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5α-reduced progestogens ameliorate mood-related behavioral pathology, neurotoxicity, and microgliosis associated with exposure to HIV-1 Tat

Jason J. Paris [a,*], ShiPing Zou [b], Yun K. Hahn [b], Pamela E. Knapp [a,b,c], Kurt F. Hauser [a,b,c,*]

[a] Department of Pharmacology and Toxicology, Virginia Commonwealth University, Medical College of Virginia (MCV) Campus, Richmond, VA 23298-0613, USA
[b] Department of Anatomy and Neurobiology, Virginia Commonwealth University, MCV Campus, Richmond, VA 23298-0709, USA
[c] Institute for Drug and Alcohol Studies, Virginia Commonwealth University, Richmond, VA, USA

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ABSTRACT

Human immunodeficiency virus (HIV) is associated with motor and mood disorders, likely influenced by reactive microgliosis and subsequent neural damage. We have recapitulated aspects of this pathology in mice that conditionally express the neurotoxic HIV-1 regulatory protein, trans-activator of transcription (Tat). Progestogens may attenuate Tat-related behavioral impairments and reduce neurotoxicity *in vitro*, perhaps via progesterone's 5α-reductase-dependent metabolism to the neuroprotective steroid, allopregnanolone. To test this, ovariectomized female mice that conditionally expressed (or did not express) central HIV-1 Tat were administered vehicle or progesterone (4 mg/kg), with or without pretreatment of a 5α-reductase inhibitor (finasteride, 50 mg/kg). Tat induction significantly increased anxiety-like behavior in an open field, elevated plus maze and a marble burying task concomitant with elevated protein oxidation in striatum. Progesterone administration attenuated anxiety-like effects in the open field and elevated plus maze, but not in conjunction with finasteride pretreatment. Progesterone also attenuated Tat-promoted protein oxidation in striatum, independent of finasteride pretreatment. Concurrent experiments *in vitro* revealed Tat (50 nM)-mediated reductions in neuronal cell survival over 60 h, as well as increased neuronal and microglial intracellular calcium, as assessed via fura-2 AM fluorescence. Co-treatment with allopregnanolone (100 nM) attenuated neuronal death in time-lapse imaging and blocked the Tat-induced exacerbation of intracellular calcium in neurons and microglia. Lastly, neuronal-glial co-cultures were labeled for Iba-1 to reveal that Tat increased microglial numbers *in vitro* and co-treatment with allopregnanolone attenuated this effect. Together, these data support the notion that 5α-reduced pregnane steroids exert protection over the neurotoxic effects of HIV-1 Tat.

1. Introduction

The advent of highly active antiretroviral therapies (HAART) has made it possible to reduce circulatory viral load of human immunodeficiency virus (HIV) to undetectable levels. However, HAART cannot eradicate HIV, in part due to cellular reservoirs that retain latent infection or low-levels of viral production residing within the central nervous system (CNS) where HAART penetration and/or accumulation are reduced (Iglesias-Ussel and Romerio, 2011). As such, neurotoxic HIV proteins continue to be produced within the CNS and are believed to underlie continuing progression of affective, cognitive, and motor dysfunction (a constellation of neurological symptoms referred to as "neuroAIDS"; Hauser et al., 2007; Hong and Banks, 2015; Nath, 2015).

Within the CNS parenchyma, microglia act as macrophages and are thought to be the primary resident cell type to harbor the virus (Gendelman and Meltzer, 1989; Meltzer and Gendelman, 1992). Given that HIV does not infect neurons, microglial activation and the production of excitotoxic and inflammatory factors likely contribute to the neurodegenerative and behavioral profile observed in neuroAIDS. One viral protein produced by infected microglia (and infected astrocytes to a lesser degree) that may particularly contribute to these effects is the HIV-1 trans-activator of transcription (Tat).

* Corresponding authors at: Department of Pharmacology and Toxicology, Virginia Commonwealth University, Hermes A. Kontos Medical Sciences Building, Room 437, 1217 East Marshall Street, P.O. Box 980613, Richmond, VA 23298-0613, USA.

E-mail addresses: jason.paris@vcuhealth.org (J.J. Paris), kurt.hauser@vcuhealth.org (K.F. Hauser).

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HIV-1 Tat acts independently or in concert with other viral proteins and inflammatory toxins to promote excitotoxic neuronal injury and/or death (Mattson et al., 2005). Among several targets, Tat activates NMDA receptors (Dreyer et al., 1990; Eugenin et al., 2007; Li et al., 2008), interrupts mitochondrial function (Brooke et al., 1998; Perry et al., 2005) and ATP production (Brooke et al., 1998; Norman et al., 2007; Turchan-Cholewo et al., 2006), and can cause focal, transient disruptions to ion homeostasis (Ca^{2+}, Na^{+}, and potentially K^{+}; Fitting et al., 2014; Greenwood and Connolly, 2007; Lee et al., 2003; Perry et al., 2005) resulting in synaptodendritic injury (Greenwood et al., 2007; Park et al., 1996). Microglia are a principal source of Tat-induced cellular toxins including cytokines, reactive oxygen and nitrogen species, and hydrolytic enzymes (King et al., 2006; Sheng et al., 2000; Turchan-Cholewo et al., 2009) as evidenced by central protein oxidation, particularly in striatum (Aksenov et al., 2001, 2003; Turchan-Cholewo et al., 2009). We have utilized a transgenic mouse model wherein HIV-1 Tat_{1-86} is conditionally expressed in a CNS-targeted manner to demonstrate Tat-driven microgliosis within the striatum of male and female mice (Hahn et al., 2015). In a similar transgenic model, conditional Tat exposure was observed to increase microglial activation throughout limbic and extra-limbic brain regions of male mice (Paris et al., 2015). However, in an examination of sex differences, the presence of a reactive nitrosative marker co-localized with microglia was significantly lower among females, compared to males (Hahn et al., 2015). This occurred concurrent with reduced neuronal cell death, astrogliosis, and reduced motor/anxiety-like pathology among females exposed to central HIV-1 Tat (Hahn et al., 2015). As such, gender may confer protection to some of Tat's neuroinflammatory and neurotoxic effects, but the mechanisms are not known.

One point of convergence between HIV-1 Tat toxicity and sex-specific neuroinflammation occurs with classic steroid hormones and their neuroprotective, non-traditionally-acting metabolites. In particular, progesterone and its 5α-reduced/3α-hydroxylated metabolite, allopregnanolone (AlloP; i.e., 5α-pregnan-3α-ol-20-one), typically fluctuate to greater concentrations within females compared to males (Kancheva et al., 2007). Progesterone can attenuate microglial activation, microgliosis, astrogliosis, and can suppress cytokine release (Labombarda et al., 2011; Munroe, 1971; Robinson and Klein, 2012). Unlike progesterone, AlloP is a potent, positive allosteric modulator of $GABA_A$ receptors (Majewska et al., 1986; Paul and Purdy, 1992), a potential negative allosteric modulator of NMDA receptors (Johansson and Le Grevès, 2005; Maurice et al., 2006), is produced in response to immune challenge (Billiards et al., 2002; Ghezzi et al., 2000), and can reduce excitotoxicity partly through rapid increases of tonic inhibition in models of CNS insult (Baulieu and Schumacher, 2000; Brunton et al., 2014; Mellon et al., 2008; Sayeed and Stein, 2009). Allopregnanolone is produced rapidly in response to stress challenges to restore sympathetic and parasympathetic tone (Barbaccia et al., 1996; Patchev et al., 1994, 1996), functions that are observed to be dysregulated among some HIV^{+} individuals (Chittiprol et al., 2007, 2008; Hurwitz et al., 2005). In ovariectomized female mice, supraphysiological progesterone attenuated the anxiety-like effects of HIV-1 Tat exposure (Paris et al., 2014a); however, the mechanisms underlying behavioral protection, the involvement of pregnane steroids on neuroinflammation, and the importance of neurosteroid formation were not assessed.

We hypothesized that HIV-1 Tat would increase both anxiety-like behavior of ovariectomized female mice and protein oxidation in brain, and progesterone administration would ameliorate these effects when unopposed by estrogens. Further, we expected that pharmacological blockade of progesterone metabolism to AlloP would attenuate behavioral protection against HIV-1 Tat *in vivo*. In concurrent experiments, we hypothesized that AlloP would ameliorate HIV-1 Tat-induced pathology *in vitro*, including effects on neurodegeneration, microgliosis, and intracellular calcium ($[Ca^{2+}]_i$).

2. Materials and methods

The use of mice in these studies was pre-approved by the Institutional Animal Care and Use Committee at Virginia Commonwealth University and the experiments were conducted in accordance with ethical guidelines defined by the National Institutes of Health (NIH Publication No. 85–23).

2.1. Subjects and housing

Adult, male ($n = 16$) and female ($n = 77$) mice expressing an HIV-1 *tat* transgene as previously described (Bruce-Keller et al., 2008; Hauser et al., 2009) were generated in the vivarium at Virginia Commonwealth University (MCV campus). Briefly, HIV-1 Tat_{1-86} is conditionally expressed in a CNS-targeted manner via a GFAP-driven Tet-on promoter (activated via consumption of chow containing doxycycline). Mice (approximately 70 days of age) were housed 4–5/cage and were maintained in a temperature- and humidity-controlled room on a 12:12 h light/dark cycle (lights off at 18:00 h) with *ad libitum* access to food and water. A subset of gonadally-intact female mice ($n = 16$) had their estrous cycles tracked as previously described (Paris et al., 2014a).

2.2. Surgical manipulation

Some female mice ($n = 61$) underwent bilateral ovariectomy (OVX) under isoflurane (4%) anesthesia as previously reported (Paris et al., 2014a). Following surgery, mice were monitored to ensure weight gain, muscle tone, and proper neurological response and general health (Crawley and Paylor, 1997). One mouse failed to recover and was excluded from the study. Mice were allowed 14 days for surgical recovery and endogenous hormone washout prior to experimental hormone manipulation.

2.3. Chemicals

To induce HIV-1 Tat_{1-86} expression, transgenic mice were placed on doxycycline chow (Dox Diet #2018; 6000 mg/kg) obtained from Harlan Laboratories (Madison, WI). In order to assess the influence of steroid hormones on experimental endpoints *in vivo*, mice were administered s.c. vehicle (10% EtOH in oil), progesterone (4 mg/kg in vehicle; Sigma–Aldrich Co., P0130; Paris et al., 2014a) or finasteride (50 mg/kg in vehicle; Sigma–Aldrich Co., F1293; Paris et al., 2011) per prior protocols.

To assess the interactions of AlloP and Tat *in vitro*, cells were treated with HIV-1 Tat_{1-86} (50 nM in ddH_2O; ImmunoDx, 1002-2) and/or AlloP (100 nM in 1:10,000 DMSO diluted in media; Sigma–Aldrich Co., P8887). The chosen Tat concentration reflects one from a range that is observed to elicit functional deficits in glia and neurons similar to those occurring in HIV infection (El-Hage et al., 2005, 2008; Kruman et al., 1998; Nath et al., 1999; Perry et al., 2010; Singh et al., 2004). The chosen AlloP dosing reflects a physiological concentration that has previously been found to confer protection from several neurotoxic insults (Ardeshiri et al., 2006; Lockhart et al., 2002; Waters et al., 1997) and is preferred to lower concentrations given the high instability of the neurosteroid due to its rapid clearance (Carter et al., 1997; Phillipps, 1975). Higher concentrations may directly activate $GABA_A$ receptors (Lambert et al., 1990) and were considered non-optimal. Moreover, others have utilized the present dosing regimen to determine potential efficacy for clinical trials aimed at assessing