphery and in the CNS, so an examination of the interaction between the opioid and chemokine system in the brain can provide valuable insights for understanding this comorbid pathology. Interestingly, DAMGO, a potent and selective μ-opioid receptor agonist, was shown to inhibit neuroprotection afforded by CXCL12 treatment in NMDA-treated neuronal cultures (Patel et al., 2006). This effect was associated with DAMGO's ability to prevent ERK and Akt phosphorylation via CXCL12 signaling. CXCL12 is also able to directly modulate NMDA receptor subunit composition on cortical neurons, by preventing the production (and likely insertion) of the NR2B receptor subunit at extrasynaptic sites (Khan et al., 2008; Nicolai, Burbassi, Rubin, & Meucci, 2010). This subunit is associated with enhanced Ca^{2+} currents and overstimulation of the neuron, resulting in a disruption of ionic homeostasis, and potential activation of caspases (Leveille et al., 2008).

3.2. Effects of opioids on neuronal and non-neuronal cells

The opioid system has varied roles in the CNS, but this section emphasizes how opioids can affect cellular processes that are dysregulated in neurocognitive disorders. Opioids can change CNS physiology by two main mechanisms, first by their neuromodulatory actions and second through their effects on immune cells. Because endogenous opioids can be released in response to a painful stimulus or an infection, these molecules are thought to be helpful in restoring homeostasis during stressful episodes. For instance, in normoxic conditions, neuronal Na^+ and K^+ homeostasis is not affected by μ or δ agonists in mouse cortical slices (Chao, Bazzy-Asaad, Balboni, & Xia, 2007). However, under hypoxic conditions, δ-opioid receptor stimulation restored normal Na^+ and K^+ ion composition in the same cortical brain slices, suggesting the receptor signal has potential neuroprotective effects (Chao et al., 2007). Exogenous opioids are typically prescribed for pain and the endogenous system can be activated under similar circumstances. Ligand-bound opioid receptors function via Gαi proteins to inhibit adenylyl cyclase and Ca^{2+} channels, which may contribute to decreased neuronal excitability. Inhibiting neurons involved in ascending pain pathways, and activating inhibitory descending pathways in specific CNS areas including the periaqueductal gray, amygdala, insula, and spinal cord are important for achieving opioid analgesia (Mansour, Fox, Akil, & Watson, 1995). However, expression of opioid receptors in other tissues, such as the gastrointestinal tract, can contribute to unwanted side effects (Ketwaroo, Cheng, & Lembo, 2013). One very interesting set of side effects of opioid treatment concerns the regulation of the immune system. In addition to neuronal opioid receptor expression, several CNS cells that exert immune-like functions also express opioid receptors and can be modulated by both endogenous and exogenous opioids. Astrocytes express all three classical isoforms of opioid receptors (Hutchinson et al., 2011), but their individual expression can change depending on developmental status and their CNS localization (Ruzicka et al., 1995). The μ-opioid receptor is most abundantly expressed in the cortex, whereas κ- and δ-opioid receptors are abundantly expressed in the cortex and hypothalamus, among other areas (Ruzicka et al., 1995). Immature astrocytes express more

κ-opioid receptors, and the expression of the other opioid receptor subtypes changes as these cells enter the cell cycle (Persson, Thorlin, Ronnback, Hansson, & Eriksson, 2000). Rat cortical microglia express all three classical opioid receptor isoforms (Turchan-Cholewo et al., 2008), but information about how the distribution of these receptors changes over cellular development is lacking. Oligodendrocytes express μ and κ isoforms, and the overall opioid receptor expression in these cells decreases as they mature (Hauser, Fitting, Dever, Podhaizer, & Knapp, 2012; Knapp, Maderspach, & Hauser, 1998).

Opioids can have effects on CNS cells via classical opioid receptors or via nonclassical receptors, such as toll-like receptor 4 (TLR4; Watkins, Hutchinson, Rice, & Maier, 2009). This pattern recognition receptor recognizes foreign bodies such as bacterial lipopolysaccharide and initiates an inflammatory response by activating its respective immune cells and facilitating the release of inflammatory cytokines and chemokines. Triple opioid receptor knockout mice are not protected from opioid-induced hyperalgesia, suggesting that another system is responsive to these compounds and may work to thwart normal opioid signaling (van Dorp et al., 2009). TLR4 may be a part of this system, but other receptors likely play a role as well. However, the effects of classical opioid receptor activation on central immune cells have been more thoroughly classified. Morphine and endogenous opioids are known to reduce proliferation of astrocytes in many different brain regions, even in the presence of epidermal growth factor (Belcheva, Tan, Heaton, Clark, & Coscia, 2003). This may contribute to a general suppression of immune activity in individuals who abuse opioids. Some reports suggest that opioids have proliferative effects as well, depending on what receptor subtype they bind and on the region in which the target cells are located. For instance, young astrocytes that highly express κ-opioid receptors are sensitive to reduced proliferation after treatment with a κ agonist, while mature astrocytes located in other CNS areas have been reported to proliferate after κ or δ stimulation (Bunn, Hanley, & Wilkin, 1985; Xu, Bruchas, Ippolito, Gendron, & Chavkin, 2007). In line with the general theme of immune suppression via opioids, morphine is able to induce apoptosis in microglial cells by activating caspase 3 and the p38 pathway (Hu, Sheng, Lokensgard, & Peterson, 2002). However, the same group demonstrated that astrocytes do not undergo apoptosis after morphine administration, suggesting integral differences in μ-opioid receptor signaling between these two cell types (Hu et al., 2002). Many interesting findings have been reported regarding opioids' ability to modulate immune cells, but further characterization is necessary to understand how specific opioids can modulate CNS cells in different CNS areas.

Generally, neurocognitive disorders are accompanied by a low-level chronic inflammation in the CNS, which can be detrimental to cellular homeostasis and result in faster disease progression. As HIV-positive patients who have abused opioids are important members of this group, understanding how opioids can enhance progression of disease is paramount in the development of future adjunctive therapies. The following section outlines what is currently known about how opioids can interact with chemokine receptors, mostly CXCR4, and the potential clinical outcomes that arise as a consequence of this interaction.

4. CXCR4 INTERACTIONS WITH OPIOIDS

The scientific literature describes many ways that the chemokine and opioid systems can differentially regulate each other in immune and neuronal cells, especially with regard to the chemokine receptor CXCR4 (please see Table 5.1 for recent examples). Classical modes of regulation, such as heterologous desensitization and transcriptional changes, have been described in addition to novel and unexpected regulatory pathways.

4.1. Heterologous desensitization

Heterologous desensitization is described as a broad desensitization of similar receptors after repeated exposure to a specific ligand (i.e., activation of receptor A by its ligand desensitizes receptor B) and it is typically a rapid event. As opioid and chemokine receptors are both G protein-coupled seven-transmembrane receptors that use the same cellular machinery to broadcast their signals, stimulation of either has been shown to reduce the subsequent signaling of the other in some cases. Moreover, these interactions have been shown to be cell- and receptor subtype- specific. For instance, in immune cells μ- and δ-opioid receptor stimulation is able to desensitize CCR1, 2, and 5 but has no effect on CXCR4 signaling (Finley et al., 2008; Szabo et al., 2003). In contrast, CXCR4 activation by CXCL12 desensitizes signals from both μ and δ receptors (Chen et al., 2007; Heinisch et al., 2011). The κ-opioid receptor is the only classical opioid receptor subtype that has been shown to desensitize subsequent CXCR4 signaling via heterologous desensitization, and Ca^{2+} signaling experiments suggest that bidirectional desensitization occurs within seconds of κ-opioid receptor or CXCR4 activation in a dose-dependent manner (Finley et al., 2008). Although total CXCR4 surface expression does not change acutely after κ stimulation by U50488H, CXCL12-mediated chemotaxis is disrupted in Jurkat T cells after pretreatment with the κ agonist. Surface expression of CXCR4 can be reduced over a longer period, but the initial desensitization of the receptor does not result from internalization. *In vivo*, dynorphin-induced analgesia is blocked by CXCL12 administration in rat periaqueductal gray, providing further evidence of these receptors' abilities to cross-desensitize each other (Finley et al., 2008). Desensitization is not limited to the receptors listed here or to the CNS, as CCL2, CCL3, CCL5, and CXCL8 can desensitize μ-opioid receptor signals in peripheral sensory neurons (Zhang, Rogers, Caterina, & Oppenheim, 2004).

4.2. Transcriptional regulation/changes in expression

Peripheral blood mononuclear cells have been shown to reduce total CXCR4 protein and transcript levels after a 30-min treatment with the κ agonist U50488H, and this effect was associated with reduced X4 HIV infection in these cells (Finley et al., 2011). This process was shown to be dependent on the JAK/STAT pathway and on interferon regulatory factor 2 (IRF2), as blocking the phosphorylation of JAK2 or STAT3 prevented the activation of IRF1 and 2 and their subsequent binding to the CXCR4 gene promoter. Although both isoforms of IRF are activated by κ stimulation, and both bind to the CXCR4 promoter, only IRF2 is necessary for CXCR4 down-regulation, an interaction confirmed *in vivo* by chromatin immunoprecipitation analysis (Finley et al., 2011). Other opioids also have effects on the translation and expression of both chemokine receptors and ligands in various CNS cell types. In rat dorsal root ganglion (DRG), morphine administration for 5 days at 10

mg/kg resulted in marked CXCR4 downregulation in neurons and satellite glia cells, an effect lasting for at least 21 days after the last treatment (Wilson et al., 2011). However, the expression of CXCL12 actually increases after chronic morphine administration in the DRG, although it is unclear whether this is a direct effect of morphine treatment or an indirect feedback mechanism (Wilson et al., 2011). Additionally, this morphine dosing regimen led to opioid-induced hyperalgesia in some of the animals. Treatment with the CXCR4 antagonist AMD3100 caused an upregulation of total CXCR4 expression in these animals that was accompanied by reduced hyperalgesia as measured by von Frey filaments (Wilson et al., 2011). Again, this interaction is mechanistically unclear, as it could be influenced by other receptors that bind morphine at high doses, such as TLR4.

4.3. Receptor dimerization

As both CXCR4 and all three classical opioid receptors are seven-transmembrane GPCRs, they have the ability to interact with each other on the cell surface. These interactions can dramatically change the way that both receptors normally bind their ligands and therefore regulate intracellular signaling. One example involves heterodimer formation between CXCR4 and δ-opioid receptors, which is dynamically regulated by the ligands of both receptors (Pello et al., 2008). Cell lines and primary monocytes that express both receptors display heterodimerization when treated with CXCL12 and D-penicillamine(2,5)-enkephalin (DPDPE) simultaneously, and this effect is associated with negative regulation of both receptors but not with heterologous desensitization (Pello et al., 2008). Fluorescence resonance energy transfer studies suggest that heterodimers can form in cell lines and primary monocytes in the absence of ligands as well. Formation of these heterodimers is disruptive to CXCR4 homodimer formation, but upon treatment with DPDPE alone, CXCR4 homodimer formation is restored (Pello et al., 2008). As homodimer formation is thought to occur during CXCR4 signaling (Toth, Ren, & Miller, 2004), this interaction demonstrated an additional avenue by which the opioid system can regulate chemokine receptor signaling. The μ-opioid receptor may also form heterodimers with CXCR4, but this has not been investigated as extensively as the δ-opioid receptor- CXCR4 heterodimer formation. In 3-week-old rats, guanosine 5'-*O*-[gamma-thio]triphosphate (GTPγS) studies show that μ-opioid receptors and CXCR4 are co-expressed in several different brain areas, including the medial and lateral cortex and the hippocampus (Burbassi, Aloyo, Simansky, & Meucci, 2008). Pretreatment with morphine or the μ-opioid receptor agonist DAMGO resulted in reduced GTPγS coupling after CXCL12 treatment (Burbassi et al., 2008). Interestingly, in μ-opioid receptor knockout glia, CXCL12-induced CXCR4 G protein coupling and other downstream signals are reduced, suggesting that regulatory mechanisms between these systems may differ depending on the cell type and the opioid receptor subtype (Burbassi, Sengupta, & Meucci, 2010).

4.4. Novel regulatory mechanisms: Ferritin heavy chain

Additional exploration of opioid–chemokine interactions has revealed that the heavy chain subunit of the ubiquitously expressed iron-binding protein ferritin (FHC) can act as a negative regulator of CXCR4 signaling (Festa & Meucci, 2012; Pitcher et al., 2014; Sengupta et al., 2009). This interaction is somewhat unusual, as ferritin has classically been described for its ability to sequester iron and for its role in iron homeostasis (Wang,

Knovich, Coffman, Torti, & Torti, 2010). Unlike heterologous desensitization and dimer formation, FHC-mediated inhibition occurs over a long-time period, i.e., hours, from receptor stimulation rather than within minutes or fractions of seconds and can persist for days. The first evidence of the FHC–CXCR4 interaction was provided by Li and colleagues, who showed that this interaction was dependent on ligand binding to CXCR4 in transfected HEK293 and HeLa cells (Li, Luo, Mines, Zhang, & Fan, 2006). They also showed that upregulation of FHC in these cells prior to treatment with the natural ligand CXCL12 was able to inhibit CXCR4 downstream signals, including ERK1/2 activation, and chemotaxis (Li et al., 2006). FHC was also shown to negatively regulate CXCR4 in Jurkat T cells, which natively express the receptor, and was able to affect other chemokine receptor signals, such as ERK1/2 activation via CXCR2 (Li et al., 2006).

In primary rat cortical neurons, and *ex vivo* rat cortical slices, morphine, and DAMGO treatment increased protein levels of FHC. This is associated with decreased activation of CXCR4 by CXCL12 and decreased activation of downstream signals, including ERK1/2 and Akt (Sengupta et al., 2009). Interestingly, FHC co-immunoprecipitates with CXCR4 after morphine treatment (Sengupta et al., 2009), which corresponds to reduced coupling of CXCR4 to G proteins (Burbassi et al., 2008). These findings suggest that FHC may interfere with G protein-mediated signals from CXCR4. The specific μ-opioid receptor antagonist CTAP (D-Phe-Cys-Tyr-D-Trp-Arg-Thr-Pen-Thr-NH2) is able to block upregulation of FHC in morphine-treated neurons and restore ERK1/2 and Akt activity, suggesting that other receptors that can potentially bind opioids are not involved in this mechanism. This is also supported by studies in μ-opioid receptor-deficient animals (Burbassi et al., 2010). Interestingly Rhesus macaques chronically treated with morphine and opioid-abusing human patients show increased FHC protein levels in cortical neurons (Pitcher et al., 2014), which is in agreement with current *in vitro* data. Of note, increased expression of FHC in both human and macaque neurons positively correlates with reduced CXCR4 activation and with neurocognitive impairment in human patients, suggesting that CXCR4 activation is crucial for maintaining cognitive function (Pitcher et al., 2014). Additionally, morphine-treated rats show decreased dendritic spine density in cortical neurons compared with controls, whereas RNAi-mediated knockdown of FHC was able to maintain dendritic spines at control levels in the presence of morphine (Pitcher et al., 2014). Cognitive decline is often associated with reduced dendritic spine density (Ellis et al., 2007; Morrison & Baxter, 2012), and ferritin may be an important player in this process through its regulation of CXCR4.

5. CHEMOKINE AND OPIOID INTERACTIONS IN HAND

HAND affect the prognosis and quality of life of HIV-positive patients to varying degrees (McArthur, Steiner, Sacktor, & Nath, 2010). With the advent of combination antiretroviral therapy, the incidence of the more severe forms of the disease has declined, but the overall prevalence of HAND has increased (Heaton et al., 2010). HIV-positive patients who use drugs, and opioids in particular, seem to show faster disease progression, as demonstrated by higher viral loads, decreased immune function, and potential noncompliance with standard therapies (Nath, 2010). Several studies support the capability of opioids to enhance HIV progression in general and HAND in particular, even in patients who are already on combined antiretroviral therapy (Fitting et al., 2010; Hauser et al., 2012; Hu et al., 2012;

Malik, Khalique, Buch, & Seth, 2011; Pitcher et al., 2014). As opioid drug abuse is a major mechanism of transmission of HIV (10% of all diagnoses in 2011), a large population of HIV-infected individuals will be faced with increasing cognitive impairment, resulting in increased healthcare costs, and poorer overall prognosis of the disease (Centers for Disease Control, 2013).

The chemokine receptors CCR5 and CXCR4 (also known as HIV co-receptors) play a major role in HIV infection of target cells. CCR5-using viruses are usually predominant in the brain and responsible for initial CNS invasion, which is due to the ability of specific subsets of peripheral monocytes to normally penetrate the brain parenchyma (Gonzalez-Scarano & Martin-Garcia, 2005). These cells can then spread infection to resident CNS cells (mainly microglia and perivascular macrophages). CXCR4-using viruses can also enter the brain, particularly at more advanced stages of disease as other immune cells can easily cross the damaged BBB. While both host and viral factors are implicated in HIV neuropathology, different mechanisms may prevail in different groups of patients (Gelman et al., 2013) thus explaining, at least in part, the limited success of antiretrovirals in reverting neurocognitive impairment. Although neurons are not infected by HIV, the neurological deficits are ultimately consequence of neuronal alterations (mainly synaptodendritic damage; Ellis et al., 2007) or loss. Direct and indirect actions of viral proteins (mostly gp120 and tat) as well as immune/inflammatory mediators that trigger oxidative stress and excitotoxicity lead to this outcome. In line with this, many studies have shown that gp120s from either X4 or R5 viruses are highly neurotoxic. This effect is likely mediated by abnormal engaging of the respective chemokine receptor and competition with endogenous chemokine ligands. However, as discussed above, chemokine receptors exert a number of physiological functions in the CNS (Li & Ransohoff, 2008). CXCR4, in particular, is essential to CNS homeostasis and activation of neuroprotective signals. Through its ability to regulate cell-cycle proteins (Khan et al., 2003, 2008), dendritic spines (Pitcher et al., 2014), excitatory and inhibitory neurotransmission (Miller et al., 2008; Nicolai et al., 2010), and neuronal glial communication (Réaux-Le Goazigo et al., 2013), among other pathways, CXCR4 may significantly contribute to natural repair mechanisms and the ability of neurons to overcome toxic insults. Therefore, alteration of the neuronal CXCL12/CXCR4 axis is thought to be involved in neurodegenerative conditions. For instance, excessive cleavage of the endogenous CXCR4 ligand CXCL12 (which leads to inability of the cleaved product to properly engage CXCR4) has been implicated in HIV-induced neuronal damage (Vergote et al., 2006; Zhang et al., 2003), while deficits in CXCR4 signaling caused by opioids are linked to HAND (Pitcher et al., 2014; Sengupta et al., 2009). On the other hand, opioids usually enhance HIV infection of target cells, which support a neurotoxic environment. Examples of CXCL12/CXCR4 alterations in the context of other CNS disorders also exist (Parachikova & Cotman, 2007; Patel, McCandless, Dorsey, & Klein, 2010).

6. GAPS AND FUTURE CHALLENGES

Many important questions remain in regards to elucidating opioid–chemokine system interactions and their potential clinical relevance. Recently developed biased agonist opioid ligands can be used to further describe mechanistic details for opioid–chemokine regulation by their ability to only activate G protein-mediated signals from the μ-opioid receptor.

Because some of the clinically relevant side effects of opioid treatments, such as constipation, are mediated through β-arrestin signaling pathways, perhaps the negative regulation of CXCR4 and subsequent pathology can be precisely localized to one arm of the μ-opioid receptor signaling pathway (DeWire et al., 2013). Many gaps remain regarding the regulation of CXCR4 by FHC as well as the mechanism of μ-opioid receptor-induced FHC upregulation. Unpublished data from our group (Ponnuru P. et al., unpublished data) suggest that the latter process may be driven by morphine-induced changes in cellular iron levels and posttranscriptional regulation of FHC; preliminary findings also suggest that morphine may interfere with iron homeostasis in select neuronal populations. Elucidation of the molecular events implicated in these steps is required to better understand morphine control of FHC. Furthermore, identification of the neuronal subpopulations and other populations of CNS cells that are susceptible to increased FHC after morphine remains an important avenue of research that has yet to be fully characterized. Rat cortical astroglia do not appear to upregulate FHC after morphine treatment, while some but not all of the rat cortical neurons are susceptible (Nash B. et al., unpublished data). This suggests different mechanisms of opioid-induced chemokine regulation in the distinct CNS cell types, which is in agreement with data reported in other non-neuronal cells, and brain regions. Examination of morphine-induced changes in iron-related proteins in different CNS cells may also provide important clues regarding changes in iron metabolism in these cells. These studies may be instrumental in finding new drug targets for HAND and other neurocognitive disorders and help elucidate the pathophysiological regulatory mechanisms between these systems.

In closure, understanding how the chemokine and opioid systems interact with each other in neuronal and immune cells will provide insight into potential dysregulated signaling pathways in HAND patients that abuse opioids. Chemokine and opioid interactions seem to be complex and receptor/cells specific, but the general trend that emerges from the work summarized above suggests that these interactions generally are inhibitory towards one another. Therefore, opioids may disrupt homeostatic chemokine signals in the CNS that are crucial for neuronal protection and repair mechanisms (including neurogenesis), which are likely implicated in cognitive decline.

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Table 5.1

Examples of chemokine-opioid receptor interactions

Receptor	Regulation	Involved receptors	Cell type	References
CXCR4	Heterologous desensitization	μOR and δOR	Rat PAG	Chen, Geller, Rogers, and Adler (2007)
	Heterodimer formation	δOR	Murine PBMCs	Pello et al. (2008)
CCR5	Heterologous desensitization	μOR and δOR	Rat PAG	Chen et al. (2007)
CX_3CR1	Heterologous desensitization	μOR and δOR	Rat PAG	Heinisch, Palma, and Kirby (2011)
κOR	Heterologous desensitization	CXCR4	Jurkat cells	Finley et al. (2008)
	mRNA expression inhibition	CXCR4	Human PBMCs, CHME-3	Finley, Steele, Cornwell, and Rogers (2011)
μOR	Heterologous desensitization	CCR1, 2, and 5	Human PBMCs	Szabo et al. (2003)
	Cell surface downregulation	CXCR4	Rat DRG	Wilson, Jung, Ripsch, Miller, and White (2011)
	Cell surface upregulation	Chronic μOR > CXCR4	Rat DRG	Wilson et al. (2011)
	FHC upregulation	μOR > CXCR4	Human/monkey/rat cortical neurons	Sengupta et al. (2009), Pitcher et al. (2014)
δOR	Heterologous desensitization	CCR1, 2, and 5	Human PBMCs	Szabo et al. (2003)