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Polyvanadate acts at the level of plasma membranes through α-adrenergic receptor and affects cellular calcium distribution and some oxidation activities

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Abstract. The activities of calcium-stimulated respiration, calcium uptake, α-glycerophosphate dehydrogenase and rates of oxidation in state 3 and of H_2O_2 generation, were found to increase and that of pyruvate dehydrogenase decrease in mitochondria isolated from livers of rats administered intraperitoneally or perfused with polyvanadate. Phenoxybenzamine, an antagonist of α–adrenergic receptor, effectively prevented these changes. It was also found that perfusion of the liver with polyvanadate reproduced one of the best characterized events of α-adrenergic activation-stimulation of protein kinase C in plasma membrane accompanied by its decrease in cytosol. These experiments indicate for the first time the α-adrenergic mimetic action of polyvanadate.

Keywords. Polyvanadate; α-adrenergic receptor; calcium; oxidation.

Introduction

Consistent with vanadium being a micronutrient, salts of vanadate are shown to influence metabolic processes (Ramasarma and Crane, 1981; Boyd and Kustin, 1984) and to possess insulin-mimetic (Tamura *et al.*, 1984) and noradrenaline mimetic (Schawabe *et al.*, 1979) properties. These actions of vanadate are obtained through its abilities other than the well known inhibition of a variety of phosphatases, including Na, K-ATPase (Cantley *et al.*, 1977).

A role of calcium in the mechanism of action of α-adrenergic receptor system is now well-documented (Taylor *et. al.*, 1987). Based on pharmacological potency and radioligand binding, the catecholamine receptors are classified as *α*- and *β*-types. While the activation of *β* -adrenergic receptor is now identified to act through adenyl cyclase, cAMP and cascade of phosphorylations, the action of *α*-type receptor is unclear. A number of effects of glycogenolysis, gluconeogenesis, respiratory activity and phosphatidylinositol turnover obtained on treatment with noradrenaline or the agonists are considered to be due to redistribution of calcium, both extra and intracellular stores. All these effects are prevented by pretreatment with phenoxybenzamine, a general antagonist of *α*-adrenergic system.

We found that 3 mitochondrial activities—H_2O_2 generation, α-glycerophosphate dehydrogenase and calcium-stimulated oxygen uptake-were stimulated, and also depletion of mitochondrial calcium on treatment of rats with noradrenaline (Sivaramakrishnan and Ramasarma, 1983; Swaroop *et al.,* 1983) or vanadate (Gullapalli *et al.*, 1989a, b). These effects were prevented by phenoxybenzamine. These results suggest that vanadate can act as an agonist of α-adrenergic receptor.

Calcium-stimulated oxygen uptake, calcium redistribution and mitochondrial enzymes in polyvanadate-treated animals

A transient (about 20 s) spurt of oxygen uptake occurs when small amounts of calcium ions are added to tightly coupled mitochondria. This is accompanied by

calcium uptake by the organelle (Carafoli and Sottocosa, 1984). Mitochondria isolated from livers of rats injected intraperitoneally with polyvanadate (4 μmol/rat, 1 h), and also metavanadate, showed significantly higher rates of this activity and the net oxygen uptake (table 1). This increase was prevented by pretreatment with phenoxybenzamine, but not propranolol or in sympathectomized rats (table 1), These results suggested that polyvanadate is acting through α-adrenergic receptor, and probably not *via* increasing endogenous noradrenaline.

The increase in calcium-stimulated respiration is an indication of depletion of calcium from mitochondria. On measuring the distribution of calcium in mitochondrial and cytosolic fractions obtained by differentia centrifugation in sucrose medium or rapidly in percoll gradient, decrease in mitochondria and increase in cytosol were demonstrated (table 1). The redistribution pattern was further confirmed by increase of ^{45}Ca-uptake in mitochondria with corresponding decrease in cytosol (table 1).

Table 1. Effect of treatment with vanadate on calcium redistribution and mitochondrial activities.

	Control	Vanadate	% control
Ca^{2+}-stimulated oxygen uptake			
Polyvanadate (PV)	7·3 ± 1·0	13·1 ± 1·5	179
Metavanadate	7·4 ± 1·2	13·2 ± 2·5	178
PV + phenoxybenzamine	7·5 ± 0·8	7·2 ± 1·5	96
PV + propranolol	5·0 ± 1·6	10·1 ± 2·2	202
PV in sympathectomy	6·9 ± 1·1	11·0 ± 0·9	159
Calcium distribution			
Mitochondrial (sucrose)	17·7 ± 1·5	11·3 ± 0·4	63
Cytosolic (sucrose)	9·1 ± 1·6	14·1 ± 2·6	154
Mitochondrial (percoll)	18·9 ± 2·0	9·7 ± 1·8	51
Cytosolic (percoll)	11·9 ± 1·4	19·7 ± 2·4	168
^{45}Ca-uptake			
Mitochondrial	30·8 ± 1·4	40·3 ± 0·6	130
Cytosolic	33·8 ± 2·8	21·6 ± 0·7	63
Mitochondrial enzymes			
State 3 respiration (succinate)	92 ± 10	125 ± 16	136
+ phenoxybenzamine	100 ± 4	96 ± 15	96
α-Glycerophosphate dehydrogenase	11·6 ± 2·1	17·7 ± 2·4	152
+ phenoxybenzamine	14·7 ± 1·5	13·3 ± 1·4	90
Pyruvate dehydrogenase	10·8 ± 1·5	5·3 ± 0·9	49
+ phenoxybenzamine	12·7 ± 4·2	11·3 ± 3·2	83
H_2O_2 generation (succinate)*	0·12 ± 0·03	0·20 ± 0·03	167

The experimental details are given in Gullapalli *et al.* (1989a,b). Polyvanadate (or metavanadate) was given intraperitoneally at a dose of 4 μmol/rat, 1 h before killing and Rivers were processed for various estimations. Where mentioned, phenoxybenzamine (2·5 mg/rat) or propranolol (1·7 mg/rat) was given 20 min before polyvanadate.

*Experiments with phenoxybenzamine in this case were not done.

The mitochondrial enzyme activities of state 3 respiration with succinate as the substrate, α-glycerophosphate dehydrogenase and succinate-dependent H_2O_2

generation increased and that of pyruvate dehydrogenase decreased on vanadate treatment (table 1). These changes were also obtained on treatment with noradrenaline and were sensitive to pretreatment with phenoxybenzamine, pointing to the participation of α-adrenergic receptor.

Experiments on perfusion with polyvanadate confirm direct action on liver tissue

Perfusion of livers at slow rates with unbuffered 0·25 M sucrose ensured removal of blood from the organ without affecting the structural integrity of subcellular organelles and avoided any possible salt effects. Perfusion of livers with a medium containing 100 μM of polyvanadate for short periods of 5–10 min gave effects similar to the experiments with intraperitoneal administration. Polyvanadate, but not metavanadate, increased calcium-stimulated oxygen uptake and this effect was sensitive to phenoxybenzamine, but not propranolol (table 2).

Table 2. Effect of perfusion of livers with polyvanadate on calcium distribution, mitochondrial activities and protein kinase C.

	Control	Polyvanadate	% control
A. Ca^{2+}-stimulated oxygen uptake	ng atom 0/mg protein		
Polyvanadate (PV)	8·2 ± 0·9	11·8 ± 1·4	144
Metavanadate	7·1 ± 1·0	7·0 ± 1·3	99
PV + phenoxybenzamine	7·3 ± 0·7	7·3 ± 0·8	100
PV + propranolol	8·2 ± 0·8	11·5 ± 1·0	140
B. Mitochondrial enzymes	nmol/min/mg protein		
State 3 respiration (succinate)	87 ± 9	82 ± 5	95
+ phenoxybenzamine	87 ± 8	85 ± 9	98
α-Glycerophosphate dehydrogenase	13·5 ± 1·2	21·3 ± 2·3	158
+ phenoxybenzamine	15·1 ± 1·4	14·4 ± 2·4	95
Pyruvate dehydrogenase	6·3 ± 0·4	3·2 ± 1·0	51
C. Protein kinase C	pmol/min/mg protein		
Plasma membrane	27 ± 6	165 ± 20	611
+ Phenoxybenzamine	13 ± 4	11 ± 2	84
+ Propranolol	17 ± 8	125 ± 12	735
Cytosol	132 ± 22	19 ± 2	14
+ Phenoxybenzamine	116 ± 21	121 ± 25	104
+ Propranolol	97 ± 27	11 ± 3	11

The livers were perfused with 100 μM polyvanadate (or metavanadate) in 0·25 M sucrose for 10 min in experiments A and B and for 5 min in experiment C. The experimental details are given in Gullapalli *et al.* (1988a, b, 1990). Where mentioned, the blocking agents were present in the perfusion medium at a concentration of 70 μg/ml. Protein kinase C activity is measured by the transfer of [γ-^{32}P]-ATP to histone H_1 in the presence of Ca^{2+}, phosphatidyl serine and diglyceride.

Of the mitochondrial enzyme activities, state 3 respiration with succinate as the substrate did not respond to polyvanadate perfusion, unlike intraperitoneal experiments. Phenoxybenzamine-sensitive increase in α-glycerophosphate dehydrogenase and decrease in pyruvate dehydrogenase were reproduced (table 2).

Increase in plasma membrane and decrease in cytosol of the activity of the calcium- and lipid-dependent protein kinase C are the well-characterized events of activation of α-adrenergic receptor (Kikkawa and Nishizuka, 1986). On brief perfusion for 5 min, polyvanadate mimicked these effects apparently acting as an agonist (table 2). Phenoxybenzamine prevented the changes of both the increase in plasma membrane and decrease in cytosol of protein kinase C while propranolol was unable to do so (table 2). These changes in protein kinase C activity are consistent with a plasma membrane-level of action of polyvanadate similar to that of an α-adrenergic agonist (Gullapalli *et al.*, 1990).

Polyvanadate must be acting at the level of plasma membrane

One other compelling reason to believe plasma membrane level of action is the finding that vanadium could not be detected in the liver tissue by atomic absorption spectroscopic analysis under the experimental conditions used. Also, addition of polyvanadate to mitochondria *in vitro* did not elicit similar responses. One interesting difference with vanadate treatment is the lack of mobilization of calcium from endoplasmic reticulum (Taylor *et al.*, 1987). It appears that the high accumulation of calcium in cytosol is due to inhibition of calcium pump by vanadate (Delfert and McDonald, 1985). The effects on redistribution of calcium and other redox enzymes appear to be indirect and the intracellular signals responsible must be generated by activation of the α-adrenergic receptor.

We tested the effect of vanadate treatment on incorporation of ^{32}P (100 μCi/rat intraperitoneally 1 h before vanadate) into inositol phosphatides (PIP2, PIP, PI) and phosphatidic acid and found little change in the pattern (data not shown) indicating that their altered turnover was not involved. Some other mechanisms of signal transduction may be utilized in vanadate action.

Vanadate-stimulated NADH oxidation in plasma membranes

An example of a polyvanadate-specific activity in plasma membranes in NADH oxidation discovered in our laboratories (Ramasarma *et al.,* 1981; Vijaya *et al.*, 1984). The properties of this system in rat liver plasma membranes are summarized in table 3. This activity will generate H_2O_2 with high rates and can be turned on only if vanadate is converted to the polymeric form by local acid conditions possible to obtain by proton gradients. Independently it appears vanadate-NADH system can generate hydroxyl radicals as the ESR spectra of DMPO-OH adducts

Table 3. Vanadate-stimulated NADH oxidation in rat liver plasma membrane.

Specific to NADH, K_m 36 μM; low activity with NADPH
Stoichiometry of NADH : O_2 : H_2O_2 is 1 : 1 : 1; rapid generation of H_2O_2
Phosphate (50 mM) is required for maximal activity
Activity increases in acid pH up to 5·0
Decavanadate, a polymeric form of vanadate produced in acid pH, is most active; maximal activity obtained at 5 μM (V_{10})
Hydroxyl radicals are produced during reaction

The activity of NDAH oxidation was measured by the decrease in absorbance at 340 nm. The experimental details are given in Ramasarma et al. (1981).

identified by us earlier (Vijaya *et al.*, 1984) are now considered to be due to hydroxyl radicals but not superoxide (Kuppusamy and Zweir, 1989). Will some of the vanadate-specific reactions occur on using H_2O_2 or hydroxyl radicals as secondary signals?

In this context it is interesting to recall some related effects of vanadate and H_2O_2 such as insulin mimetic action (Tamura *et al.*, 1984), insulin receptor protein-tyrosine phosphorylation (Haffetz and Zick, 1989), selective activation and inactivation by H_2O_2 of protein-kinase C (Gopalakrishna and Anderson, 1989).

Comparison of effects and structures of noradrenaline and decavanadate

We have been able to identify that a number of metabolic effects obtained by vanadate are similar to that by noradrenaline (Sivaramakrishnan and Ramasarma, 1983; Swaroop *et al.*, 1983). Among these are increases in calcium stimulated oxygen uptake in mitochondria, calcium concentration in cytosol, α-glycero-phosphate dehydrogenase and H_2O_2 generation in mitochondria (Gullapalli *et al.,* 1989a,b) and protein kinase C in plasma membrane, and also decreases of calcium concentration and pyruvate dehydrogenase in mitochondria and of protein kinase C in cytosol. All these effects appear to be mediated by α-adrenergic receptor, possibly the α_1-type, albeit not necessarily through inositol phosphatides. The effective form of vanadate is believed to be decavanadate ($V_{10}\ O_{28}^{6-}$) which

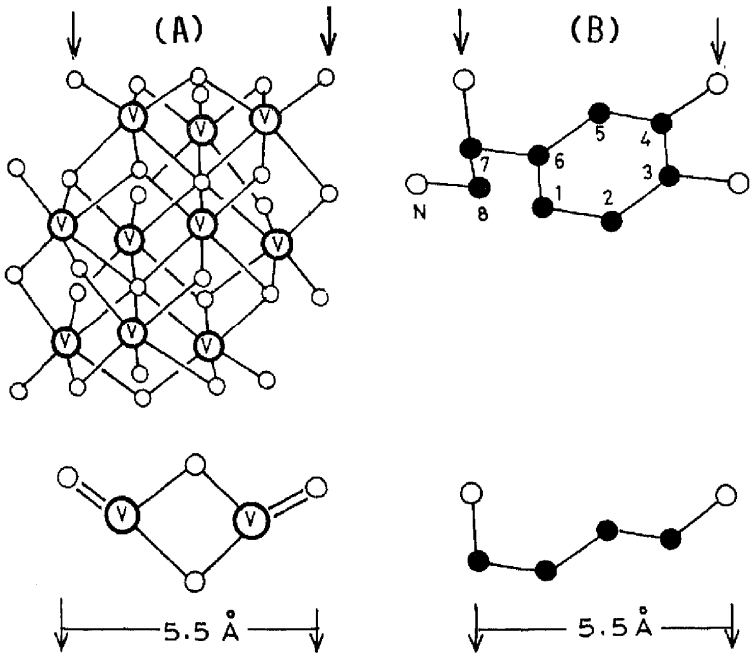

Figure 1. Comparison of structures of decavanadate (A) and noradrenaline (B). The structures were adapted from Debaerdemaekar *et al.* (1982) and Carlstrom and Bergin (1967) for decavanadate and noradrenaline, respectively. The pair of oxygens marked by arrows are at an approximate distance of 5·5 Å as shown in the abstracted portions shown below each structure and offer a common feature.

acquires on polymerization the characteristic structural feature O = V-O-V = 0 which seems to account for the 985 cm^{-1} IR band and a 3 banded NMR spectra. It is difficulty to explain how a simple inorganic polymer with nothing more than V-0 groups can stimulate actions of a catecholamine. On comparison of the crystal structures of decavanadate (Debaerdemaeker *et al.*, 1982) and noradrenaline (Carlstrom and Bergin, 1967), already available in literature, we found the presence of two oxygens at a distance of 5·5 Å (figure 1). Will these positioned pair of oxygens be responsible for ionic or H-bonded interactions with a similarly placed counter-atoms in a plasma membrane protein? Do the different activities in response of polyvanadate have a common transducing mechanism? Will H_2O_2 or hydroxyl radicals be used as signal transducers in the redistribution of calcium and protein kinase C? Investigations on these and other questions will unravel the multifaceted biological functions of vanadate.

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