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MEETING ABSTRACT

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The impact of phosphatidylinositol-4,5-bisphosphate (PIP_2) on serotonin transport function

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Background: The serotonin transporter (SERT) plays a key function in the termination of serotonergic neurotransmission. SERT is the main pharmacological target in treating depressive disorders and also a target for various drugs of abuse. Drugs like amphetamine-type stimulants reverse the direction of transport which finally leads to an increased serotonin concentration in the synaptic cleft. Trans-membrane proteins get in close contact with the lipid environment and are partitioned in specific lipid microdomains. In a previous study we already implicated the phosphatidylinositol PIP_2, a major signaling molecule, to influence amphetamine effects at SERT and elucidated a specific binding interface [1]. We explored a positively charged SERT area using a computational approach and identified a putative second binding site which is close to the inner leaflet of the plasma membrane.

Methods: ***Amino acid exchange*** was done by introduction of single point mutation into a YFP-tagged human SERT (wild-type) construct. ***Uptake and release assays:*** 0.2 µM [^{3}H]5-HT at increasing 5-HT concentrations (1–60 µM) was added for 1 min; 10 µM paroxetine was used to determine nonspecific uptake. 30 µM m-3M3FBS and 30 µM PAO or DMSO respectively 10 µM Pal-peptide were incubated for 20 min at room temperature. Substrate efflux was measured after cells were preloaded with 0.1 µM [^{3}H]MPP+ for 20 min at 37 °C. Cells were then transferred into chambers and a stable baseline was established by superfusion with Krebs-Ringer-Hepes buffer for 40 min. Efflux was induced using 3 µM *para*-chloramphetamine (*p*CA). Two-minute fractions were collected and samples were counted in a beta counter. ***Cell surface biotinylation:*** After 4 h starvation, cells were incubated with sulfo-NHS-SS-biotin (1 mg/ml). Excessive biotin was quenched (100 mM glycine). 100 µg protein was loaded on 30 µl streptavidin-agarose beads. Samples were analysed via western blot. ***Lipid overlay assay:*** PIP strips (Avanti®) were blocked with 3% BSA (fatty acid free) and incubated with protein in TBS o/n at 4 °C.

Results: Manipulating cellular PIP_2 levels had an effect on amphetamine-induced substrate efflux. Neutralizing this positively charged SERT area led to a loss of this effect.

Discussion: We could show that both binding sites are necessary for a stable PIP_2–SERT interaction. Neutralization of positive charges within the binding sites abolished PIP_2 modulation of amphetamine-induced efflux. By drastically reducing intracellular PIP_2 levels we could show a decreased amphetamine-induced efflux in SERT. This effect could not be observed in mutant SERT, indicating a loss of PIP_2-mediated effect on substrate efflux. Furthermore we could show that SERT not only interacts with PIP_2 but also with other phosphatidylinositol species. This interaction is almost lost upon neutralization of both binding sites.

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Reference

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