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Combined chronic blockade of hyper-active L-type calcium channels and NMDA receptors ameliorates HIV-1 associated hyper-excitability of mPFC pyramidal neurons

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ABSTRACT

Human Immunodeficiency Virus type 1 (HIV-1) infection induces neurological and neuropsychological deficits, which are associated with dysregulation of the medial prefrontal cortex (mPFC) and other vulnerable brain regions. We evaluated the impact of HIV infection in the mPFC and the therapeutic potential of targeting over-active voltage-gated L-type Ca^{2+} channels (L-channel) and NMDA receptors (NMDAR), as modeled in HIV-1 transgenic (Tg) rats. Whole-cell patch-clamp recording was used to assess the membrane properties and voltage-sensitive Ca^{2+} potentials (Ca^{2+} influx) in mPFC pyramidal neurons. Neurons from HIV-1 Tg rats displayed reduced rheobase, spike amplitude and inwardly-rectifying K^+ influx, increased numbers of action potentials, and a trend of aberrant firing compared to those from non-Tg control rats. Neuronal hyper-excitation was associated with abnormally-enhanced Ca^{2+} influx (independent of NMDAR), which was eliminated by acute L-channel blockade. Combined chronic blockade of over-active L-channels and NMDARs with open-channel blockers abolished HIV effects on spiking, aberrant firing and Ca^{2+} potential half-amplitude duration, though not the reduced inward rectification. In contrast, individual chronic blockade of over-active L-channels or NMDARs did not alleviate HIV-induced mPFC hyper-excitability. These studies demonstrate that HIV alters mPFC neuronal activity by dysregulating membrane excitability and Ca^{2+} influx through the L-channels. This renders these neurons more susceptible and vulnerable to excitatory stimuli, and could contribute to HIV-associated neuropathogenesis. Combined targeting of over-active L-channels/NMDARs alleviates HIV-induced dysfunction of mPFC pyramidal neurons, emphasizing a potential novel therapeutic strategy that may effectively decrease HIV-induced Ca^{2+} dysregulation in the mPFC.

1. Introduction

The introduction of combined antiretroviral therapy (cART) transformed HIV from a death sentence to a chronic disease with several co-morbid complications, including HIV-Associated Neurocognitive Disorders (HAND) (Antinori et al., 2007). Despite cART, HAND affects ~50% of HIV-infected individuals (Heaton et al., 2010; Simioni et al., 2010) and its prevalence is expected to increase as the HIV-infected population ages. This underscores the need to elucidate cellular/molecular mechanisms driving HIV-mediated neuropathogenesis to devise effective strategies that prevent and/or treat HAND.

HIV affects many brain regions, including the vulnerable medial prefrontal cortex (mPFC) (Ferris et al., 2008), a key regulator of cognition, emotion and motivation-driven behavior, from which glutamatergic pyramidal neurons (80–90% of mPFC neurons) (Yuste, 2005) provide excitatory inputs to the HIV-vulnerable striatum and midbrain (Ferris et al., 2008; Sesack et al., 1989). Although neurons are not infected, HIV-infected leukocytes invade the brain where the virus is transmitted to glial cells, which release neurotoxic viral proteins and inflammatory molecules that cause neuronal dysregulation, injury and loss. Such neuronal dysfunction involves Ca^{2+} dysregulation, excitotoxicity, oxidative stress, and mitochondrial dysfunction, but the cellular/molecular mechanisms underlying these pathogenic cascades are not fully understood (Mattson et al., 2005).

Because Ca^{2+} regulates many fundamental signaling and gene expression pathways, severe alterations in Ca^{2+} homeostasis can be damaging. HIV-1 proteins and HIV-induced inflammatory processes alter Ca^{2+} homeostasis by excessively increasing intracellular Ca^{2+} levels ($[Ca^{2+}]_{in}$) (Chami et al., 2006; Hu, 2015). Much emphasis has been placed on dysfunction of the NMDAR, a Ca^{2+}-permeable ligand-gated

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ionotropic glutamatergic receptor, which is well-established to participate in HIV-induced Ca^{2+} dysregulation (Haughey and Mattson, 2002). However, HIV-induced $[Ca^{2+}]_{in}$ increases are also regulated by intracellular Ca^{2+} release and voltage-gated Ca^{2+} channels (VGCCs) (Brini et al., 2014; Hu, 2015). In previous clinical trials, individual blockade of NMDARs or voltage-gated L-type Ca^{2+} channels (L-channels) failed to improve the progression of HIV-associated dementia (Navia et al., 1998; Schifitto et al., 2007).

Our recent research has focused on the impact of HIV on the L-channel, which mediates intrinsic excitability of neurons and regulates Ca^{2+}-induced signal transduction and gene expression (Brini et al., 2014; Ikeda, 2001). We have demonstrated that HIV-1 Tat abnormally increases L-channel activity and expression in the mPFC (Hu, 2015; Napier et al., 2014; Wayman et al., 2012), and that chronic exposure to multiple HIV-1 proteins for 6 months increases L-channel expression in vivo (Wayman et al., 2015b). These findings, in combination with the established involvement of NMDAR in HIV neuropathogenesis (Haughey and Mattson, 2002; Mattson et al., 2005), led us to hypothesize that combined repetitive targeting of both over-active L-channels and NMDARs would decrease HIV-induced mPFC neuronal hyperexcitability.

In the present study, we evaluated the impact of combined chronic blockade of over-active L-channels/NMDARs in HAND, as modeled in the HIV-1 Tg rat (Reid et al., 2001). HIV-1 Tg rats, expressing 7 of the 9 HIV-1 genes (*gag*/*pol*-deleted), have been used extensively to model HIV-1 effects on the brain, and recapitulate many features of HAND including neuronal injury and pro-inflammatory responses in the brain (Peng et al., 2010; Royal, III et al., 2012). Here, we assessed HIV-induced functional alterations in the membrane properties and Ca^{2+} influx of mPFC pyramidal neurons and determined whether combined (or individual) chronic blockade of over-active L-channels and/or NMDAR alleviates these alterations. Two selective (use-dependent) "open-channel" blockers, diltiazem (Dilt, for the L-channel) (Niimi et al., 2003) and memantine (Mem, for the NMDAR) (Chen et al., 1992) were used, which preferentially block over-activated L-channels and NMDARs, respectively, but reserve "house-keeping" channel activity. We report here that (1) mPFC pyramidal neurons in HIV-1 Tg rats are hyper-excitable, exhibiting an abnormal increase of firing and voltage-sensitive Ca^{2+} influx, due partly to L-channel over-activation, independent of NMDAR, and (2) combined, but not individual, chronic blockade of over-active L-channels and NMDAR significantly reduces these HIV-induced functional deficits. Our studies point to a therapeutic potential for targeting both L-channels and NMDARs to ameliorate and/or reduce HAND.

2. Materials and methods

2.1. Animals

Male HIV-1 Tg and non-Tg F344 rats, purchased from Envigo (Indianapolis, IN) at 3–4 weeks of age, were housed at the Rush University Comparative Research Center on a 12 h light/dark cycle with food and water available ad libitum. Rats received daily subcutaneous injections of saline (SAL, 0.1 ml), the selective L-channel blocker, Dilt (15 mg/kg), the selective NMDAR antagonist, Mem (10 mg/kg), or combined Dilt/Mem for 2 weeks, and were used for electrophysiological experiments at 6–7 weeks of age. Animal care and use procedures were conducted with Institutional Animal Care and Use Committee (IACUC) approval and in accordance with NIH, USDA and institutional guidelines.

2.2. Whole-cell patch-clamp recording from ex vivo brain slices

Chloral hydrate-sedated rats (400 mg/kg, i.p.) were transcardially perfused with ice-cold cutting solution (in mM: 248 sucrose, 2.9 KCl, 2 $MgSO_4$, 1.25 NaH_2PO_4, 26 $NaHCO_3$, 0.1 $CaCl_2$, 10 glucose, 3 kynurenic acid, 1 ascorbic acid; pH = 7.4–7.45). Brains were removed, immersed in ice-cold cutting solution, then coronally sectioned at 300 μm using a vibratome (Leica VT1000S, Buffalo Grove, IL). Slices were incubated in artificial cerebrospinal fluid (aCSF; in mM: 125 NaCl, 2.5 KCl, 25 $NaHCO_3$, 1.25 NaH_2PO_4, 1 $MgCl_2$, 2 $CaCl_2$, 15 glucose; pH = 7.35–7.4, 305–315 mOsm) for 1 h at RT before anchored in an aCSF-perfused recording chamber at ~34 °C. All solutions that brains/brain slices were placed in were oxygenated (95% O_2, 5% CO_2).

Pyramidal neurons in layers V-VI of the prelimbic area of the mPFC (Paxinos and Watson, 1998) were identified using differential interference contrast microscopy on a Nikon Eclipse E600FN microscope (Nikon Instruments Inc., Melville, NY) for recordings (Nasif et al., 2005b). Recording electrodes were made from glass pipettes using a horizontal pipette puller (p-97 Sutter Instrument Co., Novato, CA) and filled with appropriate internal solution adjusted to pH = 7.3–7.35 and 280–285 mOsm [for evoked action potentials (in mM): 120 K-glucontate, 10 HEPES, 0.1 EGTA, 20 KCl, 2 $MgCl_2$, 3 Na_2ATP, and 0.3 NaGTP; for voltage-sensitive Ca^{2+} potentials (in mM): 140 Cs-gluconate, 10 HEPES, 2 $MgCl_2$, 3 Na_2ATP and 0.3 NaGTP]. Whole-cell configuration was obtained under voltage-clamp mode settings using internal solution-filled electrodes (4–6 MΩ); then the settings were changed to current-clamp mode for studying changes in the membrane potential (V_m) of neurons. Signals were filtered, amplified and digitized with a Multiclamp 700B amplifier (Molecular Devices, Sunnyvale, CA) and a Digidata 1440 interface (Axon Instruments) and stored on a PC.

To measure evoked V_m responses, hyperpolarizing and depolarizing current pulses (−400 to +300 pA) were applied for 500 ms at 25 pA intervals to mimic inhibitory and excitatory inputs. Membrane hyperpolarization was first applied to remove inactivation, thereby allowing activation of low voltage-activated (LVA) Ca^{2+} channels (Huguenard, 1996). The membrane properties of neurons were determined from the initial action potential evoked by the minimal depolarizing current (rheobase). The input resistance (R_{in}) was calculated by fitting the V_m response from the −100 pA current step with the Boltzman charge-voltage equation. The inclusion criteria for data analysis were (1) a stable resting membrane potential (RMP) more hyperpolarized than −60 mV for neurons from non-Tg control rats, and (2) the amplitude of initial Na^+-dependent action potential (evoked by rheobase) >60 mV and 40 mV, respectively, for neurons from non-Tg and HIV-1 Tg rats (Napier et al., 2014; Wayman et al., 2015a, 2015b). The contribution of L-channels to action potential generation (neuronal excitability) was assessed by selectively blocking L-channel activity with acute perfusion of 5 μM nifedipine (Nif) (Nasif et al., 2005a).

To isolate VGCC activity, the perfusing aCSF contained inhibitors to block Na^+ channels (0.5 μM tetrodotoxin), K^+ channels (20 mM extracellular tetraethylammonium, 140 mM intracellular Cs-gluconate), ionotropic glutamate receptors (2.5 mM kynurenic acid) and $GABA_A$ receptors (100 μM picrotoxin). V_m was held at ~−70 mV (around average RMP) to compensate for K^+ channel blockade-induced depolarization of RMP (Hu et al., 2004). Ca^{2+} plateau potentials (reflecting Ca^{2+} influx through voltage-gated Ca^{2+} channels) were evoked with 40 ms rheobase currents. L-channels were selectively blocked with acute perfusion of 5 μM Nif and all voltage-gated Ca^{2+} influx was blocked with acute perfusion of 400 μM cadmium (Cd) in the bath. The Ca^{2+} potential properties were determined after at least 10 min of perfusion with aCSF containing ion channel blockers/receptor inhibitors, and when rheobase-evoked Ca^{2+} spike recordings became consistent. The Ca^{2+} potential area is the integrated area under the potential (mV x ms), demarcated at the beginning/end by the holding V_m.

2.3. Statistical analysis

Data were analyzed using SigmaPlot (Systat Software Inc., San Jose, CA) and SPSS (IBM Corporation, Armonk, NY). When appropriate, student's *t*-tests and two-way ANOVA were used to compare HIV-1 Tg and non-Tg (Ca^{2+} potential properties and membrane properties). A *Chi*-squared test was used for categorical comparison (aberrant firing