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DIFFERENT SENSIBILITY OF ECTO-ATPase FROM BRAIN AND OVARY TO Cu^{2+} AND Zn^{2+}

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Abstract

Ions of zinc and cooper represents micro elements present in all organisms but they can be accumulated in various tissues from the environment by food or pollution. In this work we examined the effects of chloride salts of Cu^{2+} and Zn^{2+} on the activity of ecto-ATPase, integral plasma membrane protein, in brain and ovarian cells. Both ions exhibit similar effects on brain or ovarian enzyme activity. Cooper in brain and ovary totally inhibits enzyme activity at the concentration of 1 mM and 0.1 M respectively. IC50 for brain is 36 μM while in ovary it is 192 μM. Zinc inhibits in both tissues 50% of the control enzyme activity at concentrations of 1 mM in brain and 0.1 M in ovary with IC50 of 1mM and 14 mM respectively. These metals possess an affinity for -SH groups in the enzyme protein, may replace Mg^{2+} in enzyme substrate, MgATP or to bind for enzyme substrate site. According to the total inhibition of ecto-ATPase activity by Cu^{2+}, it may be proposed that its site of action is on the substrate or substrate binding site, while Zn^{2+} modulates enzyme activity by acting on the –SH or S-S groups of the enzyme. Inhibiting or decreasing ecto-ATPase activity in brain and ovary, these two metal ions may be toxic and seriously disturb the proper functioning of the investigated tissues.

Introduction

The ecto adenosine triphosphatase (ecto-ATPase, EC 3.6.1.3) is an integral membrane protein that, in the presence of divalent cations (Ca^{2+} or Mg^{2+}), hydrolyses extra cellular nucleosides, since the nucleotide-hydrolysing site is outwardly orientated. By hydrolysing ATP, this enzyme is the major inactivating agent in purine triphosphate signalling. In the central nervous system, as in other tissues, these enzymes have multiple roles. By controlling the concentration of the extra cellular ATP and adenosine, along with 5`-nucleotydase, it influences a large variety of P1 and P2 receptor-mediated processes [1]. In brain as well as ovarian cells, the existence of P2X and P2Y purinoceptors were detected. ATP, as a neurotransmitter and neuromodulator, is stored within vesicles and co-released with neurotransmitters. When released, ATP may modulate the release and influence other neurotransmitters in the brain or influence maturation of ovarian cells and synthesis of gonadal hormones. The specific inhibitor(s) of ecto-ATPase has not been found up to now. In numerous tissues, the activities of ecto-ATPase may be influenced by different endogenous modulators [1] and may be modulated by a variety of agents such are detergents, lectins, ATP-

analogues and drugs [2]. In order to determine if this enzyme may be modulated by micro elements, we tested the effects of Cu^{2+} and Zn^{2+} ions in the rat brain and ovary.

Experimental Procedure

Experiments were performed on 3-month-old (300-350 g) female Wistar albino rats obtained from the local colony. Synaptosomal plasma membranes (SPM) and ovarian plasma membranes (OPM) were isolated from the rat brain and ovary as described previously [3]. The activity of ecto-ATPase was determined by the spectrophotometric method by measuring the inorganic phosphate liberated from the hydrolysis of ATP. SPM (20 μg) or OPM (70 μg) were preincubated at 37° C without or in the presence of increasing concentrations of $CuCl_2$ and $ZnCl_2$ for 20 min in an enzyme assay medium containing (in mM) 50 Tris-HCl, pH 7.4; 5 $MgCl_2$ and 1 EDTA. After incubation, the enzyme reaction was started by the addition of 2 mM ATP, allowed to proceed for 15 min and stopped by the addition of 3 mol/l perchloric acid. The results are expressed as the mean percent of enzyme activity compared to the corresponding control performed in triplicate.

Results and Discusion

Chloride salts of metals were added to the reaction mixture in the concentration range from $1x10^{-7}$ to 1 M. Effects of increasing concentrations of metal salts on SPM as well as on OPM ecto-ATPase activity shows inhibition activity relative to the control samples. Concentrations of metals for 50% of enzyme activity inhibition (IC50) were calculated from the Hill analysis of the experimental results. Cu^{2+} ions exert sigmoidal inhibition of enzyme activity for both membrane preparations (Fig. 1.). According to the IC50, brain ecto-ATPase possesses greater sensibility to this ion than ovarian enzyme (36 μM for brain, 192 μM for ovary). These results imply tissue specifity in abundance of the enzyme, which confirms specific activity of ecto-ATPase in SPM and OPM under control conditions (0.253 μmol Pi/mg/min and 0.130 μmol Pi/mg/min respectively).

Inhibition of ecto-ATPase by Zn^{2+} ions is maximum about 55% in both preparations. In the case of Zn^{2+} inhibition, brain ecto-ATPase possess higher sensibility, like as for Cu^{2+}, with IC50 of 1 mM in comparison to OPM enzyme inhibition with IC50 of 14 mM. According to the Hill coefficient, n, Cu^{2+} exerts no cooperativity (n=1) in binding to the enzyme, while Zn^{2+} ($n<1$) exerts negative cooperativity.

Conclusion

Cu^{2+} and Zn^{2+} are metals from first transition group, they exert similar inhibition in ovarian and brain ecto-ATPase activity, but there is distinction between the action of copper and zinc in the same tissue. Therefore, we may assume that their sites of action are different. These metals possess affinity for –SH is on the substrate or substrate binding site, while Zn^{2+} modulates the enzyme activity by acting on –SH

and/or S-S groups of the enzyme. Inhibiting or decreasing ecto-ATPase activity in the brain and ovary, these two metal ions increase extra cellular ATP, which may seriously disturb proper functioning of the investigated tissues.

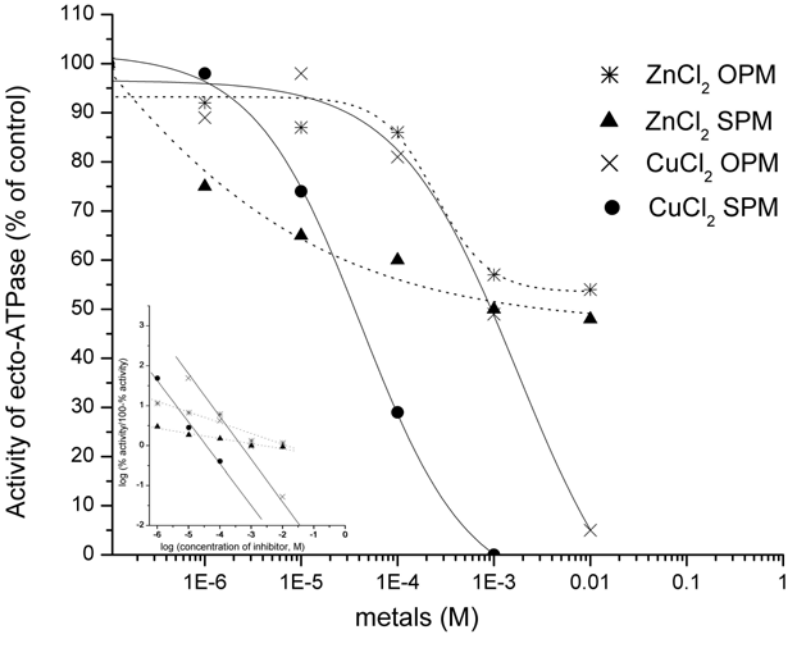

Fig. 1. Inhibition of ecto-ATPase activity from SPM by $CuCl_2$ (•) and $ZnCl_2$ (▲) as well as from OPM by $CuCl_2$ (×) and $ZnCl_2$ (∗) ions. Hill graph presented as inset.

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