PŘÍLOHY

Animal model of Alzheimer disease (Tg McGill-R-Thy1-APP rats) and mitochondrial dysfunction

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INTRODUCTION

Double transgenic rats McGill-R-Thy1-APP are one of the best animal models of Alzheimer disease (AD). Intraneuronal pathology (observed already in 3-month old rats) is accompanied by a significant elevation of soluble amyloid β 1-42 (Aβ 1-42) and by cognitive deficits, several months prior to amyloid plaque deposition (1, 2). Mitochondrial dysfunction has not been studied yet in more details, however, mitochondrial bioenergetic capacity is not fully conserved via defects in complex I enzymatic activity already in 6-month old hemizygous animals (3).

The nucleus-encoded mitochondrial matrix protein 17β-hydroxysteroid dehydrogenase type 10 (17β-HSD10) operates via multiple enzymatic as well as non-enzymatic functions. Its deficiency, overexpression or loss of function is associated with various pathologies. Experiments on transgenic animals overexpressing 17β-HSD10 and displaying higher baseline ATP levels have demonstrated a protective phenotype in models of oxidative/metabolic stress or in a pharmacological model of Parkinson disease. In people with AD or in animal models of AD, 17β-HSD10 overexpression in the brain and the enhanced concentrations in cerebrospinal fluid have been reported, too (4, 5).

Cytosolic 17β-HSD10 is imported into the mitochondrial matrix via the translocase of the outer mitochondrial membrane (TOM) and the translocase of the inner mitochondrial membrane (TIM), and its regulation by Parkin probably occurs through PINK1. PINK1-Parkin-TOM/TIM pathway participates in clearance of dysfunctional mitochondria and 17β-HSD10 levels in mitochondria could be one of the mechanisms by which Parkin preserves mitochondrial quality (6). 17β-HSD10 is known as a binding partner of intracellular Aβ accumulated in mitochondria of AD people. Under normal conditions, 17β-HSD10 localised in the mitochondrial matrix binds cyclophilin D (CycD) and, by preventing its translocation to the inner mitochondrial membrane, can regulate the opening of the mitochondrial permeability transition pore (PTP) mediated by CycD. Under conditions of increased accumulation of mitochondrial Aβ leading to dysfunctional mitochondria, interactions of Aβ and 17β-HSD10 or those of Aβ and CycD could eliminate the regulation of CycD by 17β-HSD10. Therefore, the translocation of CycD from the matrix and its interactions with mitochondrial PTP could be enhanced, which should lead to apoptosis/necrosis (7).

AIMS OF THE STUDY

To evaluate the ability of 17β-HSD10 to regulate CycD in mitochondria isolated from the brains of Tg McGill-R-Thy1-APP rats.

MATERIALS AND METHODS

Animals: 11-month old homozygous Tg McGill-R-Thy1-APP male rats (10x) and age- and sex-related WT animals (10x), 7-month old male Wistar rats as control rats (2x)

Isolation of mitochondria: Mitochondria were isolated via Percoll gradient from the left hemisphere (WT and Tg rats) or from both hemispheres (controls). All samples contained 9 mg/ml of mitochondrial proteins.

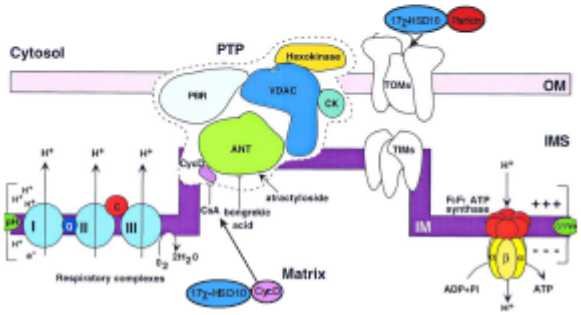
ELISA measurements: 17β-HSD10 (quantitative competitive ELISA by recombinant full length rat protein and polyclonal rabbit anti-17β-HSD10, both from Flarebio), CycD (quantitative sandwich ELISA, by a kit from MyBioSource), 17β-HSD10 – CycD complexes (semiquantitative sandwich ELISA, by polyclonal rabbit anti-17β-HSD10 (Flarebio) as a capture antibody and mouse monoclonal anti-CycD (Abnova) as primary antibodody).

Statistical significance: BMDP statistical software was used. Results are presented as means ± S.D. Levels of complexes were related to those from control mitochondria and expressed in %.

Table 1:
Results of experiments performed on mitochondria isolated from the brains of Tg McGill-R-Thy1-APP rats

	17β-HSD10 ng/μg of proteins	CycD ng/ml	17β-HSD10 – CycD %
WT rats	3.51 ± 0.73	2.98 ± 0.14	103.2 ± 20.9
Tg rats	3.25 ± 1.02	3.21 ± 0.36	78.4 ± 19.6*
ANOVA	p = 0.5067	p = 0.0828	p = 0.0160

Fig. 1:
Scheme of Pink1-Parkin-TOM/TIM – mediated transport of cytosolic 17β-HSD10 and of regulation of mitochondrial PTP via interactions of CycD and 17β-HSD10 in mitochondrial matrix



RESULTS AND DISCUSSION

I) Concentrations of 17β-HSD10 are not elevated in brain mitochondria isolated from Tg compared to WT rats. The result indicates that the up-regulation of 17β-HSD10 protein (observed in people with AD and in many animal models of AD) does not have to be followed by its increased transport into mitochondrial matrix via the PINK1-Parkin-TOM/TIM pathway.

ii) 17β-HSD10 – CycD complexes are significantly decreased but levels of 17β-HSD10 and CycD are not altered. The result can be interpreted via a weakened ability of 17β-HSD10 to regulate CycD in mitochondrial matrix in AD.

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