β-Amyloid-Induced Cytotoxicity, Peroxide Generation and Blockade of Glutamate Uptake in Cultured Astrocytes

Maria L. de Ceballos¹, Begoña Brera¹ and Maria Paz Fernández-Tomé²

¹ Cajal Institute, CSIC, Madrid, Spain
² Institute of Pharmacology and Toxicology, CSIC, Faculty of Medicine, Complutense University, Spain

β-Amyloid (βA) is cytotoxic to neurons in culture by increasing hydrogen peroxide and altering calcium homeostasis. We have evaluated βA-induced cytotoxicity, peroxide generation and glutamate (Glu) uptake in cultured astrocytes. Twenty-four hours after a single addition of either βA25–35 or βA1–40 there was a concentration-dependent decrease in viability. Catalase or vitamin E showed no protective effect against βA25–35. Dithiothreitol (DTT), N-acetylcysteine (NAC) and cyclosporine A significantly prevented the toxic effects of both βA25–35 and peroxide, while inhibition of peroxide detoxifying enzymes enhanced toxicity. Exposure to βA25–35 or βA1–40 increased peroxides at 2 h and 24 h, which was prevented by DTT and NAC, but not vitamin E. βA25–35 inhibited Glu uptake in astrocytes and neurons in culture. Following exposure of neurons to βA for 24 h there was decreased uptake and increased Glu levels in the culture medium, that resulted in gradual excitotoxicity.

Key words: Alzheimer’s disease; β-Amyloid; Glutamate; Peroxides.

Abbreviations: AD, Alzheimer’s disease; βA, β-Amyloid; CNS, central nervous system; DHK, dihydrokainic acid; DTT, dithiothreitol; Glu, glutamate; MTT, 3-[4,5-dimethyl-thiazol-2-yl]-2,5-diphenyltetrazolium bromide; NAC, N-acetylcysteine; SCR, scrambled peptide.

β-Amyloid (βA) is the major component of senile plaques and vascular deposits present in Alzheimer’s disease (AD). This 39-42 amino acid peptide is cleaved from the amyloid precursor protein and several lines of evidence indicate that βA plays a crucial role in AD pathogenesis (1). βA is cytotoxic to neurons and clonal cell lines in culture by increasing hydrogen peroxide and altering calcium homeostasis (2, 3).

Astrocytes are crucial for normal central nervous system (CNS) function, providing ion homeostasis and metabolic substrates, controlling extracellular concentrations of glutamate (Glu), synthesizing neurotrophic factors, and finally, protecting neurons from stress. We have evaluated the cytotoxicity of βA peptides (βA25–35 and βA1–40; 3-[4,5-dimethyl-thiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay) and generation of hydrogen peroxide (dichlorofluorescein fluorescence) on cortical cultured astrocytes. Since Glu transport is sensitive to oxidants (4) it was also studied in primary neuronal and astrocyte cultures (5). Glu uptake was studied along that of dihydrokainic acid (DHK), prototype of astrocyte Glu transport inhibitors. βA was more potent than DHK in inhibiting Glu uptake and the effect of both was more marked on astrocytes than on neurons (Figure 2). Following experiments done in quintuplicate. *p<0.05 different vs. untreated cultures or SCR; **p<0.05 different vs. single treatments (Student’s t test).

Fig. 1 Interference with free radical formation prevented βA and peroxide toxicity. Astrocyte cultures were exposed to the drugs and viability measured at 24 h. Open columns: scrambled peptide (SCR), hatched columns: βA25–35, cross-hatched columns: hydrogen peroxide. Results are mean±SEM of two experiments done in quintuplicate. *p<0.05 different vs. untreated cultures or SCR; **p<0.05 different vs. single treatments (Student’s t test).
Following exposure to βA for 24 h there was decreased uptake and increased Glu levels in the culture medium. Interestingly, βA gradually (2–3 days after βA addition) induced toxicity in neuronal cultures, while DHK, which at the highest concentration tested slightly increased Glu levels in the medium, was not toxic. In agreement with previous work (6) we have shown that βA inhibits Glu uptake in astrocytes but, more importantly, we here demonstrate that it inhibits the uptake in neurons as well. Furthermore, this results in Glu excitotoxic levels that may be involved in the gradual toxicity of βA to neurons in culture. In summary, the effects of βA on astrocytes may play an important role in the neuronal function and survival and may be relevant to AD pathology where oxidative stress has been demonstrated.

Acknowledgements

Funded by the Ministry of Education and the European San doz Foundation for Gerontological Research.

References


Received 22 November 2000, revised 13 February 2001, accepted 26 February 2001

Corresponding author: Dr. María L. de Ceballos, Cajal Institute, CSIC, Doctor Arce, 37, 28002 Madrid, Spain Tel:+34 91 5854716, Fax: +34 91 5854754 E-mail: mceballos@cajal.csic.es