EFFECTS OF POTASSIUM ON VANADATE INHIBITION OF SARCOPLASMIC RETICULUM Ca^{2+}-ATPase FROM DOG CARDIAC AND RABBIT SKELETAL MUSCLE

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SUMMARY: The concentration of vanadate for half maximal inhibition of dog cardiac and rabbit skeletal SR Ca^{2+}-ATPase was approximately 5 μM. Preincubation of the enzyme with vanadate resulted in greater inhibition. Effects of potassium on the inhibition were studied under various conditions.

INTRODUCTION

Recently, a potent Na^+,K^+-ATPase inhibitor copurified with ATP from equine and rabbit muscle was found (1) and identified as vanadate (vanadium oxo-anion in the V^{+5} oxidation state) (2). The finding has stimulated a considerable amount of research with respect to the possible role that vanadate may play as an _in vivo_ counterpart of ouabain, a positive inotropic agent that inhibits Na^+,K^+-ATPase. In fact, studies on papillary muscle from cat (3), ventricular strips from cat, rat, rabbit, and guinea pig and atrial muscle from rat and rabbit (4) have shown that vanadate produces a positive inotropic effect similar to that of ouabain. In contrast, however, vanadate has a negative inotropic effect on the atria of cat and guinea pig (4). It is pertinent to note that the pharmacological influence of vanadium on the contractile state of myocardium were first observed nearly seventy years ago (5). Vanadate inhibition of isolated membrane-bound Na^+,K^+-ATPase has been investigated in detail (1,2,6,7).

The high potency of vanadate in inhibiting Na^+,K^+-ATPase (Ki 10^{-2} to 10^{-1} μM) (1,2), presumably functioning as a transition-state analog of phosphate, and its interesting effects on the contractile state of

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myocardium (3-5) prompted us to study the effects of vanadate on sarcoplasmic reticulum, the membrane system associated with relaxation of muscle. Furthermore, the SR Ca^{2+}-ATPase is very similar to the Na^+, K^+-ATPase in its reaction mechanism (8). We report in this communication that, unlike previous suggestions of the specificity of vanadate inhibition of Na^+,K^+-ATPase (1), vanadate at μM concentrations inhibits SR Ca^{2+}-ATPase. Potassium enhances the vanadate inhibition. The effects were studied with and without pre-incubation of the enzyme with vanadate.

MATERIALS AND METHODS

Disodium ATP was purchased from Boehringer Manheim and sodium vanadate was from Fisher Scientific. A formula of $Na_3VO_4 4H_2O$ was used in computation of the vanadate concentration, and the solution was used within one week after preparation. Other chemicals were of reagent grade.

SR vesicles from dog cardiac and rabbit skeletal muscle were prepared as previously described (9) with a slight modification. An additional centrifugation at 143,000 x g in the final stage improved the stability of the SR preparations with less than 10% loss in yield. The enzyme was stored on ice in solution containing either 10 mM Tris/maleate plus 100 mM K^+ or 30 mM Tris/maleate without K^+(pH 6.8) and was used within 24 hours.

SR Ca^{2+}-ATPase activity was measured at 37°C in an incubation shaker. The assay medium was 1 ml, consisting of 25 mM histidine (pH 7.1), 5 mM ATP, 5 mM $MgCl_2$, 5 mM NaN_3, 100 μM EGTA - 100 μM $CaCl_2$ (Ca^{2+}, 14 μM (10)) with or without 100 mM KCl and 5 mM oxalate. In general, 50 μg/ml cardiac SR or 20 μg/ml skeletal SR vesicles were incubated with chosen ligands and reagents for 8 minutes before adding ATP (with or without vanadate and/or calcium) to initiate the reaction. The reaction was allowed to proceed for 8 minutes and was quenched with 10% cold trichloroacetic acid. The phosphate formed was assayed against a standard, using a fresh solution of ammonium molybdate (1.25%) and ferrous sulfate (50 mg/ml) in 1 N H_2SO_4(11). The specific Ca^{2+}-ATPase activity was the total ATPase activity less basal Mg^{2+}-ATPase obtained without added $CaCl_2$ in the assay medium.

RESULTS AND DISCUSSION

The effects of K^+ on the vanadate inhibition of cardiac and skeletal SR Ca^{2+}-ATPase activity are significant under various ligand conditions. As shown in Figures 1A and 1B, when the SR was preincubated with vanadate, K^+ enhances the inhibition, reducing the vanadate concentration for 50% inhibition (I_{50}) from 50 μM to 5 μM in both cardiac and skeletal SR. In the presence of 100 mM K^+, oxalate at 5 mM does not affect the vanadate inhibition; however, when the K^+ is absent, oxalate enhances the inhibition.

The effects of pre-incubation of the enzyme with vanadate was examined under the same ligand conditions. In the presence of K^{+}, normal inhibition curves were observed and I_{50} of vanadate (which was added with ATP to initiate the reaction) increased to 30-50 μM for both cardiac and skeletal SR; whereas in the absence of K^{+}, the effectiveness of vanadate (over the same reaction period) was greatly reduced. For cardiac SR, the I_{50} was higher and, in some experiments, <u>activation</u> occured at higher than 50 μM vanadate.

Without pre-incubation of the enzyme with vanadate, the effects of oxalate on the vanadate inhibition are very different from those observed under the pre-incubation conditions. As shown in Figures 2A and 2B, oxalate greatly reduces the effectiveness of vanadate inhibition of cardiac SR Ca^{2+}-ATPase in the presence or absence of 100 mM K^{+}. However, the effects of oxalate diminishes when Ca^{2+} is omitted from the SR Pre-incubation medium (Ca^{2+} is added with vanadate and ATP to initiate the reaction). In contrast, for skeletal SR Ca^{2+}-ATPase, marked differences in the effects of non-preincubation of the enzyme with vanadate was observed. In the absence of K^{+}, oxalate enhances the vanadate inhibition ($I_{50} \approx 100$ μM) of a SR preparation on which vanadate had no effect in the absence of oxalate. Without Ca^{2+} in the incubation medium, small but significant inhibition by vanadate (20% inhibition at 150 μM) is observed. Oxalate also enhances the inhibition, but to a smaller extent (35% inhibition at 150 μM) compared to the degree of enhancement with the enzyme pre-incubated with Ca^{2+}.

The previous claim of the specificity of vanadate inhibition of Na^{+},K^{+}-ATPase (1,2) is justified only on the basis of the exceptional <u>effectiveness</u> (i.e. high affinity, Ki 10^{-2} to 10^{-1} μM) of vanadate in inhibiting the enzyme. As we have shown vanadate inhibits dog cardiac and rabbit skeletal SR Ca^{2+}-ATPase, and the inhibition can occur with I_{50} of 5 μM. Vanadate inhibition of skeletal SR Ca^{2+}-ATPase and other membrane ATPases has also recently been reported (12).

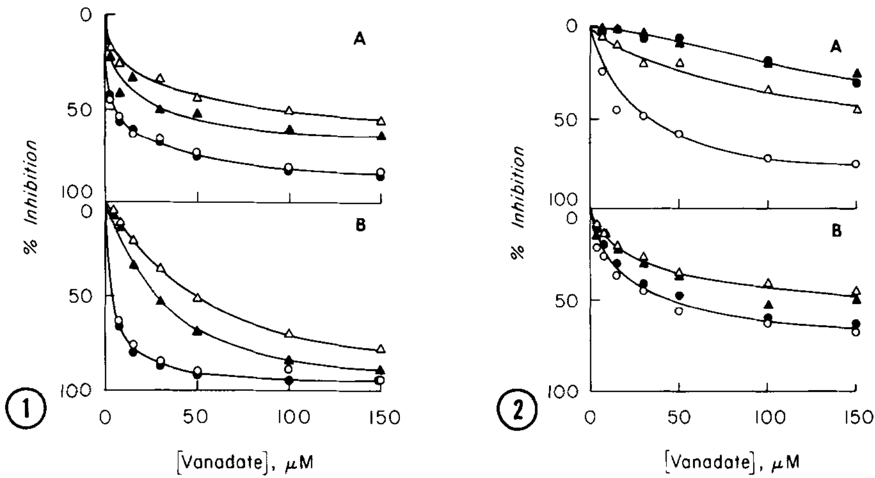

Figure 1. Effects of K^+ on vanadate inhibition of SR Ca^{2+}-ATPase in the presence and absence of oxalate. The enzyme was pre-incubated with vanadate for 8 minutes at 37°C in 25 mM histidine buffer solution (pH 7.1) consisting of 5 mM $MgCl_2$, 5 mM NaN_3, 100 μM EGTA, 100 μM $CaCl_2$. ATP (final concentration 5 mM) was added to initiate the reaction. Time for reaction, 8 minutes. Other ligands are: •, 100 mM K^+, 5 mM oxalate; o, 100 mM K^+, no oxalate; ▲, no K^+, 5 mM oxalate; Δ, no K^+, no oxalate. Experiments were done in duplicates. (A) Dog cardiac SR Ca^{2+}-ATPase; (B) Rabbit skeletal SR Ca^{2+}-ATPase.

Figure 2. Vanadate inhibition of cardiac SR Ca^{2+}-ATPase in the presence and absence of K^+ and oxalate. The enzyme was not pre-incubated with vanadate. (A) The enzyme was pre-incubated for 8 minutes in 25 mM histidine buffer solution (pH 7.1) consisting of 5 mM $MgCl_2$, 5 mM NaN_3, 100 μM EGTA, and 100 μM $CaCl_2$. A mixture of ATP and vanadate was added to initiate the reaction. Time for reaction, 8 minutes. Other ligands are: •, 100 mM K^+, 5 mM oxalate; o, 100 mM K^+, no oxalate; ▲, no K^+, 5 mM oxalate; Δ, no K^+, no oxalate. Experiments were done in duplicates. (B) The enzyme was pre-incubated in the medium without Ca^{2+}. A mixture of ATP, vanadate, and Ca^{2+} was added to initiate the reaction. Other reaction conditions and symbols are the same as in (A).

Pre-incubation of SR Ca^{2+}-ATPase with vanadate enhances considerably the effectiveness of vanadate. Such conditions for the inhibition indicate that vanadate binding to the enzyme is slow. Therefore, the effects of various ligands and reaction conditions used in the present work are at a non-equilibrium state of vanadate binding to the enzyme.

Potassium in the medium facilitates the binding of vanadate, whether or not the enzyme is incubated with vanadate for an additional time before Ca^{2+}-ATPase reaction takes place. Additional effects of ATP (5 mM) and Ca^{2+} (14 μM) under different incubation conditions are relatively small.

In both cardiac and skeletal SR, when the enzyme is pre-incubated with vanadate, oxalate (5 mM) appears to enhance the vanadate binding in the absence of potassium. One explanation of this is that Ca^{2+} at 14 μM protects the enzyme against vanadate inhibition; a lowering of the calcium concentration due to passive inward leakage effected by oxalate during pre-incubation period would therefore enhance the vanadate binding. In the presence of potassium no such effect of oxalate was observed, presumably because the effect of potassium at 100 mM is maximum.

The combined effects of Ca^{2+} and oxalate are further shown by the results that, when the enzyme is not pre-incubated with vanadate, oxalate reduces the effectiveness of vanadate inhibition even in the presence of 100 mM potassium. The drastic decrease in the potassium enhancement of vanadate inhibition may be a result of effective competition of ATP with vanadate for the enzyme at reduced calcium concentration. When the enzyme is pre-incubated with oxalate and calcium is added with vanadate and ATP, there is no such effect of ATP and the enhancing effects of potassium on the vanadate inhibition are predominant and similar in the presence and absence of oxalate.

In this regard, skeletal SR Ca^{2+}-ATPase behaves differently from cardiac SR CA^{2+}-ATPase, probably because of different requirements for calcium.

During the study we found that vanadate inhibition of SR varied in the presence or absence of potassium and oxalate. this is largely due to the freshness of the enzyme and various times of pre-incubation with vanadate.These conditions might explain the lack of effect reported previously (1,2).

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