Effect of Drug Resistance Modulator, NO Donor, on Membrane Structure and Function of Membrane-Bound Ca^{2+}-Activated Mg^{2+}-Dependent ATPase

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 146, No. 8, pp. 165-167, August, 2008
Original article submitted December 28, 2007

Exogenous NO donor 3,3-bis-(nitroxymethyl)oxetane (NMO) was synthesized at the Institute for Problems of Chemical Physics (Russian Academy of Sciences). This compound was shown to inhibit Ca^{2+}-ATPase isolated from normal muscular cells and tumor cells. Both hydrolytic and transport functions of the enzyme were inhibited under these conditions. These changes were probably related to changes in membrane structure caused by NO donor. Our results suggest that changes in intracellular Ca^{2+} concentration can modulate the formation of tumor drug resistance.

Key Words: *drug resistance; nitric oxide donors; membrane structure; Ca^{2+}-ATPase*

Drug resistance of tumors seriously limits the use of chemotherapeutic agents. Various molecular and genetic mechanisms underlying the development of tumor drug resistance are now identified and approaches to reversion of drug resistance were developed. However, they are not used in clinical practice due to high toxicity. Therefore, the search for new modulators of tumor drug resistance and evaluation of the mechanisms of their action are now in progress.

It was shown during the last decade that nitric oxide (NO) plays an important role in various biological processes under normal and pathological conditions, including tumor diseases [1,3]. NO donors can potentiate the effect of some antitumor drugs [4]. Published data show that NO plays a role in statin-induced reversion of doxorubicin resistance in human malignant mesothelioma [11]. Our previous studies showed that NO donor 3,3-bis-(nitroxymethyl)oxetane (NMO) prevents the development of cyclophosphamide resistance in P388 mouse leukemia and improves the effectiveness of cytostatic therapy [5].

Here we studied the effect of NMO on the structure of biological membrane and functions of membrane-bound enzyme Ca^{2+}-ATPase of sarcoplasmic reticulum (SR) and endoplasmic reticulum (ER) isolated from rabbit muscles and tumor cells.

MATERIALS AND METHODS

SR membranes were studied using a fluorescence probe pyrene located in hydrophobic sites of the membrane lipid bilayer. The probe molecules were excited with a pulse nitrogen laser (λ_{excit}=334 nm). Fluorescence spectra were recorded on an Aminco Bouman spectrofluorometer.

Hydrolytic and transport functions of Ca^{2+}-ATPase were studied using the enzyme from SR and ER

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0007-4888/08/1462-0200

of rabbit muscles and P388 mouse leukemia cells, respectively. Hydrolytic activity of the enzyme was estimated from the specific rate of inorganic phosphate formation (μmol P_i/mg protein/min). Transmembrane Ca^{2+} transport was determined from the specific rate of ion transport (μmol Ca^{2+}/mg protein/min). The reaction medium contained 4 mM $MgCl_2$, 2.5 mM imidazole, 100 mM NaCl, 5 mM sodium oxalate, 0.05 mg protein, and 3 mM ATP (100 μl, pH 7.0). The total volume of the test sample was 5.0 ml. NMO in the corresponding concentrations was added to experimental samples. Preincubation was performed in the presence of Ca^{2+}-ATPase at 37°C for 5 min. The reaction was induced by adding 1000 μM $CaCl_2$. Ca^{2+}-ATPase activity was evaluated from the kinetics of pH changes in the medium, because the reaction yields protons and phosphate ions in 1:1 ratio. Ca^{2+}-ATPase activity was calculated from the slope of the kinetic curve. The rate of variations in Ca^{2+} concentration was estimated from the time of absorption by SR vesicles, which resulted in the termination of ATP hydrolysis. Relative enzyme activity was calculated as follows:

$$I=100(A_0\text{-}A)/A_0,$$

where I is relative activity of SR Ca^{2+}-ATPase; A_0 is specific content of inorganic phosphorus in the control sample; and A is relative content of inorganic phosphorus in the experimental sample.

RESULTS

Sequential titration of the SR suspension with NMO was accompanied by an increase in the intensity of excimer and monomer regions in the pyrene fluorescence spectrum (Fig. 1).

Increasing the concentration of NMO was accompanied by an increase in the excimer/monomer fluorescence ratio (Fig. 1). Variations in this ratio were described by a hyperbolic curve with the saturation profile (Fig. 2). These data indicate that NMO causes a decrease in microviscosity of the membrane lipid bilayer in SR. The majority of SR proteins (90%) correspond to Ca^{2+}-ATPase. Hence, we studied the hydrolytic and transport function of this enzyme.

The effect of NMO on SR membrane-bound enzyme Ca^{2+}-ATPase from rabbit muscle was evaluated. Hydrolytic activity of the enzyme significantly decreased after treatment with NMO in high concentration (10^{-4} M). The inhibitory effect became less pronounced at lower NMO concentration. The inhibition of Ca^{2+} transport did not depend on NMO concentration (Fig. 3).

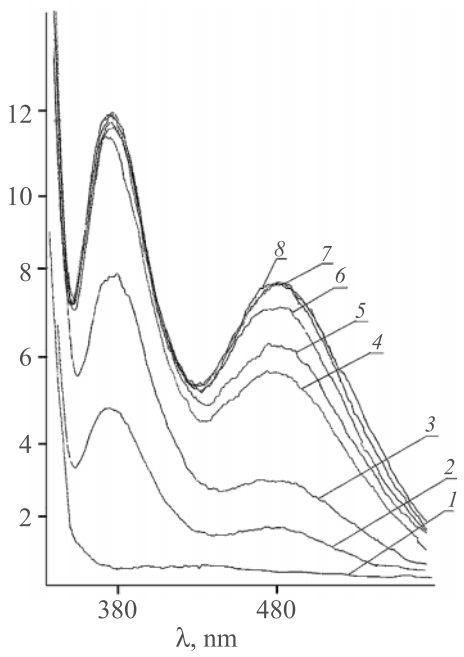

Fig. 1. Pyrene fluorescence spectra in the SR membrane (λ_{ex}=334 nm) after addition of NMO in various concentrations: monomer region of the pyrene fluorescence spectrum (λ_{fl}), 380 nm; excimer region of the pyrene fluorescence spectrum (λ_{fl}), 480 nm. SR (*1*); SR+0.02 pyrene (10^{-3} M). NMO concentrations: 2×10^{-5} (*3*), 4×10^{-5} (*4*), 6×10^{-5} (*5*), 9×10^{-5} (*6*), 12×10^{-5} (*7*), and 18×10^{-5} M (*8*). Ordinate: pyrene fluorescence, arb. units.

Further studies were performed to evaluate the effect of NMO on the function of SR Ca^{2+}-ATPase from tumor cells. NMO treatment was followed by a significant decrease in hydrolytic and transport activity of Ca^{2+}-ATPase. However, the observed changes were less pronounced compared to variations in activity of the SR enzyme (Fig. 3).

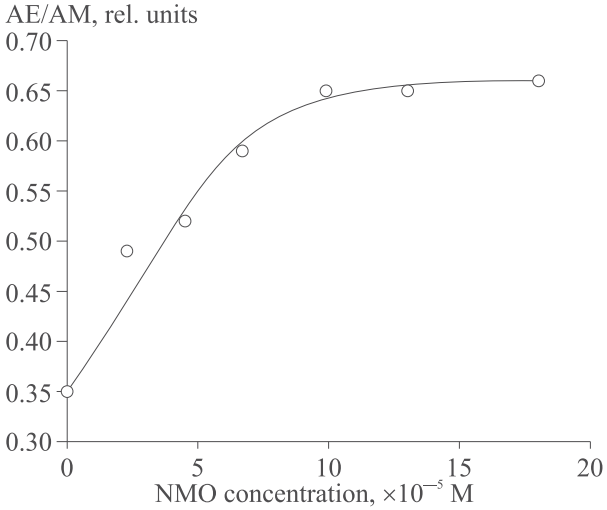

Fig. 2. Effect of NMO concentration on the ratio between excimer (A_E, λ_{max}=480 nm) and monomer regions (A_M, λ_{max}=380 nm) in the fluorescence spectrum of SR membrane-incorporated pyrene (10^{-5} M).

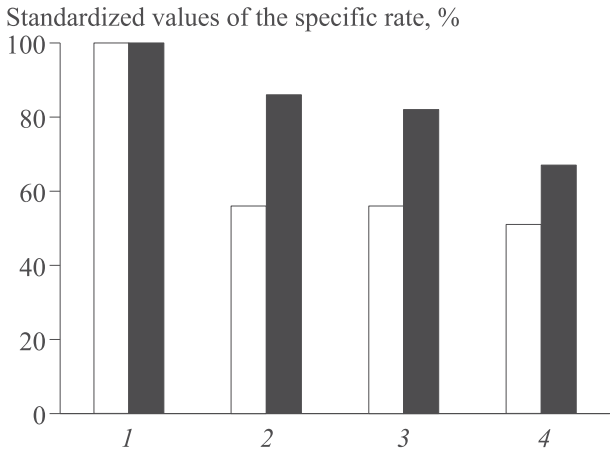

Fig. 3. Decrease in hydrolytic and transport activity of SR Ca^{2+}-ATPase from rabbit muscle. NMO concentration: control (*1*); 10^{-6} (*2*), 10^{-5} (*3*), and 10^{-4} M (*4*). Light bars, active transport of Ca^{2+}; dark bars, ATP hydrolysis.

Our results indicate that ATP hydrolysis and Ca^{2+} transport are suppressed in the presence of NMO. It should be emphasized that Ca^{2+} transport is inhibited in normal mouse cells and tumor cells. This effect is probably associated with NMO-induced structural changes in the SR membrane, which results in dysfunction of Ca^{2+}-ATPase.

It can be hypothesized that the action of NO donor on Ca^{2+}-ATPase causes an imbalance between intracellular and extracellular Ca^{2+}, which contributes to the modulatory effect of NO on drug resistance.

Little is known about the relationship between drug resistance and NO. The concentrations of endothelial and inducible NO synthase, as well as NO synthesis in resistant MCF-7 breast cancer cells are lower than in sensitive cells [6]. Moreover, inhibition of endogenous NO synthesis during hypoxia increased resistance of tumor cells. However, treatment with NO donors prevents the development of drug resistance in MDA-MB 231 and B16F10 cells [7]. Studying the reversion of doxorubicin resistance in human colonic epithelial cell line HT29-dx after incubation with NO synthesis inductors revealed a relationship between multidrug resistance and decrease in NO synthesis [10].

Calcium ions play a role in cell activity. NO regulates intracellular Ca^{2+} concentration, which is related to activation of soluble guanylate cyclase. This enzyme catalyzes the formation of cyclic guanosine monophosphate, which serves a regulator of cell activity [2].

A relationship exists between intracellular Ca^{2+} concentration and drug resistance. Changes in intracellular Ca^{2+} concentration contribute to the development of Taxol resistance in non-small cell lung carcinoma cells [9]. Ca^{2+} concentration is high in cells carrying the multidrug resistance phenotype [8]. Verapamil, which is extensively used as a revertant of multidrug resistance, serves as a calcium channel blocker. Calcium channel blockers significantly increase the effectiveness of P-glycoprotein-independent antitumor compounds [13]. Verapamil-induced apoptosis in multidrug-resistant cells was more pronounced than in sensitive cells. It should be emphasized that this effect did not depend on p53 activity [12].

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