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Soluble Beta-Amyloid Leads to Mitochondrial Defects in Amyloid Precursor Protein and Tau Transgenic Mice

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Key Words
β-Amyloid · P301L mutant tau · Alzheimer's disease · Mitochondrial dysfunction · Energy deficit · β-Amyloid oligomers · Transgenic mice

Abstract
Background: Mitochondrial dysfunction has been identified in neurodegenerative disorders including Alzheimer's disease, where accumulation of β-amyloid (Aβ) and oxidative stress seem to play central roles in the pathogenesis, by probably directly leading to mitochondrial dysfunction. ***Objective:*** In order to study the in vivo effect of Aβ load during aging, we evaluated the mitochondrial function of brain cells from transgenic mice bearing either mutant amyloid precursor protein (tgAPP) or mutant amyloid precursor protein and mutant PS1 (tgAPP/PS1) as well as from nontransgenic wild-type littermates. tgAPP mice exhibit onset of Aβ plaques at an age of 6 months, but the intracellular soluble Aβ load is already increased at 3 months of age. In contrast, onset of Aβ plaques starts at an age of 3 months in tgAPP/PS1 mice. In addition, we investigated the effects of different Aβ preparations on mitochondrial function of brain cells from tau transgenic mice. ***Results:*** Of note, mitochondrial damage such as reduced mitochondrial membrane potential and ATP levels can already be detected in the brains from these mice before the onset of plaques. In agreement with our findings in tgAPP mice, soluble Aβ induced mitochondrial dysfunction in brain cells from tau transgenic mice. ***Conclusion:*** Our results indicate that mitochondrial dysfunction is exacerbated by the presence of soluble Aβ species as a very early event during pathogenesis.

Introduction

Alzheimer's disease (AD) is the most frequent form of dementia among the elderly and is characterized by the neuropathological hallmarks of extracellular amyloid plaques and intracellular neurofibrillary tangles in the brain of AD patients. Amyloid plaques are composed of the β-amyloid (Aβ) protein, derived from its precursor protein APP. Neurofibrillary lesions are formed from paired helical filaments composed of hyperphosphorylated tau protein, a microtubule-associated protein.

In recent years, attempts have been undertaken to identify the toxic species of Aβ. The focus of attention has since shifted from fibrillar to oligomeric species of Aβ as the large, insoluble Aβ deposits which form the amyloid plaques in the limbic and association cortices of AD patients are in equilibrium with small, diffusible oligomers of the peptide that appear capable of interfering with hippocampal synaptic function and memory [1, 2]. In addi-

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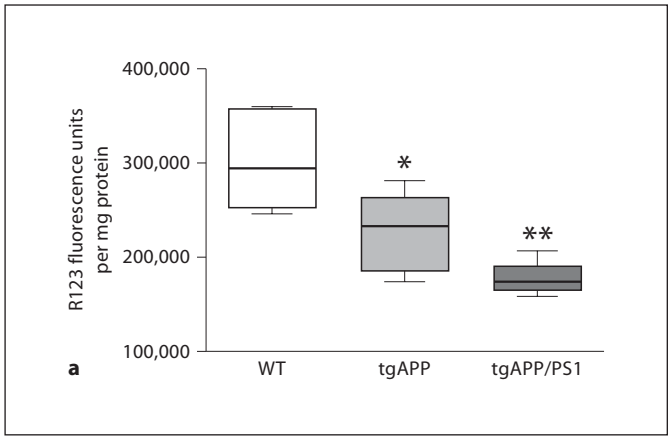

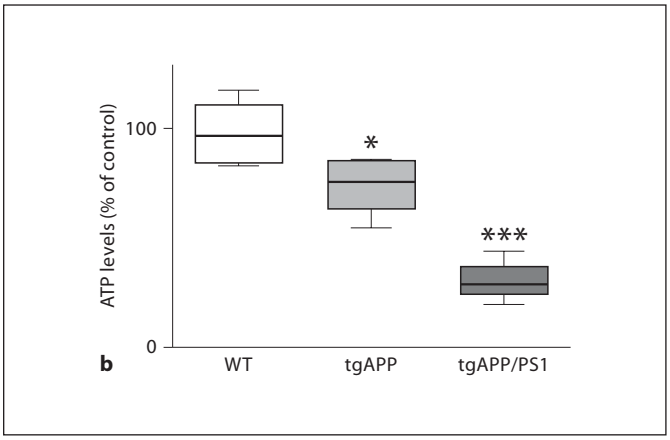

Fig. 1. a Decreased mitochondrial membrane potential in cortical brain cells from transgenic mice. Mitochondrial membrane potential in brain cells from 3-month-old tgAPP and tgAPP/PS1 mice after 4 h of in vitro incubation was significantly reduced compared to WT mice. Data are presented as a box-and-whisker graph: the box extends from the 25th percentile to the 75th percentile, with a line at the median, the whiskers extend above and below the box to show the highest and lowest values [n = 4–6; median: 294,500 (WT), 234,580 (tgAPP), and 175,365 (tgAPP/PS1) R123 fluorescence units/mg]. One-way ANOVA ($p = 0.001$) followed by Tukey's post hoc test: * $p < 0.05$, ** $p < 0.01$ versus WT mice. **b** Reduced ATP levels in transgenic mice. Levels of ATP in cortical brain cells of 3-month-old non-tgAPP, tgAPP and tgAPP/PS1 mice after 4 h of in vitro incubation were significantly reduced compared to WT mice. Data are presented as a box-and-whisker graph [n = 4–6; median: 96.8% (WT), 75.49% (tgAPP), and 29.45% (tgAPP/PS1) of control]. One-way ANOVA ($p < 0.001$) followed by Tukey's post hoc test: *** $p < 0.001$ versus tgAPP and WT mice, * $p < 0.05$ versus WT mice.

tion, mitochondrial dysfunction and energy metabolism deficiencies are recognized as earliest events and correlated with impairments of cognitive abilities in AD [3–6]. Nevertheless, the specific mechanisms leading to mitochondrial failure in AD are not well understood.

In order to elucidate the impact of Aβ during the course of AD pathogenesis in vivo, we investigated the brains from APP transgenic animals before and at the age of onset of Aβ plaques. Moreover, we previously provided evidence for a mitochondrial dysfunction in P301L tau transgenic mice, a strain modelling the tau pathology of AD [7]. In light of recent studies suggesting that soluble rather than fibrillar aggregated Aβ might be the actual toxic species, the toxicity of different preparations of Aβ is currently under investigation.

Methods

C57BL/6 mice aged 3 months bearing the human Swedish (KM595/596NL) and London (V717I) mutations in the 751-amino-acid form of the human amyloid precursor protein (tgAPP) under the control of a murine Thy-1 promoter, 3-month-old tgAPP/PS1 mutant mice [by crossing the tgAPP mice with HMG-CoA reductase promoter-driven PS1 (M146L) transgenic mice], as well as age-matched nontransgenic wild-type (WT) littermates were used for the experiments [8, 9]. The tgAPP mice exhibit onset of Aβ plaques at an age of 6 months, but the intracellular soluble Aβ load is already detectable at 3 months of age [8, 10], whereas in tgAPP/PS1 mice plaque onset starts at an age of 3 months [10, 11]. The tau transgenic mice express the human pathogenic mutation P301L of tau together with the longest human brain tau isoform (htau40) under the control of the neuron-specific murine Thy-1.2 promoter [12, 13]. P301L tau mice show tau hyperphosphorylation already at 3 months. Neurofibrillary tangle formation starts at 6 months of age. The mice were analyzed at 13–15 months of age. Cortical brain cell preparation, determination of mitochondrial membrane potential, and determination of ATP levels were obtained as previously described [7]. Soluble $A\beta_{42}$ preparations were performed according to Gonghard and coworkers [14].

Results and Conclusions

To further clarify the synergistic effects of aging and Aβ pathology, we investigated mitochondrial function of cortical brain cells from transgenic mice (tgAPP and tgAPP/PS1) and WT mice. Of note, pronounced mitochondrial dysfunction in adult tgAPP mice, such as a drop in mitochondrial membrane potential (fig. 1a) and reduced ATP levels (fig. 1b), already appeared at 3 months when elevated Aβ levels but not yet Aβ-containing

plaques are present. In tgAPP/PS1 mice with a high Aβ load exhibiting Aβ plaques already at an age of 3 months, the strongest reductions in mitochondrial membrane potential and ATP levels were found (fig. 1a, b). We conclude that Aβ-depending mitochondrial dysfunction starts already at a very young age and accelerates substantially with increasing age and Aβ load as well as accumulation.

In addition, we determined whether soluble $A\beta_{42}$ would exert a more pronounced toxicity towards P301L tau transgenic mitochondria when compared to fibrillar Aβ. Interestingly, our preliminary results indicate that oligomeric and fibrillar $A\beta_{42}$ caused a similar decrease in mitochondrial membrane potential in cortical brain cells obtained from P301L tau transgenic mice (data not shown) suggesting that in developing treatment strategies, it may not be sufficient to target either oligomeric or fibrillar Aβ species, but that the best approach is either to prevent formation of excess Aβ at all or to assist in its rapid clearance. Rigorous scientific research has identified multiple interactive mechanisms that parallel and are likely causative for the development of AD. Evidence is provided that AD is triggered by soluble, neurotoxic assemblies of Aβ, while the late-stage pathology landmarks of amyloid plaques and tangles potentiate toxicity by driving a vicious cycle of Aβ generation and oxidation, mitochondrial damage, glucose hypometabolism, energy deficiency, each accelerating the other, since a deficiency in energy can, in turn, induce BACE1 activation leading to an increased production of Aβ [15].

Although the advancement in our understanding of the molecular mechanisms of AD has resulted in a dramatic enhancement of our ability to diagnose and treat the disorder, there is still a constant need for the identification of AD-specific abnormalities finally leading to neurodegeneration, which might open new ways for the development of more efficacious therapies. Based on this assumption, strategies involving efforts to protect cells at the mitochondrial level by stabilizing or restoring mitochondrial function or to interfere with the energy metabolism appear to be promising in preventing AD, besides strategies with regard to the treatment and/or removal of both Aβ and tau pathology.

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References

1 Selkoe DJ: Alzheimer's disease is a synaptic failure. Science 2002;298:789–791.
2 Haass C, Selkoe DJ: Soluble protein oligomers in neurodegeneration: lessons from the Alzheimer's amyloid beta-peptide. Nat Rev Mol Cell Biol 2007;8:101–112.
3 Eckert A, Keil U, Marques CA, et al: Mitochondrial dysfunction, apoptotic cell death, and Alzheimer's disease. Biochem Pharmacol 2003;66:1627–1634.
4 Hauptmann S, Keil U, Scherping I, Bonert A, Eckert A, Muller WE: Mitochondrial dysfunction in sporadic and genetic Alzheimer's disease. Exp Gerontol 2006;41:668–673.
5 Keil U, Hauptmann S, Bonert A, Scherping I, Eckert A, Muller WE: Mitochondrial dysfunction induced by disease relevant AbetaPP and tau protein mutations. J Alzheimers Dis 2006;9:139–146.
6 Blass JP: Cerebrometabolic abnormalities in Alzheimer's disease. Neurol Res 2003;25:556–566.
7 David DC, Hauptmann S, Scherping I, et al: Proteomic and functional analyses reveal a mitochondrial dysfunction in P301L tau transgenic mice. J Biol Chem 2005;280:23802–23814.
8 Schuessel K, Schafer S, Bayer TA, et al: Impaired Cu/Zn-SOD activity contributes to increased oxidative damage in APP transgenic mice. Neurobiol Dis 2005;18:89–99.
9 Leutner S, Czech C, Schindowski K, Touchet N, Eckert A, Muller WE: Reduced antioxidant enzyme activity in brains of mice transgenic for human presenilin-1 with single or multiple mutations. Neurosci Lett 2000;292:87–90.
10 Wirths O, Multhaup G, Czech C, et al: Intraneuronal Abeta accumulation precedes plaque formation in beta-amyloid precursor protein and presenilin-1 double-transgenic mice. Neurosci Lett 2001;306:116–120.
11 Blanchard V, Moussaoui S, Czech C, et al: Time sequence of maturation of dystrophic neurites associated with Abeta deposits in APP/PS1 transgenic mice. Exp Neurol 2003;184:247–263.
12 Gotz J, Chen F, Barmettler R, Nitsch RM: Tau filament formation in transgenic mice expressing P301L tau. J Biol Chem 2001;276:529–534.
13 Gotz J, Chen F, van Dorpe J, Nitsch RM: Formation of neurofibrillary tangles in P301l tau transgenic mice induced by Abeta 42 fibrils. Science 2001;293:1491–1495.
14 Barghorn S, Nimmrich V, Striebinger A, et al: Globular amyloid beta-peptide oligomer – A homogenous and stable neuropathological protein in Alzheimer's disease. J Neurochem 2005;95:834–847.
15 Rhein V, Eckert A: Effects of Alzheimer's amyloid-beta and tau protein on mitochondrial function – Role of glucose metabolism and insulin signalling. Arch Physiol Biochem 2007;113:131–141.