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INTRINSIC ACTIVITY

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MEETING ABSTRACT

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Elucidating the functional role of extracellular loop 4 in the transport cycle of the serotonin transporter

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Background: The serotonin transporter (SERT), a member of the solute carrier 6 (SLC6) family, is a monoamine transporter that mediates the reuptake of serotonin (5-HT) from the extracellular space. Thus, SERT is the key player in the termination of serotonergic signalling and is involved in the replenishment of synaptic 5-HT stores. SERT is an integral membrane protein comprising 12 transmembrane segments that are linked by 6 extracellular (EL) and 5 intracellular loops (IL). Studies on the functional role of structural motifs mostly rely on site-directed mutagenesis. Structural knockdown by mutagenesis, however, often results in a loss of function as a consequence of impaired protein folding and plasma membrane trafficking. Hence, a tool is required that allows to address the functional role of a structural motif of interest while protein folding and trafficking remains unaffected.

Methods: Whole-cell patch-clamp experiments were performed in human SERT (hSERT) expressing HEK 293 cells. Fast exchange of the bath solution (exchange rate ~100 ms) was ensured by a pressure-driven application device (Octaflow). 5-HT-induced currents were recorded in hSERT-expressing cells in the absence and presence of anti-SERT-EL4 antibody. In addition, ligand-binding (5-HT and anti-SERT-EL4) to SERT was assessed by capacitance measurements.

Results: Binding of anti-SERT-EL4 antibody to SERT reduced the membrane capacitance and blocked the 5-HT transport in a dose-dependent manner. The association and dissociation rate constants (k_{on} and k_{off}) of the antibody were calculated as 3.055 ± 0.631 $M^{-1}s^{-1} \cdot 10^7$ and 1.352 ± 0.082 s^{-1}, respectively.

Discussion: Our data indicate that employing antibodies as tools is a promising approach to study structural motifs by electrophysiological techniques. Blockade of the 5-HT transport upon the restriction of EL4 movement in SERT by antibody binding shows that EL4 has a role in the conformational changes on SERT required for 5-HT transport. Further experiments are planned by producing Fabs from anti-SERT-EL4 antibody to eliminate dimerization possibility.

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