In vitro and *in silico* examination of the effect of Zinc ions on 5-HT7 receptor

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INTRODUCTION

Zinc, as an essential trace element in living organisms, has many functions, including participation in various processes within the central nervous system [1]. The role of zinc in depression and its therapy is emphasized by numerous preclinical and clinical studies, however, the exact mechanism of its action is still not fully understood [2]. Our interests are focused on its effects mediated by serotonin receptors, which are key players in the etiology of anxiety and mood disorders [3].

MATERIALS and METHODS

Chemicals [^{3}H]5-CT (spec. act. 39.2 Ci/mmol), [^{3}H]SB-269970 (62.7 Ci/mol), were purchased from PerkinElmer. Other chemicals were obtained from commercial sources and were of analytical grade.

Biological material Membranes were prepared from human embryonic kidney (HEK) 293 cells stably transfected with human 5-HT$_7$ receptor cDNA.

Ligand binding assays For [^{3}H]5-CT and [^{3}H]SB-269970 bindings crude membrane preparations were incubated in two volumes of assay buffer (0.25 ml) in 37°C for 1 h. Composition of assay buffer was: 50 mM Tris–HCl pH 7.7, 5 mM $MgCl_2$, 0.1 mM pargyline and 0.5 mM ascorbic acid. Non-specific binding was defined with the use of 10 µM serotonin. The incubations were terminated by the rapid filtration through Unifilter GF/B plates (PerkinElmer) and subsequent washing with ice-cold buffer using Unifilter harvester. Scintillation cocktail was added and the radioactivity determined in scintillation MicroBeta counter.

Analysis of in vitro data Analysis of the saturation binding data obtained for the agonist [^{3}H]5-CT and antagonist [^{3}H]SB-269970 using the program GraphPad PRISM, according to equations [5-8]:

1. „Affinity-shift"-1=[A](1-α)/(α[A]+K_A)
2. pK_{DApp}=-log([A]+10^{logKA})+log(α[A]+10^{logKA})-log*d*

where log*d* is a fitting constant; K_A denotes the affinity of zinc for the allosteric site; [A] the Zn^{2+} concentration; α defines the cooperativity factor, the magnitude by which the equilibrium dissociation constant of either ligand for its site on the receptor is modified by the concomitant presence of the other ligand. Values of α less than 1 (but greater than zero) denote negative cooperativity, values greater than 1 denote positive cooperativity, and values not significantly diffirent from 1 indicate neutral cooperativity.

Molecular dynamics 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. Calculations were performed with Desmond GPU 3.9 with MD duration set to 200 ns.

RESULTS

Analysis of saturation isotherms obtained for seven increasing concentrations of zinc (10 µM — 5 mM) **[Fig 1. and Fig 2.]** revealed decrease in radioligand binding (increased K_d values in relation to K_d of [^{3}H]5-CT and [^{3}H]SB-269970 binding without zinc) which achieved plateau at the highest concentration of zinc used (5 mM). The mechanism by which zinc inhibits the binding of radioligands was further evaluated by a Schild-type plot analysis. Data showed a negative cooperativity of zinc, which indicates allostery.

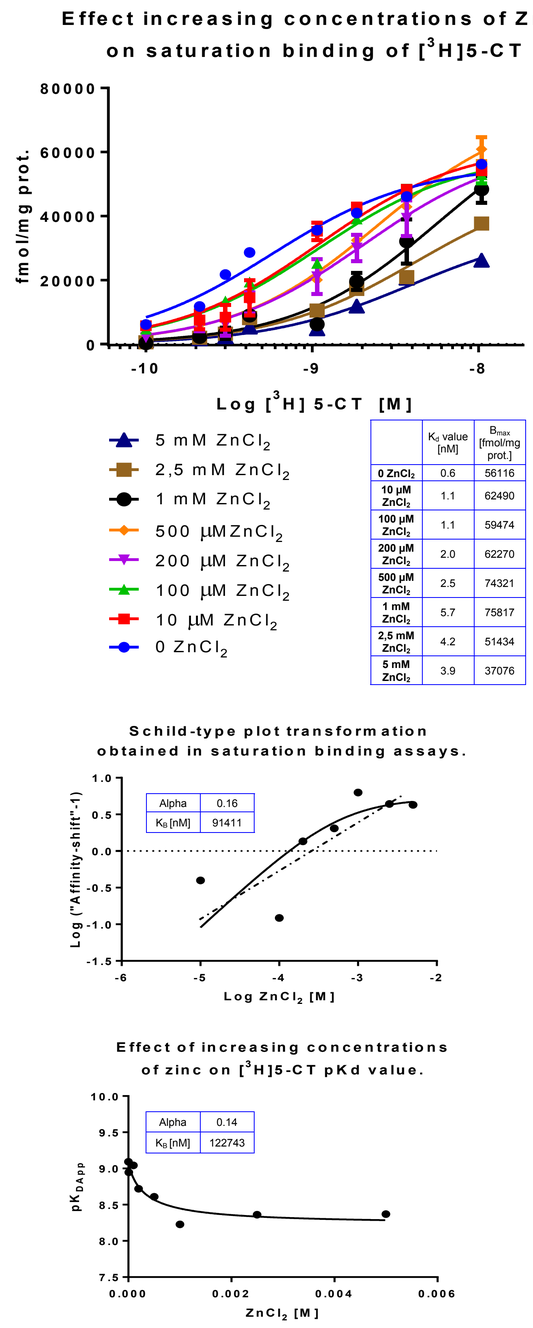
In order to further evaluate the nature of the interaction of Zn^{2+} with the 5-HT$_7$ receptor, dissociation kinetic assays for [^{3}H]5-CT (0,5 nM) in the absence and presence of 500 µM Zn^{2+} were carried out [**Fig 3.**]. The dissociation rate (K_{off}) kinetics for [^{3}H]5-CT binding to the 5-HT$_7$ receptor was assayed at 37°C by addition of serotonin (10 µM) to prevent the reassociation. The presence of 500 µM Zn^{2+} produced a significant increase of the dissociation kinetic rate of [^{3}H]5-CT. The effect of zinc upon the dissociation kinetic rate indicates that the noncompetitive interaction of Zn^{2+} at 5-HT$_7$ receptor may be classified as negative allosteric modulation.

Figure 4 shows results of control experiment, i.e. the screening of other ions on [^{3}H]5-CT binding to 5-HT$_7$ receptor. The specific action of zinc, compared to other ions, is evident.

Figure 5 shows the differences in interactions with Zinc ions between antagonist (left) and agonist (right) bound models of 5-HT$_7$. 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.

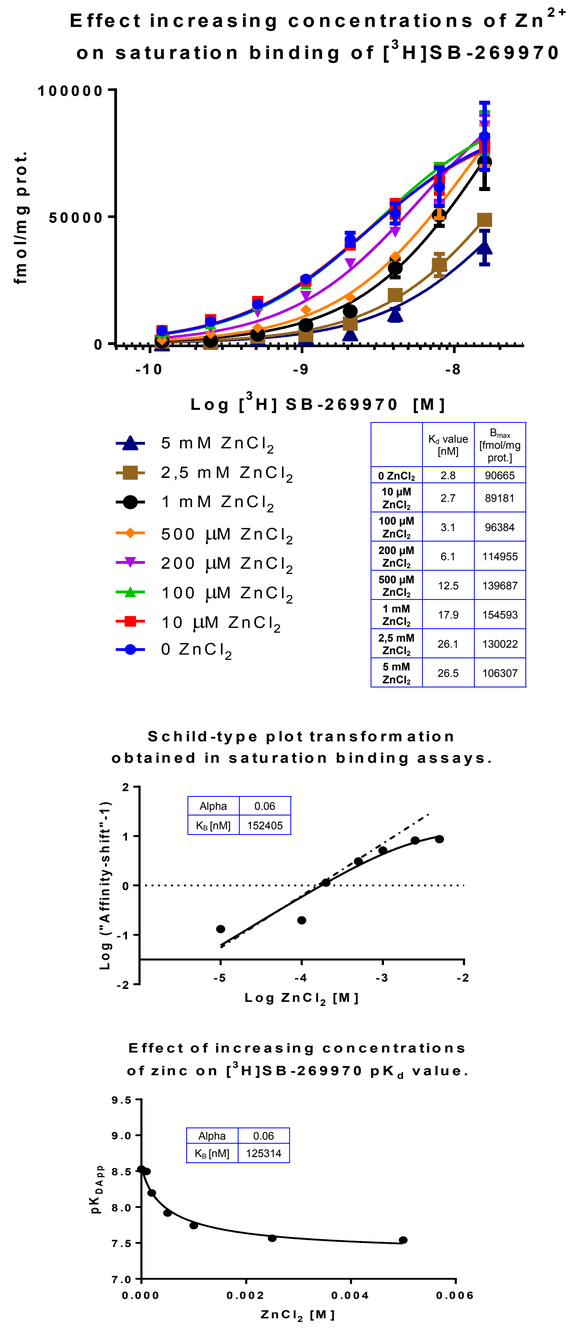
Summing up, the *in vitro* experiments clearly showed that Zn^{2+} ions act as negative allosteric modulator (NAM) at 5-HT$_7$ receptors. *In silico* experiments provide hints on possible mechanism of this effect - Zinc bound to TM3 of the receptor hinders the binding of the agonist, therefore making it less energeticaly favourable.

Figure 1.



	K_d value [nM]	B_{max} [fmol/mg prot.]
0 $ZnCl_2$	0.6	56116
10 µM $ZnCl_2$	1.1	62490
100 µM $ZnCl_2$	1.1	59474
200 µM $ZnCl_2$	2.0	62270
500 µM $ZnCl_2$	2.5	74321
1 mM $ZnCl_2$	5.7	75817
2,5 mM $ZnCl_2$	4.2	51434
5 mM $ZnCl_2$	3.9	37076

Figure 2.



	K_d value [nM]	B_{max} [fmol/mg prot.]
0 $ZnCl_2$	2.8	90665
10 µM $ZnCl_2$	2.7	89181
100 µM $ZnCl_2$	3.1	96384
200 µM $ZnCl_2$	6.1	114955
500 µM $ZnCl_2$	12.5	139687
1 mM $ZnCl_2$	17.9	154593
2,5 mM $ZnCl_2$	26.1	130022
5 mM $ZnCl_2$	26.5	106307

Figure 3.

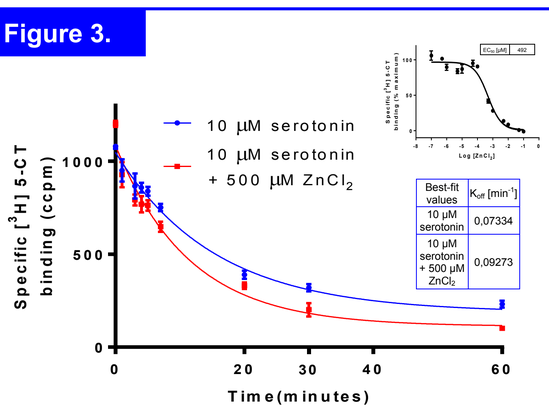


Figure 4.

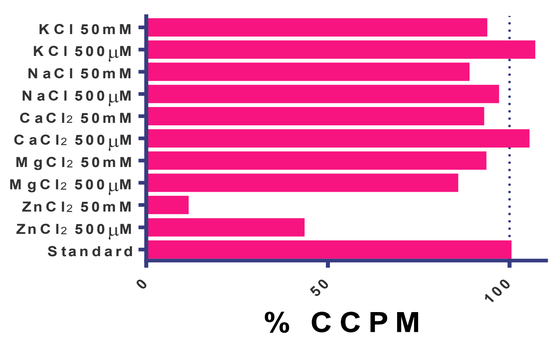
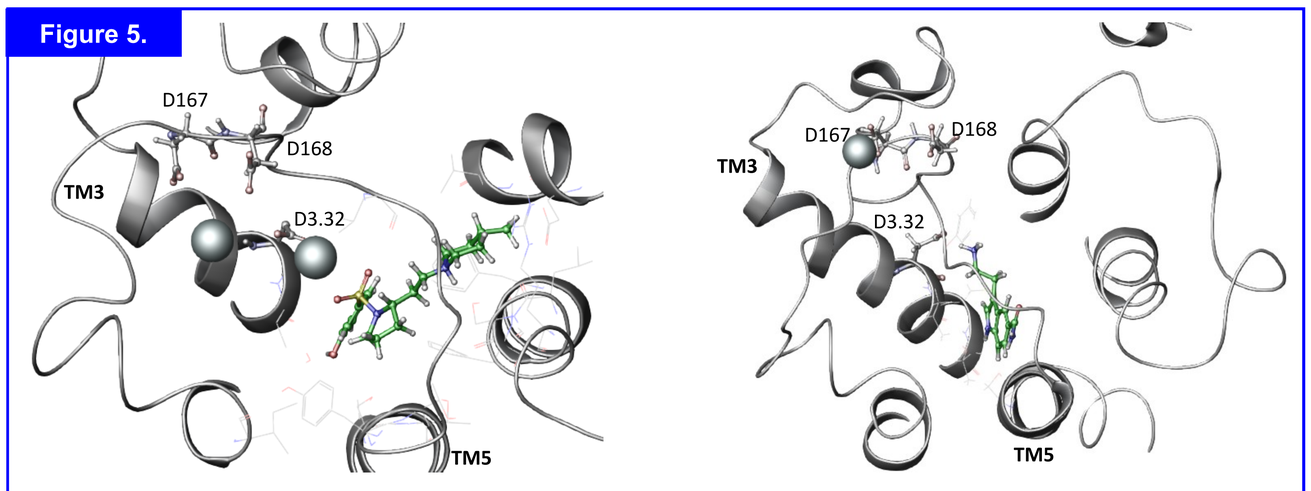


Figure 5.



5-HT$_7$R complexes with antagonist SB-269970 (right) and agonist 5-CT (left) received from MD simulations experiments.

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Acknowledgements:

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