Ca^{2+}-Induced Inhibition of Sodium Pump: Noncompetitive Inhibition in Respect of Magnesium and Sodium Cations

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Abstract. Calcium inhibits the activity of the (Na^+/K^+)-ATPase from dog kidney in a dose-dependent manner. Other 2A group cations of the periodic table such as Sr^{2+} and Ba^{2+} were able to inhibit the ATPase activity but to a lesser degree. Any considerable competition between Ca^{2+} (Ba^{2+}, Sr^{2+}) ions and magnesium or sodium ions could not be detected using enzyme kinetic analysis. Thus, the above three inhibitory acting ions depress the ATPase activity of sodium pump by interaction with loci distant from the sodium and potassium binding sites. This suggests that the (Na^+/K^+)-ATPase molecule contains an inhibitory acting binding site for calcium. This putative binding site could recognize magnesium ions as well as calcium, strontium and barium ions. The specificity of the binding site may describe herein be secured by a structure complementary to the coordination structure of Ca^{2+}, Ba^{2+} and Sr^{2+} ions characterized by coordination number 8. Mg^{2+} ions can form coordination structure with a maximum coordination number 6, and do not interact specifically with this binding site.

Key words: (Na^+/K^+)-ATPase — Ca^{2+}, Ba^{2+}, Sr^{2+} induced inhibition — Coordination bounds

Introduction

Calcium ions are involved in the regulation of many processes in the animal cells (Račav and Lehotský 1996; Račay et al. 1996; Maco et al. 1997; Sobol and Nesterov 1997; Stroffekova and Heiny 1997). In respect of sodium pump it has been found

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that Ca^{2+} in millimolar concentrations considerably inhibits the ATPase activity (Lindenmayer and Schwartz 1975 Huang and Askari 1982 Yingst 1983 Yingst and Marcovitz 1983 Yingst and Polasek 1985 Yingst et al 1986 1992 Vrbjar et al 1986) as well as the transport (and/or electrogenic) activities of this transport system (Hagane et al 1989 Stankovičova et al 1995) One possibility to explain this inhibition is the assumption that there is a competition between Ca^{2+} with cation cofactors of (Na^+/K^+)-ATPase in the respective cation binding site (Tobin et al 1973 Huang and Askari 1982) In a previous paper we could demonstrate that calcium induced inhibition of the electrogenic activity of the sodium pump is based on intracellular interaction of calcium with (Na^+/K^+)-ATPase lipoprotein complex (Stankovicova et al 1995) Thus if it is actually the case that this inhibition is of competetive nature only sodium- and magnesium- but not potassium-binding site of the enzyme may be involved On the other hand the existence of an inhibitory acting calcium binding site on the enzyme molecule (Ziegelhoffer et al 1986) is another possibility to explain the inhibitory action of Ca^{2+} on (Na^+/K^+)-ATPase activity The latter possibility is supported by the fact that the concentration at which calcium was observed to inhibit (Na^+/K^+)-ATPase activity fixes the secondary structure of heart sarcolemmal membrane proteins to a state that was found to be unfavorable for (Na^+/K^+)-ATPase activity manifestation This binding site may interact with calcium either directly or through intracellular calcium binding proteins The latter possibility was verified by Yingst 1983 1988, Yingst and Marcovitz 1983 Yingst and Polasek 1985 Yingst et al 1986 1992 who showed that application of calmodulin and 'calnactin" shifted the effective calcium concentration necessary for (Na^+/K^+)-ATPase activity inhibition from submillimolar to submicromolar level Calnactin is a putative calcium binding protein that has been proposed to modulate the effect of calcium on sodium pump activity The present work was aimed to answering the question whether there is competition between calcium and sodium or magnesium ions for the respective cation binding sites

Materials and Methods

(Na^+/K^+)-ATPase from dog kidney outer medulla was isolated according to Jorgensen (1988) using centrifugation in a fixed angle rotor (Na^+/K^+)-ATPase activity was determined as the difference in the amounts of phosphate liberated during splitting of ATP (2 mmol/l) in the presence of each 1 100 mmol/l NaCl, 10 mmol/l KCl and 0 1 2 0 mmol/l $MgCl_2$, or in the presence of 0 1 2 mmol/l $MgCl_2$ only Enzyme reaction was run in 0 5 ml of incubation medium containing 50 mmol/l imidazole-HCl buffer (pH 7 0) and 2 5 mg of pure enzyme protein at 37 °C usually for 10 min The reaction was started by adding the substrate and it was stopped by ice-cold trichloroacetic acid (0 73 mol/l) All details about the estimation of

the enzyme activity were described previously (Dzurba et al 1996) Ca^{2+}, Sr^{2+} and Ba^{2+} ions were left to interact with the enzyme during 10 min preincubation prior to starting the enzyme by ATP Sodium dodecylsulphate polyacrylamide electrophoresis (SDS-PAGE) used on 12 5% gel with Phast system (Pharmacia Uppsala Sweden) The proteins separated were visualized with Coomasie Blue R by a standard procedure according to the instrument program All chemicals were obtained from Sigma (St Louis USA) and Lachema (Brno, Czech Republic) and were of analytical purity Experimental data of (Na^+/K^+)-ATPase activity stimulation by sodium and magnesium cations in the presence of calcium were fitted as a function of two independent variables (concentrations of calcium and sodium or magnesium) according to equation (1) which is based on Michaelis-Menten relationship equipped with Hill cooperativity constant (n) and inhibitory constants K_i^{nc} and K_i^c for both noncompetitive and competitive mode of inhibition respectively (Eq 1 Breier et al 1996)

$$v = \frac{V_{max}}{1 + (i/K_i^{nc})} \frac{s^n}{(s^n + K_m^n)[1 + (i/K_i^c)]} \tag{1}$$

where v represents (Na^+/K^+)-ATPase activity when concentrations of cation cofactor of the enzyme (Na^+ and Mg^{2+}) are equal to s, and concentration of Ca^{2+} is equal to i V_{max} and K_m represent the Michaelis constant and maximal velocity of enzyme reaction respectively Experimental data on (Na^+/K^+)-ATPase inhibition by calcium in the presence or absence of magnesium were fitted according to equation (2) which represents the Dixon equations for inhibition consisting of two parts

$$v = \frac{V}{[1 + (i/IC'_{50})][1 + (i/IC''_{50})]} \tag{2}$$

where v is the (Na^+/K^+)-ATPase activity when calcium concentration is equal to i V is (Na^+/K^+)-ATPase activity in the absence of calcium IC'_{50} and IC''_{50} are median inhibitory concentrations for both parts of biphasic inhibitions

The effect of Ca^{2+} Sr^{2+} and Ba^{2+} on stimulation by magnesium was compute using Lineweaver Burk transformation of Michaelis-Menten equation All computations were done using SigmaPlot 5 0 All other details about the isolation of (Na^+/K^+)-ATPase measurements of enzyme kinetics as well as data processing were described previously (Breier et al 1996)

Results

Isolation of (Na^+/K^+)-ATPase from dog kidney according to Jorgensen (1988) yielded enzyme preparations with activity around 10 mmol/mg min The protein profile of this preparation in SDS-PAGE contained two bands with molecular weight

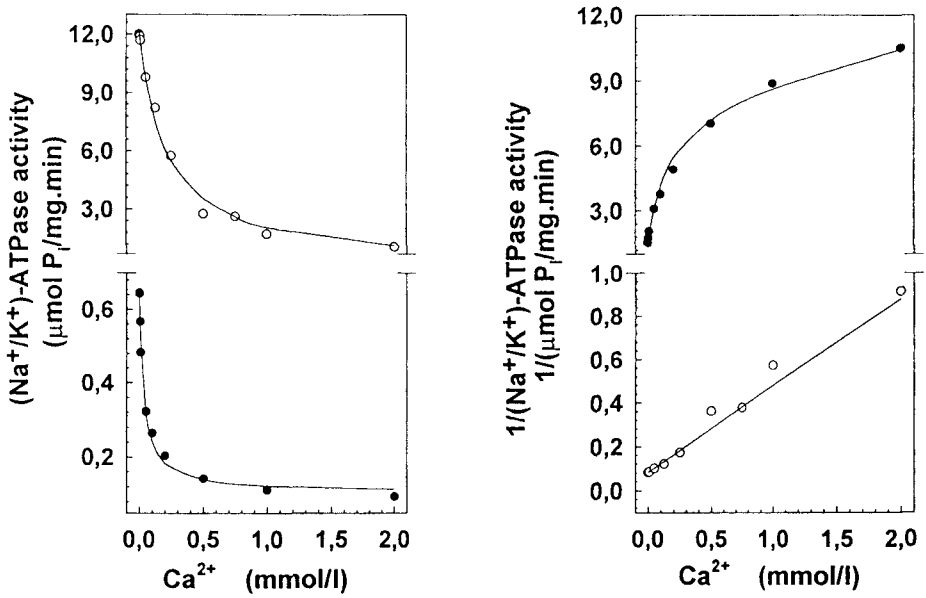

Figure 1. Calcium induced inhibition of (Na^+/K^+)-ATPase activity in the presence (○) and absence (●) of magnesium (4 mmol/l) Left panel direct plot of experimental data right panel experimental data in Dixon plot The experimental data represent means from three independent experiments and respective S E M values never exceeded 5% of the mean The data were fitted by nonlinear regression using Eq 2 For kinetic variables obtained see Results

characteristic for α and β subunits of (Na^+/K^+)-ATPase (not shown) Calcium ions in the concentration range 0 1 1 0 mmol/l considerably inhibited the (Na^+/K^+)-ATPase activity (Fig 1) When magnesium (2 mmol/l) was present in the reaction medium this inhibition could be described by simple monophasic dependency ($IC'_{50} = 0\ 205 \pm 0\ 026$ mmol/l) When magnesium was not present in the reaction medium a decrease of ATPase activity more than one order of magnitude was observed Calcium-induced inhibition of this "Mg^{2+}-independent" ATPase activity had to be fitted by biphasic dependency (Fig 1) Values of $IC'_{50} = 0\ 209 \pm 0\ 024$ mmol/l and $IC''_{50} = 0\ 034 + 0\ 005$ mmol/l were obtained by nonlinear fitting according to Eq 2 While effect of Ca^{2+} on (Na^+/K^+)-ATPase gave a straight line in the Dixon plot when magnesium ions were present a concave curvature in Dixon plot was observed in the absence of the ions (Fig 1) The effect of Ca^{2+} Sr^{2+} and Ba^{2+} ions on stimulation of (Na^+/K^+)-ATPase activity by magnesium ions is shown in Fig 2 All three bivalent cations inhibited the ATPase activity of the Na-pump with potencious decreating in the order of $Ca^{2+} > Sr^{2+} \sim Ba^{2+}$ The Lineweaver

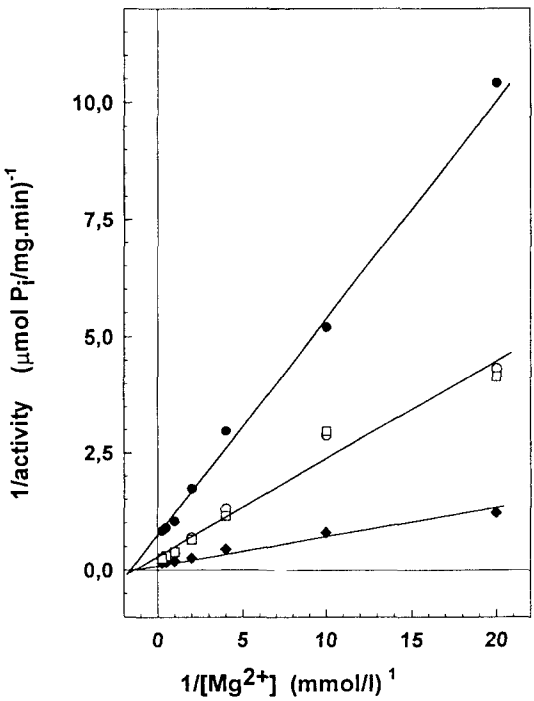

Figure 2. Noncompetitive mode of Ca^{2+} (●), Sr^{2+} (○) and Ba^{2+} (□) induced inhibition of (Na^+/K^+)-ATPase activity with respect to stimulation with magnesium ions (◆) documented in Lineweaver Burke plots. The experimental data represent means from three independent experiments and the respective S.E.M. values never exceeded 5% of the mean.

Burke plots of this inhibition (Fig. 2) revealed noncompetitive type of inhibition characterized by decrease of V_{max} only. The noncompetitive way of calcium induced depression of stimulation of (Na^+/K^+)-ATPase activity by magnesium was additionally proved by experimental data shown in Fig. 3. Fitting of these data according to Equation (1) gave the following values: V_{max}=14.25 µmol/min mg, K_m = 0.464 mmol/l, K_i^{nc} = 0.214 mmol/l, K_i' = 12.70 mmol/l and n = 1. Thus, calcium induced significant changes of V_{max} value because K_i^{nc} was found to be in the range of the calcium concentration applied. The value of K_i' was found to exceed the highest calcium concentration used by about one order of magnitude, thus it could not induce significant changes in K_m value. In contrast to simple hyperbolic noncooperative mode of magnesium stimulation of (Na^+/K^+)-ATPase activity (n = 1, Fig. 3), sodium stimulated the enzyme activity in a sigmoidal cooperative mode (n = 2.05, Fig. 4). Sodium stimulation of (Na^+/K^+)-ATPase was

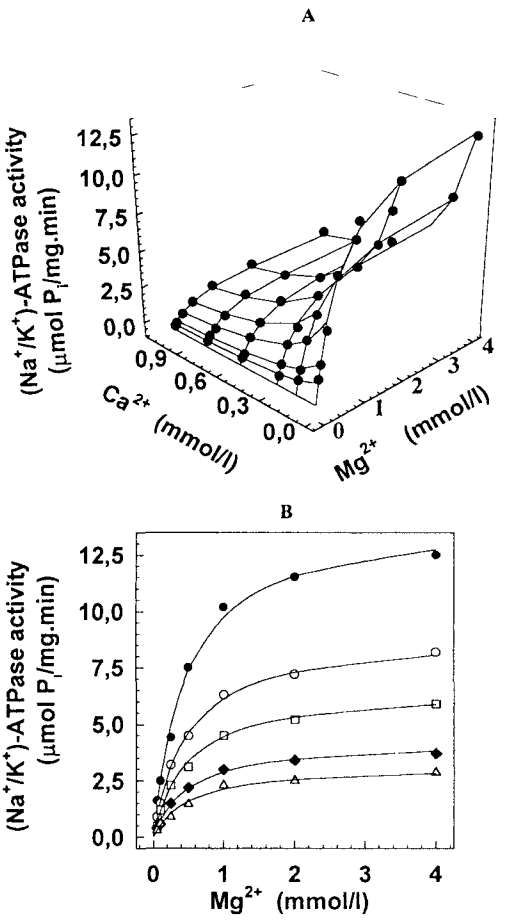

Figure 3. Calcium induced inhibition of stimulation of (Na^+/K^+)-ATPase by Mg^{2+} ions Panel *A* Three dimensional plot of (Na^+/K^+)-ATPase activity versus calcium and magnesium ions concentrations as two independent variables Panel *B* Two dimensional plot of (Na^+/K^+)-ATPase activity in the absence (●) or in the presence (○ 0 125 □ 0 250 ◆ 0 500 △ 1 000 mmol/l) of calcium ions as function of magnesium ions The experimental data represent means from three independent experiments and the respective S E M values never exceeded 5% of the mean The data were fitted by nonlinear regression using Eq 1 For kinetic variables obtained see Results

inhibited by calcium noncompetitively as it could be deduced from the following kinetics variables obtained from nonlinear regression of the data in Fig 4 using Eq 1 V_{max}=10 47 μmol Pi/min mg, K_m = 3 21 mmol/l K_m^{Ca} = 0 46 mmol/l K_i^{Ca} = 105 12 mmol/l and n = 2 05 Thus only the parameter V_{max} was influenced by calcium in this case

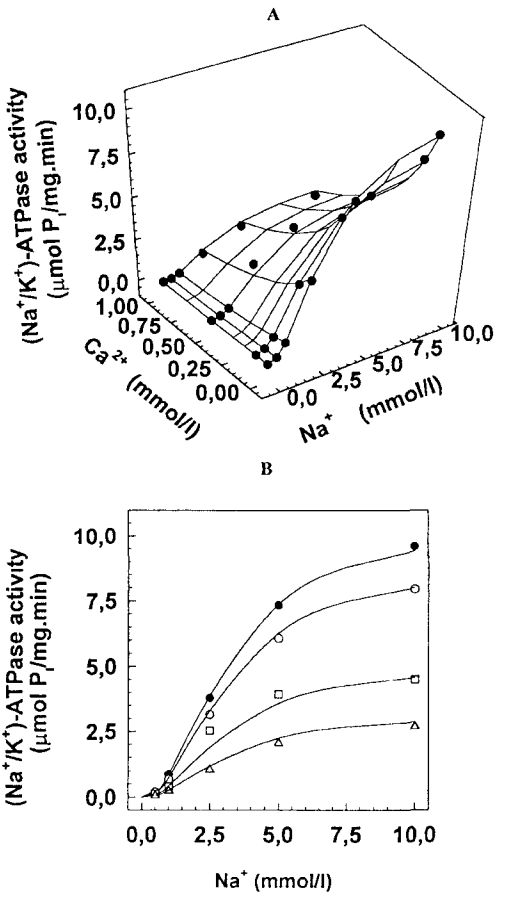

Figure 4. Calcium induced inhibition of Na^+ stimulation of (Na^+/K^+)-ATPase Panel *A* Three-dimensional plot of (Na^+/K^+)-ATPase activity versus calcium and sodium ions concentrations as two independent variables Panel *B* Two-dimensional plot of (Na^+/K^+)-ATPase activity in the absence (●) or in the presence (○ 0 1, □ 0 5, △ 1 0 mmol/l) of calcium ions as function of sodium ions The experimental data represent means from three independent experiments and the respective S E M values never exceeded 5% of mean The data were fitted by nonlinear regression using Eq 1 For kinetic variables obtained, see Results

Discussion

It has been well documented that calcium inhibits the ATPase activity of Na-pump (Lindenmayer and Schwartz 1975 Huang and Askari 1982, Yingst 1983 1988 Yingst and Marcovitz 1983 Yingst and Polasek 1985, Yingst et al 1986 1992

Vrbjar et al 1986) Moreover an increase of calcium in the extracellular medium has been reported to cause a significant depression of the transport and/or electrogenic activity of this enzyme (Hagane et al 1989 Stankovičova et al 1995) Using several calcium entry blockers we demonstrated in a previous work that calcium inhibits electrogenic activity of the sodium pump from the intracellular side of the plasma membrane (Stankovičova et al 1995) In the present work, inhibition of (Na^+/K^+)-ATPase activity was observed in the concentration range of Ca^{2+} of 0 05 1 0 mmol/l (Fig 1) which corresponds to the data published elsewhere (Lindenmayer and Schwartz 1975, Huang and Askari 1982 Yingst 1983 1988 Yingst and Marcovitz 1983 Yingst and Polasek 1985 Yingst et al 1986, 1992 Vrbjar et al 1986) In the presence of magnesium, the concentration dependence of Ca^{2+} induced inhibition of (Na^+/K^+)-ATPase activity may be described by simple Dixon equation with one value of $ID'_{50} = 0\ 205$ mmol/l When magnesium was not present or was present only as an impurity in bidistilled deionized water and/or used chemicals the activity of the enzyme could be considered as Mg^{2+}-independent' In such case the Na^+ and K^+ stimulated ATPase activity was by two orders of magnitude below that found in the presence of Mg^{2+} (Fig 1) The effect of increasing concentrations of calcium on this "Mg^{2+}-independent ATPase activity was described by equation consisting of two parts corresponding to the biphasic course of this dependence This indicated that calcium may interact with (Na^+/K^+)-ATPase at two binding loci having different affinities to calcium ions Inhibition of the enzyme by calcium binding to the high affinity binding site (characterized by $ID'_{50} = 0\ 034$ mmol/l) was observed only in the absence of magnesium ions This binding site may be considered the binding site for magnesium However, calcium may substitute magnesium in this binding site but only in the absence of magnesium or if magnesium is present in a very low concentration In contrast to Mg^{2+} ions the binding of calcium ions to this binding site, inhibits Mg^{2+} independent" ATPase activity Inhibition of the enzyme mediated by the binding of Ca^{2+} to the second binding site was characterized by $ID'_{50} = 0\ 209$ mmol/l The latter value is similar to the corresponding $ID'_{50} = 0\ 205$ mmol/l value obtained for calcium-induced inhibition of (Na^+/K^+)- ATPase activity in the presence of magnesium Thus when calcium interacts with this site, it subsequently inhibits the enzyme independently of the presence or absence of magnesium Moreover, calcium inhibited magnesium stimulated (Na^+/K^+)-ATPase activity in a noncompetitive manner (Figs 2 and 3) The noncompetitive type of this inhibition indicated that inhibition of the enzyme is mediated by the binding of calcium to a locus different from magnesium binding sites Nevertheless the interaction of the magnesium binding site with calcium observed in the absence of magnesium may take place at lower magnesium concentrations (below 0 05 mmol/l) This is how the observation may bee explained (Tobin et al 1973 Huang and Askari 1982) that there is some competition between Ca^{2+} and Mg^{2+} at the same binding locus Nevertheless under normal condi-

tions i e magnesium concentration in mmol/l range, no considerable competition between calcium and magnesium ions could be observed Moreover calcium was found to inhibit the sodium stimulated (Na^+/K^+)-ATPase activity (Fig 4) again in a noncompetitive manner Therefore it should be stressed that no considerable competition between cation cofactors of (Na^+/K^+)-ATPase and calcium ions could be expected According to Vrbjar et al (1986) the interaction of calcium with the sarcolemmal membrane causes a decrease of the content of membrane proteins in α-helical structure This decrease was associated with a proportional decrease of (Na^+/K^+)-ATPase activity Thus interaction of calcium with (Na^+/K^+)-ATPase at a binding site different from the binding site for magnesium induced changes of membrane proteins the resulting conformations of which are unsuitable for ATPase reaction From this point of view the inhibition of (Na^+/K^+)-ATPase by calcium under these conditions may be considered as allosterical and may be modulated by calmodulin and calnactin Because calcium interacts with this binding site with similar affinity in the presence or absence of magnesium, any considerable competition between both ions for this calcium binding site is improbable Thus, the putative calcium-binding site on the (Na^+/K^+)-ATPase molecule may bind strontium or barium ions but not magnesium ions The specificity of this site could be explained by the nature of the cations considered The main difference between Mg^{2+} ions and Ca^{2+} (Sr^{2+}, Ba^{2+}) ions is the inability of the former ions to form coordination bounds with coordination number 8, the coordination configuration typical of Ca^{2+} Sr^{2+} and Ba^{2+} (Hughes 1981) Thus when the putative binding site for calcium on the (Na^+/K^+)-ATPase molecule is complementary to the coordination structure of calcium with coordination number 8 magnesium ions will not be able to interact with this locus A similar principle has been suggested for the selective recognition of monovalent cations at potassium-binding site on the (Na^+/K^+)-ATPase molecule (Breier et al 1988)

Acknowledgements. This work was supported by Slovak grant agency for science (grant No 95/5305/515VT)

References

Breier A, Turi Nagy L, Ziegelhoffer A, Monošikova R (1988) Principles of selectivity of sodium and potassium binding sites of the Na/K-ATPase A corollary hypothesis Biochim Biophys Acta **946,** 129 134

Breier A, Vrbanova A, Docolomansky P, Bohacova V, Ziegelhoffer A (1996) Competitive inhibition of (Na/K) ATPase by furylethylenes with respect to potassium ions Gen Physiol Biophys **15,** 1 –17

Dzurba A, Vrbjar N, Breier A, Ziegelhoffer A (1996) The membrane effect of benfluron modulation of the heart sarcolemmal (Na^+ K^+)-ATPase and Mg^{2+} ATPase activities Gen Physiol Biophys **15,** 71 75

Hagane K, Akera T, Stemmer P (1989) Effect of Ca^{2+} on the sodium pump observed in cardiac myocytes isolated from guinea pigs Biochim Biophys Acta **982,** 279 287

Huang W-H Askari A (1982) Ca dependent activities of (Na^+ K^+)-ATPase Arch Biochem Biophys **216**, 741 750

Hughes M N (1981) The Inorganic Chemistry of Biological Processes John Wiley and Sons Ltd New York

Jorgensen P L (1988) Purification of Na^+, K^+-ATPase Enzyme sources preparative problems and preparation from mammalian kidney In Methods in Enzymology Vol **156** (Eds S Fleischer and B Fleischer) pp 29 43 Academic Press London

Maco B Brezova A Schafer B W Uhrik B Heizmann C W (1997) Localization of the Ca^{2+}-binding S100A1 protein in slow and fast skeletal muscles of the rat Gen Physiol Biophys **16**, 373 377

Lindenmayer G E Schwartz A (1975) Kinetic characterization of calcium ion (Na^+ K^+)-ATPase and its potential role as link between extracellular and intracellular events hypothesis for digitalis-induced inotropism J Mol Cell Cardiol **7**, 591 612

Racay P Lehotsky J (1996) Intracellular and molecular aspects of Ca^{2+}-mediated signal transduction in neuronal cells Gen Physiol Biophys **15**, 273 289

Racay P Kaplan P Lehotsky J (1996) Control of Ca^{2+} homeostasis in neuronal cells Gen Physiol Biophys **15**, 193 210

Sobol C V Nesterov V P (1997) Tension-Ca^{2+} concentration relationship in chemically skinned vascular smooth muscle of the frog Gen Physiol Biophys **16**, 189 192

Stankovicova T Zemkova H Breier A, Amler E Burkhard M Vyskočil F (1995) The effects of calcium and calcium channel blockers on sodium pump Pflugers Arch **429**, 711 721

Stroffekova K Herny J A (1997) Triadic Ca^{2+} modulates charge movement in skeletal muscle cells Gen Physiol Biophys **16**, 59 77

Tobin T Akera T Baskin S I Brody T M (1973) Calcium ion and sodium-potassium-dependent adenosine triphosphatase Mol Pharmacol **9**, 336 349

Vrbjar N Breier A Ziegelhoffer A Dzurba A Soos J (1986) Effect of calcium on the structure-function relationship of (Na+K)-ATPase in cardiac sarcolemma Gen Physiol Biophys **5**, 545 550

Yingst D R (1983) Hemolysate increases Ca inhibition of Na K-pump of released human red cell ghosts Biochim Biophys Acta **732**, 312 –315

Yingst D R (1988) Modulation of the Na K-ATPase activity by Ca and intracellular proteins Annu Rev Physiol **50**, 291 303

Yingst D R Marcovitz M J (1983) Effect of hemolysate on calcium inhibition of the (Na^+ K^+)-ATPase of human red blood cells Biochem Biophys Res Commun **111**, 970 979

Yingst D R Polasek P M (1985) Sensitivity and reversibility of Ca-dependent inhibitor of Na K-ATPase of human red blood cells Biochim Biophys Acta **813**, 282 286

Yingst D R Jones R M Polasek P M (1986) The effect of heart kidney and brain extracts on Ca-dependent inhibition of the Na K-ATPase Biophys J **49**, 549 551

Yingst D R Ye-Hu J Chen H Barrett V (1992) Calmodulin increases Ca^{2+}-dependent inhibition of the Na^+/K^+-ATPase in human red blod cells Arch Biochem Biophys **295**, 49 – 54

Ziegelhoffer A Vrbjar N Breier A (1986) How do the ATPase in cardiac cell membranes work? Biomed Biochim Acta **45**, S211 S214

Final version accepted May 7 1998