Effect of L-Arginine-NO System on the Erythrocyte ATPase Activity and LPO

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny,* Vol. 128, No. 9, pp. 321-323, September, 1999
Original article submitted February 2, 1998

Activity of Na,K-ATPase in erythrocytes of children with asthmatic bronchitis and cerebral palsy rapidly changed under the effect of NO synthesis inhibitor L-NAME and did not depend on the plasma Ca^{2+} concentration. Enzyme activities correlated with the content of Mg^{2+}; and a tendency toward accumulation of LPO substrates and primary molecular LPO products was noted. The relative content of Schiff bases increased almost 2-fold.

Key Words: *ATPases; LPO; nitric oxide; erythrocytes*

Nitric oxide (NO) in low concentrations is a unique second messenger in the majority of cells. NO is produced in many, if not all cells of the organism from L-arginine in the reaction catalyzed by NO-synthase (NOS). Several forms of NOS are known, the principal of which are inducible and constitutive (iNOS and cNOS, respectively).

iNOS is a calcium-calmodulin-independent enzyme responsible for the formation of high (nM) NO concentrations. iNOS is activated by bacterial lipopolysaccharides, endotoxins, interferon, *etc.* NO generated by iNOS is involved in nonspecific antiviral and antibacterial defense; however in very high concentrations NO can provoke the development of pathological processes. cNOS is a calcium-dependent enzyme stimulating the production of NO in low amounts (nM), thus playing a regulatory role. Blood cells, including erythrocytes, are actively involved in NO metabolism [3,6,7].

MATERIALS AND METHODS

Effects of various doses of NOS inhibitor L-NAME (N-nitro-L-arginine methyl ester) on ATPase activity and LPO intensity were studied in children with allergic diseases (asthmatic bronchitis, n=12) and cerebral palsy (n=10). Blood was collected in heparinized tubes, plasma was removed by centrifugation, erythrocytes were washed twice with cold 0.85% NaCl, and erythrocyte pellet was suspended in a medium containing histidine, sucrose, and albumin. Erythrocytes in this medium were stored in a refrigerator for no more than 2-3 days (the enzymes retained their activity for 7 days). The enzyme activity was evaluated by the increment in inorganic phosphorus (P_i) in the incubation medium in the presence (Ca,Mg-ATPase) or absence (total ATPase) of ouabain, and the activity of Na,K-ATPase was calculated from the difference between these values. LPO was evaluated as described previously [2], the extinction at 220 nm being a function of the number of isolated double bonds in esterified lipids, LPO substrates. The extinction ratio 232/220 nm reflects the content of primary molecular LPO products, the 278-220 nm ratio characterized the content of secondary LPO products, and the extinction ratio 440-220 nm showed the relative content of Schiff bases. The concentrations of Ca^{2+}, Mg^{2+}, and triglycerides in the plasma were measured using Lachema kits.

Blood content of L-arginine is stable and little depends on the diet, being 1.5-1.7 mg/100 ml (10-12 μM). Erythrocyte suspension in buffer was preincubated with L-NAME dissolved deionized water immediately before use (5, 1, 0.5 μM) for 5 min at 37°C, and then 40 min with 1 mM ATP; the reaction was stopped by glacial trichloroacetic acid. The results were statistically processed using Student's t test.

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0007-4888/99/0009-0936$22.00

RESULTS

ATPase activity is significantly higher in children with allergic diseases (Ca,Mg-ATPase 624.4±70,7, Na,K-ATPase 465±57.9 μM $P_i/10^{12}$ erg/h) than in children with cerebral palsy (Ca,Mg-ATPase 300.66±69.3, Na,K-ATPase 157.0±63.2 μM $P_i/10^{12}$ erg/h). The effects of L-NAME were similar in these two groups, which allowed us to pool them. In low (subphysiological) doses L-NAME significantly inhibited of Na,K-ATPase in a dose-dependent manner (Fig. 1). A higher concentration of L-NAME (5 μM) led to appearance of negative activity of Na,K-ATPase, *i. e.*, the ouabain+ L-NAME complex activated, but not inhibited the enzyme due to ouabain. L-arginine affected arterial pressure by modifying sodium excretion [5]. Moreover, there are data that low doses of ouabain activated, but not inhibited Na,K-ATPase, and therefore ouabain receptors can be bound or modified by L-NAME. In our experiments Ca,Mg-ATPase activity negatively correlated with the plasma content of Mg^{2+} (r=-0.52) and triglycerides (r=-0.505) and did not correlate with plasma Ca^{2+}.

In ischemia vasodilatation can decrease or paradoxical vasoconstriction can develop in response to endothelium-dependent vasodilator, which may be due to abnormal regulation of the main NO pathway [5]. In our study children with spastic palsy and asthmatic bronchitis undoubtedly had cell ischemia. iNOS can be markedly induced by mixed viral infection. Children with cerebral palsy are known to be carriers of numerous viruses, such as rubella, measles, influenza C, herpes, Coxsackie, and poliomyelitis viruses. It is logical to presume that NOS activity and, hence, NO synthesis are notably enhanced in these children. NO and prostacyclin exert synergic effects on platelet adhesion and aggregation [3]. The L-arginine-NO conversion acts as a negative feedback mechanism of regulation of platelet aggregation. NOS can utilize exogenous L-arginine as a substrate only after activation [4]. Previously we demonstrated negative activity of Na,K-ATPase in rats under the effect of *in vitro* added carbacyclin, a synthetic analog of prostacyclin [1]. This can confirm the hypothesis that the effect of L-arginine on Na,K-ATPase activity is mediated by NO and that the production of NO is initially enhanced in children with cerebral palsy and asthmatic bronchitis, there are no conditions for NOS activation, or L-NAME acts via the negative feedback mechanism by inhibiting NO synthesis.

It was found that L-NAME increased the content of LPO substrates and primary molecular LPO products (Fig. 2). The content of secondary LPO products decreased and the relative content of Schiff bases increased significantly (almost 2-fold). We revealed a

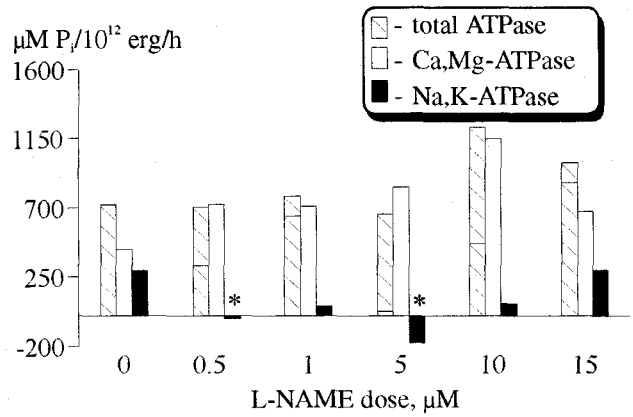

Fig. 1. Effect of N-nitro-L-arginine methyl ether (L-NAME) on ATPase activity. Here and on Fig. 2: *p<0.05 *vs.* the control.

significant correlation between Ca,Mg-ATPase activity and the concentrations of substrates (r=-0.892) and final products (r=-0.984) of LPO. The addition of L-NAME converts the positive correlation into negative. Hence, we detected no direct effect of L-NAME on LPO intensity. However, our *in vitro* studies were rather short-term and it cannot be excluded that long-term accumulation of LPO substrates and Schiff bases may lead to cell disorders.

Since Na,K-ATPase activity changed rapidly and did not depend on the concentration of Ca^{2+}, it can be caused by changes in iNOS activity, which was indirectly confirmed by high urinary excretion of viruses in examined children. The observed strict correlation between enzyme activities and Mg^{2+} content suggests that Mg^{2+} is involved in the realization of iNOS activity and/or its activation. Presumably, free radicals formed during NO synthesis, do not modify the intensity of total LPO but even in very low amounts accelerate transformation of peroxides into final LPO products Schiff bases. They modify the lipid environ-

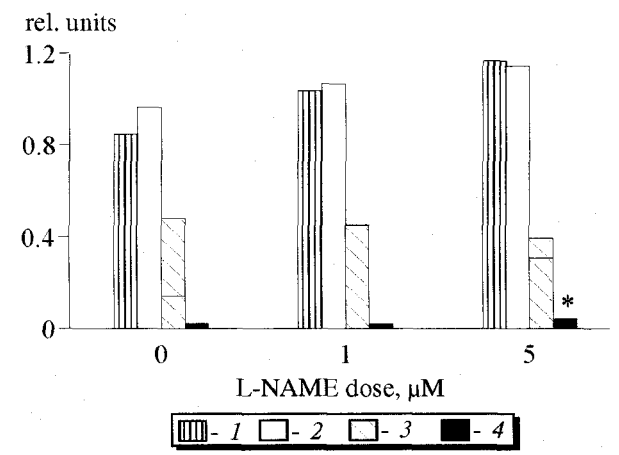

Fig. 2. Effect of N-nitro-L-arginine methyl ether (L-NAME) on LPO: extinction at 220 nm (*1*); 232/220 (*2*), 278/220 (*3*), and 440/220 (*4*) ratios.

ment of the enzyme in the erythrocyte membrane and/or compete for ouabain binding sites, thus modulating Na,K-ATPase activity.

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