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EFFECT OF VANADIUM IN THE +5, +4 AND +3 OXIDATION STATES ON CARDIAC FORCE OF CONTRACTION, ADENYLATE CYCLASE AND $(Na^+ + K^+)$-ATPase ACTIVITY

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Abstract—The influence of vanadium in the nominally +5 (NH_4VO_3; referred to as V^{5+}), +4 ($C_{10}H_{14}O_5V$ and $VOSO_4$; V^{4+}) and +3 oxidation states (VCl_3; V^{3+}) on cardiac force of contraction, adenylate cyclase and $(Na^+ + K^+)$-ATPase activity was investigated in order to determine which form of vanadium mediates the cardiac effects. V^{5+}, V^{4+} and V^{3+} (300 μM each) increased the force of contraction of isolated electrically driven cat papillary muscles by about 100%. In the presence of the reducing agent ascorbic acid (5 mM) none of the three compounds led to any distinct increase in force of contraction. On the particulate adenylate cyclase preparation from feline right ventricles only V^{5+} stimulated the enzyme activity by about 100%, whereas V^{4+} and V^{3+} were ineffective. In the presence of 5 mM ascorbic acid all three compounds were ineffective. In contrast, in the presence of the oxidizing agent diamide (azodicarboxylic acid-bis-dimethylamide; 1 mM) all three compounds became stimulatory. On the isolated $(Na^+ + K^+)$-ATPase V^{5+} (500 μM) alone reduced the basal activity by about 95%. In the presence of ascorbic acid the inhibitory effect of V^{5+} was greatly diminished. Similar results were obtained with V^{4+} . V^{3+} (100 μM) alone inhibited $(Na^+ + K^+)$-ATPase activity only by about 40%. In the presence of ascorbic acid V^{3+} was ineffective. From the results it is concluded that positive inotropism, stimulation of adenylate cyclase and inhibition of $(Na^+ + K^+)$-ATPase by vanadium compounds likewise result from an action of vanadium in the +5 oxidation state.

The occurrence of vanadium in tissues and serum of animals and man (see [1]) and the finding that vanadate (vanadium in the +5 oxidation state; V^{5+}) inhibits $(Na^+ + K^+)$-ATPase activity [2–9] and stimulates adenylate cyclase activity from various tissues [10–14] suggest that vanadium compounds may play a physiological role in regulating these enzyme activities.

Among the physiological effects of vanadate (for review see [15, 16]) the positive inotropic effect in ventricular cardiac muscle [8, 11, 17, 18] might be explained by both an inhibition of the $(Na^+ + K^+)$-ATPase and/or a stimulation of the adenylate cyclase activity with a subsequent increase in myocardial c-AMP content [14]. However, the precise mechanism underlying vanadate's positive inotropic effect is still obscure. Moreover, vanadium is known to exhibit various valence states and to be oxidized and reduced easily [19–21] but the biologically active form of vanadium which produces the cardiac effects is not known.

The present paper was designed to further elucidate the mechanism of the positive inotropic action of vanadate and especially to define biologically the form(s) in which vanadium exerts its effects on the heart. Therefore we studied the effects of various vanadium compounds in various oxidation states (V^{5+}, V^{4+}, V^{3+}) on myocardial force of contraction, on adenylate cyclase activity and on $(Na^+ + K^+)$-ATPase activity. Part of the results has been presented at the 21st Spring Meeting of the Deutsche Pharmakologische Gesellschaft in Mainz [22].

MATERIALS AND METHODS

Physiological studies. Cats (0.9–1.9, mean 1.3 kg) were anaesthetized with sodium pentobarbital (30 mg per kg i.p.), their hearts were quickly excised, and the papillary muscles (cross-sectional area 0.67 ± 0.04 mm^2; length 6.3 ± 0.4 mm; $n = 23$) were dissected from the right ventricles in aerated bathing solution (composition see below) at room temperature. The papillary muscles were attached to a bipolar platinum stimulating electrode and suspended individually in glass tissue chambers for recording isometric contractions as described in detail by Meinertz *et al.* [23]. The bathing solution (75 ml) containing (mM) NaCl 136.9, KCl 5.4, $CaCl_2$ 1.8, $MgCl_2$ 1.05, NaH_2PO_4 0.42, $NaHCO_3$ 11.9, glucose 5.5 was continuously gassed with 95% O_2 + 5% CO_2 and its temperature was maintained at 35°. The

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pH was 7.4. The force of contraction was measured with an inductive force displacement tranducer (W. Fleck, Mainz) attached to a Hellige Helco Scriptor recorder. Each muscle was stretched to the length at which force of contraction was maximal. The resting force (approximately 15 mN/mm^2) was kept constant throughout the experiment. The preparations were electrically paced at 0.2 Hz with rectangular pulses of 5 ms duration (Grass stimulator SD 9); the voltage was about 10% greater than threshold. All preparations were allowed to equilibrate for at least 30 min in drug-free bathing solution until complete stabilization. Then the drug was applicated by changing the whole bathing solution. After a 15 min incubation time the drug was washed out and the papillary muscles were again allowed to equilibrate, now in bathing solution containing 5 mM ascorbic acid. After reaching a new equilibrium (after approximately 30 min) the drug was again applicated by changing the whole bathing solution.

The vanadium compounds tested (NH_4VO_3; $C_{10}H_{14}O_5V$, $VOSO_4$; VCl_3) are deliberately referred to as V^{5+}, V^{4+} and V^{3+}, respectively. A detailed discussion of the oxidation state of the individual compounds under the experimental conditions used is given in the "Discussion". The agents were dissolved in the following way. Appropriate amounts of the substances to yield the final concentrations were suspended in bathing solution and the mixtures were warmed (35°) and gassed with 95% O_2 and 5% CO_2 for 30 min prior to application. Under these conditions all vanadium compounds yielded a colorless solution. In the experiments in which the vanadium compounds in the presence of ascorbic acid were tested, 5 mM ascorbic acid was added to the prewarmed and pregassed solutions and the solutions were warmed and gassed for a further 15 min prior to application to the organ bath. After this time all vanadium compounds gave a light blue colored solution. Isoprenaline and dihydro-ouabain were dissolved exactly in the same manner.

Adenylate cyclase assay. After the papillary muscles had been dissected, washed particulate fractions (10,000 *g* sediment) were prepared from homogenates of the same ventricular tissue and adenylate cyclase activity (EC 4.6.1.1.) was assayed as described previously [24], using the protein binding assay of Gilman [25] to determine the cyclic AMP formed. The assay contained 40 mM Tris HCl, pH 7.4, 5 mM $MgCl_2$, 0.3 mM ATP, 0.01 mM EGTA, 7 μM 5'-guanylylimidodiphosphate, 0.3 mM papaverine HCl, 5 mM creatine phosphate, 0.1 mg/ml creatine kinase, 1 mg/ml bovine serum albumin, 200 μM $KHCO_3$ and 20 μl enzyme suspension (approximately 40 μg protein) in a total volume of 100 μl. The reaction mixture was preincubated for 5 min at 37°. The reaction was started with the enzyme preparation, carried out for 10 min at 37° and was terminated by the addition of 500 μl ice-cold sodium acetate buffer, 200 μM, pH 4.0. The time course of the reaction was linear during this time. Cyclic AMP was assayed after 60-fold dilution of the samples. Under these conditions, the vanadium compounds studied did not interfere with the assay procedure. All assays were carried out in duplicate or triplicate.

$(Na^+ + K^+)$-ATPase assay. After the papillary muscles had been dissected from the hearts and after removing about 25 mg of tissue for the adenylate cyclase assay, remaining ventricular tissue was frozen and kept at −60° until use. After thawing, $(Na^+ + K^+)$-ATPase (EC 3.6.1.3.) was prepared as described by Pitts and Schwartz [26] but without glycerol treatment. Enzyme activity was determined by the coupled optical assay in an ATP-regenerating system [27]. The incubation medium containing (mM) NaCl 136.9, KCl 5.4, $CaCl_2$ 1.8, $MgCl_2$ 1.05, NaH_2PO_4 0.42, glucose 5.5 was buffered with $NaHCO_3$ to pH 7.4; the total volume was 2 ml. The enzyme protein was incubated in the medium for 15 min at 37° in each test tube with 5 mM ascorbic acid and different concentrations of the drugs. Then 0.4 ml of this mixture were assayed rapidly for ATPase activity in the coupled optical assay with continuous recording for 10 min at least. The oxidation of NADH was linear during this time. Under these conditions, 97% of total ATPase activity could be inhibited by 100 μM ouabain and thus was defined as $(Na^+ + K^+)$-ATPase. All assays were carried out in duplicate.

Protein determination. Protein concentrations were determined by the method of Lowry *et al.* [28] using bovine serum albumin as standard.

Materials. The drugs used were ammonium vanadate (NH_4VO_3, V^{5+}; Merck, Darmstadt), vanadium (IV) oxide acetylacetonate ($C_{10}H_{14}O_5V$, V^{4+}; Riedel-de Haen, Seelze-Hannover), vanadyl (IV) sulfate ($VOSO_4$, V^{4+}; Fisher Scientific Co., München), vanadium (III) chloride (VCl_3, V^{3+}; Riedel-de Haen, Seelze-Hannover), (±)-isoprenaline hydrochloride (Boehringer, Ingelheim), dihydro-ouabain (Boehringer, Mannheim), cyclic adenosine-^{3}H(G)-3',5'-monophosphate (^{3}H-c-AMP; 41.1 Ci/mmol; NEN Chemicals, Dreieich), cyclic adenosine 3',5'-monophosphate (c-AMP; Boehringer, Mannheim), adenosine-5'-triphosphate (Serva, Heidelberg), Quickzint 402 (Zinsser, Frankfurt), (+)-ascorbic acid (Merck, Darmstadt), diamide (azodicarboxycyclic acid-bis-dimethylamide; Sigma Chem. Co.). All other chemicals were of analytical or best commercial grade available. Deionized and twice-distilled water was used throughout.

Statistics. Values presented are means ± S.E.M. Statistical significance was estimated using Student's *t*-test for paired or unpaired observations. A P-value of less than 0.05 was considered significant.

RESULTS

Effects on force of contraction

The results are illustrated in Fig. 1 by original recordings from typical experiments. 300 μM NH_4VO_3 (which is a submaximally effective concentration as far as the positive inotropic effect in cat papillary muscles is concerned; [17]) increased the force of contraction within 9 min by about 100% (Fig. 1A left part). On the average 300 μM NH_4VO_3 (referred to as V^{5+}; a detailed discussion on the oxidation states of vanadium under the various experimental conditions used is given below) led to an increase in force of contraction from 13.4

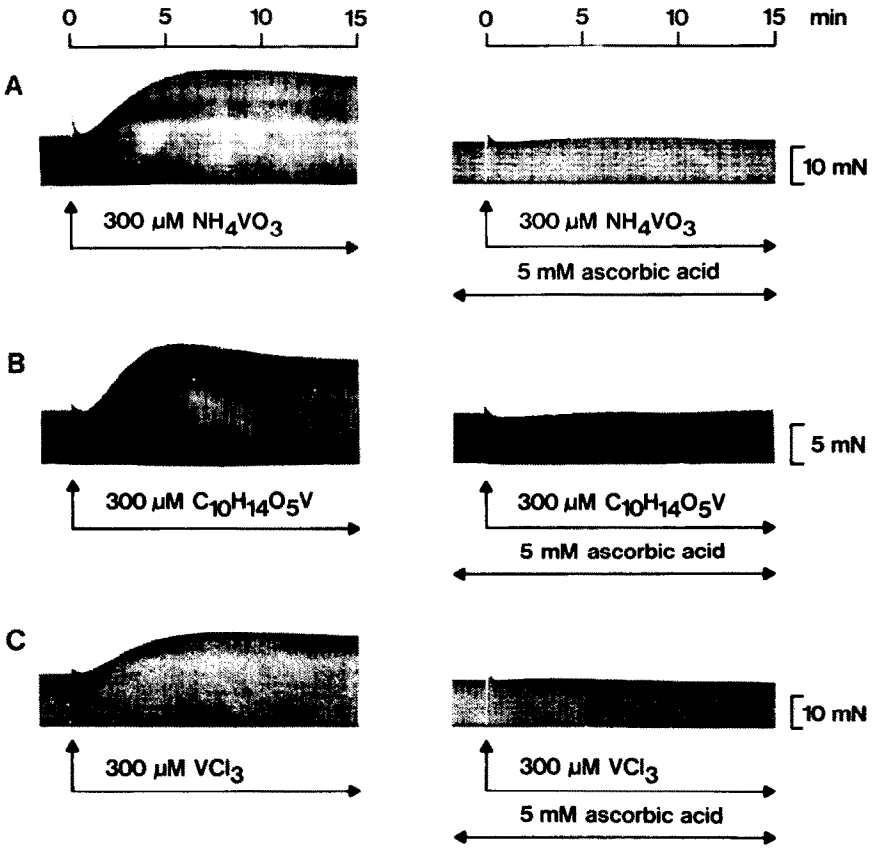

Fig. 1. Effect of ammonium vanadate (A; V^{5+}), vanadium (IV) oxide acetylacetonate (B; V^{4+}) and vanadium (III) chloride (C; V^{3+}) at concentrations of 300 μM each on force of contraction of cat isolated papillary muscles in the absence (left) and in the presence (right) of 5 mM ascorbic acid. The substances were applicated at zero time as indicated by arrows.

± 2.8 mN/mm² to 27.2 ± 6.0 mN/mm² ($n = 4$). 300 μM of the organic vanadyl complex $C_{10}H_{14}O_5V$ (V^{4+}) and 300 μM VCl_3 (V^{3+}) caused a similar increase in force of contraction from 12.3 ± 1.9 mN/mm² to 28.6 ± 5.0 mN/mm² and from 12.8 ± 3.4 mN/mm² to 24.0 ± 6.2 mN/mm² ($n = 4$, respectively, Figs. 1B and C). As is illustrated in the right part of Fig. 1, none of the three compounds led to any distinct increase in force of contraction in the presence of the reducing agent ascorbic acid (5 mM). Similar results as with the vanadyl complex $C_{10}H_{14}O_5V$ (V^{4+}) were obtained with vanadyl sulfate ($VOSO_4$, V^{4+}; data not shown). Ascorbic acid by itself did not affect force of contraction.

In order to exclude that ascorbic acid prevents the effect of any positively inotropic acting drug we investigated the influence of 5 mM ascorbic acid on the positive inotropic effect of 0.03 μM isoprenaline and 2 μM dihydro-ouabain. The concentrations of isoprenaline and dihydro-ouabain were chosen to be approximately equieffective to the concentrations of the vanadium compounds. As can be seen from Figs. 2A and B, 5 mM ascorbic acid did not influence the positive inotropic effect of isoprenaline and dihydro-ouabain.

Effects on adenylate cyclase activity

Figure 3 shows the effects of NH_4VO_3 (V^{5+}), $C_{10}H_{14}O_5V$ (V^{4+}) and VCl_3 (V^{3+}) on myocardial adenylate cyclase activity in the absence and presence of 5 mM ascorbic acid. 1000 μM V^{5+} (a submaximally effective concentration concerning the stimulatory effect on adenylate cyclase activity; [29]) increased adenylate cyclase activity by about 100% (Fig. 3, upper panel). 5 mM ascorbic acid totally abolished the stimulatory effect of V^{5+}. 5 mM ascorbic acid by itself did not affect adenylate cyclase activity.

The middle panel of Fig. 3 shows that vanadium in the nominally +4 and +3 oxidation state at concentrations of 1000 μM did not stimulate adenylate cyclase activity, even in the absence of ascorbic acid. Instead, these substances caused a slight but insignificant decrease of the basal activity. To exclude that ascorbic acid makes the adenylate cyclase unresponsive to any stimulatory intervention we studied the effect of isoprenaline (1 μM) in the absence and presence of ascorbic acid. The lower panel of Fig. 3 shows that 5 mM ascorbic acid did not alter the isoprenaline-induced stimulation of adenylate cyclase activity.

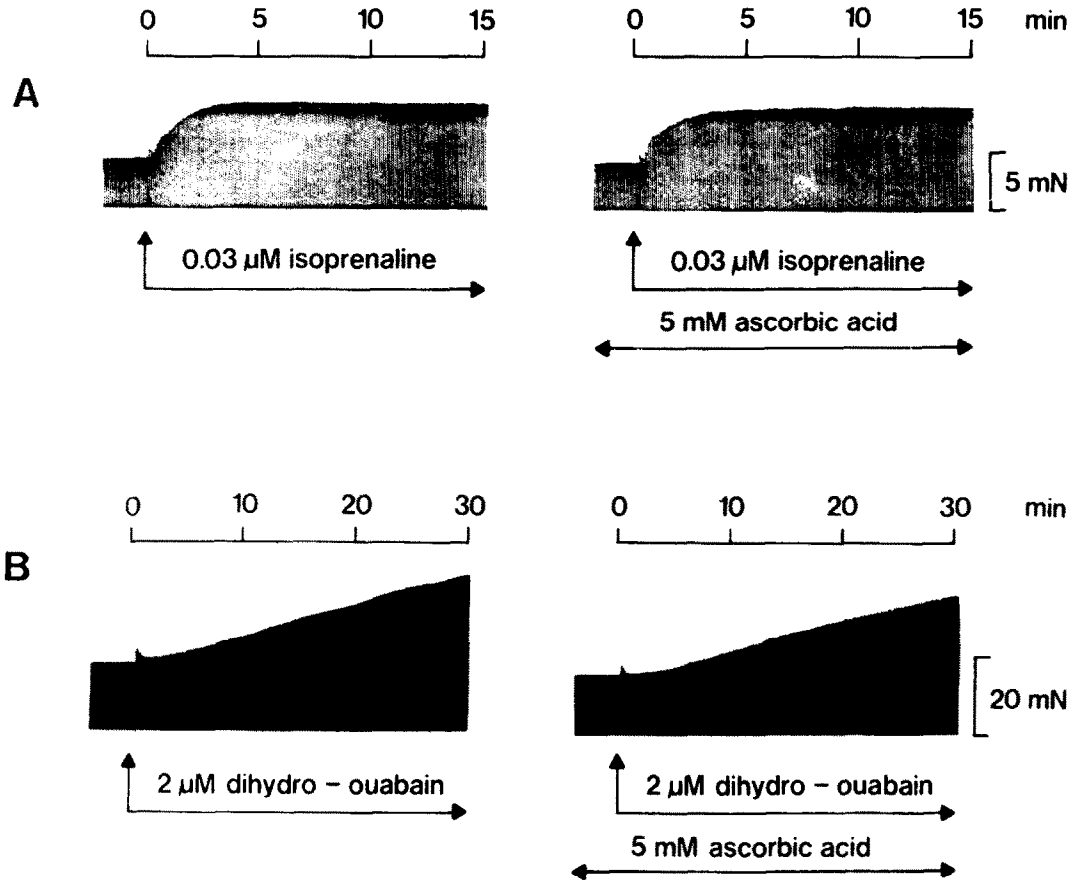

Fig. 2. Effect of 0.03 μM isoprenaline (A) and 2 μM dihydro-ouabain (B) on force of contraction of cat isolated papillary muscles in the absence (left) and presence (right) of 5 mM ascorbic acid.

The failure of V^{4+} and V^{3+} to stimulate adenylate cyclase activity (even in the absence of ascorbic acid) prompted us to study the effects of the vanadium compounds in the presence of diamide which is capable of oxidizing vanadium compounds [30]. Figure 4 shows that 1 mM diamide by itself decreased basal adenylate cyclase activity to about 50%. Despite the reduction of basal adenylate cyclase activity by diamide, 1000 μM V^{5+} caused an about 100% increase in activity in the presence of diamide as it did in the absence of the oxidizing agent (Fig. 4 upper panel). In contrast to the slight (but insignificant) inhibition of adenylate cyclase activity by V^{4+} and V^{3+} seen without the oxidizing agent diamide, these substances became stimulatory in the presence of 1 mM diamide (Fig. 4 middle panel). V^{4+} increased basal activity by about 40% and V^{3+} by about 90%. Similar results as with the organic vanadyl complex $C_{10}H_{14}O_5V$ (V^{4+}) were again obtained with vanadyl sulfate ($VOSO_4$, V^{4+}; data not shown). The lower panel of Fig. 4 shows that the stimulatory effect of 1 μM isoprenaline was about 2.2 fold under both conditions, i.e. with or without diamide.

Effects on $(Na^+ + K^+)$*-ATPase activity*

NH_4VO_3 (V^{5+}) alone inhibited myocardial $(Na^+ + K^+)$-ATPase activity in a concentration-dependent manner (0.1–500 μM) by about 95% (Fig. 5A). 5 mM ascorbic acid by itself reduced the basal $(Na^+ + K^+)$-ATPase activity by about 15%. In the presence of 5 mM ascorbic acid, the inhibitory effect on $(Na^+ + K^+)$-ATPase activity of V^{5+} was almost abolished. 500 μM V^{5+}, the highest concentration used, reduced the $(Na^+ + K^+)$-ATPase activity only by about 15%. Similar results as with V^{5+} were obtained with the vanadyl complex $C_{10}H_{14}O_5V$ (V^{4+}; Fig. 5B) and with the vanadyl sulfate ($VOSO_4$, V^{4+}; data not shown). VCl_3 (V^{3+}) inhibited the $(Na^+ + K^+)$-ATPase activity at 100 μM in the absence of ascorbic acid by about 40% (Fig. 5C). This effect was abolished in the presence of 5 mM ascorbic acid.

Figure 5D shows that the inhibitory effect of dihydro-ouabain (0.01–50 μM) was not influenced by 5 mM ascorbic acid.

DISCUSSION

Before discussing the results of the present paper from the biological point of view it is worth recalling the complicated chemical behaviour of vanadium compounds in aqueous solutions [20, 21, 30–34]. Firstly, vanadium readily changes its oxidation state. In the presence of O_2 or air (i.e. under conditions prevalent at experimentation with physiological methods) or oxidizing agents (e.g. diamide) vana-

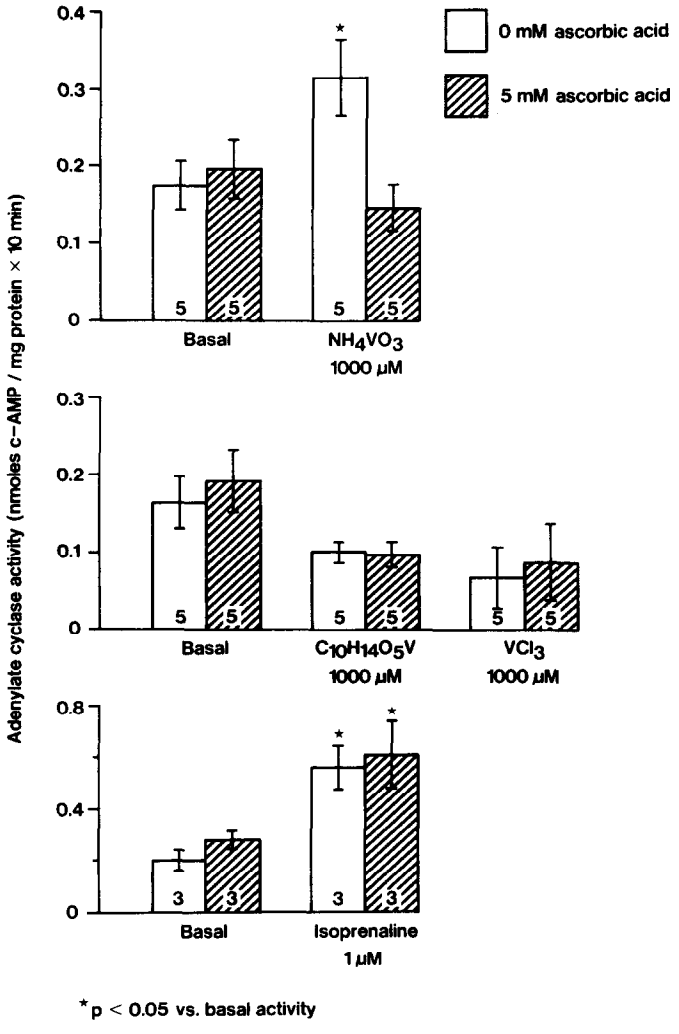

Fig. 3. Effect of ammonium vanadate (V^{5+}; upper panel), vanadium (IV) oxide acetylacetonate (V^{4+}) and vanadium (III) chloride (V^{3+}; middle panel) at concentrations of 1000 μM each on adenylate cyclase activity of particulate fractions from cat right ventricles in the absence and presence of 5 mM ascorbic acid. The effect of 1 μM isoprenaline is shown for comparison (lower panel). Significant differences from basal activity are marked with asterisks. The number of preparations is given in the columns.

dium is always in the +5 oxidation state, irrespective of the nature of the original compound. In contrast, in the presence of reducing agents such as ascorbic acid, V-compounds are in the +4 oxidation state. Secondly, one has to consider simultaneous changes in charge. These are mainly dependent on the ambient pH. Under physiological conditions at pH 7.4, vanadium in the +5 oxidation state is in the form of vanadate (VO_3^-, VO_4^{3-} or its isopolyanions), i.e. as a negatively charged anion while vanadium in the +4 oxidation state at pH 7.4 is predominantly in the form of vanadyl (VO^{2+}), i.e. as a positively charged cation. V^{5+}-cationic and V^{4+}-anionic forms are only existent under strongly basic and acid conditions, respectively, and can thus be excluded from the present discussion.

The main result of the present study is that vanadate (i.e. V^{5+}) similarly increased force of contraction, stimulated adenylate cyclase and impaired ($Na^+ + K^+$)-ATPase activity. All these effects were abolished or strongly reduced in the presence of the mild reducing agent ascorbic acid which keeps vanadium in the +4 oxidation state. These observations allow the clear-cut (positive) conclusion that the biologically active form of vanadium in the systems tested at physiological pH is the +5 oxidation state in the form of a negatively charged anion, i.e. VO_3^-, VO_4^{3-} or its isopolyanions as mentioned above. However, from the foregoing discussion on the chemical behaviour of vanadium compounds it is also clear that the correspondent negative result, namely that vanadium in the presence of ascorbic acid (that is in the +4 oxidation state) failed to exert biological effects, cannot be explained so easily. It must remain open if this lack of effect solely results from a change in the oxidation state because the reduction of vanadate (VO_3^-) to vanadyl (VO^{2+}) is accompanied by a change in charge [20, 21, 33, 35].

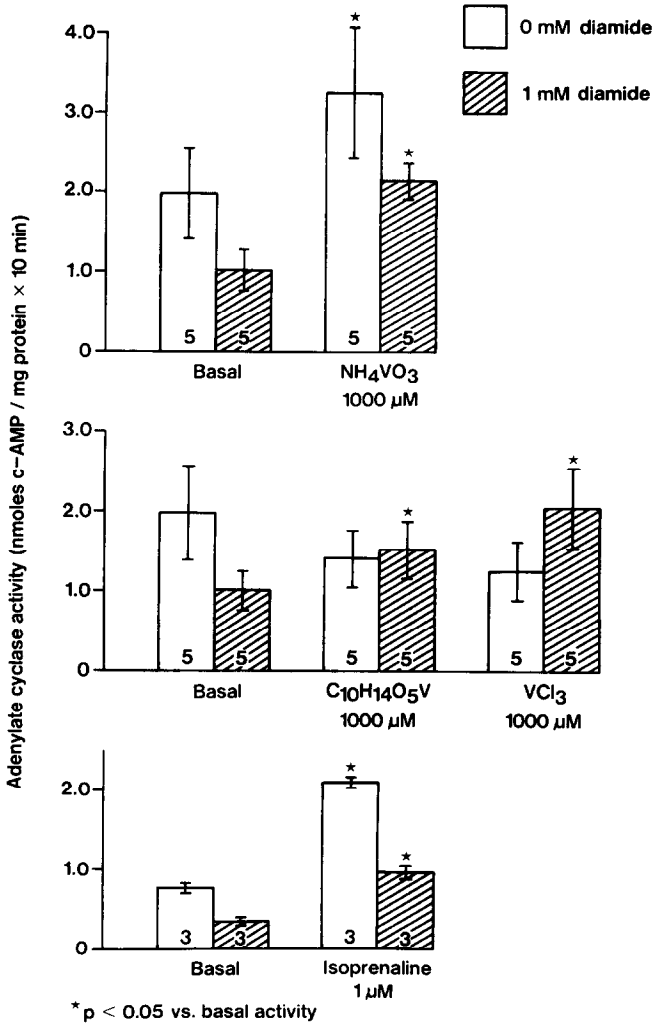

Fig. 4. Effect of ammonium vanadate (V^{5+}; upper panel), vanadium (IV) oxide acetylacetonate (V^{4+}) and vanadium (III) chloride (V^{3+}; middle panel) at concentrations of 1000 μM each on adenylate cyclase activity of particular fractions from cat right ventricles in the absence and presence of 1 mM diamide. The effect of 1 μM isoprenaline is shown for comparison (lower panel). Significant differences from basal activity are marked with asterisks. The number of preparations is given in the columns.

Thus, it may be possible that the failure of the reduced vanadium (V^{4+}; VO^{2+}) to exert biological effects is not or not only due to the reduced oxidation state itself but instead may also result from the change to a positively charged cation.

The conclusion that vanadate (i.e. V^{5+}) is the biologically active form is supported by the experiments performed with the vanadium compounds in the nominally +4 ($C_{10}H_{14}O_5V$ and $VOSO_4$) and +3 (VCl_3) oxidation state.

In the contraction experiments the positive inotropic effects of all vanadium compounds were similarly abolished in the presence of ascorbic acid. In turn, all compounds did produce a likewise similar effect on force of contraction when applied without a reducing agent. This can be easily explained by the fact that vanadium compounds (V^{4+}, V^{3+}) are oxidized spontaneously as mentioned above. This is very likely to occur in our contraction experiments where the bathing solution is continuously gassed with 95% O_2 and 5% CO_2. Under these conditions V^{4+} and V^{3+} may be oxidized readily by the high oxygen content of the bathing solution [19, 33, 36] and thus most of the vanadium added probably is in the +5 oxidation state. Accordingly, ascorbic acid prevented the effect of V^{4+} and V^{3+} on force of contraction. As the positive inotropic effect of isoprenaline and dihydro-ouabain was not influenced by ascorbic acid it can be excluded that ascorbic acid unspecifically prevents the effect of any positive inotropic intervention. Thus, in summary, it is reasonable to conclude that vanadium (irrespective of the compound added to the organ bath) under the conditions employed in our experiments finally exerts its inotropic actions as vanadium in the +5 oxidation state (i.e. as vanadate) and that these

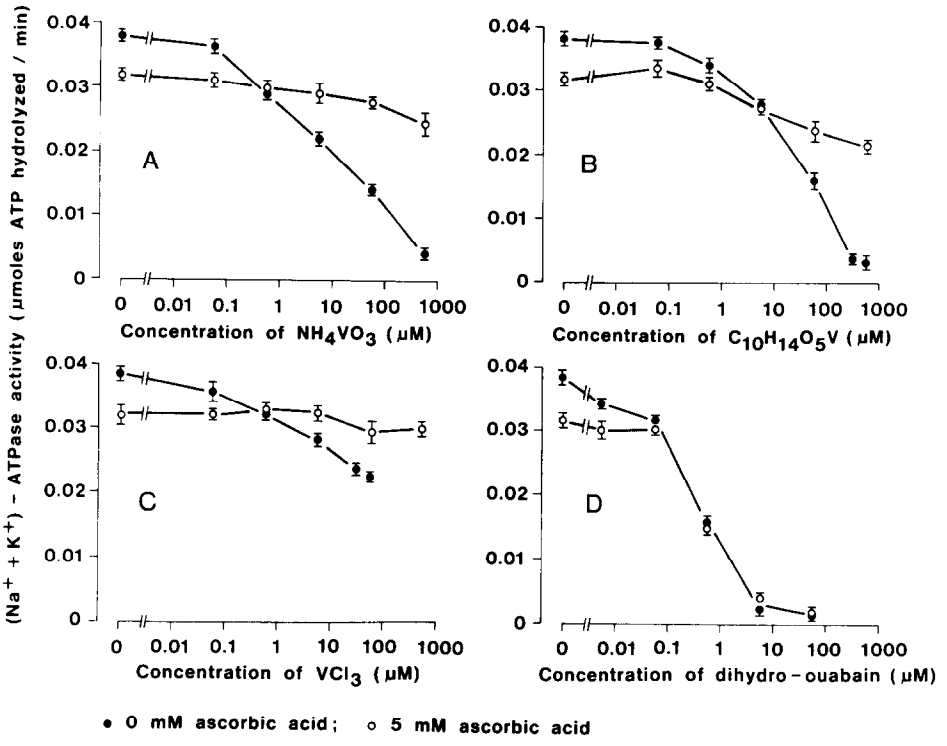

Fig. 5. Effect of ammonium vanadate (V^{5+}; A), vanadium (IV) oxide acetylacetonate (V^{4+}; B) and vanadium (III) chloride (V^{3+}; C) at concentrations of 0.01–1000 μM each on ($Na^+ + K^+$)-ATPase activity of a membrane preparation (60 mg protein) from cat hearts in the absence (●) and presence (○) of 5 mM ascorbic acid. The effect of dihydro-ouabain is shown for comparison (D). Each point represents the mean ± S.E.M. of 3 determinations.

biological effects can be prevented by reducing agents.

Similar conclusions can be drawn from the adenylate cyclase experiments. The well-known stimulatory effect of V^{5+} (i.e. vanadate) [10–12, 29] on adenylate cyclase activity could also be prevented by ascorbic acid. This prevention was again not an unspecific effect of the ascorbic acid because the isoprenaline-induced stimulation was not affected. V^{4+} and V^{3+} had no effect even in the absence of a reducing agent (Fig. 3). Thus, under the enzyme assay conditions (no gassing with the O_2/CO_2 mixture!) V^{4+} and V^{3+} are obviously not oxidized spontaneously to V^{5+} in amounts sufficient to produce biological effects. In these particular experiments another point must be considered. One could argue that V^{4+} was inactive in these experiments because the vanadyl is bound in the organic complex ($C_{10}H_{14}O_5V$) and is not sufficiently released as free vanadyl. However, as vanadyl sulfate ($VOSO_4$) yielded similar results this seems to be rather unlikely. Moreoever, as VCl_3 is an unstable compound which is already oxidized to vanadyl (VO^{2+}, V^{4+}) upon dissolving in aqueous solutions [33], the failure of VCl_3 to produce an effect also supports the conclusion that free vanadyl does not stimulate adenylate cyclase. The finding that all compounds similarly stimulated adenylate cyclase activity in the presence of the oxidizing agent diamide is in line with the conclusion that oxidation–reduction reactions may regulate the amount of biologically active vanadium. Thus, these results clearly indicate that vanadium in the +5 oxidation state (i.e. vanadate) is also the form that causes the stimulation of adenylate cyclase activity.

($Na^+ + K^+$)-ATPase activity was inhibited by V^{5+} and V^{4+} to nearly the same extent while V^{3+} was less effective. This indicates that in these experiments V^{4+} and to a lesser degree V^{3+} were oxidized spontaneously to V^{5+} in amounts sufficient to inhibit the ($Na^+ + K^+$)-ATPase activity. It is in accordance with this that the inhibitory action of all vanadium compounds (VO_3^-; $C_{10}H_{14}O_5V$; $VOSO_4$; VCl_3) was abolished or strongly reduced in the presence of ascorbic acid. This was again not due to an unspecific effect of ascorbic acid because the effect of dihydro-ouabain remained unaffected by ascorbic acid.

Thus, the results provide evidence that vanadium in the +5 oxidation state is the active form that inhibits ($Na^+ + K^+$)-ATPase activity. This is in accord with the data of Cantley and Aisen [19]. These authors showed that V^{4+} is much less effective than V^{5+} in inhibiting red cell ($Na^+ + K^+$)-ATPase activity. Moreover, in renal ($Na^+ + K^+$)-ATPase it has also been found that the reducing agents glutathione and ascorbic acid reduced the inhibitory effect of vanadate [30].

Thus, all responses to vanadium tested, the increase in force of contraction, the stimulation of adenylate cyclase and the inhibition of ($Na^+ + K^+$)-ATPase activity appear to result from an action of vanadium in the +5 oxidation state (i.e.

vanadate) and can be prevented by reduction of vanadate to vanadyl.

The observation that the inhibitory effect of vanadate on $(Na^+ + K^+)$-ATPase is greatly diminished by reducing agents led several authors to suggest that oxidation–reduction reactions in tissues may play a role in the regulation of the $(Na^+ + K^+)$-ATPase activity by vanadate [21, 30, 35]. Intracellular glutathione or ascorbic acid were thought to be candidates involved in such oxidation–reduction reactions in biological systems [21, 30, 35]. Moreover, we have previously described a NADH-vanadate-oxidoreductase in cardiac and erythrocyte membrane preparations [37, 38]. This enzyme converts vanadate (V^{5+}) to vanadyl (V^{4+}) in the presence of NADH. The existence of an enzyme that may regulate the oxidation state of vanadium compounds in cardiac tissue underlines the importance of oxidation–reduction reactions involved in the effects of vanadium compounds. The NADH-oxidoreductase may govern the amount of biologically active vanadium within the cell and as a result the activity of enzyme systems and finally physiological functions of the cell. In line with this it has been shown by electron spin resonance measurements that vanadate exogenously added to erythrocytes is found intracellularly as vanadyl (V^{4+}) or vanadyl hydroxide bound to hemoglobin [19, 35].

Although our experiments were not specifically designed to clarify the mechanism through which vanadate exerts its positive inotropic effect, the finding that positive inotropism and stimulation of cardiac adenylate cyclase are probably due to the same form of vanadium is in line with our previous conclusion that the reported small increase in cardiac c-AMP tissue levels may contribute to the positive inotropic effect of exogenously added vanadate [14, 29]. Since V^{5+} appears to be the form that most effectively inhibits $(Na^+ + K^+)$-ATPase activity in cardiac membrane preparations (Fig. 5), one could assume that this mechanism is also involved in the positive inotropic effect of exogenously added vanadate although in intact heart cells no inhibition of the ouabain-sensitive ^{86}Rb-uptake (a measure of the $(Na^+ + K^+)$-ATPase activity) was detectable [7, 39].

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