Molecular and Cellular Neuroscience 52 (2013) 1–8

Contents lists available at SciVerse ScienceDirect

Molecular and Cellular Neuroscience

journal homepage: www.elsevier.com/locate/ymcne

Inhibition of mitochondrial cytochrome *c* oxidase potentiates Aβ-induced ER stress and cell death in cortical neurons

Rui O. Costa [a], Elisabete Ferreiro [a], Catarina R. Oliveira [a,b], Cláudia M.F. Pereira [a,b,*]

[a] Center for Neuroscience and Cell Biology, University of Coimbra, Portugal
[b] Faculty of Medicine, University of Coimbra, Portugal

ARTICLE INFO

Article history:
Received 26 June 2011
Revised 31 August 2012
Accepted 21 September 2012
Available online 28 September 2012

Keywords:
Alzheimer's disease
Amyloid-β
Cytochrome *c* oxidase
Endoplasmic reticulum
Mitochondria
Electron transport chain

ABSTRACT

Previously we reported that amyloid-β (Aβ) leads to endoplasmic reticulum (ER) stress in cultured cortical neurons and that ER-mitochondria Ca^{2+} transfer is involved in Aβ-induced apoptotic neuronal cell death. In cybrid cells which recreate the defect in mitochondrial cytochrome *c* oxidase (COX) activity observed in platelets from Alzheimer's disease (AD) patients, we have shown that mitochondrial dysfunction affects the ER stress response triggered by Aβ. Here, we further investigated the impact of COX inhibition on Aβ-induced ER dysfunction using a neuronal model. Primary cultures of cortical neurons were challenged with toxic concentrations of Aβ upon chemical inhibition of COX with potassium cyanide (KCN). ER Ca^{2+} homeostasis was evaluated under these conditions, together with the levels of ER stress markers, namely the chaperone GRP78 and XBP-1, a mediator of the ER unfolded protein response (UPR). We demonstrated that COX inhibition potentiates the Aβ-induced depletion of ER Ca^{2+} content. KCN pre-treatment was also shown to enhance the rise of cytosolic Ca^{2+} levels triggered by Aβ and thapsigargin, a widely used ER stressor. This effect was reverted in the presence of dantrolene, an inhibitor of ER Ca^{2+} release through ryanodine receptors. Similarly, the increase in GRP78 and XBP-1 protein levels was shown to be higher in neurons treated with Aβ or thapsigargin in the presence of KCN in comparison with levels determined in neurons treated with the neurotoxins alone. Although the decrease in cell survival, the activation of caspase-9- and -3-mediated apoptotic cell death observed in Aβ- and thapsigargin-treated neurons were also potentiated by KCN, this effect is less pronounced than that observed in Ca^{2+} signalling and UPR. Furthermore, in neurons treated with Aβ, the potentiating effect of the COX inhibitor in cell survival and death was not prevented by dantrolene. These results show that inhibition of mitochondrial COX activity potentiates Aβ-induced ER dysfunction and, to a less extent, neuronal cell death. Furthermore, data supports that the effect of impaired COX on Aβ-induced cell death occurs independently of Ca^{2+} release through ER ryanodine receptors. Together, our data demonstrate that mitochondria dysfunction in AD enhances the neuronal susceptibility to toxic insults, namely to Aβ-induced ER stress, and strongly suggest that the close communication between ER and mitochondria can be a valuable future therapeutic target in AD.

Introduction

Alzheimer's disease (AD) is the most widespread form of dementia in the elderly that results from synaptic and neuronal loss in the hippocampus and cerebral cortex. AD patients present extracellular senile plaques in susceptible brain regions, mainly composed of fibrillar amyloid-beta peptide (Aβ) that constitute one of the neuropathological hallmarks of this neurodegenerative disorder (Selkoe, 2001).

Mitochondria play a crucial role not only in providing energy to cells through ATP synthesis in the electron transport chain (ETC.), but also in activation of cell death pathways. The ETC. modulates apoptotic cell death because its inhibition leads to energy failure and also because toxic insults interact with ETC. components increasing the cellular vulnerability to apoptosis (Kwong et al., 2007). Accumulation of mitochondrial DNA (mtDNA) mutations that lead to ETC. malfunction has been shown to increase the susceptibility for several pathologies, including AD (Kruger et al., 2010; Lin and Beal, 2006; Lin et al., 2002). Recent evidence demonstrates that mtDNA mutations are associated with early pathological events in AD (Ienco et al., 2011). Accordingly, multiple defects in energy metabolism are associated with AD neuropathology and cognitive deficits (reviewed in Ferreira et al., 2010). In addition, brain glucose hypometabolism, together with alterations in the activity of several mitochondrial metabolic enzymes, were reported in individuals with mild cognitive impairment (MCI) (Valla et al., 2006). The metabolic alterations during this pre-clinical stage of the disease and in diagnosed AD patients (Ferrer, 2009; Mosconi, 2005) involve the impairment of the mitochondrial ETC., in particular of cytochrome *c* oxidase (COX) activity

* Corresponding author at: Center for Neuroscience and Cell Biology, Largo Marquês de Pombal, University of Coimbra, 3004-517 Coimbra, Portugal. Fax: +351 239822776.
E-mail address: claudia.mf.pereira@gmail.com (C.M.F. Pereira).

1044-7431/$ – see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.mcn.2012.09.005

(Pickrell et al., 2009; Small et al., 1995). Compromised energy metabolism has been reported to increase the production of Aβ (Velliquette et al., 2005). Along with these evidences, mitochondrial-associated secretases were recently proved to be involved in amyloid precursor protein (APP) processing and Aβ generation, showing that this peptide can be produced locally in the mitochondria (Pavlov et al., 2010). In AD transgenic mice, the presence of Aβ in mitochondria has been demonstrated (Caspersen et al., 2005; Hansson Petersen et al., 2008) and it correlates with the extent of mitochondrial dysfunction due to inhibition of ETC. complexes and with the degree of cognitive impairment (reviewed in Chen and Yan, 2010; Dragicevic et al., 2010).

In addition to mitochondria, dysfunction of other cellular organelles such as the endoplasmic reticulum (ER) has been implicated in AD pathogenesis and the presence of ER stress markers has been described in the brain of AD patients (Hoozemans et al., 2005). Previously, we provided evidences that Aβ can be a trigger for ER stress and demonstrated that ER-to-mitochondria Ca^{2+} transfer plays an essential role during Aβ-induced neuronal apoptosis (Costa et al., 2010, 2012a; Ferreiro et al., 2006). Recently, we used cybrids that recapitulate the COX inhibition present in AD platelets to demonstrate that mitochondrial impairment potentiates the ER stress-mediated Aβ-induced cell death (Costa et al. 2012a). Using primary cultured rat brain cortical neurons as a neuronal model, here we investigated the role of this AD-associated mitochondrial defect in Aβ-induced ER stress and subsequent activation of apoptotic cell death. For this purpose, COX inhibition was induced by KCN treatment before neuronal exposure to toxic Aβ concentrations. As a positive control for ER stress, a parallel study was conducted using thapsigargin, a classical disruptor of ER Ca^{2+} homeostasis and ER stress inducer (Rogers et al., 1995).

Overall, we demonstrated that mitochondrial impairment due to COX inhibition increases the susceptibility to Aβ-induced ER stress-mediated apoptosis, supporting that ER/mitochondria crosstalk plays a crucial role during Aβ-induced neuronal cell death in AD.

Results

In the present study, we used primary cultures of cortical neurons treated with $Aβ_{1–40}$ upon mitochondrial ETC. inhibition to further disclose the role of ER/mitochondria crosstalk in neuronal cell death that occurs in AD. In order to mimic the mitochondrial defect (COX inhibition) associated with pre-clinical and clinical AD, we used the chemical COX inhibitor KCN. The effect of Aβ in neuronal cells was compared with that of thapsigargin, a widely used ER stressor that disrupts ER Ca^{2+} homeostasis.

Mitochondrial COX inhibition enhances $Aβ_{1–40}$-induced ER Ca^{2+} dyshomeostasis and stress

The levels of ER Ca^{2+} were monitored in cortical neurons treated with $Aβ_{1–40}$ (1 μM) during 3 h, in the absence or in the presence of the COX inhibitor KCN (1 mM). KCN alone did not significantly affect ER Ca^{2+} levels. However, we found that the decrease in ER Ca^{2+} levels observed in $Aβ_{1–40}$-treated neurons is enhanced in cells pre-incubated with KCN (Fig. 1A,B). The ER Ca^{2+} depletion was also demonstrated to be followed by an increment of cytosolic Ca^{2+} concentration measured with the fluorescent probe Indo-1/AM after 6 h of incubation with $Aβ_{1–40}$ (Fig. 1C). Similar results were obtained in neurons treated with thapsigargin (2 μM) for 6 h (Fig. 1C). Pre-incubation with KCN before $Aβ_{1–40}$ or thapsigargin treatment potentiated the increase of cytosolic Ca^{2+} levels, which involves ER Ca^{2+} release through channels coupled to the ER ryanodine receptor (RyR) since dantrolene (10 μM) significantly prevented the effect of KCN (Fig. 1C).

To further assess the role of mitochondrial COX inhibition on ER dysfunction in neurons exposed to toxic $Aβ_{1–40}$, we measured by WB the protein levels of ER stress markers. This included XBP-1, a

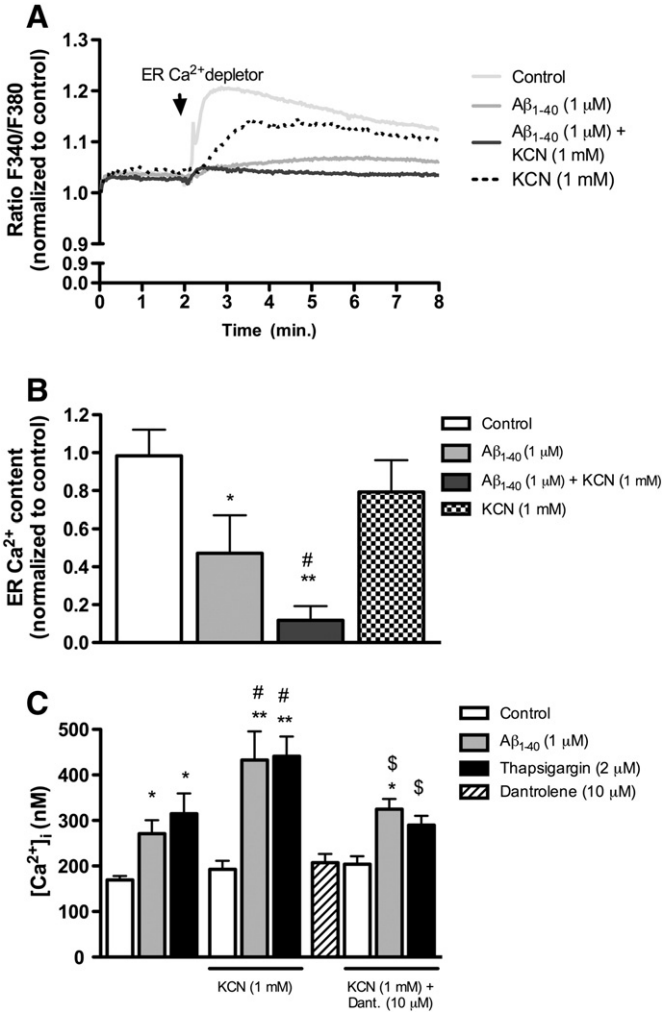

Fig. 1. COX inhibition potentiates $Aβ_{1–40}$-induced depletion of ER Ca^{2+} content in cortical neurons and also cytosolic Ca^{2+} deregulation induced by $Aβ_{1–40}$ and thapsigargin. Primary cultures of rat brain cortical neurons were incubated for 3 h with $Aβ_{1–40}$ (1 μM), in the absence or in the presence of KCN (1 mM), which was added 1 h before $Aβ_{1–40}$ or thapsigargin (2 μM). When used, dantrolene (10 μM) was also added 1 h before $Aβ_{1–40}$ or thapsigargin. Levels of ER Ca^{2+} were evaluated by monitoring the fluorescence of Fura-2 in the absence of external Ca^{2+} (A, B). At t = 2 min an ER Ca^{2+} depletor was added to expel ER Ca^{2+}. The difference between Fura-2 fluorescence ratio at 340/380 nm before and after the complete depletion of ER Ca^{2+} was used to evaluate ER Ca^{2+} content in each experimental condition. Results are presented as normalized values of Fura-2 fluorescence ratio (A) or as ER Ca^{2+} content values (the difference between the Ca^{2+} values obtained after ER Ca^{2+} depletion induction and the basal levels), normalized to control values (B). Cytosolic free Ca^{2+} concentration ($[Ca^{2+}]_i$) was determined using the fluorescent Ca^{2+} indicator Indo-1/AM at 350 nm excitation and 410 nm emission (C). Results are the means ± SEM of values corresponding to at least 3 experiments, each value being the mean of duplicate assays. $^{*}p<0.05$, $^{**}p<0.01$, significantly different when compared to control values. $^{\#}p<0.05$, significantly different when compared to the respective $Aβ_{1–40}$- or thapsigargin-treated condition. $^{\$}p<0.05$, significantly different when compared to KCN pre-treated cells.

mediator of the ER unfolded protein response (UPR), and the ER chaperone Grp78. Results showed that $Aβ_{1–40}$ and thapsigargin significantly increase the levels of XBP-1 and Grp78 after 6 h incubation and that this effect is potentiated by pre-incubation with KCN (Fig. 2A,B).

$Aβ_{1–40}$- and thapsigargin-induced apoptotic cell death is enhanced by COX inhibition

In order to better understand the role of mitochondrial COX inhibition in the toxic effect of Aβ in cortical neurons, we analyzed cell survival, activation of apoptosis-associated caspases, namely caspase-9 and