Central HIV-1 Tat exposure elevates anxiety and fear conditioned responses of male mice concurrent with altered mu-opioid receptor-mediated G-protein activation and β-arrestin 2 activity in the forebrain

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ABSTRACT

Co-exposure to opiates and HIV/HIV proteins results in enhanced CNS morphological and behavioral deficits in HIV$^+$ individuals and in animal models. Opiates with abuse liability, such as heroin and morphine, bind preferentially to and have pharmacological actions through μ-opioid-receptors (MORs). The mechanisms underlying opiate-HIV interactions are not understood. Exposure to the HIV-1 transactivator of transcription (Tat) protein causes neurodegenerative outcomes that parallel many aspects of the human disease. We have also observed that in vivo exposure to Tat results in apparent changes in morphine efficacy, and thus have hypothesized that HIV proteins might alter MOR activation. To test our hypothesis, MOR-mediated G-protein activation was determined in neuroAIDS-relevant forebrain regions of transgenic mice with inducible CNS expression of HIV-1 Tat. G-protein activation was assessed by MOR agonist-stimulated [^{35}S]guanosine-5′-O-(3-thio)triphosphate ([^{35}S]GTPγS) autoradiography in brain sections, and in concentration-effect curves of MOR agonist-stimulated [^{35}S]GTPγS binding in membranes isolated from specific brain regions. Comparative studies were done using the MOR-selective agonist DAMGO ([D-Ala^2, N-MePhe^4, Gly-ol]-enkephalin) and a more clinically relevant agonist, morphine. Tat exposure reduced MOR-mediated G-protein activation in an agonist, time, and regionally dependent manner. Levels of the GPCR regulatory protein β-arrestin-2, which is involved in MOR desensitization, were found to be elevated in only one affected brain region, the amygdala; amygdalar β-arrestin-2 also showed a significantly increased association with MOR by co-immunoprecipitation, suggesting decreased availability of MOR. Interestingly, this correlated with changes in anxiety and fear-conditioned extinction, behaviors that have substantial amygdalar input. We propose that HIV-1 Tat alters the intrinsic capacity of MOR to signal in response to agonist binding, possibly via a mechanism involving altered expression and/or function of β-arrestin-2.

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1. Introduction

Interactions between opiates and HIV/viral proteins that enhance neuro-acquired immune deficiency syndrome (neuroAIDS) pathology are well documented, both in animal models and in HIV$^+$ individuals (Anthony et al., 2008; Bell et al., 2002; Bokhari et al., 2011; Bruce-Keller et al., 2008; Byrd et al., 2011; Dougherty et al., 2002; Fitting et al., 2014; Fitting et al., 2010; Hauser and Knapp, 2014; Pitcher et al., 2014). The prototypical opiate used in animal studies has been morphine, which is also a major bioactive metabolite of heroin (diacetylmorphine). Interactions between HIV and morphine may also have important consequences for patients using prescribed opiates, because morphine is routinely administered in clinical settings for pain management. Co-administration of morphine and HIV/viral

proteins enhances central nervous system (CNS) inflammation and the synaptodendritic damage that is the presumed substrate of HIV-associated neurocognitive disorders (HAND), a spectrum of cognitive and motor deficits that are seen in many HIV patients despite antiretroviral therapy (Antinori et al., 2007; Heaton et al., 2010; Robertson et al., 2007). Morphine binds preferentially to μ-opioid-receptors (MOR), through which most of its major pharmacological actions are directed. While findings from our work and others have generally shown that morphine augments the neurodegenerative actions of the HIV-1 transactivator of transcription (Tat) protein, it is unknown whether Tat affects MOR function. We have hypothesized that some morphine–HIV interactive effects might be due to an effect of Tat protein on functional activation of the MOR, since an apparent decline in morphine efficacy has been seen in previous studies. To test this hypothesis, MOR-mediated G-protein activation was determined in neuro-AIDS-relevant forebrain regions of transgenic mice inducibly expressing the HIV-1 *tat* transgene in the CNS. G-protein activation was examined by both agonist-stimulated [-^{35}S]guanosine-5′-O-(3-thio)triphosphate ([^{35}S]GTPγS) autoradiography in brain sections, and concentration-effect curves of agonist-stimulated [^{35}S]GTPγS binding in isolated membranes, using both the selective MOR agonist [D-Ala^2, N-MePhe^4, Gly-ol]-enkephalin (DAMGO) and the clinically relevant opioid agonist morphine. In addition to MOR selectivity, these ligands also differ in relative efficacy for G-protein activation, in that DAMGO is a full agonist whereas morphine is a partial agonist relative to DAMGO, as reflected in different E$_{max}$ values in GTPγS binding assays (Selley et al., 1998; Selley et al., 1997).

Our results generally show a Tat exposure time-related and brain region-dependent reduction in MOR-mediated G-protein activation, with the greatest magnitude of reductions observed in the striatum and amygdala. These findings suggest that either MOR expression is reduced or coupling to G-proteins is desensitized, either of which could contribute to the apparent reduction in agonist efficacy. Interestingly, levels of β-arrestin-2 (β-Arr2), a regulatory protein important in G-protein coupled receptor (GPCR) desensitization and internalization (including the MOR) and which has been shown to play a role in morphine tolerance (Bohn et al., 2000; Dang and Christie, 2012; Shenoy and Lefkowitz, 2011) was elevated only in the amygdala of Tat(+) mice. Co-immunoprecipitation showed significantly increased binding of MOR to β-Arr2 specifically in the amygdala. These findings correlated with significant alterations in behaviors related to amygdalar function, such as fear conditioning and anxiety (open field and elevated plus maze), in the absence of any changes in baseline motor behaviors. We propose that exposure to HIV-1 Tat decreases MOR agonist efficacy to activate G-proteins, possibly via a mechanism involving altered expression and/or function of β-Arr2.

2. Materials and methods

Animal studies were approved by the Institutional Animal Care and Use Committee at Virginia Commonwealth University.

2.1. Subjects and housing

The inducible Tat transgenic mice used here are a well-accepted model for neuroAIDS since their neuropathology and behavioral deficits tend to mirror those observed in HIV patients with HAND. These include structural abnormalities in neurons/dendrites including reduced spine density and changes in synaptic proteins (Fitting et al., 2010; Hahn et al., 2015), disrupted hippocampal circuitry (Fitting et al., 2013), as well as glial abnormalities including microglial activation and micro/astrogliosis (Bruce-Keller et al., 2008; Hahn et al., 2015). Importantly, these mice and a related Tat-transgenic mouse also develop changes in learning/memory and motor behaviors relevant for HIV patients (Carey et al., 2012;

Fitting et al., 2013; Mediouni et al., 2015; Paris et al., 2014a; Paris et al., 2014b), although some of these effects develop only after chronic Tat expression (Hahn et al., 2015).

Adult, male, Tat-transgenic mice that express Tat$_{1-86}$, a major CNS variant of the full-length Tat$_{1-101}$ protein, were generated as previously described (Bruce-Keller et al., 2008; Fitting et al., 2010; Hauser et al., 2009). Tat transgene activity is inducibly controlled via the reverse tetracycline transactivator (rtTa) system driven by a glial fibrillary acidic protein (GFAP) promoter, which restricts Tat protein expression in the CNS to astroglia. Mice were genotyped to confirm that *tat* and *rtTA* transgenes were present. Chronic CNS Tat expression was induced starting at 2.5–3 months of age by feeding chow that contains doxycycline (DOX) (Harlan Laboratories, Inc., Indianapolis, IN; 6 g/kg) ad libitum. Tat protein expression in striatum and whole brain has previously been documented in this and a similar inducible transgenic model by our lab (Fitting et al., 2010) and others (Carey et al., 2012) using both immunostaining and western blot. Control mice (Tat−/rtta+) received the same chow to control for potential off-target DOX effects. Autoradiography and membrane binding assays were conducted after 4-day and/or 30-day DOX exposure, to capture changes at early times of exposure when we have seen in vivo inflammatory changes (Bruce-Keller et al., 2008) and at a more chronic stage of the neurodegenerative process. Motor behavioral studies were conducted weekly for 4 weeks. Anxiety and fear-conditioned responses were tested after 4 weeks of DOX exposure. To specifically assess MOR agonist effects on anxiety, some Tat(−) and Tat(+) mice (n = 6 and n = 10, respectively) were administered an injection of saline and assessed in an open field 15 min later, and then administered an acute injection of morphine (10 mg/kg, i.p.) and assessed in an open field after 15 min. Open field activity was assessed 15 min post-injection given that we have observed this time-point to be the peak of morphine-stimulated locomotion, consistent with reports using some rodent models (Kalinichev et al., 2002; Steidl and Yeomans, 2009).

2.2. Agonist-stimulated [^{35}S]GTPγS autoradiography

Agonist-stimulated [^{35}S]GTPγS autoradiography was performed on triplicate serial sections as described previously (Sim et al., 1995) with minor modifications. Coronal brain sections (20 μm) were cut on a cryostat maintained at −20 °C and thaw-mounted onto gelatin-coated slides. Sections were collected at levels that included 1) prefrontal cortex (PFC), 2) striatum, including nucleus accumbens (NAc) and caudate–putamen (CPu), and, 3) hippocampus and amygdala. Slides were stored desiccated at −80 °C until use, then brought to room temperature, and incubated in 50 mM Tris–HCl buffer (pH 7.4) with 3 mM MgCl$_2$, 0.2 mM EGTA and 100 mM NaCl (TME buffer) for 10 min at 25 °C. Slides were transferred to TME buffer +0.5% bovine serum albumin (BSA) with 2 mM GDP and 10 mU/ml adenosine deaminase for 15 min at 25 °C. Slides were then incubated in TME Buffer +0.5% BSA containing 0.04 nM [^{35}S]GTPγS, 2 mM GDP, and 10 mU/ml adenosine deaminase with/without agonist for 2 h at 25 °C. Basal binding was determined in the absence of agonist and MOR-stimulated [^{35}S]GTPγS binding was measured using maximally effective concentrations (20 μM) of DAMGO or morphine. After final incubation, slides were rinsed twice in 50 mM Tris buffer (pH 7.4) at 4 °C and then briefly in deionized water (4 °C). Slides were dried and exposed to Kodak Biomax MR film with [^{14}C] microscales for 18 h. Films were digitized at 8-bits per pixel with a Sony XC-77 video camera. Brain regions of interest were determined using The Mouse Brain Atlas (Franklin and Paxinos, 1997). Images were analyzed using NIH Image J software, and resulting values are expressed as nanocuries (nCi) of [^{35}S] per gram of tissue [net stimulation (nCi/g) = (agonist-stimulated − basal)] as previously published (Sim et al., 1996).