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RESEARCH ARTICLE

Brain and liver mitochondria isolated from diabetic Goto-Kakizaki rats show different susceptibility to induced oxidative stress

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Abstract

Background Increased oxidative stress and changes in antioxidant capacity observed in both clinical and experimental diabetes mellitus have been implicated in the etiology of chronic diabetic complications. Many authors have shown that hyperglycemia leads to an increase in lipid peroxidation in diabetic patients and animals reflecting a rise in reactive oxygen species production. The aim of the study was to compare the susceptibility of mitochondria from brain and liver of Goto-Kakizaki (12-month-old diabetic) rats (GK rats), a model of non-insulin dependent diabetes mellitus, to oxidative stress and antioxidant defenses.

Methods Brain and liver mitochondrial preparations were obtained by differential centrifugation. Oxidative damage injury was induced *in vitro* by the oxidant pair ADP/Fe^{2+} and the extent of membrane oxidation was assessed by oxygen consumption, malondialdehyde (MDA) and thiobarbituric acid reactive substances (TBARS) formation. Coenzyme Q and α-tocopherol contents were measured by high-performance liquid chromatography (HPLC).

Results Brain mitochondria isolated from 12-month-old control rats displayed a higher susceptibility to lipid peroxidation, as assessed by oxygen consumption and formation of MDA and TBARS, compared to liver mitochondria. In GK rats, mitochondria isolated from brain were more susceptible to *in vitro* oxidative damage than brain mitochondria from normal rats. In contrast, liver mitochondria from diabetic rats were less susceptible to oxidative damage than mitochondria from normal rats. This decreased susceptibility was inversely related to their α-tocopherol and coenzyme Q (CoQ) content.

Conclusions The present results indicate that the diabetic state can result in an elevation of both α-tocopherol and CoQ content in liver, which may be involved in the elimination of mitochondrially generated reactive oxygen species. The difference in the antioxidant defense mechanisms in the brain and liver mitochondrial preparations of moderately hyperglycemic diabetic GK rats may correspond to a different adaptive response of the cells to the increased oxidative damage in diabetes. Copyright © 2001 John Wiley & Sons, Ltd.

Keywords mitochondria; coenzyme Q; diabetes; oxidative stress; α-tocopherol; Goto-Kakizaki rat

Introduction

Diabetes is associated with an increased production of reactive oxygen species (ROS) in both humans and animals. Enhanced oxidative stress and changes in antioxidant capacity are considered to play an important role in the pathogenesis of chronic diabetic complications [1–4]. Evidence indicates that free oxygen radicals and membrane lipid peroxidation are significantly increased in diabetic patients and in rats used as models of diabetes [5–7]. Glucose autoxidation and non-enzymatic glycation of proteins are an important source of oxidative species, which may attack cellular components including nucleic acids, proteins and membrane lipids thus promoting cellular damage [3]. A decrease in the endogenous ROS scavenging compounds that normally protect cells from oxidative insults has also been observed in diabetic patients and animals [8,9].

Evidence associating diabetic pathology to mitochondrial dysfunction and oxidative stress has been reported previously [10–14]. Mitochondria are generally considered to be the major endogenous source of cellular ROS, under normal and pathological conditions, and perhaps of oxidative stress in general [15–17]. It has been estimated that during normal metabolism 1–2% of the electrons that flow into the redox chain catalyze the incomplete reduction of O_2 generating superoxide anion and hydrogen peroxide. Under normal physiological conditions mitochondrial antioxidant systems (enzymatic and non-enzymatic) scavenge free radicals and preserve mitochondrial integrity [18]. Moreover, under certain pathophysiological conditions the generation of superoxide radical/hydrogen peroxide dramatically increases, leading to an imbalance between the pro-oxidant factors and the antioxidant systems. Since mitochondria are a major site of ROS generation and have a high concentration of heme and non-heme iron bound to proteins in addition to nucleotide–iron and carboxylic acid–iron complexes that can participate in Haber–Weiss reactions, the mitochondrial membrane is a primary target for ROS-induced damage [18]. The high content of polyunsaturated fatty acids in mitochondrial membranes enhances mitochondrial susceptibility to lipid peroxidation, leading to membrane dysfunction and alterations in the structural and functional integrity of the membrane [19]. As brain and liver heavily depend on mitochondrial oxidative catabolism for the majority of their ATP requirements, elevated levels of ROS are particularly dangerous because they mediate mitochondrial damage, which in turn can generate further oxidative stress in the cells [20].

The Goto-Kakizaki (GK) rat represents a non-obese animal model of type 2 diabetes [21] which was obtained by selective breeding of normal Wistar rats, using glucose intolerance as the selection index [22,23]. This genetic model is particularly relevant to understanding human type 2 diabetes since moderate but stable fasting hyperglycemia has been demonstrated as early as 2–4 weeks after birth, which does not progress to the ketotic state. Therefore, in the initial stages of diabetes GK rats do not present severe complications associated with the disease, and are thus an appropriate model in which to study the events at the onset of diabetes, as compared to obese diabetic rats which present severe hyperglycemia and hyperlipidemia [21].

There is currently considerable interest in the role of vitamin E and coenzyme Q (CoQ) compounds in the protection of membrane lipids against oxidative stress. CoQ is present in membranes with α-tocopherols [24] and has been recognized as an important antioxidant in the inner mitochondrial membrane, where it scavenges radicals directly and/or regenerates α-tocopherol from the α-tocopheroxyl radical [25,26]. The possibility that CoQ content may be one of the factors controlling the susceptibility of mitochondria to oxidative stress in diabetic GK rats was examined. Data from our previous experiments have suggested that GK rats might develop enhanced defense systems against oxidative stress, which is believed to be an important factor in the development of diabetic complications [27]. The ADP/Fe^{2+} was used to induce oxidative damage injury in isolated mitochondrial preparations and the extent of membrane oxidation was assessed by oxygen consumption, malondialdehyde (MDA) and thiobarbituric acid (TBA) reactive species formation. Furthermore, α-tocopherol and CoQ (CoQ_9 and CoQ_{10}) contents were determined and studies were conducted to compare liver and brain mitochondrial susceptibility to oxidative stress and antioxidant defenses in both GK and Wistar rats.

Materials and methods

Animals

Male spontaneously diabetic GK rats (46–54 weeks of age; body weights 384.7 ± 2.4 g; non-fasting blood glucose levels 198.6 ± 13.5 mg/dl) were obtained from a local breeding colony of Coimbra, established in 1995 with breeding couples from the colony at the Tohoku University School of Medicine (Sendai, Japan; courtesy of Dr K. I. Susuki). Control animals were non-diabetic male Wistar rats of similar age (44–53 weeks of age; body weights 672.5 ± 60.5 g; non-fasting blood glucose levels 93.4 ± 2.9 mg/dl) obtained from our local colony. Animals were kept under controlled light and humidity conditions, and with free access to powdered rodent chow (diet CRF 20; Charles Rivers, France) and water. The animals were killed by decapitation and their livers and brains removed and washed in the respective homogenization medium.

Materials

Ubiquinone 10 was obtained from Fluka and ubiquinone 9 and α-tocopherol were obtained from Sigma Chemical Co. (St Louis, MO, USA). All other chemicals used were of analytical grade.

Mitochondrial preparations

Liver mitochondria were isolated according to a previously established method [28] with some modifications [29]. Homogenization medium contained 0.25 mM sucrose, 5 mM Hepes (pH 7.4) 0.2 mM EGTA, 0.1 mM EDTA and 0.1% de-fated bovine serum albumin (BSA). EGTA, EDTA and BSA were omitted from the final washing medium, adjusted to pH 7.2. The mitochondrial pellet was washed twice and suspended in washing medium.

Brain mitochondria (crude mitochondrial preparation) were isolated using the method of Lai and Clark [30]. All homogenization and centrifugation steps were performed in isolation medium (0.32 mM sucrose, 1 mM K^+-EDTA, 5 mM Hepes-Tris, pH 7.4). After isolation, the mitochondrial suspensions of liver and brain were used after a 30 min recovery and within 4 h of preparation.

Mitochondrial protein was determined by the biuret method, using BSA as a standard.

Measurement of lipid peroxidation

Lipid peroxidation was determined as described by Sassa *et al*. [31]. The oxygen consumption was measured in 1 ml of medium (175 mM KCl, 10 mM Tris-Cl pH 7.4, supplemented with 3 μM rotenone) containing 1 mg protein, using a Clark-type electrode (YSI Model 5331; Yellow Springs Institute) in a glass chamber equipped with magnetic stirring and a thermostat set at 30°C [32]. Reactions were started by the addition of 1 mM ADP and 0.1 mM $FeSO_4$, after a 2-min incubation period. The saturated concentration of O_2 in the incubation medium was assumed to be 232 μM at 30°C.

The extent of lipid peroxidation in the brain and liver mitochondria was also determined by measuring the amounts of thiobarbituric acid reactive substances (TBARS) and MDA formed by colorimetric assay and high-performance liquid chromatography (HPLC), respectively. The amount of TBARS formed was determined using the TBA test according to a modified procedure described by Ernster and Nordenbrand [33]. Mitochondrial protein (1 mg/ml) was incubated at 30°C for 15 min, in a medium containing 175 mM KCl, 10 mM Tris-Cl pH 7.4, supplemented with 3 μM rotenone. Membrane lipid peroxidation was started by adding simultaneously ADP/$FeSO_4$ (1 mM/0.1 mM). The reaction was stopped by lowering the temperature to 0–4°C by placing the tubes in ice. To measure lipid peroxidation, 0.5 ml cold 40% trichloroacetic acid and 2 ml 0.67% TBA containing 6.8 mM 2,6-diterbutyl-4-methylphenol (BHT) were added to 0.5 ml test material, which was then heated for 10 min in a boiling water bath and cooled on ice for 10 min before centrifugation in an Eppendorf centrifuge (1500 *g* for 5 min). The supernatants were collected and the absorbance measured at 535 nm. The amount of TBARS formed was calculated using a molar extinction coefficient of $1.56 \times 10^5\ mol^{-1}cm^{-1}$ and expressed as nmol TBARS/mg protein [34].

The assay procedure used for MDA determination by HPLC was that of Wong *et al*. [35]. Liquid chromatography was performed using Gilson HPLC apparatus with a reverse phase column (RP18; Spherisorb; S5 ODS_2). The samples were eluted from the column at a flow rate of 1 ml/min and detection was performed at 532 nm. The MDA content of the samples was calculated from the standard curve prepared using the TBA–MDA complex and expressed in nmol/mg protein.

Extraction and quantification of CoQ and α-tocopherol

Aliquots of mitochondria containing 1 mg protein/ml were extracted according to the method described previously [36]. The extract was evaporated to dryness under a stream of N_2, and suspended in ethanol for HPLC analysis. Liquid chromatography was performed using Gilson HPLC apparatus with a reverse phase column (RP18; Spherisorb; S5 ODS_2) as described previously [37]. Samples were eluted from the column with methanol : heptane (10 : 2 by volume) at a flow rate of 2 ml/min. Detection was performed using a UV detector at 269 nm. Identification and quantification were based on pure standards by their retention times and peak areas, respectively. The levels of CoQ (CoQ_9 and CoQ_{10}) in mitochondrial membranes were expressed in pmol/mg protein.

The extraction and separation of α-tocopherol were performed following the method described by Vatassery *et al*. [38]. The extract, evaporated to dryness under a stream of N_2 and kept at –80C, was dissolved in *n*-hexane and α-tocopherol content was analyzed by reverse phase HPLC. A 4.6×200 mm Spherisorb S10w column was eluted with *n*-hexane modified with 0.9% methanol, at a flow rate of 1.5 ml/min. Detection was performed using a UV detector at 287 nm. The levels of vitamin E in mitochondrial membranes were expressed in pmol/mg protein.

Data analysis and statistics

Data are expressed as means ± SEM of the indicated number of experiments, each obtained with a different animal. Statistical significance was determined by using the paired Student's *t*-test and by using the one-way ANOVA Student–Newmann–Keuls post-test for multiple comparisons. A p value < 0.05 was considered significant.

Results

Oxidative stress induced by ADP/Fe^{2+}

Brain mitochondria displayed a higher susceptibility to lipid peroxidation induced by ADP/Fe^{2+}, as assessed by oxygen consumption and the formation of TBARS, as compared to liver mitochondria (Figures 1 and 2). As shown in Figure 1, the time-dependent change in the

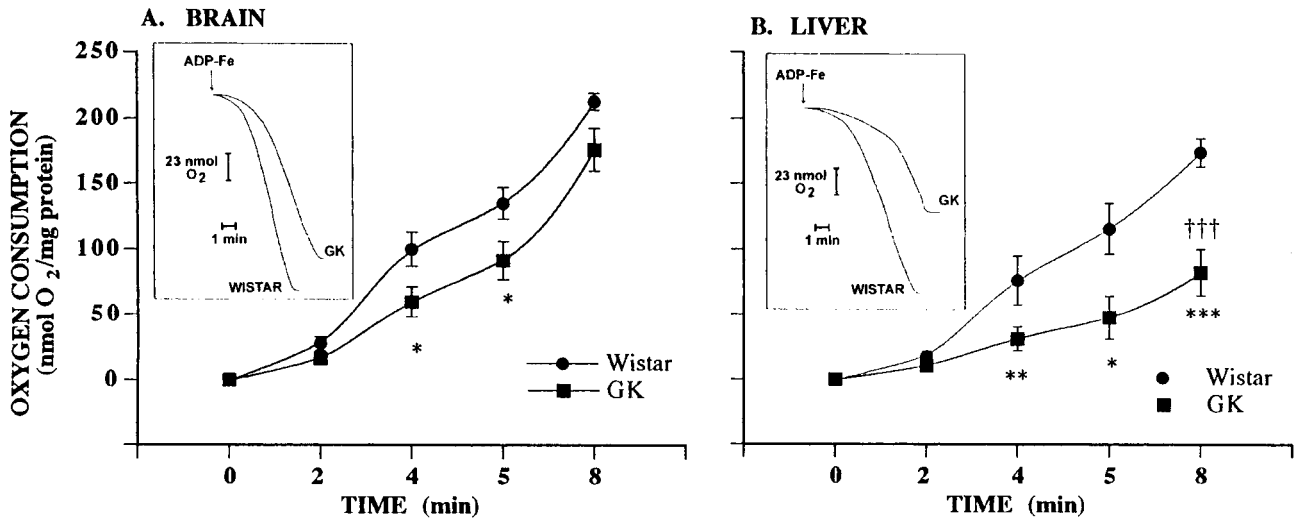

Figure 1. Comparison of ADP/Fe^{2+}-induced mitochondrial O_2 consumption in rat brain (A) and liver (B) mitochondria isolated from Wistar and GK rats. Oxidative stress was induced in mitochondria isolated from the brains or livers of control and diabetic rats. Mitochondria (1 mg) were incubated in 1 ml medium consisting of 175 mM KCl, 10 mM Tris (pH 7.4), for 30 min at 30°C, supplemented with 2 μM rotenone. Peroxidation was started by adding 1 mM ADP and 0.1 mM $FeSO_4$. The inserts represent typical recordings of oxygen consumption. Data are the means ± SEM of seven different experiments. Statistical significance: $^{***}p<0.001$, $^{**}p<0.01$ or $^{*}p<0.05$ as compared to control Wistar rats; $^{\dagger\dagger\dagger}p<0.001$ as compared to brain mitochondria

peroxidation related to oxygen concentrations in the suspensions of brain and liver mitochondria are different in preparations isolated from diabetic rats as compared to control rats. In diabetic mitochondria, at first slow oxygen consumption occurs for a few minutes after the addition of ADP/Fe^{2+} and then the consumption of oxygen increases. In addition, when mitochondria of GK rats did begin to peroxidize they did so at a slower rate than control mitochondria. The time associated with the slow oxygen consumption that follows the addition of ADP/Fe^{2+} until the rapid oxygen uptake (lag time) is considered to be the time required for the generation of a sufficient amount of reactive oxygen species derived from ADP/Fe^{2+}, such as the perferryl complex ADP-Fe^{3+}-$O_2^{\cdot -}$ responsible for the induction of lipid peroxidation. The rapid oxygen consumption after the lag phase follows the peroxidative radical chain proliferation of membrane lipids by ROS.

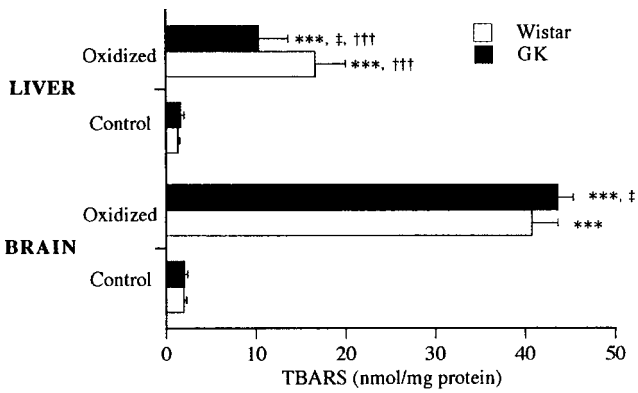

Figure 2. Effect of diabetes on the extent of mitochondrial oxidation induced by ADP/Fe^{2+}. Mitochondria (1 mg) were incubated in 1 ml medium consisting of 175 mM KCl, 10 mM Tris (pH 7.4), for 30 min at 30°C, supplemented with 2 μM rotenone. Peroxidation was started by adding 1 mM ADP and 0.1 mM $FeSO_4$. In controls mitochondria were incubated in the absence of the oxidizing agents. The extent of synaptosomal oxidation was determined by the TBA method and expressed as nmol/mg protein. Data are the means ± SEM of seven experiments run in duplicate. Statistical significance: $^{***}p<0.001$ as compared to respective controls not treated with ADP/Fe^{2+}; $^{\ddagger}p<0.05$ as compared to Wistar mitochondria treated with ADP/Fe^{2+}; $^{\dagger\dagger\dagger}p<0.001$ as compared to data obtained for oxidized brain mitochondria

Figure 2 shows that in the absence of oxidants the levels of TBARS were similar in brain and liver mitochondria isolated from control (1.97 ± 0.31 and 1.33 ± 0.23 nmol/mg protein, respectively) or GK rats (2.03 ± 0.37 and 1.67 ± 0.36 nmol/mg protein, respectively). In control rats the oxidation by ADP/Fe^{2+} increased significantly the production of TBARS in brain and liver to 40.77 ± 2.9 and 16.66 ± 3.36 nmol/mg protein, respectively. A significant increase in the extent of oxidation was observed in mitochondria isolated from the brains of GK rats as compared with mitochondria from Wistar rats. In contrast, liver mitochondria from diabetic rats were less susceptible to oxidative damage than mitochondria from normal rats.

As the validity of the TBA test for the assessment of lipid peroxidation has been questioned many times [39], the formation of lipid peroxides (as MDA) was also measured by a more specific HPLC method. The results obtained indicated that this procedure yielded lower values of MDA in control and oxidized mitochondria when compared to the respective TBARS values (Table 1 and Figure 2). In Wistar rats, ADP/Fe^{2+} increased the formation of MDA in brain and liver to 10.89 ± 0.37 and 11.97 ± 0.17 nmol MDA/mg protein, respectively, values significantly different from those determined in control conditions (1.80 ± 0.35 and 1.14 ± 0.18 nmol MDA/mg protein,

Table 1. Levels of MDA in brain and liver mitochondria oxidized with ADP/Fe^{2+}

Experimental condition	Brain	Liver
Wistar rats		
Control	1.80 ± 0.35	1.14 ± 0.18
Oxidized	10.89 ± 0.37***	11.97 ± 0.17***
GK rats		
Control	1.34 ± 0.34	0.44 ± 0.11§
Oxidized	12.44 ± 0.40***‡	9.91 ± 0.36***†††

The experimental conditions were similar to those described in the legend to Figure 2. MDA levels were determined by HPLC and were expressed as nmol MDA/mg protein. Data are the means ± SEM from three different samples, each obtained from a different rat. Statistical significance: ***$p<0.001$ as compared to respective controls not treated with ADP/Fe^{2+}; §$p<0.05$ as compared to respective Wistar control; †††$p<0.001$ and ‡$p<0.05$ as compared to Wistar mitochondria treated with ADP/Fe^{2+}.

respectively). A significant increase in MDA formation was observed in brain mitochondria isolated from GK rats submitted to ADP/Fe^{2+} (12.44 ± 0.40 nmol/mg protein) as compared with mitochondria from Wistar rats (10.89 ± 0.39 nmol/mg protein). The levels of MDA in control and oxidized liver mitochondria from GK rats (0.44 ± 0.11 and 9.91 ± 0.36 nmol/mg protein, respectively) were lower than those in Wistar mitochondria (1.14 ± 0.18 and 11.97 ± 0.17 nmol/mg protein, respectively; Table 1).

CoQ and α-tocopherol content in mitochondria

CoQ in rat mitochondria consisted of two main homologues, CoQ_9 and CoQ_{10}. In the brains of control Wistar rats, CoQ_9 represented about 80% and CoQ_{10} 20% of the total amount of mitochondrial CoQ (1.14 ± 0.07 nmol/mg protein). In liver mitochondria, CoQ_9 constituted about 97% of the total CoQ content (0.89 ± 0.04 nmol/mg protein). Brain mitochondria of GK and control Wistar rats contained similar levels of α-tocopherol, but the CoQ_9 content in the GK mitochondria was lower than that in the Wistar mitochondria. In contrast, there was a remarkable increase of about 100% in the α-tocopherol and CoQ content in the liver mitochondria isolated from diabetic rats as compared to control rats (Figures 3 and 4). In brain and liver the changes in CoQ_9 content paralleled that of the total CoQ content (Figure 4).

Discussion

One of the main findings of the present study was the observation of differential content of CoQ and α-tocopherol in mitochondria isolated from diabetic animals. Thus, diabetes increased the levels of CoQ and α-tocopherol in liver mitochondria isolated from diabetic GK rats but not in brain mitochondria. A small decrease in CoQ content was observed in brain mitochondria. Another point of interest was the observation that there is an inverse correlation between the levels of the antioxidants

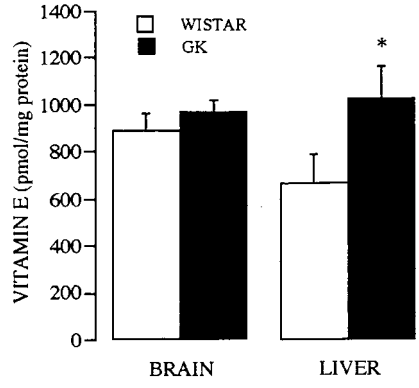

Figure 3. Vitamin E levels in brain and liver mitochondria isolated from Wistar and GK rats. Vitamin E (α-tocopherol) was measured by reverse-phase HPLC as described in Materials and methods. The data are the mean ± SEM for seven different samples, each obtained from a different rat. Statistical significance: *$p<0.05$ as compared to the levels of vitamin E in control Wistar rats

α-tocopherol and CoQ and the susceptibility of mitochondria to oxidative damage induced by ADP/Fe^{2+}.

Many authors have associated hyperglycemia-induced changes in oxidative state with diabetic complications. Evidence for oxidative stress in diabetes includes reports of increased free radical generated plasma lipid peroxides and observations of decreased antioxidant plasma concentrations in both humans and animal models of diabetes [7]. Although, the concentrations of vitamin E in plasma and in tissues, in both diabetic patients and experimental diabetes, are controversial, an inverse association between serum α-tocopherol concentration and subsequent occurrence of the disease has been

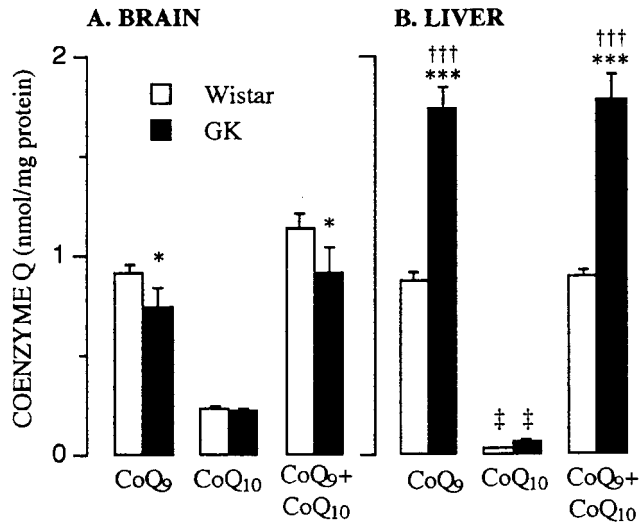

Figure 4. Coenzyme Q_9 and Q_{10} levels in brain and liver mitochondria isolated from Wistar and GK rats. Coenzyme Q levels were measured by HPLC as described in Materials and methods. Data are the means ± SEM for seven different samples, each obtained from a different rat. Statistical significance: ***$p<0.001$ or *$p<0.05$ as compared to control Wistar rats; †††$p<0.005$ as compared to GK brain mitochondria; ‡$p<0.005$ as compared to brain values

reported [40]. A beneficial effect of vitamin E on the complications of diabetes was also reported [41–43].

An increase in α-tocopherol might be expected to render the mitochondria more resistant to *in vitro* lipid peroxidation. This is illustrated by the time course experiments on oxygen consumption. Diabetes induced a significant increase in the initiation phase of the lipid peroxidation analyzed as the lag time that accompanies the oxygen uptake burst. In addition the mitochondrial fractions of GK peroxidized slowly as compared to the controls. The observed increase in the lag phase and the decrease in oxygen consumption, TBARS and MDA formation are probably due to the higher content of α-tocopherol in GK diabetic animals. It has been observed that liver microsomes from vitamin-E deficient rats peroxidized at a greater rate than control microsomes [44]. Vitamin E (α-tocopherol) is well established as one of the most important lipid-soluble, chain reaction-breaking antioxidants protecting the integrity of lipid structures in membranes against oxidative damage [45]. Increased levels of vitamin E in liver [12,46] and heart [46,47] of streptozotocin-treated diabetic rats and in brain membranes of db/db mice [48] have been reported. Sukalski *et al*. [12] suggested that the increased vitamin E levels associated with diabetes may be due to alterations in the metabolism and storage of vitamin E. The elevation of vitamin E content in membranes may be partially explained by the fact that plasma vitamin E may be increased by mobilization with lipids from the adipose tissue and the liver to other tissues [12]. Besides its effects linked to antioxidative properties, vitamin E has been shown to reduce protein glycosylation both *in vivo* and *in vitro* [49,50] and to improve insulin action in non-diabetic and non-insulin-dependent diabetic patients [51] and β-cell function [52]. Vatassery *et al*. [37] found that plasma and platelet vitamin E levels were higher in patients with types 1 and 2 diabetes as compared to the control group.

In recent years there has been considerably interest in the protective role of CoQ against oxidative stress. Besides its well-established function as a component of the mitochondrial respiratory chain, in its reduced form CoQ may function as an antioxidant, protecting membrane phospholipids and serum low-density lipoprotein from lipid peroxidation by quenching lipid radicals or lipid peroxidation initiating species and, as recent data indicate, it also protects mitochondrial membrane proteins and DNA from free radical-induced oxidative damage [25,26,53]. These effects seem to be independent of those of exogenous antioxidants, such as vitamin E, although CoQ can also potentiate the effect of α-tocopherol by regenerating it from its oxidized form [54]. Coenzyme Q is endogenously synthesized in most mammalian cells and organs and its levels in membranes are regulated, through the mevalonate pathway, by physiological factors that are related to oxidative activity of the organism. Evidence that the increase in CoQ content occurred after the increase in metabolic rate, and thus free radical production (e.g. during physical exercise, cold adaptation or thyroid hormone treatment) suggests that the increase in CoQ may an adaptation to or a response to, rather than a cause of, increased oxidative activity [25,54]. Boveris *et al*. [55] have also shown that CoQ levels significantly increased in liver mitochondria isolated from rats made diabetic by pancreatectomy. It is interesting to note that the increase in CoQ content in liver mitochondria of GK rats may be related to the increased efficiency of mitochondrial FAD-linked glycerol-3-phosphate dehydrogenase, the key enzyme in the glycerol phosphate shuttle, and to an increased efficiency rate of oxidative metabolism, as a result of the increased efficiency in the oxidative phosphorylation of diabetic rats as compared with control animals, features that are exclusive to the liver [56,57]. This may explain why CoQ levels increase in diabetes only in the liver and not in brain mitochondria.

The present results constitute the first evidence for possible differential metabolism of CoQ in diabetic animals and augment earlier reports on the higher content of α-tocopherol in several preparations isolated from diabetic animals. The simultaneous increase of CoQ and α-tocopherol in rat liver mitochondria isolated from GK rats suggests an interaction between CoQ and α-tocopherol. Sohal and colleagues reported recently that the recycling of α-tocopherol in mitochondrial membranes is directly dependent of the molar ratios of α-tocopherol and CoQ [58,59]. In fact, and in contrast to liver mitochondria, an insufficient amount of CoQ may explain why the levels of α-tocopherol in brain mitochondria were unchanged in GK diabetic animals. Recent studies have indicated that the generation of superoxide anion by submitochondrial particles was inversely related to their α-tocopherol content and unrelated to CoQ, and that the antioxidative role of CoQ in mitochondria may be due to a 'sparing/regenerating effect' of CoQ on α-tocopherol rather than a direct effect as radical scavenger [58,60–62].

In conclusion, the present findings show that liver preparations of diabetic GK rats were more resistant to oxidative stress than those of control rats, supporting the idea that the liver may be protected from the injury induced by radical species involved in diabetic complications. The significant increase in vitamin E and CoQ in liver of GK rats is consistent with the observed decreased in the susceptibility to *in vitro* lipid peroxidation. Since some of the important biochemical reactions determining the metabolic disorders observed in diabetes are localized in liver mitochondria, and the liver is a central organ in glucose homeostasis, the observed increase in antioxidant defenses and decreased susceptibility to oxidative stress in GK liver mitochondria could have developed as a biochemical response or as an adaptation to metabolic disturbances associated with type 2 diabetes. The difference in the antioxidant defense mechanisms in the brain and liver mitochondrial preparations of moderately hyperglycemic diabetic GK rats may correspond to a different adaptive response of the cells to the increased oxidative damage in diabetes.

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