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Allosteric modulatory effects on HIV-1 Tat protein-induced inhibition of human dopamine transporter function

J Zhu
University of South Carolina, USA

The inducible HIV-1 Tat Transgenic (iTat) mouse model recapitulates many aspects of neurocognitive impairments observed in HIV infected individuals. Tat and cocaine synergistically increase synaptic Dopamine (DA) levels by directly inhibiting DA Transporter (DAT) activity, ultimately leading to dopaminergic neuron damage. This study determined allosteric modulatory effects of SRI-30827 on HIV-1 Tat protein-mediated regulation of human DAT and Cocaine Condition Place Preference (CPP) in iTat mice. Results show that SRI-30827 attenuated Tat-induced inhibition of [3H]DA uptake and [3H] WIN35,428 binding in PC12 cells expressing human DAT. After a 7-d doxycycline (Dox) treatment, HPLC analysis revealed that DA content in the Prefrontal Cortex (PFC) and Nucleus accumbens (NAc) of iTat-Tg mice were increased by 92% and 37%, respectively, compared to control mice. Consistently, DA/DOPAC in the PFC and NAc of iTat-Tg mice was increased by 44% and 26%, respectively. We performed the patch clamp recording to measure Medium Spine Neurons (MSN) firing in brain NAc slices of iTat mice in the presence of DA and cocaine. Results show that that action potential frequency of NAc shell MSN was significantly increased in iTat mice compared to control mice. Further, action potential frequency of NAc shell neurons was decreased in response to 5 μM cocaine and further decreased when cocaine and 5 μM were applied together, which were completely attenuated in iTat mice. Finally, we found that ICV infusion of SRI-30827, a novel allosteric modulator, partially attenuated the potentiated cocaine-CPP in iTat mice. These findings suggest the hypothesis that Tat potentiates cocaine rewarding effect and allosteric modulator has potential for treatment of Tat-induced drug reward.

Dopamine transporter (DAT) is the target of cocaine and HIV-1 transactivator of transcription (Tat) protein. Identifying allosteric modulatory molecules with potential attenuation of cocaine and Tat binding to DAT are of great scientific and clinical interest. We demonstrated that tyrosine 470 and 88 act as functional recognition residues in human DAT (hDAT) for Tat-induced inhibition of DA transport and transporter conformational transitions. Here we investigated the allosteric modulatory effects of two allosteric ligands, SRI-20041 and SRI-30827 on cocaine binding on wild type (WT) hDAT, Y470 H and Y88 F mutants. Effect of SRI-30827 on Tat-induced inhibition of [3H]WIN35,428 binding was also determined. Compared to a competitive DAT inhibitor indatraline, both SRI-compounds displayed a similar decrease (30%) in IC50 for inhibition of [3H]DA uptake by cocaine in WT hDAT. The addition of SRI-20041 or SRI-30827 following cocaine slowed the dissociation rate of [3H]WIN35,428 binding in WT hDAT relative to cocaine alone. Moreover, Y470H and Y88F hDAT potentiate the inhibitory effect of cocaine on DA uptake and attenuate the effects of SRI-compounds on cocaine-mediated dissociation rate. SRI-30827 attenuated Tat-induced inhibition of [3H]WIN35,428 binding. These observations demonstrate that tyrosine 470 and 88 are critical for allosteric modulatory effects of SRI-compounds on the interaction of cocaine with hDAT.

The current study reports the allosteric modulatory effects of SRI-compounds on cocaine and Tat binding to hDAT. This study is an extension of our previous work demonstrating that mutations of Y470 and Y88 of hDAT are critical for Tat-induced inhibition of DA transport and conformational transporter transitions. There are three major findings from the current study. First, using indatraline to assess competitive DAT inhibition, we found that the SRI-compounds and indatraline produce a similar increase in the IC50 value of inhibition of [3H]DA uptake by cocaine in WT hDAT; however, the SRI-compounds-induced change in the IC50 of cocaine is attenuated in Y470H and Y88F. Second, using SRI-compounds to assess allosteric modulation of DAT function, the addition of either SRI-20041 or SRI-30827 following the addition of cocaine significantly slowed the dissociation rate of [3H]WIN35,428 binding compared to cocaine alone, which was totally and partially blocked in Y470H and Y88F, respectively. Third, SRI-30827 attenuates Tat-induced inhibition of [3H]WIN35,827 binding in WT hDAT. The significance of the current observations is two-fold: 1) this is the first report on the key residues responsible for the allosteric modulatory effects on cocaine and Tat binding to hDAT, and 2) the Y470 and Y88 may be related to the allosteric modulatory effects for the SRI-compounds and the Tat binding to hDAT. These findings provide a novel mechanistic basis for developing compounds that specifically attenuate cocaine and Tat binding site(s) in hDAT to normalize DA transmission to physiological levels in HIV-infected cocaine-using patients.In conclusion, we have identified that Y470 and Y88 may act as allosteric modulatory sites on hDAT responsible for the effects of cocaine binding displayed by the two allosteric ligands, SRI-20041 and SRI-30827. Given that mutation of these two residues (Y470H and Y88F) attenuates Tat-induced inhibition of DAT function44, 47, our current study also demonstrates an allosteric modulatory effect of SRI-30827 on attenuation of Tat-induced inhibition of DAT binding sites. These findings presented herein raise the exciting possibility of potential therapeutic intervention for HIV infected patients with concurrent cocaine abuse. Proof of this concept could emerge from efforts directed toward discovery and development of candidate in vivo probe molecules with the desired allosteric modulation profiles coupled with favorable drug-like attributes.

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