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Short communication

SHORT COMMUNICATION

The influence of transition and heavy metal ions on ATP-ases activity in rat synaptic plasma membranes

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Abstract: The influence of transition metal (Cu^{2+}, Zn^{2+}, Fe^{2+} and Co^{2+}) and heavy metal ions (Hg^{2+}, Pb^{2+} and Cd^{2+}) on the activities of Na^+/K^+-ATPase and Mg^{2+}-ATPase isolated from rat synaptic plasma membranes (SPM) was investigated. The aim of the study was to elucidate the inhibition of both ATPase activities by exposure to the considered metal ions as a function of their affinity to bind to the –SH containing ligand L-cysteine, as a model system. The half-maximum inhibitory activities (IC_{50}) of the enzymes were determined as parameters of rectangular hyperbolas and correlated with the stability constant (K_s) of the respective metal-ion-L-cysteine complex. The linear Dixon plots indicate equilibrium binding of the investigated ions to both enzymes.

Keywords: transition metal ions, heavy metal ions, Na^+/K^+-ATPase, Mg^{2+}-ATPase, inhibiton.

INTRODUCTION

Sodium-potassium-adenosine triphosphatase (Na^+/K^+-ATPase) and magnesium-adenosine triphosphatase (Mg^{2+}-ATPase) are membrane bound enzymes that mediate active transport of ions across the plasma membranes of most animal cells.[1–4] A great number of metal ions alter the ATPase activity in a concentration-dependent manner[5–7] that depends on their nature. The metals of the first transition series (Fe, Co, Cu, Zn) are apparently necessary to some physiological processes,[8–13] but at higher concentrations they are usually toxic and cause some diseases.[14]

The effects of some metal ions on the ouabain sensitive Na^+/K^+-ATPase have been fairly well characterized. On the contrary, there is a lack of literature data concerning the interference of metal ions with Mg^{2+}-ATPase activity. Recent results[5–7] show that Mg^{2+}-ATPase apparently consists of at least two forms with different sensitivity to metal ions.

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Recently, the *in vitro* influence of some metal ions (Cu^{2+}, Hg^{2+}, Cd^{2+}) on the activity of Na^+/K^+-ATPase and Mg^{2+}-ATPase in rat synaptic plasma membranes (SPM) was investigated.[5,6,15] The present work continues this study and deals with the investigation of the effect of some first transition series elements (Zn^{2+}, Fe^{2+}, Co^{2+}) on the activity of Na^+/K^+-ATPase and Mg^{2+}-ATPase. Since it is now generally accepted that the inhibition of Na^+/K^+-ATPase by metal ions is usually due to their binding to sulfhydryl groups,[16–18] the aim of this work was to correlate the inhibitor efficiency of metal ions towards both enzymes with their affinity to form complexes with L-cysteine, as a model compound.

MATERIALS AND METHODS

Chemicals

All chemicals were of analytical grade and were purchased from Sigma Chemicals Co.

Synaptic plasma membrane preparation and ATPase assay

The synaptic plasma membranes were isolated from three-moth-old Wistar male rats according to the standard method.[19,20] The standard assay medium for determining the ATPases activity contained (in mM): 50 Tris-HCl, pH 7.4; 100 NaCl; 20 KCl; 5 $MgCl_2$; 2 ATP; 25 μg SPM proteins. After preincubation for 10 min at 37 ºC, the reaction was initiated by addition of ATP and stopped after 5 min by addition of 22 μl of ice cold $HClO_4$ and immediately cooling on ice. The inorganic orthophosphate (Pi) released from the hydrolysis of ATP was determined by a modified spectrophotometric method.[21] The activity obtained in the presence of Mg^{2+} alone was attributed to Mg^{2+}-ATPase activity. The Na^+/K^+-ATPase activity was calculated by subtracting the Mg^{2+}-ATPase activity from the total ATPase activity in the presence of Na^+, K^+ and Mg^{2+} ions. All experiments were performed at 37 ºC in the presence of various concentrations of $ZnSO_4$, $FeSO_4$ and $CoSO_4$.

TABLE I. IC_{50} values of the investigated ions for Na^+/K^+-ATPase and Mg^{2+}-ATPase

Metal ion	IC_{50} values/μM	
	Na^+/K^+-ATPase	Mg^{2+}-ATPase
Zn^{2+}	22	108
Fe^{2+}	34	170
Co^{2+}	50	202
[a]Cu^{2+}	7.1	41.9
[b]Hg^{2+}	0.7	3.6
[b]Cd^{2+}	12.7	80
[c]Pb^{2+}	15	125

IC_{50} values: a) Ref. 6, b) Ref. 15, c) Ref. 25

The results are expressed as mean % enzyme activity compared to the corresponding control value ± S.E.M. of at least three independent experiments done in triplicate.

RESULTS AND DISCUSSION

To investigate the influence of metal ions (Zn^{2+}, Fe^{2+} and Co^{2+}) on the Na^+/K^+-ATPase and Mg^{2+}-ATPase activity, sulfate salts were added to the reaction mixture in the concentration range from 1×10^{-7} to 1×10^{-2} M. Increasing the concentration of metal ions had the effect of inhibiting the activity of SPM Na^+/K^+-ATPase and Mg^{2+}-ATPase relative to the

control samples which were incubated with the same volume of bidistilled water. To establish whether the binding of the metal ions was in equilibrium with the inhibitory sites on the en-

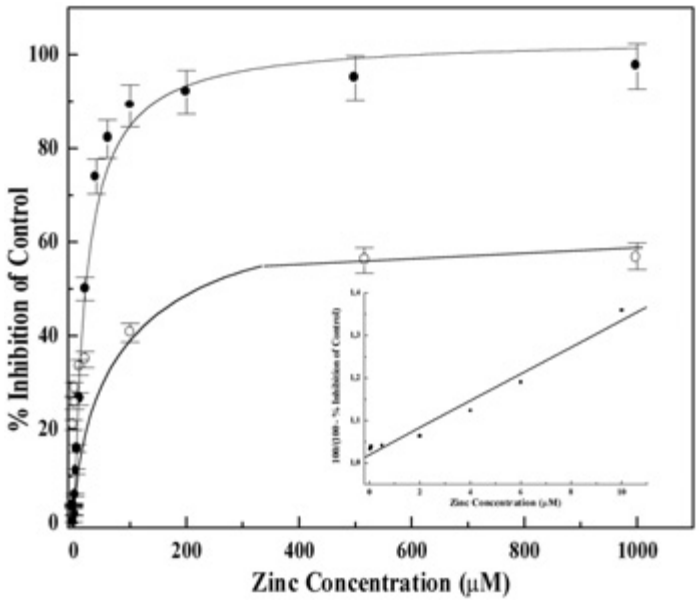

Fig. 1. Effects of Zn^{2+} on the activity of Na^+/K^+-ATPase (solid symbols) and Mg^{2+}-ATPase (open symbols) as a function of the concentration of added $ZnSO_4$. The experimental values are given as the means of at least three experiments ± S.E.M. The corresponding Dixon plot is shown in the insert.

zyme, Dixon plots,[22] *i.e.*, 100/(100 – % inhibition) *vs.* metal ion concentration were used. The obtained linear plots for both enzymes implied equilibrium binding (insert in Figs. 1, 2, 3).

Inhibition of both enzymatic activities, as previously reported,[5,17] was concentration dependent in a hyperbolic fashion (Figs. 1, 2, 3). The half-maximum inhibitory activities (IC_{50}) of the enzymes were determined as the parameters of rectangular hyperbolas (Table I). The obtained IC_{50} values for Zn^{2+} and Fe^{2+} inhibition of Na^+/K^+-ATPase are in the same order of magnitude as the IC_{50} values reported for bovine cerebral cortex Na^+/K^+-ATPase.[17]

Results of the study of the effect of the considered metal ions on ATPase activity (Table I) showed that Na^+/K^+-ATPase was more sensitive to all the investigated metals than Mg^{2+}-ATPase. It is interesting to note that the investigated ions did not inhibit Mg^{2+}-ATPase completely, even when present in concentrations above 1×10^{-3} M. The inhibition of Mg^{2+}-ATPase activity asymptotically approaches 57 % for Zn^{2+}, 80 % for Fe^{2+} and 78 % for Co^{2+} in contrast to 100 % for Na^+/K^+-ATPase. These results are in accordance with a previously reported[6] kinetic analysis, and confirm the existence of two Mg^{2+}-ATPase subtypes, differing in their sensitivity to metal ions.[23]

It is now generally accepted that inhibition by the metal ions is due to their binding to sulfhydryl groups of Na^+/K^+-ATPase.[16–18] All the investigated metals are well-known potent reagents for thiol groups. Consequently, the mechanism of enzyme inhibition caused by these ions may involve non-specific binding to enzymatic –SH groups. However, it is known that

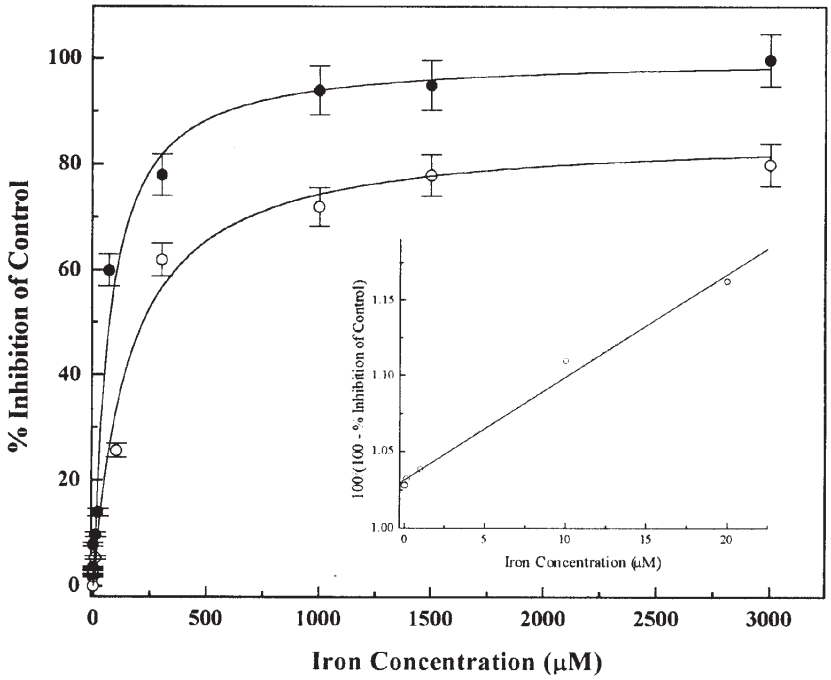

Fig. 2. Effects of Fe^{2+} on the activity of Na^+/K^+-ATPase (solid symbols) and Mg^{2+}-ATPase (open symbols) as a function of the concentration of added $FeSO_4$. The experimental values are given as the means of at least three experiments ± S.E.M. The corresponding Dixon plot is shown in the insert.

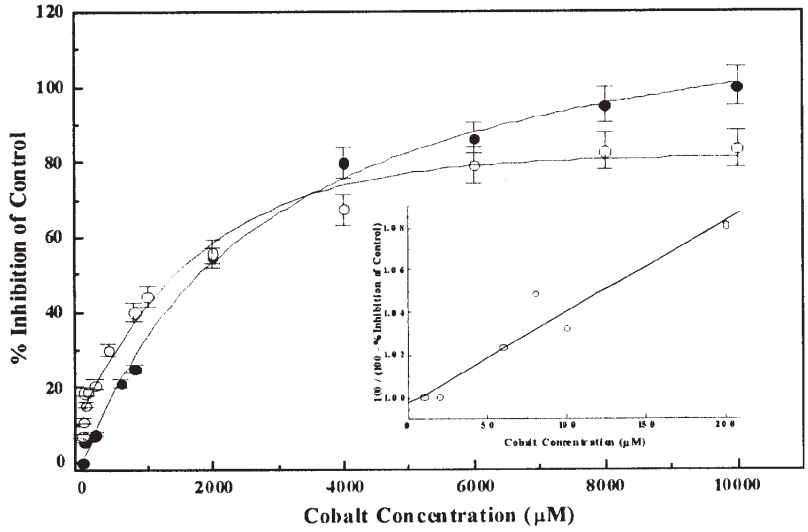

Fig. 3. Effects of Co^{2+} on the activity of Na^+/K^+-ATPase (solid symbols) and Mg^{2+}-ATPase (open symbols) as a function of the concentration of added $CoSO_4$. The experimental values are given as the means of at least three experiments ± S.E.M. Dixon plot is shown in the insert.

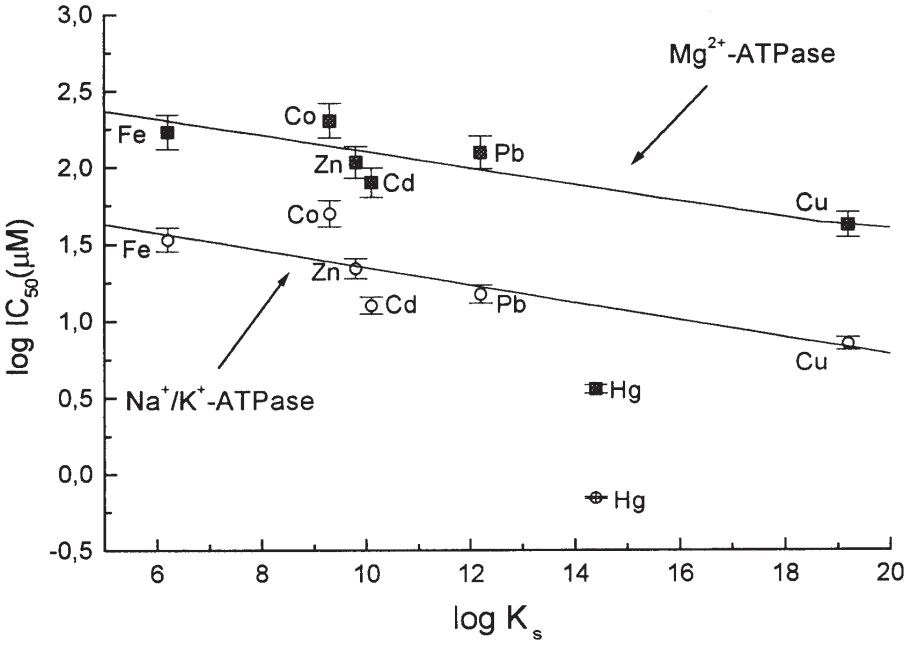

Fig. 4. Dependence of IC_{50} values of metal induced inhibition of Na^+/K^+-ATPase (circles) and Mg^{2+}-ATPase (squares) on the metal-ion – L-cysteine stability constants.

Na^+/K^+-ATPase from the kidney contains 36 sulfhydryl groups with 34 of them found in the catalytically active α subunit.[18] It seems reasonable to correlate the induced inhibition of Na^+/K^+-ATPase and Mg^{2+}-ATPase activity, expressed as IC_{50} values, with the stability constants of the corresponding metal ion complex with –SH containing ligands.[24] Therefore, L-cysteine was chosen as a model compound. The log IC_{50} *vs.* –log K_s (K_s is the stability constant of the corresponding metal ion – L-cysteine complex) plot, shown in Fig. 4, compares all the inhibition data in a single graph. Two parallel linear dependences of the toxicity *vs.* stability constants were obtained, one for each enzyme. These results suggest that the metal ion induced inhibition of Mg^{2+}-ATPase is also probably due to metal binding to the –SH groups of this enzyme. Moreover, the mechanism of inhibition of both enzymes is similar, *i.e.*, it involves a non-specific binding to the –SH groups of the enzymes.

As is obvious from the plots in Fig. 4, the IC_{50} values of the heavy metal ions fit well in the curve obtained for the K_s values of the first transition series metal ions. However, Hg^{2+} which was found to be the most toxic one, does not show the highest affinity to the sulfhydryl group.[24] This result suggests that Hg^{2+} induced inhibition may involve some other mechanism besides the non-specific binding of Hg^{2+} to the sulfhydryl groups of the proteins.

In conclusion, the presented results confirm that all the investigated metals inhibit ATPase activity in a dose-dependent manner and that their inhibitor efficiency depends on the affinity of the metal ion to bind to the –SH groups of the protein. Further work is underway to investigate the recovery effect of –SH containing ligands (L-cysteine, glutathione) on metal ions induced inhibition of rat brain Na^+/K^+-ATPase and Mg^{2+}-ATPase.

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ИЗВОД

УТИЦАЈ ЈОНА ПРЕЛАЗНИХ И ТЕШКИХ МЕТАЛА НА АКТИВНОСТ АТРаза У СИНАПТОЗОМАЛНИМ ПЛАЗМА МЕМБРАНАМА ПАЦОВА

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Испитан је утицај јона прелазних метала (Cu^{2+}, Zn^{2+}, Fe^{2+} и Co^{2+}) и јона тешких метала (Hg^{2+}, Pb^{2+} и Cd^{2+}) на активност Na^+/K^+-АТРазе и Mg^{2+}-АТРазе, ензима изолованих из синаптозомалних плазма мембрана пацова. Циљ рада је испитивање инхибиције активности АТРаза након излагања металним јонима у зависности од њиховог афинитета за везивање са SH-групама L-цистеина, као модел система. IC_{50} вредности (концентрација инхибитора која доводи до смањења ензимске активности за 50 %) су одређене као параметри ректангуларне хиперболе и корелиране са константом стабилности (K_s) комплекса металних јона са L-цистеином. Добијене Диксонове криве су линеарне, што указује да је везивање испитиваних јона за оба ензима равнотежно.

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