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L-CYSTEINE MODULATES THE ecto-ATPase ACTIVITY INHIBITION IN PRESENCE OF CADMIUM (II) AND MERCURY (II) IONS

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Abstract

L-cysteine is used as effective oral chelating agent due to property of its sulfhydryl group to bind heavy metal ions. The aim of this study was to investigate ability of L-cysteine to prevent mercury (II) and cadmium (II) induced ecto-ATPase activity inhibition of rat uterus plasma membranes. Results show that 10 mmol/l L-cysteine have protective effect on enzyme activity in the presence of cadmium and mercury ions.

Introduction

Heavy metals could be accumulated in the body during low, chronic exposure, originate from various sources such as drinking water, cigarette smoke or even from tooth fillings made of silver-mercury amalgam. According to numerous data cadmium and mercury are classified as reproductive toxicants [1] and their effects on uterine plasma membrane ecto-ATPase activity were reported previously [2].

During the years, the importance of purinergic signaling in mammalian reproductive tract has been emphasized [3]. Extracellular nucleotides and/or their degradation products affect via purinoceptors various functions of uterine tissue such as contraction, growth, remodeling and immunity. In uterine tract, ATP is co-released from autonomic nerves with neurotransmitters, but physiological sources of extracellular nucleotides are also platelets and semen. Ecto-ATPase is a member of E-NTPDases family (EC 3.6.1.5), the most abundant enzyme family among ectonucleotidases. Ectonucleotidases are surface plasma membrane bound enzymes that modulate purinergic signaling by hydrolyzing nucleotide di- and tri phosphates.

Experimental procedure

Animals: Experiments were performed on 3-month-old female Wistar albino rats obtained from the local colony. Animals were maintained under standard conditions with *ad libidum* access to food and water. Myometrial plasma membranes (MPM) were isolated as previously described [4].

ATP assay: Incubation medium contained: 50 mmol/l Tris-HCl buffer (pH 7.4), 5 mmol/l $MgCl_2$, increasing concentration of metal ions in absence or presence of 10 mmol/l L-cysteine and 7μg MPM protein. The enzyme reaction was started by addition of 2 mmol/l ATP, allowed to proceed for additional 10 min and stopped with 22μl of the ice cold 3 mol/l perchloric acid. The inorganic phosphate (Pi) liberated from the ATP hydrolysis was determined by the spectrophotometric method. All measurements were performed in triplicate.

Results and discussion

Cadmium chloride ($CdCl_2$) and mercury chloride ($HgCl_2$) were added to the reaction mixture in concentration ranges from $1x10^{-7}$ to $1x10^{-1}$ mol/l. The effects of increasing metal ion concentrations were measured in the absence and in the presence of 10 mmol/l L-cysteine.

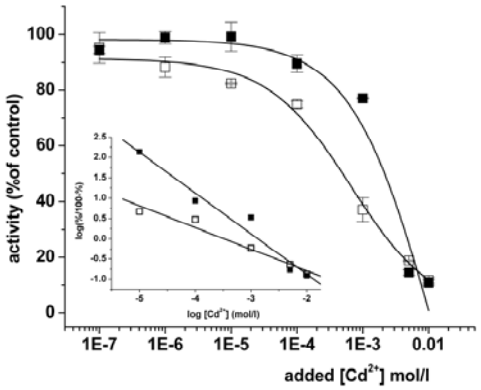

Fig.1. Inhibition of ecto-ATPase activity by $CdCl_2$ in the absence (open symbol) and in the presence (solid symbols) of 1×10^{-2} mol/l L-cysteine.
The values given are the mean ±S.E.M of tree independent MPM isolation done in triplicate.

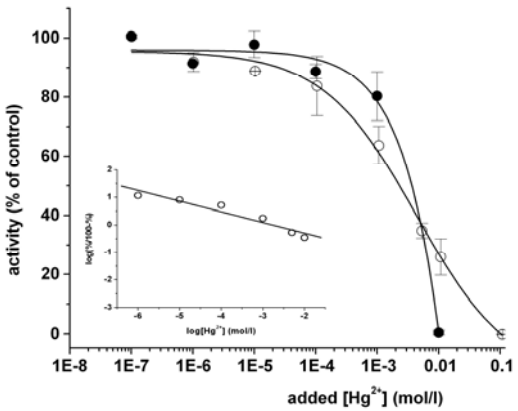

Fig.2. Inhibition of ecto-ATPase activity by $HgCl_2$ in the absence (open symbol) and in the presence (solid symbols) of 1×10^{-2} mol/l L-cysteine.
The values given are the mean ±S.E.M of three independent MPM isolation done in triplicate.

Compared to the control samples, in the absence of L-cysteine and in the presence of 0.01 mol/l of $CdCl_2$ (Fig 1.) ecto-ATPase activity showed up to 90% inhibition, while total enzyme inhibition was detected in presence of 0.1 mol/l $HgCl_2$ (Fig 2.). The half-maximum inhibitory activities (IC_{50}) determined by Hill

analysis of experimental curves in the absence or presence of L-cysteine were: 3.49×10^{-4} and 1.34×10^{-3} mol/l for cadmium (II) (Fig.1 inset) and 1.74×10^{-3} and 2.06×10^{-3} for mercury (II) (Fig.2 inset), respectively. The IC_{50} value for mercury (II) ions in the presence of 10 mmol/l L-cysteine was determined directly from inhibition curve. When cysteine and mercuric chloride mole ratio approached 2:1, white precipitation was formed and enzyme activity disappeared. Precipitation occurs due to formation of $Hg(Cys)_2$ and $Hg(Cys)_2x2HgCl_2$ [5].

L-cysteine is an important constituent of proteins, largely by virtue of its sulfhydryl group to form disulfide bonds and stabilize protein molecular configuration. Cadmium and mercury have high affinity for sulfur and sulfhydryls and therefore potential of binding to proteins throughout the body, disrupting their structure and function. Binding tightly to glutathione (GSH), they might deplete cell's antioxidant capacity. At the same time, cadmium and mercury promote formation of reactive oxygen species that alter plasma membrane structure as a result of lipid peroxides formation.

Ecto-ATPase contains 10 cysteine residues on its extracellular domain but has no free sulpfhidryl groups, so this might explain relatively high IC_{50} value for cadmium and mercury in the absence of chelator.

Conclusion

According to the results presented in this study, 10 mmol/l L-cysteine increased by one order of magnitude $CdCl_2$ concentration for half-maximal inhibition (IC_{50}), as a consequence of decreasing metal ions concentrations capable to interact with enzyme amino acid residues. In the presence of mercuric ions, L-cysteine was less efficient in prevention of enzyme activity, probably because of complex compounds formation at higher concentrations of metal ions.

Acknowledgements

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