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β-Amyloid Fragment 25–35 Causes Mitochondrial Dysfunction in Primary Cortical Neurons

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β-Amyloid deposition and compromised energy metabolism both occur in vulnerable brain regions in Alzheimer's disease. It is not known whether β-amyloid is the cause of impairment of energy metabolism, nor whether impaired energy metabolism is specific to neurons. Our results, using primary neuronal cultures, show that 24-h incubation with $A\beta_{25-35}$ caused a generalized decrease in the specific activity of mitochondrial enzymes per milligram of cellular protein, induced mitochondrial swelling, and decreased total mitochondrial number. Incubation with $A\beta_{25-35}$ decreased ATP concentration to 58% of control in neurons and 71% of control in astrocytes. Levels of reduced glutathione were also lowered by $A\beta_{25-35}$ in both neurons (from 5.1 to 2.9 nmol/mg protein) and astrocytes (from 25.2 to 14.9 nmol/mg protein). We conclude that 24-h treatment with extracellular $A\beta_{25-35}$ causes mitochondrial dysfunction in both astrocytes and neurons, the latter being more seriously affected. In astrocytes mitochondrial impairment was confined to complex I inhibition, whereas in neurons a generalized loss of mitochondria was seen.

Key Words: **β-Amyloid; Alzheimer's disease; mitochondria; neuron; astrocyte.**

INTRODUCTION

Alzheimer's disease (AD) is characterized by the accumulation of neurofibrillary tangles and neuritic plaques in the brain. The plaques contain a dense core of β-amyloid (Aβ), surrounded by dystrophic neurites and activated glial cells. A substantial deficit of energy metabolism is also a feature of AD. Impaired glucose metabolism, independent of brain atrophy, has been identified using brain imaging techniques (Ibanez *et al.*, 1998). There is evidence for a profound deficit of mitochondrial metabolism including reduced activity of the enzyme complexes cytochrome oxidase (Kish *et al.*, 1992; Mutisya *et al.*, 1994; Parker, Jr. *et al.*, 1994), pyruvate dehydrogenase (PDHC) (Sorbi *et al.*, 1983), and α-ketoglutarate dehydrogenase (α-KGDHC) (Butterworth and Besnard, 1990; Mastrogiacoma *et al.*, 1996). In addition, recent studies have provided evidence of a specific loss of functional mitochondria in affected brain regions of AD patients (de la Monte *et al.*, 2000; Hirai *et al.*, 2001).

The role of Aβ in the disruption of energy metabolism observed in AD brain is not well-defined. Cytotoxic concentrations of Aβ have consistently been shown to inhibit the chemical reduction of 3-(4,5-dimethylthiazol-2-*yl*)-2,5-diphenyltetrazolium bromide (MTT) in a variety of cultured cell types. Although this could possibly be interpreted as evidence for Aβ-induced mitochondrial impairment, this conclusion has been called into question by Liu and colleagues (Liu and Schubert, 1997), who showed that Aβ inhibits MTT reduction by enhancing MTT formazan exocytosis, not by inhibiting mitochondrial redox activity.

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Direct evidence that Aβ can inhibit mitochondrial energy metabolism in the neuronal cell line PC12 has been reported by Pereira and colleagues. PC12 cells treated with Aβ were shown to have reduced ATP levels, decreased respiratory chain complex activities, depolarized mitochondrial membrane and decreased oxygen consumption (Pereira *et al.*, 1998, 1999). These changes preceded loss of cell viability. More recently, it was reported that functional mitochondria were required for Aβ toxicity in NT2 cells (Cardoso *et al.*, 2001), suggesting that Aβ toxicity was mediated via effects on mitochondria. However, the energy metabolism of these cell lines differs from that of primary neurons in that, for example, they are less dependent upon oxidative phosphorylation. Therefore we have examined the effects of Aβ on energy metabolism in primary cultures of neurons and astrocytes.

Recent studies have identified significant mitochondrial abnormalities in AD brain. In particular, morphometry of biopsy brain tissue from AD patients and controls indicated a significant decrease in the number of intact mitochondria and an increase in lipofuscin, a marker of autophagic mitochondrial breakdown (Hirai *et al.*, 2001). Studies in our laboratory (Canevari *et al.*, 1999) and others (Parks *et al.*, 2001) have demonstrated that Aβ directly inhibits cytochrome oxidase in isolated mitochondria and in the latter study Aβ was shown to induce a cyclosporin A-sensitive mitochondrial permeability transition.

$A\beta_{25-35}$ has been used in a number of studies and found to have many effects in common with full length $A\beta_{1-40/42}$ (Pike *et al.*, 1995; Mark *et al.*, 1997b; Pereira *et al.*, 1999). The shortened fragment, 25–35, contains the residues essential for aggregation and toxicity, including the methionine 35 (Pike *et al.*, 1995). $A\beta_{25-35}$ aggregates rapidly to form β-pleated sheet structure without the need for aging procedures required for $A\beta_{1-40/42}$ aggregation. The toxicity of $A\beta_{25-35}$ is very similar to that of $A\beta_{1-42}$ on hippocampal neuronal cultures as assessed by percentage cell loss (Pike *et al.*, 1993). Moreover, we have found similar effects of $A\beta_{25-35}$ and $A\beta_{1-42}$ upon isolated mitochondria and mitochondrial enzymes (Casley *et al.*, 2002). In this study we have examined the effects of $A\beta_{25-35}$ on mitochondrial energy metabolism in cultured neurons and astrocytes.

MATERIALS AND METHODS

Materials

$A\beta_{25-35}$ and the soluble, nonaggregating reverse sequence peptide, $A\beta_{35-25}$, were purchased from Bachem Ltd. (Merseyside, UK). Stock solutions of the peptides were dissolved in ultra pure water (Milli-Q standard, Millipore, Watford, UK) at a concentration of 5 mM. Aliquots were stored at −70°C. Tissue culture reagents were purchased from Gibco (Paisley, UK). Acetoxymethyl calcein (calcein-AM) and tetramethylrhodamine methyl ester (TMRM) were purchased from Molecular Probes Europe BV (Leiden, The Netherlands). Unless stated otherwise, all chemicals were obtained from Sigma Chemical Company (Poole, UK).

Cell Culture

Isolation of cells was performed essentially as described in (Tabernero *et al.*, 1993) and (Vicario *et al.*, 1993). Astrocytes: newborn rat pups (1- to 2-day-old) were decapitated and cortex was removed. Tissue was dissociated physically and by trypsin digestion. Cells were grown in minimum essential medium supplemented with 10% (v/v) fetal bovine serum and 20 μM l-glutamine. After 13 days in culture cells were removed from flasks by trypsin digestion and seeded onto tissue culture plates at a density of 10^6 cells/well (1500 cells/mm^2). Cells were treated 24 h later.

Neurons: embryonic day 17 foetuses were removed from the uterus and the forebrains dissected. Tissue was dissociated as above. Cells were seeded onto poly-ornithine-coated cell culture plates or glass coverslips at a density of 2.5×10^6 cells/well (3750 cells/mm^2). After 3 days in culture, cytosine arabinofuranoside (10 μM final concentration) was added to the medium in order to kill non-neuronal cells such as glia. Cells were maintained in culture for 6 days before treatment.

Enzyme Activity Determination

All enzyme activities were measured spectrophotometrically at 30°C. Cell suspensions were subjected to three freeze-thaw cycles to permeabilise membranes prior to enzyme assays. Lactate dehydrogenase (LDH; EC 1.1.1.27) activity from the supernatant of cell cultures and from solubilised cell homogenates was determined using the method of (Vassault, 1983). Mitochondrial respiratory chain complex activities were measured by the following methods: Complex I (EC 1.6.99.3) (Ragan *et al.*, 1987), complex II–III (EC 1.8.3.1) (King, 1967), complex IV (cytochrome oxidase) (EC 1.9.3.1) (Wharton and Tzagoloff, 1967). The mitochondrial matrix enzyme citrate synthase (EC 4.1.3.7) was measured by the method of (Shepherd and Garland, 1969).

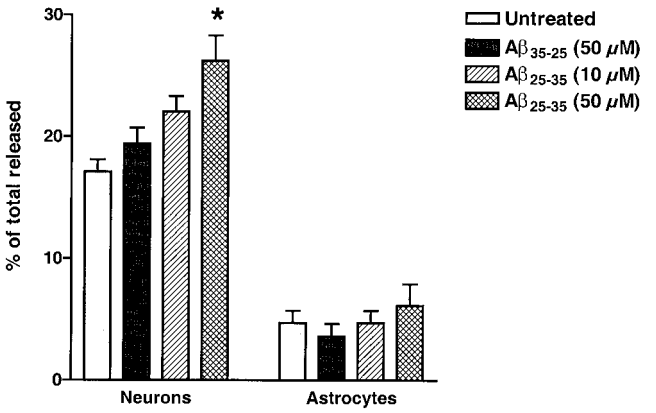

FIG. 1. Astrocyte and neuron cultures were incubated for 24 h under the following conditions: untreated, $A\beta_{35-25}$ (50 μM), $A\beta_{25-35}$ (10 μM), or $A\beta_{25-35}$ (50 μM). The LDH activity in the extracellular medium is expressed as a percentage of the total activity of the solubilised cells. *$P < 0.05$ compared with untreated neurons, ANOVA with Dunnett's post hoc test, mean ± SEM, $n = 5–8$.

Confocal Microscopy

Mitochondrial membrane potential was measured by confocal microscopy. Neurons were grown on poly-l-ornithine-coated glass coverslips and treated for 24 h. The cells were then loaded with calcein-AM (500 nM for 10 min) and then washed and bathed in 30 nM TMRM, which was allowed to equilibrate for 30 min. Coverslips were then mounted in a chamber on the stage of a confocal laser scanning microscope (model 510; Carl Zeiss Ltd., Hertfordshire, UK). Images were acquired using a 488-nm argon laser to excite calcein fluorescence and a 543-nm HeNe laser to excite TMRM fluorescence. The signals were collected at 505–530 nm (calcein) and at >570 nm (TMRM). The calcein filled the cytosol, allowing definition of the cell boundaries, while TMRM accumulated in mitochondria in response to the mitochondrial membrane potential $\Delta\psi_{mito}$. Signal from control cells (treated with $A\beta_{35-25}$) and cells treated with $A\beta_{25-35}$ were compared using identical settings for laser power, confocal thickness (1 μm) and detector sensitivity. The images were analyzed in two ways: First, the mean TMRM signal was measured per live cell. Second, the mitochondrial volume fraction was calculated by applying a signal threshold to the images, which were then binarized. Thus, the value of every pixel containing a mitochondrial structure was set to one, every empty pixel was set to zero. A similar routine was used on the calcein image, giving a value for the whole cell volume. The ratio of these two measurements then gives the fraction of the cell occupied by mitochondria. All images were constructed using projections through the z axis of the cells and therefore include all signal from each cell. Analysis was performed using the software packages, Lucida 5 (Kinetic Imaging Ltd., Wirral, UK), LSM browser 2.8 (Carl Zeiss Ltd.), and Photoshop 5 LE (Adobe, CA). At least 20 cells were measured per experiment. Results are an average of four experiments.

Electron Microscopy

Neurons were treated with $A\beta_{25-35}$ or $A\beta_{35-25}$ 50 μM for 24 h as described previously. The cells were then washed twice with HBSS and fixed for 2 h at 4°C in 3% glutaraldehyde, 0.1 M sodium cacodylate buffer, pH 7.4. After rinsing in PBS and postfixation in 1% aqueous osmium tetroxide at room temperature for 30 min, samples were dehydrated in graded ethanol solutions and embedded in epoxy resin. Thin sections (70 nm) were stained with methanolic uranyl acetate and aqueous lead citrate and examined by transmission EM on a JOEL 1200EX electron microscope.

Determination of Adenine Nucleotides

Adenosine triphosphate (ATP) was measured as in (Smolenski and Yacoub, 1993). Samples were deproteinized using 0.4 M perchloric acid and then neutralized to pH 6 using 3 M K_3PO_4. Reverse phase HPLC employed a mobile phase gradient (150 mM KH_2PO_4 with acetonitrile varying from 0 to 15%) and UV detection at 254 nm.

Determination of Reduced Glutathione

Intracellular reduced glutathione (GSH) was determined using reverse phase HPLC with coulometric electrochemical detection based on the method of (Riederer *et al.*, 1989). The mobile phase consisted of orthophosphoric acid (15 mM, pH 2.6). Cells were deproteinised with an equal volume of 30 mM orthophosphoric acid and the supernatants used for analysis.

Protein Determination

Protein concentration was determined by the method of (Lowry *et al.*, 1951) using bovine serum albumin (BSA) as protein standard.

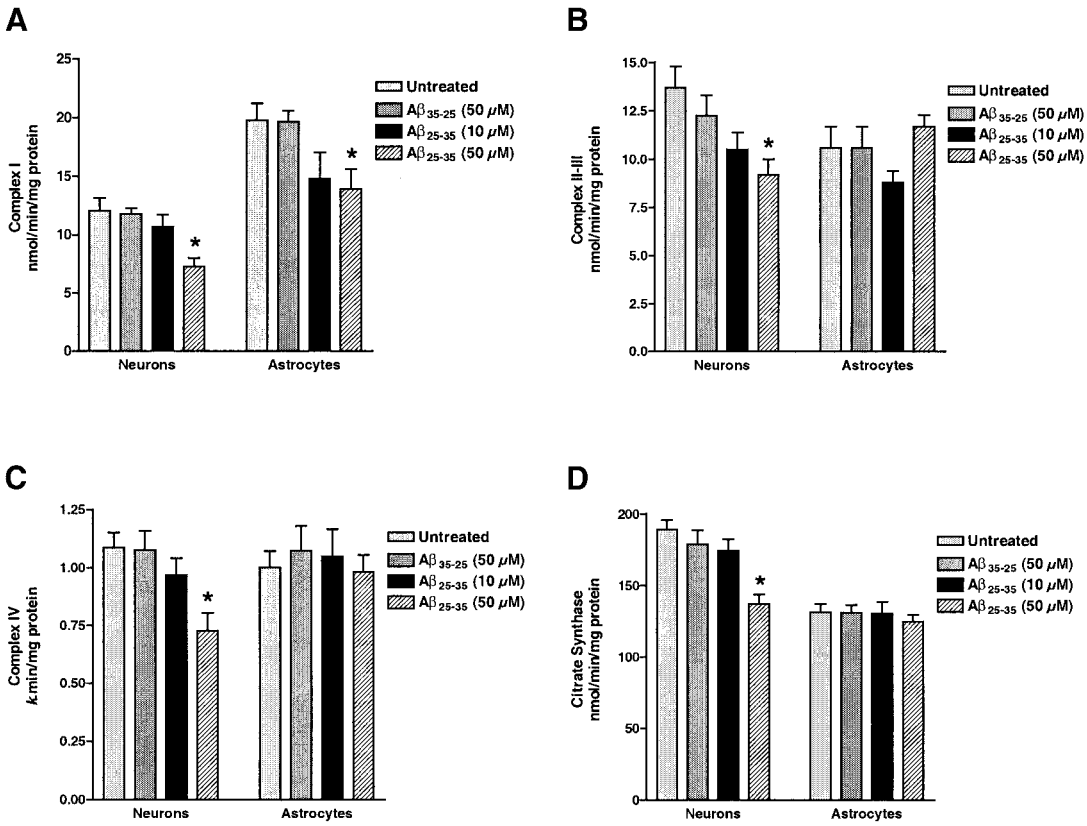

FIG. 2. Astrocytes and neurons were incubated for 24 h with the following treatments: untreated, 50 μM A$\beta_{35–25}$, 10 μM A$\beta_{25–35}$, and 50 μM A$\beta_{25–35}$. Results are expressed as nmol.min^{-1}.mg protein^{-1} for complex I (A), complex II–III (B), citrate synthase (D), and as rate constant k.min^{-1}.mg protein^{-1} for complex IV (C). *$P < 0.05$ compared with untreated control of the same cell type, ANOVA with Dunnett's post hoc test. Mean ± SEM, $n = 5$.

Statistical Analysis

Data is presented as mean ± SEM. One-way or two-way ANOVA was used for statistical comparison. Where significant differences were detected, the Dunnett's post-test for multiple comparisons was applied. For single comparisons the two-tailed t test was applied. Statistical analysis was performed using the Graphpad Prism package (Graphpad Software Inc., CA).

RESULTS

Toxicity of A$\beta_{25–35}$

Toxicity of A$\beta_{25–35}$ in both neuronal and astrocytic cultures was assessed by LDH release. Consistent with previous reports (Yankner *et al.*, 1990; Mattson *et al.*, 1992), 24-h incubation with A$\beta_{25–35}$ was toxic to neuronal cultures (Fig. 1). However, LDH release was not significantly increased by A$\beta_{25–35}$ treatment of astrocyte cultures (Fig. 1). Astrocyte cultures also displayed a significantly lower basal LDH release. Incubation with the reverse peptide, A$\beta_{35–25}$ (50 μM), did not alter LDH release in either cell type.

Effects of Aβ on Mitochondria

In order to investigate A$\beta_{25–35}$-induced mitochondrial damage, the activities of the respiratory chain complexes were measured in cultured cortical astrocytes and neurons following 24-h incubation with A$\beta_{25–35}$. In neuron cultures, the activity of citrate synthase and all respiratory chain complexes expressed relative to cellular protein was significantly decreased by treatment with 50 μM A$\beta_{25–35}$ (Fig. 2). Incubation with A$\beta_{35–25}$ (50 μM) did not have any effect on the activity of any of the enzymes (Fig. 2). The uniformity

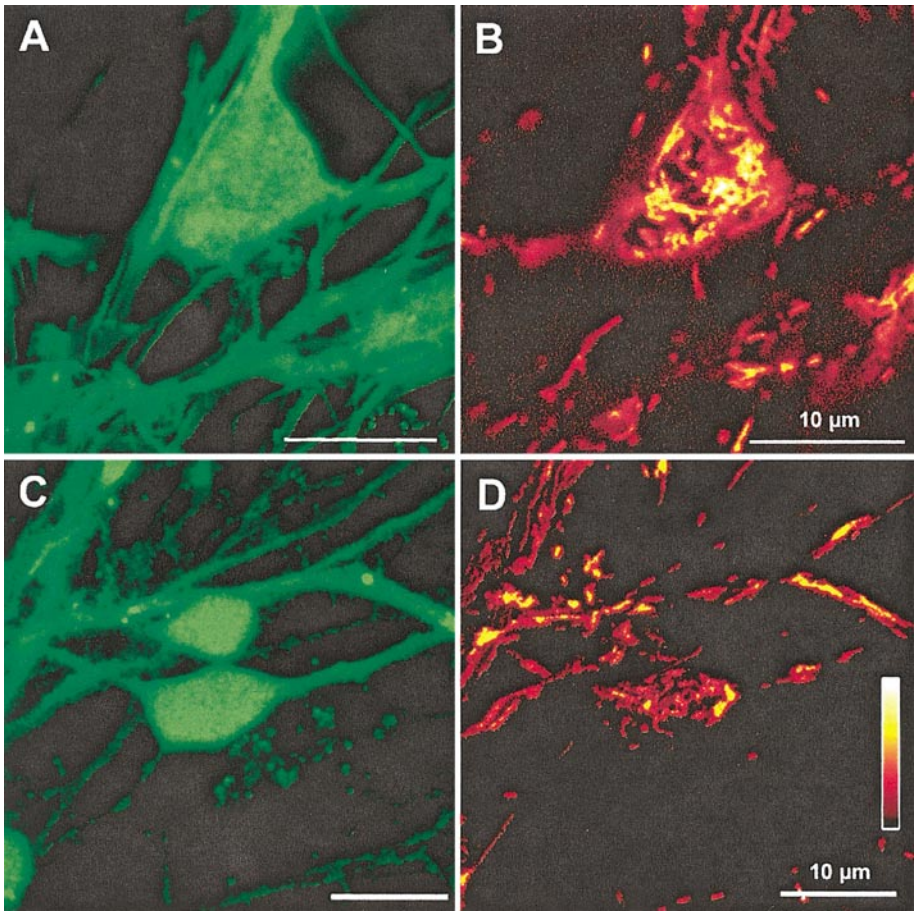

FIG. 3. Representative confocal fluorescence micrographs of cortical neurons treated for 24 h with 50 μM Aβ_{25-35} or Aβ_{35-25}. Neuronal cultures were incubated with 500 nM calcein-AM and 30 nM TMRM prior to imaging. (A) Calcein fluorescence micrograph of neurons treated with Aβ_{35-25}. (B) The same field as shown in (A) displaying TMRM fluorescence signal. (C) Calcein fluorescence micrograph of neurons treated with Aβ_{25-35}. (D) The same field as shown in (C) displaying TMRM fluorescence signal. Scale bar equals 10 μm. All images are projections through the entire z-axis of the cells.

of the inhibition of the complexes (complex I down 40%, complex II–III down 33%, complex IV down 33%) together with a similar loss of citrate synthase activity (down 27%) suggests that Aβ_{25-35} caused a generalized loss of mitochondria in neurons. The loss of mitochondrial enzyme activity cannot be explained by the cell death alone, as this concentration of Aβ_{25-35} caused no more than 10% cell loss. In addition, the majority of the dead cells detached from the substrate and were washed away prior to measurement of enzyme activities.

There was no evidence for a generalised decrease in the activity of mitochondrial enzymes in astrocyte cultures treated with Aβ_{25-35}. Incubation with 50 μM Aβ_{25-35} for 24 h significantly inhibited complex I, but no effects were seen on complexes II–III, IV or citrate synthase activity (Fig. 2). Aβ_{35-25} was also without effect.

In order to further characterise the Aβ_{25-35}-induced loss of mitochondria in neurons, we examined Aβ_{25-35}-induced changes in mitochondrial membrane potential ($\Delta\psi_{mito}$) and mitochondrial volume fraction using confocal imaging. Figure 3 shows representative fluorescence micrographs of neurons treated for 24 h with 50 μM Aβ_{25-35} (Figs. 3A and 3B) or 50 μM Aβ_{25-35} (Figs. 3C and 3D). Cells were loaded with calcein-AM and TMRM as described under Materials and Methods. The images were constructed by summation of all slices through the z axis and therefore include fluorescence of the entire cell volume. The mean TMRM signal per cell was significantly lower in Aβ_{25-35}-treated neurons (Fig. 4). This result indicates that either Aβ_{25-35}-treatment caused depo-

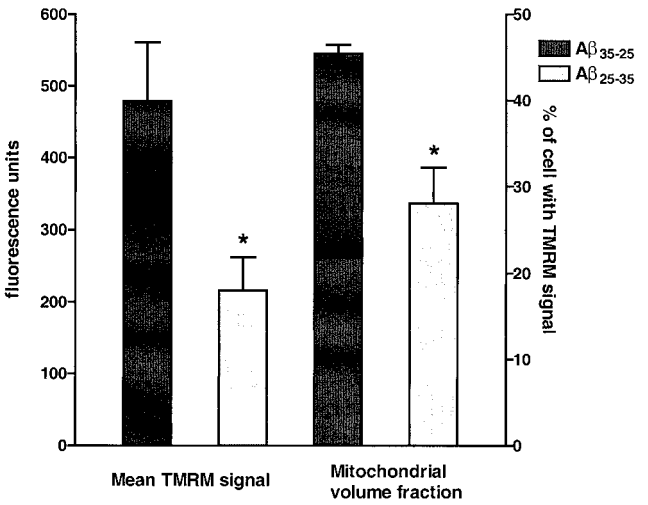

FIG. 4. TMRM signal from cortical neuronal cultures treated for 24 h with 50 μM Aβ_{25-35} or Aβ_{35-25}. The mean TMRM signal per live cell is shown in arbitrary units of fluorescence (left bars; left y-axis). The average mitochondrial volume fraction per cell is also shown (right bars; right y-axis). *$P < 0.05$ compared with Aβ_{35-25}-treated, Student's t test, $n = 4$, at least 20 cells examined per experiment.

larisation of mitochondria or it caused a loss of mitochondria. In order to distinguish between these two possibilities we measured the proportion of the cell occupied by mitochondria. We set a low threshold of TMRM signal, above which we took to indicate presence of mitochondria. Using this analysis we were able to examine changes in total mitochondrial volume fraction independently from changes in $\Delta\psi_{mito}$. We found that Aβ_{25-35}-treated neurons had a significantly decreased percentage of the cell occupied by mitochondria (Fig. 4).

We also examined mitochondrial morphology in treated neuron cultures using electron microscopy. Figure 5 shows representative electron micrographs of neurons following 24 hour treatment with either 50 μM Aβ_{35-25} (A) or 50 μM Aβ_{25-35} (B). Aβ_{35-25}-treated neurons displayed dark elongated mitochondria (Fig. 3A), typical of normal neuronal mitochondria (personal communication, Professor D. Landon, Electron Microscopy Unit, Institute of Neurology). Mitochondria in Aβ_{25-35}-treated neurons, by comparison, were swollen, an appearance typical of mitochondria that have undergone permeability transition (see for review (Bernardi *et al.*, 1999)).

Effects of Aβ on Cellular ATP Levels

Incubation with 50 μM Aβ_{25-35} for 24 h caused a significant decrease in ATP concentration in both neurons and astrocytes (Fig. 6). The magnitude of the decrease was greater in neurons (down 42% in neurons vs 29% in astrocytes). Aβ_{35-25} had no effect on ATP concentration in either cell type (Fig. 6).

Effects of Aβ on Intracellular GSH Levels

Neuronal GSH concentration was significantly decreased by 24 h incubation with 50 μM Aβ_{25-35} (down 43%), while 50 μM Aβ_{35-25} was without effect (Fig. 7). Intracellular GSH concentration was also decreased in astrocytes following 24 h treatment with 50 μM Aβ_{25-35} (down 41%) (Fig. 7). However, the absolute concentration of GSH in treated astrocytes remained higher than that of neurons.

DISCUSSION

Aβ_{25-35} treatment of neurons induced a uniform loss of activity of the mitochondrial respiratory chain complexes and the mitochondrial matrix enzyme, citrate synthase. These data are consistent with a generalised loss of neuronal mitochondria. Further support for Aβ_{25-35}-induced mitochondrial loss in neurons comes from our findings that average TMRM fluorescence per cell and the proportion of cytosol occupied by mitochondria were both decreased in Aβ_{25-35}-treated neurons compared with Aβ_{35-25}-treated controls. The decrease in average TMRM fluorescence per cell could not be entirely accounted for by loss of mitochondria, which indicates that those mitochondria that remained in Aβ_{25-35}-treated neurons were also partially depolarised. Examination of mitochondrial morphology in this study using electron microscopy of Aβ_{25-35}-treated neurons showed mitochondrial swelling, an indication that Aβ_{25-35} causes mitochondrial damage in neurons. The fact that we observed a decrease in the mitochondrial volume fraction, despite the swelling of individual mitochondria, is further evidence that Aβ_{25-35}-treatment of neurons caused a significant loss of mitochondria. It has been reported that the number of mitochondria is decreased in affected brain regions of AD patients (de la Monte *et al.*, 2000; Hirai *et al.*, 2001). Hirai and colleagues (2001) reported that this loss was due to an increase in autophagic breakdown of neuronal mitochondria in AD brain, as assessed by levels of lipofuscin. Our data support the hypothesis that Aβ may be the cause of the mitochondrial dysfunction and mitochondrial loss observed in AD brain. Although Aβ_{25-35} is not found in significant quantities in the AD brain or CSF, its ability to form aggregates of similar β-sheet structure to that of full-length A$\beta_{1-40/42}$ suggests

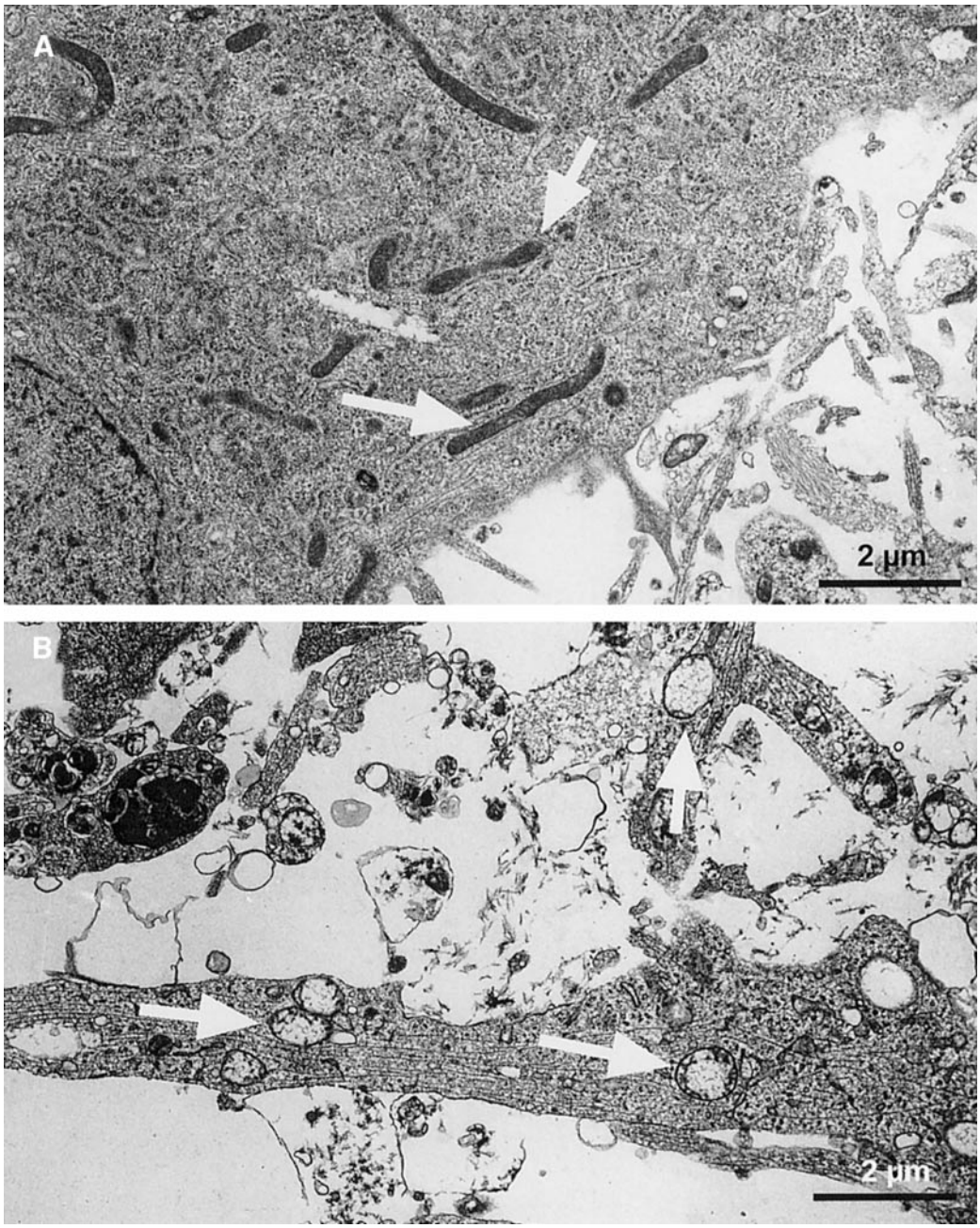

FIG. 5. Representative electron micrograph of cultured cortical neurons treated for 24 h with 50 μM Aβ_{35-25} (A) or 50 μM Aβ_{25-35} (B). Scale bar equals 2 μm. Arrows indicate mitochondria. Note the swollen mitochondria evident in B.

that Aβ_{25-35} is a useful tool for predicting the possible effects of full-length Aβ peptides. However, further work is necessary in order to verify whether endogenous Aβ peptides also cause mitochondrial loss in cortical neurons.

The mechanism by which Aβ damages mitochondria is not known. One possibility is that Aβ peptides are internalized by neurons and come into direct contact with mitochondria. Aβ has been shown to inhibit cytochrome oxidase in isolated brain mitochondria (Canevari *et al.*, 1999) and isolated liver mitochondria (Parks *et al.*, 2001). We have recently shown that Aβ directly inhibits mitochondrial respiration and key mitochondrial enzymes in isolated brain mitochondria

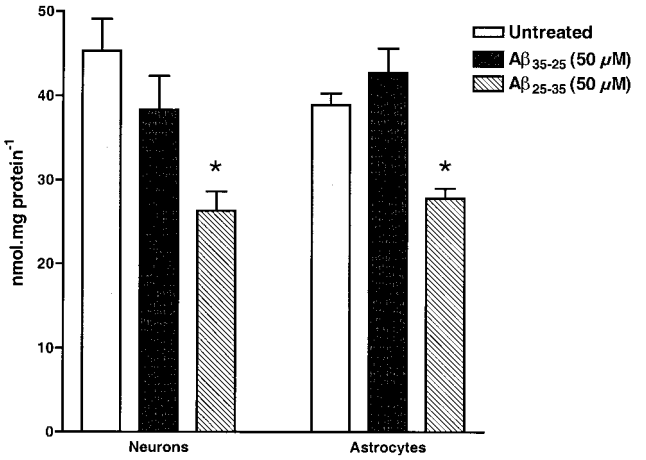

FIG. 6. Intracellular ATP concentration in cortical neuron and astrocyte cultures following 24 hour incubation without treatment or treated with $A\beta_{35-25}$ or $A\beta_{25-35}$ (both at 50 μM concentration). Expressed as nmol.mg protein^{-1}. *$P < 0.05$ compared with control of the same cell type, ANOVA with Dunnett's post hoc test, Mean ± SEM, $n = 5–8$.

(Casley *et al.*, 2002). However, in the present study, we did not observe selective loss of cytochrome oxidase activity, but a generalised loss of mitochondria, suggesting that $A\beta_{25-35}$ added extracellularly does not directly interact with mitochondria. This finding highlights the difference in the direct and indirect effects of Aβ on mitochondria, which may have important implications for the neurobiology of AD. Mitochondrial damage due to extracellular Aβ could be caused by 4-hydroxy-2-nonenal (HNE), generated as a result of Aβ-induced lipid peroxidation at the plasma membrane. HNE formation due to interaction of Aβ with membrane has been observed (Mark *et al.*, 1997a) and was suggested to mediate toxic effects of Aβ on hippocampal neurons. Aβ has also been shown to induce HNE formation in synaptosomes at concentrations that also caused significant depletion of ATP (Keller *et al.*, 1997). Further work is necessary to determine whether HNE has a role in Aβ-induced mitochondrial loss in neurons.

The disruption of neuronal mitochondrial function reported here was reflected in a significant decrease in ATP concentration. $A\beta_{25-35}$ has also been shown to decrease ATP levels in PC12 cells by causing mitochondrial dysfunction (Pereira *et al.*, 1999). However, Aβ is able to affect energy metabolism at a number of other sites. For example, Aβ inhibited glucose uptake in cortical synaptosomes (Keller *et al.*, 1997), cultured hippocampal and cortical neurons (Mark *et al.*, 1997b), and cultured astrocytes (Parpura-Gill *et al.*, 1997). Aβ also directly inhibited phosphofructokinase, one of the key enzymes of glycolysis (Bigl and Eschrich, 1995). It is therefore not surprising that $A\beta_{25-35}$ caused a decrease in ATP levels in astrocytes, despite having only a small effect on respiratory chain activity. ATP was decreased more in neurons than astrocytes, a result that may be explained by the Aβ-induced mitochondrial dysfunction in neurons.

The differential susceptibility of astrocytes and neurons to Aβ toxicity may be explained at least in part by the significantly higher levels of GSH found in cultured astrocytes compared with neurons. Higher astrocytic GSH concentration has been suggested to explain the differential susceptibility of neurons and astrocytes to peroxynitrite-mediated mitochondrial damage and toxicity (Bolaños *et al.*, 1995). There is considerable evidence from other studies that the toxicity of Aβ can be ameliorated by exogenous GSH. Elevation of GSH concentration by using cell-permeant GSH ethyl ester was sufficient to protect cultured hippocampal neurons against the toxicity of both 50 μM $A\beta_{25-35}$ and 10 μM HNE (Mark *et al.* 1997a). GSH ethyl ester also prevented inhibition of mitochondrial respiratory chain complexes in PC12 cells treated with $A\beta_{25-35}$ (Pereira *et al.*, 1999). GSH was shown to detoxify HNE by direct reaction (Hartley *et al.*, 1995), which may partly explain its ability to protect against the toxicity of Aβ. Although both cell types examined in this study displayed similar per-

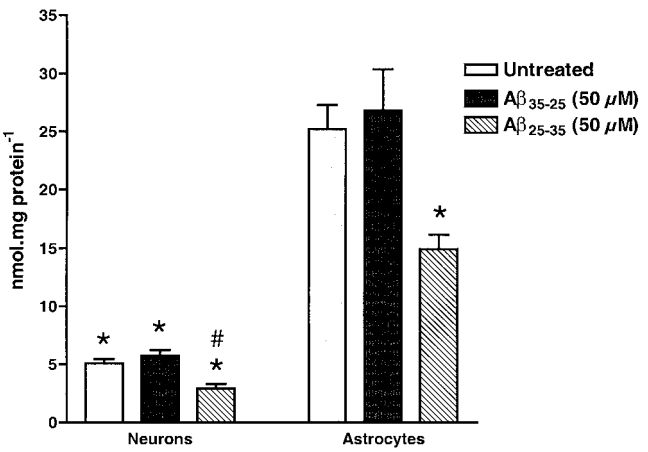

FIG. 7. Cultured astrocytes and neurons were incubated for 24 h without treatment or with $A\beta_{35-25}$ or $A\beta_{25-35}$ (both at 50 μM concentration). Intracellular glutathione concentration is expressed as nmol of GSH per milligram of protein. *$P < 0.05$ compared with untreated astrocytes; #$P < 0.05$ compared with untreated neurons, ANOVA with Tukey post hoc test. Mean ± SEM, $n = 3–4$.

centage loss of GSH upon treatment with $A\beta_{25-35}$, astrocytes maintained an absolute GSH concentration above that of control neurons. Therefore astrocytes may be capable of retaining GSH levels above a threshold, which affords them protection against Aβ toxicity. Other, as yet unidentified, cell type-specific differences between neurons and astrocytes may also contribute to their differential susceptibility to Aβ toxicity and Aβ-induced mitochondrial dysfunction.

This study not only highlights the importance of mitochondrial dysfunction in the toxicity of Aβ, but also provides an explanation for the selective toxicity of Aβ to neurons. Our results indicate that neurons are particularly susceptible to mitochondrial dysfunction, whereas astrocytes are more resistant. We suggest that extracellular Aβ causes mitochondrial loss in neurons, while intracellular Aβ, which is able to directly interact with mitochondria, could be partly responsible for specific enzyme defects associated with AD.

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