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Human immunodeficiency virus protein Tat induces oligodendrocyte injury by enhancing outward K^+ current conducted by $K_V1.3$

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ABSTRACT

Brain white matter damage is frequently detected in patients infected with human immunodeficiency virus type 1 (HIV-1). White matter is composed of neuronal axons sheathed by oligodendrocytes (Ols), the myelin-forming cells in central nervous system. Ols are susceptible to HIV-1 viral trans-activator of transcription (Tat) and injury of Ols results in myelin sheath damage. It has been demonstrated that activation of voltage-gated K^+ (K_V) channels induces cell apoptosis and Ols predominantly express K^+ channel $K_V1.3$. It is our hypothesis that Tat injures Ols via activation of $K_V1.3$. To test this hypothesis, we studied the involvement of $K_V1.3$ in Tat-induced Ol/myelin injury both in vitro and ex vivo. Application of Tat to primary rat Ol cultures enhanced whole-cell $K_V1.3$ current recorded under voltage clamp configuration and confirmed by specific $K_V1.3$ antagonists Margatoxin (MgTx) and 5-(4-phenoxybutoxy) psoralen (PAP). The Tat enhancement of $K_V1.3$ current was associated with Tat-induced Ol apoptosis, which was blocked by MgTx and PAP or by siRNA knockdown of $K_V1.3$ gene. The Tat-induced Ol injury was validated in cultured rat brain slices, particularly in corpus callosum and striatum, that incubation of the slices with Tat resulted in myelin damage and reduction of myelin basic protein which were also blocked by aforementioned $K_V1.3$ antagonists. Further studies revealed that Tat interacts with $K_V1.3$ as determined by protein pull-down of recombinant GST-Tat with $K_V1.3$ expressed in rat brains and HEK293 cells. Such protein-protein interaction may alter channel protein phosphorylation, resultant channel activity and consequent Ol/myelin injury. Taken together, these results demonstrate an involvement of $K_V1.3$ in Tat- induced Ol/myelin injury, a potential mechanism for the pathogenesis of HIV-1-associated white matter damage.

1. Introduction

Neurologic complications of human immunodeficiency virus type 1 (HIV-1) infection remain common in the era of effective combination antiretroviral therapy (cART). Up to half of infected individuals develop HIV-1-associated neurocognitive disorders (HAND), the cause(s) remain obscure. Many studies have revealed a preferential damage to cerebral white matter in HIV-1-infected brain (Gongvatana et al., 2009; Hoare et al., 2011; Sarma et al., 2014; Wohlschlaeger et al., 2009), and such damage is prevalent even in the era of cART and more severe in patients with HAND (Chen et al., 2009b; Gosztonyi et al., 1994). Structure magnetic resonance imaging and diffusion tensor imaging studies in HIV-1-infected individuals have revealed a subcortical white matter damage mainly in the regions of the corpus callosum, internal capsule and other brain regions (Chang et al., 2008; Chen et al., 2009b; Gongvatana et al., 2009; Pomara et al., 2001; Sarma et al., 2014; Wu et al., 2006). Moreover, cognitive impairment in HIV-1-infected individuals with AIDS was found to be associated with white matter injury in the corpus callosum, internal capsule, and superior longitudinal fasciculus (Gongvatana et al., 2009; Wu et al., 2006). It appears that subcortical damage in white matter plays a more important role than cortical damage in the mediation of HAND symptoms which are predominantly of the subcortical type (Chen et al., 2009a; Navia et al., 1986; Peavy et al., 1994; Price et al., 1988).

Cerebral white matter consists mostly of myelinated axons and axonal myelination is formed by oligodendrocytes (Ols). The integrity of the myelin sheaths is essential for the propagation of nerve impulses along axons. It has been demonstrated that myelin pallor, an abnormality that could reflect a decrease in myelin components (Glass et al., 1993; Power et al., 1993), is frequently seen in patients with HIV-1 encephalitis and in cART naïve subjects. Myelin sheath damage and changes in numbers of Ols have also been observed in HIV-1-infected individuals (Esiri and Morris, 1996; Esiri et al., 1991). In vitro studies have shown that exposure to recombinant viral envelope protein gp120 resulted in alterations of Ol functional activity and myelin formation in rat Ols maintained in a cell culture system (Bernardo et al., 1997;

Abbreviations: cART, combination antiretroviral therapy; GxTx, Guangxi toxin; HIV-1, human immunodeficiency virus type 1; HAND, HIV-1-associated neurocognitive disorders; MgTx, Margatoxin; Ol, oligodendrocyte; PAP, 5-(4-phenoxybutoxy) psoralen; PVD, Pervanadate.

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Kimura-Kuroda et al., 1994), exampling HIV-1 protein impairment of Ol/myelin, which may lead to axonal injury, demyelination and ultimately white matter damage. Nevertheless, how HIV-1 proteins induce Ol/myelin injury is not fully understood.

Increasing evidence indicates that activation of voltage-gated K^+ channels (K_V) is an essential pathway in programmed cell death (Burg et al., 2006; Remillard and Yuan, 2004) and enhancement of outward K^+ current results in neural cell apoptosis (Yu, 2003; Yu et al., 1997). Ols express several subtypes of K_V channels including a predominant $K_V1.3$ (Attali et al., 1997; Schmidt et al., 1999). A decrease of $K_V1.3$ expression or outward K^+ current in Ols is essential for synthesis of myelin structural proteins and suppression of outward K^+ current promotes Ol maturation and survival. These results suggest a role of $K_V1.3$ in the regulation of Ol functionality (Chittajallu et al., 2002; Tegla et al., 2011). Moreover, activation of p53 has been detected in the Ol lineage cells in the postmortem brains of HAND patients, but not in control brains (Jayadev et al., 2007), suggesting Ols undergo apoptosis in HIV-1-infected brains. Thus, it is our hypothesis that continued viral replication and viral proteins induce Ol/myelin injury by activation of Ol $K_V1.3$ channels, leading to myelin/white matter damage and HAND pathogenesis. To test this hypothesis, we studied how HIV-1 protein Tat induces Ol/myelin injury, as infected brain cells continuously express and release Tat protein despite the controlled viral replication (Johnson and Nath, 2014; Johnson et al., 2013). Our results showed that HIV-1 Tat enhances outward K^+ current conducted by $K_V1.3$ leading to Ol/myelin injury.

2. Materials and methods

2.1. Animals

Pregnant Sprague-Dawley rats were purchased from Charles River (Wilmington, MA) and maintained under the ethical guidelines for the care of laboratory animals, and all animal procedures were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of University of Nebraska Medical Center.

2.2. Ol preparation and culture

OI cell cultures were prepared as described previously (Chen et al., 2007) and all culture materials were purchased from Sigma-Aldrich (St. Louis, MO) unless otherwise stated. Briefly, cerebral cortical tissues were dissociated from *P*1–2 neonatal pups and incubated in Hank's buffered salt solution contained in 0.25% trypsin and 200 U DNAase at 37 °C for 15 min. Collected cells were suspended in DMEM (with L-glutamine and sodium pyruvate, Cellgro, Manassas, VA) supplemented with 20% FBS (Gibco, Grand Island, NY) and 1% penicillin/streptomycin (Gibco). Mixed glia cultures were grown on poly-D-lysine-coated T75 flasks (Thermo, Nazareth, PA) for 10 d and Ols were isolated by shaking overnight at 200 r.p.m. at 37 °C. Cell suspensions were transferred onto non-treated petri dishes for 30 min to further separate Ols by differential adhesion. Ol-contained supernatant was collected by slightly swirling petri dish and passed through 40 μm nylon cell strainers into a sterile 50-ml tube. Ols were collected by centrifugation at 800 r.p.m. for 5 min and suspended in proliferating medium (described below). Ols were plated onto poly-D-lysine-coated coverslips, culture dishes, or plates in different culture media depending on the developmental stage. Basal chemically defined medium (BDM) was made of DMEM containing 0.1% BSA, 1% Insulin-Transferrin-Selenium (Gibco), 10 nM D-biotin, and 10 nM hydrocortisone. Isolated Ols were maintained in proliferating medium (BDM supplemented with 10 ng/ml PDGF-AA and 10 ng/ml bFGF (both from Peprotech, Rocky Hill, NJ) for 7 d. Myelin basic protein $(MBP)^+$ mature Ols were obtained by transferring cells to differentiating medium (BDM, 15 nM triiodothyronine, 10 ng/ml CNTF (Peprotech) and 5 μg/ml *N*-acetyl-L-cysteine) for 2–3 d.

2.3. Electrophysiology

Cells were seeded onto 35 mm culture dishes for whole-cell recording of ionic currents. Recording electrodes made from borosilicate glass micropipettes (WPI Inc., Sarasota, FL) with a P-97 horizontal micropipette puller (Sutter Instruments, Novato, CA) had a tip resistance of 5–8 MΩ. The electrodes solution contained (in mM): 140 KCl, 2 $CaCl_2$, 2 $MgCl_2$, 11 EGTA, 10 HEPES/KOH, pH 7.3, and had an osmolarity of 300 mOsm, as measured by a vapor pressure osmometer (WESCOR, Logan, UT). The standard bath solution contained (in mM): 140 NaCl, 5.4 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 10 HEPES/NaOH, pH 7.3. D-sucrose was used to adjust the osmolarity of this solution to 305 mOsm prior to experiments. Stock solutions of $K_V1.3$ specific inhibitors, 5-(4-phenoxybutoxy) psoralen (PAP, Sigma) and Margatoxin (MgTx, Sigma), were prepared in deionized water, stored at −20 °C freezer and diluted to a working concentration with bath solution immediately before being applied to cells via bath perfusion at a constant flow rate of 1 ml/min. A Burleigh micromanipulator (PC-5000, EXFO, Canada) was used to position the recording electrode. Whole-cell K^+ currents were induced by applying voltage steps from −150 mV to +60 mV in increments of 15 mV, and current amplitudes were measured at the peak for each test potential. Current density (pA/pF) was calculated by dividing the whole-cell capacitance (pF), which represents cell membrane surface area, from the peak current amplitude (pA). All experiments were done at room temperature (22–23 °C). Whole cell currents were amplified with an Axopatch-200B amplifier (Molecular Devices, Sunnyvale, CA), filtered at 1 kHz, digitized at 5 kHz using a Digidata 1440A interface (Molecular Devices), displayed and recorded on a desktop computer. pCLAMP 10.0 (Molecular Devices) was employed for on-line data acquisition and off-line data analyses. All final graphics in the present work were constructed using Origin 8.5 (OriginLab, Northampton, MA).

2.4. siRNA knockdown of $K_V1.3$ gene

Pre-designed ON-target plus SMARTpool siRNA against rat KCNA3 ($K_V1.3$, NM-019270) mRNA was purchased from Dharmacon, Inc. (Chicago, IL). Ols were seeded at a density of 0.35×10^6/well into 6-well plates or 0.1×10^6/well into 12-well plates. According to the manufacturer instruction, $K_V1.3$-siRNA and NT-siRNA were transfected at the final concentration of 25 nM for 72 h to gain the protein level knockdown in presence of Dharma FECT Transfection Reagent (Dharmacon, Inc). Transfected cells were then treated with 50 ng/ml Tat for 48 h.

2.5. Apoptosis assay

Terminal deoxynucleotidyl transferase dUTP nick end labeling (Tunel) staining was used to measure apoptotic Ols. Cultures plated on coverslips were fixed in 4% paraformaldehyde for 1 h at room temperature and permeabilized with 0.1% Triton X-100 for 30 min. Tunel was performed according to manufacturer's instructions for the in situ cell death detection kit (Fluorescein, Roche Applied Science, Indianapolis, IN). Cells were incubated for 90 min at 37 °C in Tunel reaction mixture contained terminal deoxynucleotidyl transferase and fluorescein-labeled nucleotides (1:50). After washing, coverslips were mounted in vectashield mounting medium with Dapi stain (Vector Laboratories, Burlingame, CA) and Ols were visualized by a fluorescent microscope. Apoptosis was assessed in each of 3 experimental preparations by examining 10 visual fields per experimental group.

2.6. Brain slices cultures

Coronal brain slices were prepared from 21 day old Sprague-Dawley rats. The slice culture was performed as described by Stoppini et al. (Stoppini et al., 1991). Briefly, after anesthesia with isoflurane, the rats were quickly decapitated by small animal decapitator (Stoelting, 51330). The whole brain was dissected and fixed onto the stage of