Pharmacological characterization of zinc interaction with 5-HT$_{1A}$

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INTRODUCTION

Zinc, as an essential trace element in living organisms, plays an important role in the number of biological processes, especially within the central nervous system [1]. There is an evidence for the involvement of Zn ions in depression and so constitutes potential angle of therapy, as emphasized by numerous preclinical and clinical trials. However, its exact molecular mechanism of 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].

Here we present radioligand binding assays used to characterization of the pharmacological profile of Zn^{2+} at serotonin 5-HT$_{1A}$ receptor (5-HT$_{1A}$R). The direct influence of Zn^{2+} on agonist binding to human 5-HT$_{1A}$R, stably expressed in HEK293 cells, was investigated by a set of *in vitro* radioligand binding methods (saturation, competition and both association and dissociation kinetic studies) using [^{3}H]8-OH-DPAT, as a selective agonist tool compound.

MATERIALS and METHODS

Chemicals [^{3}H]-8-OH-DPAT (spec. act. 141 Ci/mmol) was 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$_{1A}$ receptor cDNA.

Ligand binding assays The membrane preparations were incubated for 1 h at room temperature in a total reaction volume of 0.25 ml in 96-well microtitre plates with [^{3}H]-8-OH-DPAT as selective 5-HT$_{1A}$R radioligand of agonistic activity. The incubation buffer consisted of 50 mM Tris–HCl pH 7.7, 5 mM $MgCl_2$, 0.2 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/C 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 data Analysis of the saturation binding data obtained for the agonist [^{3}H]-8-OH-DPAT was performed using the program GraphPad PRISM, according to equation [4]:

$$pK_{DApp}=-\log([A]+10^{logK_A})+\log(\alpha[A]+10^{logK_A})-\log d$$

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.

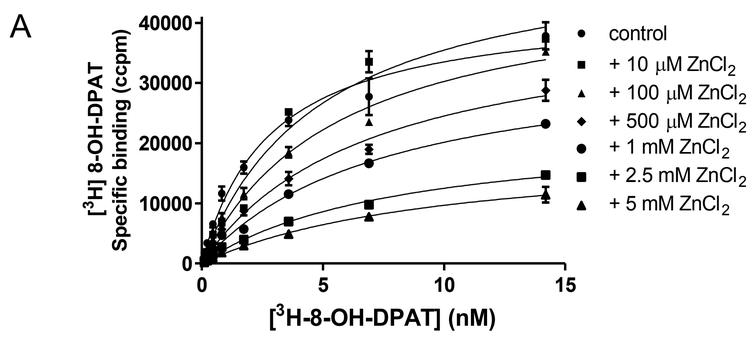
RESULTS

Analysis of saturation isotherms obtained for seven increasing concentrations of zinc (10 µM — 5 mM) **[Fig. 1]** revealed decrease in radioligand binding (increased K_d values in relation to K_d of [^{3}H]-8-OH-DPAT 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. The results of the saturation experiments suggest negative allosteric modulation of Zn on [^{3}H]-8-OH-DPAT binding (α = 0.37). Nevertheless, model of negatively cooperative interactions, does not account for all of the data obtained further in the present study.

At first, Zn tested alone in competition-like assay with [^{3}H]-8-OH-DPAT yielded bell-shaped binding curve, with marked increase in agonist radioligand binding at low modulator concentrations and a decrease in binding at high concentrations **[Fig. 2]**.

Likewise, the association kinetics of [^{3}H]-8-OH-DPAT showed an increase in association rate (k_{on}) at low (10 µM) concentration of Zn ions and the observed curve trend was typical for the presence of positive allosteric modulation of agonist radioligand binding **[Fig. 3]**. Despite the lack of statistically significant effects on k_{on} values at higher Zn concentration (500 µM) there was a tendency of reduction of association rate of [^{3}H]-8-OH-DPAT binding which was visible in the obtained curve with plateau lower than control **[Fig. 3]**. Similarly, results of the dissociation kinetic experiments showed that Zn^{2+} at both concentrations used, significantly slowed radioligand dissociation rates which is also characteristic for positive allosteric modulation **[Fig. 3]**.

Summing up, the *in vitro* experiments clearly showed that in addition to previously reported negative allosteric modulation [5], for both antagonist and agonist binding, Zn^{2+} at low concentration may also potentiate binding of agonist to 5-HT$_{1A}$R.



Zn^{2+} [µM]	K_D [nM]	B_{max} [pM/ mg prot]
0	2.9 ± 0.1	4.5 ± 0.3
10	5.0 ± 0.1	5.8 ± 0.7
100	6.0 ± 0.4	6.2 ± 2.0
500	6.9 ± 0.2	4.7 ± 0.7
1000	8.2 ± 0.9	4.0 ± 0.6
2500	8.6 ± 0.6	3.1 ± 1.2
5000	9.6 ± 0.6	2.5 ± 0.8

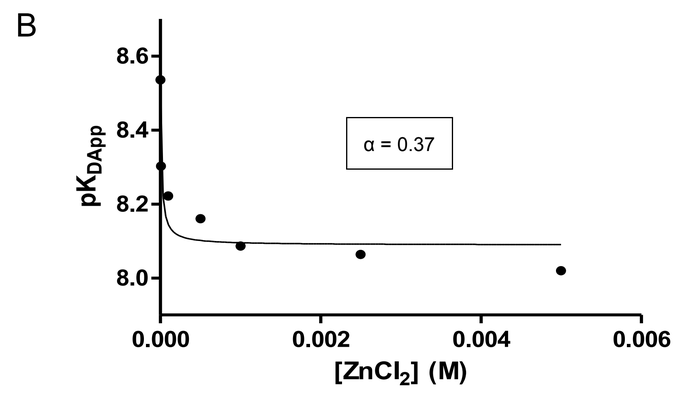


Figure 1. Effect of increasing concentrations of Zn^{2+} on saturation binding of [^{3}H]-8-OH-DPAT in h5-HT$_{1A}$ receptors in HEK293 cells. A: Representative set of radioligand saturation binding curves obtained in the absence and presence of Zn^{2+}; B: Nonlinear regression analysis of the saturation data .

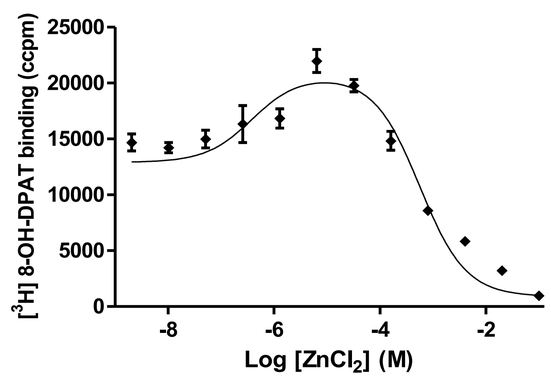


Figure 2. The "bell shaped" zinc titration curve obtained in competition-like experiments with the enhancement (~10 µM of Zn^{2+}) and inhibition (above 100 µM of Zn^{2+}) of [^{3}H]-8-OH-DPAT binding at h5-HT$_{1A}$ receptors.

	k_{on} [$M^{-1}min^{-1}$]	$k_{off\ fast}$ [min^{-1}]	$k_{off\ slow}$ [min^{-1}]
Control	3.4 ± 0.4 x10^8	2.33 ± 0.31	0.21 ± 0.02
+ 10 µM Zn^{2+}	6.4 ± 0.8 x10^8	0.25 ± 0.04 one phase	
+ 500 µM Zn^{2+}	3.6 ± 0.3 x10^8	0.58 ± 0.07	0.06 ± 0.01

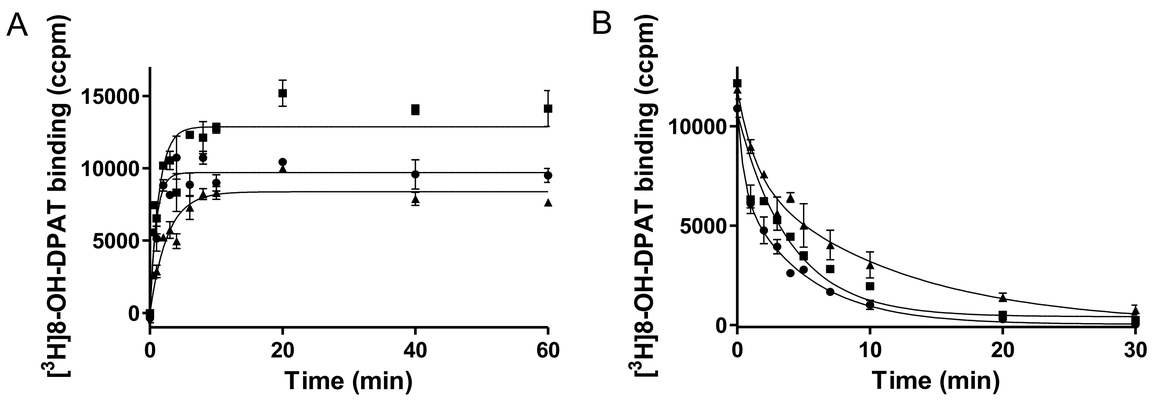


Figure 3. Effect of Zn^{2+} on association (A) and dissociation (B) rates of [^{3}H]-8-OH-DPAT in the absence (●) or presence of 10 µM (■) and 500 µM (▲) of Zn^{2+}.

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Acknowledgements:

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