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MEETING ABSTRACT

A2.1

Exocyst-dependent trafficking of the wild-type dopamine transporter (DAT) and folding-deficient DAT mutants

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Background: Uptake through the dopamine transporter (DAT) represents the primary mechanism used to terminate dopaminergic transmission in the brain. Synaptic function depends on the targeting and delivery of a large number of different proteins at the presynaptic membrane, including neurotransmitter transporters, ion channels, anchoring and cell adhesion molecules, and a variety of signal transduction modulators. DAT requires an intact C-terminal PDZ-binding motif to reach the cell surface; the closely related serotonin transporter SERT does not. Previous experiments showed that the PDZ-binding motif of GAT1 engaged the exocyst. The exocyst is a multiprotein complex required by many membrane proteins for delivery to and insertion into the plasma membrane. Here, we tested the hypothesis that DAT requires the exocyst for reaching the cell surface.

Methods: Briefly, the cells were transiently transfected with plasmids encoding DAT, SERT or NET, along with different amounts of the plasmid encoding Exo70; 48 h after transfection, uptake of radio-labelled substrate was determined to quantify surface expression of transporters.

Results: DAT relied on the exocyst to reach the cell surface. Surprisingly, SERT did not require the exocyst complex to reach the cell surface, regardless of whether the experiments were performed in HEK 293 cells (a cell line of fibroblast origin) or in CAD cells. We examined the effects of exocyst components on transporter expression by performing radiolabelled substrate uptake assays in HEK 293 and CAD cells.

Discussion: Exo70 mediates DAT targeting to presynaptic membranes. Identification of proteins as DAT interactors along with the molecular bases and physiological significance of such inter-actions will result in a better understanding of the role that DAT plays in regulating DA homeostasis in the brain.

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