Volume 267, number 1, 93–95 FEBS 08560 July 1990

Decavanadate acts like an α-adrenergic agonist in redistributing protein kinase C activity

Sharada Gullapalli, C.K. Ramakrishna Kurup and T. Ramasarma

Department of Biochemistry, Indian Institute of Science, Bangalore 560 012, India

Received 9 April 1990

Perfusion of rat livers with polyvanadate, but not metavanadate, was found to increase in plasma membrane and decrease in cytosol protein kinase C activity, similar to that obtained with phenylephrine, an α-adrenergic agonist The effect was prevented by phenoxybenzamine, but not by propranolol implicating α-adrenergic receptor activation Comparison of crystal structures of decavanadate and nonadrenaline revealed the occurrence of a structural feature of O–O–O(N) with distances of 5 5 Å and 2.9 Å

Decavanadate; Protein kinase C; α-Adrenergic agonist

1. INTRODUCTION

A variety of physiological processes and metabolic reactions are affected on treatment of animals or cells with salts of vanadium, known to be an essential trace element in some organisms (reviewed in [1–3]). Vanadate is found to mimic activities of noradrenaline of increase in the concentration of cyclic AMP in intact cardiac muscle [4], of contraction pulmonary artery [5], and of reduction of muscle tension [6]. It is not known whether such actions are obtained through activation of adrenergic receptors. Noradrenaline and adrenergic agonists are now established to act through α-adrenergic receptor for mobilization of intracellular pools of calcium resulting in its decrease in mitochondria and increase in cytosol in experiments with rat liver with isolated hepatocytes [7] or by perfusion [8]. The changes in calcium concentrations are accredited with the metabolic responses of activation of α-adrenergic receptor (reviewed in [9]).

We reported that intraperitoneal treatment of rats with noradrenaline [10,11] or decavanadate [12] showed a number of effects on hepatic mitochondria, including increases in activities of α-glycerophosphate dehydrogenase and substrate-dependent H_2O_2 generation. Increase in calcium-dependent respiration, depletion of mitochondrial calcium and decrease in pyruvate dehydrogenase activity, known to occur on activation of α-adrenergic receptors [13,14], also were obtained on treatment with decavanadate [15]. Under these conditions, the concentration of cytosolic calcium increased due to inhibition of plasma membrane Ca^{2+}-ATPase by vanadate [16], preventing its exit from the cells. These vanadate-induced changes were also obtained in sympathectomized animals excluding the possibility of intervention of endogenous noradrenaline, but were prevented by phenoxybenzamine, an α-adrenergic blocking agent. Vanadate added in vitro had no effect on these mitochondrial activities. Since its concentrations did not build up in the tissue under the experimental conditions, the action of vanadate is considered to be at the level of plasma membrane as a possible agonist of α-adrenergic receptor. We now show that the intracellular changes in protein kinase C are obtained on perfusion of livers with decavanadate, similar to phenylephrine, an α-adrenergic agonist.

Correspondence address: S. Gullapalli, Dept of Biochemistry, Indian Institute of Science, Bangalore 560 012, India

2. MATERIALS AND METHODS

Male albino rats (120–130 g) of the Wistar strain were anaesthetized with ethyl ether and the livers were perfused with 0.25 M sucrose at 37°C in a non-circulating mode at a flow rate of 4 ml/min. Decavanadate (50 μM) and other compounds were added to the sucrose solution where indicated. The liver blanched in about 2 min and perfusion was continued for the time periods indicated. The slow rate of perfusion with unbuffered sucrose ensured removal of blood from the organ without affecting the structural integrity of subcellular organelles and avoided any possible salt effects.

Liver plasma membrane vesicles were prepared by the method of centrifugation on Percoll gradient described by Prpic et al. [17]. The membrane fraction identified by 5′-nucleotidase activity (560 nmol AMP hydrolyzed/min per mg protein) was washed twice with 0.25 M sucrose containing Tris buffer (50 mM, pH 8.0), and suspended in Tris buffer (50 mM, pH 8.0), containing glycerol (10% w/v) and Triton X-100 (0.05% w/v) and stirred for 1 h in an ice-bath. The mixture was centrifuged at 15000 × g for 30 min and the supernatant fraction was used for the assay of protein kinase C as described by Kikkawa et al. [18] by measuring the Ca^{2+}-dependent transfer of ^{32}P from [γ-^{32}P]ATP to histone H_1 in the presence of phosphatidyl serine and diglyceride.

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/90/$3.50

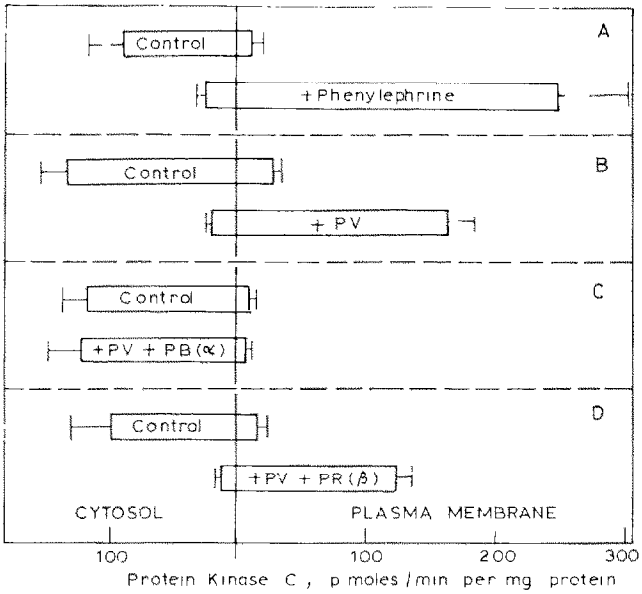

Fig. 1. Livers were perfused with 0.25 M sucrose (at 37°C) for 5 min in all the cases. Where mentioned, the medium contained phenylephrine (1 μM) (A) or decavanadate (50 μM) (B). The adrenergic blocking agents, phenoxybenzamine [PB(α)] (C) or propranolol [PR(β)] (D), were included along with decavanadate at a concentration of 70 mg/ml. Plasma membrane and cytosol fractions were prepared and assayed for the activity of protein kinase C. The values of calcium-dependent activity expressed as pmoles of ^{32}P transferred from [γ-^{32}P]ATP to histone H_1/min per mg plasma membrane protein, are means and SD of independent determinations of livers of 4 rats in each group. The changes in all the cases except in expt C with phenoxybenzamine are significant ($P < 0.01$).

Decavanadate was prepared by stirring excess V_2O_5 in 0.3 M NaOH for 12 h. The filtered orange-yellow solution (pH 7) contained predominantly the deca-form (as judged by NMR) and is taken to be equivalent to 0.05 M concentration of $Na_6V_{10}O_{28}$.

3. RESULTS

One of the well-characterized events of activation of α-adrenergic receptor is the increase in the plasma membrane and the decrease in cytosol of the activity of Ca^{2+}- and lipid-dependent protein kinase C (reviewed in [19]). Brief perfusion of livers for 5 min with decavanadate mimicked the effect of the α-adrenergic agonist, phenylephrine, in redistributing the activities of protein kinase C in plasma membrane and in cytosol (Fig.1A and B). In other experiments (data not shown) increase in perfusion with decavanadate for 10 min produced further increase in plasma membrane activity and the effect was not obtained with metavanadate (100 μM). The data on preventing these effects by phenoxybenzamine, an α-adrenergic blocking agent, but not by propranolol, a β-adrenergic blocking agent, when added in the perfusion medium along with polyvanadate (Fig.1C and D) are consistent with membrane-level of action of polyvanadate in activating the α-adrenergic receptor.

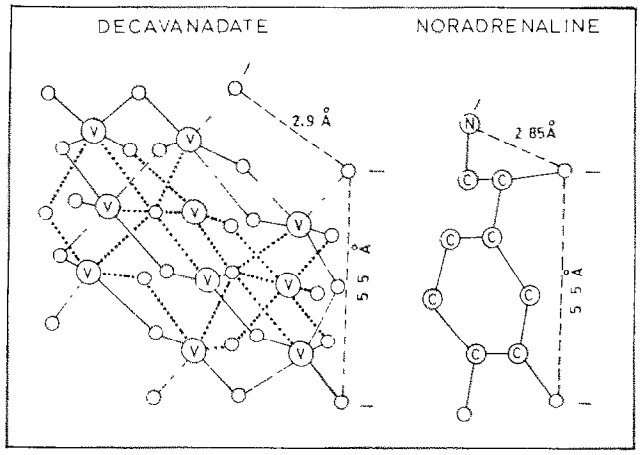

Fig. 2. Comparison of structures of decavanadate and noradrenaline. The structures were adapted from crystal structures given by Evans Jr. [20] for decavanadate and by Carlstrom and Bergin [21] for noradrenaline. The atoms are marked within the circles by V, C, N and open for vanadium, carbon, nitrogen and oxygen, respectively. The approximate distances between the atoms O–O and O–N marked along the broken lines are obtained from the distances and angles provided in the two references.

It is puzzling to see how the two unrelated chemical compounds, noradrenaline, a catecholamine, and decavanadate, an inorganic polymer, can produce similar effects at the plasma membrane level. It is implicit that the two active compounds are interacting with an appropriate domain of the receptor protein. We therefore searched for a common structural feature in them. The only apparent structural feature in decavanadate is the presence of oxygens linked to vanadium in three ways: V=O, V–OH or V–O–V. In noradrenaline, the *meta*-position ring oxygen and the side-chain oxygen and nitrogen seem to be essential, but not the *para*-position ring oxygen, absent in the active agonist, phenylephrine. On comparison of the crystal structures of decavanadate [20] and noradrenaline [21], we found the presence of two oxygens at about 5.5 Å and of a third oxygen or nitrogen at about 2.9 Å from one of the above oxygens, shown by dotted lines in Fig. 2. The formation of these three oxygens in decavanadate, not existing in the inactive monomer, is made possible because of polymerization to the cage-like structure characteristic of decavanadate [20]. Admittedly, there is no proof that this three atom configuration of O–O–O(N) makes these molecules active but there is little else structurally that these molecules offer.

4. DISCUSSION

To the list of many effects of vanadate treatment of whole animals or cells we have added the redistribution of intracellular calcium and protein kinase C activity, the two characteristic effectors of signal transduction in

α-adrenergic stimulation [9]. We are encouraged by these results to suggest that vanadate in polymeric, but not monomeric, form is able to act directly on the liver plasma membrane as an agonist of α-adrenergic system. This explains in some measure the multiplicity of its effects. A number of other direct actions of vanadate on plasma membrane-associated enzyme systems are known: inhibition of Na,K-ATPase [22], Ca^{2+}-ATPase [16,23], and 5′-nucleotidase [1]; stimulation of adenyl cyclase [24,25], phosphorylation of protein [26] and protein-tyrosine [27] and phosphatidylinositol [28]; and interaction with transducin of the visual excitation system [29]. Another example of selective action of decavanadate on plasma membrane is the stimulation of NADH oxidation accompanied by oxygen reduction to H_2O_2 [30,31]. The relationship between these two decavanadate-specific reactions is not known. Selective oxidative modification of the regulatory domain of protein kinase C by H_2O_2, both activation and inactivation depending on concentration, has been recently reported [32]. Would H_2O_2 become a link between the two actions and thus participate in signal transduction?

Acknowledgements: Financial support from the Department of Science and Technology, Government of India, New Delhi is acknowledged.

REFERENCES

[1] Ramasarma, T. and Crane, F.L. (1981) Curr. Top. Cell. Regul. 20, 248–301.
[2] Jandhyala, B.S. and Hom, G.S. (1983) Life Sci. 33, 1325–1340.
[3] Boyd, D.W. and Kustin, K. (1984) Adv. Inorg. Biochem. 6, 311–365.
[4] Hackbarth, F., Schmitz, W., Scholtz, H., Wetzel, E., Erdmann, E., Kraweitz, W. and Philipp, G. (1980) Biochem. Pharmacol. 29, 1429–1431.
[5] Ozaki, H. and Urakawa, N. (1980) Eur. J. Pharmacol. 68, 339–344.
[6] Delamere, N.A. and Williams, R.N. (1986) Invest. Ophthalmol. Visual Sci. 27, 1336–1340.
[7] Babcock, D.F., Chen, J.H., Yip, B.P. and Lardy, H.A. (1979) J. Biol. Chem. 254, 8117–8120.
[8] Rainhart, P.H., Taylor, H.M. and Bygrave, F.L. (1982) Biochem. J. 208, 619–630.
[9] Taylor, W.M., Reinhart, P.H. and Bygrave, F.L. (1987) in: The Role of Calcium in Drug Action (Denborough, M.A. ed.) pp. 139–155, Pergamon, Oxford, UK.
[10] Sivaramakrishnan, S. and Ramasarma, T. (1983) Indian J. Biochem. Biophys. 20, 16–22.
[11] Swaroop, A., Patole, M.S., Puranam, R.S. and Ramasarma, T. (1983) Biochem. J. 214, 745–750.
[12] Gullapalli, S., Shivaswamy, V., Ramasarma, T. and Ramakrishna Kurup, C.K. (1989) Indian J. Biochem. Biophys. 26, 227–233.
[13] McCormack, J.G. and Denton, R.M. (1980) Biochem. J. 190, 95–105.
[14] Reinhart, P.H., Taylor, W.M. and Bygrave, F.L. (1984) Biochem. J. 223, 1–13.
[15] Gullapalli, S., Shivaswamy, V., Ramasarma, T. and Ramakrishna Kurup, C.K. (1989) Mol. Cell. Biochem. 90, 155–164.
[16] Delfert, D.M. and McDonald, J.M. (1985) Arch. Biochem. Biophys. 241, 665–672.
[17] Prpic, V., Green, K.C., Blackmore, P.F. and Exton, J.H. (1984) J. Biol. Chem. 259, 1382–1385.
[18] Kikkawa, U., Minakuchi, R., Takai, Y. and Nishizuka, Y. (1983) Methods Enzymol. 99, 288–298.
[19] Kikkawa, U. and Nishizuka, Y. (1986) Annu. Rev. Cell Biol. 2, 149–178.
[20] Evans, H.T. Jr (1966) J. Inorg. Chem. 5, 967–977.
[21] Carlstrom, D. and Bergin, R. (1967) Acta Crystallogr. 23, 313–319.
[22] Cantley, L.C. Jr, Josephson, L., Warner, R., Yanagisawa, M., Lechene, C. and Guidotti, G. (1977) J. Biol. Chem. 252, 7421–7423.
[23] Wang, L., Tsai, L.I., Solaro, R.J., Grasside, G. and Schwartz, A. (1979) Biochem. Biophys. Res. Commun. 91, 356–361.
[24] Schawabe, U., Puchstein, C., Hannemann, H. and Sochtig, E. (1979) Nature (Lond.) 277, 143–145.
[25] Kraweitz, W., Werden, K. and Erdmann, E. (1979) Biochem. Pharmacol. 28, 2517–2522.
[26] Catalan, R.E., Martinez, A.M. and Aragones, M.D. (1980) Biochem. Biophys. Res. Commun. 96, 672–677.
[27] Chan, T.M., Chen, E., Tatoyan, A., Shargill, N.S., Pleta, M. and Hochstein, P. (1986) Biochem. Biophys. Res. Commun. 139, 439–445.
[28] Yang, D., Brown, A.B. and Chan, T.M. (1989) Arch. Biochem. Biophys. 274, 659–662.
[29] Kanaho, Y., Chang, P.P., Moss, J. and Vaughan, M. (1988) J. Biol. Chem. 263, 17584–17589.
[30] Ramasarma, T., Mackellar, W.C. and Crane, F.L. (1981) Biochim. Biophys. Acta 646, 88–98.
[31] Vijaya, S., Crane, F.L. and Ramasarma, T. (1984) Mol. Cell. Biochem. 62, 175–185.
[32] Rayudu, G. and Anderson, W.B. (1989) Proc. Natl. Acad. Sci. USA 86, 6758–6762.