CoQ10 therapy attenuates amyloid β-peptide toxicity in brain mitochondria isolated from aged diabetic rats

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Abstract

Using brain mitochondria isolated from 20-month-old diabetic Goto–Kakizaki rats, we evaluated the efficacy of CoQ10 treatment against mitochondrial dysfunction induced by A\textsubscript{β1–40}. For that purpose, several mitochondrial parameters were evaluated: respiratory indexes (RCR and ADP/O ratio), transmembrane potential (ΔΨm), repolarization lag phase, repolarization and ATP levels and the capacity of mitochondria to produce hydrogen peroxide. We observed that 4 μM A\textsubscript{β1–40} induced a significant decrease in the RCR and ATP content and a significant increase in hydrogen peroxide production. CoQ10 treatment attenuated the decrease in oxidative phosphorylation efficiency and avoided the increase in hydrogen peroxide production induced by the neurotoxic peptide. These results indicate that CoQ10 treatment counteracts brain mitochondrial alterations induced by A\textsubscript{β1–40} suggesting that CoQ10 therapy can help to avoid a drastic energy deficiency that characterizes diabetes and Alzheimer’s disease pathophysiology.

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Introduction

Type 2 diabetes accounts for about 90% of the existing cases of diabetes and is characterized by defects in both insulin action and secretion (Gavin et al., 1997). Many studies demonstrated that diabetes produces molecular, cellular, morphological and behavioral changes in the central nervous system (CNS) (Biessels et al., 2002). Diabetes is often associated with mitochondrial diseases characterized by defects in the mitochondrial genome (Gerbitz et al., 1995). Mitochondria play a central role in the development of type 2 diabetes by regulating energy balance and the generation of reactive oxygen species (ROS) (Wallace, 1999; Lowell and Shulman, 2005).

Defects in energy metabolism are a consistent feature of AD-affected brain. In Alzheimer’s disease (AD), a major imbalance between glucose and oxygen consumption has been found in the incipient stage, whereas in the advanced stage, both glucose and oxygen consumption are diminished (Hoyer, 1991). This could be a consequence of deviant insulin action or brain insulin receptor function, which can affect brain energy metabolism (Hoyer, 1991). These abnormalities in insulin metabolism may account for the pathological changes (formation of senile plaques and neurofibrillary tangles) found in Alzheimer’s disease (Frol-ich et al., 1998; Hoyer, 2002).

Several lines of evidence suggest that amyloid deposition in the brain contributes to neuronal degeneration in AD (Selkoe, 2001) just as amyloid formation in the pancreas is believed to contribute to β-cell loss in type 2 diabetes (Höppener et al., 2000). Hoyer (1998) argues that in late-onset AD, a disturbance in the control of neuronal glucose
metabolism consequent to impaired insulin signaling strongly resembles the pathophysiology of type 2 diabetes in nonneural tissue. Furthermore, several epidemiological studies have established that diabetes increases the risk of dementia in general (Hassing et al., 2004) and AD in particular (Leibson et al., 1997; Ott et al., 1999).

The observation that oxidative stress is increased in AD and diabetes has generated the notion that antioxidants and other regulators of oxidative stress may protect against oxidative damage. Coenzyme Q is a potent antioxidant and free radical scavenger as well as membrane stabilizer (Beyer and Ernster, 1990). Besides its antioxidant properties, coenzyme Q has an important function in mitochondrial bioenergetics. It participates as a cofactor of dehydrogenases in the transport of electrons and protons as well as in ATP production (Mitchell, 1991; Crane and Navas, 1997).

Several animal models are available for experimental investigation of type 2 diabetes. One of those models is the Goto–Kakizaki (GK) rat: a nonobese, spontaneously diabetic animal (Serradas et al., 1998) produced by selective breeding of Wistar rats and first characterized by Goto and Kakizaki (Goto and Kakizaki, 1981).

Previous data from our laboratory have shown that diabetes-related mitochondrial dysfunction is exacerbated by aging and/or by the presence of the neurotoxic Aβ peptides (Moreira et al., 2003). In this line, this study was aimed to evaluate the efficacy of coenzyme Q10 (CoQ10) treatment against the mitochondrial dysfunction induced by the neurotoxic agent Aβ1–40. For this purpose, we used brain mitochondria isolated from 20-month-old GK rats and the following mitochondrial parameters were examined: respiratory indexes (RCR and ADP/O ratio), transmembrane potential, lag phase of repolarization, repolarization and ATP levels and the production of hydrogen peroxide. Studies in an aged animal model of type 2 diabetes may identify a key metabolic feature common to that disorder and late-onset AD.

Materials and methods

Materials

Aβ1–40 was obtained from Bachem AG (Bubendorf, Germany). Protease (Subtilisin, Carlsberg) type VIII, CoQ10 and soybean oil were obtained from Sigma (Portugal). Digitonin was obtained from Calbiochem. All the other chemicals were of the highest grade of purity commercially available.

Animals and experimental protocol

Male GK and control Wistar rats with 20 months of age were housed in our animal colony (Laboratory Research Center, University Hospital, Coimbra, Portugal). They were maintained under controlled light and humidity with free access to water and powdered rodent chow (diet C.R.F. 20, Charles River, France). Glucose tolerance tests were used to select GK rats for study. GK rats were randomly divided into three groups: group 1: soybean oil (vehicle solution) (2 ml/kg i.p.), group 2: CoQ10 (20 mg/kg i.p.) and group 3: GK rats without treatment. Animals were injected each 48 h during 7 weeks. Experiments were conducted 24 h after the last injection. Adhering to procedures approved by the Institutional Animal Care and Use Committee, the animals were sacrificed by cervical displacement and decapitation.

Biochemical analysis

Blood glucose was determined immediately after animals sacrifice by a glucose oxidase reaction, using a glucometer (Glucometer-Elite, Bayer) and compatible reactive tests. Hemoglobin A1c (HbA1c) levels were determined through ionic change chromatographic assay (Abbott Imx Glicohemoglobin, Abbott Laboratories, Portugal). Levels of urinary 8-hydroxydeoxyguanosine (8-OHdG) were determined using a commercially available enzyme-linked immuno-sorbent assay (Bioxytech 8-OHdG-EIA Kit, Oxis Health Products, Portland, USA).

Isolation of brain mitochondria

Brain mitochondria were isolated from male Wistar and GK rats by the method of Rosenthal et al. (1987), with slight modifications, using 0.02% digitonin to free mitochondria from the synaptosomal fraction. In brief, a rat was decapitated, and the whole brain minus the cerebellum was rapidly removed, washed, minced and homogenized at 4°C in 10 ml of isolation medium (225 mM mannitol, 75 mM sucrose, 5 mM Hepes, 1 mM EGTA, 1 mg/ml bovine serum albumin, pH 7.4) containing 5 mg of the bacterial protease. Single brain homogenates were brought to 30 ml with the isolation medium and then centrifuged at 2000×g for 3 min. The pellet, including the fluffy synaptosomal layer, was resuspended in 10 ml of the isolation medium containing 0.02% digitonin and centrifuged at 12,000×g for 8 min. The brown mitochondrial pellet without the synaptosomal layer was then resuspended again in 10 ml of medium and recentrifuged at 12,000×g for 10 min. The mitochondrial pellet was resuspended in 300 μl of resuspension medium (225 mM mannitol, 75 mM sucrose, 5 mM Hepes, pH 7.4). Mitochondrial protein was determined by the biuret method calibrated with bovine serum albumin (Gornall et al., 1949).

Membrane potential (ΔΨm) measurements

The mitochondrial transmembrane potential was monitored by evaluating the transmembrane distribution of TPP+ (tetraphenylphosphonium) with a TPP+-selective electrode prepared according to Kamo et al. (1979) using a Ag/AgCl2 electrode as reference.
Reactions were carried out in a chamber with magnetic stirring in 1 ml of reaction medium (100 mM sucrose, 100 mM KCl, 2 mM KH₂PO₄, 5 mM Hepes, 10 μM EGTA, pH 7.4) supplemented with 3 μM TPP⁺. The experiments were started by adding 5 mM succinate to mitochondria in suspension at 0.8 mg protein/ml. After a steady-state distribution of TPP⁺ had been reached (ca. 2 min of recording), Ca²⁺ was added and ∆Ψᵐ recorded. Membrane potential was estimated from the decrease of TPP⁺ concentration in the reaction medium as described elsewhere (Moreno and Madeira, 1991). Homogenates were incubated with 4 μM Aβ₁₋₄₀ for 5 min before succinate addition.

**Mitochondrial respiration**

Oxygen consumption of isolated mitochondria was monitored polarographically with a Clark oxygen electrode (Estabrook, 1967) connected to a suitable recorder in a 1 ml, thermostated, water-jacketed closed chamber, with magnetic stirring. The reactions were carried out at 30°C in 1 ml of the reaction medium with 0.8 mg protein. Homogenates were incubated with 4 μM Aβ₁₋₄₀ for 5 min before succinate addition.

**Analysis of ATP**

At the end of the ∆Ψᵐ experiments, each mitochondrial suspension was rapidly centrifuged at 14,000 rpm for 6 min with 0.3 M perchloric acid. The supernatants were neutralized with 10 M KOH in 5 M Tris and centrifuged at 14,000 rpm for 5 min. The resulting supernatants were assayed for ATP by separation in a reverse-phase high performance liquid chromatography. The chromatography apparatus was a Beckman-System Gold, consisting of a 126 Binary Pump Model and 166 Variable UV detector controlled by a computer. The detection wavelength was 254 nm, and the excitation wavelength and 420 nm as emission wavelength. The resulting supernatants were assayed for ATP by separation in a reverse-phase high performance liquid chromatography. The chromatography apparatus was a Beckman-System Gold, consisting of a 126 Binary Pump Model and 166 Variable UV detector controlled by a computer. The detection wavelength was 254 nm, and the excitation wavelength and 420 nm as emission wavelength. The rate of H₂O₂ production was calculated using a standard curve of H₂O₂.

**Measurement of H₂O₂ production**

The rate of hydrogen peroxide (H₂O₂) production was measured fluorimetrically using a modification of the method described by Barja (1999). Briefly, mitochondria were incubated at 37°C with 10 mM succinate in 1.5 ml of phosphate buffer, pH 7.4, containing 0.1 mM EGTA, 5 mM KH₂PO₄, 3 mM MgCl₂, 145 mM KCl, 30 mM Hepes, 0.1 mM homovalinic acid and 6 U/ml horseradish peroxidase in the presence or absence of 4 μM Aβ₁₋₄₀. The incubation was stopped at 15 min with 0.5 ml of cold 2 M glycine buffer containing 25 mM EDTA and NaOH, pH 12. The fluorescence of supernatants was measured at 312 nm as excitation wavelength and 420 nm as emission wavelength. The rate of H₂O₂ production was calculated using a standard curve of H₂O₂.

**Statistical analysis**

Results are presented as mean ± SEM of the indicated number of experiments. Statistical significance was determined using the one-way ANOVA test for multiple comparisons, followed by the post hoc Tukey–Kramer test. A P value < 0.05 was considered significant.

**Results**

**CoQ10 treatment decreases glycemia levels**

To confirm diabetes mellitus in GK rats glycemia, glycated hemoglobin, body weight and the urinary levels of 8-OHdG were determined (Table 1). The percentage of glycated hemoglobin (HbA₁C), glycemia and levels of 8-OHdG were significantly higher in GK rats (66.38% ± 4.89, 158.75% ± 10.52, 172.74% ± 12.1, respectively) when compared with control animals. However, body weight was significantly lower in diabetic animals (49.54% ± 2.18) when compared with control animals. CoQ10 as well as soybean oil (vehicle solution) induced a slight improvement in glycemia levels (17.45% ± 1.01 and 13.05% ± 2.83, respectively) when compared to GK rats without treatment. However, both CoQ10 and soybean oil did not induce any significant alteration in body weight, HbA₁C and 8-OHdG levels when compared to GK rats without treatment (Table 1).

**CoQ10 avoids a drastic decrease in respiratory control ratio (RCR) induced by the amyloid β-peptide**

The RCR is defined as the ratio between the states 3 (consumption of oxygen in the presence of substrate and

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**Table 1**

Characterization of Wistar control and GK diabetic rats

<table>
<thead>
<tr>
<th>Glycemia (mg/dl)</th>
<th>HbA₁C (%)</th>
<th>Body weight (g)</th>
<th>8OHdG (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wistar</td>
<td>114.9 ± 28.1</td>
<td>5.01 ± 0.08</td>
<td>760.9 ± 28.0</td>
</tr>
<tr>
<td>GK</td>
<td>297.3 ± 16.1***</td>
<td>8.34 ± 0.33***</td>
<td>383.9 ± 11.4***</td>
</tr>
<tr>
<td>GK vehicle</td>
<td>258.5 ± 7.6**</td>
<td>7.87 ± 0.06***</td>
<td>387.7 ± 3.32***</td>
</tr>
<tr>
<td>GK CoQ10</td>
<td>245.4 ± 19.1**</td>
<td>7.62 ± 0.35***</td>
<td>398.6 ± 9.03***</td>
</tr>
</tbody>
</table>

Data shown represent mean ± SEM from 6 animals for each condition studied. ***P < 0.001; **P < 0.01, statistically significant when compared with Wistar control rats.
ADP) and 4 (consumption of oxygen after ADP has been consumed) of respiration. At basal conditions, no significant alterations were induced by CoQ10 treatment (Fig. 1A). The presence of 4 μM Aβ1–40 induced a significant decrease in all the conditions tested, however, less pronounced in diabetic animals treated with both CoQ10 (15.09% ± 2.03) and soybean oil (17.12% ± 1.01) than in GK rats without treatment (19.63% ± 0.24), when compared to GK rats without treatment and in the absence of the neurotoxic peptide (Fig. 1A).

ADP/O ratio, an indicator of oxidative phosphorylation efficiency, is expressed by the ratio between the amount of ADP added and the oxygen consumed during state 3 respiration. We did not observe any significant alteration in this respiratory parameter induced by CoQ10 treatment and/or the presence of 4 μM Aβ1–40 (Fig. 1B).

CoQ10 treatment prevents the decrease in oxidative phosphorylation efficiency induced by amyloid β-peptide

ΔΨm is fundamental for the phenomenon of oxidative phosphorylation, the conversion of ADP to ATP via ATP synthase. Mitochondrial respiratory chain pumps H⁺ out of the mitochondrial matrix across the inner mitochondrial membrane. The H⁺ gradient establishes an electrochemical potential (∆ψ) resulting in a pH (∆pH) and a voltage gradient (∆Ψm) across the mitochondrial inner membrane. We observed that neither CoQ10 treatment nor Aβ1–40 exposure induced any significant alteration in the ∆Ψm as well as in the repolarization lag phase (Table 2). In this study, repolarization lag phase is defined as the time required for mitochondria to phosphorylate the ADP added to the reaction medium. Concerning the repolarization level (capacity of mitochondria to re-establish the ∆Ψm, after ADP phosphorylation), we observed that the presence of Aβ1–40 induced a significant decrease of this parameter in GK rats without treatment when compared with GK rats at basal conditions (9.55% ± 0.97) as well as when compared with GK rats treated with CoQ10 and soybean oil (10.92% ± 0.08 and 11.64% ± 0.95, respectively) (Table 2). Mitochondria isolated from diabetic animals treated with soybean oil and CoQ10 did not suffer any significant alteration of this parameter when exposed to the neurotoxic peptide.

At basal conditions, CoQ10 and soybean oil did not induce any significant alteration on ATP content of brain mitochondria. However, the presence of Aβ1–40 induced a significant decrease of this parameter in mitochondria isolated from diabetic rats without treatment (10.65% ± 3.7) when compared with GK rats at basal condition. However, CoQ10 treatment prevented the decrease in ATP levels induced by Aβ1–40 (Table 2).

CoQ10 treatment prevents the increase in hydrogen peroxide levels induced by amyloid β-peptide

The production of H₂O₂ by mitochondria gives an indication of the propensity of mitochondria to originate and/or exacerbate oxidative stress. We observed that diabetic rats mitochondria exposed to Aβ1–40 produced a significantly higher level of H₂O₂ when compared with brain mitochondria isolated from CoQ10 treated animals in the presence (127.15% ± 2.5) or absence (83.68% ± 4.4) of Aβ1–40 indicating that CoQ treatment avoids the increase of H₂O₂ production (Fig. 2).

Discussion

Neuron viability and defense against neurodegenerative injury can be achieved by targeting mitochondrial function to reduce oxidative stress, increase mitochondrial defense mechanisms or promote energetic metabolism. In this study, we observed that GK rats submitted to CoQ10 treatment...
possess brain mitochondria more resistant to \( A\beta_{1-40} \) neurotoxicity.

The characterization of diabetes mellitus in GK rats was performed by determining glycemia, glycated hemoglobin (HbA1c), urinary levels of 8-OHdG and body weight. A significant increase in glycated hemoglobin, glycemia and 8-OHdG levels (DNA oxidation) was observed in GK rats when compared with control animals (Table 1). A considerable amount of evidence suggests that oxidative stress may play an important role in the pathogenesis and complications of diabetes (Baynes, 1991, 1995; Seghrouchni et al., 2002). Different mechanisms can contribute to the enhanced oxidative stress in diabetic patients, in particular in subjects with poor glycemic control, and hypertriglyceridemia (Kitahara et al., 1980; Armstrong and Al-Awadi, 1991; Leinonen et al., 1997; Collins et al., 1998; Hinokio et al., 1999). Increased urinary excretion and higher levels of 8-OHdG in mononuclear leukocyte DNA have been found in types 1 and 2 diabetic patients (Dandona et al., 1996; Leinonen et al., 1997; Hinokio et al., 1999). Studies with the comet assay have shown increased levels of DNA breakage in peripheral blood cells of diabetic patients with poor glycemic control, but not in patients with normal glycemia (Collins et al., 1998; Anderson et al., 1998). Furthermore, we observed that GK rats present a lower body weight when compared with control animals (Table 1). The pathogenesis of diabetes in the GK rat involves an impaired insulin secretion, insulin resistance, an abnormal glucose metabolism as well as an impaired ontogenetic development of pancreatic islet cells. However, in contrast to many other rodent models of type 2 diabetes, GK rats do not become obese and do not develop hyperlipidemia (for review see Janssen et al., 2004).

Accumulating evidence suggests that mitochondrial dysfunction is intimately associated with diabetes and AD pathophysiology. Studies from our laboratory showed that \( A\beta \) inhibits the respiratory chain complexes and reduces ATP levels in PC12 cells (Pereira et al., 1998, 1999). We also reported that \( A\beta \) exacerbates the Ca\(^{2+}\)-induced opening of the mitochondrial permeability transition in isolated brain mitochondria, without inducing the permeability per se (Moreira et al., 2001, 2002). In addition, we have demonstrated that a functional mitochondria is required for \( A\beta \)-induced neurotoxicity, as investigated using \( p^+ \) and \( p^0 \) mitochondrial DNA depleted cells (Cardoso et al., 2001). Interestingly, several antioxidants (vitamin E, idebenone and reduced glutathione), melatonin and nicotine showed protective effects by maintaining the mitochondrial membrane potential, improving the activity of the respiratory complexes and the cellular energetic levels (Cardoso et al., 2001). Recently, Lustbader et al. (2004) reported that \( A\beta \) interacts with \( A\beta \)-binding dehydrogenase (ABAD) in mitochondria obtained from brains of AD patients and transgenic mice, which suggests that ABAD is a direct molecular link from \( A\beta \) to mitochondrial toxicity. Furthermore, we found that type 2 diabetes-related mitochondrial dysfunction is exacerbated by aging and/or by the

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**Table 2**

Effect of CoQ10 treatment and \( \beta \)-amyloid exposure on mitochondrial transmembrane potential (\( \Delta \Psi \)), repolarization level, repolarization lag phase and ATP content of brain mitochondria isolated from diabetic rats

<table>
<thead>
<tr>
<th></th>
<th>( \Delta \Psi \mbox{m} ) ( (-mV) )</th>
<th>Repolarization level ( (-mV) )</th>
<th>Repolarization lag phase (min)</th>
<th>ATP content (nmol/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wistar</td>
<td>178.1 ± 3.09</td>
<td>147.7 ± 2.23</td>
<td>0.668 ± 0.043</td>
<td>318.0 ± 14.72</td>
</tr>
<tr>
<td>GK</td>
<td>180.5 ± 3.35</td>
<td>152.8 ± 2.14</td>
<td>0.676 ± 0.056</td>
<td>309.8 ± 18.85</td>
</tr>
<tr>
<td>GK vehicle</td>
<td>182.0 ± 2.87</td>
<td>156.0 ± 2.96</td>
<td>0.613 ± 0.038</td>
<td>296.1 ± 16.81</td>
</tr>
<tr>
<td>GK CoQ10</td>
<td>180.7 ± 2.89</td>
<td>155.1 ± 3.50</td>
<td>0.629 ± 0.027</td>
<td>293.2 ± 12.08</td>
</tr>
<tr>
<td>GK + ( A\beta_{1-40} )</td>
<td>165.5 ± 3.58</td>
<td>138.2 ± 3.62**</td>
<td>0.721 ± 0.075</td>
<td>276.8 ± 7.40*</td>
</tr>
<tr>
<td>GK vehicle + ( A\beta_{1-40} )</td>
<td>170.9 ± 3.19</td>
<td>145.2 ± 3.49</td>
<td>0.670 ± 0.039</td>
<td>231.5 ± 19.70*</td>
</tr>
<tr>
<td>GK CoQ10 + ( A\beta_{1-40} )</td>
<td>183.0 ± 3.21</td>
<td>143.6 ± 3.61</td>
<td>0.726 ± 0.067</td>
<td>294.2 ± 12.28</td>
</tr>
</tbody>
</table>

Freshly isolated brain mitochondria (0.6 mg) in 1 ml of the reaction medium supplemented with 3 \( \mu \)M TPP and 2 \( \mu \)M rotenone were energized with 5 mM succinate. *\( P < 0.05 \) statistically significant when compared with Wistar rats. \#P < 0.05 statistically significant when compared with GK rats. \&P < 0.05 statistically significant when compared with GK rats treated with the vehicle solution. \&\&P < 0.01 statistically significant when compared with GK rats treated with CoQ10. Data shown represent mean ± SEM from 6 animals for each condition studied.
Coenzyme Q (CoQ) alterations have been suggested to be involved in diabetes (Jameson, 1991; McCarthy, 1999) and in neurodegenerative diseases such as Huntington’s disease (HD) (Koroshetz et al., 1997), Parkinson’s disease (PD) (Ebadi et al., 2001), Alzheimer’s disease (AD) (Edlund et al., 1992), and amyotrophic lateral sclerosis (Sohmiya et al., 2005). Under nonpathological processes, the capability of tissues to synthesize CoQ apparently decreases during aging (Kalen et al., 1989); however, it has been reported that CoQ biosynthesis can be affected by dietary supplementation (Kwong et al., 2002). Supplementation with CoQ10 or analogues has shown benefits in neurodegenerative processes such as PD (Sharma et al., 2004), HD (Beal and Shults, 2003) and AD (Gutzmann and Hadler, 1998), and also in mitochondrial disorders (Sobreira et al., 1997; Di Giovanni et al., 2001).

In this study, we choose the intraperitoneal administration of CoQ10, instead of CoQ10 supplemented chow, because this experimental procedure allows us to control the exact amount of CoQ10 administered to each animal. Recently, Fernández-Ayala et al. (2005) reported that HL-60 cells incorporate exogenously added CoQ10 into mitochondria increasing complex I + III and complex II + III activities. Although, we did not observe any statistical difference in mitochondrial content of CoQ10 in the three experimental groups (data not shown), the improvement of mitochondrial function can be explained by the better performance of CoQ10 as mitochondrial electron carrier. The primary role of coenzyme Q is to transfer electrons between redox components of the electron transport chain, to create a proton gradient across the inner mitochondrial membrane and drive ATP formation (Ebadi et al., 2001). Besides its role in electron transfer reactions, CoQ10 is a powerful antioxidant that has been shown to be protective against oxidative stress (Singh et al., 1998; Hoppe et al., 1999; Tomasetti et al., 1999). In this line, McCarthy et al. (2004) reported that pre-incubation of SHSY-5Y cells with water-soluble CoQ10 inhibits ROS production induced by paraquat, a nonselective herbicide. Pretreatment with CoQ10 also significantly reduced the number of apoptotic cells and DNA fragmentation. Furthermore, CoQ10 was able to inhibit mitochondrial ROS generation and inner mitochondrial depolarization induced by paraquat. Furthermore, it has been shown that supplementing the diet of rats with CoQ10 does not increase ROS production in mitochondria (Lass and Sohal, 2000; Kwong et al., 2002) but increases plasma membrane protection against oxidative stress (Gomez-Diaz et al., 2003), and extends life span (Quiles et al., 2004). In accordance, our results show that CoQ treatment avoids the increase in H2O2 production (Fig. 2) and the decrease in oxidative phosphorylation efficiency (Table 2) induced by Aβ1–40. Recently, a CoQ10 binding site has been proposed to be located on the permeability transition pore where it may inhibit its opening and thus prevent collapse of mitochondrial membrane potential (Papucci et al., 2003).

We observed that the effects of the vehicle solution (soybean oil) and CoQ10 were similar concerning glycemia (Table 1), RCR (Fig. 1) and repolarization levels (Table 2) suggesting that the effects of both components (soybean oil and CoQ10) are not synergistic nor potentiated since their coadministration did not increase the protection observed. The protective effect exerted by the soybean oil can be explained by the fact that it is a triglyceride derived from soybean that is rich in C-18 unsaturated components, tocopherols (specially γ-tocopherol, α-tocopherol is present in modest amounts), ubiquinones and other antioxidants such as bioflavonoids and aromatic compounds (Cabrin et al., 2001; Lee et al., 2000). Several studies in animals and humans have shown that the consumption of soybean has beneficial effects in a variety of diseases including diabetes and obesity (Bhathena and Velasquez, 2002) and neurodegenerative disorders (Kim et al., 2000).

In conclusion, our results show that CoQ10 treatment exerts a partial protection against Aβ1–40-induced mitochondrial dysfunction. Given the importance of mitochondria as primary source of oxidative stress in AD and diabetes, the use of CoQ10 may be useful. However, the broad occurrence of both diseases, the nonregenerative nature of the CNS and the fact that AD diagnosis often does not occur until late in disease progression, suggest that the ideal antioxidant should be used as prophylactic treatment in aged population. Due to their low toxicity, low cost and their ability to target the earliest sources of oxidative stress, CoQ treatment alone or in combination with other antioxidants is particularly attractive.

References
Barja, G., 1999. Mitochondrial oxygen radical generation and leak: sites of


Pereira, C., Santos, M.S., Oliveira, C., 1998. Mitochondrial function

Papucci, L., Schiavone, N., Witort, E., Donnini, M., Lapucci, A.,

Ott, A., Stolk, R.P., van Harskamp, F., Pols, H.A., Hofman, A., Breteler,

Moreno, A.J.M., Madeira, V.M.C., 2001. Amyloid beta-


Moreira, P.I., Santos, M.S., Moreno, A., Rego, A.C., Oliveira, C., 2001. Amyloid beta-


