Effect of Calcium on the Structure-Function Relationship of $(Na^+ + K^+)$-ATPase in Cardiac Sarcolemma

N. VRBJAR[1], A. BREIER[2], A. ZIEGELHÖFFER[1], A. DŽURBA[1] and J. SOÓS[3]

1 *Department of Biochemistry, Institute of Experimental Surgery, Centre of Physiological Sciences, Slovak Academy of Sciences, Dúbravská cesta 9, 842 33 Bratislava, Czechoslovakia*
2 *Centre of Physiological Sciences, Slovak Academy of Sciences, Vlárska 3, 833 06 Bratislava, Czechoslovakia*
3 *Institute of Biophysics, Biological Research Centre, Hungarian Academy of Sciences, H-6701 Szeged, P. O. Box 521, Hungary*

Abstract. Calcium-induced changes in $(Na^+ + K^+)$-ATPase activity and structural changes of membrane bound proteins in rat heart sarcolemma were investigated. Increasing concentrations of Ca^{2+} (0.1—8.0 mmol . l^{-1}) gradually inhibited the $(Na^+ + K^+)$-ATPase activity and decreased the α-helix content of sarcolemmal proteins. Mathematical and graphical analysis of observed data yielded a quantitative relationship between Ca^{2+}-induced changes in $(Na^+ + K^+)$-ATPase activity and the secondary structure of membrane proteins in cardiac sarcolemma.

Introduction

$(Na^+ + K^+)$-ATPase (E.C.3.6.1.3) is responsible for the active transport of Na^+ and K^+ across the plasma membrane using energy derived from ATP hydrolysis (Skou 1965). During a turnover cycle two major conformational forms of the enzyme, a sodium bound form (E_1Na) and a potassium bound form (E_2K), have been identified (Jørgensen 1975; Karlish and Yates 1978; Matsui 1982). Approximately 80 amino acid residues are involved in the transition between these two enzyme conformations as has been determined on the basis of circular dichroism spectra of membrane bound $(Na^+ + K^+)$-ATPase isolated from guinea pig kidney (Gresalfi and Wallace 1984). It has been also shown that similar conformational changes in heart sarcolemmal proteins may be already induced by the interaction of sarcolemma with Na^+ and K^+ when combined with Mg^{2+} in concentrations stimulatory to the activity of membrane bound $(Na^+ + K^+)$-ATPase (Vrbjar et al. 1984). All this suggests changes in the secondary structure of the corresponding membrane proteins during the turnover cycle of the enzyme. Nevertheless, at optimal stimulation by K^+ and Na^+ in the pressence of Mg^{2+}, $(Na^+ + K^+)$-ATPase from various sources was found to be inhibited by Ca^{2+} (Huang and Askari 1982; Lindenmayer and Schwartz 1975; Yingst and Marcovitz 1983). This ion might, in

addition to the known competition for cation binding sites (Lindenmayer and Schwartz 1975) also interfere with the establishment of an optimal protein conformation for enzyme activity. In the present study a possible link between Ca^{2+}-induced changes in protein conformation and the corresponding inhibition of $(Na^+ + K^+)$-ATPase in cardiac sarcolemma was investigated.

Materials and Methods

Sarcolemmal membrane fraction from rat hearts was prepared using hypotonic shock and additional treatments with 0.6 mol . l^{-1} NaI and 1 mmol . l^{-1} EDTA as described earlier (Vrbjar et al. 1984; Ziegelhöffer et al. 1983).

Protein concentration was determined according to Lowry et al. (1951). $(Na^+ + K^+)$-ATPase activity was measured at 37 °C in 1 ml of medium containing 50 mmol . l^{-1} TRIS-HCl, pH — 7.4, 5 mmol . l^{-1} $MgCl_2$, 100 mmol . l^{-1} NaCl, 10 mmol . l^{-1} KCl and 100 μg of membrane proteins. After 5 min of preincubation the reaction was started by adding ATP (final concentration 4 mmol . l^{-1}), and it was terminated by adding 1 ml of 0.73 mol . l^{-1} of ice-cold trichloroacetic acid. The inorganic phosphorus liberated during 10 min reaction was determined by the method of Taussky and Shorr (1953). The effect of Ca^{2+} on $(Na^+ + K^+)$-ATPase activity was studied within a $CaCl_2$ concentration range of 0.1—8.0 mmol . l^{-1}.

The secondary structure of sarcolemmal proteins was determined by evaluating circular dichroism (CD) spectra of membrane vesicles recorded at 210 to 240 nm using a Jasco 40 c dichrograph calibrated with d-10-camphorsulphonic acid. The results obtained using integrated sarcolemmal vesicles were corrected for absorption flattening, to get spectra corresponding to pure proteins as chromophores. The method of correction applied was described earlier (Vrbjar et al. 1984). The alpha-helix contents of membrane proteins in the absence and presence of various Ca^{2+} concentrations were calculated from the corrected CD spectra using the method of Siegel et al. (1980).

Results and Discussion

It has been recognized that calcium inhibits the activity of $(Na^+ + K^+)$-ATPase by several ways, including competition with Mg^{2+} and/or Na^+ in the activation mechanism and interaction of K^+ with the enzyme (Lindenmayer and Schwartz 1975). Our results presented in Fig. 1*A* and 1*B* show that interaction of Ca^{2+} with the sarcolemma at Ca^{2+} concentrations which are able to inhibit $(Na^+ + K^+)$-ATPase also induces a diminution of the α-helix content in sarcolemmal membrane proteins. Transformation of curves from Fig. 1 into the semilogarithmic coordinate system provided straight lines (Fig. 2*A*, *B*) enabling mathematical processing. The relationship between $(Na^+ + K^+)$-ATPase activity in cardiac sarcolemma and calcium concentration could be characterized by equation

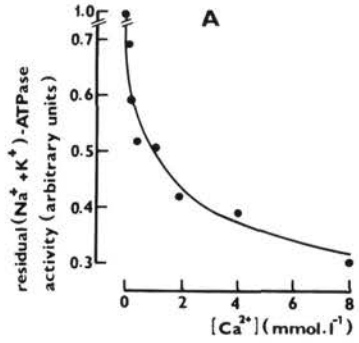
$$V = a_1 - b_1 \ln [Ca^{2+}] \quad (1)$$

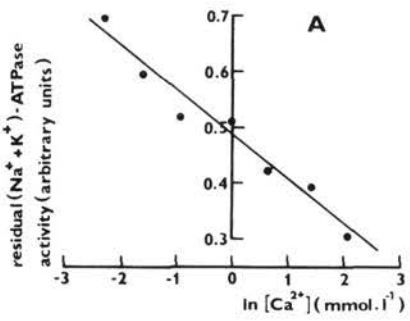
where V represents the actual enzyme activity, a_1 represents the residual $(Na^+ + K^+)$-ATPase activity at a unit concentration of Ca^{2+}, and b_1 represents the slope of the straight line.

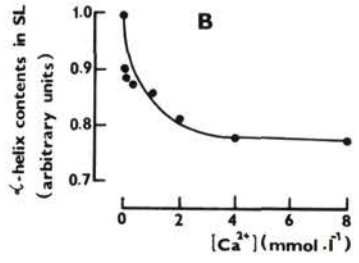
The relationship between the secondary structure of sarcolemmal proteins and the calcium concentration could similarly be described by equation

$$[\alpha_H] = a_2 - b_2 \ln [Ca^{2+}] \quad (2)$$

where $[\alpha_H]$ represents the α-helix contents of sarcolemmal proteins, a_2 represents the α-helix contents of sarcolemmal proteins at a unit concentration of Ca^{2+}, and b_2 represents the slope of the straight line.







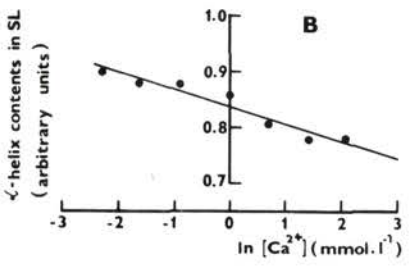


Fig. 1. Effect of Ca^{2+} on sarcolemmal membrane. *A:* Inhibitory effect of Ca^{2+} on the sarcolemmal $(Na^+ + K^+)$-ATPase activity. The reference $(Na^+ + K^+)$-ATPase activity (value taken for 1) established in the absence of Ca^{2+} was 12.84 (μmol P_i . mg^{-1} proteins . hr^{-1}). *B:* Ca^{2+} -induced changes in secondary structure of sarcolemmal proteins. The basal value of the α-helix contents in sarcolemmal proteins (value taken for 1) established in TRIS buffer (pH = 7.4) in the absence of Ca^{2+} was 79.6 per cent.

Fig. 2. Semilogarithmic plots of the effect of Ca^{2+} on sarcolemmal membrane. *A:* Inhibitory effect of Ca^{2+} on the sarcolemmal $(Na^+ + K^+)$-ATPase. The straight line is characterized by the equation: $V = 0.483 - 0.08 \ln [Ca^{2+}]$, where V represents $(Na^+ + K^+)$-ATPase activity. The correlation coefficient of the straight line is 0.981, $p < 0.01$. *B:* Ca^{2+}-induced changes in secondary structure of sarcolemmal proteins. The straight line is characterized by the equation $[\alpha_H] = 0.837 - 0.031 \ln [Ca^{2+}]$, where $[\alpha_H]$ represents the α-helix contents of sarcolemmal proteins. The correlation coefficient of the straight line is 0.973, $p < 0.01$.

Combining equations (1) and (2) yields

$$V = \left(a_1 - \frac{b_1}{b_2} a_2 \right) + \frac{b_1}{b_2} [\alpha_H] \tag{3}$$

expressing the quantitative relationship between $(Na^+ + K^+)$-ATPase activity and the α-helix content of sarcolemmal membrane proteins at different calcium concentrations (Fig. 3). The above quantitative relationship between Ca^{2+} induced modulation of $(Na^+ + K^+)$-ATPase activity and the secondary structure of membrane proteins proved to be highly significant (probability exceeding 99 %). This suggest that the Ca^{2+}-induced conformation of sarcolemmal proteins will play an important role in the regulation of $(Na^+ + K^+)$-ATPase activity. Thus, in addition to the competition for binding sites for Mg^{2+} and Na^+ on the $(Na^+ + K^+)$-ATPase molecule, binding of calcium ions to membrane seems to interfere with oscillations between the E_1Na and E_2K conformation states of the enzyme during its action.

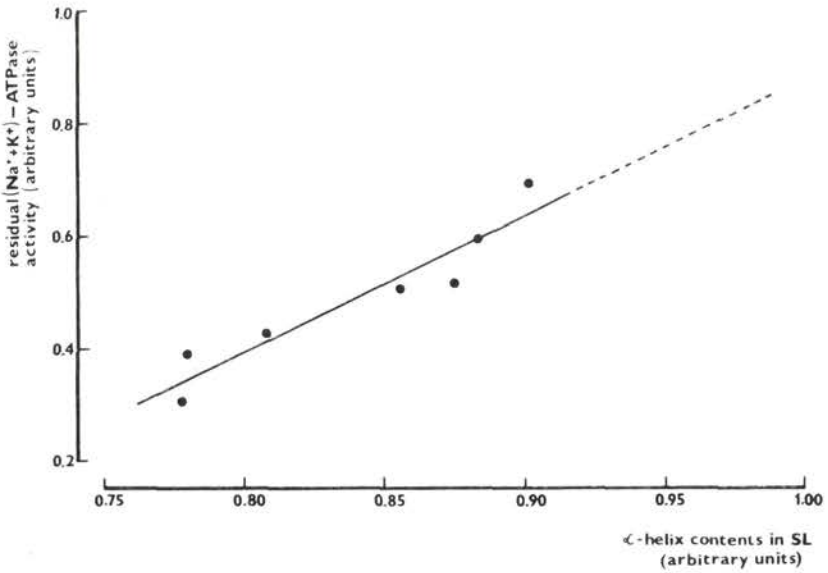


Fig. 3. Relationship between $(Na^+ + K^+)$-ATPase activity and the secondary structure of proteins in cardiac sarcolemma. The straight line is characterized by the equation: $V = 1.55 + 2.432\,[\alpha_H]$, where V represents $(Na^+ + K^+)$-ATPase activity and $[\alpha_H]$ the α-helix contents of sarcolemmal proteins. The correlation coefficient of the straight line is 0.948, $p < 0.01$.

State E_1Na, contrary to state E_2K, is namely characterized by a higher α-helix content in proteins as it has been demonstrated on both, the enzyme and the sarcolemmal level (Gresalfi and Wallace 1984; Vrbjar et al. 1984). Hence, the nature of the above interference is probably based on a diminution of the α-helix content of membrane proteins by Ca^{2+} to a state close to E_2K of $(Na^+ + K^+)$-ATPase thus preserving the latter at the expense of E_1Na.

It is evident that the Ca-induced changes observed in protein helicity also concern membrane proteins other than $(Na^+ + K^+)$-ATPase. Consequently only a part of the decrease in α-helix content observed may be relevant to the $(Na^+ + K^+)$-ATPase molecule. Nevertheless, a mathematical analysis of the investigated structure-activity relationship revealed that the Ca-induced modulation of the secondary structure of membrane proteins is in its nature highly regulatory to $(Na^+ + K^+)$-ATPase activity in cardiac sarcolemma.

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