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Partial releasers rescue misfolded serotonin transporters by conformational trapping
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Background: Transporters for serotonin (SERT) and dopamine (DAT) display promiscuous binding to a vast array of exogenous ligands. These include naphypyropene-2-amine analogues (PAL series): some of these have been characterised as ‘partial releasers’ because their efficacy in inducing neurotransmitter efflux through SERT and DAT is lower than that of amphetamines. We hypothesize that this occurs due to the ability of partial releasers to trap SERT/DAT in certain conformational states during the transport cycle. This conformational trapping may also favour protein folding, conferring them the ability to rescue function of misfolded transporters. We explored this conjecture by analysing the effect of PAL binding on (i) SERT transport cycle using electrophysiological recordings, and (ii) rescuing surface expression and transport activity of the folding-deficient mutant SERT.601PG602-AA.

Methods: SERT undergoes a series of conformational changes during the transport cycle, which can be inferred from analysing transporter currents. Substrate-induced SERT currents consist of a peak component, which reflects substrate-induced charge movement, and a steady-state component, which represents a conducting state of the transporter associated with completion of the transport cycle. These currents were measured in HEK 293 cells stably expressing human SERT in whole-cell patch clamp recordings. The currents induced by PAL1045 (a partial releaser for SERT/DAT) were compared to those induced by the full releasers PAL287, PAL1046 and para-chloroamphetamine (pCA) with serotonin (5-HT)-induced currents as a reference. Rescue of transporter function by PAL compounds on SERT.601PG602-AA was checked by combining radioactive transport assays with immunoblotting and confocal microscopy.

Results: At physiological pH, all PALs stimulated steady-state currents with a bell-shaped concentration response curve over the range of 0.3–30 µM as opposed to a saturation hyperbola for 5-HT and pCA. This is indicative of internal PAL accumulation and rebinding due to substrate diffusion. This interpretation was confirmed by lowering the extracellular pH to 5.5 during electrophysiological recordings, a manipulation which eliminated the descending limb at high concentrations of PALs. We hypothesized that PAL compounds were ligands, which bound to both outward-open and inward-open states of SERT with high affinity. SERT binding-affinity estimates calculated separately from binding assays and kinetic characterization of substrate-induced peak currents revealed PAL1045 having low nM affinity followed by PAL1046, PAL287 and pCA with increasing IC50s. This high affinity is governed by poor dissociation rates and thus consistent with conformational trapping. Owing to high-affinity binding to multiple conformational SERT states, all 3 PALs restored surface expression and transporter activity of the non-functional folding-deficient mutant SERT.601PG602-AA, with PAL1045 being the most efficacious.

Discussion: PAL1045 acts as a partial releaser and a pharmacochaperone by virtue of its high-affinity binding to SERT. Slow dissociation rates result in longer dwell time at the binding site. This not only precludes intracellular 5-HT binding and subsequent efflux but also facilitates proper folding of the otherwise ER-retained 601PG602-AA mutant form of SERT, leading to restoration of surface expression and transporter function.

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