Identification of Critical Residues in the Carboxy Terminus of the Dopamine Transporter Involved in the G Protein βγ-Induced Dopamine Efflux

Pino, J.A.
Nuñez-Vivanco, G.
Hidalgo, G.
Reyes Parada, M.
Khoshbouei, H.
Torres, G.E.

Abstract
The dopamine transporter (DAT) plays a crucial role in the regulation of brain dopamine (DA) homeostasis through the re-uptake of DA back into the presynaptic terminal. In addition to re-uptake, DAT is also able to release DA through a process referred to as DAT-mediated DA efflux. This is the mechanism by which potent and highly addictive psychostimulants, such as amphetamine (AMPH) and its analogues, increase extracellular DA levels in motivational and reward areas of the brain. Recently, we discovered that G protein βγ subunits (Gβγ) binds to the DAT, and that activation of Gβγ results in DAT-mediated efflux - a similar mechanism as AMPH. Previously, we have shown that Gβγ binds directly to a stretch of 15 residues within the intracellular carboxy terminus of DAT (residues 582–596). Additionally, a TAT peptide containing residues 582 to 596 of DAT was able to block the Gβγ-induced DA efflux through DAT. Here, we use a combination of computational biology, mutagenesis, biochemical, and functional assays to identify the amino acid residues within the 582–596 sequence of the DAT carboxy terminus involved in the DAT-Gβγ interaction and Gβγ-induced DA efflux. Our in-silico protein-protein docking analysis predicted the importance of F587 and R588 residues in a network of interactions with residues in Gβγ. In addition, we observed that mutating R588 to alanine residue resulted in a mutant DAT which exhibited attenuated DA efflux induced by Gβγ activation. We demonstrate that R588, and to a lesser extent F5837, located within the carboxy terminus of DAT play a critical role in the DAT-Gβγ physical interaction and promotion of DA efflux. These results identify a potential new pharmacological target for the treatment of neuropsychiatric conditions in which DAT functionality is implicated including ADHD and substance use disorder.

Author keywords
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