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MEETING ABSTRACT

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Mechanism of low-efficacy substrate efflux at the human serotonin transporter

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

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

Background: The dopamine transporter (DAT) and the serotonin transporter (SERT) terminate dopaminergic and serotoninergic synaptic transmission by reuptake of their cognate neurotransmitters from the synaptic cleft. Mutations in SERT and DAT lead to their misfolding and ER retention. This is of clinical relevance: several mutations have been identified in DAT, which give rise to a syndrome of infantile/juvenile dystonia and Parkinsonism. Misfolding of proteins can be rescued by their cognate ligands, provided that they act as scaffolding molecules and assist in proper protein folding by lowering the energy barrier between folding intermediates. Compounds, which exert this action, are referred to as pharmacochaperones. For SERT, the folding trajectory is thought to proceed through the inward-facing conformation. SERT and DAT have a rich pharmacology, because they are also important targets for illicit substances derived from amphetamine and cathinone. We tapped into a phenethylamine library of compounds (PAL) to search for low efficacy in inducing neurotransmitter efflux through SERT and DAT when compared to amphetamines. This indicates that the compounds trap SERT and DAT in a conformational state in the transport cycle. Thus, these compounds are of interest as candidate pharmacochaperones: they are predicted to rescue folding-deficient SERT and DAT mutants, if this state is visited during the folding trajectory. Thus, the aim of our study was to identify the conformational state, to which PAL compounds bind, by analysing their effects on the transport cycle.
Methods: Substrate translocation through neurotransmitter transporters require a series of conformational changes which can be inferred from electrophysiological analysis of substrate-induced currents that are carried through the transporter: the peak current reflects substrate-induced charge movement; the steady-state current indicates inward-facing conformation visited by the transporter during the conformational cycle. These currents were measured by whole-cell patch clamping of HEK 293 cells stably expressing hSERT. The compound PAL-1045 was studied as an example of a partial releaser for SERT (and DAT) and currents induced by this compound were compared to 5-hydroxytryptamine-induced currents.
Results: Steady-state amplitudes of currents though SERT decreased with increasing concentrations of PAL-1045. This suggests that PAL-1045 readily diffuses through the cell plasma membrane and displays high affinity for the inward-facing conformation of SERT. This was confirmed by increased steady-state amplitudes with increasing concentrations of PAL-1045 when pH of the external solution was lowered from 7.4 to 5.5 decreasing its membrane diffusibility. Slow recovery of 5-HT-induced peak currents on PAL-1045 application and subsequent washout also argues for a longer dwell time of PAL-1045 in its binding site, which precludes intracellular serotonin binding and efflux. Thus, PAL-1045 may be a potential pharmacochaperone for rescue of folding mutants of SERT.
Discussion: Taken together, our observations provide evidence for a mechanism resulting in low-efficacy substrate efflux through SERT in the presence of PAL-1045. The results have implications for the development of low-efficacy releasers as therapeutic agents for addiction therapy and as pharmacochaperones for the treatment of folding mutants in SERT and DAT.
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*Corresponding author e-mail: *michael.freissmuth@meduniwien.ac.at*