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Meeting abstract

Importance of the carboxyl terminus for folding and trafficking of the serotonin transporter

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Background

The human serotonin transporter is the plasma membrane Na^+/Cl^--dependent transporter responsible for uptake of serotonin from the synaptic cleft.

Methods

We studied the importance of the carboxyl terminus in folding and trafficking of the serotonin transporter by generation of SERT mutants by site-directed mutagenesis (alanine scanning mutagenesis), transient expression in HEK293 cells, localization by epifluorescence microscopy and confocal laser scanning microscopy, biochemical characterization (binding studies, uptake studies) and test for possible pharmacochaperoning effect of SERT ligands.

Results

Our data show that the mutation in P601G602-AA, R607I608-AA and RII-AAA (Sec24 binding site) causes intracellular retention and abolishes uptake and binding. We could rescue the mutant (RI-AA and RII-AAA) by ibogaine (100 mM) and DMSO (2%) but not with 5-HT (100 mM), imipramine (10 mM) or low temperature (31°C). However, the mutant PG-AA could not be rescued by any of these compounds. We studied the effect of the mutations on mis-folding by expression of our mutants in a bacterial system [1] but we could not use this protocol for SERT, so we turned to co-immunopreciptation of SERT (wild-type and mutant) with calnexin antibody as has been described by Duvernay *et al.* [2], and we could immunopreciptate calnexin (preliminary data).

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References

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