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EFFECTS OF VANADATE ON THE CYCLIC AMP-PROTEIN KINASE SYSTEM IN RAT LIVER

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SUMMARY

Vanadate produced a marked increase of cyclic AMP levels in rat liver slices; a parallel scheme of variation was found in the case of protein kinase activity. Similar trends of variations were observed in in vivo experiments. These results support the idea that vanadate can affect the cyclic AMP-protein kinase system and moreover, due to the function of liver (an organ considered to play a fundamental role in the general metabolism in mammals) the same results add more data in support for a general regulatory role for vanadate.

The biological effects of vanadate have been extensively studied in the last years and although the biochemical basis for the action of vanadate is still unknown, several authors have suggested that vanadate may represent a new regulatory factor in mammalian tissues (see ref. 1 for a review). Particularly interesting is the selective inhibition of some ATPases by vanadate and in this regard it has been identified as a potent inhibitor of (Na,K)ATPases (2,3). As vanadate was originally identified as a inhibitor in muscle-derived ATP preparations (3) and in equine and rabbit skeletal mucles, its has been suggested that it is an endogenous regulator of (Na,K)ATPases (3-5). Vanadate also inhibits several other enzymes such as acid phosphatase (6), alkaline phosphatase (7), phosphofructokinase (8) and adenylate kinase (9); on the other hand it has been recently demonstrated that vanadate activates rather than inhibits adenylate cyclase (10-12).

As the effect of vanadate on the cyclic AMP system may be of general interest due to vanadate's exerting biological activities in several intact cellular systems and taking into account the scarce data on ist biochemical mode of action (particularly in relation to cyclic nucleotide system) we have studied the effects of vanadate on the cyclic AMP-protein

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kinase system in rat liver in an attempt to understand better its mechanism of action.

MATERIALS AND METHODS

Experimental conditions for animal treatment and incubation of slices of liver were carried out essentially as described (13,14). Male Wister rats weighing 180-200 g were used throughout these experiments. Animals were maintained on a standard laboratory diet with water ad libitum and killed by decapitation. The livers were rapidly removed and tissue slices (thickness 0.50 ± 0.02 mm) were obtained with a Stadie-Riggs tissue slicer and placed in a covered petri dish kept at ice temperature. The liver slices (140-160 mg) were incubated for 30 min in 2 ml of Krebs-Ringer-bicarbonate buffer (pH 7.4) (15) containing 10 mM D-glucose. Solutions were gassed throughout all incubations with 95% O_2/5% CO_2. The incubations were performed at 37°C. The incubations were terminated by collection of slices and immediate homogeneization with 3 ml of 10% TCA in a Potter-Elvehjem homogenizer at 0-4°C to measure cyclic nucleotide levels; in the case of protein kinase and cyclic AMP-binding determinations, after the incubations were terminated, slices were collected and homogenized in the appropiate buffers.

Cyclic AMP was measured by a competitive binding protein assay. Cyclic GMP was measured by radioimmunoassay. Extraction, assay conditions and linearization of the data obtained have been described previously (16,17).

Protein kinase and cyclic AMP binding protein activities were measured as described previously (18).

In vivo experiments were carried out by injecting i. p. 100 µl of vanadate (at the appropiate concentrations) dissolved in Krebs-Ringer-bicarbonate buffer (pH 7.4). After 3 h, livers were obtained and nucleotide levels and protein activities determined as described (16-18).

Protein was determined by the method of Lowry et al. (19).

A statistical analysis of the data was done using Student's t-test (20).

RESULTS AND DISCUSSION

Figure 1 shows the effects of vanadate on cyclic nucleotide levels and on protein kinase and protein binding activities

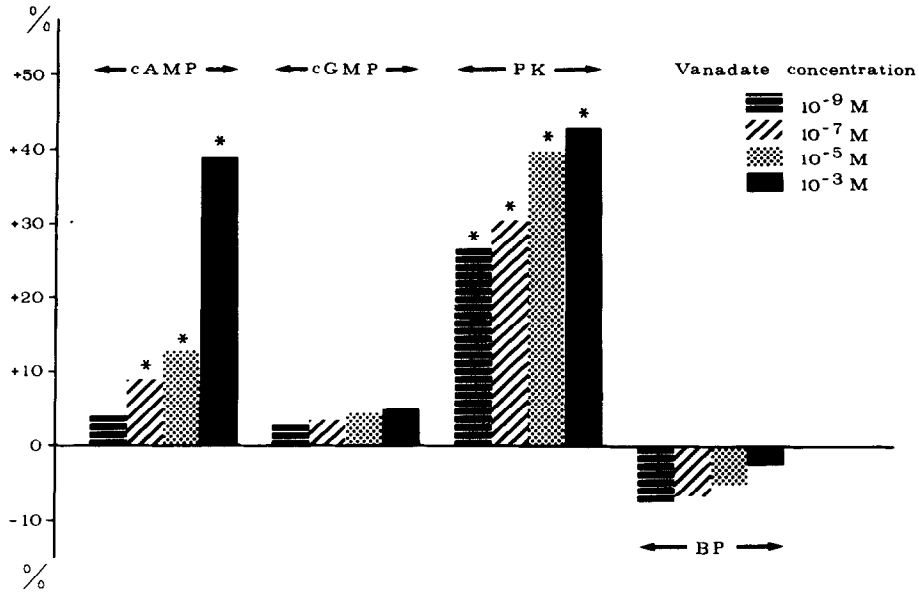

Figure 1. Effects of vanadate ($NaVO_3$) on the cyclic AMP-protein kinase system in rat liver slices. Changes are expressed as percent of the control values (=100%). Asterisks indicate statistically significant changes with respect to the control levels. Cyclic AMP (cAMP); cyclic GMP (cGMP); protein kinase (PK); cyclic AMP binding protein (BP).

in rat liver. These results, that represent, as far as we are aware, the first report on the variations of the cyclic nucleotide system in liver after vanadate treatment are , in general, in agreement with previous results described in adenylate cyclase of rat fat cell membranes (10) and of myocardial strips (11,12); moreover, the variation in protein kinase during the same experiment lends further support to this idea; the similar trends in the variation of the kinase and cyclic AMP levels agree quite well with the established concept that cyclic AMP stimulates the production of a cyclic AMP-dependent protein kinase, thereby inducing protein phosphorylation (21). With this regard, we must point out that preliminary experiments have indicated that vanadate activates a purified catalytic subunit from myocardial protein kinase (Catalán, R. E., Martínez, A. M. and Aragonés, M. D., unpublished results).

In vivo experiments (Table 1) support the ideas explained above because similar trends of variations were observed in

Table 1

In vivo effect of vanadate on the cyclic AMP levels (cAMP) and protein kinase (PK) and cyclic AMP binding protein (BP) activities in rat liver[1]

Additions	cAMP[2]	PK[3]	BP[4]
None	1.58	2498	2840
Vanadate 10^{-5} M	1.66	2748	1979
Vanadate 10^{-3} M	1.83	4766	1526

1 The figures are the average of assays run in triplicate
2 pmoles cAMP/mg protein
3 arbitrary units (cpm/mg protein)
4 arbitrary units (cpm/mg protein)

the variations of cyclic AMP levels and protein kinase and protein binding activities after vanadate treatment, a fact that can account for a site of action beyond cyclic AMP generation. We must point out that the in vivo effect of vanadate has not been described elaborately; inhibition of the sodium pump has been reported to occur in intact red blood cell (22) and vanadate has been described as a potent diuretic and natriuretic in rats (23).

Cyclic GMP levels remained unchanged and as no data are available on the guanylate cyclase variation due to vanadate, the role played by the guanylate cyclase-cyclic GMP system must await further studies although these results do not permit the suggestion of any effect of vanadate on the cyclic GMP metabolism.

The effect of vanadium compounds in the metabolism of liver has not been studied in detail; sodium metavanadate lowered the content of coenzyme A (24,25) and vanadium diminished the synthesis of cholesterol as well as the levels of triglycerides (26). The incorporation of labeled phosphate into liver phospholipids was decreased following injection of vanadyl sulfate probably by decreasing the synthesis of phospholipids (27). Our results could explain, in part, some of the results reviewed earlier. Thus, it has been demonstrated that the activity of hydroxymethylglutaryl CoA reductase (an important control point in cholesterol biosynthesis) is decreased by cyclic AMP (28) and the activation of the

hormone-sensitive lipase (that could account for the diminished levels of triglycerides above cited) has been reported (29).

Vanadium has very recently begun to interest the biochemists and studies on the physiological effects of vanadate have revealed a wide range of activity; as a matter of fact, several pharmacological effects (among them, a pheripheral vasoconstriction) are not really new (30). Although the exact mechanism of the vanadate action still remains speculative, our findings seem to be quite interesting because the liver occupies a fundamental place in the general metabolism and has perhaps the greatest metabolic flexibility of any organ. This fact and the variations found in an extremely important biochemical system such as the cyclic AMP-protein kinase system could support the concept for a general regulatory role for vanadate.

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REFERENCES

1. Simons, T. J. B. (1979) Nature, 282, 337-338.
2. Josephson, L., and Cantley, L. C. (1977) Biochemistry, 16, 4572-4578.
3. Cantley, L. C., Josephson, L., Warner, R., Yanagisowa, C., Lechene, C., and Guidotti, G. (1977) J. Biol. Chem., 252, 7421-7423.
4. Beauge, L. A., and Glynn, I. M. (1978) Nature, 272, 551-552.
5. Cantley, L. C., Resh, M. D., and Guidotti, G. (1978) Nature, 272, 552-554.
6. Van Etten, R. L., Waymack, P. P., and Rehkop, D. M. (1967) J. Am. Chem. Soc., 175, 31-39.
7. Lopez, V., Stevens, T., and Lindquist, R. N. (1976) Arch. Biochem. Biophys., 175, 31-38.
8. Choate, G. L., and Mansour, T. E. (1978) Fed. Proc.,37, 1433.
9. De Master, E. G., and Mitchell, R. A. (1973) Biochemistry, 12, 3616-3621.
10. Schwabe, U., Puchstein, C., Hannemann, H., and Sochtig, E. (1979) Nature, 277, 143-145.
11. Grupp, G., Grupp, I., Johnson, C. L., Wallick, E. T., and Schwartz, A. (1979) Biochem. Biophys. Res. Commun., 88, 440-447.

12. Kravietz, W., Werdan, K., and Erdmannn, E. (1979) Biochem. Pharmacol., 28, 2517-2520.

13. Catalán, R. E., Avila, C., Vila, T., and Castillón, M. P. (1978) Metabolism, 27, 1359-1360.

14. Catalán, R. E., Aragonés, M. D., and Martínez, A. M. (1979) Biochim. Biophys. Acta, 586, 213-216.

15. Umbreit, W. W., Burris, R. H., and Stauffer, J. F. (1949) in Manometric Techniques and Tissue Metabolism, 2nd ed., p. 185. Burgess, Minneapolis.

16. Catalán, R. E., Vila, T., and Castillón, M. P. (1976) Experientia, 32, 843-844.

17. Catalán, R. E., Castillón, M. P., and Municio, A. M. (1976) Biochem. Biophys. Res. Commun., 72, 184-189.

18. Catalán, R. E., Aragonés, M. D., and Martínez, A. M. (1979) Biochem. Biophys. Res. Commun., 89, 44-49.

19. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. (1951) J. Biol. Chem., 193, 265-275.

20. Snedecor, G. W. (1961) Statistical Methods. 5th ed., p. 45, Iowa State University, Ames, Iowa.

21. Brostrom, M. A., Reiman, E. M., Walsh, D. A., and Krebs, E. G. (1970) Adv. Enzyme Regul., 8, 191-203.

22. Cantley, L. C., and Aisen, P. (1979) J. Biol. Chem., 254, 1781-1784.

23. Balfour, W. E., Grantham, J. J., and Glynn, I. M. (1978) Nature, 275, 768.

24. Mascitelli-Coriandoli, E., and Citterio, C. (1959) Nature, 183, 1527.

25. Mascitelli-Coriandoli, E., and Citterio, C. (1950) Nature, 184, 1641.

26. Curran, G. L. (1954) J. Biol. Chem., 210, 765-771.

27. Snyder, F., and Cornatzer, W. E. (1958) Nature, 182, 462-463.

28. Bricker, L. A., and Levey, G. S. (1972) J. Biol. Chem., 247, 4914-4915.

29. Huttunen, J. K., Steinberg, D., and Mayer, S. E. (1970) Proc. Natl. Acad. Sci. USA, 67, 290-295.

30. Jackson, D. E. (1911) J. Pharmac. Exp. Ther., 13, 477-514.