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Iron uncouples oxidative phosphorylation in brain mitochondria isolated from vitamin E-deficient rats

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Abstract

Few, if any, studies have examined the effect of vitamin E deficiency on brain mitochondrial oxidative phosphorylation. The latter was studied using brain mitochondria isolated from control and vitamin E-deficient rats (13 months of deficiency) after exposure to iron, an inducer of oxidative stress. Mitochondria were treated with iron (2 to 50 μM) added as ferrous ammonium sulfate. Rates of state 3 and state 4 respiration, respiratory control ratios, and ADP/O ratios were not affected by vitamin E deficiency alone. However, iron uncoupled oxidative phosphorylation in vitamin E-deficient mitochondria, but not in controls. In vitamin E-deficient mitochondria, iron decreased ADP/O ratios and markedly stimulated state 4 respiration; iron had only a modest effect on these parameters in control mitochondria. Thus, vitamin E may have an important role in sustaining oxidative phosphorylation. Low concentrations of iron (2 to 5 μM) oxidized mitochondrial tocopherol that exists in two pools. The release of iron in brain may impair oxidative phosphorylation, which would be exacerbated by vitamin E deficiency. The results are important for understanding the pathogenesis of human brain disorders known to be associated with abnormalities in mitochondrial function as well as iron homeostasis (e.g., Parkinson's disease).

Keywords: Vitamin E; Iron; Tocopherol; Mitochondria; Uncoupling; Deficiency

1. Introduction

Vitamin E is the main chain-breaking antioxidant in most, if not all, biological membranes. Therefore, it is not surprising that a deficiency of vitamin E (mainly alpha-tocopherol in animal experiments) results in a number of pathological manifestations such as reproductive failure in both male and female rats, liver necrosis in rats and pigs, erythrocyte hemolysis, anemia, exudative diathesis, encephalomalacia, kidney degeneration, and nutritional muscular dystrophy in different species of animals [1]. Even though a deficiency of this nutrient seems to affect a number of systems, it is likely that alpha-tocopherol plays a critical role in the functioning of specific subcellular organelles or enzymatic systems. One possibility is that the antioxidant and/or another biological activity of alpha-tocopherol is especially important for maintenance of mitochondrial function. Corwin [2] reviewed some of the early observations on the effect of vitamin E deficiency on mitochondria and noted that skeletal muscle from vitamin E-deficient animals showed enhanced respiration whereas liver slices were characterized by a decline in respiration. Schwarz [3] found that liver homogenates from vitamin E-deficient rats could not maintain normal oxygen consumption upon in vitro incubation for a period of time. This phenomenon was termed respiratory decline and could be prevented by administration of alpha-tocopherol. However, as pointed out by Corwin [2], the defective respiration (respiratory

Abbreviations: RCR, respiratory control ratio (ratio of state 3 to state 4 respiration of mitochondria); State 3, rate of in vitro respiration (oxygen consumption) of mitochondria upon stimulation with ADP (active or stimulated respiration); State 4, rate of in vitro respiration (oxygen consumption) of mitochondria under basal conditions (basal or controlled respiration)

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decline) could be observed only after the tissue homogenates had been incubated in vitro for a few hours suggesting that this may be a secondary effect. Since sulfhydryl groups were lost during respiratory decline, the suggestion was that alpha-tocopherol may be protecting critical enzyme sulfhydryl groups [2]. In addition, morphological alterations in the mitochondria from gracile and cuneate nuclei were reported by Schochet [4] a number of years ago. Vitamin E-deficient mitochondria were enlarged and pleomorphic; they underwent a characteristic non-autophagic dissolution leading to accumulation of masses of granular dense material.

Several investigations have examined the role of alpha-tocopherol as an antioxidant in the mitochondrial membranes. The interaction between the antioxidant activities of ubiquinol and alpha-tocopherol in mitochondrial membranes has been the subject of several studies. Ernster et al. [5] observed that ubiquinol acted as a powerful antioxidant in submitochondrial particles from which tocopherol had been extracted by treatment with pentane. However, Kagan et al. [6] proposed that the antioxidant activity of ubiquinol is mediated by its ability to regenerate alpha-tocopherol from the alpha tocopheroxyl radical [7,8]. Maguire et al. [9] demonstrated that tocopheroxyl radical can be reduced back to tocopherol by the respiratory chain. They showed that NADH, succinate and reduced cytochrome *c* linked oxidation were able to prevent the accumulation of tocopheroxyl radical and the consumption of alpha-tocopherol. In short, these reports suggest that the oxidative consumption of alpha-tocopherol is controlled in a complex manner by various factors including the levels of reduced ubiquinone and the level of reduction of the respiratory chain.

Few reports address the changes in tissue mitochondrial oxygen consumption in vitamin E deficiency. For example, Paraidathathu et al. [10] found that mitochondria from the hearts of vitamin E-deficient and normal rats showed similar respiratory control ratios (the ratio between oxygen consumption under basal and ADP-induced conditions i.e., state 4 and 3 rates). This is in contrast to the work of Ferrari et al. [11] who found that heart mitochondria from rabbits fed a vitamin E-deficient diet had lower RCR than control mitochondria. Thus, there are conflicting data on the effects of vitamin E deficiency upon mitochondrial oxygen consumption even among the limited number of publications in the literature. Furthermore, brain mitochondria were not studied in any of these investigations. Since brain is a critical target of abnormal vitamin E status in animals and man (see reviews by Vatassery et al. [12,13]), it is important to understand the effect of vitamin E deficiency upon brain mitochondria and this is one of the goals of this report.

Oxidative stress can be induced in mitochondrial membranes by a number of agents including metal ions such as iron. Furthermore, abnormalities in iron metabolism have been reported in many human brain disorders such as Alzheimer's disease, Parkinson's disease and multiple sclerosis (see review by Connor and Benkovic [14]). The molecular mechanisms and proteins involved in processing iron in cells have been studied extensively (see reviews by Hill [15] and Eisenstein [16]). Among the many proteins involved with iron metabolism, frataxin is of special interest. It is a mitochondrial protein taking part in iron homeostasis in the mitochondria and abnormal levels of this protein is considered to be one of the major causes of pathology in Friedrich's ataxia [17]. In fact, Rustin et al. [18] have considered Friedrich's ataxia to be the most common human mitochondrial disorder with mitochondrial overloading of iron accompanied by deficiency of aconitase and reduced respiration. It is noteworthy that Friedrich's ataxia is clinically very similar to inherited ataxia with vitamin E deficiency [19]. In order to understand the influence of antioxidants and iron in the pathogenesis of disorders such as Friedrich's ataxia and Alzheimer's disease, we need to understand more fully the influence of iron upon mitochondrial metabolism. Little, if any, information is available on the influence of iron on parameters of mitochondrial function such as respiration under vitamin E-deficient conditions. Therefore, we have investigated the effect of iron on the function of brain mitochondria from control and vitamin E-deficient animals.

2. Materials and methods

2.1. Chemicals

The chemicals used were of reagent grade purity from standard sources. Solvents for chromatography were HPLC grade from Burdick and Jackson Laboratories, Inc., Muskegon, MI, USA. Other special chemicals were purchased from the following sources: α-tocopherol and α-tocopherolquinone from Kodak Laboratory Chemicals, Rochester, NY, USA; Ficoll (Type 400) from Sigma Chemicals, St. Louis, MO, USA. Absolute ethanol was obtained from Midwest Solvents Company, Pekin, IL, USA and was redistilled prior to use. Most of the other reagent grade chemicals including tris-ADP (equine muscle), L-glutamic acid and L-malic acid were from Sigma.

2.2. Treatment of animals

All procedures with animals were approved by the Subcommittee on Animal Studies of the Minneapolis Veterans Affairs Medical Center. Male Fischer 344 rats were obtained at weanling. These animals were placed on control or vitamin E-deficient diet for a period of 13 months. The diets were custom manufactured by Dyets Inc., Bethlehem, PA 18017 (catalog numbers 119614 and 119615) according to the specifications of AIN 93 purified diets for laboratory rodents [20] and slightly modified for production of vitamin E-deficient formulations. The lipid source was tocopherol-stripped lard (10%). The control diet was obtained by the

addition of 75 IU of vitamin E as alpha-tocopherol acetate per kilogram of diet.

The animals were sacrificed by decapitation and the cerebral hemispheres were quickly dissected out of two animals; the tissues were pooled and rapidly rinsed using isotonic saline buffer. These samples were used for isolation of mitochondria as described below.

2.3. Preparation of mitochondria

The subcellular fractions were isolated from cerebral hemispheres by standard centrifugation methods [21]. The Ficoll solution used was purified by dialyzing a 40% (w/v) aqueous solution against nanopure (Millipore system) water for 3 h. The final Ficoll concentration was estimated by using a graph relating density and concentration. Using a glass-glass homogenizer, rat brain hemisphere tissue was homogenized in 10 volumes of ice-cold isolation medium containing 0.3 M sucrose, 5 mM HEPES, 5 mM KH_2PO_4, 1 mM EGTA and 0.1% (w/v) fatty acid-free bovine serum albumin adjusted to pH 7.4 with KOH. The homogenate was centrifuged at 1300×*g* for 3 min and the supernatant saved. The pellet was resuspended in 10 ml of the isolation medium, rehomogenized and centrifuged at 1300×*g* for 3 min. The pooled supernatants were centrifuged at 17,000×*g* for 10 min to get the crude mitochondrial fraction. The resulting pellet was resuspended in 8 ml of isolation medium. This suspension was layered over 11 ml of 7.5% (w/v) Ficoll medium which had been layered over 11 ml of 10% (w/v) Ficoll medium. The tubes were centrifuged in a Beckman SW 28 rotor at 99,000×*g* for 45 min. The mitochondria that sedimented at the bottom were removed, diluted 1:5 with isolation medium and centrifuged for 10 min at 17,000×*g*. The mitochondria were washed once with isolation medium. The purity of mitochondria was checked by assaying for the marker enzyme succinate dehydrogenase [22], which was enriched eight- to tenfold in the mitochondria compared with crude homogenate.

2.4. Studies of mitochondrial respiration and sampling for assays of alpha-tocopherol

Mitochondrial respiration was followed using the biological oxygen monitor (Model 5300, YSI Company, Yellow Springs, OH, USA). The system had a Clark electrode and a microcell with a capacity of 0.6 ml. The mitochondria were added to buffer (isolation medium without bovine serum albumin and with only 50 μM EGTA) with substrate (10 mM glutamate and 2.5 mM malate or 5 mM succinate) in the cell kept at 28–29 °C at a final protein concentration of 400 μg/ml. Oxygen consumption was followed for 5 min prior to the addition of ADP. Whenever ferrous iron solutions were added, the mitochondria were incubated for 3 min in the presence of iron. The oxygen consumption was traced on a strip chart recorder and the state 3 and state 4 respiratory rates were determined. The respiratory control ratios (state 3 rate/state 4 rate) (RCR) were also calculated. Whenever iron was added, there is a slight increase in oxygen consumption probably due to lipid peroxidation. However, stable state 4 levels were established after the equilibration time. The rate of phosphorylation was calculated in nanomoles of ADP phosphorylated per minute per milligram of mitochondrial protein. After two cycles of ADP-induced respiration, the mitochondria were removed and stored for assays of alpha-tocopherol.

2.5. Determination of tocopherol

The method for determination of tocopherols by liquid chromatography has been published [23]. Briefly, 2-ml ethanol containing 0.025% (w/v) butylated hydroxytoluene (BHT) and 0.1 ml of 30% (w/v) ascorbic acid were pipetted into tubes containing samples for tocopherol analyses. The mixture was saponified at 60 °C for 30 min after the addition of 1 ml of 10% potassium hydroxide solution. Tubes were cooled and 2 ml of water was added followed by 2 ml of hexane containing 0.025% (w/v) BHT. Tocopherols and quinone were extracted into the hexane phase by vortexing the samples for one minute. The hexane phase was separated out and evaporated down under a stream of nitrogen. The residue was redissolved in mobile phase and analyzed by reverse phase liquid chromatography using the following conditions: column=ultrasphere ODS, 5 μm, 4.6×150 mm (Beckman Instruments, Fullerton, CA, USA); mobile phase=methanol/water, (94.5:5.5) with 7.5 mM sodium dihydrogen phosphate (final concentration); flow rate=2.7 ml/min. The tocopherols were detected electrochemically: Coulochem 5100 A detector, 5011 analytical cell with detector 1 at -0.25 V and detector 2 at +0.55 V and 5021 conditioning cell at −0.75 V.

2.6. Biochemical assays

Concentration of total protein was determined by the Lowry technique as modified by Markwell et al. [24].

3. Results

Alpha-tocopherol (the form of vitamin E present in animal diets) was depleted more rapidly in peripheral tissues such as serum and liver than in brain when rats were fed vitamin E-deficient diets, in agreement with our early report [25]. A comparison of the alpha-tocopherol levels in selected tissues from control and vitamin E-deficient rats is shown in Fig. 1. Alpha-tocopherol in the peripheral tissues of vitamin E-deficient animals were barely detectable (less than 1% of those from controls). The concentration of alpha-tocopherol in brain of vitamin E-deficient rats was reduced to 3–5% of control levels. Thus, it is important to note that brain tissue from these vitamin E-deficient animals cannot be considered to be free of alpha-tocopherol.

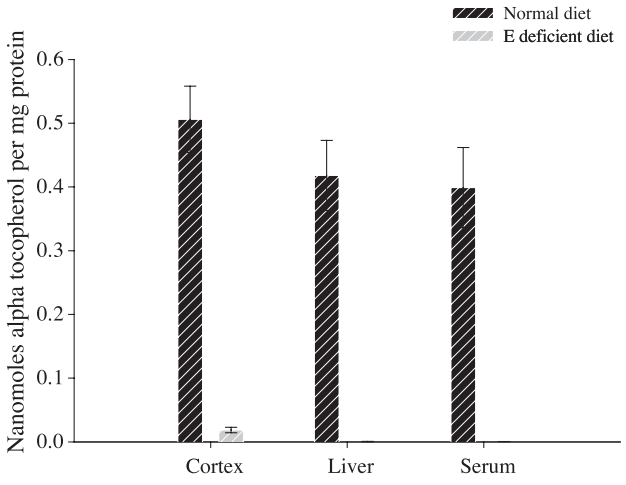

Fig. 1. Comparison of alpha-tocopherol concentrations in the brain and peripheral tissues of rats raised on control or vitamin E-deficient diets. Male Fisher 344 rats were raised on the respective diets for 13 months, the tissues isolated and analyzed for alpha-tocopherol by HPLC.

Rates of respiration and oxidative phosphorylation were determined for brain mitochondria isolated from control and vitamin E-deficient rats before and after the addition of several different concentrations of iron. Mitochondrial respiration supported by glutamate/malate was determined using a Clark electrode under basal conditions (state 4) and after addition of 208.5 nmoles of ADP (state 3 respiration). Different concentrations of iron in the form of ferrous ammonium sulfate were added to the reaction mixture before the measurement of oxygen consumption. The respiratory control ratios (RCR) (ratio of state 3 to state 4 respiration) for mitochondria from control and vitamin E-deficient rats were calculated. The results are shown in Fig. 2. Treatment of mitochondria with iron resulted in a loss of respiratory control as seen by declining respiratory control ratios with increasing concentrations of iron. Hence, the presence of iron is deleterious to mitochondria from both control and vitamin E-deficient brains. However, the vitamin E-deficient mitochondria were more susceptible to iron than control mitochondria. At an iron concentration of 50 μM, mitochondria from vitamin E-deficient brain showed a total loss of respiratory control, i.e., the rates of state 3 and state 4 respiration were the same.

The respiratory control ratios are composite indices of mitochondrial function and are a ratio of state 3 (ADP-stimulated) to state 4 (basal) respiration rates. Fig. 3A shows the changes in rates of state 3 and state 4 respiration of brain mitochondria from control rats after treatment with iron. A significant decrease in state 3 and a modest increase in state 4 rates occurred upon treatment of the control mitochondria

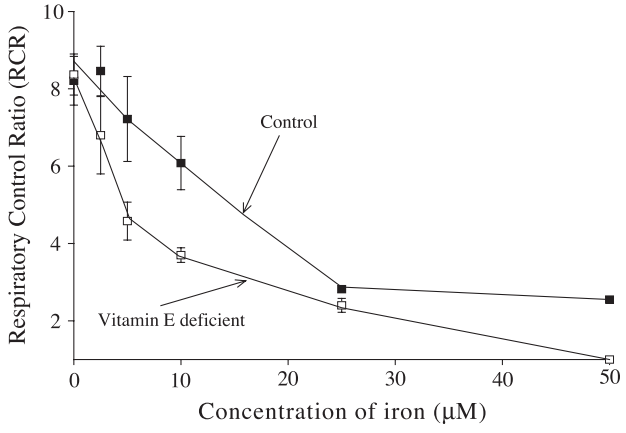

Fig. 2. The respiratory control ratios (RCR) for brain mitochondria from control and vitamin E-deficient rats. Male Fisher 344 rats were raised on control and vitamin E-deficient diets for 13 months. The cerebral hemispheres were dissected out, mitochondria isolated and incubated in 300 mM sucrose, 5 mM HEPES, 5 mM KH_2PO_4 and 50 μM EGTA. The oxygen consumption was determined under state 4 conditions with glutamate (10 mM)/malate (2.5 mM) as substrates and also under state 3 conditions after the addition of 208.5 nmol of ADP. The ratios between state 3 and state 4 respiration rates were calculated as RCR.

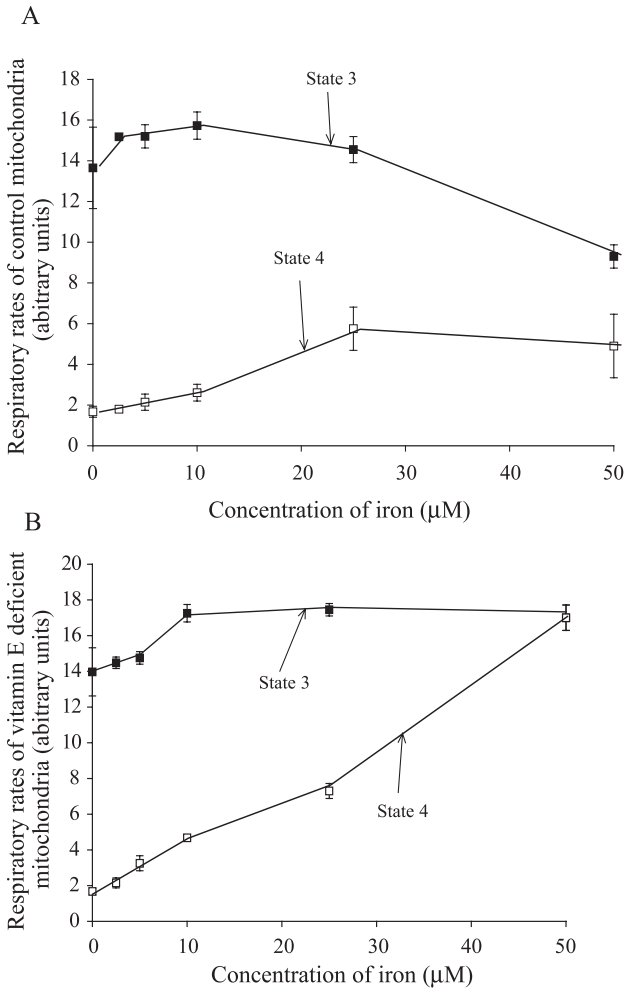

Fig. 3. Respiratory rates under state 3 and state 4 conditions of mitochondria from rat brains after exposure to different concentrations of iron: (A) control and (B) vitamin E-deficient. The rate of respiration on the ordinate is in arbitrary units: 1 unit=2.71 ng atoms oxygen. See legend of Fig. 2 for details of experimental conditions.

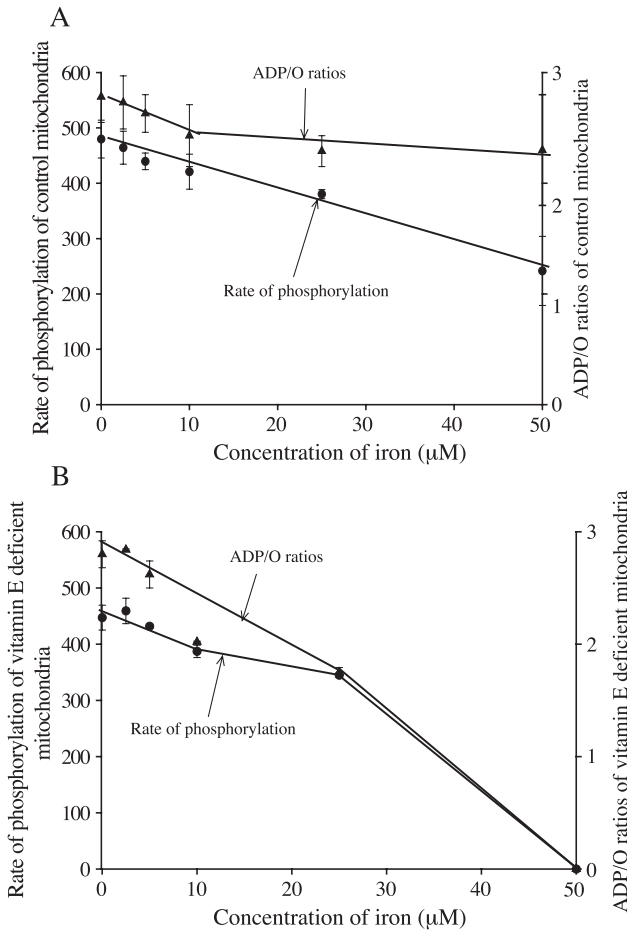

Fig. 4. Rates of phosphorylation and ADP/O ratios of mitochondria from rats after exposure to different concentrations of iron: (A) controls and (B) vitamin E-deficient. The rate of phosphorylation is in nanomoles of ADP phosphorylated per minute per milligram of mitochondrial protein. See legend of Fig. 2 for experimental details.

with increasing concentrations of iron. In contrast, iron induced a small increase in state 3 and a substantial increase in state 4 respiration in vitamin E-deficient mitochondria as shown in Fig. 3B.

The rates of phosphorylation and ADP/O ratios were also calculated using the data from the above experiment and are shown in Fig. 4A (control) and B (vitamin E-deficient). Treatment of mitochondria from control animals with varying concentrations of iron resulted in a progressive decline in phosphorylation rates and a lesser decline in ADP/O ratios (Fig. 4A). Vitamin E-deficient mitochondria underwent much more remarkable declines in both rates of phosphorylation and ADP/O ratios compared with control mitochondria (Fig. 4A and B). At 50 μM iron, the ADP/O ratio for control mitochondria was still >2, whereas the ADP/O ratio for vitamin E-deficient mitochondria was zero. These data show that iron treatment results in minimal uncoupling of oxidative phosphorylation in control mitochondria, while causing complete uncoupling in vitamin E-deficient mitochondria.

The effects of iron upon these mitochondrial functions were also studied using succinate as substrate (data not shown). The data were similar to those described above with glutamate/malate as substrates. Again, iron inhibited state 3 respiration in control mitochondria, but not in vitamin E-deficient mitochondria and iron markedly increased state 4 respiration in vitamin E-deficient mitochondria with a much smaller increase observed with control mitochondria. Treatment with iron decreased ADP/O ratios in vitamin E-deficient, but not in control mitochondria.

We also examined whether treatment with iron resulted in any changes in the alpha-tocopherol content of the brain mitochondria under conditions identical to those used in oxygen consumption studies. After two cycles of basal and ADP-stimulated respiration, samples of mitochondria were removed from the Clark electrode cell and assayed for alpha-tocopherol as well as its oxidation product, tocopherolquinone, by HPLC. The results are shown in Fig. 5. Alpha-tocopherol is lost from the mitochondria upon incubation with iron at concentrations as low as 2.5 μM (Fig. 5). Even the extremely small amount of alpha-tocopherol present in the mitochondria from vitamin E-deficient diet was lost after treatment with low concentrations of iron. Thus, the vitamin E-deficient, iron-treated mitochondria could be considered free of alpha-tocopherol. This may contribute to the uncoupling of vitamin E-deficient mitochondria by iron. Note that vitamin E deficiency alone did not result in uncoupling or alterations in RCR, rates of state 3 and 4 respiration, rate of phosphorylation or ADP/O ratios. The changes in these parameters were observed only after the vitamin E-deficient mitochondria were exposed to iron in the medium.

The production of tocopherolquinone, the oxidation product of tocopherol, was also monitored in the same

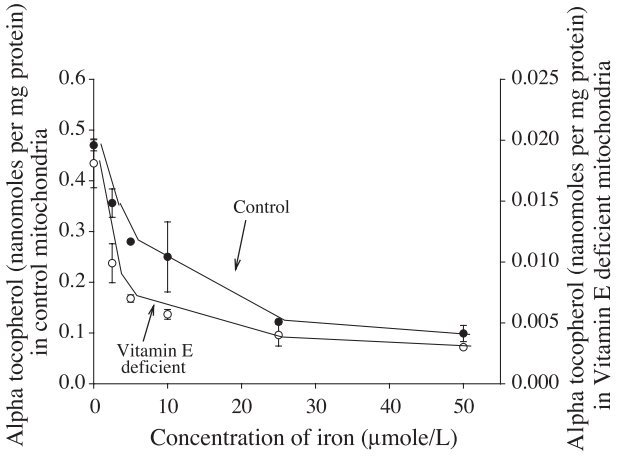

Fig. 5. Changes in alpha-tocopherol concentrations of mitochondria from brains of control and vitamin E-deficient rats. Mitochondria were isolated from the cerebral hemispheres of control and vitamin E-deficient rats. They were then suspended in buffer and placed in Clark electrode cell. After study of respiration, the mitochondria were recovered and the alpha-tocopherol concentrations determined by HPLC.

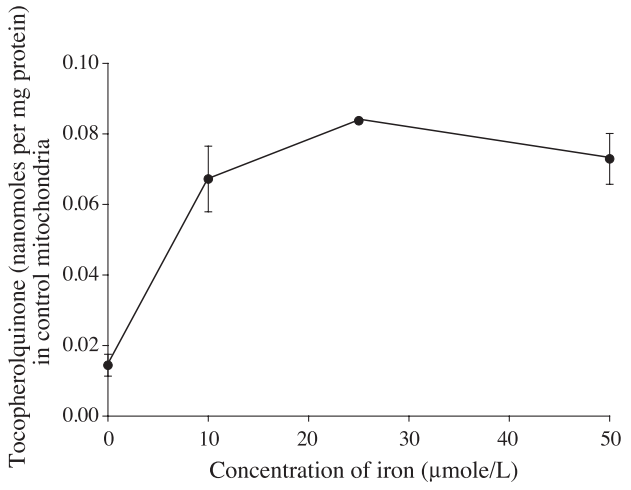

Fig. 6. Concentrations of tocopherolquinone in mitochondria treated with iron. See legend of Fig. 5 for experimental details.

samples. Data are provided only with control mitochondria since the quinone levels in vitamin E-deficient mitochondria treated with iron were too low to quantify. The results in Fig. 6 show a steady increase in the production of tocopherolquinone until the concentration of iron reached 25 μM, at which point the tocopherolquinone production reached a limiting level. Only a small fraction of alpha-tocopherol lost was converted into its quinone with most of the remainder being converted to other compounds that have not been characterized. The data in Figs. 5 and 6 demonstrate that oxidative stress was induced by incubating mitochondria with iron and that a major portion of alpha-tocopherol was consumed during this process.

4. Discussion

Mitochondria are the major sites of utilization of cellular iron and, hence, this subcellular organelle is likely to encounter the most traffic in iron in various forms. A pool of iron that has been referred to as the "intermediate or chelatable iron" has been described by many investigators and some suggest that almost 20% of the intracellular iron may be in this compartment with mitochondria reported to contain 40% of its iron content in this form [26,27]. The range of the non-chelated (redox-active) pool of mitochondrial iron is reported to be about 12.2 μM in rat hepatocytes [28]. This level falls within the range of iron concentrations used in this investigation.

During this study, we have determined RCR, state 3 and state 4 rates, ADP/O ratios and rates of phosphorylation using mitochondria from vitamin E-deficient and control animals before the addition of iron (see the initial data points at zero concentrations of iron in Figs. 2–4). Invariably, we found that vitamin E deficiency per se did not alter the different respiratory properties of mitochondria. This observation agrees with that of Paraidathathu et al. [10] using heart mitochondria from rats. Hence, a deficiency of vitamin E per se does not seem to alter oxidative phosphorylation of brain mitochondria. It is possible that the animals on the long-term vitamin E-deficient diets could have developed compensatory mechanisms to overcome the effects of lack of alpha-tocopherol upon mitochondrial metabolism.

Our results demonstrate that substantial amounts of mitochondrial alpha-tocopherol are consumed upon exposure to low concentrations of iron. Examination of data for control mitochondria shows that mitochondrial alpha-tocopherol is quite sensitive to the presence of iron in the medium (Fig. 5). In fact, the decline in alpha-tocopherol induced by low concentrations of iron (2 to 5 μM) can be used as a sensitive indicator of iron-induced oxidative stress in mitochondria (Fig. 5). The levels of alpha-tocopherol within the mitochondria in the controls go down to a limiting level when the iron concentration in the medium is 25 μM or more. This suggests the existence of two different compartments (one labile and the other more stable) of alpha-tocopherol within brain mitochondria. We have observed similar results when brain subcellular fractions were exposed to the synthetic free radical generator 2,2′ -azobis(2-amidinopropane) dihydrochloride (ABAPH) [29]. Melhorn et al. [30] have also found that when rat liver microsomes and mitochondria were oxidized by enzymatically generated free radicals, a portion of the tocopherol remained unoxidized. One could speculate that the labile compartment is functionally more important even though we do not know the location and the conditions of utilization of the different pools of tocopherol.

In vivo investigations involving ingestion of iron by vitamin E-deficient animals have been reported by a couple of groups. In one study, Bacon et al. [31] found that when vitamin E-deficient rats were given chronic oral doses of iron, there was a reduction in RCR in isolated liver mitochondria. This experiment can be considered to be an in vivo counterpart of our studies even though the source of the mitochondria was the liver whereas we used brain mitochondria. Results of Bacon et al. [31] agree with our in vitro data showing that iron causes reductions in RCR when the mitochondria are exposed to the metal. Link et al. [32] examined the effect of vitamin E upon iron-induced changes in mitochondria using iron-loaded heart cells from the rat and found that the iron-loaded cells had substantially reduced activities of complex I plus III as well as succinate dehydrogenase. They found that alpha-tocopherol completely protected the damage to the mitochondrial enzymes induced by iron loading without altering the uptake or release of iron. Thus, the above studies show that alpha-tocopherol and iron exhibit strong functional interactions in mitochondria. Our data clearly demonstrate that the interaction between alpha-tocopherol and iron will modulate the function of brain mitochondria.

We have shown that addition of iron leads to uncoupling of mitochondrial oxidative phosphorylation in vitamin E deficiency. When vitamin E-deficient mitochondria

were treated with increasing concentrations of iron, the state 4 respiration rate was increased substantially (Fig. 3B). It is known that the rate of state 4 respiration is controlled by proton leak across the mitochondrial inner membrane [33]. Hence, vitamin E deficiency caused alterations in mitochondrial membranes such that the rate of proton leak is increased upon treatment with iron. Various factors including alterations in the biochemical composition and architecture of the membranes can alter proton leak. For example, Brand et al. [34] have shown that proton leak is strikingly correlated with the fatty acid composition of the membrane phospholipids. It is possible that the vitamin E-deficient membranes were more peroxidized upon treatment with iron and this resulted in a change in proton leak.

The susceptibility of the vitamin E-deficient mitochondria to iron-induced stress was observed whether glutamate/malate or succinate was used as substrates. Succinate-supported respiration has been shown to be more susceptible to production of reaction oxygen species [35] and one would have expected the vitamin E-deficient brain mitochondria to be more susceptible to damage from exposure to iron when succinate was used as a substrate. This was not the case. Hence, it is possible that alpha-tocopherol may have other functions in mitochondria in addition to being a powerful membrane lipid antioxidant as has been suggested by some studies [36].

The exact molecular mechanism of how iron uncouples vitamin E-deficient mitochondria is unknown. We have formulated a hypothetical scheme, which is shown in Fig. 7. The addition of iron to vitamin E-deficient mitochondria will be expected to initiate oxidative chain reactions in the membrane. Critical components will be oxidized resulting in excessive production of superoxide by the respiratory chain. This sequence of events is supported by studies of Cornejo et al. [37] who showed that submitochondrial particles from the liver of iron-overloaded rats produce significantly more superoxide than controls. Superoxide formed can activate uncoupling protein(s) and induce uncoupling as reported by Echtay et al. [38] from the Brand group. In addition, superoxide can also inhibit dehydratases such as aconitase [39] and cause abnormalities in oxidative metabolism which in turn can result in uncoupling. Finally, studies of Putvinsky et al. [40] have established more than two decades ago that lipid peroxidation can induce increases in electrical conductance; this can also cause uncoupling. The changes in uncoupling protein activity, generation of lipid peroxidation products, alterations in enzyme activity or increased electrical conductance could all be ultimately responsible for increased proton leak leading to uncoupling. Vitamin E is a very potent, lipid-soluble antioxidant ideally located within the membranes preventing most of these changes under normal physiological conditions.

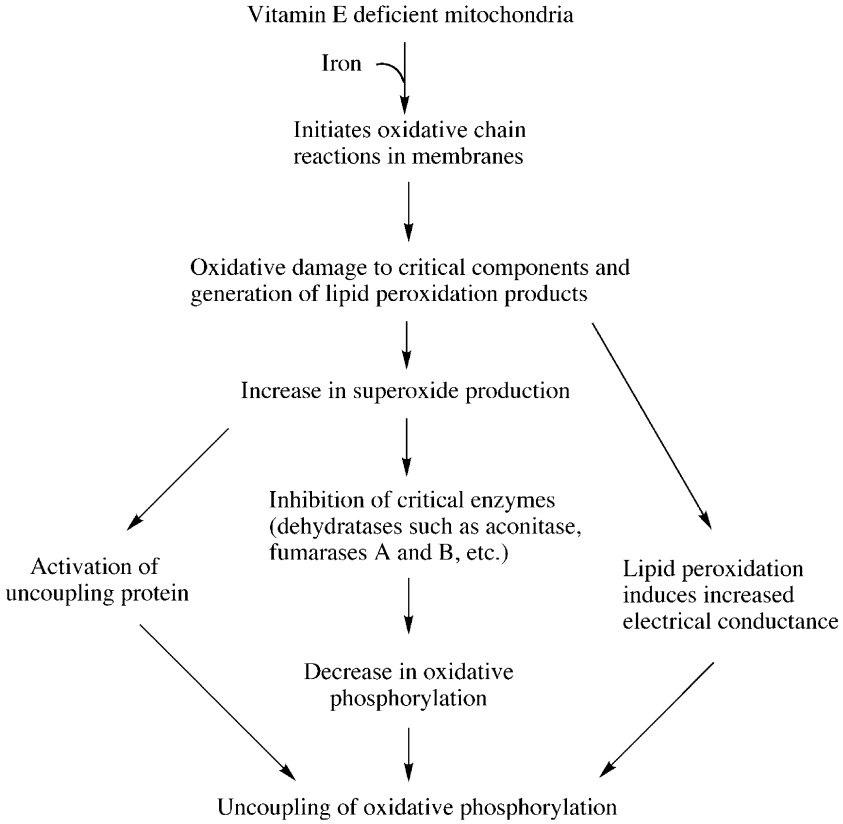

Fig. 7. A hypothetical scheme of the mechanism of induction of mitochondrial uncoupling by iron in vitamin E deficiency.

In conclusion, our experiments show that exposure to iron results in uncoupling of oxidative phosphorylation of vitamin E-deficient mitochondria. The concentrations of iron used were within the range of the non-chelated (redox-active) pool of mitochondrial iron, which is reported to be about 12.2 μM in rat hepatocytes [28]. The results suggest that alpha-tocopherol may have a specific role in maintaining oxidative phosphorylation especially under oxidative stress induced by iron. It can be postulated that alpha-tocopherol may have a role in modulating mitochondrial proton leak. Whenever there is an imbalance in cellular iron homeostasis, the antioxidant status of mitochondria (especially with respect to vitamin E) needs to be carefully considered when assessing iron toxicity. Thus, our findings would be particularly relevant for understanding the pathophysiological mechanisms involved in neurodegenerative disorders such as Alzheimer's and Parkinson's diseases and Friedrich's ataxia where abnormal iron metabolism and/or oxidative stress has been demonstrated to be present.

Acknowledgements

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