Naunyn-Schmiedeberg's Archives of Pharmacology (2018) 391:1157–1161
https://doi.org/10.1007/s00210-018-1533-1

BRIEF COMMUNICATION

Supraspinal interaction between HIV-1-gp120 and cannabinoid analgesic effectiveness

Jonathan Palma[1] · Madhusudhanan Narasimhan[2] · Josée Guindon[2] · Khalid Benamar[2]

Received: 16 April 2018 / Accepted: 5 July 2018 / Published online: 15 July 2018
© Springer-Verlag GmbH Germany, part of Springer Nature 2018

Abstract

The growing therapeutic use (self-medication) of cannabinoids by HIV-1 infected people and the recent interest in the possible medicinal use of cannabinoids, particularly in pain management, create an urgent need to identify their potential interactions with HIV-1. The goal here is to determine any interaction between proteins of HIV-1 and the analgesic effectiveness of cannabinoid at supraspinal level. Young adult male rats (Sprague-Dawley) were stereotaxically pretreated with HIV-1 envelope glycoprotein 120 (gp120) into the periaqueductal gray (PAG) area, the primary control center of pain modulation. Then, we examined its effect on cannabinoid receptor agonist WIN55,212-2-induced analgesia. Our results demonstrated that gp120 in PAG diminished the analgesic effectiveness of this cannabinoid agonist. These results suggest that gp120 may interact with the cannabinoid system through the descending modulatory pain pathways centered in the PAG to impair the analgesic effectiveness of cannabinoids.

Keywords Periaqueductal gray · Cannabinoid · WIN55,212-2 · HIV-1 · gp120 · Analgesia

Introduction

The periaqueductal gray (PAG), a brainstem area and key element of the descending pain control system modulates nociceptive neurotransmission at the level of the spinal cord dorsal horn via the rostral ventromedial medulla (RVM) (Fields et al. 1991; Heinricher et al. 2009; Ossipov et al. 2000). The PAG with its direct projection to RVM (Bandler et al. 2000) is considered a key element of the descending pain modulatory pathway that projects from PAG to RVM and then to the spinal cord (Heinricher and Ingram 2008). The PAG-RVM neuraxis can exert pain inhibitory and facilitatory influences on dorsal horn neurons involved in nociceptive function (Heinricher et al. 2009). For instance, intense stress and fear are associated with descending inhibition and hypoalgesia, whereas inflammation and nerve injury is correlated with descending facilitation and hyperalgesia (Heinricher et al. 2009; Porreca et al. 2002). The PAG is a site of action of cannabinoid-induced analgesia (Lichtman et al. 1996; Benamar et al. 2008).

The brain is one of the first site targeted by HIV-1. This virus enters the brain early in the disease and then continues to produce central nervous system (CNS) dysfunction as the disease progresses. HIV-1 enters targeted cells by binding its envelope glycoprotein gp120 (gp120) to the CD4 receptor and co-receptors such as C-C chemokine receptor type 5 (CCR5) and C-X-C chemokine receptor type 4 (CXCR4) depending of strains. Gp120 has been found in the brain of patients with HIV-1 encephalitis who also have dementia (Jones et al. 2000). It produces several neurobehavioral effects in rodents that are characteristic of HIV-1/AIDS (Barak et al. 2002; Benamar et al. 2010; Opp et al. 1996). Neurodegeneration and gliosis similar to that seen in patients with HIV-1-associated dementia (HAD) have been reported in brain-targeted gp120 transgenic mice (Toggas et al. 1994).

With the urgent need to identify potential interactions between HIV-1 and cannabinoids, the present work is designed to determine if the presence of gp120 in PAG interacts with the analgesic effectiveness of the cannabinoid agonist WIN55,212-2.

✉ Khalid Benamar
khalid.benamar@ttuhsc.edu; kbenamar@temple.edu

[1] Center for Substance Abuse Research, Temple University School of Medicine, Philadelphia, PA 19140, USA

[2] Department of Pharmacology and Neuroscience, School of Medicine, Texas Tech University Health Sciences Center, Lubbock, TX 79430, USA

Materials and methods

Animals

Sprague-Dawley male rats (Envigo, Somerset, New Jersey, USA), weighing 250–300 g, were housed in groups of three for at least 1 week in an animal room maintained at 22 ± 1 °C and approximately 50 ± 5% relative humidity. Lighting was on a 12/12-h light/dark cycle (lights on at 7:00 and off at 19:00). The animals were allowed free access to food and water. All experimental procedures were approved by the Institutional Animal Care and Use Committees at Texas Tech University Health Sciences Center and conform to the guidelines of the International Association for the Study of Pain and of the National Institutes of Health.

Surgery and histological procedures

Rats were anesthetized with 4% isoflurane for induction and 3% for maintenance. A sterilized stainless steel C313G guide cannula (22-gauge, Plastics One Inc., Roanoke, Virginia, USA) was implanted bilaterally into the ventrolateral periaqueductal grey (vlPAG) (Benamar et al. 2004). The stereotaxic coordinates were as follows: 7.8 mm posterior to bregma, 0.5 mm from midline, and 4.5 mm ventral to the dura mater (Paxinos and Watson 2007). A C313DC cannula dummy (Plastics One Inc., Roanoke, Virginia, USA) of identical length was inserted into the guide tube to prevent its occlusion. Experiments began 1 week postoperatively. At the end of the behavioral experiments, standard histological procedures were used to verify the site of injection (Benamar et al. 2004).

Nociceptive test

A 52 °C hot-plate (Ugo Basile, Varese, Italy) was used. 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 60 min by using either jumping or hind-paw licking as the nociceptive endpoint and 30 s as the cutoff point (to avoid tissue damage).

The percentage of maximal possible antinociception (%MPA) for each animal at each time point was calculated using the formula: %MPA = [(test latency − baseline latency) / (30 − baseline latency)] × 100.

Hot-plate is a useful nociceptive test that allows the detection of anti-nociception mediated by supraspinal structures such as PAG (South and Smith 1998). It has been used successfully to study anti-nociceptive effects of various analgesic drugs (Palma et al. 2011; Chen et al. 2011; Benamar et al. 2008), including WIN 55,212-2 (Benamar et al. 2008).

Stereotaxic injections

After a 7-day recovery period, rats were allowed to habituate to the test chambers for 1 h before testing. Vehicle, gp120IIIB (gp120 of T cell line tropic HIV strain IIIB), gp120IIIB inactivated (90 °C for 30 min), or AMD3100 (A CXCR4 antagonist) was microinjected into the PAG in a volume of 0.5 μl via a C313I internal cannula (28-gauge, Plastics One Inc., Roanoke, VA) as we previously described (Benamar et al. 2004). The C313I internal cannula was connected to a 10-μl Hamilton syringe by polyethylene tubing. A volume of 0.5 μl of drug or vehicle was delivered at a rate of 0.5 μl per min (Pump 11 Elite Programmable Syringe Pumps, Harvard Apparatus, Harvard Apparatus, Massachusetts, USA) and the internal cannula left in place an additional 90 s to allow diffusion. Immediately thereafter, a dummy cannula (C313DC) was inserted into the cannula guide to prevent any contamination. WIN 55,212-2 was injected subcutaneously (s.c.).

Histologic and statistical analyses

At the conclusion of experiments, each rat was checked for confirmation of the correct site of the injection according to our standard histologic procedures (Benamar et al. 2008). Only data from animals in which the site of injection was clearly located and identified within the vlPAG regions were included in the studies.

The data are expressed as the mean and standard error of the mean (S.E.M.). Statistical analysis of the difference between groups was assessed with repeated measures (ANOVA) followed by Bonferroni multiple comparison test. $P < 0.05$ was taken as the significant level of difference.

Drugs

The cannabinoid agonist WIN 55,212-2: (R)-(+)-[2,3-dihydro-5-methyl-3-(4-morpholinylmethyl)pyrrolo[1,2,3-de]-1,4-benzoxazin-6-yl]-1-naphthalenylmethanone mesylate was obtained from Sigma-Aldrich (St. Louis, MO, USA) and dissolved in Cremophor, dimethylsulphoxide, and saline (1,1,18). AMD3100: 1,1′-[1,4-Phenylenebis(methylene)]bis-1,4,8,11-tetraazacyclotetradecane octahydrochloride was obtained from Sigma-Aldrich (St. Louis, MO, USA) and dissolved in pyrogen-free saline. Gp120IIIB (T-tropic strain) was obtained from Advanced Biotechnologies Inc. (Columbia, Maryland, USA).

Experimental design

Experiment 1. After a 60-min baseline interval, WIN 55,212-2 (0.25–1 mg/kg, s.c.) or vehicle was injected into the vlPAG at

time 0, and nociception was measured for 60 min using the hot-plate test (Fig. 1).

Experiment 2. To assess the effect of gp120IIIB on WIN 55,212-2-induced analgesia, this viral protein was administered directly into the vlPAG 30 min before WIN 55,212-2. The nociception was measured for 60 min (Fig. 1).

Experiment 3. We determined whether CXCR4 mediates the inhibitory effect of gp120IIIB on WIN 55,212-2-induced analgesia. After a 60-min baseline interval, AMD 3100 (100 ng) was injected into the vlPAG. Thirty minutes later, gp120IIIB was injected into the vlPAG followed 30 min later by WIN 55,212-2. The nociception was measured for 60 min (Fig. 1).

The dose and treatment time of gp120 and AMD3100 are based on our previous study (Chen et al. 2011; Benamar et al. 2008).

Results

The effect of the intra-PAG gp120IIIB on WIN 55,212-2-induced analgesia

First, we established a dose-response study of WIN 55,212-2 in hot-plate test. At dose of 0.25 mg/kg, WIN 55,212-2 induced an increase of 30 ± 7.3% MPA at 30 min compared to vehicle (Fig. 2a, $P < 0.001$). At doses of 0.5 and 1 mg, the increase was 46.6 ± 7.4 (Fig. 2a, $P < 0.001$) and 57.7 ± 2.7 %MPA (Fig. 2a, $P < 0.001$) respectively compared to vehicle.

To allow for possible potentiation or antagonism of the analgesic effect, we sought to determine a dose of WIN 55,212-2 that would give approximately a 50% MPA. The dose of 1 mg/kg WIN was used in the subsequent experiments (Fig. 2a).

Second, we examined the effect of stereotaxic administration of gp120 into PAG on WIN 55,212-2-induced analgesia. By itself, gp120IIIB (100 ng/0.5 μl, vlPAG) did not produce any change in pain threshold in hot-plate test (Fig. 2b, $P > 0.05$). However, gp120IIIB (100 ng/0.5 μl) given 30 min before s.c. injection of WIN 55,212-2 reduced the maximal analgesic effect of WIN 55,212-2 (1 mg/kg) from 58.4 ± 5 to 28.6 ± 9% MPA (Fig. 2b, $P < 0.001$, $n = 6$).

To rule out that the effects of gp120IIIB were due to non-specific interactions with hydrophobic regions of functional proteins, their lipid surroundings in the cell membrane, or contamination, heat-inactivated gp120IIIB was tested. Heat-inactivated gp120IIIB (100 ng/0.5 μl) did not affect WIN 55,212-2-induced analgesia (Fig. 2b, $P > 0.05$).

The effect of AMD3100 on the gp120IIIB antagonism of WIN 55,212-2-induced analgesia

To examine if the gp120IIIB effect was mediated by CXCR4 mechanism, we pretreated the animals with a CXCR4

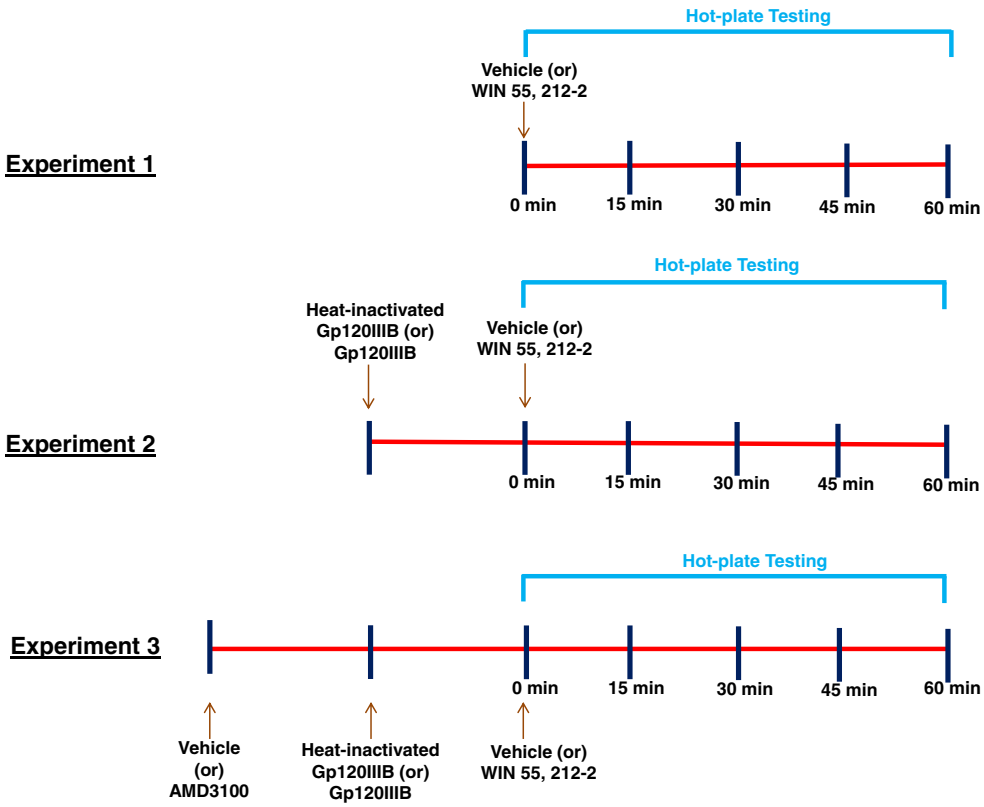

Fig. 1 Schema of experimental design

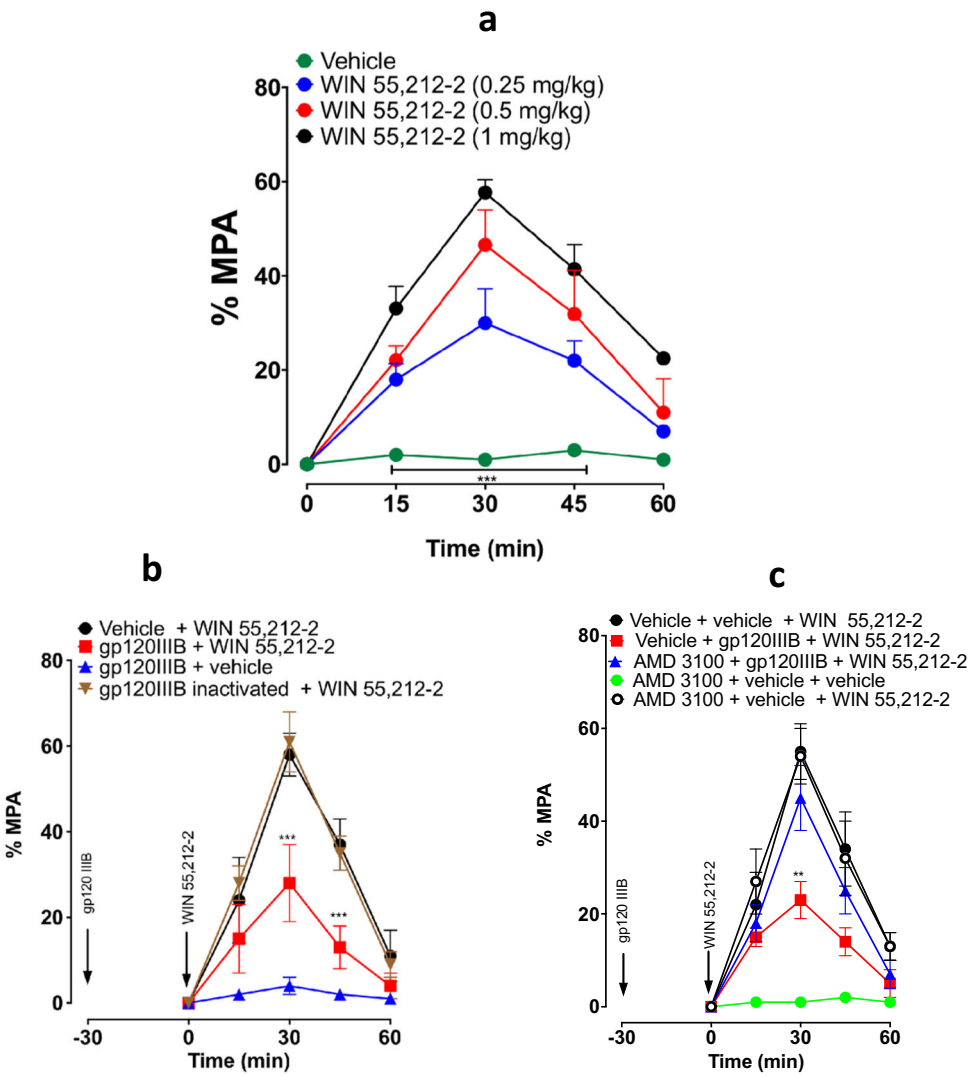

Fig. 2 **a** WIN 55,212-2 (0.25–1 mg/kg, s.c.) induced analgesia dose-dependent in hot-plate test. **b**) vIPAG pretreatment with gp120IIIB (100 ng/0.5 μl) attenuates the analgesic effect of WIN 55,212-2 (1 mg/kg, s.c.). Gp120IIIB was injected 30 min before WIN 55,212-2. ***$P <$ 0.001 vehicle + WIN 55,212-2 vs gp120IIIB + WIN 55,212-2 at 30 and 45 min. **c** vIPAG pretreatment with AMD 3100 (100 ng/0.5 μl, 30 min before gp120IIIB) reverses gp120IIIB effect on WIN 55,212-2-induced analgesia. %MPA percent of maximal possible analgesia. Data are presented as mean ± SEM. **$P < 0.003$ AMD 3100 + gp120IIIB + WIN 55,212-2 vs vehicle + gp120IIIB + WIN 55,212-2 at 30 min

antagonist (AMD 3100). AMD 3100 (100 ng) given 30 min before the injection of gp120 prevented the attenuation of WIN 55,212-2-induced analgesia by gp120IIIB (Fig. 2c, $P < 0.003$, $n = 6$). AMD3100, by itself has no effect on pain threshold in hot-plate test (Fig. 2c, $P > 0.05$).

Discussion

Here, we report the first in vivo evidence for an adverse effect of HIV-1 component on cannabinoid-induced analgesia at supraspinal level. We demonstrated that stereotaxic administration of gp120IIIB into PAG reduce the analgesic effectiveness of WIN 55,212-2. This suggests that gp120IIIB may interact with the cannabinoid system at the level of the descending pain modulatory system localized in the PAG to impair the analgesic effectiveness.

In an attempt to establish the contribution of CXCR4 in the antagonistic effect of gp120IIIB (T-tropic strain) on WIN 55,212-2-induced analgesia, AMD3100 (CXCR4 antagonist) was administered directly into the PAG prior to gp120 and WIN 55,212-2. Our present data show that pretreatment with AMD3100 prevents the effect of gp120 on WIN 55,212-2-induced analgesia 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 WIN 55,212-2 suggests that chemokine blockers might be used as a strategy to restore cannabinoid functions in the context of HIV-1.

The fact that gp120 has no effect on pain threshold, a physiological antagonism is unlikely to explain the reduced analgesic effectiveness of WIN 55,212-2. However, one likely mechanism by which gp120 impairs WIN 55,212-2-induced analgesia may be via a CXCR4 and CB1 cross-talk, particularly a heterologous desensitization between CXCR4 and cannabinoid type 1 (CB1), that could cause CB1 desensitization and/or downregulation, and consequently preventing WIN 55,212-2 analgesic effect. The hypothesis of a cross-talk is

supported by the fact that CXCR4 and CB1 are GPCRs and expressed in the PAG (Banisadr et al. 2002; Cristino et al. 2006), (2) the anti-nociceptive effect of WIN 55,212–2 in PAG is mediated via CB1 (Benamar et al. 2008), and (3) gp120IIIB via CXCR4 reduced the analgesic effect of WIN 55,212-2 (current study; Fig. 2c).

A number of other questions arise from the results of this study that warrant further research. While our data show an interaction of gp120 and WIN 55,212-2-induced analgesia in male rats, it will be important to determine if such interaction can occur under HIV-1 neuropathic pain model and if there is any sex-difference. And while our focus is in PAG, it will be important to determine the role of gp120 and cannabinoid in other components of descending pain modulatory system (e.g., RVM).

The intriguing concept that HIV-1/components such as gp120 may interact with the cannabinoid system at the level of the descending pain modulatory system centered on the PAG to impair the cannabinoid analgesic effectiveness will impact the field of cannabinoid analgesia and HIV-1.

Author's contribution KB designed research. JP and MN conducted experiments. JG and MN analyzed data. KB and MN wrote the manuscript. All authors read and approved the manuscript.

Funding This work was supported by grants DA031605 (to KB) and DA029414 (to KB) from the National Institutes of Health.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

References

Bandler R, Keay KA, Floyd N, Price J (2000) Central circuits mediating patterned autonomic activity during active vs. passive emotional coping. Brain Res Bull 53:95–104

Banisadr G, Fontanges P, Haour F, Kitabgi P, Rostène W, Mélik Parsadaniantz S (2002) Neuroanatomical distribution of CXCR4 in adult rat brain and its localization in cholinergic and dopaminergic neurons. Eur J Neurosci 16:1661–1671

Barak O, Goshen I, Ben-Hur T, Weidenfeld J, Taylor AN, Yirmiya R (2002) Involvement of brain cytokines in the neurobehavioral disturbances induced by HIV-1 glycoprotein120. Brain Res 19:98–108

Benamar K, Rawls SM, Geller EB, Adler MW (2004) Intrahypothalamic injection of deltorphin-II alters body temperature in rats. Brain Res 1019:22–27

Benamar K, Geller EB, Adler MW (2008) First in vivo evidence for a functional interaction between chemokine and cannabinoid systems in the brain. J Pharmacol Exp Ther 325:641–645

Benamar K, Addou S, Yondorf M, Geller EB, Eisenstein TK, Adler MW (2010) Intrahypothalamic injection of the HIV-1 envelope glycoprotein induces fever via interaction with the chemokine system. J Pharmacol Exp Ther 332:549–553

Benamar K, Palma J, Cowan A, Geller EB, Adler MW (2011) Analgesic efficacy of buprenorphine in the presence of high levels of SDF-1α/CXCL12 in the brain. Drug Alcohol Depend 114:246–248

Chen X, Kirby LG, Palma J, Benamar K, Geller EB, Eisenstein TK, Adler MW (2011) The effect of gp120 on morphine's antinociceptive and neurophysiological actions. Brain Behav Immun 25:1434–1443

Cristino L, de Petrocellis L, Pryce G, Baker D, Guglielmotti V, Di Marzo V (2006) Immunohistochemical localization of cannabinoid type 1 and vanilloid transient receptor potential vanilloid type 1 receptors in the mouse brain. Neuroscience 139:1405–1415

Fields HL, Heinricher MM, Mason P (1991) Neurotransmitters in nociceptive modulatory circuits. Annu Rev Neurosci 14:219–245

Heinricher MM, Ingram SL (2008) The senses: a comprehensive reference, the brainstem and nociceptive modulation. Academic, San Diego, pp 593–626

Heinricher MM, Tavares I, Leith JL, Lumb BM (2009) Descending control of nociception: specificity, recruitment and plasticity. Brain Res Rev 60:214–225

Jones MV, Bell JE, Nath A (2000) Immunolocalization of HIV envelope gp120 in HIV encephalitis with dementia. AIDS 4:2709–2713

Lichtman AH, Cook SA, Martin BR (1996) Investigation of brain sites mediating cannabinoid-induced antinociception in rats: evidence supporting periaqueductal gray involvement. J Pharmacol Exp Ther 276(121):585–593

Opp MR, Rady PL, Hughes TK Jr, Cadet P, Tyring SK, Smith EM (1996) Human immunodeficiency virus envelope glycoprotein 120 alters sleep and induces cytokine mRNA expression in rats. Am J Physiol 270(5 Pt 2):R963–70

Ossipov MH, Lai J, Malan TP Jr, Porreca F (2000) Spinal and supraspinal mechanisms of neuropathic pain. Ann N Y Acad Sci 909:12–24

Palma J, Cowan A, Geller EB, Adler MW, Benamar K (2011) Differential antinociceptive effects of buprenorphine and methadone in the presence of HIV-gp120. Drug Alcohol Depend 118(2-3):497–9

Palma J, Abood ME, Benamar K (2015) HIV-gp120 and physical dependence to buprenorphine. Drug Alcohol Depend 150:175–178

Paxinos G, Watson C (2007) The rat brain in stereotaxic coordinates. Academic, San Diego

Porreca F, Ossipov MH, Gebhart GF (2002) Chronic pain and medullary descending facilitation. Trends Neurosci 25:319–325

South SM, Smith MT (1998) Apparent insensitivity of the hotplate latency test for detection of antinociception following intraperitoneal, intravenous or intracerebroventricular M6G administration to rats. J Pharmacol Exp Ther 286:1326–1332

Toggas SM, Masliah E, Rockenstein EM, Rall GF, Abraham CR, Mucke L (1994) Central nervous system damage produced by expression of the HIV-1 coat protein gp120 in transgenic mice. Nature 367:188–193