Vol. 96, No. 1, 1980 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
September 16, 1980 Pages 39-46

Interactions of Ouabain and Vanadate with (Na^+,K^+)ATPase and Isolated Cardiac Muscle

Timothy D. Myers* and Robert C. Boerth

Departments of Pharmacology and Pediatrics
Vanderbilt University School of Medicine
Nashville,Tennessee 37232

Received May 15,1980

Summary: The interactions of ouabain and vanadate with (Na^+,K^+)ATPase were investigated at different potassium concentrations.Also,the contractile effects of a mixture of these two inhibitors were compared to those produced by ouabain or vanadate alone.The results from the enzyme and contractile studies suggested that inhibition of sarcolemmal (Na^+,K^+)ATPase was involved in mediating the positive inotropic effect of vanadate.

INTRODUCTION

Vanadate and ouabain are inhibitors of (Na^+,K^+)ATPase acting at different sites on the enzyme(1,2). Ouabain inhibits from the extracellular side of the plasma membrane, and vanadate binds from the intracellular side(3,4). Since vanadium levels in the micromolar range have been documented in mammalian tissues, a two inhibitor system is conceivably operating in instances where a person is exposed to the cardiac glycosides(5). An intriguing observation is that potassium antagonizes ouabain inhibition but facilitates vanadate inhibition. This suggests that not only can potassium determine the power of these ligands as inhibitors but it also controls the amount of inhibition by one versus the other when they are present together(6,7). Experiments were designed to test the effects of potassium on the inhibition of (Na^+,K^+)ATPase by these two inhibitors together.

Since the positive inotropic effect of ouabain has been linked to inhibition of sarcolemmal (Na^+,K^+)ATPase, there is a tendency to associate the positive inotropic effect of vanadate with inhibition of this enzyme(8,9). An

*Address correspondence and reprint requests to Timothy D. Myers, Section of Cardiovascular Sciences, Department of Medicine, Baylor College of Medicine, Houston, Texas 77030

0006-291X/80/170039-08$01.00/0

assumption of this association is that ouabain and vanadate are specific inhibitors of (Na^+,K^+)ATPase. Though this appears to be true for ouabain it is not the case for vanadate. Previous studies show that vanadate inhibits a variety of enzymes and stimulates adenylate cyclase(10,11,12,13). The multitude of effects seen with vanadate implies that something other than inhibition of (Na^+,K^+)ATPase may be responsible for the positive inotropic effect. Borchard et al conclude from their experiments in cardiac muscle that alterations of transmembrane potential and(or) stimulation of adenylate cyclase may be the primary biochemical event(s) mediating the positive inotropic effect of vanadate(14). However, the results of Schmitz et al shed doubt on the involvement of adenylate cyclase by showing that concentrations of vanadate producing a maximum positive inotropic effect have virtually no effect on cAMP levels of cardiac muscle(15). In studies with the atria of guinea pigs, Akera and coworkers report that vanadate does not inhibit ^{86}Rb uptake at a concentration capable of inhibiting isolated (Na^+,K^+)ATPase(16). From this they conclude that vanadate apparently does not produce positive inotropic effects via inhibition of (Na^+,K^+)ATPase. However, the isolated guinea pig atrium responds differently to vanadate than most other cardiac muscle and may not be suitable for studying the positive inotropic effects of vanadate(14). In the light of these results, our laboratory began to investigate the effects of vanadate in cardiac tissue showing a positive inotropic response and to test whether or not these effects could be linked to (Na^+,K^+)ATPase inhibition.

METHODS

The assay for inhibition of (Na^+,K^+)ATPase by ouabain and vanadate has been previously described(17). The enzyme preparation was purchased from Sigma Chemical Co. and consisted of (Na^+,K^+)ATPase isolated from canine kidney(lot # c9750). The enzyme was received as a lyophilized powder which was suspended in 1.0ml of a buffer containing 20.0mM Tris-Cl(pH 7.4) with 1.0mM EDTA and kept in a refrigerator. The protein content of this stock solution was 6.0mg/ml,and the specific activity was 0.9μmoles Pi/min/mg protein. For each assay the enzyme was diluted 1 to 10 in a buffer containing 20.2mM Tris-Cl(pH 7.4),5.2mM $MgCl_2$,and 0.2mM EDTA. Fifty microliters of this dilution was used per ml of the reaction mixture.

Functional studies were conducted using left atrial strips of rabbits. The strips were clamped in a 25ml muscle bath and connected to an isometric force transducer by a light jewelry chain. Electrical stimulation(60 per min)

was at 10% above threshold voltage. The muscles were stretched to maintain a resting tension of 1.0gm, and a cumulative concentration-response relationship to ouabain,vanadate, or the mixture of the two was determined at 30°C. The response to any concentration of ouabain or vanadate was allowed to reach equilibrium before the next concentration was added. Any muscle exhibiting an arrhythmia was discarded from the study. The buffer was Krebs-Ringer(potassium concentration of 6.0mM) buffered with bicarbonate and gassed with $95\%O_2/5\%CO_2$.

RESULTS

Because potassium has been shown to exert a divergent effect on the binding of ouabain and vanadate to (Na^+,K^+)ATPase, we investigated the effects of this ion on enzyme activity in the presence of both inhibitors. Under conditions of rate limiting potassium(0.25mM), ouabain was a more potent inhibitor than vanadate(Figure 1). The I_{50} was 16nM for ouabain and 50μM for vanadate. In conditions which optimized vanadate binding(high $Mg^{2+}$and K^+), Cantley et al found a dissociation constant for vanadate of 4nM with the same enzyme preparation(see ref.7) suggesting that high potassium increased the potency of vanadate as an inhibitor. Adding a high concentration of potassium to enzyme preparations partially inhibited by ouabain or vanadate pointed out the dramatic difference between these two inhibitors(see panels B and C of Fig.1). The addition of potassium accelerated enzyme activity in the presence of ouabain but virtually stopped the reaction in the presence of vanadate. Ouabain and vanadate together followed the same pattern as vanadate alone suggesting that under these conditions vanadate was the dominant component of the mixture(panel D of Fig.1). The results using this combination also showed that ouabain and vanadate were producing a cumulative inhibition since the mixture had to be diluted four-fold to produce inhibition equal to that of ouabain or vanadate alone(compare the concentrations in panel D with those in panels B and C). These results combined with our previous observations(see Table 1 of ref.17) showing cumulative inhibition by ouabain and vanadate at 20mM potassium implied that this mixture could additively inhibit this enzyme over a wide range of potassium concentrations. Potassium appeared to determine the relative potency of these inhibitors when both were present.

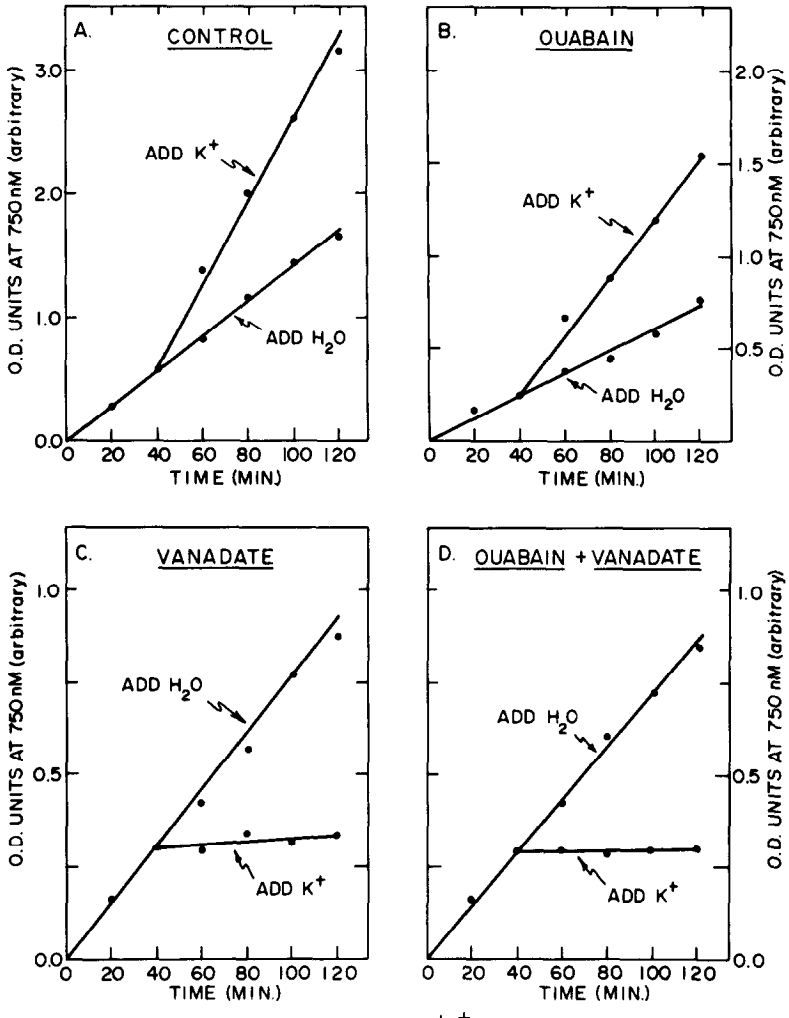

FIGURE 1. Contrasting effects of potassium on (Na^+,K^+) ATPase activity partially inhibited by ouabain, vanadate, or a combination of the two. The potassium concentration was 0.25 mM for the first forty minutes of all reactions. The ordinates represent the measurements of inorganic phosphate and are proportional to enzyme activity. Note that the ordinates vary slightly from panel to panel. The amounts of inhibition produced by ouabain, vanadate, or the combination were equal before the addition of potassium (38mM) or water at forty minutes. Panel A. Control activity in the absence of ouabain or vanadate. The reaction was stimulated by additional potassium. Panel B. Activity in the presence of ouabain (16nM). The activity was stimulated by additional potassium. Panel C. Activity in the presence of vanadate (50uM). The addition of potassium halted enzyme activity. Panel D. Activity in the presence of ouabain (4.0nM) and vanadate (12.5uM). Once again the addition of potassium halted enzyme activity.

To explore the possible link between inhibition of (Na^+,K^+)ATPase and positive inotropic responses, the responses to a mixture of ouabain and vanadate were studied in left atria isolated from rabbits and compared to those

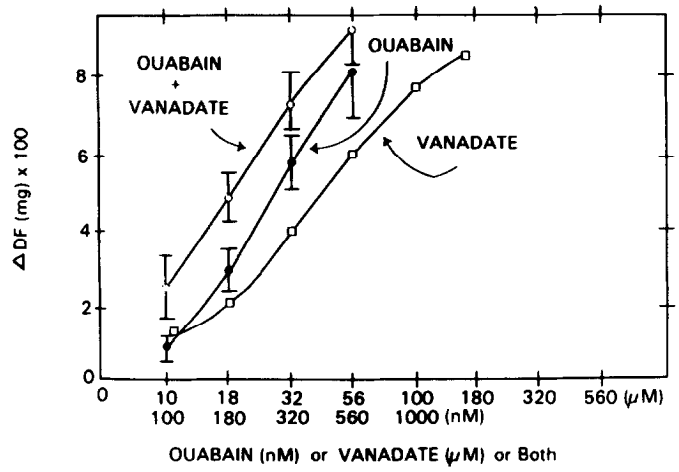

FIGURE 2. Comparison of the concentration-response relationships of ouabain, vanadate, or the combination of the two in left atrial strips of rabbits. The ordinate represents the change in developed force in milligrams (multiplied by 100) from control (neither ouabain nor vanadate present). The developed force in the control preparations did not vary significantly among the groups and averaged 830mg. The concentrations producing the peak, positive inotropic effects were 560nM for ouabain, 180uM for vanadate, and 560nM plus 56uM vanadate for the mixture. Higher concentrations produced either no further increase or a decrease in developed force. Values shown represent the mean $\pm$ S.E.M. For vanadate alone these bars were deleted for the sake of clarity but the variability was similar to the other two groups. N=6 for vanadate alone, N=7 for the mixture, and N=13 for ouabain.

responses produced by exposure to the individual inhibitors. The concentration-response relationship to the mixture of ouabain plus vanadate was to the left of those produced by exposure to either inhibitor alone(Figure 2). This finding was consistent with the idea that the greater inotropic response produced by the combination could be related to its cumulative inhibition of (Na^+,K^+)ATPase. The maximum response with the mixture of ouabain and vanadate was not different than that produced by ouabain or vanadate alone which supported a common mechanism for these inhibitors(Fig.2).

Since the enzyme studies showed that increased potassium enhanced vanadate inhibition of (Na^+,K^+)ATPase, one could assume that the inotropic response produced by vanadate would vary as the potassium concentration is altered if the binding of the inhibitor to (Na^+,K^+)ATPase is related to its positive inotropic effect. Raising the potassium concentration not only increased the potency of vanadate as an inhibitor of (Na^+,K^+)ATPase(refer to panel C of Fig.1)

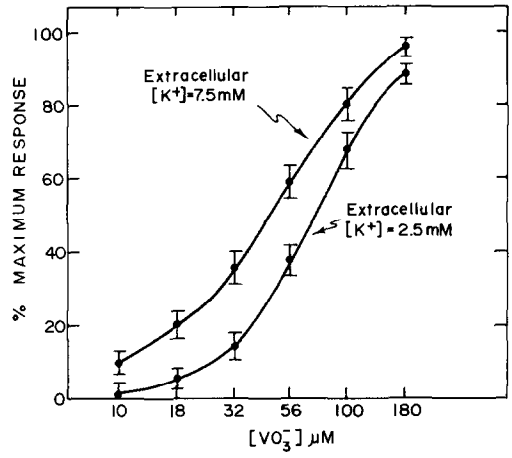

FIGURE 3. Effect of varying the potassium concentration on the concentration-response relationship of rabbit left atrial strips to vanadate. The ordinate represents the per cent of the maximal response which was defined as the difference between the developed force at the peak of the concentration-response relationship and control developed force (no vanadate present). The control developed force and the peak developed force were not significantly different than the values shown in Figure 2 for either potassium concentration. Values shown represent the mean $\pm$ S.E.M. N=6 for 7.5mM potassium, N=5 for 2.5mM potassium.

but also caused a shift to the left of its concentration-inotropic response relationship in the muscle bath(Figure 3), supporting a positive relationship between (Na^+,K^+)ATPase inhibition and this positive inotropic effect. Several investigators have reported a correlation between the potassium antagonism of the inotropic effects of ouabain and its antagonism of ouabain binding to isolated (Na^+,K^+)ATPase(9). The effect of potassium on vanadate-enhanced contractile function(Fig.3) appeared to correlate well with previous observations of its effect on vanadate inhibition of (Na^+,K^+)ATPase suggesting a cause-effect relationship between these two phenomena.

DISCUSSION

There are several effects of vanadate on enzymes that theoretically could mediate a positive inotropic effect. Some of these effects are: 1) inhibition of (Na^+,K^+)ATPase, 2) inhibition of Ca^{2+}-ATPase of the sarcolemma, and 3) stimulation of adenylate cyclase. All of these effects conceivably will deliver increased amounts of calcium to the contractile proteins. Our

results showing 1) the cumulative inhibition of (Na^+,K^+)ATPase and positive inotropic effects by the combination of ouabain plus vanadate and 2) the potassium effect on the response of cardiac muscle to vanadate lead us to conclude that inhibition of (Na^+,K^+)ATPase is the likely choice out of the list. However, our results do not exclude other mechanisms from contributing to the positive inotropic effect. Since inhibition of (Na^+,K^+)ATPase is apparently involved in mediating the positive inotropic effect of vanadate, it is conceivable that inhibition of myocardial (Na^+,K^+)ATPase by endogenous vanadate can be a determinant of both the contractile state of the heart and its sensitivity to the cardiac glycosides.

Our results do not suggest a mechanism for the negative inotropic effect of vanadate. Both Akera et al and Borchard et al show that this effect may be due to antagonism of calcium movements through the plasma membrane(14,16). It is also intriguing that ouabain can overcome this effect of vanadate in vitro which implies that inhibition of (Na^+,K^+)ATPase is not involved in the negative inotropic actions of vanadate(13).

ACKNOWLEDGEMENTS

Support for this research was supplied by Biomedical Research Support Grant RR 05424 and NIH Training Grants GM00058 and GM07628 from the National Institute of General Medical Sciences.

REFERENCES

1. Cantley,L.,Jr.,Josephson,L.,Warner,R.,Yanagisawa,M.,Lechene,C.,and Guidotti, G.(1977) J.Biol.Chem.252,7421-7423
2. Sen,A.,Tobin,T.,and Post,R.(1969) J.Biol.Chem.244,6596-6604
3. Perrone,J.and Blostein,R.(1973) Biochim.Biophys.Acta 291,680-689
4. Cantley,L.,Jr.,Resh,M.,and Guidotti,G.(1978) Nature 272,552-554
5. Post,R.,Hunt,D.,Walderhaug,M.,Perkins,R.,Park,J.,and Beth,A.(1980) Proceedings of the 2nd International Conference on the Properties and Functions of (Na^+,K^+)ATPase(eds.Skou and Norby),Academic Press
6. Inagaki,C.,Lindenmayer,G.,and Schwartz,A.(1974) J.Biol.Chem.249,5135-5140
7. Cantley,L.,Jr.,Cantley,L.,and Josephson,L.(1978) J.Biol.Chem.253,7361-7368
8. Hackbarth,I.,Schmitz,W.,Scholz,H.,Erdmann,E.,Krawietz,W.,and Philipp,G. (1978) Nature 275,67
9. Lee,K.and Klaus,W.(1971) Pharmacol.Rev. 23,193-261
10. O'Neal,S.,Rhoads,D.,and Racker,E.(1979) Biochem.Biophys.Res.Comm. 89,845-850
11. Schwabe,V.,Puchstein,C.,Hannemann,H.,and Sochtig,E.(1979) Nature 277,143-145
12. Krawietz,W.,Werdan,K.,and Erdmann,E.(1979) Biochem.Pharmacol.28,2517-2520

13. Grupp,G.,Grupp,I.,Johnson,C.,Wallick,E.,and Schwartz,A.(1979) Biochem. Biophys.Res.Comm.88,440-447
14. Borchard,V.,Fox,A.,Greef,K.,and Schlieper,P.(1979) Nature 279,339-341
15. Schmitz,W.,Hackbarth,I.,Wetzel,E.,and Scholz,H.(1979) In the Symposium on the Cardiac Effects of Vanadate;Munich,Germany(in the press)
16. Akera,T.,Takeda,K.,Yamamoto,S.,and Brody,T.(1979) Life Sci.25,1803-1812
17. Myers,T.,Boerth,R.,and Post,R.(1979) Biochim.Biophys.Acta 558,99-107