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Short-Term NO Synthase Inhibition and the Na^+-Binding Properties of Cardiac Na,K-ATPase

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Summary

It is known that hypertension is accompanied by increased $[Na^+]_i$. The functional properties of Na,K-ATPase, which transports the Na^+ out and K^+ into myocardial cells during the relaxation phase, were investigated in the left ventricle (LV), septum (SV) and the right ventricle (RV) of anesthetized dogs with moderate acute blood pressure elevation elicited by short-term (4-hour) NO synthase inhibition. The NO-insufficiency was induced by administration of an L-arginine analogue, the N^G-nitro-L-arginine methyl ester (L-NAME). Concerning the function of Na,K-ATPase under the conditions of lowered NO synthesis, we focused our attention to the binding of Na^+ to the enzyme molecule. Activation of the enzyme by increasing Na^+ concentrations revealed significant changes in both the maximal velocity (V_{max}) and the affinity for Na^+ (K_{Na}) in all investigated heart sections. The V_{max} increased by 27 % in LV, by 87 % in SV and by 58 % in RV. The K_{Na} value increased by 86 % in LV, by 105 % in SV and by 93% in RV, indicating an apparent decrease in the sensitivity of the Na^+-binding site in the Na,K-ATPase molecule. This apparently decreased pump affinity for Na^+ together with the increase of V_{max} suggest that, during the short-term inhibition of NO synthesis, the Na,K-ATPase is capable of extruding the excessive Na^+ from the myocardial cells more effectively at higher $[Na^+]_i$ as compared to the Na,K-ATPase of control animals.

Key words

Na,K-ATPase • Heart • Pressure overload • Nitric oxide • L-NAME

Introduction

Nitric oxide (NO) formed from L-arginine in endothelial cells plays a substantial role in regulation of the cardiovascular system acting as a relaxing factor (Palmer *et al.* 1987). Chronic inhibition of NO synthesis by analogues of L-arginine resulted in hypertension (Ribeiro *et al.* 1992, Dananberg *et al.* 1993, Bernátová and Pecháňová 1994, Pecháňová and Bernátová 1996). In acute experiments, the inhibition of NO synthesis lasting 4 h also elevated the blood pressure, similarly as did 4-h lasting aortic stenosis or noradrenaline infusion. The pressure overload was accompanied in all three models with significantly enhanced proteosynthesis in the myocardium as well as in epicardial conduit coronary arteries (Gerová *et al.* 1995, 1996, 1998). Since hypertension is accompanied with increased $[Na^+]_i$ (Jelicks and Gupta 1994), the activity of Na,K-ATPase might be altered in conditions of lowered NO synthesis. This enzyme involved in the active translocation of Na^+

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and K^+ ions across cell membranes during the relaxation phase, was shown to be inhibited in various tissues (Guzman *et al.* 1995) but also stimulated by nitric oxide (Gupta *et al.* 1994, 1995). Inhibition of NO synthesis in acute experiments by high doses of the L-arginine analogue decreased the activity of Na,K-ATPase (Groenendaal *et al.* 1995). In our study, we inhibited NO synthesis by administration of an L-arginine analogue, the N^G-nitro-L-arginine methyl ester (L-NAME), and we thus increased the blood pressure. Concerning the function of Na,K-ATPase under conditions with lowered synthesis of NO, we focused our attention on the question of the binding of Na^+ to the enzyme molecule.

Material and Methods

Experimental model

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. Some physiological and biochemical characteristics of this model have been described in detail previously (Gerová *et al.* 1998). The systolic blood pressure at the beginning and 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 investigations.

Na,K-ATPase measurements

Cardiac sarcolemma was prepared by the hypotonic shock-NaI treatment method (Vrbjar *et al.* 1984). The protein concentration was 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 preincubated without ATP for 15 min, then the reaction was started by addition of ATP and it was stopped after another 15 min by 12 % ice cold trichloracetic acid. The concentration of NaCl varied in the range of 2-100 mmol/l. The concentration of substrate, buffer and other cofactors was constant: ATP 4, imidazole 50, pH 7.4, $MgCl_2$ 4 and KCl 10 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, ATP hydrolysis that had occurred in the presence of Mg^{2+} only, was subtracted.

Data processing and statistical analysis

The kinetic parameters were evaluated by direct nonlinear regression of the obtained data. All results were expressed as means ± 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.

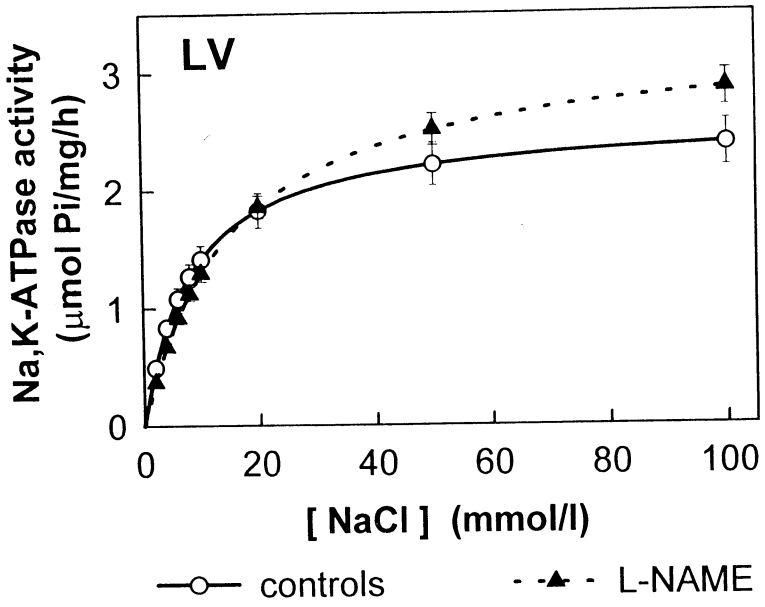

Fig. 1. *The influence of acute in vivo inhibition of the NO synthesis on the Na,K-ATPase in the left ventricle of dog heart.*

Results

Activation of the Na,K-ATPase by increasing Na^+ concentrations revealed a significant increase of the enzyme activity especially at higher concentrations of Na^+ in all investigated heart sections from dogs subjected to the inhibition of NO-synthase by L-NAME for 4 h. In the left ventricle, the activities were significantly higher from 50 mmol/l of NaCl (Fig. 1), in the septum from 10 mmol/l (Fig. 2) and in the right ventricle from 20 mmo/l of NaCl (Fig. 3).

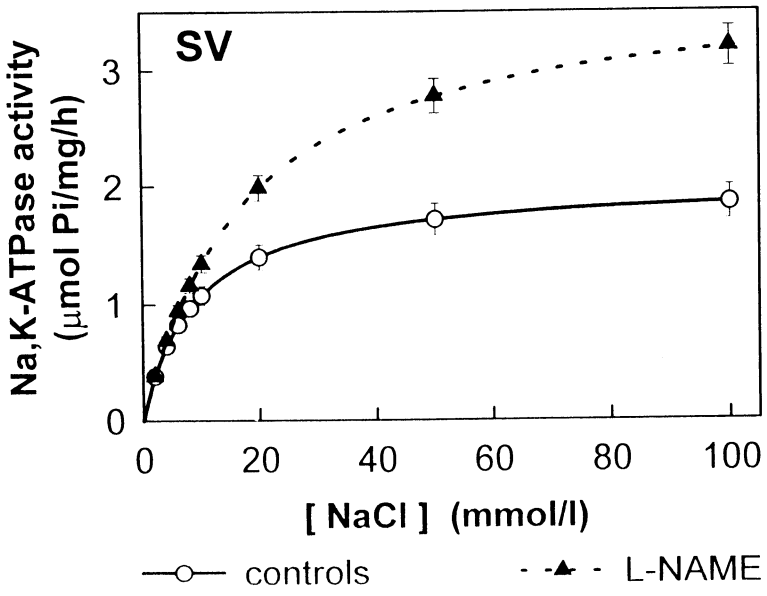

Fig. 2. *The influence of acute in vivo inhibition of the NO synthesis on the Na,K-ATPase in the ventricular septum of dog heart.*

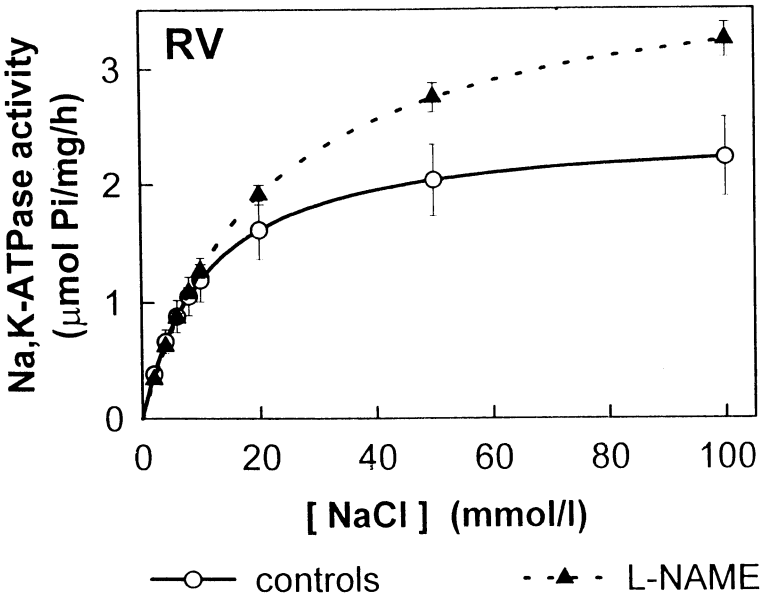

Fig. 3. *The influence of acute in vivo inhibition of the NO synthesis on the Na,K-ATPase in the right ventricle of dog heart.*

The evaluation of kinetic parameters of the Na,K-ATPase by non-linear regression revealed significant changes of the maximal velocity (V_{max}) of Na,K-ATPase, and also of its affinity for Na^+ (K_{Na}) in all investigated heart sections of dogs treated with L-NAME. The V_{max} increased by 27 % in the left ventricle, by 87 % in ventricular septum and by 58 % in the right ventricle (Table 1).

The K_{Na} value increased by 86 % in the left ventricle, by 105 % in the septum and by 93 % in the right ventricle (Table 2), indicating an apparent decrease in sensitivity of the Na^+-binding site in the Na,K-ATPase molecule.

Table 1. Effect of acute L-NAME treatment on the maximal velocity (V_{max}) of Na,K-ATPase in dog hearts.

V_{max}	Control	L-NAME
Left ventricle	2.596±0.215	3.308±0.177*
	n=9	n=8
Septum	2.021±0.154	3.770±0.205*
	n=7	n=7
Right ventricle	2.483±0.383	3.916±0.176*
	n=6	n=8

*Data represent mean ± SEM. V_{max} values are expressed in μmol P/ mg protein/h. * $p<0.05$ as compared to control*

Table 2. Effect of acute L-NAME treatment on the affinity for Na^+ (K_{Na}) of Na,K-ATPase in dog hearts.

K_{Na}	Control	L-NAME
Left ventricle	8.42±0.64	15.63±1.35*
	n=9	n=8
Septum	8.73±1.15	17.91±1.42*
	n=7	n=7
Right ventricle	10.85±1.56	20.95±1.66*
	n=6	n=8

*Data represent mean ± SEM. K_{Na} values are expressed in mmol/l of NaCl.. * $p<0.05$ as compared to control*

Discussion

Intracellular Na^+ homeostasis is based on a balance between the influx and efflux of Na^+. The influx is mediated by various pathways including Na^+ leakage through different Na^+ channels, Na^+-K^+/2Cl^- cotransport, Na^+/H^+ exchanger and Na^+/HCO_3 cotransport. In hypertension the enhanced Na^+ leakage is responsible for the increase of intracellular Na^+ concentration (Friedman 1979, Jones 1982, Zicha and Kuneš 1999).

Under these conditions the functional properties of Na,K-ATPase which mediates the Na^+ efflux from cells are also very important. Our data show that already 4 h lasting pressure overload caused by inhibition of NO-synthase induces significant changes in the functional properties of Na,K-ATPase as was also observed after the long-lasting inhibition of NO-synthesis in rats (Vrbjar *et al.* 1998, Vrbjar *et al.* 1999c). The apparently decreased affinity for Na^+ together with increased V_{max} suggest that the enzyme adapts to altered ion homeostasis induced by enhanced diastolic blood pressure during the short-term inhibition of NO synthesis. Thus the Na,K-pump of NO-deficient dogs is able to extrude the excessive Na^+ out of myocardial cells more effectively also at higher $[Na^+]_i$, while Na,K-ATPase from control animals is unable to increase its activity further.

In the hearts of dogs with inhibited synthesis of NO, the increased maximal velocity of Na,K-ATPase seems to represent a mechanism compensating for the decrease in the level of the endothelium-derived relaxing factor (NO). This hypothesis is supported by the fact that the acetylcholine hypotension remained unchanged or was even more pronounced in animals with acute or chronic inhibition of NO synthesis (Gerová 1999).

Na,K-ATPase activity is known to affect important endothelial functions, including the regulation of cytosolic Ca^{2+} and the angiotensin-converting enzyme (ACE). For example, baseline ACE levels in cultured bovine endothelial cells are decreased 10-fold by ouabain, a specific inhibitor of Na,K-ATPase, and ouabain also blocks Ca^{2+}-dependent increases in ACE (Dasarathy *et al.* 1989). This effect was not seen with inhibitors of the Na^+-K^+/2Cl cotransport or the Na^+/H^+ exchanger. The increased function of Na,K-ATPase during acute inhibition of NO synthesis in our experiments probably provides favorable conditions for persisting activity of ACE, thus ensuring the increase of blood pressure. This hypothesis is supported by the finding that administration of L-NAME was followed by unchanged or increased activity of ACE (Yang *et al.* 1996).

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