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VANADATE INHIBITION OF SARCOPLASMIC RETICULUM Ca^{2+}-ATPase AND OTHER ATPases

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SUMMARY: Vanadate is a potent inhibitor of the Ca^{2+}-ATPase activity of sarcoplasmic reticulum in the presence of A-23187. The purified enzyme is sensitive to vanadate even in the absence of the ionophore. Ca^{2+} and norepinephrine protect the enzyme against inhibition of vanadate. The nonspecificity of vanadate is emphasized by the finding of inhibition of several other ATPases including the $Ca^{2+}Mg^{2+}$-ATPases of the ascites and human red cell plasma membranes, Mg^{2+}-ATPase of the ascites plasma membrane, and the K^{+}-ATPases of E. coli and hog gastric mucosal cell membranes. The ascites plasma membrane Ca^{2+}-ATPase (an ecto ATPase) and mitochondrial ATPase are not inhibited by vanadate.

ATP extracted from equine muscle contains a potent inhibitor of $Na^{+}K^{+}$-ATPase from several sources (1-8). This inhibitor was identified as vanadate (9). While the potency of the inhibitor is increased by K^{+} and Mg^{2+} (4,7), Na^{+} reverses and catecholamines prevent inhibition (4).

The report (7) that ATP containing vanadate did not inhibit the ATPase of sarcoplasmic reticulum (SR) vesicles is inconsistent with the observation that ATP containing vanadate inhibited purified SR Ca^{2+}-ATPase (4). This discrepancy prompted us to reinvestigate the specificity of vanadate. We show in this communication that vanadate is an effective inhibitor of purified Ca^{2+}-ATPase as well as of the ATPase of SR vesicles in the presence of A-23187. Low concentrations of Ca^{2+} protect the enzyme against inhibition by vanadate. The lack of vanadate specificitiy is further documented and discussed.

MATERIALS AND METHODS

Radioactive orthophosphate ($^{32}P_i$) for the preparation of [γ-^{32}P]ATP (10) was obtained from ICN, Inc. A-23187 was a gift from Dr. R. Hosley of Eli Lilly. Sodium orthovanadate was purchased from Fisher Scientific, asolectin from Associated Concentrates (Woodside, Long Island, NY) and octyl-ß-D-glucopyranoside from Calbiochem. All other biochemicals were obtained from Sigma.

ABBREVIATION: SR, sarcoplasmic reticulum.

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Rabbit muscle SR was the R_1 preparation of MacLennan (11). Ca^{2+}-ATPase from SR was purified by octyl-β-D-glucopyranoside extraction (12). Plasma membranes were prepared from Ehrlich ascites cells as described (13) except that 10 mM $CaCl_2$ was substituted for 1 mM $ZnCl_2$ in order to increase yield and stability (Miyamoto, H. and Racker, E., unpublished observations). Human blood cell ghosts were donated by Dr. D. Sogin and K^+-ATPase from hog gastric mucosal cells by Dr. G. Sachs. The E. coli K^+-stimulated, membrane-associated ATPase of the Kdp K^+-transport system was assayed in urea-extracted French press vesicles prepared from cells of K-12 strain TK 2242 (14). Bovine heart mitochondrial ATPase (F_1) was prepared as described (15).

In each case ATPase activity was determined as the release of $^{32}P_i$ from [γ-^{32}P]ATP (10). SR vesicles or purified SR Ca^{2+}-ATPase were incubated for 4 min at 37° in a medium (1.0 ml) consisting of 50 mM Tris-HCl, pH 7.5, 100 mM KCl, 5 mM $MgCl_2$, 50 μM $CaCl_2$, 10 mM [γ-^{32}P]ATP (4.10 x 10^4 cpm/μmole), and 20 μg protein. The reactions were stopped by the addition of 50 μl 50% trichloroacetic acid. Data shown reflect only activity which is sensitive to ethyleneglycol-bis-(β-aminoethyl ether)N,N'-tetra-acetic acid. Ehrlich ascites cell ethyleneglycol-bis(β-aminoethyl ether)N,N'-tetra-acetic acid-sensitive Ca^{2+}-ATPase, Mg^{2+}-ATPase, $Ca^{2+}Mg^{2+}$-ATPase, and human red cell ghost $Ca^{2+}Mg^{2+}$-ATPase were assayed in a medium (1.0 ml) of 100 mM imidazole-HCl, pH 6.8, 1 mM ouabain, 3 mM [γ-^{32}P]ATP, plus cations and protein, as indicated. Mitochondrial ATPase was assayed for 15 min at 37° (16), gastric mucosal cell K^+-ATPase activity was assayed as described (17), and E. coli K^+-ATPase was determined by the method of Epstein et al. (14). E. coli K^+-ATPase activity was calculated as the hydrolytic activity in the presence of K^+ minus that in the presence of Na^+. Protein was determined in the presence of 0.8% deoxycholate according to Lowry et al. (18) using crystalline bovine serum albumin as standard.

RESULTS

As shown in Fig. 1 (panel A) SR Ca^{2+}-ATPase activity is sensitive to vanadate if tested in the presence of A-23187. As in the case of the Na^+K^+-ATPase, norepinephrine protects against the inhibition. Similar data were obtained with purified Ca^{2+}-ATPase in the absence of A-23187 (panel B). The presence of ionophore confers a slight increase in sensitivity to the inhibitor.

Since vanadate was much less effective on SR vesicles in the absence of A-23187, it seemed likely that low concentrations of Ca^{2+} bound to a hydrophobic site protect the enzyme. Similar observations have been made with dicyclohexylcarbodiimide which inhibits the Ca^{2+}-ATPase only in the presence of A-23187 (19). It can be seen from Table I that vanadate loses its ability to inhibit the enzyme in the presence of increasing Ca^{2+} concentrations. This experiment, however, is complicated by the fact (11) that at concentrations above 100 μM, Ca^{2+} itself inhibits the enzyme. Therefore, at 500 μM Ca^{2+}, the activity and insensitivity of the enzyme to vanadate resembles that of SR vesicles.

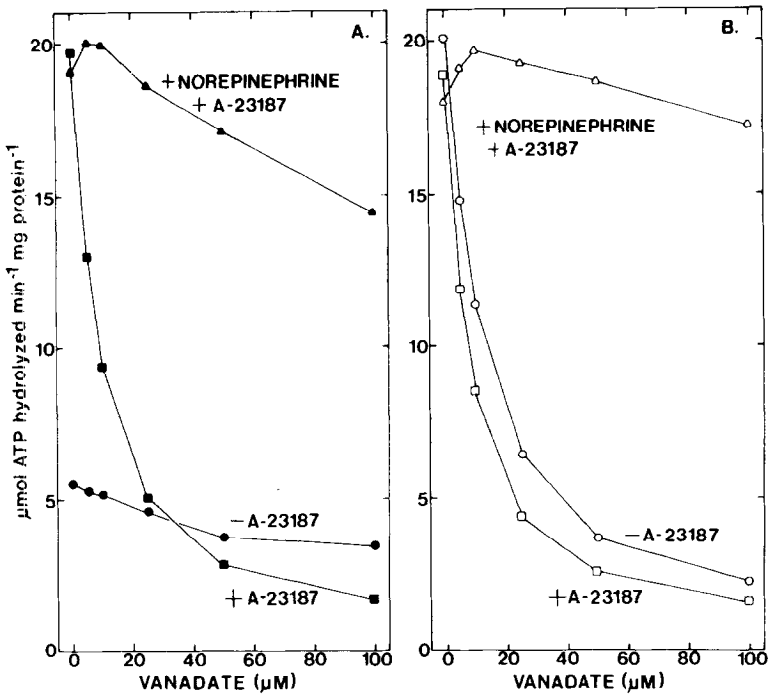

Fig. 1. Vanadate inhibition of SR Ca^{2+}-ATPase. SR vesicles (panel A) or purified Ca^{2+}-ATPase (panel B) were assayed for Ca^{2+}-ATPase activity, as described under "Materials and Methods," in the presence of varying concentrations of Na_3VO_4, plus 5 μg A-23187 and 2 mM norepinephrine where indicated.

TABLE I

Effect of Ca^{2+} Concentration on the Vanadate Inhibition of SR Ca^{2+}-ATPase

$CaCl_2$ (μM)	Specific activity μmol ATP hydrolyzed min^{-1} mg $protein^{-1}$		% Inhibition
	$- VO_4^{3-}$	$+ VO_4^{3-}$	
50	21.6	6.34	70.6
100	18.8	6.99	62.8
250	12.9	8.30	35.6
500	7.60	7.52	1.0

Assays were performed as outlined under "Materials and Methods" using the noted $CaCl_2$ and 25 μM Na_3VO_4, where indicated.

The finding that vanadate inhibited SR Ca^{2+}-ATPase prompted an examination of other ATPases (Table II). Of the enzymes tested, only the mitochondrial ATPase and the Ca^{2+}-ATPase of Ehrlich ascites tumor cell plasma membranes were resistant to vanadate inhibition.

TABLE II

Effect of Vanadate on Other ATPases

Enzyme	Cation (mM)	Specific activity μmol ATP hydrolyzed min mg protein		% Inhibition
		$-VO_4^{3-}$	$+ VO_4^{3-}$	
Ascites plasma membrane Ca-ATPase	Ca^{2+},3	0.0173	0.0169	2.3
Ascites plasma membrane Mg-ATPase	Mg^{2+},3	0.0126	0.0074	40.8
Ascites plasma membrane CaMg-ATPase	Ca^{2+},0.025			
	Mg^{2+},6	0.022	0.0076	65.6
Human red blood cell CaMg-ATPase	Ca^{2+},0.025			
	Mg^{2+},6	0.0261	0.0081	69.0
Gastric K-ATPase	K^+,2	0.689	0.276	60.0
E. coli K-ATPase	K^+,10	0.137	0.059	56.9
Mitochondrial F_1 ATPase	Mg^{2+},3	24.1	24.5	- 1.6

Ehrlich ascites cell plasma membranes (615 μg), human red blood cell ghosts (190 μg), gastric mucosal cell plasma membrane K-ATPase (131 μg), E. coli K-ATPase (200 μg), and mitochondrial F_1 ATPase (2 μg) were assayed, as described under "Materials and Methods," in the presence of the indicated cations with or without 10 μM Na_3VO_4.

DISCUSSION

Vanadate is clearly not a specific inhibitor of Na^+K^+-ATPase. Among the enzymes which are sensitive are dynein ATPase (20,21), myosin ATPase (22), Saccharomyces cerevisiae plasma membrane Mg^{2+}-ATPase (23), the plasma membrane ATPase of Neurospora crassa (24), and the ATPases reported in this paper, including the Ca^{2+}-ATPase of SR, the ascites plasma membrane $Ca^{2+}Mg^{2+}$-ATPase which functions as an ATP-driven Ca^{2+} pump in a reconstituted system (25), the ascites plasma membrane Mg^{2+}-ATPase, whose function is unknown, and the K^+-ATPases of E. coli and hog gastric mucosal cell membranes. Moreover,

several other enzymes are reportedly sensitive to vanadate, namely, ribonuclease (26), and acid (27) and alkaline (28) phosphatases.

It is of interest therefore, that mitochondrial ATPase and ascites plasma membrane Ca^{2+}-ATPase are not sensitive to vanadate. The latter, which is active in the presence of Ca^{2+} and absence of Mg^{2+}, is probably an ectoenzyme since similar activity can be obtained with intact cells (Suolinna, E-M., and Racker, E., unpublished observation). We found that Ca^{2+}-ATPase activity of intact ascites cells is also resistant to vanadate. Unlike the Ca^{2+}-ATPase of SR, the Ca^{2+}-ATPase of the ascites plasma membrane was not activated by A-23187 and did not become sensitive to vanadate in the presence of the ionophore.

Cantley et al. (29) proposed that vanadate prevents $Na^{+}K^{+}$-ATPase turnover by binding to the site of enzyme phosphorylation. If this interesting suggestion is correct, it would imply that neither the mitochondrial ATPase nor the plasma membrane Ca^{2+}-ATPase undergo the cyclic phosphorylation-dephosphorylation steps characteristic of other ATPases which are sensitive to vanadate.

Finally, it should be pointed out that since the low amounts of Ca^{2+} present in SR vesicles protect the Ca^{2+}-ATPase against vanadate, this inhibitor may be useful for studying the Ca^{2+} pump of the SR without interference caused by the $Na^{+}K^{+}$-ATPase.

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