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VANADYL- AND VANADATE-INDUCED LIPID PEROXIDATION IN MITOCHONDRIA AND IN PHOSPHATIDYLCHOLINE SUSPENSIONS

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Vanadyl ($V_{(IV)}$) was found to induce rapidly developing lipid peroxidation in intact and sonicated mitochondria as well as in phosphatidylcholine suspension. The ability of vanadate ($V_{(V)}$) to induce lipid peroxidation was much less pronounced compared to that of vanadyl. The peroxidative action of vanadate on phosphatidylcholine much increased in the presence of NADH and ascorbate. Preincubation of vanadate with glucose had the same effect.

Vanadyl-induced lipid peroxidation was not essentially influenced by SOD, catalase and ethanol but was completely inhibited by butylated hydroxytoluene.

All these effects of vanadyl and vanadate are thought to participate in the insulin-like and other biological actions of vanadium.

KEY WORDS: Vanadyl, vanadate, lipid peroxidation, mitochondria, phosphatidylcholine.

INTRODUCTION

In an earlier work[1] we have suggested that some of the biological effects of vanadium such as insulin-like activity,[2–4] inhibition of lipid[5] and cholesterol[5,6] biosynthesis, are at least partly due to the vanadyl ($V_{(IV)}$) – and vanadate ($V_{(V)}$) – catalyzed superoxide-dependent oxidation of NADPH and NADH. The mechanism of the vanadate- and vanadyl-dependent NAD(P)H oxidation in the presence and in the absence of biological and chemical generators of O_2^- and H_2O_2 has been studied in detail.[7–9]

There is a large body of research indicating the ability of the ions of transition metals (iron, copper, etc.) to catalyze lipid peroxidation (LPO) or other processes in which active oxygen species (AOS) are involved.[10] Little is, however, known about the ability of vanadium ions to catalyze LPO *in vivo* and *in vitro*.[11–16] Further investigations on the prooxidant capacity of vanadium would also contribute to the elucidation of the role of the free radical processes in the biological effects of this transition metal.

The present work was undertaken to study the ability of vanadate and vanadyl to induce LPO in intact and sonicated mitochondria as well as in phospholipid suspension. The participation of AOS in vanadyl-induced LPO and the effects of some biological substances (NADH, ascorbate and glucose) on vanadate-induced LPO were also examined.

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MATERIALS AND METHODS

Ammonium metavanadate and phosphatidylcholine (from Soy Beans) were purchased from Sigma Chemical Company, vanadyl sulfate from Aldrich Chemical Company, Inc., NADH and catalase from Boehringer Mannheim GmbH, butylated hydroxytoluene (BHT) from Fluka. Cu, Zn-SOD was a generous gift from Dr. I. Fridovich (Department of Biochemistry, Duke University Medical Center, Durham, N.C., U.S.A.). All other reagents were of analytical grade.

Mitochondria from the livers of male Wistar rats were isolated after centrifugation of a 10% liver homogenate in 0.25 M sucrose at 6500 g for 15 min and two-fold washing of the resulting pellet with 0.15 M KCl.

Sonicated mitochondria were prepared by treating mitochondria (10 mg protein/ml) suspended in 50 mM K–PO_4 buffer, pH 7.4 with a MSE 150 W ultrasonicator for 2 min (4 × 30 s with 30-s intervals). A phospholipid suspension was obtained by treating phosphatidylcholine (PC) suspended in 50 mM K–PO_4 buffer, pH 7.4 (5 mg/ml) with a MSE 150 W ultrasonicator for 2 min (4 × 30 s with 30-s intervals).

Incubation with vanadyl and vanadate of intact mitochondria suspended in 0.15 M KCl–10 mM Hepes, pH 7.4 (0.2 mg/ml) and of sonicated mitochondria and PC suspended in 50 mM K–PO_4, pH 7.4 (0.2 and 0.5 mg/ml, respectively) was carried out in a waterbath shaker at 37°C.

Vanadyl- and vanadate-induced LPO was measured by the formed TBA-reactive material in aliquots of the incubation mixture against controls without vanadium salts according to Buege and Aust.[17] No TBA-reactive material was detected in controls in the absence of lipid substrate.

Protein content was measured by the method of Lowry *et al.*[18]

RESULTS

Both vanadate and vanadyl induced lipid peroxidation (LPO) in intact mitochondria, but the effect of vanadyl was much more pronounced than that of vanadate (Figure 1A). The vanadyl and vanadate effects might depend on the membrane structure and composition, on the membrane permeability for vanadyl and vanadate as well as on the influence of some intramitochondrial components. To further elucidate the role of these factors we studied the ability of vanadyl and vanadate to catalyze LPO not only in intact mitochondria but also in sonicated mitochondria and phosphalidylcholine (PC) suspensions. It is well known that after sonication of mitochondria the resulting mitochondrial particles have inverted membranes. The use of PC suspensions allows for estimating only the effects of vanadyl and vanadate that depend on their direct interaction with phospholipids.

From Figure 1B it is seen that the effect of vanadyl in sonicated mitochondria was more pronounced compared to that of vanadate as was the case of intact mitochondria. The amount of TBA-reactive material formed after vanadyl- or vanadate treatment of intact mitochondria was higher than that in sonicated mitochondria (Figure 1A, B). This suggests that the effects of vanadyl and of vanadate depend on the membrane organization and/or on the interaction of vanadyl and vanadate with intramitochondrial components.

Both vanadyl and vanadate catalyzed LPO also in phospholipid suspension in a dose- and time-dependent manner (Figure 1C). Here again the effect of vanadyl was

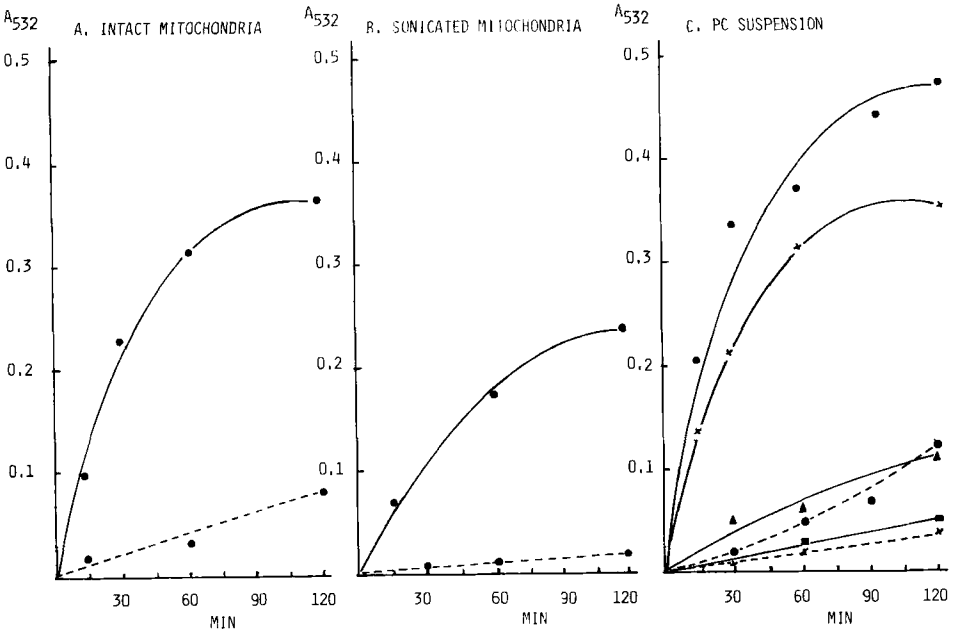

FIGURE 1 Lipid perodixation induced by vanadyl and vanadate. The incubation was carried out with 500 μM (-●---●-); 100 μM (-×---×-) vanadate or with 500 μM (-●—●-); 100 μM (-×—×-); 50 μM (-▲—▲-); 10 μM (-■—■-) vanadyl.

much more pronounced than that of vanadate. The strong prooxidant effect of vanadyl on PC suspension was proved also polarographically by O_2 consumption (data not shown). The decrease in the rate of the vanadyl-induced LPO in time (Figure 1) could be explained by the decrease in the vanadyl concentration due to its oxidation and/or by the exhaustion of unsaturated fatty acids available to oxidation. The antioxidant BHT completely inhibited the vanadyl-induced LPO, while catalase had no effect. SOD and ethanol inhibited but insignificantly this process (Figure 2), suggesting that neither O_2^- nor $^{\cdot}OH$ play much role in the vanadyl-induced LPO. The larger part of the vanadyl effect on PC suspension is probably due to a free radical process that is not dependent on O_2^- or $^{\cdot}OH$. The effects of BHT and AOS scavengers on the vanadyl-induced LPO were also studied in sonicated mitochondria. The results obtained (not shown) were similar to those with PC suspension: BHT completely prevented LPO, while SOD, catalase and ethanol were ineffective. This suggests one and the same mechanism underlying the vanadyl-induced LPO in submitochondrial particles and phospholipid suspension. The lack of SOD effect on LPO in sonicated mitochondria may be due to endogenous SOD.

As was already mentioned the stronger vanadate effect on intact mitochondria as compared to that on sonicated mitochondria might be due to interactions (most probably reduction) of vanadate with intramitochondrial components. Thus we studied the influence of some biologically important substances on the vanadate-dependent LPO in PC suspension. Figure 3B shows the ascorbate, which by itself induces LPO, greatly increased the vanadate induced LPO. The synergism between ascorbate and vanadate was observed only in the earlier period (15 min) of incubation

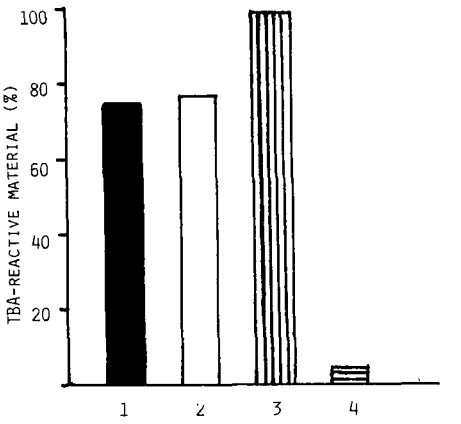

FIGURE 2 Effects of SOD, ethanol, catalase and BHT on vanadyl-induced peroxidation of phosphatidylcholine suspension. Peroxidation was induced by 100 μM vanadyl and was measured at 120 min of the incubation in the presence of: 1 – 5 μg SOD/ml; 2 – 1% ethanol; 3 – 5 μg catalase/ml; 4 – 0.5 mM BHT (ethanol solution).

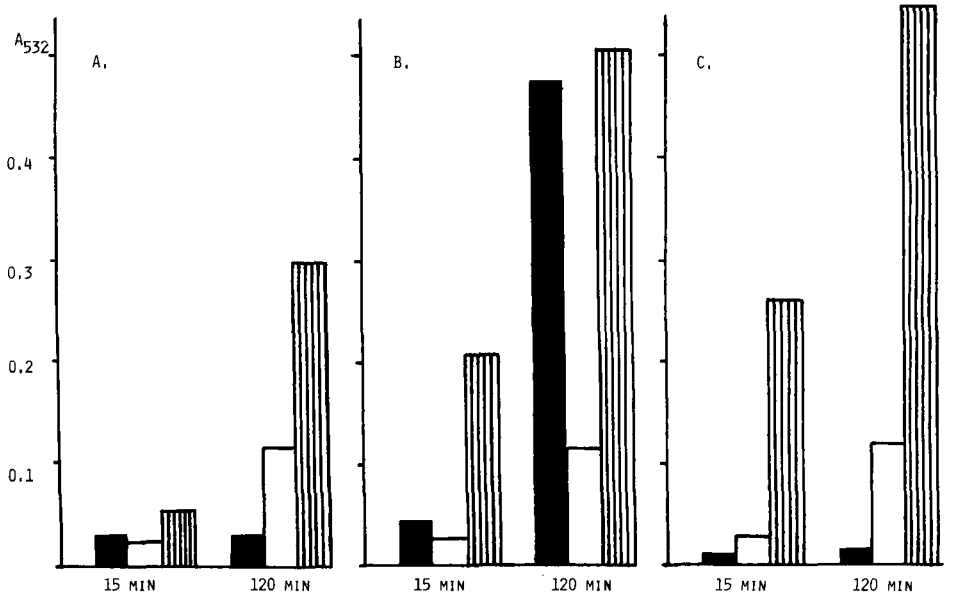

FIGURE 3 Stimulation of vanadate-induced phosphatidylcholine peroxidation by NADH, ascorbate and glucose. PC suspension was incubated in the presence of 0.5 mM vanadate (open bars); 0.5 mM NADH (A), 0.5 mM ascorbate (B), 5 mM glucose (C) – (dark bars); vanadate + NADH (A), vanadate + ascorbate (B), vanadate, preincubated with glucose (C)* – (hatched bars). The formed TBA-reactive material was measured at 15 and 120 min of incubation. *Equal volumes of 0.5 M glucose and 50 mM vanadate, both in water, were mixed and preincubated for 24 hs at room temperature.

before the appearance of the maximal prooxidant effect of ascorbate. This synergism is probably due to the vanadyl generation resulting from the reduction of vanadate by ascorbate.

Preincubation of vanadate with glucose for one night at room temperature led to the disappearance of the yellow colour of vanadate and to the development of blue-green colour, showing the reduction of vanadate to vanadyl. After incubation of PC suspension with this mixture the amount of TBA-reactive material exceeded the total amount of TBA-reactive material formed after incubation either with vanadate or with glucose (Figure 3C). No TBA-reactive material was formed in the absence of PC.

NADH also greatly increased the prooxidant effect of vanadate (Figure 3A). Our earlier data suggested that the slight NADH oxidation in the presence of vanadate is mainly due to a O_2^- -dependent chain process and a direct reduction of vanadate has not been excluded.[7,8] Innoye *et al.*[11] have observed synergism between O_2^- and vanadate with respect to their ability to catalyze LPO in cell organelles. Thus it is reasonable to believe that the observed increase in the effect of vanadate in the presence of NADH is due both to the reduction of vanadate to vanadyl and to the participation of O_2^-.

DISCUSSION

The present results showed that the effect of vanadyl was much more pronounced than that of vanadate in all three model systems studied. The ability of vanadyl to catalyze LPO was not affected by AOS scavengers, which suggested that its effect was not mediated by AOS. Mitochondrial membranes, isolated even in the presence of BHT, contain some hydroperoxides.[19,20] It is also known that vanadyl is rapidly oxidized by H_2O_2 and we found that it was also oxidized by organic peroxides (data not shown). According to up-to-date views the ions of transition metals in their lower oxidation state catalyze LPO mainly by the following reactions:

$$Me^{n+} + ROOH \longrightarrow Me^{(n+1)+} + RO^{\cdot} + OH^-$$

$$RO^{\cdot} + RH \longrightarrow ROH + R^{\cdot}$$

$$R^{\cdot} + O_2 \longrightarrow RO_2^{\cdot}$$

$$RO_2^{\cdot} + RH \longrightarrow ROOH + R^{\cdot}$$

Based on these reactions and on the present results, we suggest that vanadyl catalyzes LPO reacting directly with lipid peroxides. Previously, we have discussed that trace metals could influence the vanadyl-catalyzed NADH oxidation.[9] There are also data that some metals, such as Al^{3+} potentiate the iron-dependent LPO not being active themselves.[21] The observed ascorbate-induced LPO in the present work suggests the presence of trace iron and/or other metal ions in the reaction mixtures used. Thus, the possibility that the vanadyl-catalyzed LPO could be influenced by other metal ions should be considered, too.

Stacey and Klaasen[15] have found that sodium vanadate produces large amounts of TBA reactants in isolated hepatocytes. We, however, observed that the ability of vanadate to catalyze LPO was much lower than that of vanadyl, but it sharply

increased in the presence of some biologically important substances reducing vanadate. Reduction of vanadate to vanadyl after vanadate uptake in the cell has been reported.[3,22] All this led us to believe that the *in vitro* ability of vanadate to catalyze LPO is of no importance for the cell where the vanadium-induced LPO is due to vanadyl.

We suggest that the vanadyl-induced LPO is associated with some of the observed vanadium effects, e.g. with changes in the membrane structure and functions (for references see the review of Boyd and Kustin).[23]

LPO induced directly by vanadyl would lead to the oxidation of NADPH to $NADP^+$ as a result of the action of the coupled enzymes glutathione peroxidase and glutathione reductase. Since the hexose monophosphate shunt is limited mainly by the availability of $NADP^+$, the elevated $NADP^+$ level would increase the direct oxidative degradation of glucose. Thus we think that the vanadate- and vanadyl-catalyzed O_2^--dependent NAD(P)H oxidation[8,9] as well as the ability of vanadyl to catalyze LPO could account for a part of the insulin-like effect of vanadium. It should be mentioned that the ions of copper, another transition metal, which induce LPO in mitochondria[24] and in PC suspension[25] also have an insulin-like effect.[26] Having regard to the fact that NADPH is necessary for lipid and cholesterol biosynthesis and that vanadate inhibits both processes,[5,6] the vanadate-dependent decrease in the $NADPH/NADP^+$ ratio could partly determine these biological effects of vanadium, too.

Acknowledgement

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