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In vitro and *in silico* studies on zinc interaction with 5-HT$_7$ receptors

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Background: Zinc ions play important functions in the central nervous system (CNS) but many aspects of their action is unclear. In recent years, various aspects of GPCR allosterism were intensively studied, because the interaction with the receptor according to other than classical, competitive model of orthosteric ligand binding, creates new possibilities for its regulation. Our interests are focused on Zn^{2+} effects mediated by serotonin receptors, which are key players in the etiology of anxiety and mood disorders.
Materials and methods: The direct influence of Zn^{2+} on compound binding to human 5-HT$_7$R, stably expressed in HEK293 cells, was investigated by a set of *in vitro* functional cAMP assays and radioligand binding methods (saturation, competition and both association and dissociation kinetic studies) using [^{3}H]5-CT, [^{3}H]SB-269970 and [^{3}H]Mesulergine - an agonist and two antagonists, respectively. The MD simulations were performed on homology models of 5-HT$_7$R, created on the basis of crystal structure of 5-HT$_{1B}$R (pdb: 4IAR). Structures of reference agonist (5-CT) and antagonist (SB-269970) were docked into the model (Glide 5.5) and the ligand-receptor complexes were input for the MD with zinc ions. Simulations sytems were constructed with POPC membrane and TIP3P water model.
Results: Results of both types of *in vitro* experiments demonstrated that Zn^{2+} ions act as negative allosteric modulator (NAM) at 5-HT7 receptors. MD shows the differences in interactions with zinc ions between antagonist- and agonist-bound models of 5-HT$_7$R. In both experiments zinc was contacting with ecl2 (D167 and D168), however in complex with antagonist zinc ion is also observed interacting with D3.32.
Conclusions: Our study extends data regarding the Zn^{2+} action at 5-HT$_7$R. Both *in vitro* and *in silico* studies suggest that zinc can modulate the 5-HT$_7$R.
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