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Short-term NO Synthase Inhibition and the ATP Affinity of Cardiac Na,K-ATPase

N. VRBJAR, M. STRNISKOVÁ, O. PECHÁŇOVÁ[1], M. GEROVÁ[1]

Institute for Heart Research and [1]Institute of Normal and Pathological Physiology, Slovak Academy of Sciences, Bratislava, Slovak Republic

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Summary

It was previously shown that 4 hours´ lasting inhibition of nitric oxide synthesis by administration of an L-arginine analogue, the N^G-nitro-L-arginine methyl ester (L-NAME) changed the affinity of the Na-binding site of Na,K-ATPase thus resulting in elevation of enzyme activity especially at higher concentrations of sodium. Using the same experimental model, we focused our attention in the present study to the question of binding of ATP to the enzyme molecule in the left ventricle (LV), ventricular septum (S) and the right ventricle (RV) of the dog heart. Activation of the enzyme by increasing concentrations of ATP revealed a significant increase of the V_{max} only in septum (by 38 %). The K_M increased significantly in septum (by 40 %) and in left ventricle (by 56 %) indicating an altered sensitivity of the ATP-binding site of Na,K-ATPase in the hearts of NO-deficient animals. The alterations of Na,K-ATPase in its ability to bind and hydrolyze ATP are localized to the tissue surrounding the cavity of the left ventricle.

Key words
Sodium pump • Heart • Pressure overload • Nitric oxide • L-NAME

Introduction

The Na,K-ATPase in the heart is involved in reestablishing the transmembraneous ionic gradients, following the influx of Na^+ and Ca^{2+} as well as the efflux of K^+ ions from cardiac cells during the processes of contraction and relaxation. For the countertransport of 3 Na^+ out from the intracellular space and 2 K^+ into the cell, the enzyme utilizes the energy derived from hydrolysis of 1 molecule of ATP, originating in the heart predominantly from glycolysis (for review see Ziegelhöffer *et al.* 2000). The Na,K-ATPase was shown in various tissues to be inhibited (Guzman *et al.* 1995) and also stimulated (Gupta *et al.* 1994, 1995, Vrbjar *et al.* 1999) by nitric oxide, to which the role of endothelium-derived relaxing factor has been ascribed (Palmer *et al.* 1987, Ignarro *et al.* 1987, Khan and Furchgott 1987).

Earlier studies performed in our laboratory demonstrated that 4 hours´ lasting inhibition of nitric oxide synthesis by administration of an L-arginine analogue, the N^G-nitro-L-arginine methyl ester (L-NAME), changed the affinity of Na^+-binding site of the Na,K-ATPase thus enhancing enzyme activity especially at higher concentration of sodium. This suggested that during the short-term inhibition of NO synthesis Na,K-ATPase is capable of extruding the excessive Na^+ more effectively also at higher $[Na^+]_i$ from the subsarcolemmal compartment of myocardial cells (Vrbjar *et al.* 2000).

Fax+4202 24920590
E-mail: physres@biomed.cas.cz http://www.biomed.cas.cz/physiolres

The present study was designed to investigate the energy utilization of Na,K-ATPase and its ATP-binding properties under conditions of short-term inhibition of NO synthesis in the canine heart.

Methods

The physiological model has been described in detail previously (Gerová *et al*. 1998, Gerová 2000). Briefly, anesthetized adult mongrel dogs were treated by intravenous administration of L-NAME each hour in a dose of 50 mg/kg, amounting to a total of 200 mg/kg in the course of a 4-hour period. The blood pressure was monitored *via* a cannula introduced into the brachial artery by a Statham Manometer and registered on a Physioscript Schwarzer. The systolic blood pressure at the beginning and the end of the experiment did not differ significantly. Nevertheless, the diastolic pressure increased from 131.8±0.87 to 149.4±3.98 mm Hg. At the end of the experiment, samples of the cardiac tissue were taken, frozen in liquid nitrogen and used for further analysis.

Cardiac sarcolemma was prepared by the hypotonic shock-NaI treatment method (Vrbjar *et al*. 1984). Protein concentrations were estimated by the method of Lowry *et al*. (1951). The kinetics of Na,K-ATPase was estimated by measuring the splitting of ATP by 30 μg of sarcolemmal proteins at 37 °C. The samples were pre-incubated without ATP for 15 min, then the reaction was started by addition of ATP and it was stopped after an other 15 min by 12 % ice-cold TCA. The concentration of ATP varied in the range of 0.08-4.00 mmol/l. The concentration of cofactors and other components was constant; imidazole 50 (pH=7.4), $MgCl_2$ 4, NaCl 100 and KCl 10 (in mmol/l). The inorganic phosphorus liberated was determined according to the method of Taussky and Shorr (1953). In order to obtain the Na,K-ATPase activity, the ATP hydrolysis that occurred in the presence of Mg^{2+} only, was subtracted.

The kinetic parameters were evaluated by direct nonlinear regression of the obtained data. All results were expressed as mean ± S.E.M. The significance of differences between the individual groups was determined by the unpaired Student's t-test and the Bonferroni test. A value of $p<0.05$ was regarded as significant.

Results

Activation of Na,K-ATPase from the left ventricle with increasing concentrations of ATP revealed an inhibition of the enzyme throughout the investigated concentration range in tissues of animals which were treated with L-NAME. At the lowest investigated concentration of ATP (0.08 mmol/l) the inhibition represented 33 %. With increasing concentrations of the substrate, the inhibitory effect gradually decreased to 6 % which we observed in the presence of 4 mmol/l (Fig. 1A). These changes point to a significant alteration of K_m value as is also shown in the Hanes plot of the data (Fig. 1B). More precise evaluation of the data presented in Figure 1A by the method of nonlinear regression revealed more reliable K_m and V_{max} values, shown in Figure 4. Application of L-NAME resulted in a statistically significant rise of the K_m value by 56 % without any significant alteration of the V_{max} value as compared to the controls (Fig. 4).

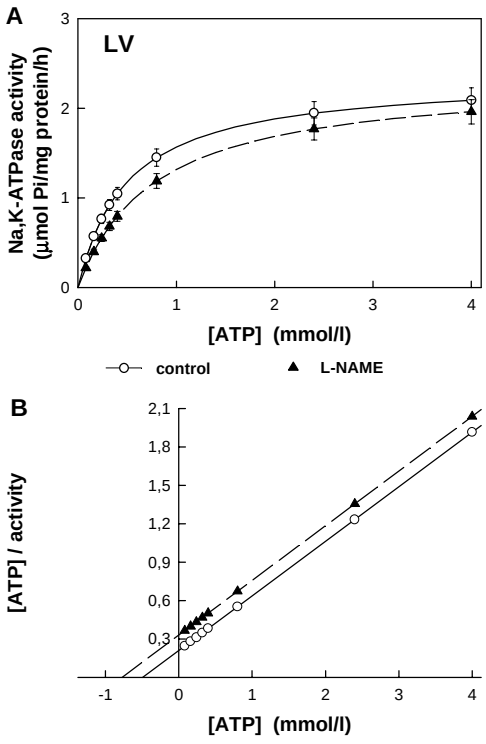

Fig. 1. *Influence of acute in vivo inhibition of NO synthesis on the Na,K-ATPase in the left ventricle of the canine heart. Panel A: Activation of the enzyme with increasing concentrations of ATP. Panel B: Transformation of the data to Hanes plot.*

In the ventricular septum the increase of ATP concentration was followed by a gradual stimulation of the Na,K-ATPase in tissues of NO-deficient dogs. At the concentration of ATP of 0.08 mmol/l, the stimulatory effect was still insignificant but in the presence of 0.16

mmol/l ATP the stimulation already represented 6 % and at 4 mmol/l ATP the simulation reached 32 % (Fig. 2A). The evaluation of kinetic parameters revealed a significant increase of both investigated values. After the application of L-NAME the V_{max} increased by 38 % and the K_m by 40 % (Fig. 4).

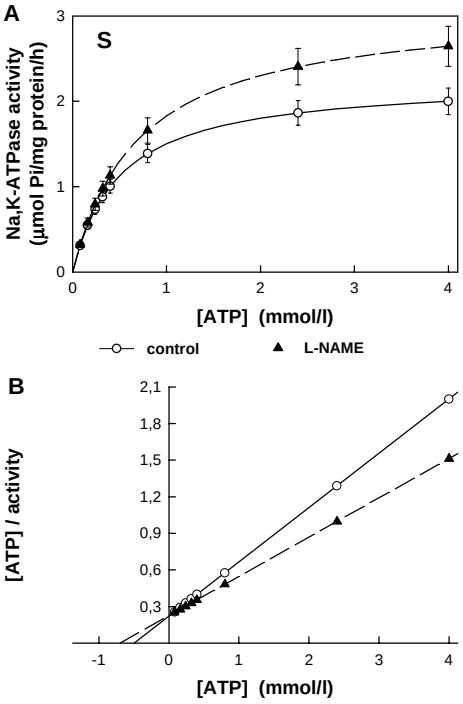

Fig. 2. *Influence of acute in vivo inhibition of NO synthesis on the Na,K-ATPase in the ventricular septum of the canine heart. Panel A: Activation of the enzyme with increasing concentrations of ATP. Panel B: Transformation of the data to Hanes plot.*

The NO-deficiency in the right ventricle did not induce any significant change in Na,K-ATPase activity throughout the investigated concentration range of ATP (Fig. 3). Consequently the V_{max} and K_m values did not differ significantly (Fig. 4).

Discussion

It was demonstrated previously that NO-deficiency induces changes in the affinity of Na^+-binding site of the Na,K-ATPase thus elevating of enzyme activity especially at higher concentrations of sodium. This suggested that during the short-term inhibition of NO synthesis Na,K-ATPase is capable of extruding the excessive Na^+ more effectively also at higher $[Na^+]_i$ from the subsarcolemmal compartment of myocardial cells. These changes were similar in all investigated parts of the heart (Vrbjar *et al*. 2000).

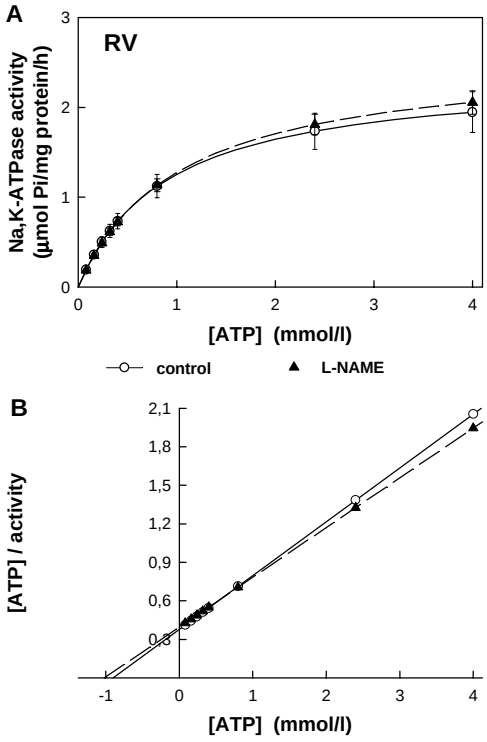

Fig. 3. *Influence of acute in vivo inhibition of NO synthesis on the Na,K-ATPase in the right ventricle of the canine heart. Panel A: Activation of the enzyme with increasing concentrations of ATP. Panel B: Transformation of the data to Hanes plot.*

Considering the energy utilization and ATP-binding properties, the 4 hours´ lasting NO-deficiency induced by application of NO-synthase inhibitor L-NAME, was accompanied by specific responses of the cardiac Na,K-ATPase in various regions of canine ventricles.

In the left ventricle the enzyme binds and hydrolyzes ATP less effectively, as revealed by the increased value of K_m. This deterioration is more significant especially in the presence of lower ATP concentrations which are of physiological relevance in the intracellular space. Increasing the concentration of substrate above this range masked the decreased sensitivity of the ATP-binding site. These enzyme alterations may have two different mechanisms. One of them might be due to the hypothetical presence of an

inhibitor competing for the ATP-binding site on the molecule of Na,K-ATPase.

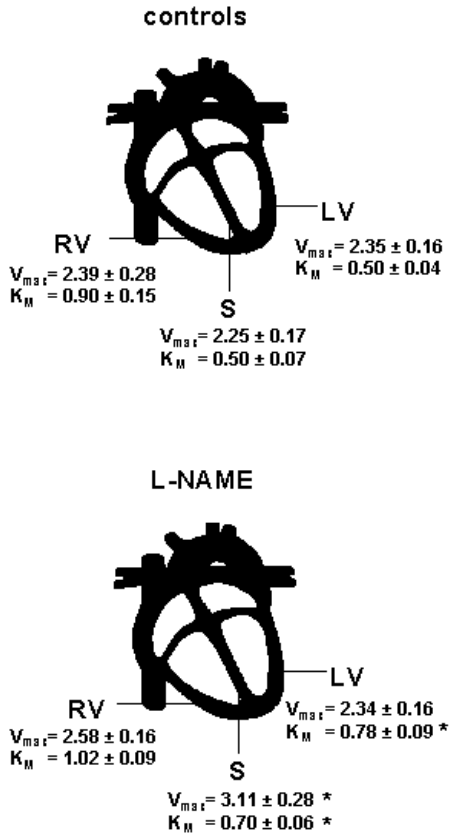

Fig. 4. *Kinetic parameters of Na,K-ATPase in canine heart after acute in vivo inhibition of NO synthesis. Number of estimations for each tissue and experimental group was the following: control group LV=15, S=8, RV=10; L-NAME group LV=12, S=6, RV=8. The V_{max} is expressed in μmol P_i/mg protein/h and the K_M in mmol/l of ATP. Data represent mean ± SEM, * $p<0.05$ as compared to controls.*

The existence of such an inhibitor seems to be unlikely since it should be washed out during preparation of the sarcolemmal membrane fraction. The second, more probable possibility might concern the fact that NO-deficiency induces a conformational change in the polypeptide chain of the enzyme in the vicinity of the ATP-binding site. These conformational alterations are situated probably within the cytoplasmic protrusion of the α subunit between the membranous loops M 4 and M 5 as the ATP binding site was localized previously in this region (Blanco and Mercer 1998, Jorgensen and Pedersen 2001).

In the ventricular septum, the response of Na,K-ATPase to short-term NO-deficiency is completely different from its response in the left ventricle. Instead of inhibition, the enzyme is stimulated in this part of the heart, especially in the presence of higher concentrations of the substrate, resulting in an apparent decrease of the affinity of ATP-binding site. Thus under conditions of NO-deficiency the Na,K-ATPase in ventricular septum is also able to utilize ATP in the presence of such high concentrations which are not followed by a significant additional increase of enzyme activity in the controls. The physiological relevance of the fact is not clear and needs further elucidation.

The alterations of the ability of Na,K-ATPase to bind and hydrolyze ATP are present in tissue surrounding the cavity of the left ventricle, as suggested by the lack of any effect on the enzyme activity in tissue sections from the right ventricle of NO-deficient hearts.

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Reprint requests

N. Vrbjar, Institute for Heart Research, Slovak Academy of Sciences, Dúbravská cesta 9, 842 33 Bratislava, Slovak Republic. Fax: +421 2 5477 6637, E-mail: usrdnorb@savba.sk