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VANADYL (VO^{2+}) AND VANADATE (VO_3^-) IONS INHIBIT THE BRAIN MICROSOMAL Na,K-ATPase WITH SIMILAR AFFINITIES. PROTECTION BY TRANSFERRIN AND NORADRENALINE

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Abstract—The activity of Na,K-ATPase was measured in brain microsomes as the function of increasing concentrations of vanadyl ($VOSO_4$, V^{4+}) and the vanadate ($NaVO_3$, V^{5+}) ions. Both forms of vanadium inhibited the Na,K-ATPase activity with high affinity $-K_i$ (vanadate) = 3×10^{-7} M and K_i (vanadyl = 1×10^{-6} M. The stability of V^{4+} in ATPase reaction media (Tris buffers) was measured by electron spin resonance spectroscopy. Without any reducing agent, V^{4+} was quickly oxidised by atmospheric oxygen. When a reducing agent such as dithiothreitol was added, the V^{4+} was stable for at least 30 min and the inhibition pattern of Na,K-ATPase by V^{4+} was not changed. The blocking effect of V^{4+} in the presence of dithiothreitol was counteracted by pre-incubation with equimolar concentrations of transferrin or 100 times excess of noradrenaline.

The regulation of brain Na,K-ATPase by vanadate may be represented by competition between low-capacity inhibitory binding sites localized on the enzyme molecule and high-capacity sites of intracellular proteins. Preferential binding of vanadyl to the latter type of sites will decrease the intracellular concentration of the free metal and thus eliminate the enzyme inhibition.

In the tissues of higher animals, two vanadium species may be present under physiological conditions (see review of Ramasarma and Crane, ref. 1). Vanadate, the +5 oxidation state of vanadium (V^{5+}), is known as a very potent inhibitor of sodium plus potassium activated, magnesium dependent adenosinetriphosphatase (Na,K-ATPase) and neutral K^+-stimulated *p*-nitrophenyl-phosphatase in membrane preparations of various tissues [2–11]. On the other hand, vanadyl, the +4 oxidation state of vanadium (V^{5+}), is considered as a poor inhibitor of the Na,K-ATPase *in vitro* [12, 13].

In a previous report [14] we also described the disparity between the effects of V^{5+} and V^{4+} ions on Na,K-ATPase and K^+-stimulated *p*-nitrophenyl-phosphatase in skeletal muscle membrane preparations. Only vanadate (V^{5+}) inhibited significantly the muscle Na,K-ATPase with $K_i = 1 \times 10^{-6}$ M, whereas the vanadyl (V^{5+}) ions were almost without effect. We also found, using electron spin resonance spectroscopy (ESR), that several psychotropic drugs such as phenothiazine derivate, chlorpromazine, and a dibenzazepine analog, imipramine, quantitatively reduced V^{5+} to V^{4+} [15]. Reduction of the inhibitory V^{5+} form to non-inhibiting V^{+4} might be the mechanism for the claimed benefit of these drugs in manic-depressive syndroms caused, according to Naylor and Smith [16, 17], by the V^{5+}-induced blockade of Na,K-ATPase. These pathological states may arise when V^{5+} accumulates near the cell membrane. This idea implies *a priori* that V^{4+} in the brain does not inhibit the Na,K-ATPase as in other tissues [12–14]. The aim of the present communication was therefore to follow the inhibitory action of both V^{4+} and V^{5+} forms of vanadium on the activity of brain microsomal Na,K-ATPase *in vitro*.

MATERIALS AND METHODS

Preparation of microsomal fractions from the rat cerebral cortex. The brain subcellular fractions were prepared according to De Robertis *et al.* [18] from the cerebral cortex of white Wistar rats (180 g) as described in detail in previous papers [19, 20]. The microsomal membrane particles were sedimented from 12,000 *g* supernatant by centrifugation for 60 min at 100,000 *g*. The resulting sediment was suspended in 0.32 M sucrose, 10 mM Tris–HCl pH 7.4 microsomes. The samples were stored at −25°.

Measurement of enzyme activities. The activity of sodium plus potassium activated, magnesium dependent adenosinetriphosphatase (Na,K-ATPase) was determined [19, 20] as inorganic phosphorus production.

For determination of Na+K+Mg-ATPase activity the brain microsomal fractions (0.03–0.05 mg membrane protein per ml) were preincubated for 5 min in 100 mM NaCl, 20 mM KCl, 100 mM Tris–HCl, pH 7.4, 5 mM $MgCl_2$. Basal, ouabain-independent Mg-ATPase was measured in 120 mM NaCl, 100 mM

Tris–HCl, pH 7.4 5 mM $MgCl_2$ and 2×10^{-4} M ouabain (Sigma) at 37°. The reaction in total volume of 2 ml was started by addition of ATP (Boehringer) to final concentration 2.5 mM, continued for 15 min at 37° and terminated by addition of 0.5 ml of 1 N $HClO_4$. The precipitated protein was removed by centrifugation and inorganic phosphate was determined according to Taussky and Shorr [21]. All chemicals tested were present in the course of preincubation period, i.e. 5 min before the addition of ATP. The ATPase activities were expressed as inorganic phosphate produced per hour per mg of membrane protein, Na,K-ATPase activity was calculated as the difference between the Na+K+Mg-ATPase and the ouabain-insensitive Mg-ATPase. Vanadyl ($VOSO_4$, Merck) and vanadate ($NaVO_3$, Merck) were applied from stock solutions of 100 mM in distilled water or 0.1 N NaOH respectively. The stock solutions were prepared freshly before assay. It was checked, that the presence of vanadate or vanadyl (10^{-9}–10^{-5} M) in ATPase reaction media in the course of enzyme assay does not interfere with the subsequent determination of inorganic phosphate.

Electron spin resonance (ESR) measurements. The measurements of paramagnetic V^{4+} were performed on a JEOL JES-PE-3X spectrometer at 100 KHz modulation frequency at 22°. Samples were measured in an LC-01 aqueous solution cell. The active volume of the sample was 0.2 ml. Immediately before the measurements, the samples were acidified with concentrated HCl (0.025 ml of HCl per 0.5 ml sample) according to Fitzgerald and Chasteen [22]. Acidification of the media was used because V^{4+} species not bound to protein or buffer molecules do not exhibit an ESR signal above pH 5.5. The analysis of ESR spectra was based on a linear relationship between peak-to-peak signal intensity of the first derivative curve of the V^{4+} concentration. The samples were compared with the standard curve measured at known V^{4+} concentrations. For more details see legend to Figs. 3 and 4.

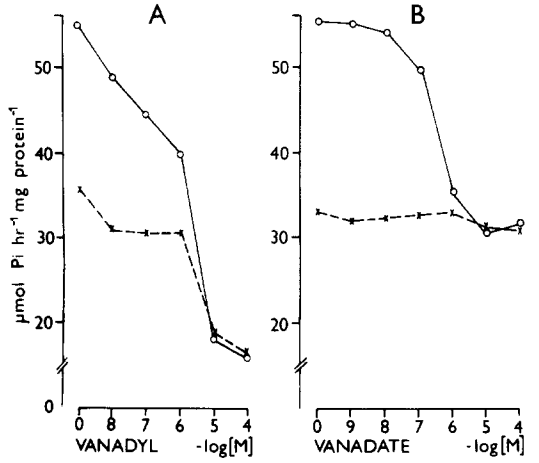


Fig. 1. Inhibition of the brain microsomal Na+K+Mg-ATPase and of Mg-ATPase by vanadyl (V^{4+} and vanadate (V^{5+}) ions. (A) Dose–response curve of vanadyl-induced inhibition of ATPase activities. (B) Dose–response curve of vanadate-induced inhibition of ATPase activities. The microsomes were preincubated for 5 min in Na+K+Mg-ATPase (100 mM NaCl, 20 mM KCl, 10 mM Tris–HCl pH 7.4, 5 mM $MgCl_2$) and in Mg-ATPase (120 mM NaCl, 100 mM Tris–HCl, pH 7.4, 5 mM $MgCl_2$, 2×10^{-4} M ouabain) reaction media with increasing concentrations of vanadium compounds ($VOSO_4$, $NaVO_3$, Merck). After addition of ATP to final concentration of 2.5 mM, the enzyme activities were determined as described in Methods. The data shown are the results of a typical experiment carried out in triplicates. O——O Na+K+Mg-ATPase activity; ×——× Basal, ouabain-independent Mg-ATPase.

RESULTS

The inhibition of the brain microsomal Na,K-*ATPase by vanadate* (V^{5+}) *and vanadyl* (V^{4+}) *ions*

To test the efficiency of vanadium compounds to inhibit the brain Na,K-ATPase, the enzyme activity was measured as a function of increasing concentrations of V^{4+} and V^{5+} ions (Fig. 1). Na,K-ATPase activity was calculated from the results presented in Fig. 1 as the difference between the Na+K+Mg-ATPase and the ouabain-insensitive Mg-ATPase. In accordance with the previously published data [2, 3, 12], a typical inhibition curve of V^{5+} was observed with 50% inhibition manifested at 3×10^{-7} M (average from 5 experiments). Total inhibition was achieved at 1×10^{-5} M of V^{5+}. In agreement with the findings of Josephson and Cantley [2] and Post *et al.* [23], EDTA at 1×10^{-4} M did not diminish the vanadate effect on the Na+K+Mg-ATPase activity (Fig. 2). In the presence of EDTA the dose-response curve of V^{5+}-inhibition was slightly but significantly shifted to higher enzyme activities. The ouabain independent Mg-ATPase was not effected (data not shown).

The complexation of vanadate with EDTA [24] may change the chemical properties of this metal.

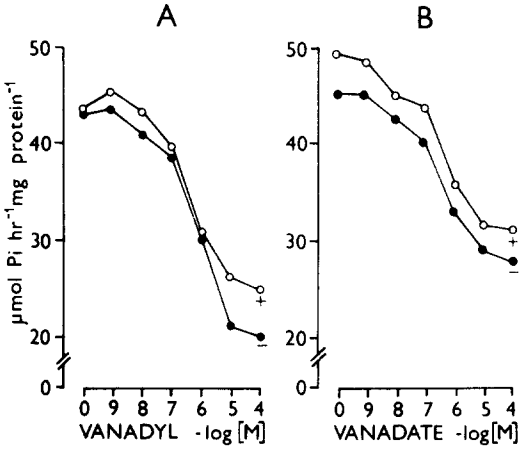
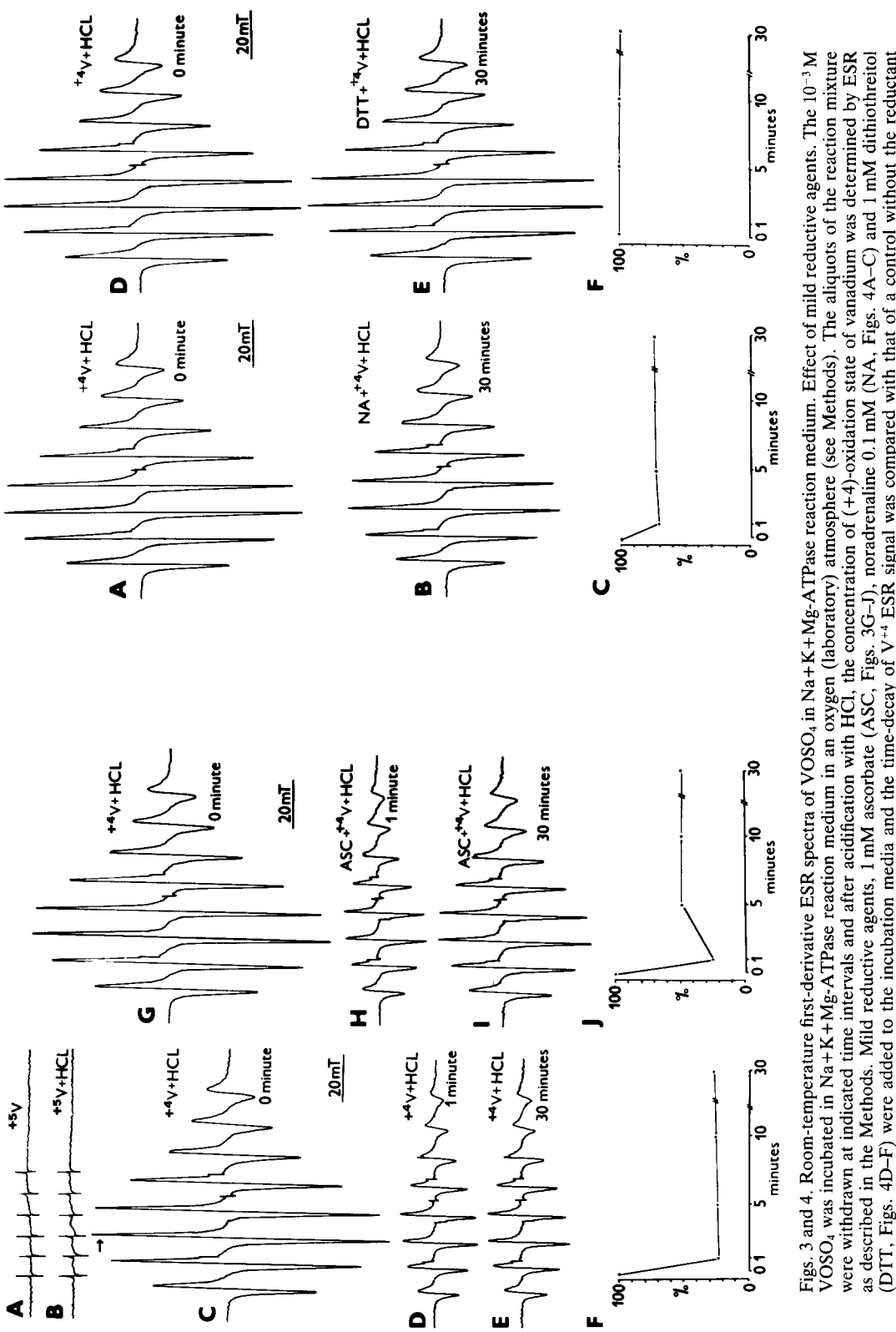


Fig. 2. The effect of EDTA on vanadyl (V^{4+}) and vanadate (V^{5+})-induced inhibition of the brain microsomal Na+K+Mg-ATPase. Na+K+Mg-ATPase activity was determined in microsomes identically as described in methods and in the legend to Fig. 1. EDTA, at final concentration of 10^{-4} M was present in the preincubation media 5 min before addition of ATP. Abscissa vanadium concentration [− log M]; ordinate ATPase activity [µmoles P_i/hr/mg]. O——O EDTA present; ●——● EDTA absent.



Figs. 3 and 4. Room-temperature first-derivative ESR spectra of $VOSO_4$ in Na+K+Mg-ATPase reaction medium. Effect of mild reductive agents. The 10^{-3} M $VOSO_4$ was incubated in Na+K+Mg-ATPase reaction medium in an oxygen (laboratory) atmosphere (see Methods). The aliquots of the reaction mixture were withdrawn at indicated time intervals and after acidification with HCl, the concentration of (+4)-oxidation state of vanadium was determined by ESR as described in the Methods. Mild reductive agents, 1 mM ascorbate (ASC, Figs. 3G–J), noradrenaline 0.1 mM (NA, Figs. 4A–C) and 1 mM dithiothreitol (DTT, Figs. 4D–F) were added to the incubation media and the time-decay of V^{+4} ESR signal was compared with that of a control without the reductant (Figs. 3A–F).

For estimation of spectral intensity, the peak-to peak amplitudes of $M_I = 3/2$ line (see Fitzgerald and Chasteen, ref. 22 and arrow in Fig. 3C of this paper) were compared. The amplitude of this line in Figs. 3(C) and (G) and 4(A) and (D) were taken as 100%. The time-decay of the V^{4+} signal as it is demonstrated in Figs. 3(F) and (J) and 4(C) and (F) was compared to this 100% level measured at time zero (0 min).

Recording conditions were as follows: Modulation frequency, 100 kHz; modulation amplitude, 10 mT; microwave power–time-constant, 0.1 s; spectrometer gain = 2.5×10^2 for $D = 4 \times 10^5$, Mangan in ZnS was used as internal standard—see lines in opposite phase clearly manifested in Figs. 3(A) and (B).

For example, the non-enzymatic oxidation of NADH by V^{5+} [25] is blocked by EDTA. This effect is apparently not significant in enzyme-dependent reactions studied here, since vanadium effects remained essentially unchanged by EDTA (Fig. 2).

Surprisingly, the +4 oxidation state of vanadium was almost as effective as V^{5+} in enzyme inhibition (Fig. 1). The 50% inhibition of Na,K-ATPase was found at 1×10^{-6} M of V^{4+} and the total blockade was achieved at 1×10^{-5} M V^{4+}. Similarly as in the case of V^{5+}, the inhibition curve was not markedly influenced by EDTA (Fig. 2). It thus appeared that vanadium compounds of both redox states inhibit the brain microsomal Na,K-ATPase in a very similar manner.

The main difference in the reactivity of the two redox states of vanadium concerned their effect on the basal, ouabain-independent Mg-ATPase. Vanadate (V^{5+}) had no influence on this enzyme activity up to a concentration of 10^{-4} M, whereas the V^{4+} exhibited a strong inhibitory action. This inhibition was relatively small up to the 10^{-6} M concentration of V^{4+}. At higher concentrations a significant portion of Mg-ATPase was diminished (50% at 10^{-4} M of V^{4+}).

The experiments described so far were performed in Tris-buffered media under an oxygen atmosphere and at slightly alkaline pH. It could be expected that under these conditions the inhibitory effect of nominally V^{4+} might be caused at least partly, by V^{5+} arising by oxidation of V^{4+} [23, 26].

ESR spectra of vanadyl (V^{4+}) *in Tris buffers.* To check the actual concentration of V^{4+} in an Na+K+Mg-ATPase reaction medium, paramagnetic V^{4+} was detected by ESR spectroscopy [22, 27]. The data presented in Figs. 3 (C)–(F) show that already after one minute of incubation at 25° in an oxygen atmosphere, 80% of vanadium added at time zero as V^{4+} was not detectable by an ESR signal. After 20 min of incubation, the same 80% of V^{4+} was still missing. The control signal of non-paramagnetic V^{5+} was not affected by acidification of the media nor by prolonged standing at laboratory temperature (Figs. 3A and B).

In view of these ESR measurements, the V^{4+} inhibition of Na,K-ATPase was of questionable value—when the rapid oxidation of V^{4+} occurs in ATPase reaction media, the V^{5+} (representing as much as 80% of nominally V^{4+}) may be the actual inhibitory form of vanadium acting during 15 min of ATPase assay. The redox transition between V^{4+} and V^{5+} is, however, a reversible process [28].

In the next set of ESR experiments, mild reductive agents such as ascorbate [22, 29], noradrenaline [30] and dithiothreitol (DTT) were therefore tested in their potency to protect V^{4+} against oxidation by air oxygen. In the presence of ascorbate (1 mM), the disappearance of the V^{4+} signal from the media was slowed down (Figs. 3 G–J), however, after 30 min of incubation, still only 50% of the original V^{4+} signal was detectable. In the first minute, transient oxidation of 70% of V^{4+} was observed. Noradrenaline (0.1 mM) exhibited a higher potency in this respect (Figs. 4 A–C). A small decrease by 30% of the V^{4+} signal was already manifested after one minute, but further incubation, which continued up to the 30 min did not result in any further oxidation and/or loss of V^{4+}.

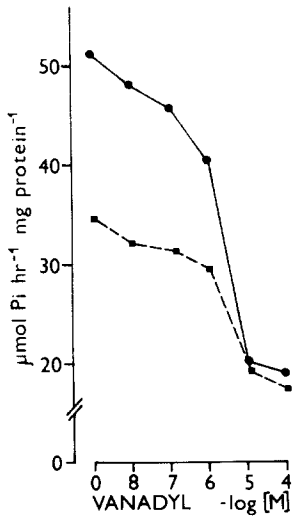


Fig. 5. The vanadyl (V^{4+}) induced inhibition of Na+K+Mg-ATPase and of Mg-ATPase measure in the presence of dithiothreitol. The dose–response curve of vanadyl inhibition of Na+K+Mg-ATPase and of ouabain-independent Mg-ATPase was measured in the presence of 10^{-4} mM dithiothreitol. ATPase activities were determined in microsomes as described in Methods and in the legend to Fig. 1. ●——● Na+K+Mg-ATPase, ■——■ Ouabain-independent Mg-ATPase.

Dithiothreitol proved to be most powerful antioxidant. The concentration of V^{4+} detected as peak amplitude of band M = −3/2 in the ESR spectra was quite stable up to the 30 min of incubation. It could therefore be concluded that dithiothreitol (1 mM) completely abolished the oxidation of V^{4+} to V^{5+} in ATPase reaction media (Figs. 4 D–F). The identical data was obtained even at lower DTT concentrations ($10^{-4} - 10^{-5}$ M) when using in equimolar ratios with V^{4+} (not shown).

Inhibition of Na,K-*ATPase by* V^{4+} *in the presence of DTT.* The vanadyl (V^{4+}) inhibition of Na,K-ATPase was verified in the presence of dithiothreitol. Na,K-ATPase was calculated from the data presented in Fig. 5 as the difference between Na+K+Mg-ATPase and the ouabain-independent Mg-ATPase. Dithiothreitol exhibited almost no effect on the control enzyme activities without V^{4+} (compare initial values in Figs. 5 and 1). Neither was V^{4+} inhibition of Na,K-ATPase activity affected by 10^{-4} M DTT. The +4 oxidation state of vanadium was therefore exclusively responsible for the inhibitory action on the brain Na,K-ATPase.

The effect of transferrin on the blocking potency of vanadium ions. The vanadium compounds may form stable complexes with proteins such as transferrin [22, 31], ferritin [32], calf intestinal alkaline phosphatase [33] and haemoglobin [12, 34]. The complexes of +4 oxidation state of vanadium are generally much stronger than of V^{5+}. The complexation of a given ion is usually associated with the elimination of its inhibitory effect on the Na,K-ATPase activity [12, 23, 35–38]. The vanadium com-

pounds were therefore shortly preincubated with Fe-free transferrin (Fig. 6) and the V^{4+} and V^{5+}-induced inhibition of Na,K-ATPase was measured. Transferrin without HCO_3^- was only partly effective in blocking the vanadyl action while with 2×10^{-3} M of bicarbonate [39] the effect was more pronounced and 85% of original activity was restored. The blockade of the enzyme by V^{5+} was not affected either by transferrin itself or when added together with a bicarbonate. Similar results (not shown) were obtained with myoglobin when it was added in equimolar concentrations with vanadium into the preincubation media.

Noradrenaline effect on V^{4+} *and* V^{5+}*-induced inhibition of the brain* Na,K-*ATPase.* The reversal of the V^{5+} inhibition of Na,K-ATPase was observed with noradrenaline and other catechol compounds through reduction and complex formation [2, 3, 30]. The influence of noradrenaline on the V^{4+}- and V^{5+}-induced inhibition of Na,K-ATPase was therefore measured and compared with the transferrin effect (see Fig. 7). The potency of noradrenaline to reverse the V^{4+} inhibition was much lower than in the case of transferrin—100 times excess was necessary to achieve the comparable (78%) recovery of enzyme activity. Noradrenaline was even less effective in reversing V^{5+} inhibition—at 100 times excess only 40% of Na,K-ATPase was protected against inhibition. This finding may be related to the results of Hudgins and Bond [40] who showed that catecholamines in a complex with V^{4+} may be oxidised and the products of this oxidation inhibited Na,K-ATPase. The complexation of vanadium ions with transferrin thus represents a preferential system to catecholamines as far as the protection of Na,K-ATPase activity is concerned.

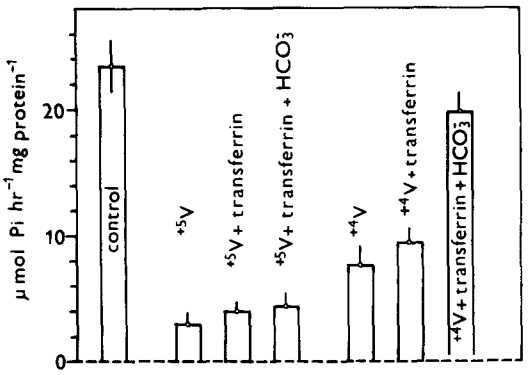


Fig. 6. Protection of vanadyl (V^{4+}) and vanadate (V^{5+})-induced inhibition of brain microsomal Na,K-ATPase by transferrin. Transferrin ± 2 mM bicarbonate (HCO_3^-) was mixed at 0° with 10^{-6} M V^{4+} or V^{5+} (1:1 molar ratio) in ATPase reaction media (see methods) approximately 10–15 min before the preincubation period which was started by addition of the enzyme (microsomes). After 5 min of preincubation at 37° the reaction was initiated by ATP. The Na,K-ATPase was determined and expressed as described in Materials and Methods. The results shown are the means ± S.E.M. of 4 experiments carried out in triplicates.

DISCUSSION

The data presented in this work indicate that vanadium of both redox species, V^{4+} as well as V^{5+}, inhibit the brain Na,K-ATPase with similar efficiency. Unlike vanadate, the vanadyl-induced inhibition of Na,K-ATPase activity may be reversed by preincubation with transferrin in equimolar concentrations or high excess of noradrenaline.

The inhibition of brain Na,K-ATPase by V^{4+} is the fact which differs from our previous finding on skeletal muscle [14], where only V^{5+} lowered the enzyme activity with $K_i = 10^{-6}$ M, but V^{4+} was ineffective. The concentration of V^{5+} needed for 50% inhibition of brain Na,K-ATPase also differs from the muscle preparation being approximately three times lower (3×10^{-7} M). This might also reflect the structural specificity of the brain enzyme.

The blocking effect of V^{4+} is somewhat surprising in the view of the general scheme of vanadium inhibition of Na,K-ATPase. In the model of Cantley *et al.* [14, 42] and of Ramasarma and Crane [1] the exclusive inhibitor of this enzyme is V^{5+} which is supposed to bind to the inner part of the plasma membrane. The binding is oriented probably to that part of the enzyme where phosphorylation and dephosphorylation take place. It is not quite reasonable to believe that positively charged vanadyl (VO^{2+}) and negative vanadate ions (VO_3^-) have an identical site of action. Rather, one can speculate, that in the brain Na,K-ATPase there are at least two different sites, one for the cationic and one for the anionic form of vanadium. In the muscle membrane or in some other tissues where V^{4+} is not effective, the V^{4+}- site of Na,K-ATPase is either absent or not accesible for V^{4+}.

This arrangement might create a more convenient

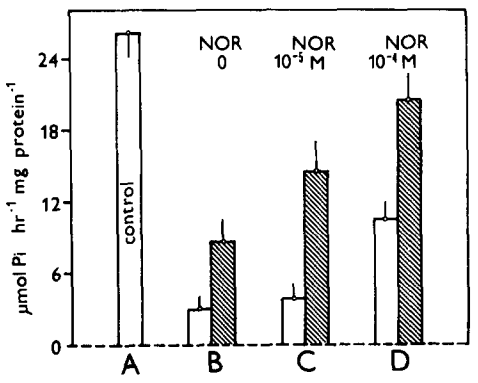


Fig. 7. The influence of noradrenaline on vanadyl (V^{4+}) and vanadate (V^{5+}) inhibition of brain microsomal Na,K-ATPase. Noradrenaline was mixed with vanadium compounds ($VOSO_4$, $NaVO_3$) in ATPase reaction media (see methods) approximately 10–15 min before the onset of 5 min preincubation period, which was started by addition of the enzyme (microsomes). The Na,K-ATPase was determined as described in Methods. The reaction mixture was shielded from the light during the whole assay. (A) Control Na,K-ATPase activity without any additions, (B) Na,K-ATPase measured in the presence of 10^{-6} M V^{5+} (□) or of 10^{-6} M V^{4+} (▨). (C) Na,K-ATPase measured in microsomes preincubated with 10^{-5} M noradrenaline and 10^{-6} M V^{5+} (□) or 10^{-6} M V^{4+} (▨). (D) Na,K-ATPase measured in microsomes preincubated with 10^{-4} M noradrenaline and with 10^{-6} M V^{5+} (□) or 10^{-6} M V^{4+} (▨). the data shown are the means ± S.E.M. of five experiments carried out in duplicates.

situation for the regulatory role of vanadium in the brain cell. After entering the cell via a more or less specific anion carrier [42], vanadium is reduced by NADH [25], glutathione and other intracellular reductants [34], but it may be again oxidised by mitochondria [43, 44]. The intracellular V^{+4} may be present in active (inhibitory) free form or inactivated by complexing with some intracellular.metal-binding proteins. High concentrated intracellular proteins may represent high-affinity binding sites which decrease the free V^{4+} concentration to zero and the inhibitory sites of V^{4+} on Na,K-ATPase remain unoccupied.

Our results indicating the loss of the inhibitory capacity of V^{4+} but not of V^{5+} in the presence of transferrin and myoglobin are in accordance with this general scheme. We also observed that bicarbonate is a necessary prerequisite for V^{4+} inactivation by transferrin [39]. Apparently, the anion neutralizes positive charges on proteins or creates a more convenient conformational orientation of the molecule for metal coordination [31]. Furthermore, V^{4+} ions may also compete with monovalent cations such as potassium for other intracellular binding sites. According to the recent data of Zemková [45], V^{4+} increases the intracellular concentrations of potassium in skeletal muscle cells incubated in a potassium free medium. This effect, which is similar to insulin, is associated with an increase of resting membrane potential.

In agreement with Josephson and Cantley [25] and Post *et al.* [14] and in contradiction to Wu and Phillis [29], the vanadate (V^{5+}) inhibition of Na,K-ATPase was not counteracted by EDTA (Fig. 2). The slight increase of Na,K-ATPase activities by EDTA as well as the parallel increase of basal and vanadium-inhibited enzyme levels in the results of Wu and Phillis [29] is not due to the removal of vanadate inhibition but most probably to removal of the endogenous cystosolic inhibitor which was described by Schaeffer [4, 5, 7]. This effect, of course, is quantitatively of more significance in the data of Wu and Phillis [29] where brain homogenates (with cytosol present) were used as an enzyme preparation. In the microsomal fraction used in this work (see Methods) only residual, small amounts of soluble inhibitor may be present.

In experiments with V^{4+}, a marked inhibitory effect was observed on the ouabain-independent Mg-ATPase which was decreased to 50% at 10^{-4} M of V^{4+}. The magnitude of the inhibition was independent of the presence or absence of dithiothreitol. In contrast to the results of Mishra *et al.* [46], we did not find any effect of V^{5+} on the activity of this enzyme. The role of Mg-ATPase in the plasma membrane is still obscure and the V^{4+} ions may therefore serve as an advantageous tool for elucidating the functional significance of this enzyme.

It has recently been reported that the activity of erythrocyte Na,K-ATPase is decreased in maniodepressive patients [47, 48] concomitantly with a 50% increase of vanadate plasma concentration (up to 3×10^{-4} M). The increased level of vanadium may be one of the ethiological factors causing the maniodepressive syndromes and some therapeutical approaches (lithium, ascorbate, antidepressive drugs) were effective in re-establishing the normal Na,K-ATPase activity as well as in treating the disease [49, 17].

The antidepressive drugs imipramine and chlorpromazine can reduce V^{5+} to V^{4+} [15]. Provided the +4 oxidation state of vanadium in non-inhibitory, this drug-induced reduction could really represent the *in vivo* mechanism for removal of Na,K-ATPase inhibition. However, the data presented in this paper exclude this simple mechanism, because the brain vanadium is an effective inhibitor of Na,K-ATPase in both oxidised and reduced forms. The situation is probably more complicated and the particular influence of vanadium on the activity of this enzyme is modulated by the actual redox state of the cell interior and by the ability of proteins to bind the various species of this element.

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