Effects of HIV-1 Tat protein on structure, function and trafficking of the dopamine transporter

Narasimha M. Midde

The alarming rise in HIV-1 associated neurocognitive disorder (HAND) is, at least in part, associated with HIV-1 viral proteins shed from infected macrophages, including transactivator of transcription (Tat) despite the success of anti-retroviral therapies. The dopamine (DA) system is greatly involved in the progression of HAND and is influenced by psychostimulants like cocaine. The DA transporter (DAT), a key regulator of neurocognitive functions, is a major molecular target for both Tat and cocaine. Our lab previously reported that exposure to Tat decreases DA uptake through allosteric regulation and alters cocaine binding sites in DAT.

In this research project the hypothesis ‘HIV-1 Tat protein via allosteric modulation of DAT induces inhibition of DA transport, leading to dysfunction of the DA system’ was tested. Initially, it was shown that Tat protein directly interacts with DAT to impair DA translocation. Based on the predictions of computational modeling and simulations, Y470, Y88 and K92 residues of the human DAT (hDAT) are essential to stabilize the compact structure of DAT and potentially recognize Tat. Mutating these residues in hDAT – Y470H, Y88F, and K92M attenuated Tat-induced inhibition of DA uptake. Additional substitutions Y470A and Y470F at 470 displayed attenuated or no effect on Tat-induced inhibition of DA uptake respectively, indicating the significant role of aromatic ring of Y470 in DAT and Tat interaction. Pharmacological characterization showed that compared to wild type hDAT, Y470H and K92M but not Y88F reduce V\text{max} with no change in the K\text{m} values for DA uptake. Moreover, Y470H, K92M, and Y88F mutants exhibited no alterations in IC\text{50} values of DA to inhibit [3H]DA uptake but increased [3H]DA uptake potency or [3H]WIN35,428 binding potency for cocaine and GBR12909, suggesting that these three Tat-recognition residues do not overlap with substrate DA binding but influence binding of small molecule inhibitors. Besides, all five mutants reversed zinc-induced increase of [3H]WIN35,428 binding and differentially altered basal DA efflux properties of the DAT, indicating that Tat protein through interaction at these recognition residues disrupts intermolecular interactions that are critical for maintenance of the outward-facing conformation of DAT.

Another study was conducted to determine the effects of Tat on DAT phosphorylation, trafficking and its influence on sequestration of [3H]DA by vesicular monoamine transporter 2 (VMAT2). We found that protein kinase C (PKC) inhibitor, bisindolylmaleimide-I eliminates Tat effects on DA uptake and Tat increases intracellular DAT immunoreactivity. Moreover, Tat also produced inhibitory effects on VMAT2 function. Collectively, these findings revealed that Tat inhibits DAT function through PKC and trafficking- dependent mechanisms; and both DAT and VMAT2 proteins may involve in Tat-induced dysregulation of the DA system.

In conclusion, Tat inhibits DA translocation process principally by altering the conformational states of the DAT through interaction at specific recognition residues. Furthermore, regulatory pathways that control the functional attributes of DAT may play a vital role in Tat-mediated impairment of the DA system. Future studies will be necessary to identify and characterize other recognition residues for Tat binding and these molecular insights will be helpful to develop adjunctive therapies to restore the impaired DA system in HIV-1 positive individuals.