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Effects of Nitrosyl Iron Complexes with Thiocarbamide and Its Aliphatic Derivatives on Activities of Ca^{2+}-ATPase of Sarcoplasmic Reticulum and cGMP Phosphodiesterase

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We studied the effects of water-soluble cationic dinitrosyl iron complexes with thiocarbamide and its aliphatic derivatives, new synthetic analogs of natural NO donors, active centers of nitrosyl [1Fe-2S]proteins, on activities of Ca^{2+}-ATPase of sarcoplasmic reticulum and cGMP phosphodiesterase. Nitrosyl iron complexes $[Fe(C_3N_2H_8S)Cl(NO)_2]^0[Fe(NO)_2(C_3N_2H_8S)_2]^+Cl^-$ **(I)**, $[Fe(SC(N(CH_3)_2)_2(NO)_2]Cl$ **(II)**, $[Fe(SC(NH_2)_2)_2(NO)_2Cl\times H_2O$ **(III)**, and $[Fe(SC(NH_2)_2)_2(NO)_2]_2SO_4\times H_2O$ **(IV)** in a concentration of 10^{-4} M completely inhibited the transporting and hydrolytic functions of Ca^{2+}-ATPase. In a concentration of 10^{-5} M, they inhibited active Ca^{2+} transport by 57±6, 75±8, 80±8, and 85±9% and ATP hydrolysis by 0, 40±4, 48±5, and 38±4%, respectively. Complex II reversibly and noncompetitively inhibited the hydrolytic function of Ca^{2+}-ATPase ($K_i=1.7\times10^{-6}$ M). All the studied iron—sulphur complexes in a concentration of 10^{-4} M inhibited cGMP phosphodiesterase function. These data suggest that the studied complexes can exhibit antimetastatic, antiaggregation, vasodilatatory, and antihypertensive activities.

Key Words: *sarcoplasmic reticulum Ca^{2+}-ATPase; cyclic guanosine monophosphate phosphodiesterase; nitrosyl iron complexes*

Nitrosyl iron complexes (NIC) of various structural types are hydrolyzed with the formation of NO in proton media [9] and constitute a new class of universal NO donors [2]. Isolation of NO and other degradation products can lead to various manifestations of biological activities of these complexes. We studied new NIC of the following composition: $[Fe(C_3N_2H_8S)Cl(NO)_2]^0[Fe(NO)_2(C_3N_2H_8S)_2]^+Cl^-$ **(I)**, $[Fe(SC(N(CH_3)_2)_2(NO)_2]Cl$ **(II)**, $[Fe(SC(NH_2)_2)_2(NO)2Cl\times H_2O$ **(III)**, and $[Fe(SC(NH_2)_2)_2(NO)_2]_2SO_4\times H_2O$ **(IV)**. Their synthesis and physicochemical characteristics, including NO donation activity, were described previously [7,8].

Active transport of calcium ions through the membrane of sarcoplasmic reticulum (SPR) is realized at the expense of energy of ATP hydrolysis catalyzed by SPR Ca^{2+}-ATPase. Inhibition of Ca^{2+} transport modifies the ratio of intra- to extracellular Ca^{2+}, this preventing the formation of clots and adhesion of metastatic cells to capillary endothelium [5] and eventually preventing metastatic growth [3,4,6].

Inhibition of cGMP phosphodiesterase (PDE) leads to accumulation of cGMP, a secondary messenger regulating vascular tome and exhibiting antiaggregation activity *in vivo* [1].

We study the effects of new NIC on activities of SPR Ca^{2+}-dependent ATPase and cGMP PDE.

MATERIALS AND METHODS

The following reagents were used: cGMP, nucleotidase (cobra venom), ATP (Sigma; without additional purification), histidine, human albumin, imidazole,

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TABLE 1. Effects of NIC in Studied Concentrations on Inhibition of SPR Ca^{2+}-ATPase Activity (% of control; *n*=3-6; *M*±*m*)

Compound index	Active Ca^{2+} transport			ATP hydrolysis		
	10^{-4} M	10^{-5} M	10^{-6} M	10^{-4} M	10^{-5} M	10^{-6} M
I	100±10*	57±6*	19±2	100±10*	0	0*
II	100±10*	75±8*	56±6*	100±10*	40±4	28±3*
III	100±10*	80±8*	19±2	100±10*	48±4*	28±3*
IV	100±10*	85±8*	28±3*	100±10*	38±3	17±2

Note. *$p<0.05$ in comparison with the control.

DMSO, EDTA, trichloroacetic acid, sucrose, $MgCl_2$, NaCl, KCl, $CaCl_2$, sodium oxalate, and ammonium molybdate ($MoNH_4$; Reachim) after appropriate additional purification.

Ca^{2+}-ATPase of SPR was isolated from rabbit hind paw white muscles. Specific activity of Ca^{2+}-ATPase was 15 μmol/mg protein/min. Hydrolytic activity of Ca^{2+}-ATPase was calculated from the slope of ATP hydrolysis kinetic curve.

Inhibition of the enzyme hydrolytic activity was calculated by the formula:

$$I=100(A_0-A)/A_0,$$

where I is activity, A_0 and A are specific content of inorganic phosphorus (P_i) in the control and experimental (in the presence of the complex) samples.

The increment of Ca^{2+} concentration was evaluated by the kinetics of their absorption by SPR vesicles. The kinetics of SPR Ca^{2+}-ATPase inhibition was evaluated by the dependence of reaction rate on substrate concentration (ATP) in the presence of 10^{-5} M NIC II and without it.

The reversibility of NIC I-IV effect was evaluated by dialysis of SPR Ca^{2+}-ATPase buffer solution containing NIC I-IV in a concentration of 10^{-5} M against a 100-fold excess of NIC-free buffer for 24 h at 4°C.

cGMP PDE was isolated from Wistar rat brain cortex. Activity of cGMP PDE was evaluated by the level of inorganic phosphorus released from GMP after addition of nucleotidase.

The effects of NIC on activities of SPR Ca^{2+}-ATPase and cGMP PDE were studied after 3-min preincubation of the preparations with the enzyme. All the studied NIC were prepared in argon medium before addition to the reaction mixture.

RESULTS

NIC I-IV in a concentration of 10^{-4} M inhibited active transport of Ca^{2+} and hydrolysis of ATP by 100% (Table 1). In a concentration of 10^{-5} M, they inhibited active Ca^{2+} transport by 57-85% and to a lesser extent (by 19-56%) hydrolysis of ATP, thus uncoupling the hydrolytic and transporting functions of the enzyme. This was paralleled by changes in the ratio of extra- to intracellular calcium ions (normally ATP:Ca 1:2), which disturbed platelet aggregation and their binding with metastatic tumor cells and prevented adhesion of these cells to the vascular walls [5].

The effects of dialysis on SPR Ca^{2+}-ATPase activity (Table 2) in the presence and absence of NIC confirmed reversible effect of the studied NIC on the

TABLE 2. Effects of NIC (0.01 mM) on Hydrolytic and Transporting Function of SPR Ca^{2+}-ATPase before and after Dialysis (% of control; *n*=3; *M*±*m*)

Compound index	Before dialysis		After dialysis	
	Active transport of Ca^{2++}	ATP hydrolysis	Active transport of Ca^{2++}	ATP hydrolysis
I	57±8	0	0*	0
II	75±8	40±4	0*	0*
III	80±8	48±4	20±2*	0*
IV	85±8	38±3	25±3*	0*

Note. *$p<0.01$ in comparison with the corresponding value before dialysis.

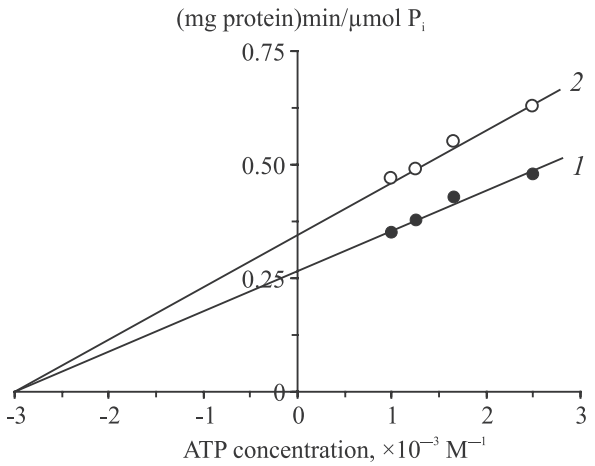

Fig. 1. Changes in the rate of ATP hydrolysis by SPR Ca^{2+}-ATPase at different substrate concentrations under the effect of NIC II (Lineweaver—Burk coordinates) at different concentrations of ATP substrate without NIC (*1*) and in the presence of NIC II in a concentration of 10^{-5} M (*2*).

TABLE 3. Effects of NIC on Inhibition of cGMP PDE Activity (% of control; *n*=3-6; *M*±*m*)

Compound index	Concentration, M		
	10^{-4}	10^{-5}	10^{-6}
I	82±8*	28±3*	15±2
II	59±6*	25±3*	0
III	87±9*	23±2*	40±4
IV	87±9*	37±4*	25±3

Note. *p<0.05 in comparison with the control.

function of SPR Ca^{2+}-ATPase, this indicating their noncovalent binding to the enzyme.

The inhibition constant (K_i) was calculated from the maximum rates of ATP hydrolysis for NIC II added to SPR Ca^{2+}-ATPase. The complex noncompetitively inhibited the hydrolytic function of SPR Ca^{2+}-ATPase with $Ki=1.7\times10^{-6}$ M (Fig. 1). This fact suggested that NIC II did not bind to the active center of the enzyme. Presumably, NIC II interacted with SPR membrane and modified its structure and function.

All the studied NIC inhibited the function of cGMP PDE (Table 3), this suggesting the antiaggregation, antihypertensive, and vasodilatation activities of these complexes determined by accumulation of secondary messenger cGMP [1].

These data recommend NIC I-IV for further studies on animals as prospective drugs with antimetastatic, antiaggregation, and antihypertensive activities [1-4].

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