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Supplementary Data

Amyloid-β Peptide-Induced Secretion of Endoplasmic Reticulum Chaperone Glycoprotein GRP94

Ricardo J.S. Viana[a], Clifford J. Steer[b,c] and Cecília M.P. Rodrigues[a,d,*]
[a]*Research Institute for Medicines and Pharmaceutical Sciences, Faculty of Pharmacy, University of Lisbon, Lisbon, Portugal*
[b]*Department of Medicine, University of Minnesota Medical School, Minneapolis, MN, USA*
[c]*Department of Genetics, Cell Biology, and Development, University of Minnesota Medical School, Minneapolis, MN, USA*
[d]*Department of Biochemistry and Human Biology, Faculty of Pharmacy, University of Lisbon, Lisbon, Portugal*

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SUPPLEMENTARY MATERIALS AND METHODS

Isolation and culture of rat cortical neurons

Primary cultures of rat cortical neurons were prepared from 17- to 18-day-old fetuses of Wistar rats as previously described [1] with minor modifications. In short, pregnant rats were ether-anesthetized and decapitated. The fetuses were collected in Hank's balanced salt solution (HBSS-1; Invitrogen, Grand Island, NY, USA) and rapidly decapitated. After removal of meninges and white matter, the brain cortex was collected in Hank's balanced salt solution without Ca^{2+} and Mg^{2+} (HBSS-2). The cortex was then mechanically fragmented, transferred to a 0.025% trypsin in HBSS-2 solution, and incubated for 15 min at 37°C. Following trypsinization, cells were washed twice in HBSS-2 containing 10% fetal calf serum and re-suspended in Neurobasal medium (Invitrogen), supplemented with 0.5 mM L-glutamine, 25 μM L-glutamic acid, 2% B-27 supplement (Invitrogen), and 12 mg/mL gentamicin. Neurons were then plated on tissue culture plates, precoated with poly-D-lysine at 1×10^6 cells/mL, and maintained at 37°C in a humidified atmosphere of 5% CO_2. All experiments were performed on cells cultured for 4 days in fresh medium without glutamic acid and B-27 supplement. Cells were characterized by phase contrast microscopy and indirect immunocytochemistry for neurofilaments and glial fibrillary acidic protein. Neuronal cultures were >95% pure.

PC12 cells and culture conditions

PC12 cells were cultured as described in Materials and methods.

Induction and assessment of apoptosis

Isolated rat neurons after 4 days in culture or PC12 cells were incubated with either 25 μM soluble $A\beta_{25\text{-}35}$, 10 μM soluble $A\beta_{1\text{-}40}$, 20 μM fibrillar $A\beta_{1\text{-}42}$ (Bachem AG, Budendorf, Switzerland), or no

*Correspondence to: Cecília M.P. Rodrigues, iMed.UL, Faculty of Pharmacy, University of Lisbon, Lisbon 1649-003, Portugal. Tel.: +351 21 794 6490; Fax: + 351 21 794 6491; E-mail: cmprodrigues@ff.ul.pt.

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Supplementary Table 1
TUDCA inhibits apoptosis induced by Aβ peptides in rat PC12 neuronal cells and in primary rat cortical neurons

	Apoptotic Cells (%)	
	Rat PC12-neuronal cells	Rat cortical neurons
Control	5.0 ± 2.1	16.3 ± 3.3
TUDCA	4.0 ± 0.4	13.0 ± 1.2
$A\beta_{25\text{-}35}$	15.1 ± 2.8*	47.9 ± 7.0*
$A\beta_{25\text{-}35}$ + TUDCA	$7.2 \pm 0.5^{\dagger}$	$24.9 \pm 3.2^{\dagger}$
$A\beta_{1\text{-}40}$	13.1 ± 3.4	46.1 ± 3.4*
$A\beta_{1\text{-}40}$ + TUDCA	$6.0 \pm 0.2^{\dagger}$	$31.8 \pm 5.9^{\dagger}$
$A\beta_{1\text{-}42}$	15.3 ± 4.9	33.1 ± 4.2*
$A\beta_{1\text{-}42}$ + TUDCA	$5.4 \pm 1.6^{\dagger}$	$15.1 \pm 2.2^{\ddagger}$

The results are expressed as mean ± SEM for 3–7 different experiments. *$p < 0.01$ and $p < 0.05$ from control; $^{\ddagger}p < 0.01$ and $^{\dagger}p < 0.05$ from Aβ alone. Aβ, amyloid β peptide; TUDCA, tauroursodeoxycholic acid.

addition (control), ±100 μM TUDCA (Sigma Chemical, St. Louis, MO, USA) for 24 h. In co-incubation experiments, TUDCA was added to neurons 12 h prior to incubation with Aβ peptides. Fibrillar $A\beta_{1\text{-}42}$ was induced to form fibrils by preincubation in culture medium [2]. In short, 0.45 mg of $A\beta_{1\text{-}42}$ peptide was dissolved in 20 μl of DMSO and diluted to a 100 μM stock solution in medium, which was then incubated with gentle shaking at room temperature for 4 days. Fibrillar $A\beta_{1\text{-}42}$ was then diluted to 20 μM and applied to neuron cultures or PC12 cells. Finally, cells were fixed and stained for microscopic assessment of apoptosis after Hoechst staining, and the percentage of apoptotic cells was determined. Cells exposed to Aβ peptides alone showed more nuclear fragmentation compared with cells pre-treated with TUDCA (Supplementary Table 1).

REFERENCES

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