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EFFECTS OF VANADATE ON CARDIAC CONTRACTION AND ADENYLATE CYCLASE

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SUMMARY: Vanadate produces a positive inotropic effect on ventricular muscle from rat, rabbit, guinea pig and cat; a positive inotropic effect on the atria of rat and rabbit, but a negative inotropic effect on the atria of guinea pig and cat. The effects of vanadate are completely reversible and occur in a concentration range of 10^{-5}M to 10^{-3}M. In this same concentration range, vanadate also causes a marked activation of cardiac adenylate cyclase suggesting that the positive inotropic action might be due in part to an elevation of cyclic AMP levels. The effects of vanadate are not influenced by alprenolol, cimetidine, or mepyramine, indicating a lack of involvement of β-adrenergic or histamine H_2 and H_1 receptors.

INTRODUCTION: Vanadium compounds inhibit Na,K-ATPase from kidney, red blood cells and heart (1-5). In solution, vanadium may exist in several oxidation states including orthovanadate VO_4^{-3}, metavanadate VO_3^{-1} and vanadyl VO^{2+}. The term "vanadate" is used here to describe the inhibitor although this may not be the form of the active component. The inhibitory effects of vanadate on Na,K-ATPase have been linked with its effects on urine formation and increased contractile force (positive inotropic effect) of the heart (6,7). Depending upon the oxidation state, vanadate is as potent an inhibitory agent of Na,K-ATPase as are cardiac glycosides (1,2). Because of possible animal species variability and differences between atria and ventricles, the effects of vanadate were studied on isolated ventricular strips, papillary muscles and atrial strips of rat, rabbit, guinea pig and cat. Since the positive inotropic effects of several hormones including histamine, catecholamines and glucagon may be mediated by cAMP, we also examined the possibility that vanadate might influence adenylate cyclase activity.

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MATERIALS & METHODS:

Physiological Studies: Isolated left atrial strips and papillary muscle preparations or strips from right ventricles of the four species mentioned were used. The animals were anesthetized with 20 mg/kg sodium pentobarbital and injected with heparin. The hearts were removed and immediately suspended for retrograde perfusion of the coronary system with warm Krebs-Henseleit solution. Atrial and ventricular strips or papillary muscles were then dissected from the suspended hearts and mounted in the same bath chamber (volume 70 ml). The Krebs-Henseleit solution contained (in mM) 118 NaCl, 4.7 KCl, 2.5 $CaCl_2$, 1.2 $MgSO_4$, 1.2 KH_2PO_4, 0.5 Ca EDTA, 25 $NaHCO_3$ and 5.5 glucose. The solution was saturated with 95% O_2 and 5% CO_2 which maintained a pH of 7.4; bath temperature was 37°C. The myocardial tissues were studied under isometric conditions (Grass FT 03C force transducer), with an initial load of 1 g and stimulated through needle electrodes at 1.5 to 2 CPS (Grass S8 stimulator) with a stimulus duration of 0.5 msec and voltage 20% above threshold. Contractile force and its first derivative dF/dt (Grass 7P20C differentiator) were recorded. An increased force of contraction is called a positive inotropic effect and decreased force of contraction, negative inotropic effect. The preparations were equilibrated for 90 to 120 minutes. All dose-response curves were obtained on a cumulative basis and ED_{50} was determined graphically.

Adenylate Cyclase Assay: Washed particulate fractions (1000 xg) were prepared from homogenates of guinea pig and rat atrium and ventricle in 0.25M sucrose, 1 mM EGTA, 5 mM Tris (pH 7.4) and assayed for adenylate cyclase activity in a medium containing 1 mM Mg_2 [α-^{32}P]ATP, 1 mM [^{3}H]cAMP, 100 mM Tris-Cl (pH 7.4), 10^{-5}M GTP, 0.1% bovine serum albumin, 12.5 mM sucrose and 50μM EGTA. The labeled cAMP was isolated by the Salomon *et al* procedure (8).

RESULTS: The atria and ventricles of rat and rabbit responded to vanadate with a positive inotropic effect (Table I). The rabbit heart, as previously noted (9), was nearly 100 times more sensitive to ouabain than the rat heart. In sharp contrast, rat atrium and ventricle were about 10 times more sensitive to vanadate than the rabbit heart. At higher concentrations of vanadate, the rat atrium (but not the ventricle) exhibited a small, transient, negative inotropy, followed by a strong and sustained positive inotropic response. The effects of solutions of NH_4-m-vanadate were indistinguishable from those of Na-o-vanadate on rat heart. Surprisingly, vanadate caused a dose-dependent negative inotropic response in the guinea pig atrium; this effect occurred at lower concentrations than the positive inotropic response of other tissues (Table I). The cat atrium also responded with a negative inotropic effect to high concentrations of vanadate (10^{-5}M, or higher) although maximum inhibition was only 40% and the effect appeared to be transient. The ventricles of both guinea pig and cat responded to vanadate with positive inotropic effects. All of the effects described above could be quickly washed out, and subsequent applications of vanadate elicited

TABLE I

EFFECTIVE DOSES (ED_{50}) OF VANADATE AND OUABAIN FOR POSITIVE AND NEGATIVE INOTROPIC EFFECTS ON ATRIA AND VENTRICLES OF FOUR SPECIES; ADENYLATE CYCLASE ACTIVITY AS PERCENT INCREASE ABOVE CONTROL

Responses	RAT		GUINEA PIG		RABBIT		CAT	
	left atrium	right ventricle	left atrium	right ventricle	left atrium	right ventricle	left atrium	right ventricle
INOTROPIC EFFECTS								
ED_{50} Ouabain	5.2×10^{-5}M	4.5×10^{-5}M	2.5×10^{-7}M	1.9×10^{-7}M	2.4×10^{-7}M	4.1×10^{-7}M	---	---
ED_{50} Na_3VO_4 (o-vanadate)	4.1×10^{-5}M	2.1×10^{-5}M	3.2×10^{-6}M (Neg. Inotropy)	7.4×10^{-5}M	4.9×10^{-4}M	5.5×10^{-4}M	2.1×10^{-5}M (Neg. Inotropy)	4.7×10^{-4}M
ED_{50} NH_4VO_3 (m-vanadate)	4.2×10^{-5}M	4.5×10^{-5}M	---	---	---	---	---	---
% STIMULATION OF ADENYLATE CYCLASE BY NH_4VO_3								
10^{-5}M	---	18%	37%	45%±11%	---	---	---	---
10^{-4}M	59%	43%	100%	117%± 7%	---	---	---	---
10^{-3}M	123%	149%	205%	310%±31%	---	---	---	---

the same responses as the control tissues. Cimetidine (10^{-5}M), alprenolol (10^{-6}M) and mepyramine (10^{-6}M) did not influence the effects of vanadate suggesting that histamine H_1 and H_2 receptors, or β-receptors are not involved in the positive or negative inotropic responses.

Since it is well known that ouabain and vanadate both inhibit Na,K-ATPase, the combined effect of these inhibitors on the heart tissues was examined. First, vanadate concentrations were increased until a higher concentration no longer increased tension in the ventricle or decreased tension in the guinea pig atrium (Figure 1). A "therapeutic" concentration of ouabain was then added. The addition of ouabain promptly increased tension still further in the ventricle, and converted the negative inotropic effect of vanadate in the atrium to a very large positive inotropic effect. In all species and tissues examined, the addition of ouabain, after the maximal effect of vanadate had been attained, elicited a marked increase in tension.

Among other factors, cyclic AMP is thought to mediate the positive inotropic effects induced by some agonists on the myocardium (10). It is possible, therefore, that vanadate may have two effects: an inhibition of Na,K-ATPase like ouabain, and an augmentation of adenylate cyclase like isoproterenol. The possible inter-relationship between adenylate cyclase and Na,K-ATPase has been discussed (11). As shown in Table I, vanadate in concentrations from 10^{-5}M to 10^{-3}M stimulated adenylate cyclase activity of rat and guinea pig atrium and ventricles. In four preparations of guinea pig ventricle, the basal cyclase activity varied from 13.9 to 51.0 pmoles cAMP formed/min/mg protein. However, the relative stimulation by NH_4VO_3 was very similar in all of these preparations. Vanadate, at 10^{-3}M, stimulated guinea pig ventricular cyclase four- to five-fold. This stimulation is similar to that achieved by saturating concentrations of histamine or NaF. Addition of vanadate to a preparation already maximally stimulated by NaF did not lead to any further increase in cyclase activity. Dose-response curves to NaF, carried out in the presence and absence of vanadate, indicated that vanadate and fluoride were not competing for the same

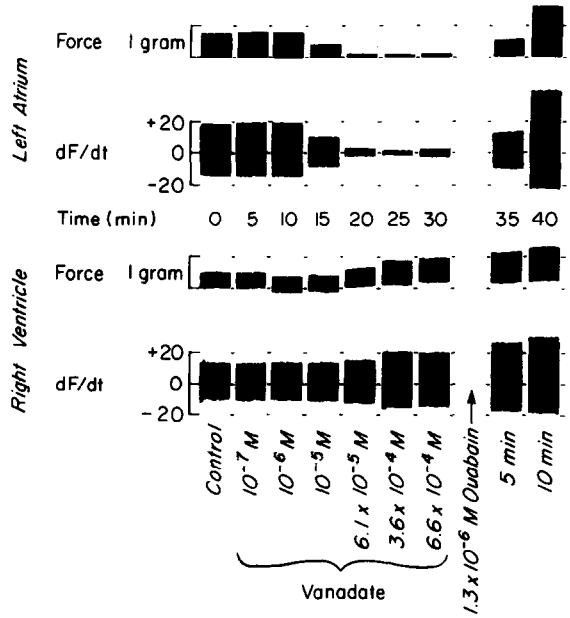

Figure 1: The Effect of Vanadate on Guinea Pig Atrial (upper 2 tracings) and Ventricular Strips (lower 2 tracings). Developed force in grams and derived dF/dt (g/sec) are shown. Increase in force denotes a positive inotropic effect, decrease a negative inotropic effect. Increasing doses of vanadate (Na_3VO_4) decreased force and dF/dt of the atrium and increased force and dF/dt of the ventricle. Vanadate's effects are maximal at 3.6×10^{-4}M. Addition, without washout of vanadate, of 1.3×10^{-6}M ouabain increased ventricular force further and converted the negative inotropic effect on the atrium to a positive one.

regulatory site on adenylate cyclase. To test the reversibility of the vanadate effects on adenylate cyclase, membranes were pretreated with 10^{-3}M NH_4VO_3 for 10 minutes at 30°C, washed by repeated centrifugation and resuspension, and tested for cyclase activity. Membranes so treated had the same activity as untreated membranes. The stimulatory effects of vanadate were not blocked by 10^{-6}M alprenolol or 10^{-4}M cimetidine, indicating that vanadate's effects are not mediated by either β or H_2 receptor activation of adenylate cyclase. Ouabain (10^{-4}M) did not influence cyclase activity in the absence or presence of 10^{-3}M vanadate. Since this concentration of the cardiac glycoside would presumably be sufficient to completely inhibit the Na,K-ATPase, these results indicate that the actions of vanadate on adenylate cyclase are not indirectly mediated by inhibition of the ATPase.

DISCUSSION: The effects of ouabain and vanadate on all ventricular tissues studied were similar with respect to onset of positive inotropic action. It is of interest that both agents show similarities in the inhibition of Na,K-ATPase (1,2). The fact that either agent still produced a significant positive inotropic effect after the response to the other agent had peaked suggests that ouabain and vanadate act at different sites on the receptor, or that a different receptor for each is involved. In contrast to ouabain, vanadate produced a negative inotropic effect on guinea pig and cat atrial tissue. It is known that the T-system in atria of several species is less prominent than that of ventricular tissues (12). This, and other as yet unknown structural characteristics of atria, may play some role in this unusual response (J. R. Sommer, personal communication).

The possibility has to be considered that vanadate might function as an endogenous modulator of myocardial contraction. Post *et al* (13) report that there is about 0.2μmole of vanadium per kg wet weight in rat heart. This is approximately the same amount of Na,K-ATPase, as estimated by ouabain binding sites, that is found in cat heart (14). Consequently, it is possible that in the normal physiological state, the sodium pump may be partially inhibited. The I_{50} for vanadate-induced inhibition of rat kidney Na,K-ATPase is about 5.5×10^{-7}M (15) which indicates a much greater sensitivity of the rat enzyme to vanadate than to ouabain (9). It is also of interest that vanadate can regulate the binding of ouabain to isolated Na,K-ATPase so that it is also possible that endogenous vanadate might modulate the therapeutic effects of cardiac glycosides (15).

The concentrations of vanadate required to elicit positive inotropic effects are orders of magnitude greater than those required to inhibit isolated Na,K-ATPases. However, the vanadate concentrations needed for stimulation of adenylate cyclase are similar to those required for positive inotropy suggesting that the latter response might be explained on the basis of increases in cAMP levels. It will be of interest to measure levels of cAMP in the tissue at the

peak of the vanadate-induced response. Since the reported endogenous vanadium levels (13) are much lower than those required for the contractile and cAMP effects, it is difficult to ascribe a role for vanadate in the physiological regulation of myocardial contraction or of adenylate cyclase activity. However, it is not possible at this time to determine the actual concentrations of the active form(s) of vanadium in the reaction media of the enzyme assays, or in the biophase at the receptor sites. Furthermore, it is clear that various substances such as ascorbic acid, oxygen and EDTA, as well as other experimental conditions, can alter the concentration and stability of the various chemical species of vanadium (13). In addition to its possible physiological significance, the stimulation of adenylate cyclase by vanadate is of interest because this agent is readily taken up by cells (1-3). Thus, vanadate, in contrast to fluoride ion, may be a useful tool for modifying cAMP levels in intact cells. After these studies were in manuscript form, a report appeared describing similar stimulatory effects of vanadate on fat cell membrane adenylate cyclase of rat (16). It is of interest also that a direct digitalis-like action of vanadate on heart muscle was noted as early as 1876 (17,18).

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