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**MEETING ABSTRACT**

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**Exploiting non-classical pharmacology of monoamine transporters to address multiple disorders associated with transporter conformational states**

Shreyas BHAT, Walter SANDTNER and Michael FREISSMUTH*

Institute of Pharmacology and the Gaston H. Glock Research Laboratories for Exploratory Drug Development, Center of Physiology and Pharmacology, Medical University of Vienna, Austria.

**Background:** Monoamine transporters (MATs) encompass transporters for serotonin (5-HT), dopamine (DA) and norepinephrine (NE) that are expressed in cognate monoaminergic neurons. They shuttle monoamines from the synaptic cleft back into nerve terminals effectively terminating synaptic transmission and ensuring vesicular replenishment. Perturbed MAT functioning leads to advent of several neurological disorders. Most MAT mutations lead to ER-retained transporters and subsequent loss of function. Such mutations in DAT cause an infantile form of parkinsonism/dystonia. MATs also have a rich pharmacology binding to a plethora of exogenous ligands that act as either non-transportable inhibitors (e.g. cocaine) or transportable substrates (e.g. amphetamines). These drugs increase extracellular monoamine levels by inhibiting MAT function. While MAT ligands have been used as medications for certain psychiatric disorders, most are abused as recreational psychostimulants often leading to addictive disorders. Recent discovery of ligands that display atypical pharmacology at MATs has been a subject of intense research for treatment against addiction. These include atypical inhibitors and partial substrates whose mechanisms of action are unknown, but are assumed to stabilize certain transporter conformational states distinct from those on binding of cocaine and standard amphetamines. We hypothesize stabilization of these unique conformational states also confer atypical MAT ligands the ability to rescue ER-retained MAT mutants.

**Methods:** We used electrophysiological recordings to probe MAT conformational states stabilised by PAL-1045, a naphthyl propan-2-amine that was shown earlier to possess partial efficacy in inducing neurotransmitter efflux through SERT and DAT when compared to standard amphetamines. Using whole-cell patch clamping of HEK293 cells stably expressing hSERT, we compared current profiles, under constant voltage, elicited by PAL-1045 in comparison to those elicited by 5-HT and a standard amphetamine para-chloroamphetamine (pCA). Binding kinetics of these substrates was also determined using electrophysiological means, and these rates were compared to those determined by standard radiotracer assays. Pharmacochaperoning abilities of PAL-1045 were tested on a SERT mutant (SERT-PG601,602AA) using a combined immunoblotting, functional uptake assays and confocal microscopy approach.

**Results:** Under physiological conditions, PAL-1045 induced currents that showed a bell-shaped concentration–response curve over a range of 0.3–30 µM as opposed to Michaelis-Menten current profiles for 5-HT and pCA. This is indicative of internal PAL accumulation on membrane diffusion and high-affinity binding to inward-open state of SERT. Binding kinetics determined by electrophysiological recordings to outward-open SERT were in excellent agreement with those determined by radiotracer assays. Both readouts indicated low nM affinities for PAL-1045 governed by poor dissociation rates. 5-HT and pCA, on the other hand, show µM affinities. Owing to high-affinity binding to multiple conformational SERT states, PAL-1045 restored surface expression and transporter activity of non-functional folding-deficient mutant SERT-PG601,602AA.

**Discussion:** The present study aims to unify anti-addictive properties of certain drugs with their previously unknown pharmacochaperoning capabilities. This hypothesis is currently being extended to rescuing of all documented DAT variants associated with infantile parkinsonism/dystonia expressed in heterologous systems with > 50 atypical DAT ligands.

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*Corresponding author e-mail: michael.freissmuth@meduniwien.ac.at