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HIV-1 Protein Tat Inhibits Vesicular Monoamine Transporter-2 Activity in Rat Striatum

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INTRODUCTION

HIV-1 neurodegeneration is often associated with symptoms of motor dysfunction (Navia et al., 1986). The basal ganglia, which are critical for the execution of voluntary motor tasks, may be a prominent target of HIV-1 in the brain (Nath et al., 2000). The HIV-1 transactivator of transcription protein (Tat) has been shown to play an active role in HIV-1-induced neurodegeneration (Theodore et al., 2007). In addition to the widely known neurotoxic actions of Tat, recent evidence has uncovered a role for Tat in altering normal function of the dopamine (DA) transporter, which is essential for reuptake of DA released into the cytoplasm (Zhu et al., 2009). It is not known whether Tat has any effects on the function of vesicular monoamine transporter-2 (VMAT-2) that is involved in sequestering DA within the synaptic vesicles. Inhibition of VMAT-2 may be of potential significance in that accumulation of cytosolic DA, which could auto-oxidize, could cause an increase in free radical-mediated injury to the terminal. In this study, therefore, we administered recombinant Tat¹⁻⁷² into rat striatum and examined the ability of Tat to inhibit VMAT-2 and alter the sequestration of DA within the vesicles.

We first examined the effect of Tat on DA uptake into synaptosomal vesicles. For this study, male Sprague Dawley rats ($n = 6$ per group) received either intrastriatal injections of Tat¹⁻⁷² (20 μ g) or vehicle (normal saline) into both the left and right striatum. Animals were sacrificed 24 h later, and a 4 mm block of striatum containing the injection site was removed from both the left and right striatum and pooled for each animal to prepare sufficient quantity of synaptic vesicles. Vesicles were incubated in the presence of varying

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concentrations (0.03–1.0 μM) of [^3H]DA isotopically diluted with nonradioactive DA, and the amount of [^3H]DA uptake determined as previously described (Teng et al., 1997). The VMAT-2 inhibitor RO 004-1284 was used at 10 μM to determine nonspecific binding at each of the four concentrations of DA. At the lowest DA concentrations, there was no difference in the amount of DA uptake in vesicles prepared from Tat-treated animals versus vehicle-injected animals (Fig. 1A). In contrast, there was an almost 35% reduction in DA uptake when vesicles obtained from Tat-injected rats were incubated in the presence of the highest (i.e., saturating) concentration of DA (Fig. 1A).

Based on the previous findings, we hypothesized that the inhibition of VMAT-2 by Tat would result in lowered storage of DA within the vesicles. To test this, we used in vivo microdialysis using a previously published procedure (Cass et al., 2003) and measured K^+ -evoked DA release in animals exposed to Tat. Because K^+ stimulates the release of vesicular DA by exocytosis, we predicted that there would be a decrease in the levels of evoked DA release in Tat-treated striatum. Rats ($n = 8$) received injections of Tat (20 μg) into the right striatum and vehicle in the left striatum. Twenty-four hours later, using the identical stereotaxic coordinates, dialysis probes were placed in the left and right striata and perfused with artificial cerebrospinal fluid. Following a 60 min equilibration period, animals were treated with nomifensine to mitigate any confounding influence that might result from Tat interactions with the DA transporter. Following a 20 min pulse of potassium chloride (100 mM) through the dialysis probes, there was an approximately ninefold increase in peak synaptic DA levels in the vehicle (control) side and this was reduced by 40% in the side injected 24 h earlier with Tat (Fig. 1B). When the areas under the curves were calculated, the overall decrease in total DA overflow in Tat-injected striata was ~30% versus vehicle-injected striata (Fig. 1C). The above data collectively suggest the ability of HIV-1 Tat to reduce the sequestration of DA within the synaptic vesicles, which could potentially result in elevated cytosolic levels of DA.

The above findings are significant in that the structures targeted by HIV-1 Tat are precisely those in which the sympathomimetic methamphetamine has its greatest effects. In fact, HIV-1 infected patients that also abuse psychostimulants present clinically with a more severe neurodegenerative condition (Bouwman et al., 1998). In experimental models, exposure of cells in culture or rats to both methamphetamine and HIV-1 Tat results in a synergistic neurotoxicity to the dopaminergic system evidenced by degeneration of DA terminals, increased cytokine production, increased oxidative stress, and loss of DA in striatum (Theodore et al., 2006a,b). The observation that the effect of Tat on VMAT-2 uptake was only seen at the highest concentration of DA suggests the possibility that DA uptake into vesicles may be reduced under conditions in which cytosolic DA levels are higher than normal. Because methamphetamine has been shown to elevate cytoplasmic levels of DA, presumably by inhibition of VMAT-2 function (Brown et al., 2000), the net result in HIV-1 infected-methamphetamine abusing patients could be the generation of pathologically elevated levels of cytosolic DA that could lead to increased free radical levels within the terminals that in turn may lead to more severe neurodegeneration in this patient population compared with HIV-1 infected individuals that do not abuse methamphetamine. It is important to point out that Tat has also been demonstrated to inhibit DA transporter

function (Zhu et al., 2009), which may have contributed to the reduction in K⁺-evoked DA release. Thus, there is likely to be a complex interplay between these two processes that require further investigation.

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Fig. 1.

A: Tat inhibits VMAT-2 function. Specific DA uptake (pmol/min/mg protein) by VMAT-2 into vesicles prepared from control (filled bars) and Tat-treated animals (open bars), ($n = 6/\text{group}$). At lower DA concentrations, there was no difference in the amount of uptake in vesicles prepared from the striata of Tat-treated animals versus vehicle-injected animals. In contrast, there was an almost 35% reduction in DA uptake when vesicles obtained from Tat-injected rats were incubated in the presence of the highest (i.e., saturating) concentration of DA (* $p < 0.05$, Student's t -test). **B and C:** Tat alters K^+ -evoked release of striatal DA. **B:**

Eight rats were injected with Tat in the right and vehicle in the left striatum and 24 h later, baseline and K⁺-evoked release of DA measured in each side. Peak evoked DA release is reduced ~40% in Tat-injected animals (thin line) versus control striatum (thick line; * $p < 0.001$ Student's t -test). C: Overall DA overflow is reduced by ~30% in Tat-injected (open bars) versus saline injected striatum (filled bars; * $p < 0.05$ Student's t -test).