Effect of β-Amyloid Peptide Fragment 25-35 on Nonselective Permeability of Mitochondria

E. F. Shevtzova, E. G. Kireeva, and S. O. Bachurin

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 132, No. 12, pp. 652-656, December, 2001
Original article submitted June 22, 2001

β-Amyloid peptide fragment 25-35 potentiated phosphate- and calcium-induced opening of mitochondrial channels and caused swelling of mitochondria (even without exogenous calcium and phosphate). These changes were accompanied by accumulation of lipid peroxidation products in mitochondria. Specific inhibitors of mitochondrial channels ADP and cyclosporine A prevented β-amyloid peptide-induced swelling of mitochondria. Our findings suggest that potentiation of mitochondrial channel opening is an important component of the neurodegenerative effect of β-amyloid.

Key Words: *mitochondria; β-amyloid; neurodegeneration; cyclosporine-sensitive mitochondrial channels; lipid peroxidation*

Recent studies showed that mitochondria play a key role in the development of neurodegenerative disorders, including Alzheimer's disease [2,5]. Selective death of brain neurons and accumulation of insoluble multicomponent plagues formed from fibrils of β-amyloid peptides containing 39-42 amino acids are the major pathomorphological signs of this disease. Published data indicate that β-amyloid plays an important role in the pathogenesis of Alzheimer's disease [8,15]. The neurotoxic effects of β-amyloid include modulation of free radical formation and lipid peroxidation (LPO) and impairment of calcium homeostasis and redox potential in cells [13]. There are data that neuronal death during Alzheimer's disease occurs via apoptosis [6]. Opening of specific calcium-dependent cyclosporine-sensitive nonselective channels in the inner mitochondrial membrane triggers and regulates apoptosis in neurons. Dysfunction of mitochondria, which is manifested in accumulation of free radicals, decrease in the transmembrane potential, and oxidative damages to mitochondrial DNA, probably underlies the early cytotoxic effect of β-amyloid [5]. These changes can result from both the interaction of extracellular β-amyloid with various targets on the neuronal membrane and toxic effects of intracellular β-amyloid oligomers [10].

β-Amyloid-induced death of nerve cells is probably mediated by its direct influence on mitochondria. Here we studied the effects of β-amyloid peptide fragment 25-35 (βA_{25-35}) on permeability of the inner mitochondrial membrane and LPO in mitochondrial membranes. βA_{25-35} contains a hydrophobic region consisting of 7 amino acids residues 29-35 arranged in a β-helix. This fragment rapidly forms soluble aggregates. The structure of fragment 25-35 determines high toxicity of abnormal β-amyloid peptides [3]. Therefore, βA_{25-35} is widely used to study the mechanisms underlying neurotoxicity of β-amyloid.

MATERIALS AND METHODS

Liver mitochondria were isolated from Wistar rats by differential centrifugation in buffer A containing 210 mM mannitol, 70 mM sucrose, 5 mM HEPES, and 1 mM EDTA (pH 7.4) [12]. Buffer B containing 210 mM sucrose, 70 mM KCl, 5 mM HEPES, and 1 mM EDTA (pH 7.4) was used for evaluation of LPO intensity and mitochondrion swelling. This buffer contained no mannitol binding hydroxyl ions. Finally, the mitochondria were washed and suspended

Laboratory of Neurochemistry, Institute of Physiologically Active Substances, Russian Academy of Sciences, Chernogolovka. ***Address for correspondence:*** shevt@ipac.ac.ru. Shevtzova E. F.

0007-4888/01/1326-1173$25.00

in EDTA-free buffers A and B. Rat brain mitochondria were obtained by centrifugation in a Percoll density gradient by the method of N. R. Sims [14]. Protein content was measured by the biuret method.

Opening of mitochondrial channels (OMC) was studied spectrophotometrically by evaluating mitochondrial swelling in EDTA-free buffers A and B containing 0.8 mM rotenone, 5 mM succinate, and isolated mitochondria (1 mg protein/ml). The measurements were performed on a Beckman DU 640 spectrophotometer at 540 nm, 25°C, and constant stirring. The rate of swelling was determined as ΔA_{540}/min/mg protein and calculated as the tangent of the slope angle of the most precipitous segment in the curve representing the time dependence of A_{540}.

LPO intensity in mitochondrial membranes was estimated spectrophotometrically by accumulation of thiobarbituric acid-reactive substances (TBARS) [7].

Experiments were performed with at least 3 preparations of isolated mitochondria. Figures represent results of one representative experimental trial.

RESULTS

Published data suggest that mitochondria are the most probable intracellular target for the cytotoxic effect of β-amyloid. Our experiments showed that peptide βA_{25-35} in concentrations of 10-20 mM significantly potentiated phosphate- and calcium-induced OMC in rat liver mitochondria (Figs. 1, *a*, *b*). Moreover, βA_{25-35} in concentrations of 25-100 mM induced dose-dependent changes in the mitochondrial shape in calcium- and phosphate-free media (Fig. 1, *c*).

βA_{25-35}-induced potentiation of reactions to phosphate and calcium and swelling of mitochondria were suppressed with 0.2 mM ADP. However, β-amy-

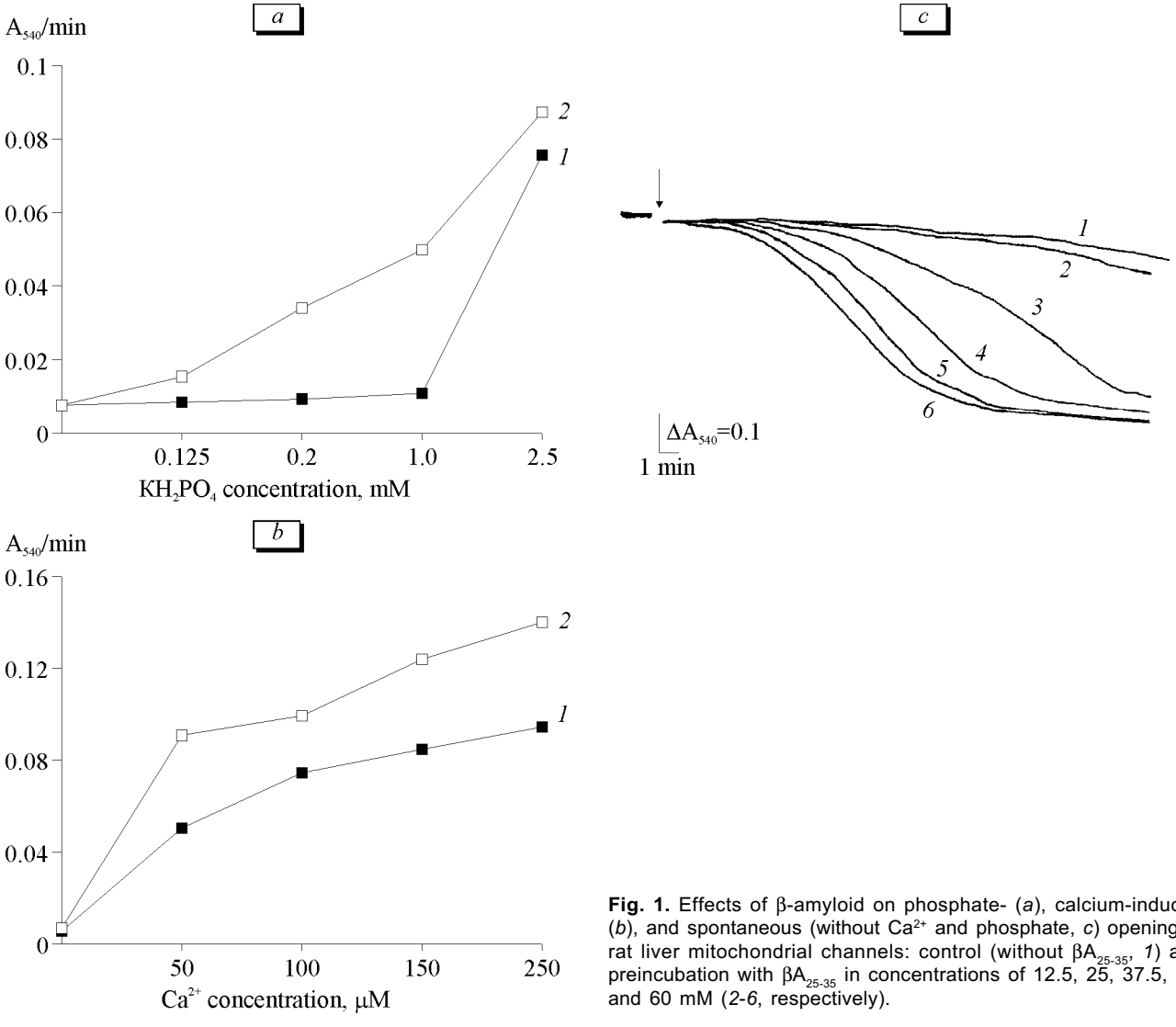

Fig. 1. Effects of β-amyloid on phosphate- (*a*), calcium-induced (*b*), and spontaneous (without Ca^{2+} and phosphate, *c*) opening of rat liver mitochondrial channels: control (without βA_{25-35}, *1*) and preincubation with βA_{25-35} in concentrations of 12.5, 25, 37.5, 50, and 60 mM (*2-6*, respectively).

loid markedly attenuated inhibition of phosphate- and calcium-induced swelling of mitochondria. Increasing the time of preincubation in the medium for measuring OMC (5, 25, and 50 min) was accompanied by progressive acceleration of mitochondrial swelling. However, addition of ADP into the incubation medium blocked OMC at various preincubation periods. βA_{25-35} in a concentration of 12.5 mM 4-fold intensified mitochondrial reactions to 1 mM KH_2PO_4 and abolished potentiation of OMC in response to prolongation of preincubation. Moreover, βA_{25-35} considerably decreased the ability of ADP to prevent changes in the shape of mitochondria induced by 1 mM KH_2PO_4. It should be emphasized that this effect depended on the time of preincubation. The rate of swelling decreased by 17 and 3 times after preincubation for 5 and 50 min, respectively.

β-Amyloid in low concentrations that did not potentiate mitochondrial reactions to phosphate and calcium decreased the ability of ADP to inhibit OMC. In the presence of 2 mM βA_{25-35}, ADP suppressed calcium-induced changes in the shape of mitochondria by 47% (*vs*. 67% without β-amyloid). At the same time, specific OMC inhibitor cyclosporine A in a concentration of 2 mM completely blocked β-amyloid-induced changes in mitochondrial shape (Fig. 2, *a*). These results suggest that βA_{25-35}-induced changes in mitochondrial shape are related to opening of specific nonselective mitochondrial channels.

In our experiments we used mainly rat liver mitochondria. However, in special experimental series we demonstrated similar effects of βA_{25-35} on brain mitochondria (Fig. 3, *a*, *b*). β-Amyloid considerably potentiated the response of brain mitochondria to phosphate and calcium. A lower sensitivity of brain mitochondria to β-amyloid and other OMC inductors can be related to heterogeneity of not only these organelles, but also of the initial cell preparation [4]. However, β-amyloid produced similar effects on mitochondria in the brain and liver.

Taking into account published data that the toxic effect of β-amyloid is associated with its ability to stimulate LPO and accumulation of free radicals, we studied TBARS formation [3]. Samples were taken after 30-min recording of mitochondrial swelling. βA_{25-35} intensified LPO in mitochondria (Fig. 2, *b*). Cyclosporine A in a concentration of 2 mM slightly inhibited TBARS formation, but completely blocked βA_{25-35}-induced changes in mitochondrial shape (Fig. 2, *a*, *b*). βA_{25-35} caused swelling of mitochondria in media containing and not containing mannitol, a hydroxyl radical trap. Probably, β-amyloid-induced OMC is only partially associated with its ability to initiate LPO. It was shown that antioxidants prevent oxidative stress in cells, but not their death induced by amyloid peptides βA_{25-35} and βA_{1-42} [11].

Our previous experiments on cerebellar granular cells demonstrated that βA_{25-35} produces a cytotoxic effect and causes apoptosis in cells [1]. It should be emphasized that βA_{25-35} in similar concentrations dose-dependently decreases neuronal survival (IC_{50}= 25 mM), induces OMC in preparations of rat liver mitochondria, and potentiates calcium- and phosphate-induced OMC in brain mitochondria.

Our results suggest that the neurodegenerative effect of β-amyloid is associated with its direct influence on mitochondria [10], opening of specific mitochondrial channels, and initiation of programmed cell death (apoptosis). Probably, β-amyloid-induced apoptosis serves as a protective physiological mechanism that underlies elimination of cells with high content of free radicals and oxidatively damaged

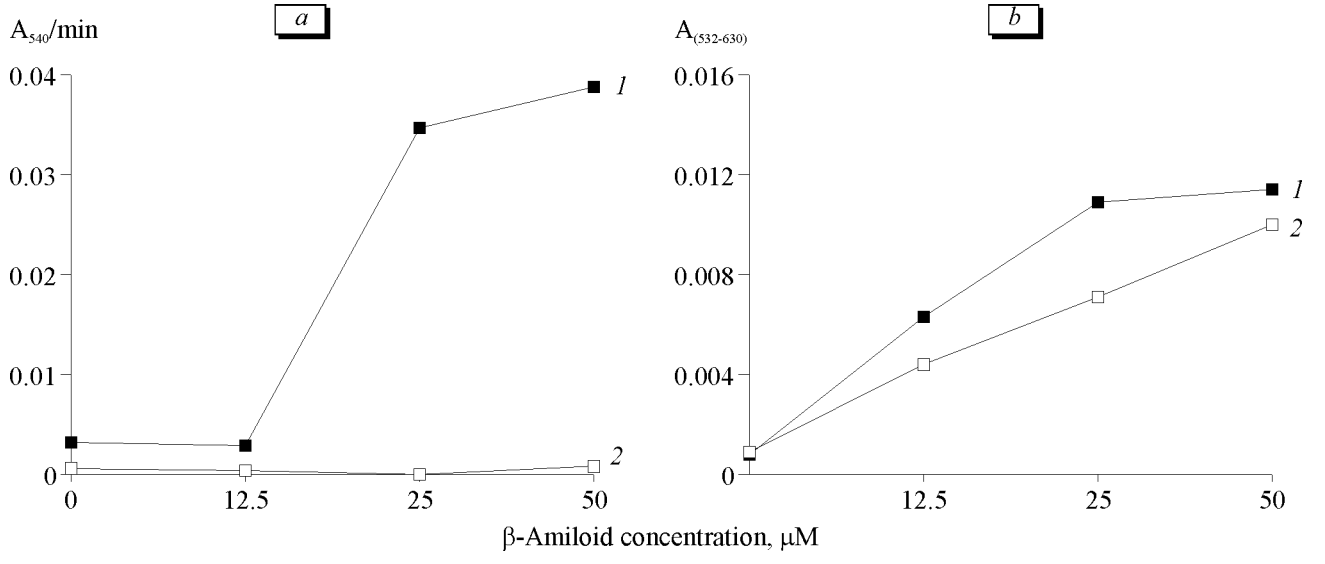

Fig. 2. Effect of cyclosporine A (2 mM) on β-amyloid-induced swelling of mitochondria (*a*) and LPO (*b*): βA_{25-35} (*1*) and βA_{25-35}+cyclosporine A (*2*).

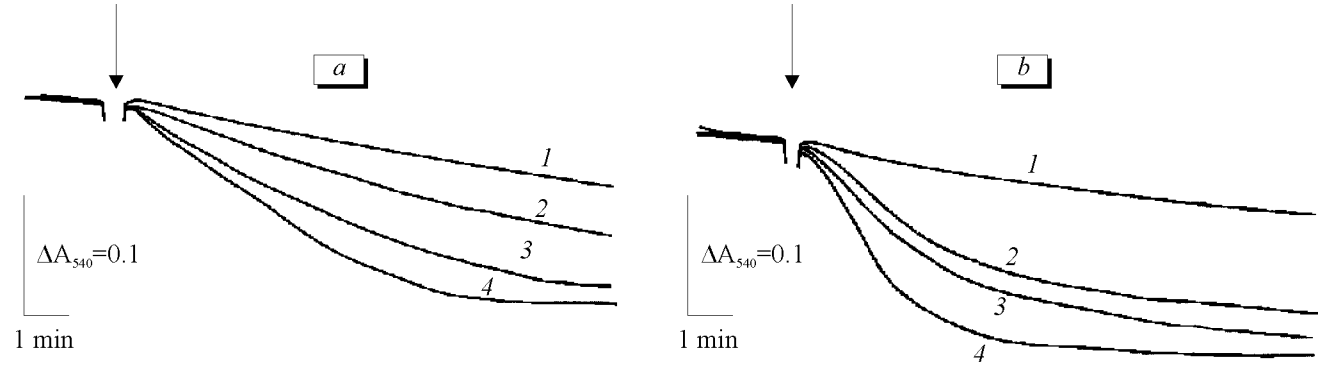

Fig. 3. Potentiation of phosphate- (*a*) and calcium-induced (*b*) changes in the shape of rat brain mitochondria by β-amyloid. Arrows indicate treatment with KH_2PO_4 (1 mM, *a*) and Ca^{2+} (0.05 mM, *b*). Spontaneous changes in the shape of mitochondria (*1*), induction of channel opening with phosphate (*a*) or Ca^{2+} (*b*, *2*), and 5-min preincubation with βA_{25-35} in concentrations of 25 (*3*) and 50 mM (*4*).

mitochondrial genome. These pathological changes are related to the appearance of aggregated β-amyloid peptides in cells. However, the number of these errors markedly increases with age. Moreover, if the mitochondrial genome includes mutations specific for Alzheimer's disease, β-amyloid itself can initiate the formation of its toxic forms by triggering apoptosis. This progressive autocatalytic cascade of cytotoxic processes contributes to neurodegeneration in the brain during Alzheimer's disease.

This work was supported by the Russian Foundation for Basic Research (grant No. 00-04-48398).

REFERENCES

1. S. Bachurin, N. Lermontova, E. Shevtzova, *et al*., *J. Neurochem*., **71**, Suppl. 1, S68 (1998).
2. M. F. Beal, *Trends Neurosci*., **23**, 298-304 (2000).
3. C. Behl, *Cell Tissue Res*., **290**, 471-480 (1997).
4. S. B. Berman, S. C. Watkins, and T. G. Hastings, *Exp. Neurol*., **164**, 415-425 (2000).
5. D. S. Cassarino and Jr. J. P. Bennett, *Brain Res. Rev*., **29**, 1-25 (1999).
6. C. W. Cotman, E. R. Whittemore, J. A. Watt, *et al*., *Ann. N. Y. Acad. Sci*., **747**, 36-49 (1994).
7. W. L. Erdahl, R. J. Krebsbach, and D. R. Pfeiffer, *Arch. Biochem. Biophys*., **285**, 252-260 (1991).
8. J. A. Hardy and G. A. Higgins, *Science*, **256**, 184-185 (1992).
9. B. L. Martin, G. Schrader-Fischer, J. Busciglio, *et al*., *J. Biol. Chem*., **270**, 26,727-26,730 (1995).
10. J. K. Parks, T. S. Smith, P. A. Trimmer, *et al*., *J. Neurochem*., **76**, 1050-1056 (2001).
11. C. J. Pike, N. Ramezan-Arab, and C. W. Cotman, *Ibid*., **69**, 1601-1611 (1997).
12. R. Ramsay, R. Mehlhorn, and T. Singer, *Biochim. Biophys. Res. Commun*., **159**, 983-990 (1989).
13. M. N. Sabbagh, D. Galashko, and L. J. Thal, *Alzheimer Dis. Rev*., **3**, 1-19 (1998).
14. N. R. Sims, *J. Neurochem*., **55**, 698-707 (1990).
15. D. H. Small and C. A. McLean, *Ibid*., **73**, 443-449 (1999).