Biochemical Pharmacology, Vol.28, pp.2517-2520.

0006-2952/79/0815-2517 $02.00/0

PRELIMINARY COMMUNICATIONS

STIMULATORY EFFECT OF VANADATE ON THE ADENYLATE CYCLASE OF CARDIAC TISSUE

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(*Received* 5 *March* 1979; *accepted* 28 *May* 1979)

We have shown that vanadate has a distinct positive ionotropic effect in cat papillary muscle (1). This positive ionotropic effect could be due to vanadate induced inhibition of $(Na^+ + K^+)$-ATPase, which has been shown to occur in red cells (2) and in membrane preparations from dog kidney (3). This enzyme inhibition has been shown to be rather specific as other ATPases were not affected under these conditions (3). Vanadate, therefore, has been suggested to be "an ideal specific regulator" of the $(Na^+ + K^+)$-ATPase (3).

However, positive ionotropic effects in the heart can also be caused by other mechanism, such as stimulation of cardiac adenylate cyclase by catecholamines (4). In fact, it has been demonstrated that vanadate does stimulate adenylate cyclase activity in fat cells, too (5). The experiments presented in this paper show that vanadate causes a marked stimulation of cardiac adenylate cyclase in membranes isolated from guinea-pig heart.

Methods: A crude membrane fraction was prepared as described by Drummond and Severson (1974) (6). The determination of adenyate cyclase activity was measured as described by Salomon et al. (1973) (7). The assay contained a total volume of 60 µl: $MgCl_2$, 1 mM; creatinephosphate, 8.7 mM; creatinekinase, 50 U/ml; cyclic AMP, 1 mM; isobutylmethylxanthine, 5.4 mM; Tris-HCl buffer, pH 7.8, 50 mM; ^{32}P-ATP, 0.3 mM (25-50 cpm/pmole), and myocardial membranes (80-160 µg protein). The chemicals used to assay adenylate cyclase were of analytical purity and were purchased from E. Merck, Darmstadt, and Boehringer, Mannheim, Germany.

Results and Discussion: Na_3VO_4 (10^{-5}- 10^{-2} M) stimulates the activity of adenylate cyclase in a concentration-dependent manner from 290 to 3050 pmoles/mg prot x 10 min, probably in a saturable manner (Fig. 1). Calculation of the K_m-value for Na_3VO_4 according to Lineweaver-Burk yields a value of 200 µM. This stimulating effect might be induced either at the ß-adrenergic-binding site, at the coupling-mechanism (8) between receptor and adenylate cyclase or at the enzyme directly.

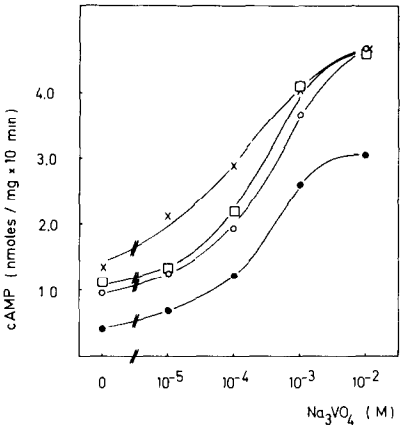

Fig. 1: Incubation of cardiac tissue with increasing concentrations of isoproterenol and vanadate: without isoproterenol ● – ●, 0.1 µM isoproterenol o - o, 1.0 µM isoproterenol □ – □, 10.0 µM isoproterenol x - x. Vanadate and isoproterenol stimulate additionally the enzyme until adenylate cyclase is maximally stimulated.

Catecholamines bind to the ß-adrenergic receptor and by this way stimulate the enzyme (4). Na_3VO_4 (10^{-5}- 10^{-2} M) has no effect on the specific binding of (-)-^{3}H-dihydroalprenolol in cardiac cell membranes (Experiments not shown). Additionally, binding inhibition of labelled dihydroalprenolol by ß-adrenergic agonists or antagonists is not affected by Na_3VO_4 (Experiments not shown). The well-known stimulating effect of isoproterenol on adenylate cyclase is apparently not influenced by Na_3VO_4 (Fig. 1). When plotting the data according to Lineweaver-Burk, there is no indication of a competitive action between isoproterenol and vanadate. Thus, isoproterenol and vanadate stimulate the enzyme independently of each other.

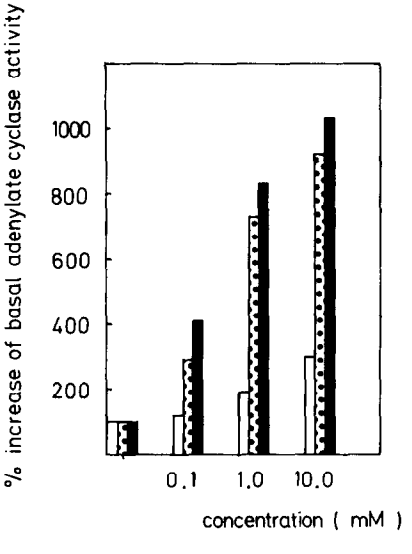

Fig. 2: Cardiac tissue is incubated with increasing concentrations of Mg^{2+}, F^- and Na_3VO_4. The basal activity of adenylate cyclase is set 100 %, the increasing different stimulation effects of Mg^{2+} (□), Na_3VO_4 (▨) and F^- (■) are shown as percentage over basal activity. Na_3VO_4 in the same concentration overcomes the effects of Mg^{2+} and F^-.

F^- and Mg^{2+} activate adenylate cyclase in cell membranes devoid of a ß-adrenergic binding site (9). The activation site for these ions is thought to be at the coupling mechanism between receptor and enzyme (10) or on the enzyme directly. At identical

concentrations, Na_3VO_4 is the most potent stimulator of adenylate cyclase among Mg^{2+} and F^- (Fig. 2). As shown (see Table 1), the enzyme may be stimulated additionally by Na_3VO_4 and F^- together up to a maximum of 4883 pmoles/mg prot x 10 min. Plotting these values according to Fig. 1, indicates that F^- and Na_3VO_4 stimulate the enzyme through different active sites.

Table 1: Incubation of increasing concentrations of F^- and Na_3VO_4 to adenylate cyclase.

	cAMP (pmoles / mg x 10 min)		
NaF	Na_3VO_4		
(mM)	0	0.1 mM	10 mM
0	450	1250	3040
0.2	979	2121	4868
2.0	2396	2852	4883

Comparing our data to Schwabe et al. (5), it may be noted that no effect to adenylate cyclase activity is obtained in fat cells when Na_3VO_4 and F^- were incubated together. Noradrenaline in their experiments together with vanadate caused only little stimulation of the adenylate cyclase of fat cells. This may be due to the different sources of tissue or to the rather high concentration of Mg^{2+} used in their assay (20 mM Mg^{2+}).

As vanadate acts directly on $(Na^+ + K^+)$-ATPase by a binding site with high affinity (3) a similar mechanism might exist at the adenylate cyclase. Mg^{2+}- and nucleotide binding sites on the adenylate cyclase have already been characterized (11,12). Gpp(NH)p, a non-hydrolizable nucleotide, binds to a specific site, and stimulates the adenylate cyclase in a concentration-dependent manner (13). Incubation together with vanadate is of no additive effect (Fig. 3). Plotting these data according to Lineweaver-Burk indicates a competitive activating process. The calculation of K_m values for Gpp(NH)p activated adenylate cyclase resulted in 0.250 µM and for Na_3VO_4 in 200 µM with an identical V_{max} of 3.0 nmoles/mg prot x 10 min.

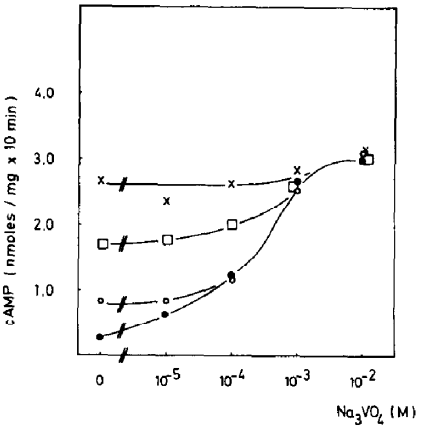

Fig. 3: Effects of Gpp(NH)p and vanadate, together, on the adenylate cyclase. Increasing concentrations of Gpp(NH)p stimulate the adenylate cyclase in a concentration-dependent way: without Gpp(NH)p • - •, 0.1 µM Gpp(NH)p o - o, 1.0 µM Gpp(NH)p □ - □, 10.0 µM Gpp(NH)p x - x. Increasing concentrations of vanadate demonstrate no additional but a competitive effect.

These experiments may indicate that the cardiac adenylate cyclase is stimulated by Na_3VO_4 via the nucleotide binding site. The real mechanism of the vanadate effect remains, how-

ever, still speculative: (a) competition at the nucleotide binding site as at the $(Na^+ + K^+)$-ATPase (14), (b) binding to the GTPase and thereby inhibiting the hydrolyzation of the endogenous GTP (15).

Further experiments with ^{48}V-Na_3VO_4 are in progress to investigate the mechanism of Na_3VO_4 action. In view of the presence of vanadate in mammalian tissue (serum concentration about 1 µM, and higher concentrations in tissues) (16) the vanadate stimulating effect on adenylate cyclase may be of physiological importance. It has therefore to be discussed as a reason for a biological effect of vanadate (ionotropic) besides the inhibition of the $(Na^+ + K^+)$-ATPase. Recent experiments have shown that $(Na^+ + K^+)$-ATPase may be stimulated under certain conditions by vanadate, too (17).

References:

1. Hackbarth, I., Schmitz, W., Scholz, H., Erdmann, E., Krawietz, W., Philipp, G. Nature 275, 67 (1978)
2. Beaugé, L.A. and Glynn, I.M. Nature 272, 551 (1978)
3. Cantley, Jr. L.C., Josephson, L., Warner, R., Yanagisawa, M., Lechene, C. and Guidotti, G. J. Biol. Chem. 252, 7421 (1977)
4. Epstein, S.E., Levey, G.S. and Skelton, C.L. Circulation 43, 437 (1971)
5. Schwabe, U., Puchstein, Ch., Hannemann, H., Söchtig, E. Nature 277, 143 (1979)
6. Drummond, G.I. and Severson, D.L. In: Methods in Enzymology 38, pp 143-149, edit. J.G. Hardman and B.W. O'Malley, New York/San Francisco/London, Academic Press (1974)
7. Salomon, Y., Londos, C. and Rodbell, M. Anal. Biochem. 58, 541 (1974)
8. Robinson, G.A., Butcher, R.W. and Sutherland, E.W. Annals of the New York Academy of Siences 139, 703 (1967)
9. Levey, G.S. and Keein, J. J. Clin. Invest. 51, 1578 (1972)
10. Pastan, J., Pricer, W. and Blanchette-Mackie, J. Metabolism 19, 809 (1970)
11. De Haen, Ch. J. Biol. Chem. 249, 2756 (1974)
12. Drummond, G.I. and Duncan, L. J. Biol. Chem. 245, 976 (1970)
13. Rodbell, M. J. Biol. Chem. 250, 5826 (1975)
14. Cantley, L.C., Cantley, L.G. and Josephson, L. J. Biol. Chem. 253, 7361 (1978)
15. Cassel, D. and Selinger, Z. Proc. Natl. Acad. Sci, USA 74, 3307 (1977)
16. Hamlyn, J.M. and Duffy, T. Biochim. Biophys. Res. Commun. 84, 458 (1978)
17. Erdmann, E., Krawietz, W., Philipp, G., Hackbarth, I., Schmitz, W., Scholz, H. Nature (in press)