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MEETING ABSTRACT

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Elucidating the mechanism for low-efficacy substrate efflux at the human serotonin transporter

Shreyas BHAT, Peter S. HASENHÜTL, Walter SANDTNER and Michael FREISSMUTH*

Institute of Pharmacology, Center of Physiology and Pharmacology, Medical University of Vienna, Austria

Background: Amphetamines and their congeners induce serotonin and dopamine efflux from presynaptic neurons through the serotonin transporter (SERT) and the dopamine transporter (DAT), respectively. Pathological consumption of amphetamine leads to drug abuse and addiction. In an attempt to design medications with low abuse potential, some compounds belonging to phenethylamine library (PAL) were recently synthesized exhibiting low efficacy in inducing neurotransmitter efflux through SERT and DAT as compared to amphetamines, though mechanistic explanation for partial efflux is unknown. We hypothesize that these 'partial releasers' trap SERT and DAT in specific conformational states, a question we address using an electrophysiological approach to investigate the effect of PAL compounds on the transport cycle of SERT.

Methods: Conformational changes in SERT and DAT on substrate binding can be inferred from analysis of substrate-induced currents carried through the transporters. These currents through SERT are comprised of two components: (i) peak currents reflecting substrate-induced charge movement, and (ii) steady-state currents indicating Na^+-conducting state associated with K^+-bound inward-facing conformation visited by the transporter during transport cycle. These currents were measured by whole-cell patch clamping of HEK 293 cells stably expressing human SERT. Currents induced by PAL-1045 (a partial releaser for SERT and DAT) were compared to currents induced by serotonin (5-HT) and the 'complete releasers' PAL-287, PAL-1046 and *para*-chloroamphetamine (pCA).

Results: The amplitudes of steady-state currents through SERT decreased with application of increasing concentrations of PAL-287, PAL-1045 and PAL-1046, indicative of a biphasic concentration response. This suggests that PALs readily diffuse through the cell plasma membrane and display high affinity to both the outward- and inward-facing conformation of SERT. This was further confirmed when these steady-state currents were rescued by lowering the pH of the external bath solution from 7.4 to 5.5, a condition that decreases membrane diffusibility of PALs. Substrate-induced peak currents under different internal pipette conditions were used as kinetic read-outs for on- and off-rates of the PALs from the outward-open conformation of the transporters. Subsequent data from these experiments point to the partial releaser PAL-1045 having comparable on-rates to, but considerably slower off-rates from, the outward-open conformation of SERT when compared to the complete releasers PAL-287 and PAL-1046.

Discussion: In their unprotonated state, PAL compounds readily diffuse through the membrane and bind with high affinity to both the inward- and outward-facing conformation of SERT. Tight binding of the partial releaser PAL-1045 to SERT, owing to fast on-rates and slow off-rates, slows down the transport cycle and precludes 5-HT binding to transporters in the inward-open conformation. This accounts for a reduction in substrate efflux and partial release.

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