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Effect of L-Arginine on Na^+,K^+-ATPase Activity in Rat Aorta Endothelium

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Abstract—The effect of L-arginine on the Na^+,K^+-ATPase activity in rat aorta endothelium was studied at its physiological concentrations in the range of 10^{-6}-10^{-3} M. The enzyme activity was 35.5% increased by low concentrations of L-arginine ($\leq 10^{-5}$ M) and its activity was 32.3-37.1% decreased at the L-arginine concentrations of 10^{-4}-10^{-3} M. A similar inhibition (by 34.5-42.8%) was also found in the presence of a NO-donor nitroglycerol (10^{-4}-10^{-3} M). An optical isomer of L-arginine, D-arginine, at the concentrations of $\leq 10^{-5}$ M also increased the enzyme activity by 37.1%, but its inhibiting effect was much less pronounced and was 15.7% at the D-arginine concentration of 10^{-3} M. An inhibitor of NO-synthase, L-NAME (N^G-nitroarginine, methyl ester), failed to inhibit Na^+,K^+-ATPase. However, the presence of L-NAME abolished the inhibition of Na^+,K^+-ATPase by high concentrations of L-arginine. Thus, the effect of L-arginine on the endothelial Na^+-pump depended on its concentration, and it is suggested that the enzyme inhibition by high concentrations of L-arginine should be associated with activation of the endogenous synthesis of NO.

Key words: Na^+,K^+-ATPase, endothelium, aorta, L-arginine, NO

At present, nitric oxide (NO) is considered to be one of the key regulators of vascular tonus during life activity. Even in early studies vascular endothelium cells were shown to synthesize NO from its biological precursor L-arginine, both endogenous and exogenous [1]. A direct stimulation by exogenous L-arginine of NO synthesis in endothelium was shown by the finding of suppression by inhibitors of NO-synthase (NOS) of the vasodilating effect of L-arginine in vessels with intact endothelium [2, 3]. Competitive systems of L-arginine synthesis and conversion [4, 5] responsible for the maintenance of a certain level of the substrate for NO synthesis by endothelial tissues under physiological conditions also provide the functioning of one of the most important regulatory mechanisms of vascular tonus in the body.

Recent studies resulted in significant progress in the comprehension of a variety of physiological functions of NO, including the functions of NO synthesized by endothelial tissues. However, the role of NO in the regulation of ionic homeostasis in endothelium remains nearly unstudied. This function of NO can be provided by its effect on one of the key transport systems, the Na^+-pump (Na^+,K^+-ATPase, EC 3.6.1.37) of endothelium, an enzyme which is present in virtually all cells and is responsible for Na^+ and K^+ transfer across plasma membranes against their concentration gradients [6].

The system of endothelial transport mechanisms which also include the Na^+-pump has been given little attention, and endothelial tissues were mainly considered to be a source of various endogenous agents involved in the regulation of vascular tonus [1]. But the contribution of the Na^+-pump to ionic homeostasis in the endothelium is important for normal functions of the enzyme systems responsible for endothelial regulation [7] of vascular tonus under physiological conditions [1] and also for direct maintenance of vascular tonus due to involvement of the enzyme in the regulation of the coupled ion transport systems in the cell, in particular, of the Na^+/Ca^{2+}-exchanger.

The effect of NO on ion transport systems, including Na^+,K^+-ATPase, has been studied in various tissues, but the data available are rather contradictory. Thus, NO was found to activate the Na^+/H^+-antiport [8] and to suppress Na^+,K^+-ATPase in renal proximal channels [9], to indirectly increase the activity of Na^+,K^+-ATPase in aorta [10], and to display an isoform-specific inhibiting effect on Na^+,K^+-ATPase in the endothelium of brain vessels [11]. According to the literature [12], α_1- and α_2-isoforms of Na^+,K^+-ATPase are expressed in the aorta endothelium. And although it has been reported [11] that the NO-

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associated inhibition only of the α_3-isoform of Na^+,K^+-ATPase occurs, the problem of a selective effect of NO on definite isoforms of Na^+,K^+-ATPase is still unclear. Thus, sodium nitroprusside was shown to inhibit Na^+,K^+-ATPase in various cells, in particular, in cells containing the enzyme α_1-isoform [13].

We have recently shown that long-term administration of L-arginine *in vivo* significantly increased the activity of Na^+,K^+-ATPase in a number of tissues, and this increase seemed to be associated with an increased production of NO. This effect was the most pronounced in the aorta endothelium [14]. Therefore, to assess the effect of L-arginine as a NO precursor on the endothelium transport system, our work was designed to study *in vitro* the effect of physiological concentrations of L-arginine on the Na^+,K^+-ATPase activity in the rat aorta endothelium.

MATERIALS AND METHODS

White Wistar rats with the body weight of 200-250 g were used. Aortas removed from the decapitated animals were carefully washed with cool (2°C) 0.02 M Tris-HCl buffer (pH 7.4). A scrape of the endothelial layer was homogenized with a tenfold volume of medium (0.25 M sucrose, 0.02 M Tris-HCl buffer (pH 7.4)) at 2°C. In some experiments the microsomal fraction of rat kidney cortex was used. To prepare the microsomal fraction, the tissue homogenate in a fivefold volume of medium (0.25 M sucrose, 0.02 M Tris-HCl buffer, 1 mM EDTA (pH 7.4)) was centrifuged at 11,000*g*, r_{av} = 6.5 cm, 15 min, 2°C. Then the supernatant was centrifuged at 48,000*g*, r_{av} = 6 cm, 50 min, 2°C, as described in [14].

The activity of Na^+,K^+-ATPase was determined in endothelium tissue homogenates in 1 ml of the following medium: 25 mM Tris-HCl, 5 mM $MgCl_2$, 100 mM NaCl, 10 mM KCl, 3 mM Na_2ATP (pH 7.4). The protein amount was determined by the Lowry method [15]. A sample of the homogenate (100-120 μg protein) was preincubated for 15 min at 37°C (pH 7.4) with given concentrations of L-arginine and of other reagents. Then other components of the medium were introduced for determination the Na^+,K^+-ATPase activity. The reaction was initiated by addition of Na_2ATP, performed for 10 min at 37°C, and stopped by addition of SDS to the final concentration of 0.3%. The activity of Na^+,K^+-ATPase was determined by an increase in inorganic phosphate (P_i) by the Fiske—Subarrow method [16] as a difference between the total Mg^{2+},Na^+,K^+-ATPase activity and the activity of Mg^{2+}-ATPase and expressed in μmol P_i/h per mg protein. No significant changes in the Mg^{2+}-ATPase activity were found under the influence of the agents under study.

The following reagents were used: SDS, Tris (base) from Serva (Germany), Na_2ATP (Reanal, Hungary), L-arginine, D-arginine, L-NAME (N^G-nitroarginine, methyl ester), L-citrulline, DL-arginine, sodium nitroprusside (Sigma, USA), and reagents of domestic production pure for analysis. All solutions were prepared with bidistilled water. The findings are presented as the mean ± standard error. The significance was evaluated using Student's *t*-test. The value of $p < 0.05$ was considered to be statistically significant.

RESULTS AND DISCUSSION

The effect of L-arginine on the activity of Na^+,K^+-ATPase was studied in the concentration range of 10^{-6}-10^{-3} M, which corresponds to L-arginine concentrations in tissues under physiological conditions [4, 5]. The table shows the inhibiting effect of L-arginine at concentrations of 10^{-4} and 10^{-3} M on Na^+,K^+-ATPase. The decrease in the Na^+,K^+-ATPase activity was by 32.3 and 37.1%, respectively. The activity of Na^+,K^+-ATPase was also decreased in the presence of an NO donor nitroglycerol at the concentrations of 10^{-4} and 10^{-3} M by 34.5 and 42.8%, respectively (Fig. 1) and by 29.8% in the presence of 10^{-3} M sodium nitroprusside. However, low concentrations of L-arginine (10^{-5} M) decreased the Na^+,K^+-ATPase activity by 35.5% (table).

The optical isomer of L-arginine, D-arginine, which is not a substrate for the synthesis of NO at low concentrations (10^{-5} M) increased by 37.1% the activity of Na^+,K^+-ATPase (table) and, unlike L-arginine, which suppressed the enzyme activity by 37.1%, at the concentration of 10^{-3} M, decreased it by only 15.7%.

The correlation found between the inhibiting effects of L-arginine and NO donors and also a significantly lower inhibition of the enzyme by D-arginine suggested that the effect of physiologically high concentrations of L-arginine should be related with an activation of the endogenous synthesis of NO. To test the hypothesis of the NO involvement in the effect of L-arginine on Na^+,K^+-ATPase in the endothelium, the effect of L-arginine on the enzyme was studied in the presence of an NOS inhibitor, L-NAME. Unlike L-arginine, L-NAME in the concentration of 10^{-4}-10^{-3} M was earlier shown not only to significantly inhibit the enzyme, but low concentrations of L-NAME (10^{-6} M) activated Na^+,K^+-ATPase by 25.6% (table). However, the inhibiting effect of L-arginine was abolished in the presence of $5\cdot10^{-4}$ M L-NAME: an addition of 10^{-4}-10^{-3} M L-arginine to the incubation medium failed to change the Na^+,K^+-ATPase activity beyond the control level (Fig. 2). Thus, the abolishment by the NOS inhibitor of the inhibiting effect of L-arginine suggested that Na^+,K^+-ATPase should be inhibited on stimulation of the NO-synthase activity in the aorta endothelium and that the endogenous NO should be involved in the L-arginine-caused inhibition of Na^+,K^+-ATPase in the aorta endothelium.

Effects of L-arginine, D-arginine, and L-NAME on the Na^+,K^+-ATPase activity in rat aorta endothelium

Reagents		Concentration, M				
		0	10^{-6}	10^{-5}	10^{-4}	10^{-3}
L-Arginine	A	6.2 ± 0.5 (16)	6.7 ± 0.4 (9)	8.4 ± 0.7 (6)	4.2 ± 0.7 (11)	3.9 ± 0.3 (10)
	Δ			+35.5*	−32.3*	−37.1*
D-Arginine	A	7.0 ± 0.4 (11)	8.7 ± 1.1 (6)	9.6 ± 0.6 (7)	7.7 ± 0.8 (9)	5.9 ± 0.7 (9)
	Δ		+24.2**	+37.1*		−15.7**
L-NAME	A	7.8 ± 0.8 (8)	9.8 ± 0.6 (8)	8.4 ± 0.8 (9)	7.8 ± 0.7 (9)	7.0 ± 1.0 (8)
	Δ		+25.6*		0.0	

Note: $M \pm m$ (m is the standard error, in parentheses the number of measurements), A is the activity in μmol P_i/h per mg protein, Δ is the change in the activity, %.

* $p \le 0.05$.

** $p \le 0.01$ (with respect to the control).

Vascular endothelium tissues are known to compete in the utilization of L-arginine, and products of its immediate conversion by the NO-synthase and arginase mechanisms in addition to NO are L-citrulline, L-ornithine, and urea, respectively [4, 5]. These products, in particular L-citrulline and L-ornithine, can also be involved in the mechanisms of the physiological effect of L-arginine that is especially displayed for a wide variety of *in vivo* situations [4]. However, even the highest concentrations studied of L-citrulline and L-ornithine failed to decrease the Na^+,K^+-ATPase activity; thus, unlike NO, neither L-citrulline, nor L-ornithine can mediate the L-arginine-induced inhibition of Na^+,K^+-ATPase.

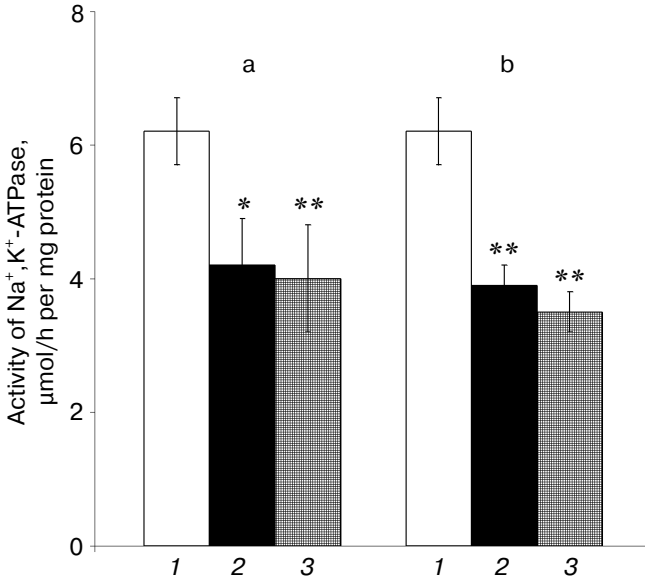

Fig. 1. Effect of L-arginine and of nitroglycerol on the Na^+,K^+-ATPase activity in rat aorta endothelium (L-arginine and of nitroglycerol concentrations are 10^{-4} (a) and 10^{-3} M (b)): *1*) control; *2*) L-arginine; *3*) nitroglycerol. $M \pm m$, $n = 10$-12, * $p \le 0.05$, ** $p \le 0.01$ (with respect to the control).

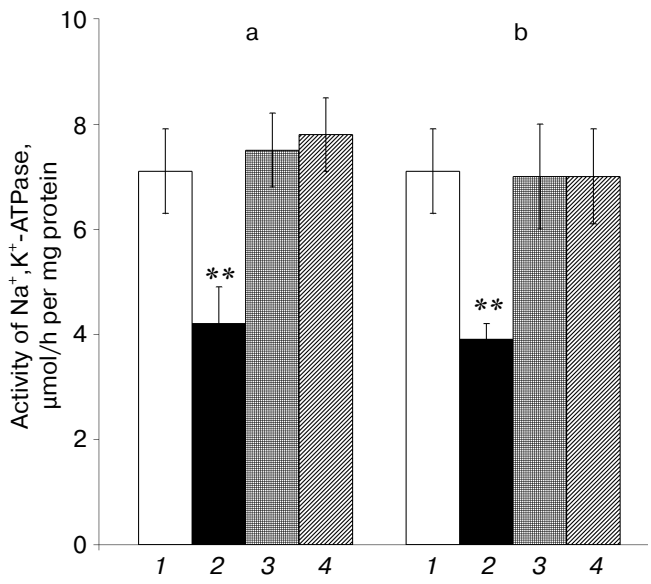

Fig. 2. Effect of L-NAME ($5 \cdot 10^{-4}$ M) on the Na^+,K^+-ATPase inhibition by L-arginine in the concentrations of 10^{-4} M (a) and 10^{-3} M (b): *1*) control; *2*) L-arginine; *3*) L-NAME + L-arginine; *4*) L-NAME. $M \pm m$, $n = 9$-11, ** $p \le 0.01$ (with respect to the control).

Low concentrations of L-arginine (10^{-5} M) cause a noticeable (35.5%) activation of Na^+,K^+-ATPase in the endothelium. The comparable activating effect (35.6%, $p < 0.001$) of low concentrations of L-arginine on Na^+,K^+-ATPase was also found in the microsomal fraction of rat kidney cortex. This activation of Na^+,K^+-ATPase was abolished by a detergent (sodium deoxycholate), and this suggested that the abolishment of the activating effect of L-arginine should be due to membrane modification by the detergent. Thus, the effect of L-arginine on membrane structures seem to partially unmask catalytic sites of Na^+,K^+-ATPase. Note that the L-arginine effect on Na^+,K^+-ATPase was not specific, because similar effects were also displayed by its structural analogs D-arginine and L-NAME, which activated the enzyme by 37.1 and 25.6%, respectively (table). These findings also suggested that the effect of low concentrations of L-arginine on Na^+,K^+-ATPase of the endothelium should not be associated with the effect of NO. Other literature data also suggest the possibility of the L-arginine effect on certain membrane structures. Thus, low concentrations of L-arginine activated ATP-dependent K^+-channels [17] and attenuated the vasopressin- and endothelin-stimulated Ca^{2+}-signal in mesangial cells [18]. And similarly to the Na^+,K^+-ATPase activation in the aorta endothelium, both effects described in these works were not caused by NO.

Note that our findings of the *in vitro* effect of L-arginine on Na^+,K^+-ATPase in the endothelium are unlike our previous data on the *in vivo* increase in the Na^+,K^+-ATPase activity in kidney endothelium and cortex by 25.0 and 15.0% ($p < 0.05$), respectively, caused by a long-term administration of L-arginine [14]. The cause of this difference is now unclear. It seems to be due to difference in the short- and long-term mechanisms of the L-arginine and NO effects on Na^+,K^+-ATPase. As shown by the *in vitro* experiments, the effect of L-arginine cannot be one-to-one explained by stimulation of the NO synthesis. It seems that this problem would be solved by finding a correlation between the increase in the Na^+,K^+-ATPase activity caused by the long-term administration of L-arginine and the activation of not only the NO-synthase pathway of its metabolism but also of other pathways of its utilization under the whole body conditions [4, 5]. However, the comparison of the *in vitro* findings with the *in vivo* experimental data [14] reveals a certain parallelism between the Na^+,K^+-ATPase *in vitro* activation by low concentrations of L-arginine and the Na^+,K^+-ATPase activation in tissue preparations by its long-term administration, and this does not contradict the decrease in the enzyme activity observed in the presence of high concentrations of L-arginine and NO-donors.

Thus, L-arginine plays rather a complicated role in the regulation of Na^+,K^+-ATPase. The effect of L-arginine on the enzyme is dual. However, only the inhibition of Na^+,K^+-ATPase by physiologically high concentrations of L-arginine was associated with the effect of NO in the system studied. Based on our findings, it is suggested that *in vivo* an increased content of L-arginine in the body (similarly to hyperproduction of nitrogen oxide under some pathological conditions) can disturb functioning of the endothelium transport system associated with the Na^+-pump. Obviously, further studies are required to comprehend more in detail the specific features of NO involvement in the short- and long-term mechanisms of the ion homeostasis regulation in tissues with different functions; this regulation is provided by the influence of NO on transport mechanisms of plasma membranes and, first of all, on the Na^+-pump and the coupled systems of ion transport.

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