Published in final edited form as:

Brain Behav Immun. 2011 October ; 25(7): 1434–1443. doi:10.1016/j.bbi.2011.04.014.

The effect of gp120 on morphine's antinociceptive and neurophysiological actions

Xiaohong Chen*, Lynn G. Kirby, Jonathan Palma, Khalid Benamar, Ellen B. Geller, Toby K. Eisenstein, and Martin W. Adler

Center for Substance Abuse Research, Temple University School of Medicine, Philadelphia, 19140, USA

Abstract

Recently, we have shown that morphine's analgesic activity can be attenuated by chemokines, specifically CCL5 and CXCL12. Because the HIV-1 coat protein, glycoprotein 120 (gp120), binds to the same receptors as do CCL5 and CXCL12, experiments were designed to investigate the effect of gp120 in the brain on antinociception induced by morphine in the cold-water (-3°C) tail-flick (CWT) and hot-plate (+54°C) tests. In addition, mu-opioid-receptor-mediated effects in brain periaqueductal grey (PAG) slices were examined with whole-cell patch-clamp recordings. The results showed that (1) pretreatment with gp120 itself (10, 25, 50, 100 or 133 ng, PAG) had no nociceptive effect in the CWT; (2) pretreatment with gp120 (25 or 100 ng) dose-dependently reduced antinociception induced by subcutaneous (sc) injection of morphine (3 or 6 mg/kg) or PAG injection of morphine (100 ng) in the CWT; (3) a PAG injection of gp120 (133 ng), given 30 min before sc injection of morphine (6 mg/kg), similarly reduced morphine antinociception in the hotplate test; (4) the inhibitory effect of gp120 on morphine-induced antinociception in the CWT was reversed by AMD3100, an antagonist of CXCR4; (5) pretreatment of slices with gp120 (200 pM) prevented morphine (10 µM)-induced hyperpolarization and reduction of input resistance in PAG neurons. Electrophysiology studies paralleled gp120-induced desensitization of a mu-opioid-receptor-mediated response in PAG neurons at the single-cell level. These studies are the first to demonstrate that the analgesic activity of morphine can be reduced by the presence of gp120 in the PAG and that pretreatment with AMD3100 is able to restore the analgesic effects of morphine.

Keywords

Morphine; gp120; CXCR4; Antinociception; Electrophysiology; PAG; Rat

© 2011 Elsevier Inc. All rights reserved.

*Corresponding Author: Xiaohong Chen, M.D., Center for Substance Abuse Research, Temple University School of Medicine, 3420 N. Broad Street, Philadelphia, PA 19140, Tel: 215-707-5305, Fax: 215-707-1904, chenxyang@temple.edu.

These studies are the first to demonstrate that the analgesic activity of morphine can be reduced by the presence of gp120 in the PAG via CXCR4 receptor activation.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

1. Introduction

Various pain syndromes have been reported in human immunodeficiency virus type 1 (HIV-1)-infected individuals. Most of these patients require medication for appropriate pain control. Although the opioids, particularly morphine, remain the best option for treating acute and chronic severe pain, they are less effective in treating some types, such as inflammatory and neuropathic pain. The entrance of HIV-1 into susceptible cells by fusion of the viral membrane with the cell plasma membrane (Dimitrov, 2000) is generally initiated by the binding of the HIV-1 envelope glycoprotein gp120 to CD4 on the host cell surface. The conformational change of the gp120 then allows it to interact with cellular surface chemokine receptors (coreceptors), of which the most common types are the CC chemokine receptor 5 (CCR5) and the CXC chemokine receptor 4 (CXCR4) (Shimizu et al., 2000, Berger et al., 1999). Chemokines are a superfamily of small cytokine-like molecules that have the ability to mediate the migration of various cell types. Zsuzsanna CXCL12/SDF-1 α binds primarily to one receptor, CXCR4, although it can also bind to CXCR7 (Hartmann et al., 2008, Thelen and Thelen, 2008). CXCR4 binds only CXCL12, whereas CCL5/RANTES binds primarily to CCR5 but also to other receptors (CCR1 and CCR3) (Murphy et al., 2000). The chemokines CCL5 or CXCL12 and their receptors have been reported to be involved in blocking the analgesic effects of opioids (Adler et al., 2006). Our laboratories were the first to report an apparent *in vivo* inactivation of opioid receptors by chemoattractant factors (Szabo et al., 2002). Pretreatment with CCL5 or CXCL12 can block the antinociceptive effect of DAMGO, a selective μ -opioid receptor agonist, or morphine (Adler and Rogers, 2005, Szabo et al., 2002).

The effects of opioids such as morphine are mediated through the opioid receptors. Opioid receptors are widely expressed both peripherally and in the CNS (Chen et al., 1993, Evans et al., 1992). The opioid agonist morphine has been shown to amplify the effect of the tubular cell-gp120 interaction on the proliferation of kidney fibroblasts (Singhal et al., 1998) and to potentiate gp120-induced neuronal apoptosis (Hu et al., 2005). Morphine significantly downregulated the gene expression of the beta chemokine, MIP-1 β , while reciprocally upregulating the gene expression of its specific receptors, CCR3 and CCR5, suggesting that μ opioids can increase HIV-1 coreceptor (Mahajan et al., 2005). HIV gp120 and morphine alter μ opiate receptor expression in human vascular endothelium (Cadet et al., 2001). Long-term exposure of human blood vessels to HIV gp120, morphine, and anandamide increases endothelial adhesion of monocytes, and decreases nitric oxide release (Stefano et al., 1998).

The PAG, a critical brain area in processing pain signals, is a primary site of action of many analgesic compounds (Basbaum and Fields, 1984, Behbehani and Field, 1979, Vaughan et al., 2003, Xin et al., 1997). CXCR4 immunoreactivity (Banisadr et al., 2003) and colocalization between CXCR4 and μ opioid receptor (Heinisch and Kirby, 2010, Heinisch et al., 2010) have been observed in the PAG. In both *in vitro* and *in vivo* experiments in rats, it was found that in PAG, binding at receptors for selected chemokines can desensitize opioid receptors, resulting in a decrease in the *in vivo* analgesic responses in rodents to morphine (Chen et al., 2007, Adler and Rogers, 2005, Zhang and Oppenheim, 2005, Zhang et al., 2004). This occurs by a process of heterologous desensitization in which activation of

the chemokine receptor blocks the signaling of the opioid receptor. Indeed, we have shown that the direct infusion of CCL5 or CXCL12 in the PAG can block analgesic actions of morphine (Chen et al., 2007, Adler and Rogers, 2005). Through the process of heterologous desensitization, chemokine ligands for CXCR4 and CCR5 can apparently inactivate the signaling pathway involved in reducing the sensation of pain. Because gp120 binds and activates these receptors, the possibility exists that gp120, via CXCR4 activation, desensitizes the opioid receptors, resulting in a reduction of morphine's effect. In addition to diminishing analgesia, the chemokine receptors may also decrease unwanted side effects of opioids through chemokine desensitization of selected opioid pathways.

Although no data are available regarding the in vivo effect of gp120 on the physiological function of morphine, our recent studies showed the capacity of chemokines, particularly CXCL12 or CCL5, to attenuate mu-opioid receptor function and diminish the analgesic effect of morphine (Adler et al., 2006). As CXCL12 and HIV-1 coat protein gp120 can bind and activate the CXCR4, we hypothesized that the presence of gp120 in the brain would diminish the analgesic activities of morphine. On the basis of the above studies showing the relationship among gp120, chemokines and opioids, investigations into whether gp120 itself has an effect and whether gp120 microinjected into the PAG interferes with the antinociception induced by morphine were conducted in the cold water tail-flick test and in the hot-plate test. Parallel electrophysiology studies were also conducted in PAG slices in order to investigate gp120-morphine interactions at the single-cell level.

2. Materials and methods

2.1. Animals

Sprague-Dawley male rats (Ace Animals, Inc. for behavioral studies; Taconic Farms, Germantown, NY for electrophysiology studies), weighing 175-200 g (behavioral studies) or 75-100 g (electrophysiology studies), were housed in groups of 3-4 for at least 1 week in an animal room maintained at $22\pm 1^{\circ}\text{C}$ and approximately $50\pm 5\%$ relative humidity. Lighting was on a 12/12-hour light/dark cycle (lights on at 7:00 and off at 19:00). The animals were allowed free access to food and water. All the experiments were conducted between 9:00 am to 3:00 pm. All animal use procedures were in strict accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee.

2.2. Surgery procedures

Rats were anesthetized with a mixture of ketamine hydrochloride (100-150 mg/kg) and acepromazine maleate (0.2 mg/kg). A sterilized stainless steel C313G guide cannula (22-gauge, Plastics One Inc., Roanoke, VA) was implanted into the PAG and fixed with dental cement. The stereotaxic coordinates were as follows: 7.8 mm posterior to bregma, 0.5 mm from midline and 5 mm ventral to the dura mater (Paxinos and Watson, 1998). A C313DC cannula dummy (Plastics One Inc., Roanoke, VA) of identical length was inserted into the guide tube to prevent its occlusion. The animals were housed individually after surgery. Experiments began 1 week postoperatively. Each rat was used only once. At the end of the behavioral experiments, each rat was injected with 0.5 μl of cresyl violet, anesthetized and

perfused transcardially with 0.9% isotonic saline, followed by phosphate-buffered saline (PBS) 4% paraformaldehyde (pH 7.4). The brain was removed, stored in the same fixative for 4 h, kept in 20% sucrose overnight, and cut into 20- μ m sections on a freezing microtome. Each coronal section was mounted according to standard histological procedures (Benamar et al., 2004), and the site of injection was verified by locating the dye.

2.3. Nociceptive tests

The latency to flick the tail in cold water (-3°C) was used as the antinociceptive index, according to a standard procedure in our laboratory (Pizziketti et al., 1985). A 1:1 mix of -3° ethylene glycol:water was maintained at -3°C with a circulating water bath (Model 9500, Fisher Scientific; Pittsburgh, PA). Rats were held over the bath with their tails submerged approximately halfway into the solution. All animals were tested at 60, 15 and 0 min before drug injection. For each animal, the first reading was discarded and the mean of the second and third readings was taken as the baseline value (range from 10 to 20 seconds). Latencies to tail flick after injection are expressed as percentage change from baseline. The percent of maximal possible antinociception (%MPA) for each animal at each time was calculated using the following formula: $\% \text{MPA} = [(\text{test latency} - \text{baseline latency}) / (60 - \text{baseline latency})] \times 100$. A cutoff limit of 60 seconds was set to avoid damage to the tail.

A 54°C hot plate (Ugo Basile, Varese, Italy) was used, according to the method of Dewey et al. (Dewey et al., 1970). The baseline response latency was obtained for each animal after two conditioning runs. Each rat was retested on the hot plate at +15 min and thereafter at 15-min intervals up to +45 min by using either jumping or hind-paw licking as the nociceptive endpoint and 30 seconds as the cutoff point. The percentage of maximal possible antinociception (%MPA) for each animal at each time point was calculated using the formula: $\% \text{MPA} = [(\text{test latency} - \text{baseline latency}) / (30 - \text{baseline latency})] \times 100$.

Rats are divided into groups of similar number for each test day, but not every animal's data can be used. Rats that showed extreme agitation, aberrant baselines, or had non-functional cannulae, were eliminated from the test group. These make the animal numbers of each group were not even.

2.4. Drugs

The opioid agonist morphine was obtained from NIDA. Drugs were dissolved in 0.9% saline. The human immunodeficiency virus type 1 (HIV-1) envelope glycoprotein gp120 recombinant viral protein (T-tropic, lot # 6D0020) was obtained from Advanced Biotechnologies Inc. (Columbia, MD). This protein, produced using the Baculovirus expression system and purified by immunoaffinity chromatography, has a molecular weight of 120 KD. The CXCR4 antagonist (Donzella et al., 1998), 1,1'-[1,4-phenylenebis(methylene)]bis-[1,4,8,11-tetraazacyclotetradecane (AMD3100), is a product from Sigma Chemical Co. (St. Louis, MO). Drugs were dissolved in phosphate-buffered saline (PBS).

2.5. Injections

After a 7-day recovery period, rats were allowed to habituate to the test chambers for 1 h before testing. Vehicle, gp120 or AMD3100 was microinjected into the PAG in a volume of

1.0 μ l via a C313I internal cannula (28-gauge, Plastics One Inc., Roanoke, VA). The C313I internal cannula was connected to a 10- μ l Hamilton syringe by polyethylene tubing. A volume of 1.0 μ l of drug or vehicle was delivered at a rate of 1.0 μ l per min (manually) and the internal cannula left in place an additional 90 sec to allow diffusion. Immediately thereafter, a dummy cannula (C313DC) was inserted into the cannula guide to prevent any contamination. For sc injection, morphine or saline was given mid-dorsally over 10 seconds.

2.6. Electrophysiology

Rats were rapidly decapitated and the head placed in ice cold artificial cerebrospinal fluid (ACSF) in which sucrose (248 mM) was substituted for NaCl. The brain was rapidly removed and trimmed to isolate the brainstem region. Slices 300 μ m thick were cut throughout the rostro-caudal extent of the PAG using a Vibratome 3000 Plus (Vibratome, St. Louis, MO) and placed in a holding vial containing ACSF at 35°C bubbled with 95% O₂ /5% CO₂ for 1 h. Slices were then maintained in room temperature ACSF bubbled with 95% O₂ /5% CO₂. The composition of the ACSF was (mM), NaCl 124, KCl 2.5, NaH₂ PO₄ 2, CaCl₂ 2.5, MgSO₄ 2, Dextrose 10 and NaHCO₃ 26.

Slices were transferred to a recording chamber (Warner Instruments, Hamden, CT) and continuously perfused with ACSF at 1.5-2.0 ml/min at 32-34°C maintained by an in-line solution heater (TC-324, Warner Instruments). Neurons were visualized using a Nikon E600 upright microscope fitted with a 40 $\times$ water-immersion objective, differential interference contrast and infrared filter (Optical Apparatus, Ardmore, PA). The image from the microscope was enhanced using a CCD camera and displayed on a computer monitor. Whole-cell recording pipettes were fashioned on a P-97 micropipette puller (Sutter Instruments, Novato, CA) using borosilicate glass capillary tubing (1.2 mm OD, 0.69 mm ID; Warner Instruments). The resistance of the electrodes was 4-8 M Ω when filled with an intracellular solution of (in mM) Kgluconate 130, NaCl 5, MgCl₂ 1, EGTA 0.02, HEPES 10, Naphosphocreatinine 10, MgATP 2, Na₂GTP 0.5, 0.1% Biocytin, pH 7.3.

Recordings were conducted in cells located in the ventrolateral subdivision of the PAG at the mid-caudal levels (corresponding to -5.88 mm to -6.84 caudal to bregma in Paxinos and Watson, 1998). A visualized cell was approached with the electrode, a gigaohm seal established and the cell membrane ruptured to obtain a whole-cell recording using a HEKA patch clamp EPC-10 amplifier (HEKA Elektronik, Pfalz, Germany). Series resistance was monitored throughout the experiment. If the series resistance was unstable or exceeded four times the electrode resistance, the cell was discarded. Once the whole-cell recording was obtained, cell membrane potential and input resistance were obtained in current-clamp mode ($I = 0$ pA).

Baseline membrane potential was initially recorded for 5 min to ensure that the cell was stable. First, gp120 (200 pM) was bath applied and the cell recorded for 6 min. Next, morphine (10 μ M) was added and the cell recorded for an additional 10 min. If a drug effect was observed, drugs were removed when the response reached steady-state for a post-drug washout period. Input resistance (IR) was calculated from voltage responses to periodic -300 pA current pulses. IR was calculated based on the average of three voltage responses at

baseline or when the drug effect had reached steady-state. Data were analyzed using pClamp 9.0 software (Molecular Devices, Sunnyvale, CA).

2.7. Statistical analysis

The data are expressed as the mean and standard error of mean (S.E.M.). Statistical analysis of difference between groups for behavioral experiments was assessed with a one-way analysis of variance (ANOVA) followed by Tukey multiple comparison test. Statistical analysis of difference between groups for electrophysiology experiments was assessed with a one-way ANOVA or ANOVA on Ranks when appropriate, followed by Newman-Keuls or Dunn's multiple comparison tests, respectively. $P < 0.05$ was taken as the significant level of difference.

3. Results

3.1. The effect of the intra-PAG gp120 and morphine in the CWT test

To determine if intra-PAG injection of HIV-1 coat protein gp120, itself, has an antinociceptive effect in the CWT test, rats were divided into 6 groups as shown in Figure 1A. After a 60-min baseline interval, gp120 or vehicle was injected into the PAG at time zero and antinociceptive threshold was measured for 120 min. The PAG administration of gp120 (10, 25, 50, 100 and 133 ng/ μ l) produced no antinociceptive effect in the CWT, as compared to the PBS control group ($p > 0.05$, ANOVA followed by Tukey test). Doses of 25 ng and 100 ng of gp120 were used in the subsequent experiments.

To allow for possible potentiation or antagonism of the antinociceptive effect, we sought to determine a dose of morphine that would give approximately a 50% MPA. To determine a moderate antinociceptive dose of morphine in the CWT test, rats were divided into 5 groups as shown in Figure 1B. The sc administration of morphine (3, 6, 10, 15 or 30 mg/kg) produced a dose-dependent analgesic effect in the CWT test. The dose of 6 mg/kg morphine, which caused a maximum increase of ~60%, was used in the subsequent experiments.

3.2. The effect of the intra-PAG gp120 on the antinociception induced by morphine in the CWT test

From previous studies with pretreatment of CXCL12/SDF-1 α at 30, 60 and 120 min before DAMGO (Szabo et al., 2002), the best antagonism of CXCL12 was shown at 30 min pretreatment before DAMGO. Because both CXCL12 and gp120 bind CXCR4 (Berger et al., 1999), the timing of gp120 injection was chosen to match that of CXCL12 (30 min pretreatment). The sc administration of 6 mg/kg of morphine produced a significant increase in antinociception at 30 min ($58.4 \pm 2.2\%$) in the CWT, as shown in Figure 2. A PAG injection of gp120 or PBS, given 30 min before sc injection of morphine, reduced the antinociception from $58.4 \pm 2.2\%$ to $17.6 \pm 2.5\%$ (gp120, 25 ng/ μ l) ($p < 0.001$) and to $10.6 \pm 0.5\%$ (gp120, 100 ng/ μ l) ($p < 0.001$). Thus, the administration of gp120 at a low dose (25 ng/ μ l) or high dose (100 ng/ μ l) blocked the antinociceptive effect induced by morphine in the CWT, as compared to the control group ($p < 0.001$). Increasing the gp120 dose did not result in an increase of its effect on morphine-induced antinociception.

We further investigated the effect of gp120 and morphine when both are given into the PAG. Rats were divided into 3 groups as shown in Figure 3. A PAG injection of gp120 or PBS was given 30 min before PAG injection of morphine (100 ng/ μ l). The results showed that PAG injection of gp120 (100 ng/ μ l) reduces the antinociception induced by morphine (100 ng/ μ l, PAG) at 30 min from $44.4 \pm 2.6\%$ to $19.2 \pm 3.1\%$ ($P < 0.001$).

3.3. The effect of AMD3100 on the gp120 antagonism of antinociception induced by PAG injection of morphine in the cold water tail-flick test

In an attempt to determine whether the effect of gp120 in morphine-induced antinociception involves CXCR4, we pretreated the rats with AMD3100, an antagonist of that receptor CXCR4 (Schols et al., 1997a, Schols et al., 1997b). As shown in Figure 4, the intra-PAG injection of AMD3100 by itself had no effect ($p > 0.05$) on nociceptive threshold. Pretreatment with AMD3100 (PAG, 100 ng) shown in Figure 5 blocked the inhibitory effect of gp120 on morphine-induced antinociception ($P < 0.001$), comparing PBS + gp120 + Morphine group to PBS + PBS + Morphine group or AMD3100 + gp120 + Morphine group at 15, 30, 45 and 60 min.

3.4. The effect of gp120 on the antinociception induced by sc injection of morphine in the hot-plate (+54°C) test

To test if intra-PAG injection of gp120 has a similar effect on morphine antinociception in the hot-plate (+54°C) test, rats were divided into 2 groups as shown in Figure 6. A PAG injection of gp120 (133 ng), given 30 min before sc injection of morphine (6 mg/kg), reduced the antinociception from $45 \pm 4.1\%$ to $21 \pm 6\%$ ($p < 0.01$) at 30 min in the hot-plate test.

3.5. The effect of gp120 on electrophysiological responses to morphine in PAG neurons

An individual PAG neuron shown in Figure 7A hyperpolarized from a baseline level of -69.4 mV to -76.8 mV in response to 10μ M morphine. The morphine-induced hyperpolarization returned to a baseline level of -69.5 mV following drug washout. Morphine hyperpolarized PAG neurons from an average baseline of -48.2 ± 3.0 mV to -57.8 ± 2.9 mV ($N = 18$), producing a mean hyperpolarization of -9.5 ± 0.6 mV (Figure 7C). 89% of PAG neurons showed at least a 7 mV hyperpolarization response to morphine. Gp120 application alone (200 pM) did not change the average membrane potential from baseline levels in PAG neurons ($N = 6$, Figure 7B). However, gp120 pretreatment blocked morphine-induced hyperpolarization ($N = 6$) in all PAG neurons examined (Figures 7C and 7D). There was a statistically significant drug effect when comparing the treatment groups by one-way ANOVA on ranks ($H(2) = 20.97$, $p < 0.001$). Post-hoc Dunn's test indicated that morphine-mediated hyperpolarization was statistically greater than gp120 ($p < 0.01$) or gp120 + morphine ($p < 0.01$).

An individual PAG neuron shown in Figure 7A demonstrated a reduction in input resistance in response to 10μ M morphine from a baseline of 180 M Ω to 153 M Ω with a return to baseline following drug washout (177 M Ω). There was a statistically significant drug effect when comparing the treatment groups by one-way ANOVA ($p < 0.05$). Post-hoc Student-Newman-Keuls tests indicated that the morphine-mediated reduction of input resistance was

greater than the effect of gp120 alone ($p < 0.05$) or in combination with morphine ($p < 0.05$). In summary, gp120 did not change the membrane potential or input resistance of PAG neurons. However, gp120 pretreatment blocked both morphine-mediated hyperpolarization as well as morphine-mediated reduction in input resistance in these neurons.

4. Discussion

An estimated more than 2 million people die from AIDS every year. Up to 80 percent of these people suffer severe pain, some for months on end. Generally, pain in patients with HIV disease may result from direct effects of HIV on the central or peripheral nervous system, immunosuppression (e.g. opportunistic infections or cancers), or therapies for HIV (e.g. antiretroviral medications). The great majority of these patients require medication for appropriate pain control.

Gp120 is implicated in the pathogenesis of neurological disorders associated with HIV and is capable of initiating neurotoxic cascades via an interaction with the CXCR4 and/or CCR5 chemokine receptors. It can mimic many of the behavioral and physiological dysfunctions that are characteristic of HIV infection, including pain (Milligan et al., 2000). It has been linked to genesis of neuropathic pain (Oh et al., 2001) and is capable of producing thermal hyperalgesia and mechanical allodynia (Milligan et al., 2000). Many studies have revealed a relationship between the opioids and the HIV type 1 coat protein, gp120. While there are many mechanisms by which opioids could affect HIV replication, there is also the possibility, heretofore only minimally explored, that HIV and its products also affect the physiological functions of opioids. To our knowledge, no one has previously examined the in vivo physiological consequences of the presence of gp120 in the brain on the effect of morphine, particularly in terms of pain. In the present studies, we investigated the effect of gp120 on morphine-induced analgesia. First, our data showed that gp120 itself microinjected directly into the PAG does not alter antinociceptive thresholds in the CWT test. Other investigators have reported that intrathecal administration of gp120 activates astrocytes and microglia to release products that induce thermal hyperalgesia (Milligan et al., 2001, Milligan et al., 2000). Those studies utilized the radiant heat tail-flick and Hargreaves tests, intrathecal injection and higher doses of gp120. However, although by itself it did not affect antinociceptive thresholds, gp120 given directly into the PAG was able to attenuate significantly morphine-induced antinociception (given either peripherally or directly into the PAG). Our present data show that gp120 diminished the morphine-induced analgesia in both the cold-water (-3°C) tail flick test and the hot plate ($+54^{\circ}\text{C}$) test.

One difficulty in studying gp120 is that it binds to more than one receptor. Understanding how cells respond to gp120 is complex because of this promiscuity. In an attempt to establish the contribution of CXCR4 in the antagonistic effect of gp120 on morphine-induced antinociception, AMD3100 was administered directly into the PAG prior to gp120 and morphine. It was found that AMD3100 (Donzella et al., 1998) interacts with and blocks CXCR4, the main co-receptor used by T-tropic viruses (Schols et al., 1997a, Schols et al., 1997b). In previous studies, AMD3100 was shown to inhibit the replication of T-tropic HIV strains or clinical isolates in T-cell lines, such as MT-4, MOLT-4, or CEM cells (De Vreese et al., 1996, De Clercq, 1994). In addition, the interaction between CXCL12 and CXCR4

can be blocked by AMD3100 (Schols et al., 1997a, Schols et al., 1997b). Our present data show that pretreatment with AMD3100 prevents the effect of gp120 on morphine-induced antinociception and indicates that the inhibitory effect of gp120 is mediated by CXCR4 in the PAG. That the pretreatment with AMD3100 was able to restore the analgesic effects of morphine suggests that chemokine blockers might be used as a strategy to restore opioid functions, particularly in neuroinflammatory conditions such as the presence of gp120 in the brain. The effect of gp120 could occur in one of two ways. The most direct mechanism would be for gp120 to bind directly to the chemokine receptors on the neurons and thereby inhibit the induction of morphine-induced analgesia. The alternative mechanism would be for gp120 to bind to microglia and release chemokines, which would bind to the chemokine receptors to cause the effect on opioid receptors. Although this study was not designed to determine which of these two pathways is in operation, it demonstrates conclusively that CXCR4 is involved in the antagonism of gp120 on morphine-induced analgesia.

Our laboratory has reported electrophysiological evidence for heterologous desensitization between the mu opioid receptor and CXCR4 in the PAG at the single-cell level (Heinisch and Kirby, 2010, Heinisch et al., 2010). Specifically, we demonstrated that chemokines, including CXCL12, can prevent mu-opioid receptor-mediated PAG neuron hyperpolarization and reduction of input resistance. In a similar manner, the current study (Figure 7) showed that gp120 pretreatment blocked both morphine-mediated hyperpolarization as well as morphine-mediated reduction in input resistance in these neurons, which strongly support the in vivo results from the antinociceptive tests (cold-water tail-flick test and hot-plate test). These findings from both behavioral experiments and electrophysiology experiments demonstrate for the first time that gp120 reduces morphine antinociception in the PAG and that morphine's effect can be restored by antagonism of CXCR4 receptors expressed in the brain of rats.

Acknowledgments

This work was supported in part by NIDA Grants DA 06650 (MWA) and DA13429 (MWA) and DA 20126 (LGK)

References Cited

- Adler MW, Geller EB, Chen X, Rogers TJ. Viewing chemokines as a third major system of communication in the brain. *AAPS J.* 2006; 7:E865–70. [PubMed: 16594639]
- Adler MW, Rogers TJ. Are chemokines the third major system in the brain? *J Leukoc Biol.* 2005; 78:1204–1209. [PubMed: 16204637]
- Banisadr G, Skrzydelski D, Kitabgi P, Rostene W, Parsadaniantz SM. Highly regionalized distribution of stromal cell-derived factor-1/CXCL12 in adult rat brain: constitutive expression in cholinergic, dopaminergic and vasopressinergic neurons. *Eur J Neurosci.* 2003; 18:1593–1606. [PubMed: 14511338]
- Basbaum AI, Fields HL. Endogenous pain control systems: brainstem spinal pathways and endorphin circuitry. *Ann Rev Neurosci.* 1984; 7:309–338. [PubMed: 6143527]
- Behbehani MM, Fields HL. Evidence that an excitatory connection between the periaqueductal gray and nucleus raphe magnus mediates stimulation produced analgesia. *Brain Res.* 1979; 170:85–93. [PubMed: 223721]
- Benamar K, Rawls SM, Geller EB, Adler MW. Intrahypothalamic injection of deltorphin-II alters body temperature in rats. *Brain Res.* 2004; 1019:22–27. [PubMed: 15306234]

- Berger EA, Murphy PM, Farber JM. Chemokine receptors as HIV-1 coreceptors: roles in viral entry, tropism, and disease. *Annu Rev Immunol.* 1999; 17:657–700. [PubMed: 10358771]
- Cadet P, Weeks BS, Bilfinger TV, Mantione KJ, Casares F, Stefano GB. HIV gp120 and morphine alter mu opiate receptor expression in human vascular endothelium. *Int J Mol Med.* 2001; 8:165–169. [PubMed: 11445868]
- Chen X, Geller EB, Rogers TJ, Adler MW. Rapid heterologous desensitization of antinociceptive activity between mu or delta opioid receptors and chemokine receptors in rats. *Drug Alcohol Depend.* 2007; 88:36–41. [PubMed: 17049756]
- Chen Y, Mestek A, Liu J, Hurley JA, Yu L. Molecular cloning and functional expression of a μ -opioid receptor from rat brain. *Mol Pharmacol.* 1993; 44:8–12. [PubMed: 8393525]
- De Clercq E. How to overcome resistance of HIV-1 to HIV-1-specific reverse transcriptase inhibitors. *AIDS.* 1994; 8:1020–1021. [PubMed: 7524543]
- De Vreese K, Reymen D, Griffin P, Steinkasserer A, Werner G, Bridger GJ, Este J, James W, Henson GW, Desmyter J, Anne J, De Clercq I. The bicyclams, a new class of potent human immunodeficiency virus inhibitors, block viral entry after binding. *Antiviral Res.* 1996; 29:209–219. [PubMed: 8739600]
- Dewey WL, Harris LS, Howes JF, Nuite JA. The effect of various neurohumoral modulators on the activity of morphine and the narcotic antagonists in the tailflick and phenylquinone tests. *J Pharmacol Exp Ther.* 1970; 175:435–442. [PubMed: 4394803]
- Dimitrov DS. Cell biology of virus entry. *Cell.* 2000; 101:697–702. [PubMed: 10892741]
- Donzella GA, Schols D, Lin SW, Esté JA, Nagashima KA, Maddon PJ, Allaway GP, Sakmar TP, Henson G, De Clercq E, Moore JP. AMD3100, a small molecule inhibitor of HIV-1 entry via the CXCR4 co-receptor. *Nat Med.* 1998; 4(1):72–7. [PubMed: 9427609]
- Evans CJ, Keith DEJ, Morrison H, Magendzo K, Edwards RH. Cloning of a delta opioid receptor by functional expression. *Science.* 1992; 258:1952–1955. [PubMed: 1335167]
- Hartmann TN, Grabovsky V, Pasvolosky R, Shulman Z, Buss EC, Spiegel A, Nagler A, Lapidot T, Thelen M, Alon R. A crosstalk between intracellular CXCR7 and CXCR4 involved in rapid CXCL12-triggered integrin activation but not in chemokine-triggered motility of human T lymphocytes and CD34+ cells. *J Leukoc Biol.* 2008; 84:1130–1140. [PubMed: 18653785]
- He J, Chen Y, Farzan M, Choe H, Ohagen A, Gartner S, Busciglio J, Yang X, Hofmann W, Newman W, Mackay CR, Sodroski J, Gabuzda D. CCR3 and CCR5 are co-receptors for HIV-1 infection of microglia. *Nature.* 1997; 385:645–649. [PubMed: 9024664]
- Heinisch S, Kirby LG. SDF-1 α /CXCL12 enhances GABA and glutamate synaptic activity at serotonin neurons in the rat dorsal raphe nucleus. *Neuropharmacology.* 2010; 58:501–514. [PubMed: 19755127]
- Heinisch S, Palma J, Kirby LG. Interactions between chemokine and mu-opioid receptors: Anatomical findings and electrophysiological studies in the rat periaqueductal grey. *Brain Behav Immun.* 2011; 25:360–372. [PubMed: 20974247]
- Hu S, Sheng WS, Lokensgard JR, Peterson PK. Morphine potentiates HIV-1 gp120-induced neuronal apoptosis. *J Infect Dis.* 2005; 191:886–889. [PubMed: 15717263]
- Lavi E, Strizki JM, Ulrich AM, Zhang W, Fu L, Wang Q, O'Connor M, Hoxie JA, Gonzalez-Scarano F. CXCR-4 (Fusin), a co-receptor for the type 1 human immunodeficiency virus (HIV-1), is expressed in the human brain in a variety of cell types including microglia and neurons. *Am J Pathol.* 1997; 151:1035–1042. [PubMed: 9327737]
- Mahajan SD, Schwartz SA, Aalink R, Chawda RP, Sykes DE, Nair MP. Morphine modulates chemokine gene regulation in normal human astrocytes. *Clinical Immunology.* 2005; 115:323–332. [PubMed: 15893700]
- Milligan ED, Mehmert KK, Hinde JL, Harvey LO, Martin D, Tracey KJ, Maier SF, Watkins LR. Thermal hyperalgesia and mechanical allodynia produced by intrathecal administration of the human immunodeficiency virus-1 (HIV-1) envelope glycoprotein, gp120. *Brain Res.* 2000; 861:105–116. [PubMed: 10751570]
- Milligan ED, O'Connor KA, Armstrong CB, Hansen MK, Martin D, Tracey KJ, Maier SF, Watkins LR. Systemic administration of CNI-1493, a p38 mitogenactivated protein kinase inhibitor, blocks

- intrathecal human immunodeficiency virus-1 gp120-induced enhanced pain states in rats. *J Pain*. 2001; 2:326–333. [PubMed: 14622812]
- Murphy PM, Baggiolini M, Charo IF, Hebert CA, Horuk R, Matsushima K, Miller LH, Oppenheim JJ, Power CA. International Union of Pharmacology. XXII. Nomenclature for chemokine receptors. *Pharmacol Rev*. 2000; 52:145–176. [PubMed: 10699158]
- Oh SB, Tran PB, Gillard SE, Hurley RW, Hammond DL, Miller RJ. Chemokines and glycoprotein120 produce pain hypersensitivity by directly exciting primary nociceptive neurons. *J Neurosci*. 2001; 21:5027–5035. [PubMed: 11438578]
- Paxinos, G.; Watson, C. *The Rat Brain in Stereotaxic Coordinates*. Academic Press; San Diego: 1998.
- Pizziketti RJ, Pressman NS, Geller EB, Cowan A, Adler MW. Rat cold water tail-flick: A novel analgesic test that distinguishes opioid agonists from mixed agonist/antagonists. *Eur J Pharmacol*. 1985; 119:23–29. [PubMed: 2867920]
- Schols D, Este JA, Henson G, De Clercq E. Bicyclams, a class of potent anti-HIV agents, are targeted at the HIV coreceptor fusin/CXCR-4. *Antiviral Res*. 1997a; 35:147–156. [PubMed: 9298754]
- Schols D, Struyf S, Van Damme J, Este JA, Henson G, De Clercq E. Inhibition of T-tropic HIV strains by selective antagonization of the chemokine receptor CXCR4. *J Exp Med*. 1997b; 186:1383–1388. [PubMed: 9334378]
- Shieh JT, Albright AV, Sharron M, Gartner S, Strizki J, Doms RW, Gonzalez-Scarano F. Chemokine receptor utilization by human immunodeficiency virus type 1 isolates that replicate in microglia. *J Virol*. 1998; 72:4243–4249. [PubMed: 9557714]
- Shimizu N, Soda Y, Kanbe K, Liu H, Mukai R, Kitamura T, Hoshino H. A putative G protein-coupled receptor, RDC1, is a novel coreceptor for human and simian immunodeficiency viruses. *J Virol*. 2000; 74:619–626. [PubMed: 10623723]
- Singhal PC, Sagar S, Reddy K, Sharma P, Ranjan R, Franki N. HIV-1 gp120 envelope protein and morphine-tubular cell interaction products modulate kidney fibroblast proliferation. *J Investig Med*. 1998; 46:243–248.
- Stefano GB, Salzet M, Bilfinger TV. Long-term exposure of human blood vessels to HIV gp120, morphine, and anandamide increases endothelial adhesion of monocytes: uncoupling of nitric oxide release. *J Cardiovasc Pharmacol*. 1998; 31:862–868. [PubMed: 9641470]
- Szabo I, Chen XH, Xin L, Adler MW, Howard OMZ, Oppenheim JJ, Rogers TJ. Heterologous desensitization of opioid receptors by chemokines inhibits chemotaxis and enhances the perception of pain. *Proc Natl Acad Sci U S A*. 2002; 99:10276–10281. [PubMed: 12130663]
- Tanabe S, Heesen M, Yoshizawa I, Berman MA, Luo Y, Bleul CC, Springer TA, Okuda K, Gerard N, Dorf ME. Functional expression of the CXC-chemokine receptor-4/fusin on mouse microglial cells and astrocytes. *J Immunol*. 1997; 159:905–911. [PubMed: 9218610]
- Thelen M, Thelen S. CXCR7, CXCR4 and CXCL12: an eccentric trio? *J Neuroimmunol*. 2008; 198:9–13. [PubMed: 18533280]
- Van der Meer P, Ulrich AM, González-Scarano F, Lavi E. Immunohistochemical analysis of CCR2, CCR3, CCR5, and CXCR4 in the human brain: potential mechanisms for HIV dementia. *Exp. Mol Pathol*. 2000; 69:192–201. 13.
- Vaughan CW, Bagley EE, Drew GM, Schuller A, Pintar JE, Hack SP, Christie MJ. Cellular actions of opioids on periaqueductal grey neurons from C57B16/J mice and mutant mice lacking MOR-1. *Br J Pharmacol*. 2003; 139(2):362–367. [PubMed: 12770941]
- Xin L, Geller EB, Liu-Chen LY, Chen C, Adler MW. Substance P release in the rat periaqueductal gray and preoptic anterior hypothalamus after noxious cold stimulation: Effect of selective mu and kappa opioid agonists. *J Pharmacol Exp Ther*. 1997; 282:1055–1063. [PubMed: 9262375]
- Zhang N, Oppenheim JJ. Crosstalk between chemokines and neuronal receptors bridges immune and nervous systems. *J Leukoc Biol*. 2005; 78:1210–1214. [PubMed: 16204635]
- Zhang N, Rogers TJ, Caterina M, Oppenheim JJ. Proinflammatory chemokines, such as C-C chemokine ligand 3, desensitize μ -opioid receptors on dorsal root ganglia neurons. *J Immunol*. 2004; 173:594–599. [PubMed: 15210821]

Fig. 1.

Effect of gp120 and morphine in the CWT test. Rats were divided into 6 groups and were administered gp120 (10, 25, 50, 100 and 133 ng/ μ l) (Figure 1A, N=6-15 per group) into PAG. There are no significant difference between any 2 groups at same time point ($p > 0.05$). The effect of sc injection of morphine (3, 6, 10, 15 or 30 mg/kg) was tested in the CWT test (Figure 1B, N=6-15 per group). Each point represents the mean + S.E.M.

Fig. 2.

Effect of gp120 on antinociception induced by sc injection of morphine in the CWT test. Rats were divided into 6 groups and were given gp120 (25 or 100 ng/ μ l) into PAG, followed by sc injection of morphine (3 or 6 mg/kg), respectively. $P < 0.01$ (at 60 min) and $p < 0.001$ (at all other time points at 15 30 and 45 min), comparing PBS + Morphine group to gp120 + Morphine group.

N=6-8 per group. Each point represents the mean + S.E.M.

Fig. 3.

Effect of gp120 on antinociception induced by PAG injection of morphine in the CWT test. Rats were divided into 3 groups and were given gp120 (100 ng/ μ l, PAG) followed by morphine (100 ng, PAG), respectively. $P < 0.001$ (at 30 and 45 min) and $p < 0.05$ (at 60 min), comparing PBS + Morphine group to gp120 + Morphine group. $N = 7-8$ per group. Each point represents the mean + S.E.M.

Fig. 4.

Effect of PAG injection of AMD3100 in the CWT test. Rats were divided into 5 groups and were given AMD3100 (100 pg, 10 ng, 100 ng and 500 ng) intra-PAG. There are no significant difference between any 2 groups at same time point ($p > 0.05$). $N=4-7$ per group. Each point represents the mean + S.E.M.

Fig. 5.

Antagonism of the effects of gp120 on morphine-induced antinociception by AMD3100 in the CWT test. Rats were treated intra-PAG with gp120 30 min before morphine. AMD3100 was administered to rats at a dose of 100 ng 45 min before gp120 (at 0 min). $P < 0.001$, comparing PBS+gp120+Morphine group to PBS + PBS + Morphine group or AMD3100 + gp120 + Morphine group at 15, 30, 45 and 60 min. N=6 per group. Data are expressed as mean + S.E.M.

Fig. 6.

The effects of gp120 on antinociception induced by sc injection of morphine in the hot-plate (+54°C) test. Rats were treated intra-PAG with gp120 30 min before morphine. $P < 0.01$, comparing PBS+Morphine group to gp120 + Morphine group at 30 min. $N=4-6$ per group. Data are expressed as mean + S.E.M.

Fig. 7.

The effect of gp120 on the antinociception induced by s.c. Pretreatment with gp120 blocks morphine-mediated hyperpolarization and reduction of input resistance in PAG neurons. Morphine (10 μ M) hyperpolarizes PAG neurons (A, C) accompanied by a reduction of input resistance (A, D). Itself, gp 120 (200 pM) has no effect on resting membrane potential or input resistance (B-D) but pretreatment with gp120 blocks morphine's effect on resting membrane potential and input resistance (B-D). Asterisks indicate significant differences from morphine alone by one-way ANOVA followed by post-hoc Student-Newman-Keuls or Dunn's tests ($p < 0.05$). Group data are represented as mean $\pm$ SEM.