Introduction

Alzheimer’s disease (AD) affects mainly people over the age of 65, suffering from progressive decline in memory, thinking, language, and learning capacity. Increasing evidence points to soluble oligomeric Aβ (AβO) aggregates/proteins as putative toxic species in Alzheimer’s disease pathogenesis, through a deficiency in protein homeostasis. Endoplasmic reticulum (ER) stress is involved in many neurodegenerative disorders and is caused by misfolded proteins and protein aggregates. ER stress activates an unfolded protein response (UPR) through PERK, ATF6 and/or IRE1 alpha proteins.

Here, we aimed to study the ER signalling pathways in primary cortical neurons and/or IRE1 alpha proteins. ER-stress regulation and effect of Guanabenz in a primary culture of cortical neurons injured with Aβ1-42 peptide, an in vitro model of Alzheimer’s disease.

Methods

Primary culture: Primary rat cortical neurons (E15) were cultured as described by Callizot et al., 2013 with modifications. The cells were seeded at a density of 25,000 per well in 96-well plates (immunostaining) or at 140,000 cells per well in 24-well plates (protein level). Cells were cultured in neurobasal medium supplemented with B27.

Pharmacological treatments: Aβ1-42 (20 µM ~2 µM of AβO, as determined by WB) and Guanabenz (5 µM) were applied on day 11. Untreated cultures served as controls.

Immunostaining: After intoxication, neurons were fixed with a solution of acetic acid (5 %) and ethanol (95 %). The cells were incubated with a rabbit polyclonal antibody anti-MAP2 (neuronal marker) and a mouse monoclonal antibody anti-phosphorylated Tau (Ser212/Thr214). Secondary Alexa488 and Alexa568 antibodies were used. Pictures (20x magnification) were acquired on an automated microscope with MetaExpress software and automatically analyzed with Custom Module Editor (Molecular Devices).

Protein level analysis: Cells were collected and lysed with CellLysis buffer (Protein Simple). Levels of GAPDH, ATF6, phosphorylated-Tau and phosphorylated-eIF2a were determined automatically analyzed with Custom Module Editor (Molecular Devices).

Results

Neurotoxicity of Aβ1-42 and protective effects of Guanabenz, in a primary culture of cortical neurons

Figure 1: Toxicity of Aβ1-42 and protective effects of Guanabenz on cortical neurons. Shown above the neuronal survival (A), total neurite network (B) and area of phosphorylated Tau (C) in MAP-2 neurons. (D) Representative pictures in control and intoxicated neurons. (E) Representative pictures of healthy neurons (left panel) and neurons with hyperphosphorylated Tau (right panel). Scale bar=100 µm.

Activation of ER stress in a culture of cortical neurons, after an injury with Aβ1-42

Figure 2: Regulation of ER stress and UPR after application of Aβ1-42 in a primary culture of cortical neurons. Protein level of phosphorylated Tau (Tau-P), phosphorylated eIF2a (eIF2a-P) and cleaved ATF6 (ATF6-c) after 8 (A) or 24 hours (B) of Aβ1-42 intoxication. (C) Picture of blot-free western analysis at 24 hours. *, p-value<0.05 versus control; §, p-value<0.05 versus Aβ1-42+Guanabenz. One-way ANOVA, followed by Dunnett’s test.

Conclusions

• Hyperphosphorylation of Tau (Ser202/Thr214) is an early event in cortical neurons in the first hours after Aβ1-42 application.

• Our data shows that activation of the ATF6 pathway is an early response to Aβ1-42 injury in a primary culture of cortical neurons while the eIF2a pathway is activated later.

• Guanabenz, a well-known inhibitor of GADD34, prevents neuronal loss in the first hours after Aβ1-42 injury, and reduced Tau hyperphosphorylation, by maintaining eIF2a in a phosphorylated state, and surprisingly by promoting ATF6 cleavage.

• Taken together, our results suggest that ER stress is strongly activated after Aβ1-42 injury and that UPR represents a therapeutic target for Alzheimer’s disease.