ELSEVIER

Biochimica et Biophysica Acta 1321 (1997) 243–251

BIOCHIMICA ET BIOPHYSICA ACTA
BBA

Effect of nitroso compounds on Na/K-ATPase

Alexander A. Boldyrev [a,*], Elena R. Bulygina [a], Galina G. Kramarenko [a], Anatoly F. Vanin [b]

[a] *Department of Biochemistry, School of Biology, International Biotechnological Center of M.V. Lomonosov Moscow State University, 119899 Moscow, Russia*

[b] *Institute of Chemical Physics, Russian Academy of Sciences, Moscow, Russia*

Received 7 May 1997; accepted 22 May 1997

Abstract

Thiol containing NO˙-derivatives were found to inhibit the activity of brain and kidney Na/K-ATPase. *S*-Nitrosoglutathione demonstrated only minor inhibiting activity, while dinitrosyl iron complexes (DNIC) with cysteine or glutathione were much more effective. Brain Na/K-ATPase is more vulnerable to inhibiting action than kidney Na/K-ATPase. Inhibition of the activity is accompanied by a decrease in amount of protein thiol groups and a change in the substrate dependence curve of the enzyme. Restoration of Na/K-ATPase activity by SH-reagent dithiothreitol or cysteine is accompanied by restoration of SH-groups of the enzyme. This suggests that blockade of SH-groups of Na/K-ATPase is responsible for the inhibition. The possibility that this blockade results in disordering of interprotomer interactions within the oligomeric complexes of Na/K-ATPase is suggested. Possible regulatory meaning of the effect of NO˙ derivatives is discussed. © 1997 Elsevier Science B.V.

Keywords: ATPase, Na^+/K^+-; NO radical; Dinitrosyl iron complex; *S*-Nitrosothiol; Brain; Kidney

1. Introduction

NO-radical is known to play diverse important roles as second messenger in biological systems, regulator of haem containing enzymes like guanylate cyclase, or inductor of ADP-ribosylation [1,2]. Some investigators reported that NO˙ can modify oxidative processes in the cell demonstrating both pro- and anti-oxidant properties [2,3].

Recently additional functions of NO radical were described — an ability to open K-channels in the plasma membrane of vascular smooth muscle cells [4] and to inactivate NMDA receptors in various areas of brain [5]. In both cases the NO˙ effect was found to be realized via reversible oxidation of SH-groups of channel proteins, and such mechanism presumably involves S-nitrosylation of protein residues [4].

These data attract an attention to Na/K-ATPase (E.C. 3.6.1.37), — an enzyme system which supports ionic homeostasis in the cell. Indirect effect of NO˙ on this enzyme mediated by activation of Na/H-exchanger [6] or protein kinase C [7] were recently

Abbreviations: DNIC-CYS, dinitrosyl iron complexes with cysteine; DNIC-GS, dinitrosyl iron complexes with glutathione; DTT, dithiothreitol; DTNB, 5,5-dithio-bis(2-nitrobenzoic) acid; EPR, electron paramagnetic resonance; GS-NO, *S*-nitrosoglutathione; NO, nitric oxide; PIPES, piperasine-*N*,*N*′-bis(2-ethanesulfonic acid); SNP, sodium nitroprusside

* Corresponding author. Fax: (7) (95) 490-2408; E-mail: aab@atpase.bio.msu.su

PII S0005-2728(97)00053-4

published using tissues slices, but sensitivity of Na/K-ATPase itself to NO radical was not characterized. Very recently Sato et al [8] have reported inhibition of pig brain Na/K-ATPase by $NO^{\cdot}$ generating drugs. However no direct effect of $NO^{\cdot}$ on kidney Na/K-ATPase was observed [9]. Na/K-ATPase is known to be an SH containing enzyme [10]. Its thiol groups could be the target not only for $NO^{\cdot}$ but also for its stable compounds which are described in living cells, namely S-nitrosothiols [11] or dinitrosyl iron complexes (DNIC) with thiol containing compounds [12]. This suggestion was examined in our experiments here.

2. Materials and methods

2.1. Reagents

ATP, ouabain, Tris-HCl, PIPES and sodium nitroprusside (SNP) were purchased from Sigma (USA), other reagents were from Reachim (Russia) of analytical grade.

Dinitrosyl-iron complexes with cysteine (DNIC-CYS) or glutathione (DNIC-GS) and S-nitrosoglutathione (GS-NO) were synthesized as described elsewhere [13,14]. Two forms of DNIC were used in the experiments: diamagnetic (dimeric) — (DNIC 1:2) or paramagnetic (monomeric) — (DNIC 1:30) being prepared at two different Fe^{2+} ions to the thiol molar ratio (1:2 and 1:30, respectively). Concentration of the nitroso compounds used was controlled spectrophotometrically or by the EPR method [13,14].

2.2. Enzyme source

In the experiments partially purified membrane bound Na/K-ATPase from two different sources — bovine brain [15] and dog kidney [16] was used.

2.3. Measurement of enzyme activity

Na/K-ATPase activity was measured by inorganic phosphate liberation as described by Rathbun and Betlach [17]. Reaction medium for activity determination contained: 3 mM ATP, 5 mM $MgCl_2$, 130 mM NaCl, 20 mM KCl, and 30 mM PIPES (pH 7.4) at 25 or 37°C (as noted in figure legends and tables) in 1 ml volume; protein amount was 2–7 μg. Na/K-ATPase activity was calculated as a difference in P_i liberation between these samples and anothers, containing 1 mM ouabain, specific inhibitor of Na/K-ATPase. Reaction was stopped by addition of 3 M sodium acetate (pH 4.3). Enzyme activity (measured under optimal conditions at 37°C) was 170–220 μmol P_i/mg per h for brain enzymes and 850–1000 μmol P_i/mg per h for kidney enzymes.

2.4. Protein determination

Protein was determined according to Lowry et al. [18] after solubilization of membrane suspension in 1% sodium deoxycholate, with bovine serum albumin as standard.

2.5. Assay of SH-groups of Na / K-ATPase

The amount of SH-groups in Na/K-ATPase preparations was determined using 5,5′-dithio bis-(2-nitrobenzoic acid), DTNB [19,20]. Absorbance of the coloured product of the reaction was measured at 412 nm on a Hitachi-557 spectrophotometer. Aliquots of 60–100 μg of protein were added to medium containing 1.0 mM EDTA, 10 mM Tris-HCl (pH 8.0), 8 M urea and 0.5% Tritone X-100. In the experiments with low-molecular weight nitrosothiols the samples after preincubation were placed on gel filtrating Eppendorf tubes (1.3 ml) and centrifuged (1000 g for 3 min). The Eppendorf tubes were filled up with Sephadex G-25 suspended in 10 mM Tris-HCl (pH 8.0). This procedure prevented interaction of low-molecular weight thiols with DTNB. In the special experiments there was found that single gel filtration procedure provides for a total removal of low-molecular weight thiols from the sample.

2.6. Chemical modification of Na / K-ATPase

The procedure of $NO^{\cdot}$ generation induced by SNP illumination was described elsewhere [21]. The rate of nitric oxide generation was determined by the simultaneous spectrophotometric measurement of ascorbic acid oxidation under the same conditions (ascorbic acid test) [9].

In order to modify Na/K-ATPase by GS-NO or DNICs the following procedure was used. ATPase protein was preincubated 1.5–40 min at 4°C with different concentrations of inhibitors (reaction was started by the addition of inhibitor to preincubation medium containing 150 mM KCl, 30 mM PIPES, pH 7.4, 1 mM EDTA and 0.2–1.3 mg/ml Na/K-ATPase). At the end of the exposure, aliquots of the preincubation medium containing appropriate amount of protein were taken up to assay ATPase activity and content of SH-groups, as described above.

3. Results

3.1. Estimation of a direct effect of NO˙ on Na / K-ATPase

In order to investigate the effect of NO˙ on Na/K-ATPase, 1–10 mM SNP was added to the preincubation medium. SNP is known to be a poor source of the radical, but illumination strongly activates NO˙ liberation [21]. However, using different illumination conditions and a broad range of SNP concentrations we found no difference in Na/K-ATPase activity with or without illumination of samples (Table 1). Thus, we conclude that under all of the conditions used there was no direct inhibition of Na/K-ATPase by the NO radical. Modest suppression of the activity in the presence of SNP itself was independent of illumination time and therefore was ascribed to an effect on the enzyme of SNP itself.

Table 1
Effect of SNP on Na/K-ATPase from bovine brain (A) or dog kidney (B) under different conditions (average results from three experiments and standard deviations are given)

Preincubation conditions	Illumination	Na/K-ATPase (% to control)
A + 1 mM SNP 30 min, 4°C	−	86 ± 5
	+	89 ± 5
A + 1 mM SNP 30 min, 20°C	−	77 ± 4
	+	77 ± 3
A + 10 mM SNP 5 min, 20°C	−	75 ± 5
	+	77 ± 4
B + 10 mM SNP 5 min, 20°C	−	70 ± 5
	+	63 ± 4

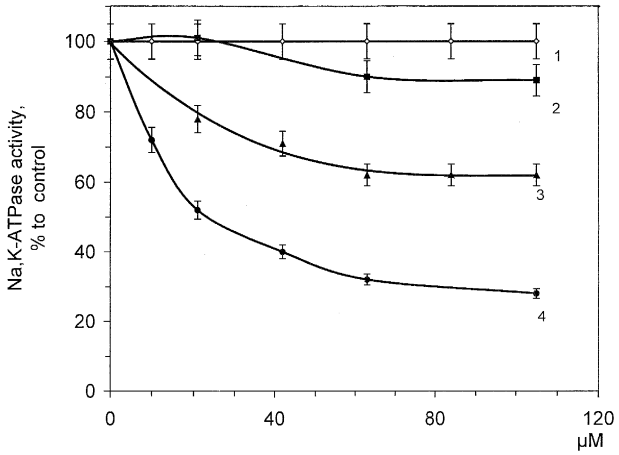


Fig. 1. Dependence of bovine brain Na/K-ATPase on concentration of NO complexes with glutathione and cysteine. Enzyme (25 μg/ml) was preincubated 15 min at 4°C with no additions (1) or in the presence of GS-NO (2), DNIC-GS (1:2) (3), or DNIC-CYS (1:2) (4). The preincubation medium consisted of 150 mM KCl, 30 mM PIPES (pH 7.4), 1 mM EDTA. After that samples of 25 μl volume were 40-fold diluted by incubation medium and use to measure enzyme activity. Na/K-ATPase activity was measured at 37°C in a medium containing 3 mM ATP, 5 mM $MgCl_2$, 130 mM NaCl, 20 mM KCl, 30 mM PIPES (pH 7.4).

It is known from the literature [2] that NO˙ generation in brain neurons being under stress conditions amounts to 100 nmol per mg protein per h. In our experiments we have followed NO˙ generation by the ascorbic acid test in parallel samples. The highest rate of NO˙ generation which we measured in the samples was to 300 nmol/h, whereas content of brain Na/K-ATPase in these samples was at least 20 times higher than that in brain homogenate [15]. However, even under these conditions, inhibition of enzyme activity was not found. Using in such experiment kidney enzyme which is characterized by a higher Na/K-ATPase content [15,16] we also did not find any direct effect of NO˙ on Na/K-ATPase activity.

3.2. Effects of NO˙ containing compounds on Na / K-ATPase

GS-NO and diamagnetic forms of DNIC-GS or DNIC-CYS demonstrated inhibiting effects on the enzyme being distinct in their efficiency (Fig. 1). GS-NO revealed the lowest action, e.g., 100 μM GS-NO suppressed enzyme activity by 12%, whereas 50 μM DNIC-GS or DNIC-CYS provided for 45% and 70% inhibition of Na/K-ATPase, respectively.

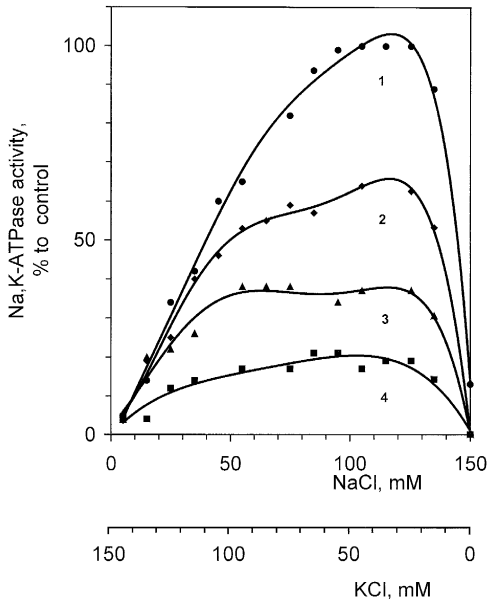


Fig. 2. Dependence of brain Na/K-ATPase modified by dimeric DNIC complexes on Na/K ratio in the incubation medium. Enzyme (25 μg/ml) was preincubated 15 min at 4°C with no additions (1) as well as in the presence of 100 μM DNIC-GS (2), 42 μM (3), or 105 μM (4) DNIC-CYS. Conditions of incubation as in Fig. 1.

One of the features of the enzyme studied is its specific dependence on the Na/K ratio in the incubation medium. It is seen from Fig. 2 that inhibition of Na/K-ATPase by these DNIC's was not accompanied with a change in profile of such dependence, thus demonstrating that a change in Na/K-ratio did not affect the action of the inhibitors. It is noteworthy that inhibition of Na/K-ATPase by H_2O_2 and hypochlorous anion likewise was not protected by sodium or potassium ions [22].

Fig. 3 characterizes the inhibiting effect of diamagnetic DNIC-CYS on the enzyme; the effect does depend on both concentration of the inhibitor and time of preincubation. The lowest concentration of the inhibitor needs 20 min for realization of the whole effect, while the highest one takes it for less than 1 min. Similar dependence was found for diamagnetic DNIC-GS.

3.3. Effect on SH-groups of the enzyme

We have compared sensitivity of the enzyme from brain and kidney to inhibiting action of diamagnetic

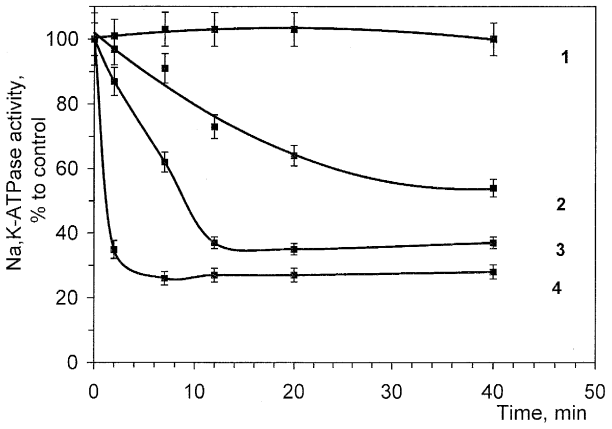


Fig. 3. Time-course of inactivation of dog kidney Na/K-ATPase by DNIC-CYS (1:2). Na/K-ATPase (300 μg/ml) was preincubated 2–40 min at 4°C in the absence (1) or presence of 30 (2), 60 (3) or 100 (4) μM DNIC-CYS, then activity was measured as described in Materials and methods.

DNIC-CYS. It was found that brain enzyme is more sensitive to inhibition than kidney enzyme (Fig. 4). This agrees with the different sensitivities of these enzymes to hydrogen peroxide and hypochlorous anion reported earlier [22] — Na/K-ATPase from brain is more vulnerable to oxidative modification than that from kidney, independent of the chemistry of the modifying molecule [23]. This presumably corresponds to a difference in the amount of SH-groups in

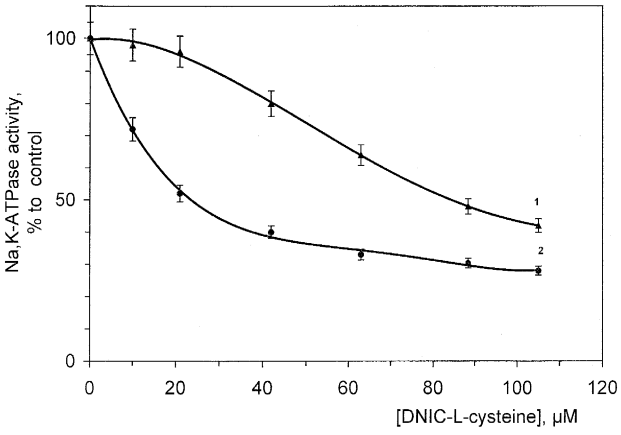


Fig. 4. Inhibiting effect of DNIC-CYS on Na/K-ATPase from dog kidney (1) and bovine brain (2). Enzyme from brain (25 μg/ml) and kidney (30 μg/ml) was preincubated 15 min with different concentrations of DNIC-CYS (0–105 μM) in the presence of 150 mM KCl, 30 mM PIPES (pH 7.4 at 4°C) and 1 mM EDTA.

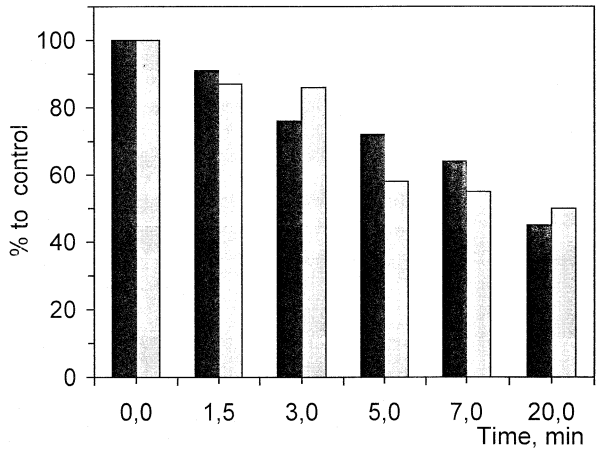


Fig. 5. Time-course of inhibition of Na/K-ATPase activity (black bars) and thiol content (grey bars) in kidney enzyme preparation during its modification by 50 μM DNIC-CYS (1:2). Na/K-ATPase (1.0 mg/ml) was preincubated with DNIC-CYS as described in Fig. 1. Sulfhydryl groups were determined as described in Section 2. Decrease in both parameters comparatively to control is statisticaly significant ($P < 0.05$).

different isoforms of Na/K-ATPase characteristic for brain and kidney tissues: brain isoforms contain more cysteine residues than the kidney isoform [24].

In kidney Na/K-ATPase 46 nmol/mg protein SH-groups accessible to DTNB was found. This is in a good correlation with an amount of Na/K-ATPase thiols in membrane preparations used: 20–23 mol per mol Na/K-ATPase of such a purity [15,16,25]. Actually, inhibition of the enzyme by oxidants is known to result in simultaneous decrease in DTNB titratable SH-groups [20,25], thus demonstrating that thiol groups directly involve in oxidative modification of Na/K-ATPase.

Following this observations, we have compared in further experiments the effect of DNIC-CYS (1:2) complex on both enzymatic activity and DTNB accessible SH-groups of kidney enzyme. As it is seen in Fig. 5, both enzyme activity and SH-groups content are suppressed simultaneously with time of preincubation. 50%-inhibition of the activity corresponds to 50%-disappearance of DTNB accessible thiols (Fig. 5).

Inhibition of Na/K-ATPase by the compounds tested suggests to be irreversible one, because enzyme activity is not restored by 40 times dilution of the sample after preincubation. The same conclusion was reached in experiments with oxidative attack on Na/K-ATPase carried out by hypochlorous anion or hydrogen peroxide [22]. In the case of $NO^{\cdot}$ complexes, however, enzyme activity suppressed by DNIC-CYS can be restored by SH-containing compounds like cysteine or dithiotreitol (DTT). Fifteen min incubation of kidney enzyme with diamagnetic form of DNIC-CYS suppresses the activity by 40% and successive 10 min incubation of the sample with 1 mM cysteine (25°C) results in complete restoration of the activity (Table 2). Use of 100 μM DTT under the same conditions recovers the activity by 94%. In this last case, the total restoration of SH-groups of the enzyme was also demonstrated (Table 3).

3.4. Substrate dependence

Substrate dependence of the enzyme is described by a complex curve with intermediary plateau [26]. This feature presumably reflects the interprotomer interaction within oligomeric complexes of the membrane bound enzyme, at least solubilization of the enzyme by non-ionic detergent (being not followed by a suppression of enzyme activity) decreases the molecular size of Na/K-ATPase close to its protomeric form with a subsequent transformation of the substrate dependence curve into one that is hyperbolic [27]. We have demonstrated recently that oxidative modification of Na/K-ATPase being accompa-

Table 2
Effect of DNIC-CYS on kidney Na/K-ATPase (from three experiments)

Conditions	Enzyme activity (mean ± S.D.) (μmol P_i/mg per h, 25°C): with no additions	Enzyme activity (mean ± S.D.) (μmol P_i/mg per h, 25°C): with 1 mM cysteine
Control	211 ± 12	240 ± 17
50 μM DNIC-CYS (1:2)	125 ± 8	245 ± 15

Table 3
Effect of 50 μM DNIC-CYS (1:2) on Na/K-ATPase activity and SH-group content in kidney enzyme preparation in the absence or presence of 100 μM DTT (from three experiments)

Conditions	Na/K-ATPase activity (mean ± S.D.) (μmol P_i/mg per h at 25°C); without DTT	Amount of SH-groups (mean ± S.D.) (nmol/mg protein), without DTT	Na/K-ATPase activity (mean ± S.D.) (μmol P_i/mg per h at 25°C); with 100 μM DTT	Amount of SH-groups (mean ± S.D.) (nmol/mg protein), with 100 μM DTT
Control	170 ± 11	46 ± 3	179 ± 9	53 ± 4
+DNIC-CYS	119 ± 10	30 ± 2	160 ± 10	46 ± 3

nied by attack to SH-groups of the protein, also resulted in simplification of the substrate dependence curve [25]. In these experiments we have found the same correlation — inhibition of the activity being accompanied with decrease in amount of SH-groups simultaneously resulted in transformation of the curve with an intermediary plateau into that close to hyperbolic one (Fig. 6).

The substrate dependence curve for the native enzyme consists of two components, a Michaelis curve and a Hill curve [25] each corresponding approximately half of the maximal activity ($V_{max\ M} + V_{max\ H} = 0.504 + 0.497 = 1.01$). The some substrate dependence for the oxidazed enzyme (40 μM DNIC-Cys 1:2, 15 min at 4°C) is consisted of only first part ($V_{max\ M}$) which is equal to 0.525 arbitrary units. Thus the Hill component disappeared after oxidative modification with subsequent decrease in affinity of the enzyme to ATP (K_m increased from 0.075 mM to 0.816 mM) (Fig. 6).

3.5. Modification of S-nitroso glutathione efficiency

Effect of mononitroso glutathione on the enzyme was strongly increased when 36 μM $FeSO_4$ and 72 μM cysteine were simultaneously added to 100 μM GS-NO. In these experiments brain enzyme was preincubated 15 min at 4°C with either 100 μM GS-NO or 100 μM GS-NO + 36 μM $FeSO_4$ + 76 μM cysteine; then Na/K-ATPase activity was tested. In the former case only 11% inhibition was observed, while in the latter one the enzyme was inhibited by

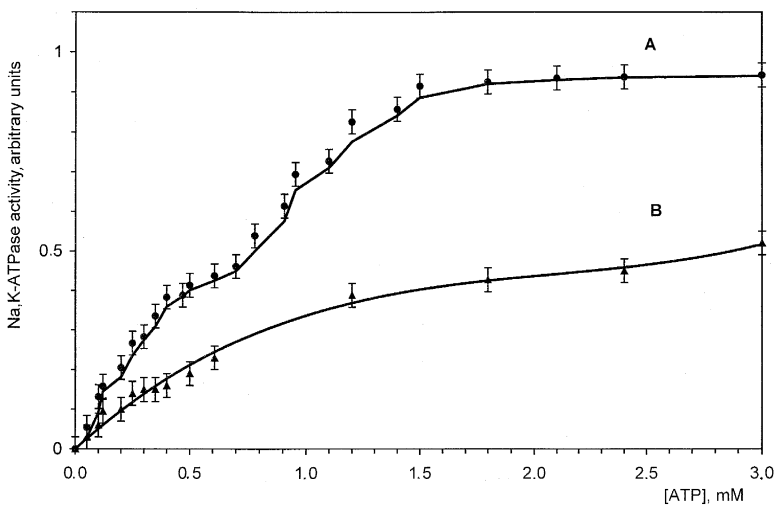


Fig. 6. Dependence of dog kidney Na/K-ATPase on ATP concentration. A: native enzyme; B: sample of enzyme after 30 min preincubation with 42 μM DNIC-CYS (1:2). Na/K-ATPase (130 μg/ml) was treated with DNIC-CYS as described in Fig. 1. Activity was measured at 37°C in medium containing 0.03–3 mM ATP, 5 mM $MgCl_2$, 130 mM NaCl, 20 mM KCl, 30 mM PIPES (pH 7.4) and expressed in arbitrary units.

about 70%. We suggest that in excess of ferrous ions and cysteine, some portion of GS-NO· was transformed into diamagnetic DNIC-CYS complex. Such transformation was recently demonstrated in model experiments when $FeSO_4$ and cysteine were added to S-nitrosothiol solution [14,28].

Actually, simultaneous addition of 36 μM Fe^{2+} and 72 μM cysteine to 100 μM GS-NO resulted in the decomposition of GS-NO and formation of diamagnetic DNIC-GS in concentrations corresponding to the amount of NO· released and ferrous ions accessible (no more than 36 μM). We have measured the amount of GS-NO breaking down under these conditions, using its characteristic absorbtion spectrum (band at 338 nm) and calculated that under conditions used, 70–80% of GS-NO is dissipated. As a result of interaction of NO· formed with 36 μM $FeSO_4$ and 72 μM cysteine added, about 36 μM of diamagnetic DNIC-CYS has to be formed. Thus, the mixture of low active GS-NO and highly active DNIC-CYS was characterised by increased inhibitory activity than GS-NO alone.

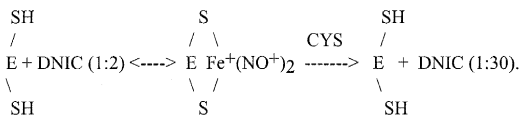
Contrary to these data, paramagnetic DNIC (1:30) complex has no inhibiting effect on Na/K-ATPase activity but even slightly (by 12–15%) activated the enzyme. The results presented suggest that transformation of the NO-thiol derivatives in the cell can exert diverse effects on SH-containing proteins depending on environmental concentration of low-molecular weight thiols.

4. Discussion

It was demonstrated in the literature that Na/K-ATPase of different tissues can be regulated by protein kinases [29,30]. On the other side, cellular effects of NO· is known to be result in cGMP formation and protein kinase C activation [7,31]. In our enzyme preparations, however this way of regulation can not be realyzed because of lack of protein kinase activities which was demonstrated by the absence of enzyme phosphorylation without addition of exogenous protein kinases (our unpublished observation). Thus, effect of the compounds studied on Na/K-ATPase activity is presumably mediated by not the regulatory phosphorylation as in the case of protein kinase but modification of sulfhydryl groups of the enzyme by nitroso compounds.

Of all the inhibitors used the most effective in modification of Na/K-ATPase were diamagnetic DNIC containing thiol ligands glutathione or cysteine, the latter possessing the highest efficiency. The following mechanism of their action can be proposed consisting of transfer of $Fe^{+}(NO^{+})_2$ from the complex used onto thiol groups of the protein. This blocks participation of the groups modified in catalytic processes and/or interprotomer interactions.

This possibility is illustrated by an interaction of bovine serum albumin and DNIC (1:2) with low-molecular thiol containing ligands [28]. The reaction was expressed in a quantitative transfer of $Fe^{+}(NO^{+})_2$ group from DNIC to the protein. Further addition to this mixture of low-molecular weight thiols, namely cysteine, in excess, resulted in dissociation of the complex formed. Under high concentration of cysteine a reversal process took place — transfer of $Fe^{+}(NO^{+})_2$ onto cysteine. It is likely that a similar processes took place when restoration of Na/K-ATPase activity inhibited by diamagnetic highly active DNIC-CYS was demonstrated after addition of cysteine or DTT to reaction mixture (Tables 2 and 3). The common mechanism of Na/K-ATPase inhibition by DNIC is suggested to be described by following reaction:



GS-NO is much less effective as inhibitor of Na/K-ATPase than DNIC. In agreement with present days understanding, interaction of the S-nitrosyl group with proteins results in S-*trans*-nitrosylation, e.g., transfer of NO^{+} group from S-nitroso thiols to SH-groups of protein. However the low effect of GS-NO on Na/K-ATPase and the increase of the inhibitory action after transformation of GS-NO into DNIC suggests that more reliable mechanism of SH-groups inactivation includes the above reaction.

It is well known that Fe ions and S-nitroso thiols easily form thiol-containing DNIC in the presence of cysteine [14,28]. In agreement with data presented, formation of the dimeric DNIC complex will inhibit SH-containing proteins more efficiently than in the

case of S-nitroso thiols. Transformation of S-nitroso thiols into DNIC was demonstrated in living cells [28,32]. This attracts an attention to mechanism(s) of regulation of SH-containing proteins like K-channels, NMDA receptors or Na/K-ATPase by nitroso compounds in vivo.

As we noted, Sato et al. [8] have demonstrated inhibition of commertially available Na/K-ATPase from cerebral cortex by a number of $NO^{\cdot}$ generating drugs. The inhibiting action of these drugs (but not of SNP) was eliminated by reduced glutathione, DTT and superoxide dismutase, the last being used as $NO^{\cdot}$ scavenger. The inhibiting effect of SNP was accelerated by DTT. The conclusion was that NO-derived products may attack SH-groups of the enzyme to induce their oxidation [8]. Our data directly demonstrate this possibility. The inhibiting effect of $NO^{\cdot}$ on membrane bound Na/K-ATPase in our experiments is realised by formation of nitroso compounds which attack protein SH-groups resulting in suppression of the activity.

At first sight, a recent publication of Gupta et al. [33] is in contradiction with these conclusion. Gupta et al. have found that SNP or GS-NO possesses a dose-dependent activating action on $^{86}Rb^{+}$ uptake and relaxation of smooth muscle. This effect was not mimicked by 8-bromocyclic GMP evidencing that it was realized not via guanilate cyclase, but was suppressed by ouabain and methylene blue. The authors concluded that the NO radical can activate (not inhibit) Na/K-ATPase, thus stimulating relaxation of smooth muscle cells [33].

Among several explanations of these data obtained on such complex system as the whole muscle, the likelyhood of the direct $NO^{\cdot}$ effect on Na/K-ATPase is the less possible one. Na/K-ATPase of smooth muscle membranes is presented by the same isoforms as brain enzyme [24], which, as we demonstrated is not sensitive to direct addition of SNP. More likely, the reason for activation of the ouabain dependent $^{86}Rb^{+}$ uptake by $NO^{\cdot}$ lies in activation of antioxidant defense of the cells which was recently demonstrated [2]. This effect could supposingly result in mobilization of cell thiols protecting SH-containing proteins. This opportunity reflecting indirect effects of $NO^{\cdot}$ other than that directed toward protein kinase system can be realyzed in the whole muscle. Additional regulatory way consisting in the inhibiting effect of $NO^{\cdot}$ on Na/K-ATPase was described here which is realyzed via formation of dinitrosyl complexes and their attack of SH groups of protein.

Thus, interpretation of multiple effects of S-nitroso thiols in biological systems has to be due regard to their secondary effects (namely, DNIC formation). The study presented here leads to the conclusion that Na/K-ATPase, the key enzyme regulating asymmetric distribution of monovalent ions across outer cell membranes, could be regulated in situ by nitroso thiols but not by the $NO^{\cdot}$ radical alone. The further step of our study has to be demonstration of the effect of these compounds on Na/K-ATPase in intact tissue.

Acknowledgements

The work is supported by Russian Foundation for Basic Research, Nos. 96-04-49078a and No. 96-04-48066.

References

[1] J.F. Kervin, J.R. Lancaster, P. Feldman, J. Med. Chem. 38 (1995) 4343–4361.
[2] C.C. Chiueh, D.L. Gilbert, A.C. Carol (Eds.), The Neurobiology of NO and OH Radicals, New York, 1993.
[3] V. Darley-Usmar, H. Wiseman, B. Halliwell, FEBS Lett. 369 (1995) 131–135.
[4] V.M. Bolotina, S. Najibi, J.J. Palacino, P.J. Pagano, R.A. Cohen, Nature 368 (1994) 850–853.
[5] S.A. Lipton, Trends Neurosci. 16 (1993) 527–532.
[6] S. Gupta, C. McArthur, Ch. Grady, N. Ruderman, Am. J. Physiol. 266 (1994) H2146–H2151.
[7] M. McKee, C. Scavone, J. Nathanson, Proc. Nat. Acad. Sci. USA 91 (1994) 12056–12060.
[8] T. Sato, Y. Kamata, M. Irufine, T. Nishikawa, Brain Res. 704 (1995) 117–120.
[9] A. Boldyrev, E. Bulygina, G. Kramarenko, Byull. Exp. Biol. Med. (Rus) 3 (1996) 275–278.
[10] W. Coetzee, H. Ichikawa, D. Hearse, Am. J. Physiol. 266 (1994) H909–H919.
[11] J.S. Stamler, Cell 78 (1994) 931–936.
[12] Y. Henry, M. Lepoivre, J.-C. Drapier, C. Ducrocq, J.L. Boucher, A. Guissani, FASEB J., vol. 7, 1993, pp. 1124-1134.
[13] A.F. Vanin, Biochemistry (Moscow) 60 (1995) 308–314.
[14] A.F. Vanin, Biochemistry (Moscow) 60 (1995) 441–447.
[15] I. Klodos, P. Ottolenghi, A. Boldyrev, Anal. Biochem. 67 (1975) 397–403.

[16] W.-H. Huang, Y. Wang, A. Askari, N. Zolotarjeva, M. Ganjeizadeh, Biochim. Biophys. Acta 1190 (1994) 108–114.
[17] W.B. Rathbun, M.V. Betlach, Anal. Biochem. 28 (1969) 436–441.
[18] O.H. Lowry, N.Y. Rosebrough, A.L. Farr, R.J. Randall, J. Biol. Chem. 193 (1951) 265–273.
[19] G.L. Ellman, Arch. Biochem. Biophys. 82 (1959) 70–77.
[20] E. Kurella, A. Osipov, R. Goldman, A. Boldyrev, V. Kagan, Biochim. Biophys. Acta 1232 (1995) 52–58.
[21] J. Joseph, B. Kalyanaraman, J.S. Hyde, Biochem. Biophys. Res. Commun. 192 (1993) 926–934.
[22] A. Boldyrev, E. Bulygina, E. Volynskaya, E. Kurella, O. Tyulina, Biochemistry (Moscow) 60 (1995) 1293–1298.
[23] Z. Xie, M. Jack-Hays, Y. Wang, S.M. Periyasamy, G. Blanco, W.-H. Huang, A. Askari, Biochem. Biophys. Res. Commun. 207 (1995) 155–159.
[24] K.J. Sweadner, Biochim. Biophys. Acta 988 (1989) 185–220.
[25] A. Boldyrev, E. Kurella, Biochem. Biophys. Res. Commun. 222 (1996) 483–487.
[26] O. Lopina, N. Sarvazyan, A. Askari, A. Boldyrev, Arch. Biochem. Biophys. 321 (1995) 429–433.
[27] A. Boldyrev, O. Lopina, N. Fedosova, Biochim. Int. 21 (1990) 45–52.
[28] A.F. Vanin, I.V. Malenkova, P.I. Mordvintcev, A. Mulsh, Biochemistry (Moscow), vol. 58, 1993, pp. 1094-1103.
[29] M. Fesenko, K. Sweadner, J. Biol. Chem. 269 (1994) 30436–30444.
[30] C. Scavone, C. Scanlon, M. McKee, J.A. Nathanson, J. Pharmacol. Exp. Ther. 272 (1995) 1036–10443.
[31] J.A. Nathanson, C. Scavone, C. Scanlon, M. McKee, Neuron 14 (1995) 781–794.
[32] B. Roy, M. Lepoivre, Y. Henry, M. Fortecare, Biochemistry 34 (1995) 5411–5420.
[33] S. Gupta, R.B. Moreland, R. Munarriz, J. Daley, I. Goldstein, I. Saenz de Tejada, Br. J. Pharmacol. 116 (1995) 2201–2206.