Effect of Amyloid β-Peptide On Permeability Transition Pore: A Comparative Study

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A potentially central factor in neurodegeneration is the permeability transition pore (PTP). Because of the tissue-specific differences in pore properties, we directly compared isolated brain and liver mitochondria responses to the neurotoxic Aβ peptides. For this purpose, the following parameters were examined: mitochondrial membrane potential (ΔΨm), respiration, swelling, ultrastructural morphology, and content of cytochrome c. Both peptides, Aβ25–35 (50 μM) and Aβ1–40 (2 μM), had a similar toxicity, exacerbating the effects of Ca2+; although, per se, they did not induce (PTP). In liver mitochondria, Aβ led to a drop in ΔΨm and potentiated matrix swelling and disruption induced by Ca2+. In contrast, brain mitochondria, exposed to the same conditions, demonstrated a higher capacity to accumulate Ca2+ before the ΔΨm drop and a slight increase of mitochondrial swelling compared with liver mitochondria. Furthermore, mitochondrial respiratory state 3 was depressed in the presence of Aβ, whereas state 4 was unaltered, resulting in an uncoupling of respiration. In both types of mitochondria, Aβ did not affect the content of cytochrome c. The ΔΨm drop was reversed when Ca2+ was removed by EGTA or when ADP plus oligomycin was present. Pretreatment with cyclosporin A or ADP plus oligomycin prevented the deleterious effects promoted by Aβ and/or Ca2+. It can be concluded that brain and liver mitochondria show a different susceptibility to the deleterious effect of Aβ peptide, brain mitochondria being more resistant to the potentiation by Aβ of Ca2+-induced PTP.

Key words: β-amyloid peptide; permeability transition pore; mitochondria membrane potential; neurodegeneration

The main constituents of senile plaques, one of the hallmarks of Alzheimer’s disease (AD), are deposits of 39–43 amino acids long, abnormal protein aggregates called β-amyloid (Aβ) (Glenn and Wong, 1984; Selkoe et al., 1996). A smaller, 11-residue fragment of Aβ (Aβ25–35) possesses much of the biological activity of the full-length peptide, including the capacity to generate free radicals. Oxidative stress transforms soluble Aβ into insoluble and, consequently, toxic Aβ aggregates in vitro (Dyrs et al., 1992). Aβ itself has been shown to be a source of free radicals, probably because of distortions of the electronic structure of Aβ, resulting in “radicalization” of the peptide (Hensley et al., 1994). It has been suggested that internalized Aβ, through intimate contacts with intracellular recognition sites, directly damages membranes of intracellular organelles, namely, the mitochondria internal membrane associated with the mitochondria respiratory chain (MRC) (Pike and Cotman, 1993). The consequent dysfunction of MRC enhances the production of reactive oxygen species (ROS) and compromises neurons' viability as a result of energy depletion. Pereira et al. (1999) showed, in PC12 cells, that Aβ induces mitochondrial dysfunction and impairment of glycolysis, leading to ATP depletion, by a mechanism involving the generation of ROS.

Mitochondrial oxidative damage is a major factor in many human disorders, including neurodegenerative diseases and aging (Shigenaga et al., 1994; Beal, 1995, 1996). The continuous electron leak from the respiratory chain leads to the generation of damaging ROS, whose first target is mitochondria (Richter and Kass, 1991; Beal, 1996; Cay and Jones, 1999). Mitochondrial oxidative damage leads to the impairment of ATP synthesis and calcium homeostasis and induces the permeability transition pore (PTP) (Shigenaga et al., 1994; Broekemeier and Pfeiffer, 1995; Berman et al., 2000), leading to necrotic or apoptotic cell death (Gunter et al., 1994; Zoratti and Szabo, 1995). The PTP is a nonselective, high-conductance channel that spans the inner and outer mi-

Contract grant sponsor: FCT (Portuguese Research Council).
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Received 14 January 2002; Revised 18 March 2002; Accepted 18 March 2002
to the mitochondrial membrane (Bernardi et al., 1994; Zoratti and Szabo, 1995; Bernardi and Petronilli, 1996). Experimentally, induction of the PTP is characterized by an abrupt swelling and depolarization of mitochondrial membrane, accompanied by the efflux of mitochondrial calcium, reduced glutathione (GSH), and NAD(P)H (Bernardi, 1992; Petronilli et al., 1994), which are inhibited by the immuno-suppessant cyclosporin A (CsA) (Broekemeier et al., 1989). Most of the studies relative to PTP have been obtained by using mitochondria isolated from tissues such as heart, liver, and skeletal muscle. Recent studies give support to the hypothesis that, at least under certain circumstances, a PTP is also assembled in brain mitochondria (Andreyev et al., 1998; Berman et al., 2000).

In several studies (Cassarino et al., 1998, 1999), data obtained with liver mitochondria have been extrapolated to brain. However, it is well known that metabolic differences between tissues do in fact exist, leading to controversial conclusions. Accordingly, the goal of our study was to investigate and compare the effects of Aβ on permeability transition induced by Ca2+ on brain and liver mitochondria. Mitochondrial transmembrane potential (ΔΨm), respiration, swelling, ultrastructural morphology, and cytochrome c content were the parameters studied.

**MATERIALS AND METHODS**

**Chemicals**

Aβ peptide fragments (Aβ25–35 and Aβ1–40) were obtained from Bachem AG (Bubendorf, Germany). Protease (Subtilisin, Carlsberg) type VIII was obtained from Sigma (Portugal). Digitonin was obtained from Calbiochem (Portugal). Cytochrome c antibodies, against denatured forms, were obtained from PharMingen (San Diego, CA). All the other chemicals were of the highest grade of purity commercially available.

**Animals**

Male Wistar rats (6 weeks old) were kept under controlled conditions with respect to light, temperature, and humidity and with free access to water and food. Experimental animals were sacrificed by cervical displacement and decapitation. All animal procedures were approved by the Institutional Animal Care and Use Committee.

**Isolation of Mitochondria**

**Brain mitochondria.** Brain mitochondria were isolated from male Wistar rats (6 weeks old) by a method previously described (Rosenthal et al., 1987), with slight modifications. The rats were killed by decapitation, and the brains were rapidly removed 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 type VIII (Subtilisin). Brain homogenates were brought to 30 ml and centrifuged at 2,000 g for 3 min. The pellet was resuspended in 10 ml of the isolation medium containing 0.02% digitonin (which sets free mitochondria from the synaptosomal fraction) and centrifuged at 12,000 g for 8 min. The mitochondrial pellet was resuspended in 10 ml of medium and recentrifuged at 12,000 g for 10 min.

Finally, the mitochondrial pellet was resuspended in 200 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).

**Liver mitochondria.** Wistar rats (6 weeks old) were killed by cervical displacement, and mitochondria were isolated by conventional methods (Gazzotti et al., 1979), with slight modifications. Briefly, liver mitochondria were isolated in a medium containing 210 mM mannitol, 70 mM sucrose, 5 mM HEPES, 0.2 mM EGTA, 0.1 mM EDTA, and 0.1% bovine serum albumin (pH 7.2). EDTA, EGTA, and bovine serum albumin were omitted from the final washing medium. The mitochondrial pellet was washed twice and suspended in the washing medium. Mitochondrial protein was determined by the biuret method, calibrated with bovine serum albumin (Gornall et al., 1949). The functionality of isolated mitochondria was evaluated by the determination of respiratory control ratio (RCR).

**ΔΨm Measurements**

The ΔΨm was monitored by evaluating transmembrane distribution of the lipophilic cation tetraphenylphosphonium ion (TPP+) with a TPP+-selective electrode prepared according to Kamo et al. (1979) using a calomel electrode as reference. TPP+ uptake has been measured from the decreased TPP+ concentration in the medium sensed by the electrode. The potential difference between the selective electrode and the reference electrode was measured with an electrometer and recorded continuously in a Linear 1200 recorder. The voltage response of the TPP+ electrode to log[TPP+] was linear, with a slope of 59 ± 1, in good agreement with the Nernst equation. Reactions were carried out in a chamber with magnetic stirring in 1 ml of the standard medium (100 mM sucrose, 100 mM KCl, 2 mM KH2PO4, 10 μM EGTA, 5 mM HEPES, pH 7.4, supplemented with 2 μM rotenone) containing 3 μM TPP+. This TPP+ concentration was chosen to achieve high sensitivity in measurements and to avoid possible toxic effects on mitochondria (Jensen and Gunter, 1984; Wingrove and Gunter, 1985). The ΔΨm was estimated by the equation:

\[
\Delta \Psi_m (mV) = 59 \log(v/V) - 59 \log(10^{DE/59} - 1),
\]

as indicated by Kamo et al. (1979) and Muratsugu et al. (1977). v, V, and ΔE stand for mitochondrial volume, volume of the incubation medium, and deflection of the electrode potential from the baseline, respectively. This equation was derived assuming that TPP+ distribution between the mitochondria and the medium follows the Nernst equation and that the law of mass conservation is applicable. A matrix volume of 1.1 μl/mg protein was assumed. No correction was made for the “passive” binding contribution of TPP+ to the mitochondrial membranes, because the purpose of the experiments was to show relative changes in potentials rather than absolute values. As a consequence, we can anticipate a slight overestimation on ΔΨm values. However, the overestimation is significant only at ΔΨm values below 90 mV and,
therefore, far from our measurements. Nevertheless, the nonspecific binding of TPP+ to the membrane was checked by using appropriate deenergizing inhibitors to demonstrate that Aβ peptides do not affect this binding.

The assays 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 (~2 min of recording), Ca2+ was added and ΔΨm fluctuations were recorded. Aβ25–35 and Aβ1–40 were incubated 5 min before succinate addition, whereas 0.85 μM CsA and 2 μg/ml oligomycin plus 1 mM ADP were added 2 min prior to Aβ preincubation or mitochondria energization.

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 thermostatted, water-jacketed closed chamber with magnetic stirring. The reactions were carried out at 30°C in 1 ml of the standard medium with 0.8 mg protein. Aβ25–35 (50 or 100 μM) was incubated 5 min before 5 mM succinate addition, whereas 0.85 μM CsA and 2 μg/ml oligomycin plus 1 mM ADP were added 2 min prior to Aβ preincubation or mitochondria energization.

State 3 respiration is defined as consumption of oxygen in the presence of substrate and ADP. State 4 respiration is defined as consumption of oxygen after the ADP has been consumed; RCR = state 3/state 4.

Mitochondrial Swelling

Mitochondrial osmotic volume changes were estimated by changes in light scattering (apparent absorbance changes) as monitored spectrophotometrically at 540 nm (Henry et al., 1995; Palmeira and Wallace, 1997). The reactions, stirred continuously, were carried out at 30°C in 2 ml of the standard medium. Mitochondria were suspended at 0.5 mg protein/ml of standard medium. The reaction was stirred continuously and the temperature maintained at 30°C. Mitochondria were energized with 5 mM succinate for 2 min before adding Ca2+ at varying concentrations. Where indicated, Aβ25–35 (50 or 100 μM) was incubated for 5 min before succinate addition, whereas 0.85 μM CsA and 2 μg/ml oligomycin plus 1 mM ADP were added 2 min prior to Aβ25–35 preincubation or mitochondria energization. None of these reagents interfered with the spectrophotometric analysis.

Electron Microscopy

Mitochondria were fixed for electron microscopy by the addition of 3% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.3, and incubated for 2 hr at 4°C. After centrifugation (15,000g, 3 min), pellets were washed with 0.1 M sodium cacodylate buffer, pH 7.3. The fixed and washed pellet was resuspended in 1% osmium tetroxide (OsO4) buffered with sodium cacodylate 0.1 M, pH 7.3. After 2 hr of incubation, membranes were washed with cacodylate buffer (pH 7.3; without OsO4). After preincubation in 1% agar, the samples were dehydrated in grade ethanol and embedded in Spurr. The ultrathin sections were obtained in an LKB ultramicrotome Ultratome III and stained with methanolic uranyl acetate, followed by lead citrate, and examined with a Jeol Jem-100SV electron microscope operated at 80 kV.

Cytochrome c Content

At the end of the ΔΨm experiments, each mitochondrial suspension was rapidly centrifuged at 14,000 rpm for 6 min. The resulting pellets were resolved on 15% gels for SDS-PAGE, after denaturation at 100°C, for 5 min, in buffer containing 100 mM Tris–HCl, pH 6.8, 200 mM dithiothreitol (DTT), 4% SDS, 0.2% bromophenol blue, and 20% glycerol. The proteins were electrotransferred onto PVDF membranes (Hybond-P), and, after transfer, the membranes were incubated overnight at 0–4°C in blocking buffer (25 mM Tris–HCl, pH 7.6, 150 mM NaCl, and 0.05% Tween-20) containing 5% fat-free milk. Then, the blots were incubated with the antibody anticytochrome c (PharMingen) against the denatured form of the protein (1:1,000 dilution in blocking buffer containing 1% fat-free milk) for 2 hr at room temperature (RT) with gentle shaking. After washes in blocking buffer (three times, 5 min each), the immunoblots were incubated for 1 hr at RT with anti-mouse antibody alkaline phosphatase (1:1,000 dilution in blocking buffer containing 1% fat-free milk) (Amersham Pharmacia Biotech, Arlington Heights, IL), followed by an ECF substrate detection using a Storm (860 Molecular Analysis) scanner for band visualization. Cytochrome c content was quantified by determining the areas of each band; these quantifications were automatically performed by a computer program.

Statistical Analysis

Results are presented as mean (percentage of control) ± SEM of the indicated number of experiments. Statistical significance was determined using Student’s t-test, with P < 0.05 considered significant.

RESULTS

Effect of Aβ25–35 and Aβ1–40 on ΔΨm

The ΔΨm drop is a typical phenomenon that follows the induction of PTP. Figure 1 shows the alteration of ΔΨm induced by Aβ25–35 preincubated for 5 min at 30°C with mitochondria. Under brain control conditions (Fig. 1Aa), the addition of 5 mM succinate produced a ΔΨm of ~190 mV (negative inside mitochondria), corresponding to respiratory state 4. Then, the first and second pulses of Ca2+ led to rapid depolarizations (decrease of ΔΨm), followed by repolarizations (recovery of ΔΨm). The depolarizations were due to the entry of Ca2+ into the electronegative mitochondrial matrix, followed by efflux of H+ and restoration of ΔΨm. However, a third pulse of Ca2+ led to a total depolarization of mitochondria after a smaller repolarization. Mitochondria can tolerate some depolarization (50 or 100 μM) was incubated for 5 min before succinate addition, whereas 0.85 μM CsA and 2 μg/ml oligomycin plus 1 mM ADP were added 2 min prior to Aβ25–35 preincubation or mitochondria energization. None of these reagents interfered with the spectrophotometric analysis.

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of respiratory state 4 (i.e., before Ca\(^{2+}\) addition). \(\Delta \Psi \text{m}\) measured under control conditions, with or without 5 min of preincubation at 30\(^\circ\)C, did not present significant alterations (data not shown). In the presence of \(\text{A} \beta_{25-35}\) (100 \(\mu\)M) (Fig. 1Ab), a significant decrease of \(\Delta \Psi \text{m}\), measured after mitochondria energization, occurred. In the presence of \(\text{A} \beta_{25-35}\), the mitochondria were more susceptible to the amount of Ca\(^{2+}\) added, undergoing PTP in the presence of lower Ca\(^{2+}\) concentrations (after two Ca\(^{2+}\) challenges) compared to the control mitochondria. Figure 1B shows the alteration of \(\Delta \Psi \text{m}\) induced by \(\text{A} \beta_{25-35}\) in liver mitochondria. Under control conditions (Fig. 1Ba), after the energization of liver mitochondria with succinate, the first pulse of Ca\(^{2+}\) led to a rapid depolarization, followed by repolarization. However, this repolarization was not sustained, and the \(\Delta \Psi \text{m}\) dropped to basal values. The collapse of \(\Delta \Psi \text{m}\) was prevented by adding EGTA or oligomycin plus ADP, which further led to a complete restoration of the \(\Delta \Psi \text{m}\) to levels observed at respiratory state 4 (i.e., before Ca\(^{2+}\) addition) (data not shown). In the presence of \(\text{A} \beta_{25-35}\) (100 \(\mu\)M) (Fig. 1Bb), a significant decrease of \(\Delta \Psi \text{m}\), measured after mitochondria energization, occurred. Figure 2A shows that 2 \(\mu\)M \(\text{A} \beta_{1-40}\) had a similar effect on brain \(\Delta \Psi \text{m}\) compared with that induced by 50 \(\mu\)M \(\text{A} \beta_{25-35}\) (Fig. 2B), indicative of the higher toxicity inherent to \(\text{A} \beta_{1-40}\) compared with \(\text{A} \beta_{25-35}\). The preincubation (2 min) of mitochondria with 1 mM ADP plus 2 \(\mu\)g/ml oligomycin completely prevented mitochondrial membrane depolarization, by increasing dramatically the repolarization capacity of mitochondrial membrane after Ca\(^{2+}\) accumulation (Fig. 3Aa). Additionally, the presence of 0.85 \(\mu\)M cyclosporin A (CsA) (a specific inhibitor of PTP) added 2 min prior to \(\text{A} \beta_{25-35}\) and/or Ca\(^{2+}\) afforded a clear protection of mitochondria, because it prevents the depolarization induced by Ca\(^{2+}\) when \(\text{A} \beta_{25-35}\) is present (Fig. 3Ab, Ba).

**Effect of \(\text{A} \beta_{25-35}\) on Mitochondrial Respiration**

Induction of the mitochondrial permeability transition by \(\text{A} \beta_{25-35}\) is further evidenced by the effect on mitochondrial respiration (Fig. 4A, B). Addition of 100 \(\mu\)M Ca\(^{2+}\) (Fig. 4Aa) to brain mitochondria caused a transient increase in the rate of oxygen consumption, followed by a rapid returning to state 4. However, when the mitochondria were preincubated for 5 min with 50 \(\mu\)M (Fig. 4Ab) and 100 \(\mu\)M \(\text{A} \beta_{25-35}\) (Fig. 4Ac), addition of 100 \(\mu\)M Ca\(^{2+}\) resulted in a complete uncoupling of respiration; i.e., in the presence of Ca\(^{2+}\), mitochondria shown no difference in state 3 and state 4 of
respiration. In liver mitochondria, the addition of 100 μM Ca\(^{2+}\) is sufficient to cause the uncoupling of respiration (Fig. 4Ba), independently of the presence or absence of Aβ\(_{25-35}\) (Fig. 4Bb).

Preincubation (2 min) of both types of mitochondria with 1 mM ADP plus 2 μg/ml oligomycin or 0.85 μM CsA afforded clear protection, because they prevent the uncoupling of mitochondrial respiration induced by Ca\(^{2+}\) and Aβ\(_{25-35}\) (Fig. 4Ad,e,Bc,d). The effects of CsA or ADP/oligomycin in eliminating Ca\(^{2+}\) and Aβ\(_{25-35}\) depolarization were remarkable in that mitochondria could withstand successive additions of Ca\(^{2+}\), without becoming uncoupled. Respiratory rates characteristic of state 4 (succinate alone) and state 3 (ADP-stimulated respiration) are summarized in the Figure 4A,B insets. In both types of mitochondria, state 3 respiration was shown to be decreased in the presence of Aβ\(_{25-35}\) compared with control conditions, whereas state 4 remained unaltered.

**Effect of Aβ\(_{25-35}\) on Mitochondrial Swelling**

Usually, induction of the PTP is monitored by following the decrease in absorbance associated with mito-

![Fig. 2. Effect of Aβ\(_{1-40}\) on the mitochondrial membrane potential (ΔΨm). Freshly isolated brain mitochondria (0.8 mg) in 1 ml of the standard medium supplemented with 3 μM TPP\(^{+}\) and 2 μM rotenone were energized with 5 mM succinate. B: 50 μM Aβ\(_{25-35}\) was preincubated 5 min at 30°C before mitochondria energization. A: 2 μM Aβ\(_{1-40}\) was preincubated 5 min at 30°C before mitochondria energization. The traces represent typical direct recordings from three independent experiments with different mitochondrial preparations.](image1)

![Fig. 3. Inhibitory effect of CsA and oligomycin plus ADP on Aβ\(_{25-35}\)-dependent permeability transition pore opening. Brain (A) and liver (B) mitochondria were incubated at 0.8 mg protein/ml under standard conditions, as described in Materials and Methods. CsA at 0.85 μM (Ab,Ba) and 1 mM ADP plus 2 μg/ml oligomycin (Aa) were incubated with mitochondria for 2 min before adding Aβ\(_{25-35}\). Aβ\(_{25-35}\) was added 5 min before energizing the mitochondria with 5 mM succinate. The traces are typical of four or five experiments.](image2)
chondrial swelling. The addition of increasing concentrations of Ca²⁺ resulted in a decrement of the absorbance (mitochondrial swelling), which is indicative that mitochondria have a finite capacity to accumulate Ca²⁺ before undergoing PTP (Fig. 5Bd,g). Aβ25–35 potentiated the effect of Ca²⁺ on swelling also in a concentration-dependent manner (Fig. 5Be,f,h), and CsA (0.85 μM) protected against the decrease in absorbance induced by Aβ25–35 and/or Ca²⁺ (Fig. 5Bc). Aβ25–35 per se did not induce a significant decrement of absorbance (Fig. 5Bb).

Effect of Aβ25–35 on Mitochondrial Ultrastructural Morphology

Figure 6Aa shows a heterogeneous population of brain mitochondria, some mitochondria being smaller than others. All mitochondria were also shown to contain dense cristae. Incubation with 100 μM Ca²⁺ induced only a small increase in mitochondrial volume (Fig. 6Ab). In the presence of 100 μM Aβ25–35 plus 100 μM Ca²⁺ (Fig.

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This text is an excerpt from a scientific paper discussing mitochondrial swelling and the effects of Aβ25–35 on mitochondrial function and morphology. The paper examines how increasing concentrations of Ca²⁺ affect mitochondrial swelling and how Aβ25–35 influences these effects. It also investigates the role of CsA in protecting against the decrease in absorbance induced by Aβ25–35 and Ca²⁺.

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AB Promotes Permeability Transition

A. Brain

6Ac), an enlargement and deformation of brain mitochondria, with cristae disruption, were observed. ADP (1 mM) plus oligomycin (2 μg/ml) (Fig. 6Ad) protected mitochondria against the effects induced by these toxic agents, and mitochondria seemed to adopt a conformation with more compact cristae compared with control mitochondria. A similar protective effect was observed in the presence of 0.85 μM CsA (data not shown).

Under control conditions (Fig. 6Ba), rat liver mitochondria appeared as a homogeneous population of larger mitochondria compared with control brain mitochondria. Addition of 100 μM Ca²⁺ induced mitochondria enlargement, with disruption of some cristae (Fig. 6Bb). AB₂₅₋₃₅ (100 μM) potentiated the effect exerted by 100 μM Ca²⁺, leading to mitochondria swelling and less compacted cristae (Fig. 6Bc). CsA (0.85 μM) (Fig. 6Bd) prevented the swelling and cristae destruction induced by 100 μM AB₂₅₋₃₅ plus 100 μM Ca²⁺, and, similarly to brain mitochondria, liver mitochondria seemed to acquire a more compact organization of the cristae. ADP (1 mM) plus oligomycin (2 μg/ml) showed a similar protective effect (data not shown).

Fig. 6. Effect of AB₂₅₋₃₅ on mitochondrial ultrastructural morphology. At the end of incubations such as those described for Figure 1, suspensions of either rat brain (A) or rat liver (B) mitochondria were centrifuged and processed for electron microscopy as described in Materials and Methods. Aa,Ba: Control; Ab,Bb: 100 μM Ca²⁺; Ac,Bc: 100 μM AB₂₅₋₃₅ plus 100 μM Ca²⁺; Ad: 1 mM ADP plus 2 μg/ml oligomycin plus 100 μM AB₂₅₋₃₅ plus 100 μM Ca²⁺; Bd: 0.85 μM CsA plus 100 μM AB₂₅₋₃₅ plus 100 μM Ca²⁺.
Effect of Aβ25-35 on Cytochrome c Content

At the end of the experiments, such as those presented in Figures 1 and 2, each mitochondrial suspension was rapidly centrifuged, and the pellets were used for immunoblot determinations of cytochrome c content. Addition of 100 μM Ca2+ to brain (Fig. 7Ab) and liver (Fig. 7Bg) mitochondria led to a large decrement in cytochrome c content. However, 50 μM Aβ25-35 either alone (Fig. 7Bn) or in the presence of 100 μM Ca2+ (Fig. 7Ac,Bh) did not affect cytochrome c content. The release of cytochrome c was prevented in the presence of CsA (0.85 μM) (Fig. 7Bj) or ADP (1 mM) plus oligomycin (2 μg/ml) (Fig. 7Ac). Furthermore, those agents, per se, did not affect cytochrome c content. (Fig. 7Bo,p).

DISCUSSION

The existence of tissue-specific differences in mitochondria pore properties has been described (Berman et al., 2000). Accordingly, a comparative study of the responses of isolated brain and liver mitochondria to Aβ, was performed. The ΔΨm, mitochondrial respiration, swelling, ultrastructural morphology, and cytochrome c content were the parameters analyzed.

Our study clearly demonstrates that Aβ potentiates the permeability transition in brain and liver mitochondria induced by Ca2+. However, its capacity to promote PTP is different in the two types of mitochondria, depending on the resistance of the brain and liver mitochondria populations to toxic insults. Brain mitochondria possess a higher capacity to resist to the deleterious effects promoted by Aβ peptide than liver mitochondria.

A strict requirement for matrix Ca2+ and a selective inhibition by CsA (Bernardi, 1992; Zoratti and Szabo, 1995) have been recognized to be key features of PTP. Similarly to what has been described from other studies (Andreyev et al., 1998; Berman et al., 2000), brain mitochondria were shown to be more resistant to Ca2+ insults than liver mitochondria. Accordingly, we observed a large, CsA-inhibited decrease in absorbance (Fig. 5Bd,g) and a drastic alteration in the ultrastructural morphology of liver mitochondria (Fig. 6Bb). In contrast, brain mitochondria did not undergo large-amplitude swelling (Fig. 6Ab), and an increase in spectrophotometric absorbance was further observed (Fig. 5Ab,c). Similar observations were previously associated with Ca2+ precipitation within brain mitochondria (Andreyev et al., 1998). The formation of Ca2+ crystals was suggested to obscure a decrease in spectrophotometric absorbance. However, other studies demonstrated the existence of brain mitochondrial swelling (Kristal and Dubinsky, 1997; Berman et al., 2000; Brustovetsky and Dubinsky, 2000), suggesting that the differences in these results probably were due to the use of different methodologies in mitochondria isolation.

A higher resistance of brain mitochondria to the exposure to Ca2+ has been suggested to be due to the heterogeneity of the brain mitochondria population (Fig. 6Aa), composed of neuronal and glial mitochondria (Kristián et al., 2000; Berman et al., 2000). This suggestion can also be supported by the ΔΨm data (Fig. 1Aa) showing that, after a first Ca2+ challenge, a small, transient release of mitochondrial Ca2+ occurred. This phenomenon suggests that some mitochondria underwent an induction of PTP and that Ca2+ released from this subpopulation was then accumulated by other mitochondria that did not release the accumulated Ca2+.

According to the different characteristics of mitochondria reported above, the potentiation of Aβ peptide or Ca2+-induced depolarization was higher in liver mitochondria. Aβ induced a decrease in the ΔΨm (Figs. 1Ab,Bb, 2A,B) and mitochondrial light scattering (only in liver mitochondria; Fig. 5Be,f,h), accelerating the induction of PTP. Parks et al. (2001) demonstrated that the induction of the mitochondrial permeability transition in
isolated liver mitochondria by Aβ_{25–35} is concentration-dependent and requires calcium. In brain mitochondria, the increase in light scattering may be due to the exacerbation of Ca^{2+} precipitation by this peptide (Fig. 5Ad,e). These results are in agreement with other studies demonstrating that Aβ peptides can cause a harmful elevation of intracellular calcium (Mattson et al., 1992; Weis et al., 1994; Mogensen et al., 1998). Potentiation of PTP induction in the presence of Aβ suggests that, in the presence of this peptide, conditions promote Ca^{2+} cycling exist (Figs. 1Ab,Bb, 2A,B, 5B).

Although Aβ per se did not trigger the PTP, ΔΨm data indicate that Aβ alone decreased ΔΨm (Figs. 1Ab,Bb, 2A,B) This may be due to the capacity of Aβ to interact with the mitochondrial redox chain and confirms the impairment of electron transfer along the respiratory chain (Fig. 4Ab,c,Bb). The study of ΔΨm is essential for an integrated evaluation of mitochondrial function, because it reflects differences in electrical potential and represents the main component of the proton electrochemical gradient, accounting for more than 90% of the total available respiratory energy (Nicholls, 1982). Moreover, the control of respiration in isolated mitochondria is well understood (Tager et al., 1983; Balaban, 1990). Resting coupled respiration (state 4) is controlled by the proton leak through the mitochondrial inner membrane and, to a lesser extent, by the respiratory chain (Brand et al., 1988). Conversely, the respiratory chain essentially controls uncoupled respiration. Therefore, changes in both parameters may indicate that the mitochondrial processes were affected. It was previously shown that other agents, such as protonophore uncouplers, decrease ΔΨm and promote the induction of PTP.

Although our results show that Aβ promotes PTP opening, they did not refute the channel-forming ability of Aβ. As shown by others, Aβ also can operate as a membrane channel, allowing the entry of ions, such as Ca^{2+} (Mizzbekov et al., 1994; Zhu et al., 2000), thus having an effect similar to that of other ion channel-forming peptides, such as the protonophore gramicidin A (Cohen, 1975). However, the action of these compounds on mitochondrial permeability transition has not been evaluated.

A reduced rate of brain metabolism is one of the best-documented abnormalities in AD. The activities of several important enzymes of energy metabolism have been shown to be reduced in the AD brain. These include the pyruvate dehydrogenase complex, the α-ketoglutarate dehydrogenase complex, and the cytochrome oxidase complex (Blass, 1997; Blass et al., 1997; Poirier et al., 1999). There is also strong evidence showing an increase in oxygen consumption in comparison with glucose utilization, in brain, from positron emission tomography (Fukuyama et al., 1994).

Our data also showed that 2 μM Aβ_{1–40} (Fig. 2A) had a similar toxic effect compared with that of 50 μM Aβ_{25–35} (Fig. 2B). The higher toxicity inherent to Aβ_{1–40} comparatively to Aβ_{25–35} was also shown by others (Her-
sant peptide and specific inhibitor of PTP CsA. The effect of CsA on the ability of brain mitochondria to accumulate Ca\textsuperscript{2+} was only moderate compared with the effects described for CsA on liver or muscle mitochondria (Fontaine et al., 1998; Brustovetsky and Dubinsky, 2000; Dubinsky et al., 1999; Kristian et al., 2000). Similar effects of CsA were also found with cell lines (Murphy et al., 1996) and neuronal primary cultures (Dubinsky and Levi, 1998). Although CsA does not give total protection against PTP induction, it increases the capacity of brain mitochondria to accumulate Ca\textsuperscript{2+} (Fig. 3Aa). However, when ADP and oligomycin were present initially, the Aβ25-35- and Ca\textsuperscript{2+}-induced depolarization was prevented. Brustovetsky and Dubinsky (2000) reported previously the protection exerted by ADP plus oligomycin on brain mitochondria before calcium challenge.

As observed by electron microscopy, CsA (Fig. 6Bd) and ADP plus oligomycin (Fig. 6Ad) induced protection against mitochondrial swelling. Furthermore, we observed that those agents rendered the cristae more compacted, suggesting a defense mechanism against toxic insults.

These findings demonstrate that the neurotoxic peptide Aβ is shown to act as a respiratory chain uncoupler, capable of promoting the induction of PTP in both brain and liver mitochondria. Accordingly, the modulators of mitochondrial permeability transition and cytochrome c release can be envisaged as the targets in novel therapies for neurodegenerative disorders.

ACKNOWLEDGMENT

P.I.M. is the recipient of grant PRAXIS XXI/BM/20952/99.

REFERENCES


