# Enzyme Kinetics and the Activation Energy of (Na,K)-ATPase in Ischaemic Hearts: Influence of the Duration of Ischaemia

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**Abstract.** Hearts from male rats were incubated at 37 °C for variable periods of global ischaemia. Estimation of kinetic parameters of (Na,K)-ATPase at 37 °C in the presence of increasing concentrations of ATP revealed a significant decrease of $V_{max}$ in the first 15 minutes of ischaemia with further stabilization at the lowest level in 45–60 minutes of ischaemia. The changes in ATP binding site occurred later after 45 minutes of ischaemia as showed by the decrease of the $K_m$ value. As to the activation energy, there were no significant differences between control and ischaemic hearts.

**Key words:** (Na,K)-ATPase — Activation energy — Cardiac sarcolemma — Ischaemia

## Introduction

(Na,K)-ATPase (EC 3.6.1.3), an enzyme involved in the active translocation of Na and K ions across cell membranes, is in cardiac tissue localized in sarcolemmal membranes (Asano et al. 1980, Tribulová et al. 1982). Regional long-lasting ischaemia of the rat heart induced by left coronary artery occlusion resulted in depression of (Na,K)-ATPase activity. This depression was observed 8–16 weeks after the operation and was characterized by decrease in $V_{max}$ with no changes in the affinities of the enzyme neither for Mg – ATP, $Na^+$ nor $K^+$ (Dixon et al. 1992). The authors explained these findings on the basis of enzyme inactivation, reduction in the number of enzyme molecules, and/or changes in the characteristics of the enzyme due to alterations in the lipid-protein as well as protein-protein interactions (Dhalla et al. 1978). In acute experiments, using the model of global ischaemia, diminution of intracellular concentration of ATP in ischaemic myocardium (Fedelešová et al. 1978, Reimer et al. 1981) was followed by significant decrease in (Na,K)-ATPase activity in a time-dependent manner (Kim et al. 1989). To clarify the molecular

basis of the enzyme inhibition induced by global ischaemia, the present study was undertaken to investigate the ATP-binding site by enzyme kinetics and the energy barrier of (Na,K)-ATPase reaction in ischaemic myocardium.

## Materials and Methods

Quickly excised hearts from male rats (200–250 g) were incubated at 37 °C for variable periods of global ischaemia. Cardiac sarcolemma was prepared by the hypotonic shock-NaI treatment method as described previously (Vrbjar et al. 1984). The protein content was assayed by the procedure of Lowry et al. (1951) using bovine serum albumin as a standard.

Kinetic parameters of (Na,K)-ATPase were estimated measuring the splitting of ATP by 30–50 $\mu$g sarcolemmal proteins at 37 °C in the presence of increasing concentrations of ATP in the range of 0.08–6.0 mmol/l in a total volume of 0.5 ml of medium containing 50 mmol/l imidazole (pH 7.4), 4 mmol/l $MgCl_2$, 10 mmol/l KCl and 100 mmol/l NaCl. Following 10 minutes of preincubation in the substrate free medium the reaction was started by addition of ATP and after 20 minutes was terminated by 1 ml of 12% solution of trichloroacetic acid. The inorganic phosphorus liberated was determined according to Taussky and Shorr (1953). The (Na,K)-ATPase activity was expressed as the difference between $P_i$ split in the presence of $Na^+$, $K^+$ and $Mg^{2+}$, and in the presence of $Mg^{2+}$ ions alone.

The activation energy of (Na,K)-ATPase reaction was estimated analysing the temperature dependence of ATP splitting in the range of 1–45 °C at constant concentration of ATP (4 mmol/l).

All results were expressed as mean ± S.E.M.. The significance of differences between the individual groups was determined with the use of the unpaired Student's $t$-test.

## Results

The activity of sarcolemmal (Na,K)-ATPase in the presence of a constant amount of cofactors Na and K was determined at eight concentrations of ATP in the range of 0.08 to 6.0 mmol/l. In all cases investigated, ischaemia in duration from 15 to 60 minutes induced a significant decrease in the enzyme activity (Fig. 1).

Non-linear regression analysis of the obtained data according to Michaelis-Menten equation revealed significant decrease of maximum velocities ($V_{max}$) of ATP hydrolysis in ischaemic hearts. The most significant change was observed 15 minutes after the onset of ischaemia. Prolongation of ischaemia was characterized by further diminution of $V_{max}$ value (Table 1). The value of $V_{max}$ reached after 45 minutes of ischaemia remained unchanged also after 60 minutes of ischaemia. In contrary, the value of $K_m$ was not changed significantly up to 15 minutes of ischaemia. However, prolongation of ischaemia to 30 minutes caused a considerable decrease in the $K_m$ value as compared to controls. The decrease became statistically significant after prolongation of ischaemia to 45 and/or 60 minutes (Table 1). Comparison of $K_m$ values after 45, 60 minutes of ischaemia against the value ob-

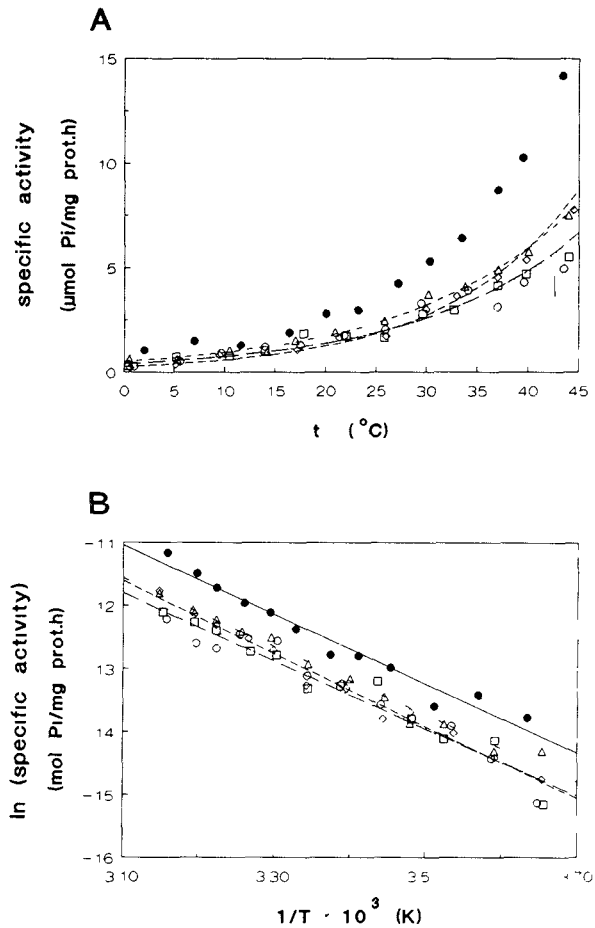


**Figure 1.** Activation by ATP of the (Na,K)-ATPase in cardiac sarcolemma in respect to duration of global ischaemia of the heart. Panel *A*: Actual data of representative measurements. Panel *B*: Lineweaver-Burk plot for the ATPase reaction. Duration of ischaemia (min): ● – contr; △ – 15; ◇ – 30; □ – 45; ○ – 60

tained after 30 minutes did not reveal any statistically significant difference.

Measurements of temperature dependence showed that activity of (Na,K)-ATPase was increasing up to 45 °C in control also in ischaemic hearts (Fig. 2*A*). Evaluation of temperature dependence of (Na,K)-ATPase activity (Fig. 2*B*) re-

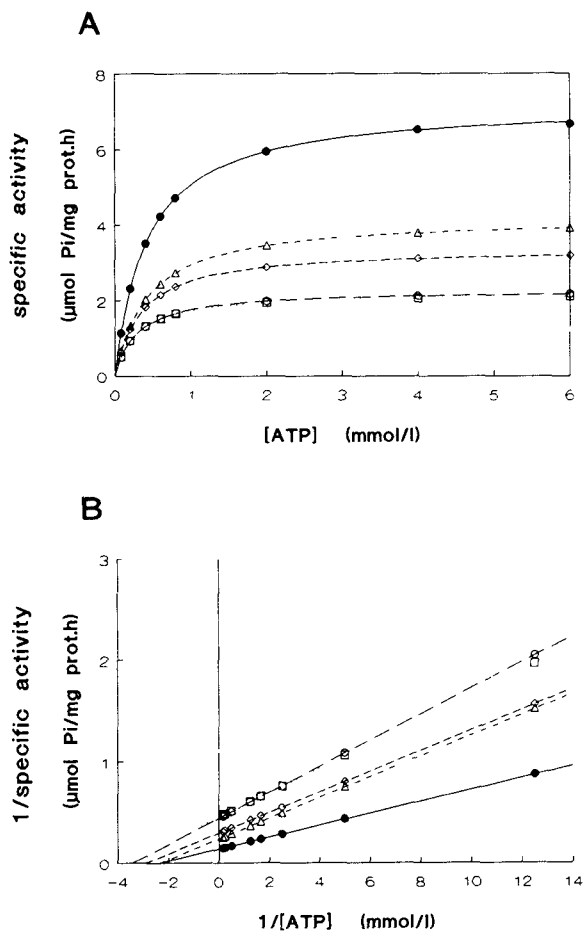


**Figure 2.** Panel *A*: Ischaemia and temperature dependence of (Na,K)-ATPase activity in cardiac sarcolemma. Representative measurements. Panel *B*: Temperature and the (Na,K)-ATPase activity; Arrhenius plot. Duration of ischaemia (min): ● – contr; △ – 15; ◇ – 30; □ – 45; ○ – 60

vealed a slightly lower value of activation energy ($E_a$) in hearts undergoing global ischaemia for 15, 45 and 60 minutes as compared to control hearts (Table 1). Ischaemia lasting 30 minutes induced an insignificant increase of $E_a$ above the value observed for controls.

**Table 1.** Kinetic parameters and activation energy of (Na,K)-ATPase from cardiac sarcolemma in hearts subjected to global ischaemia

| Ischaemia (min) | $V_{max}$ ($\mu$mol $P_i$/mg.h) | S.E.M. | $K_m$ [ATP] (mmol/l) | S.E.M. | $E_a$ (kJ/mol) | S.E.M. |
|---|---|---|---|---|---|---|
| Control | 7.20 | 0.63 | 0.422 | 0.035 | 48.45 | 1.92 |
| 15 | $4.20^2$ | 0.48 | 0.429 | 0.049 | 46.58 | 0.65 |
| 30 | $3.38^3$ | 0.70 | 0.342 | 0.046 | 50.06 | 1.91 |
| 45 | $2.20^3$ | 0.27 | $0.265^2$ | 0.039 | 45.77 | 1.39 |
| 60 | $2.28^3$ | 0.34 | $0.292^1$ | 0.027 | 44.52 | 1.73 |

Results are means of 10–18 separate measurements each performed in triplicates. Statistical significance: 1 – $p < 0.05$, 2 – $p < 0.01$, 3 – $p < 0.001$

## Discussion

It was reported previously (Vrbjar et al. 1990) that in enzyme reactions the activation energy depends on substrate concentration. A theoretical analysis of the above relationship by combination of Arrhenius and Michaelis-Menten equations revealed a biphasic relationship between the concentration of substrate and the $E_a$. Low concentrations of substrate influence the $E_a$. On the other hand, substrate at concentrations exceeding the $K_m$ value does not influence the value of $E_a$ (Vrbjar et al. 1990). Therefore, for correct estimation of $E_a$ that in ischaemic conditions would reflect the properties of the enzyme itself, it is necessary to evaluate the activation energy at high non-limiting concentrations of ATP. Accordingly, the temperature dependence of (Na,K)-ATPase reaction was estimated at the concentration of ATP of 4 mmol/l, that is 10–13 times exceeding the $K_m$ value in control and ischaemic hearts. Concentration of ionic cofactors sodium (100 mmol/l) and potassium (10 mmol/l) exceeded 7–8 times the respective $K_a$ values for activation of the (Na,K)-ATPase (Džurba et al. 1993).

Previous studies indicated that Arrhenius plots of (Na,K)-ATPase activity of cardiac sarcolemma could be resolved into two straight lines with different slopes (Matsuda and Iwata 1985). The corresponding break temperature for the enzyme from rat hearts was 25.4 °C (Matsuda and Iwata 1985). Our investigation on (Na,K)-ATPase in the temperature range of 1–45 °C produced linear plots without discontinuities in ischaemic and also in control hearts. When calculating two straight lines for the regions below and above 25 °C, the obtained slopes were practically the same with the sum of S.D. much larger than the S.D. for one line calculated over the whole temperature range (data not shown). This allowed the conclusion that in our experiments the hydrolysis of ATP catalyzed by (Na,K)-ATPase can be characterized by one value of activation energy ($E_a$) over the investigated range

of temperature. In control and ischaemic hearts the lines were almost parallel (Fig. 2*B*) suggesting thus that the energy barrier of the process in ischaemic conditions was not changed significantly when compared to control conditions.

Our observation that the decrease of $V_{max}$ of ATP hydrolysis in ischaemic hearts is most significant in the first 15 minutes agrees with the findings about the dynamics of decrease in ATP content in the heart (Fedelešová et al. 1978, Reimer et al. 1981). This suggests that diminution of activities of enzymes utilizing ATP is a response to the decreased synthesis of ATP in ischaemia. Because cardiac (Na,K)-ATPase is an essential part of the (Na,K)-pump which is suggested to participate in the maintenance of cellular resting potential (Glitsch 1979), a decline of (Na,K)-ATPase activity could cause disturbance in the electrical behaviour of the ischaemic myocardium. The decrease of $K_m$ value detected 30–45 minutes after the onset of ischaemia indicates an enhancement in apparent affinity of the enzyme for ATP. This may indicate that after 30–45 minutes of ischaemia the decrease in turnover of the (Na,K)-ATPase (reduced $V_{max}$) is already compensated by changes in the structure of ATP-binding site. The latter phenomenon may be interpreted as a mechanism securing the partial maintenance of function of the (Na,K)-ATPase in cardiac sarcolemma even in conditions with permanently insufficient supply of ATP. Similar changes in kinetic properties of sarcolemmal ATPases were also detected in hearts acclimatized to high altitude hypoxia (Ziegelhöffer et al. 1987) indicating that the same mechanism of adaptation on enzyme level might be involved in various physiological and pathophysiological situations accompanied by the decrease of intracellular ATP content. The fact that structural changes of (Na,K)-ATPase in the vicinity of ATP binding site occurred between 15–30 minutes of ischaemia is supported by cytochemical observations showing the most profound changes in distribution of (Na,K)-ATPase in the same period which is the time interval believed when ischaemia-induced changes tend to be irreversible (Miura et al. 1991). The ability of the (Na,K)-ATPase to increase the affinity of its ATP-binding site observed in our acute experiments is presumably sensitive to the duration of the ischaemia. When the heart was subjected to long-lasting persistent ischaemia, no changes in $K_m$ value were observed (Dixon et al. 1992).

**Acknowledgements.** This research was supported by the Slovak Grant Agency (grant No. 999241). The authors thank Mrs. E. Havránková for her technical assistance.

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Final version accepted October 28, 1994