Functional Alterations of Cardiac (Na,K)-ATPase in L-NAME Induced Hypertension

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Abstract. (Na,K)-ATPase, an enzyme involved in the active translocation of Na^+ and K^+ ions across cell membranes was shown to be affected by nitric oxide (NO) in various tissues In the present study the functional alterations of (Na,K)-ATPase after chronic inhibition of nitric oxide synthesis were studied in rat hearts Four weeks lasting administration of an L-arginine analogue, the N^G-nitro-L-arginine methyl ester (L-NAME) induced an increase in the systolic blood pressure of about 36% In this hypertension the kinetic parameters K_m and V_{max} for ATP-activation of the (Na,K)-ATPase did not show any significant changes Activation of the enzyme by its cofactor Na^+ revealed no change in the V_{max}, but the K_{Na} increased by 50% Two weeks after terminating the administration of L-NAME the blood pressure returned to control values In these conditions the activity of (Na,K)-ATPase increased, due to enlarged affinity of the ATP-binding site as revealed from the diminished K_m value for ATP The K_{Na} value for activation with Na^+ returned to control value

Our findings indicate that there is no change in energy utilization by the (Na,K)-ATPase during L-NAME induced hypertension in the heart The transport properties of the enzyme are deteriorated, due to its decreased sensitivity to Na^+ This inhibition of the (Na,K)-ATPase might be responsible for the increase of $[Na^+]_i$ during lowered NO synthesis In hearts from rats that recovered from the hypertension, the (Na,K)-ATPase increases its activity due to improved ATP binding properties

Key words: (Na,K)-ATPase — Nitric oxide — L-NAME — Heart

Introduction

Nitric oxide (NO) is widely recognized as a second messenger, and it has been shown to participate in physiological as well as pathophysiological functions of many organ systems Inhibition of NO-synthesis in acute experiment by high doses of L-arginine analogue decreased the activity of (Na,K)-ATPase (Groennedaal *et al* 1995) This enzyme involved in the active translocation of Na^+ and K^+ ions across cell membranes, was shown in various tissues to be inhibited (Groenendaal *et al* 1995, Guzman *et al* 1995) and also stimulated

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(Gupta *et al* 1994, Gupta *et al* 1995) by nitric oxide In our study we applied an analogue of L-arginine L-NAME using a model of chronic inhibition of NO synthase which is well characterized from physiological, morphological and biochemical point of view (Pecháňová and Bernátová 1996, Holécyová *et al* 1996, Babál *et al* 1997) Concerning the function of (Na,K)-ATPase at conditions with various blood pressure we focused our attention to two main questions One concerned the energy supply by ATP and the other involved the binding of Na^+ to the enzyme molecule

Materials and Methods

15 weeks old male Wistar Kyoto rats were divided into three groups First group served as controls, the second group "L-NAME group" was treated with analogue of L-arginine the N^G-nitro-L-arginine methyl ester (L-NAME) in a dose 40 mg/kg/day in the drinking water for 4 weeks, the third group "recovery group" was also treated with the same dose of L-NAME for four weeks with following two weeks without any treatment with the aim to allow to the animals to recover from the inhibition of NO synthase The systolic blood pressure (SBP) was measured by a noninvasive method – tail cuff pletysmography Cardiac sarcolemma was prepared by the hypotonic shock-NaI treatment method (Vrbjar *et al* 1984) The kinetics of (Na,K)-ATPase was estimated measuring the splitting of ATP by 30 μg of sarcolemmal proteins at 37 °C For substrate kinetics the ATP varied in the range of 0 08–4 0 mmol/l with constant concentration of NaCl 100 mmol/l For sodium kinetics the concentrations of NaCl varied in the range 2 100 mmol/l using constant amount of ATP 4 mmol/l

Results and Discussion

Four weeks lasting administration of NO-synthase inhibitor L-NAME induced a significant increase of the SBP from 120 ± 3 to 170 ± 8 mm Hg in the L-NAME group Two weeks after terminating the application of L-NAME the SBP decreased in the recovery group to 116 ± 5 mm Hg This value was similar to value observed in the control group

At the activation of the (Na,K)-ATPase with increasing concentrations of ATP the hypertension induced by L-NAME did not provoke significant changes in both, the V_{max} and the K_m value (Table 1) On the other side the V_{max} increased significantly in the recovery group probably due to increased affinity of the ATP binding site as it revealed from the profound decrease of the K_m value (Table 1)

Table 1. Kinetic parameters of (Na,K)-ATPase in the heart at normal conditions, at L NAME induced hypertension and after recovery

	activation with ATP		activation with Na^+	
	K_m ± SEM	V_{max} ± SEM	K_{Na} ± SEM	V_{max} ± SEM
	[ATP] (mmol/l)	(nmol P_i/mg/min)	[ATP] (mmol/l)	(nmol P_i/mg/min)
controls	0 54 ± 0 05	86 70 ± 2 95	8 18 ± 0 83	65 70 ± 5 90
L-NAME	0 53 ± 0 05	71 60 ± 5 83	12 98 ± 1 02 [a]	66 55 ± 7 15
recovery	0 31 + 0 02 [a b]	137 38 + 5 37 [a b]	7 01 + 1 42 [b]	157 63 + 3 20 [a b]

Statistical significance $p < 0\ 05$, a vs controls, b vs L-NAME group

Activation of the (Na,K)-ATPase by its cofactor Na^+ revealed an inhibition of the enzyme at all investigated concentrations in hearts from the L-NAME group Evaluation of the nature of this inhibition resulted in unchanged V_{max} value but significantly increased K_{Na} value for the activation with Na^+ (Table 1) In the recovery group the V_{max} increased significantly as compared to controls and also to the L-NAME group with lowered synthesis of NO The value of K_{Na} was similar in controls and in recovery group but it increased significantly in conditions with L-NAME induced hypertension

Our findings indicate that there is no change in energy utilization by the (Na,K)-ATPase during L-NAME induced hypertension in the heart The transport properties of the enzyme are deteriorated, due to its decreased sensitivity to Na^+ This inhibition of the (Na,K)-ATPase might be responsible for the increase of $[Na^+]_i$ during hypertension In hearts from rats that recovered from the hypertension, the (Na,K)-ATPase increases its activity due to improved ATP binding properties

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