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1521-0103/384/2/306–314$35.00
THE JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS
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dx.doi.org/10.1124/jpet.122.001291
J Pharmacol Exp Ther 384:306–314, February 2023

The Journal of PHARMACOLOGY And Experimental Therapeutics

Novel Allosteric Modulator Southern Research Institute-32743 Reverses HIV-1 Transactivator of Transcription-Induced Increase in Dopamine Release in the Caudate Putamen of Inducible Transactivator of Transcription Transgenic Mice[S]

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Received May 5, 2022; accepted November 7, 2022

ABSTRACT

Development of neurocognitive disorder in human immunodeficiency virus (HIV)-infected patients has been linked to dysregulation of dopamine by the HIV-1 transactivator of transcription (Tat) protein, a negative allosteric modulator of dopamine transporter (DAT). Using fast scan cyclic voltammetry, the present study determined the effects of in vivo Tat expression on dopamine release in the caudate putamen of inducible Tat transgenic (iTat-tg) mice and the impact of a novel DAT allosteric modulator, Southern Research Institute (SRI)-32743, on the Tat effect. We found that 7- or 14-day doxycycline (Dox)-induced Tat expression in iTat-tg mice resulted in a 2-fold increase in phasic but not tonic stimulated baseline dopamine release relative to saline control mice. To determine whether the Tat-induced increase in dopamine release is mediated by DAT regulation, we examined the effect of an in vitro applied DAT inhibitor, nomifensine, on the dopamine release. Nomifensine (1 nM–10 μM) concentration-dependently enhanced phasic stimulated dopamine release in both saline- and Dox-treated iTat-tg mice, while the magnitude of the nomifensine-mediated dopamine release was unchanged between saline and Dox treatment groups. A single systemic administration of SRI-32743 prior to animal sacrifice reversed the increased dopamine release in the baseline of phasic dopamine release and nomifensine-augmented dopamine levels in Dox-treated iTat-tg mice, while SRI-32743 alone did not alter baseline of dopamine release. These findings suggest that Tat expression induced an increase in extracellular dopamine levels by not only inhibiting DAT-mediated dopamine transport but also stimulating synaptic dopamine release. Thus, DAT allosteric modulators may serve as a potential therapeutic intervention for HIV infection-dysregulated dopamine system observed in HIV-1 positive individuals.

SIGNIFICANCE STATEMENT

HIV infection-induced dysregulation of the dopaminergic system has been implicated in the development of neurocognitive impairments observed in HIV positive patients. Understanding the mechanisms underlying HIV-1 Tat protein-induced alteration of extracellular dopamine levels will provide insights into the development of molecules that can attenuate Tat interaction with targets in the dopaminergic system. Here, we determined whether Tat alters dopamine release and how the novel DAT allosteric modulator, SRI-32743, impacts dopamine neurotransmission to attenuate Tat-induced effects on extracellular dopamine dynamics.

Introduction

Despite the use of combined antiretroviral therapies (cART), human immunodeficiency virus (HIV)-1 associated neurocognitive disorders (HANDs) persist, affecting 50% of HIV-1 seropositive individuals (Gannon et al., 2011). HAND presents a group of neurocognitive disorders including HIV-associated dementia and mild neurocognitive impairment (Heaton et al., 2010). HIV-1 infected macrophages can cross the blood brain barrier and serve as reservoirs to produce HIV-1 viral proteins, resulting in pathophysiological damages in the central nervous system (CNS) (King et al., 2006). HIV-1 viral RNA has been detected in cerebrospinal fluid (CSF) as early as eight days after initial infection (Valcour et al., 2012), with associated cognitive impairments occurring within months of HIV-1 infection, thus supporting a long therapeutic

This work was supported by the SPARC grant through the University of South Carolina Office of the Vice President for Research to SD and National Institutes of Health [Grant DA035714] (to J.Z.) and [Grant DA047924] (to C.A.S.).
No author has an actual or perceived conflict of interest with the contents of this article.
dx.doi.org/10.1124/jpet.122.001291.
[S] This article has supplemental material available at jpet.aspetjournals.org.

ABBREVIATIONS: cART, combined antiretroviral therapies; CNS, central nervous system; CPu, caudate putamen; CSF, cerebrospinal fluid; DA, dopamine; DAT, dopamine transporter; Dox, doxycycline; FSCV, fast scan cyclic voltammetry; HAND, human immunodeficiency virus-associated neurocognitive disorders; HIV, human immunodeficiency virus; HPLC, high-performance liquid chromatography; iTat-tg, inducible Tat transgenic mouse; Tat, transactivator of transcription; SRI, Southern Research Institute; VMAT2, vesicular monoamine transporter 2.

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window for treatment (Saylor et al., 2016). Although acute viral replication has been well controlled with cART in early HIV infection, long-term viral protein exposure within the CNS causes dopaminergic deficits, which may act as a mediating factor in development of HAND (Gaskill et al., 2009; Heaton et al., 2010; Soontornniyomkij et al., 2016). Therefore, there is a need for developing a therapeutic intervention for development of HAND by targeting the initial drivers of HAND pathogenesis.

Neurocognitive deficits in HIV-1-infected persons are associated with HIV-1 viral proteins (Frankel and Young, 1998; Power et al., 1998; Brack-Werner, 1999; Johnston et al., 2001). In particular, the HIV-1 transactivator of transcription (Tat) protein, which promotes viral replication in early HIV infection, has been associated with the pathophysiology of HAND development (Rappaport et al., 1999; King et al., 2006). Over time, exposure to Tat induces damage to dopaminergic brain regions (Nath et al., 1987; Berger and Arendt, 2000; Koutsilieri et al., 2002a) and thus alters motivation pathways (Wise and Bozarth, 1987; Everitt and Robbins, 2005; Berridge, 2007). Relevant levels of Tat have been detected in dopamine (DA)-rich brain areas (Del Valle et al., 2000; Hudson et al., 2000; Lamers et al., 2010) and the sera (Westendorp et al., 1995; Xiao et al., 2000) of HIV-1 infected individuals despite the use of cART (Johnson et al., 2013). Specifically, increased levels of DA and decreased DA turnover are present in the CSF of therapy-naïve HIV patients in asymptomatic infection (Scheller et al., 2010), whereas in advanced stages of infection, there are decreased levels of DA in DA-rich brain regions (Sardar et al., 1996; Kumar et al., 2009; Kumar et al., 2011). Furthermore, the transgenic expression of HIV-1 viral proteins in animal models results in distinct alterations of DA levels in the CSF (Berger et al., 1994; di Rocco et al., 2000; Scheller et al., 2010) and in different brain regions (Koutsilieri et al., 2002b; Kumar et al., 2011; Javadi-Paydar et al., 2017). We and another group have reported that induction of Tat expression in inducible Tat transgenic (iTat-tg) mice by a systemic administration of doxycycline increases DA tissue contents in the caudate putamen (CPu) (Kesby et al., 2016b) and the prefrontal cortex (Strauss et al., 2020) as assessed by high-performance liquid chromatography (HPLC). This study used fast-scan cyclic voltammetry (FSCV) to determine extracellular DA dynamics in the brain slices of iTat-tg mice. Compared with traditional measurements of DA changes through HPLC and microdialysis, FSCV can detect rapid neurotransmitter dynamics in the brain (Robinson et al., 2003).

The dopamine transporter (DAT) transports extracellular DA into the synaptic cytosolic space and is critical for DA homeostasis. Tat interacts with DAT as a negative allosteric modulator (Zhu et al., 2009; Zhu et al., 2011), causing a decrease in DAT-mediated DA uptake in cells expressing human DAT (Midde et al., 2013; Midde et al., 2015; Quizon et al., 2016) and in the prefrontal cortex of iTat-tg mice (Strauss et al., 2020). This study aims to determine whether DAT mediates Tat effects on extracellular DA dynamics or Tat directly stimulates vesicular DA release. Further, our recent study demonstrated that a novel quinazoline structure-based compound, SRI-32743, functions as an allosteric modulator of DAT (Zhu et al., 2022). Southern Research Institute (SRI)-32743 reverses Tat-induced inhibition of DAT-mediated DA uptake in cells expressing human DAT and ameliorates in vivo Tat expression-potentiated cocaine conditioned place preference in iTat-tg mice (Zhu et al., 2022). Thus, the current study was also undertaken to determine whether Tat effects on extracellular DA can be reversed by SRI-32743. Investigating whether SRI-32743 attenuates Tat-induced dynamic changes in synaptic DA release in iTat-tg mice may provide insight into the mechanism underlying the development of cognitive deficits in HIV-1 infected persons.

Materials and Methods

Animals. The iTat-tg mouse line expresses a "tetracycline-on (TETON)" system under the control of the astrocyte-specific glial fibrillary acidic protein promoter, allowing for astrocyte-specific (CNS) expression of the Tat_{1-86} gene (Kim et al., 2003). The tetracycline derivative doxycycline (Dox) was used to induce expression of Tat protein in the CNS. A previous study shows that detectable Tat levels by Tat western blotting was found on day 5 after 14-day administration of Dox (100 mg/kg) until day 14, but no difference in Tat levels was found between 7- and 14-day Dox treatment groups (Carey et al., 2012). Importantly, 7-day injection of 100 mg/kg of Dox is sufficient to induce relevant cognitive impairment in iTat-tg mice working memory (Carey et al., 2012). Furthermore, we have recently reported that both 7- and 14-day administration of Dox (100 mg/kg) induces an equivalent reduction of DAT-mediated DA uptake in iTat-tg mice (Strauss et al., 2020). Therefore, for this study, mice were injected once daily for 7 or 14 days with either Dox (100 mg/kg) or saline (0.9%) to determine the dose-dependent effect of Tat on extracellular DA release.

Male iTat-tg mice were provided by Dr. Jay Mclaughlin (College of Pharmacy at the University of Florida, Gainesville, FL). Mice used for all experiments were between the ages of 9 and 12 weeks. This age range was chosen based on the previous reports, which found both physiologic neuroadaptive (Carey et al., 2013; Cirino et al., 2020) and behavioral deficits (Carey et al., 2012; Paris et al., 2014; Paris et al., 2015) in the iTat-tg mice using an identical age range. Male mice were derived as described previously (Strauss et al., 2020). Mice were housed (3–5/cage) on a 12:12h light/dark cycle (lights on at 7:00 AM), in temperature (21 ± 2°C), humidity (50 ± 10%), with ad libitum food and water. Animals were housed in accordance with the University of South Carolina Animal Care and Use Committee guidelines. Male animals were chosen because our recent report demonstrates that 14-day Dox-induced Tat expression decreases DAT-mediated DA uptake in male iTat-tg mice (Strauss et al., 2020). Consistently, the current study used male mice to further determine the impact of 14-day Tat expression on extracellular DA dynamics.

Chemicals. The following commercially available reagents were used and purchased from Sigma Aldrich: ascorbic acid (A5960), D-(+)-glucose (G7528), sodium bicarbonate (S6297), potassium chloride (P5405), sodium chloride (S3014), sodium phosphate monobasic monohydrate (S9638), calcium chloride dihydrate (C-3881), dimethyl sulfoxide (D8418), and doxycycline hyclate (D9891). The following commercially available reagent was purchased from Fisher Scientific: magnesium chloride hexahydrate (593292). The novel DAT allosteric modulators, SRI-32743, was synthesized at Southern Research Institute (Birmingham, AL).

Drug Administration. Mice were randomly assigned to four treatment groups: saline (0.9%)/vehicle (90% saline/10% DMSO), saline/SRI-32743 (10 mg/kg in 90% saline/10% DMSO), Dox (100 mg/kg)/vehicle, and Dox/SRI-32743. Mice received administration of SRI-32743 (10 mg/kg in 90% saline/10% DMSO, i.p.) 30 minutes prior to mice being sacrificed. The dosage and paradigm were chosen because systemic administration (i.p.) of SRI-32743 (at a dose of 10 mg/kg) 60 minutes prior to cocaine-conditioned place preference (cocaine-CPP) testing in iTat-tg mice ameliorated 14-day Tat expression-induced potentiation of cocaine-CPP, while SRI-32743 alone did not affect baseline cocaine-CPP behavior (Zhu et al., 2022). Therefore, the current study was to determine whether altered extracellular DA levels through the ex vivo FSCV contribute to the Tat-

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induced potentiated cocaine-CPP behavior. SRI-32743 was initially dissolved in 100% DMSO (10 mg/ml) and sonicated for 10 minutes in a 50°C water bath, then diluted with 0.9% saline (1 mg/ml). SRI-32743 was obtained from the Southern Research Institute in Birmingham, AL. Doxycycline, nomifensine, and all other chemicals needed were purchased from Sigma-Aldrich.

Fast Scan Cyclic Voltammetry. All slice voltammetry experiments were conducted in mice 24 hours after their last administration of saline or doxycycline. Mouse brains were removed by rapid decapitation and 400 μm thick coronal sections of brain tissue were obtained using a Campden Instruments 5100 mz microtome (Loughborough, Leics., LE12 7TJ. U.K.). A single slice was transferred to a chamber with flowing oxygenated (95% O_2/5% CO_2) artificial CSF (aCSF) at approximately 1 ml/min (aCSF, 0.4 mM ascorbic acid, 11 mM dextrose, 25 mM $NaHCO_3$, 126 mM NaCl, 2.5 mM KCl, 1.2 mM NaH_2O_4, 2.4 $CaCl_2$, 1.2 $MgCl_2$, pH = 7.4) at 32°C. A carbon fiber working electrode was fabricated using 7 μm diameter (100–200 μm length, validated using AmScope camera MD500, AmScope, Irvine, California) T-650 carbon fiber (Cytec Engineering, Woodland Park, NJ) sealed in paraffin (Ramsson et al., 2015) and filled with 1 mM KCl to provide electrochemical contact with the stainless steel lead. The carbon fiber electrode was cycled in aCSF for 15 minutes at 60 Hz, then 5 minutes at 10 Hz, prior to use, during which the slice recovered in the electrophysiological recording chamber. A twisted bipolar electrode was used for stimulation of DA release. The carbon fiber electrode was inserted into the slice tissues at ~75 μm depth, whereas the stimulating electrode (P1 Technologies, Roanoke, VA, part # MS303/3-B/SPC) was placed on the surface of the slice tissues 100 μm away from the carbon fiber electrode. Electrode placement was done using the aid of a MEIJI EMZ stereoscope (MEIJI TECHNO, Santa Clara, California) with an eyepiece (SWF10X) with a grated ruler. Electrodes were placed consistently in the medial CPu as illustrated in Fig. 1A. All brain slices used were located within bregma limits 1.33 mm (anterior) to 0.85 mm (posterior). Data acquisition was collected using a ChemClamp potentiostat (Dagan, Minneapolis, MN). All experiments were performed using the following parameters: potential window (-0.4–1.2 V), scan rate (400 V/s), versus Ag/AgCl. A stable baseline was obtained, data collected for 8 files (15 seconds long, stimulation at 5 seconds) with 180-second intervals between each file. To determine the role of DAT in mediating effects on extracellular DA, pharmacological response to the selective DAT inhibitor, nomifensine, was determined (Tatsumi et al., 1997). Our pilot study has identified that Dox-induced Tat expression altered phasic-like (20 Hz, 5 pulses, 7.5 V amplitude, 4.00 millisecond pulse width) stimulated DA, but not tonic-like DA release (1 pulse, Supplemental Fig. 1); thus, in the current study, we measured Tat-induced increase in extracellular DA using phasic-like stimulated DA release. The CPu region was the focus of the current study because the CPu expresses higher density of DAT, which can clarify whether Tat-induced alteration of extracellular DA release is mediated through Tat-induced inhibition of DAT-mediated DA uptake. Once a stable baseline was achieved, nomifensine was added in increasing concentration order (1, 10, 100 nM, 1, 10 μM). Data for nomifensine treatment was collected the same as baseline (8 files, 15-second collection window, 180-second wait time between collections). The final file collected for each treatment was used for data analysis. Electrode calibration factors (nA/μM) were derived in situ as previously described (Roberts et al., 2013). The in situ--derived calibration factor for each experiment was determined by the area under curve for the background current for the last data file collected at the end of the experiment.

FSCV Data Acquisition and Analysis. Data acquisition and analysis was performed using DEMON voltammetry software (Yorgason et al., 2011). Five electrochemical signal parameters were measured from FSCV experiments. The peak of DA signal (μM) is defined as maximal height of the DA oxidation signal after tonic or phasic stimulation. T_{20} and T_{80} (seconds) are defined as time required for the DA signal to decay by 20% and 80%, respectively, from its maximal DA signal. Tau is derived from an exponential curve fit to the uptake portion of the release event, and full width at half height refers to the width of the release event at half the peak height during the event. Data are presented as mean ± S.E.M.; n represents number of mice. Data from FSCV experiments are presented as: 1) the peak of oxidation values for DA signal, T_{20}, and T_{80} at baseline that were collected from the last 15-second recording of DA release, 2) DA signal versus time trace, 3) representative heatmap, and 4) % change in T_{20} and T_{80} versus their respective baseline.

Data are presented as mean ± S.E.M.; n represents number of mice. To analyze the baseline DA signal parameter (Table 1 and Fig. 1B), separate three-way mixed factorial ANOVAs with Tat induction (saline versus doxycycline) and treatment (vehicle versus SRI-32743) as between-group factors were performed. To analyze the effect of nomifensine on DA signal, T_{20}, and T_{80}, separate two-way repeated measurement ANOVA with nomifensine concentration as a within group factor and Tat induction and treatment as two between-group factors were performed. Where appropriate, Tukey's post hoc tests and simple main effect analyses were performed. All statistical analyses were performed using IBM SPSS software version 28. Differences were considered statistically significant at $p < 0.05$.

Results

Induction of Tat Expression in iTat-tg mice by 7- or 14-day Administration of Dox Results in an Increase in Baseline Extracellular DA and SRI-32743 Attenuates the Tat-Increased DA Release. After a 7-day administration of Dox or saline, the effects of Dox-induced Tat expression and systemic administration of SRI-32743 on phasic-stimulated DA release were determined in the CPu of iTat-tg mice (Table 1 and Fig. 1B). Two-way ANOVA analysis revealed that significant main effects of Tat expression ($F_{(1,21)} = 8.2$, $p = 0.009$), SRI-32743 treatment ($F_{(1,21)} = 21.8$, $p < 0.001$), and interaction between Tat expression × SRI-32743 treatment ($F_{(1,21)} = 6.3$, $p = 0.02$). Post-Hoc analysis using simple main effect comparison showed that a significant increase in phasic DA release was observed in the CPu of Dox-treated iTat-tg mice (0.423 ± 0.078 μM) compared with saline control mice (0.830 ± 0.093 μM) [($F_{(1,11)} = 10.7$, $p = 0.007$)]. Systemic administration of SRI-32743 alone did not alter DA in saline- and SRI-32743-treated iTat-tg mice (0.260 ± 0.059 μM) compared with saline- and vehicle-treated mice [($F_{(1,10)} = 2.76$, $p = 0.128$)]; however, SRI-32743 attenuated the Tat-increased DA release in Dox- and SRI-32743-treated iTat-tg mice (0.287 ± 0.055 μM) [($F_{(1,11)} = 23.0$, $p < 0.001$)]. Representative DA versus time graphs and color plots (Fig. 1 C-F) are shown for all four treatment groups.

We further determined the effects of 14-day administration of Dox on the phasic-stimulated DA release in the CPu of iTat-tg mice (Table 1 and Fig. 2B). Two-way ANOVA analysis revealed that significant main effects of Tat expression ($F_{(1,20)} = 11.1$, $p = 0.003$), SRI-32743 treatment ($F_{(1,20)} = 19.7$, $p < 0.001$), and interaction between Tat expression × SRI-32743 treatment ($F_{(1,20)} = 7.5$, $p = 0.0013$). Post hoc analysis revealed that a significant increase in DA release was observed in Dox- and vehicle-treated mice (1.185 ± 0.166 μM) compared with saline- and vehicle-treated mice (0.587 ± 0.073 μM) [($F_{(1,10)} = 10.8$, $p = 0.008$)]. The Tat-increased DA release observed in 14-day Dox-treated iTat-tg mice was attenuated in Dox- and SRI-32743-treated iTat-tg mice (0.467 ± 0.068 μM) [($F_{(1,10)} = 15.6$, $p = 0.008$)], while SRI-32743 itself did not alter DA release relative to saline- and vehicle-treated mice [($F_{1,10)} = 4.2$, $p =$

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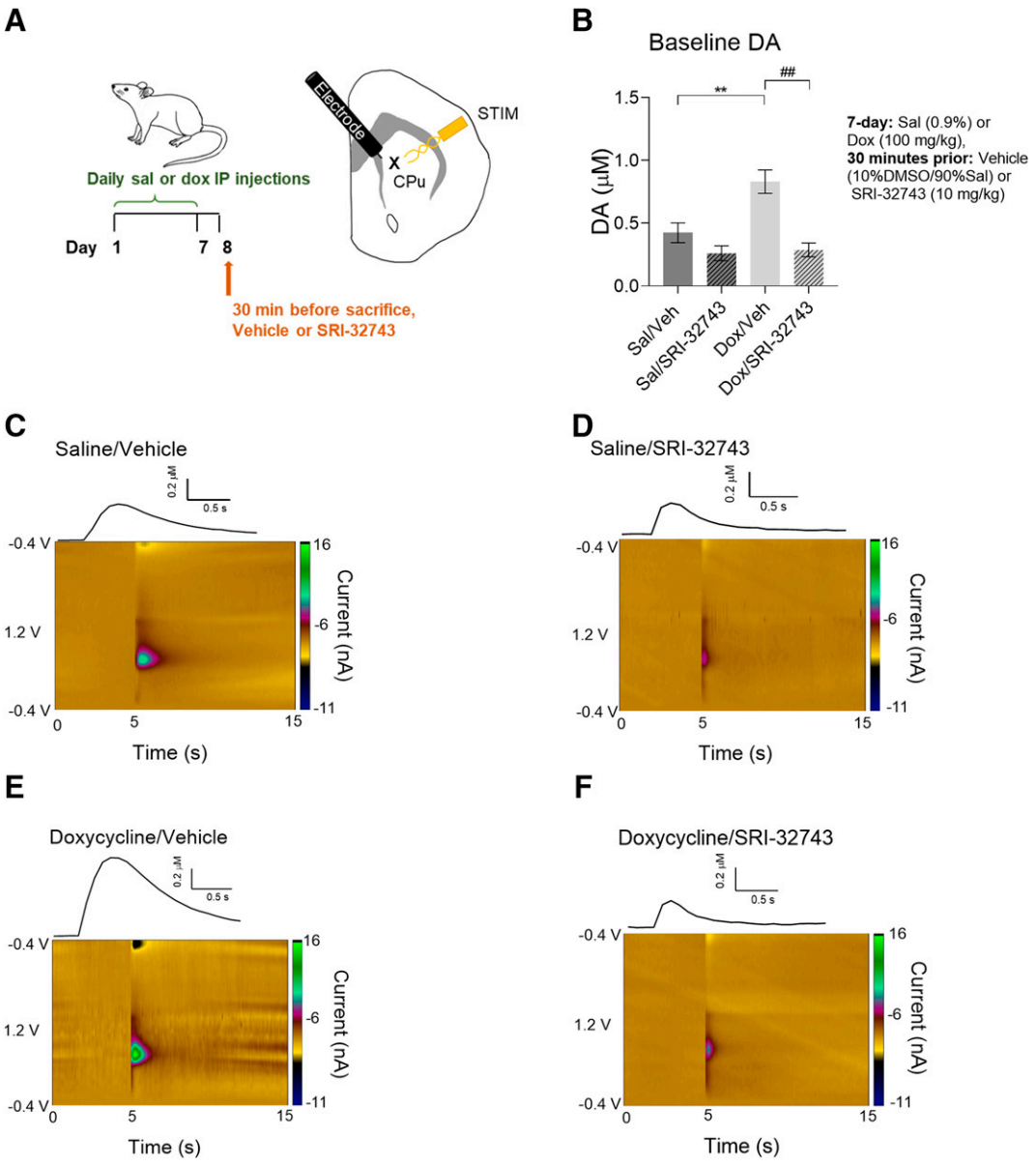

Fig. 1. Effect of SRI-32743 on Tat-induced alteration of extracellular DA levels in iTat-tg mice following 7-day administration of doxycycline or saline. iTat-tg mice were treated daily for 7 days with either saline (Sal) or doxycycline (Dox, 100 mg/kg) to induce expression of Tat. On day 8, mice were administered vehicle (Veh, 10% DMSO/90% saline, i.p.) or SRI-32743 (10 mg/kg, i.p.) 30 minutes prior to mice being sacrificed. Mice were sacrificed and 400 μm thick slices from the CPu were prepared. (A) A carbon fiber electrode placement in the CPu is indicated by an ×. (B) Baseline DA level in the CPu under phasic like-stimulation (20 Hz, 5 pulses) for iTat-tg mice treated with Sal/Veh, Sal/SRI-32743, Dox/Veh or Dox/SRI-32743. (C-F) Real-time current, indicated by its oxidation and reduction across time, is represented as pseudocolor plots for all four treatment groups. Data are expressed in μM and represent mean ± S.E.M. from six to seven independent experiments (6–7 mice/group). ** $p < 0.01$, Dox/Veh compared to Sal/Veh. $^{\#\#}$ $p < 0.01$, Dox/SRI-32743 compared to Dox/Veh.

TABLE 1

Effects of SRI-32743 and Tat expression on the phasic-stimulated baseline DA release in the CPu of iTat-tg mice following 7- and 14-day administration of doxycycline

	DA (μM)			
Dosage	Saline/Vehicle	Saline/SRI-32743	Doxycycline/Vehicle	Doxycycline/SRI-32743
7-day saline or doxycycline, 10 mg/kg SRI-32743	0.423 ± 0.078	0.260 ± 0.059	0.830 ± 0.093	0.287 ± 0.055
14-day saline or doxycycline, 10 mg/kg SRI-32743	0.587 ± 0.073	0.418 ± 0.037	1.185 ± 0.166	0.467 ± 0.068

Data are expressed as mean ± S.E.M. with values from 6–7 independent experiments for each treatment group.

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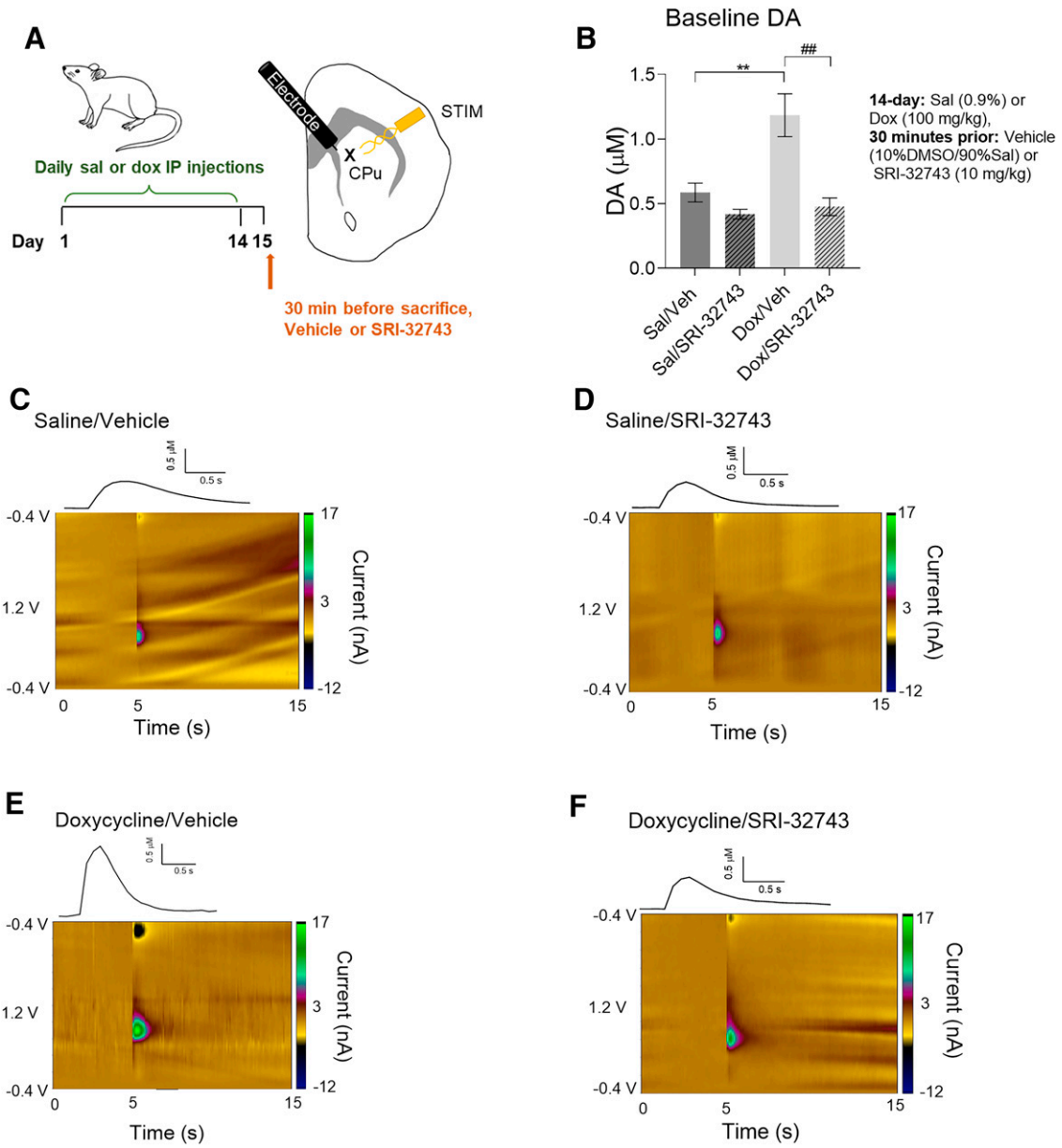

Fig. 2. Effect of SRI-32743 on Tat-induced alteration of extracellular DA levels in iTat-tg mice following 14-day administration of doxycycline or saline. iTat-tg mice were treated daily for 14 days with either saline (Sal) or doxycycline (Dox, 100 mg/kg) to induce expression of Tat. On day 15, mice were administered vehicle (Veh, 10% DMSO/90% saline, i.p.) or SRI-32743 (10 mg/kg, i.p.) 30 minutes prior to mice being sacrificed. Mice were sacrificed and 400 μm thick slices from the CPu were prepared. (A) Carbon fiber electrode placement in the CPu is indicated by an ×. (B) Baseline DA level in the CPu under phasic-like stimulation (20 Hz, 5 pulses) for iTat-tg mice treated with Sal/Veh, Sal/SRI-32743, Dox/Veh or Dox/SRI-32743. (C-F) Real-time current, indicated by its oxidation and reduction across time, is represented as pseudocolor plots for all four treatment groups. Data are expressed in μM and represent mean ± S.E.M. from six independent experiments (6 mice/group). ** $p < 0.01$, Dox/Veh compared to Sal/Veh. $^{\#\#}$ $p < 0.01$, Dox/SRI-32743 compared to Dox/Veh.

0.067)]. Additionally, no difference was observed between saline/SRI-32743 and Dox/SRI-32743 ($F_{(1,10)} = 0.567$, $p = 0.469$). Representative DA versus time graphs and color plots (Fig. 2 C-F) are shown for all four treatment groups.

Effects of SRI-32743 on Dox-Induced Changes in DA Release in Response to DAT Inhibitor Nomifensine in iTat-tg Mice. To determine the role of DAT in the Tat-increased DA release observed in Dox-treated iTat-tg mice, we examined the effect of nomifensine, a selective DAT inhibitor, on phasic-stimulated DA release in the CPu of iTat-tg mice following 7- or 14-day administration of Dox or saline. After the last 15-second recording of baseline DA release, peak oxidation values for the DA signal at baseline in response to a range of nomifensine (1 nM–10 μM) were collected from four treatment groups of iTat-tg mice after 7-day Dox or saline (Fig. 3). Fig. 3A shows the representative DA versus time traces in response to 1 μM of nomifensine in four treatment groups of iTat-tg mice. Three-way repeated measurement ANOVA for DA release in response to nomifensine revealed significant main effects of Tat expression ($F_{(1,19)} = 14.4$, $p = 0.001$), SRI-32743 treatment ($F_{(1,19)} = 58.1$, $p < 0.001$), and nomifensine concentration ($F_{(5,95)} = 122.3$, $p < 0.001$). There were significant interactions of Tat expression × treatment ($F_{(1,19)} = 5.15$, $p = 0.035$), Tat expression × nomifensine concentration ($F_{(5,95)} = 4.63$, $p < 0.001$)

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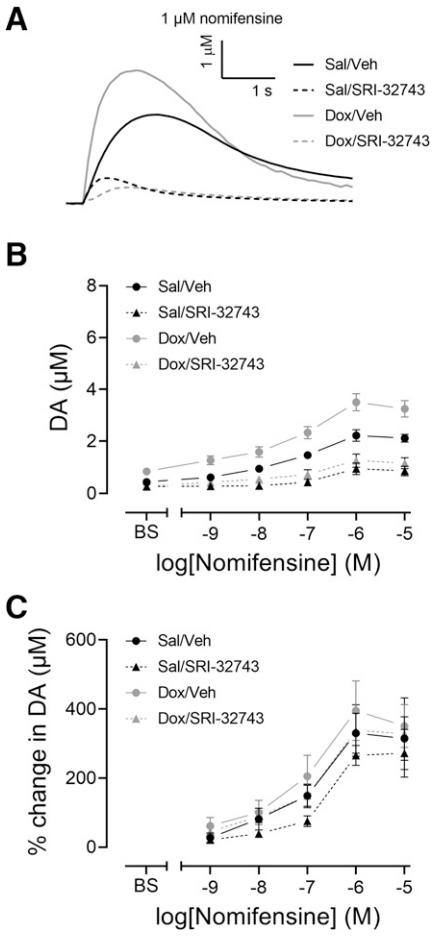

Fig. 3. Effect of nomifensine on Tat-induced alteration of extracellular DA levels in iTat-tg mice following 7-day administration of doxycycline or saline. iTat-tg mice were treated daily for 7 days with either saline (Sal) or doxycycline (Dox, 100 mg/kg) to induce expression of Tat. On day 8, mice were administered vehicle (Veh, 10% DMSO/90% saline, i.p.) or SRI-32743 (10 mg/kg, i.p.) 30 minutes prior to mice being sacrificed. Mice were sacrificed and 400 μm thick slices from the CPu were prepared. (A) Phasic-stimulated DA signal versus time trace in response to 1 μM of nomifensine in four treatment groups. (B) Dose-response curve of DA signal (μM) in response to a range of nomifensine concentrations (1 nM–10 μM) in four treatment groups. (C) Dose-response curve of % change in DA signal versus baseline in response to a range of nomifensine concentrations (1 nM–10 μM) in four treatment groups.

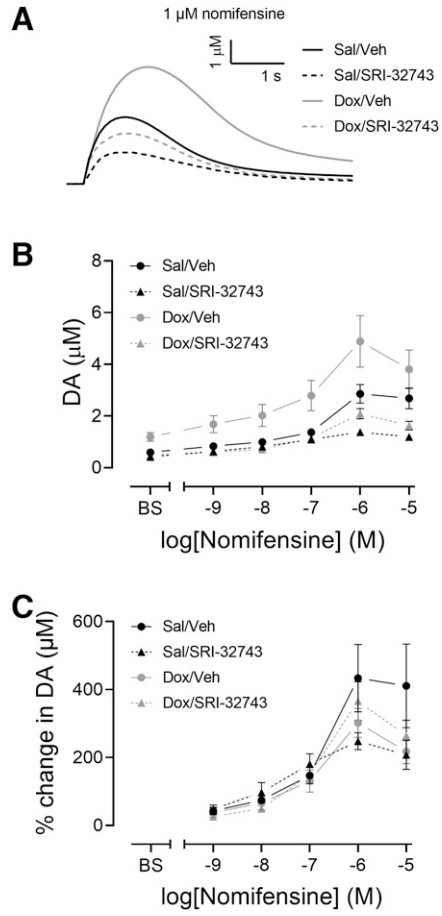

Fig. 4. Effect of nomifensine on Tat-induced alteration of extracellular DA levels in iTat-tg mice following 14-day administration of doxycycline or saline. iTat-tg mice were treated daily for 14 days with either saline (Sal) or doxycycline (Dox, 100 mg/kg) to induce expression of Tat. On day 8, mice were administered vehicle (Veh, 10% DMSO/90% saline, i.p.) or SRI-32743 (10 mg/kg, i.p.) 30 minutes prior to mice being sacrificed. Mice were sacrificed and 400 μm thick slices from the CPu were prepared. (A) Phasic-stimulated DA signal versus time trace in response to 1 μM of nomifensine in four treatment groups. (B) Dose-response curve of DA signal (μM) in response to a range of nomifensine concentrations (1 nM–10 μM) in four treatment groups. (C) Dose-response curve of % change in DA signal versus baseline in response to a range of nomifensine concentrations (1 nM–10 μM) in four treatment groups.

and treatment × nomifensine concentration ($F_{(5,95)} = 24.6$, $p < 0.001$), indicating that nomifensine enhances DA signal in a concentration-dependent manner. No significant three-way interaction of Tat expression × treatment × nomifensine was found ($F_{(5,95)} = 1.57$, $p = 0.177$). Post hoc analysis with separate two-way ANOVA repeat measurements were performed to further determine the differences in collapsed DA signal across nomifensine concentrations among the four groups. Compared with the saline/vehicle group, the DA signal in the Dox/vehicle group was significantly increased ($F_{(1,19)} = 15.3$, $p = 0.004$), which was attenuated in the Dox/SRI-32743 group ($F_{(1,10)} = 35.9$, $p < 0.001$). Nomifensine at 1 μM induced a 57% increase in DA release in the Dox/vehicle group compared with the saline/vehicle group. There was no difference in baseline and nomifensine-induced DA release between saline/SRI-32743 and saline/vehicle groups. When nomifensine data were normalized to baseline as shown in Fig. 3C, no difference in DA release in response to a range of nomifensine concentrations was found among the four treatment groups.

We further determined the effects of nomifensine on phasic-stimulated DA release in the CPu of 14-day Dox-administered iTat-tg mice. As shown in Fig. 4A, the representative DA versus time traces in response to 1 μM nomifensine in four treatment groups of iTat-tg mice show that DA release was enhanced in the Dox/vehicle group relative to the saline/vehicle group, which was attenuated in the saline/SRI-32743 group. Fig. 4B shows the peak oxidation values for the DA signal at baseline in response to a range of nomifensine (1 nM–10 μM) that were collected after the last 15-second recording of baseline DA from four treatment groups. Three-way repeated measurement ANOVA for DA release in response to nomifensine (Fig. 4B) revealed significant main effects of Tat expression ($F_{(1,20)} = 6.3$, $p = 0.021$), SRI-32743 treatment ($F_{(1,20)} = 16.8$, $p = 0.001$), and nomifensine concentration ($F_{(5,100)} = 67.2$, $p < 0.001$). There were significant interactions

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of Tat expression × nomifensine concentration ($F_{(5,100)} = 4.2$, $p < 0.001$) and treatment × nomifensine concentration ($F_{(5,100)} = 12.9$, $p < 0.001$), indicating that the effects of Tat expression and SRI-32743 on DA release occur in a nomifensine concentration-dependent manner. However, no significant three-way interaction of Tat expression × treatment × nomifensine concentration was found ($F_{(5,100)} = 0.62$, $p > 0.05$). To further determine the between-group difference, separate two-way ANOVAs with repeated measurement for DA release in response to nomifensine were conducted for the four treatment groups. Within vehicle groups, Dox-induced Tat expression increased DA release in the Dox/vehicle group compared with the saline/vehicle group ($F_{(1,10)} = 4.5$, $p < 0.05$). Within Dox treatment groups, Tat expression-increased DA release in the Dox/vehicle group was attenuated in the Dox/SRI-32743 group ($F_{(1,10)} = 4.6$, $p < 0.05$). Nomifensine at 1 μM induced a 70% increase in DA release in the Dox/vehicle group compared with the saline/vehicle group. There was no difference in baseline and nomifensine-induced DA release between the saline/SRI-32743 and saline/vehicle groups. Finally, there were no significant effects of nomifensine or treatment of the normalized response to nomifensine (% change in DA versus baseline, Fig. 4C).

Effects of Tat Expression and SRI-32743 on the Parameters Associated with DA Uptake in the Four Treatment Groups. Parameters associated with DA uptake, including T_{20}, T_{80}, tau, and full width at half height were analyzed in baseline and response to nomifensine for iTat-tg mice treated with 7- or 14-day saline versus doxycycline, respectively. Neither significant main effects of Tat expression and SRI-32743 or their interactions were observed among the four treatment groups (Supplemental Figs. 2 and 3).

Discussion

The current study determined the effects of inducible Tat expression on dynamic changes in synaptic DA release by phasic stimulation in the CPu of iTat-tg mice. To accomplish this, we used FSCV to measure extracellular DA dynamics in the CPu slices of iTat-tg mice after 7- or 14-day administration of Dox or saline. Our findings provide the first evidence that in vivo Tat expression resulted in a 2-fold increase in baseline phasic stimulation-induced DA release relative to saline control mice. Our observation is consistent with one previous report showing an increase in DA tissue content by HPLC measurement, which was observed in the CPu of 3-day Dox-treated iTat-tg mice compared with G-tg (Tat null) mice, whereas a trend for decrease in DA tissue content was found in the nucleus accumbens (Kesby et al., 2016a). A previous study using in vivo microdialysis followed by HPLC measurement showed that intrastriatal infusion of recombinant Tat_{1-86} into the rat nucleus accumbens reduced extracellular DA levels (Ferris et al., 2009). However, compared with HPLC measurement, the FSCV used in the current study provides real-time detection of neurotransmitters on a subsecond time scale. In this study, an optimized dose (100 mg/kg. i.p.) of Dox was chosen for 7- or 14-day administration, which was previously proven efficacious for induction of Tat expression (Zou et al., 2007; Carey et al., 2012; Paris et al., 2014). Tat expression levels in the brains of Dox-treated iTat-tg mice were calculated to be in the range of 1–5 ng/ml (Kim et al., 2003; Langford et al., 2018), which is close to the reported Tat levels in the brains of HIV-infected individuals (Westendorp et al., 1995; Xiao et al., 2000). Further, a Tat immunoreactivity study showed that the Dox-induced Tat is widely expressed throughout the brains of iTat-tg mice and correlates with the duration and dose of Dox administered (Carey et al., 2012; Paris et al., 2014). Our results show that 7- or 14-day Tat expression increases phasic stimulation DA release in the CPu of Dox-treated iTat-tg mice; however, our ongoing study shows 14-day, but not 7-day Tat expression decreases phasic-like DA release in the nucleus accumbens of Dox-treated iTat-tg mice (unpublished data). Thus, Dox-induced Tat expression may influence DA release in a region-specific manner. The possibilities for Tat differential profiles in different brain regions could be differences in transporter or receptor expression, neuronal circuitry pathways, or differences in mechanisms associated with vesicular DA release. Determining the effects of Tat expression on the mesocorticolimbic brain regions in future study will allow us further understanding of the mechanism(s) underlying Tat-induced dysregulation of dopaminergic transmission. Importantly, the current findings correlate with clinical observation that increased DA levels are detected in the cerebrospinal fluid of therapy-naïve HIV-positive patients in early HIV infection (Scheller et al., 2010), which has been implicated as a mediating factor in the abnormal neurocognitive function observed in HAND (Berger and Arendt, 2000; Purohit et al., 2011).

Several possibilities may underlie the Tat expression-mediated increase in DA release in the CPu of Dox-treated iTat-tg mice. One possibility is that Tat expression may induce a compensatory redistribution of vesicular monoamine transporter-2 (VMAT-2) in cytosolic-enriched fraction to plasma membrane enriched fraction, which results in more vesicles ready for release. Previously, we reported that exposure of rat synaptosomes to Tat decreases DAT-mediated DA transport and induces DAT redistribution from the plasma membrane fraction to the vesicular-enriched fraction with a reduction of vesicular DA uptake through VMAT2 (Midde et al., 2012). Given that DA D2 receptor activity is preferentially vulnerable to HIV-1 viral proteins (Gaskill et al., 2009; Schier et al., 2017), another possibility is that Tat may increase synaptic DA release by deactivating presynaptic D2 autoreceptors because the striatal D2 receptor-expressing medium spiny neurons were found to be disrupted in iTat-tg mice (Barbour et al., 2021). Together, these findings suggest that Tat expression may, however, affect DA release through a variety of bidirectional effects on DA neurons. Nevertheless, investigating these potential mechanisms will be interesting topics for future studies.

We demonstrate here that Tat expression increases phasic but not tonic stimulated DA release in the CPu of Dox-treated iTat-tg mice, suggesting an excitatory effect of Tat on DA transmission. Previous ex vivo studies have shown that phasic stimulation produces larger DA release than tonic stimulation (Zhang et al., 2009), which then activates autoreceptors (Bello et al., 2011). Mouse striatal dopaminergic D1 or D2 medium spiny neurons are modulated by GABAergic and cholinergic interneurons (Boccalaro et al., 2019). Further, deactivation of GABAergic neurons increases phasic but not tonic stimulated DA release (Rice et al., 2011). The Tat protein has been shown to decrease GABAergic activity in mouse prefrontal cortex slices by whole-cell recordings (Xu et al., 2016). Thus, another explanation for the Tat-induced increases in DA release under phasic stimulation could be the deactivation of GABAergic inhibition of DA neurons.

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Another key finding in this study is that Tat-induced inhibition of DAT-mediated DA uptake is not responsible for the increased phasic-like DA release observed in Dox-treated iTat-tg mice. Although the absolute phasic DA release levels in response to nomifensine in Dox-treated iTat-tg mice are greater than those observed in saline control mice, no significant difference was found between Dox- and saline-treated groups when the data were normalized to baseline DA release. We have demonstrated that the Tat protein acts as a negative allosteric modulator by keeping DAT in an outward-facing conformation state (Zhu et al., 2011; Yuan et al., 2015), thereby reducing DAT-mediated DA transport into cells expressing human DAT (Midde et al., 2013; Midde et al., 2015; Quizon et al., 2016) and in the prefrontal cortex of iTat-tg mice (Strauss et al., 2020). Nomifensine has been shown to impact on DA release dynamics by directly stimulating vesicular DA release in rat striatal slices using FSCV (Hoffman et al., 2016) or inhibiting DAT-mediated DA uptake (Butcher et al., 1991; Adachi et al., 2001; Lee et al., 2001; Garris et al., 2003; Ngo et al., 2017). Therefore, results from this study suggest that Tat-induced increases in baseline DA release are not mediated by DAT but directly reflect Tat-stimulated vesicular DA release. Indeed, our previous studies also show that Tat inhibits VMAT2-mediated DA uptake in isolated mouse brain vesicles with in vitro Tat exposure (Midde et al., 2012) and in Dox-treated iTat-tg mice (unpublished data). Thus, we hypothesize that Tat-induced increases in DA release may be mediated by Tat effects on VMAT2. Exploring whether VMAT2 mediates Tat-induced effects on presynaptic DA release is an important future direction for this work. Interestingly, all FSCV parameters associated with DA uptake, including T_{20}, T_{80}, tau, and full width at half height in baseline and nomifensine-induced DA release, are not changed among the treatment groups.

The most intriguing observation is that systemic administration of SRI-32743 reversed the Tat-induced increase in baseline and nomifensine-mediated phasic-like DA release observed in iTat-tg mice after 7- or 14-day Dox treatment. Our pilot study found that Dox-induced Tat expression only affects phasic-like DA release but not tonic DA release, indicating that Tat does not alter spontaneous firing mode from DA neurons but affects the extra firing activity of DA neurons. Since the current study focuses only on phasic-like DA release, it is not clear whether SRI-32743 alters tonic DA release. Our recent study shows that 50 nM SRI-32743 attenuates Tat-induced inhibition of DAT-mediated DA uptake, while SRI-32743 alone shows no inhibitory effect on baseline DAT-mediated DA uptake (Zhu et al., 2022). Further, our behavior study shows that systemic administration of SRI-32743 (at doses of 1 or 10 mg/kg, i.p.) one hour prior to behavior testing alleviates the Tat-induced potentiation of cocaine CPP observed in iTat-tg mice following a 14-day Dox treatment, while SRI-32743 alone does not alter cocaine-CPP in control mice (Zhu et al., 2022). Thus, these findings suggest that SRI-32743 alone may not affect tonic like DA release. Therefore, systemic administration of SRI-32743 prior to these FSCV and cocaine-CPP experiments may reverse Tat expression-induced dysregulation of the DA system through SRI-32743-induced attenuation of Tat's effects on DA release and cocaine reward. Our ongoing study shows that SRI-32743 in vitro attenuates Tat-induced inhibition of VMAT2-mediated DA uptake in isolated mouse brain vesicles (data not shown). Thus, determining the role of SRI-32743 in Tat-induced dysregulation of vesicular DA release will provide direct insight into its underlying mechanisms and reveal the potential for therapeutic use of novel DAT/VMAT2 allosteric modulators in the context of HAND.

In conclusion, these findings provide new evidence that HIV-1 Tat protein increases extracellular DA levels by stimulating vesicular DA release, which greatly impacts the role of the Tat protein on HIV-1 induced dysregulation of dopaminergic transmission in early HIV infection and development of HAND. The attenuation of the Tat-induced increase in DA release by systemic administration of SRI-32743 provides a biologic basis for developing allosteric modulators that specifically reverse Tat-dysregulated dopaminergic transmission with minimal effect on the physiologic DA system. This study highlights a potential mechanism for addressing Tat-induced dysregulation of dopaminergic transmission and its associated cognitive deficits observed in HIV-1 infected patients.

Acknowledgments

The authors would like to thank Dr. Eric Ramsson (Grand Valley State University, Allendale, MI) for the gift of the T-650 carbon fiber. The authors would also like to thank Dr. Taylor Stowe and Lacey Sexton (Wake Forest University, Winston-Salem, NC) for assistance in assembling the voltammetry equipment and providing training.

Authorship Contributions

Participated in research design: Davis, Ferris, Zhu.

Conducted experiments: Davis.

Contributed new reagents or analytic tools: Ananthan, Augelli-Szafran.

Performed data analysis: Davis, Zhu.

Wrote or contributed to the writing of the manuscript: Davis, Ferris, Ananthan, Augelli-Szafran, Zhu.

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