Functional regulation of serotonin transporter by SNARE protein Syntaxin 3

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The serotonin transporter (SERT) is a membrane protein that terminates serotonergic neural transmission. We have found that SKF-10047(SKF), a prototype sigma-1receptor (SigR1) agonist, promoted the membrane trafficking of SERT and SERT C-terminus deleted mutant, retained in the endoplasmic reticulum (ER). Since these effects of SKF on SERT are independent of SigR1 function, cDNA array was performed on AD 293 cells treated with SKF for 24 h. As a result, we identified Syntaxin 3 (STX3), a member of the SNARE proteins, as a target gene for the SKF effects. The purpose of this study was to elucidate whether STX3 could regulate the SERT function.

We used FLAG-SERT-expressing AD293, COS-7 cells and colon cancer-derived Caco-2 cells that endogenously express SERT and STX3. STX3 siRNA and plasmid were transfected by electroporation.

The STX3 knockdown caused the upward band shift of maturely-glycosylated SERT, while it did not affect SERT uptake activity. Immunohistochemical studies revealed that HA-SERT and Myc-DDK-STX3 expressed on COS-7 cells were colocalized mainly in the ER and Golgi apparatus. In Caco-2 cells, the knockdown of STX3 decreased the SERT 5-HT uptake activity. Immunofluorescent staining revealed that both SERT and STX3 were colocalized in the microvillus-like structures. These results suggest that STX3 may have some effect on glycosylation of SERT. Moreover, in Caco-2 cells, the SERT uptake activity is suggested to be upregulated by STX3. Taken together, STX3 may regulate SERT function during SERT membrane trafficking by interacting with SERT.