α-synuclein: Cause or consequence of cytopathological lesions observed in in vitro models of Parkinson’s disease.

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Introduction

Parkinson’s disease (PD) is characterized by loss of dopaminergic neurons in the substantia nigra pars compacta and intraneuronal protein aggregates (Lewy bodies (LB)). Mitochondrial involvement has been postulated based on observations with mitochondrial toxins, which cause Parkinson’s like syndromes. α-synuclein (α-syn) mutations, major LB component, cause autosomal dominant familial PD. Additionally, α-syn null mice demonstrate increased resistance to MPTP whereas transgenic α-syn animals treated with MPTP present morphological abnormal mitochondria. Studies have demonstrated that extracellular LB and nigral aggregates immunoreactive to α-syn were often surrounded by activated microglia or inflammatory mediators. Altogether, these observations raise the question of the α-syn role in PD. To investigate this point, we used rodent primary mesencephalic neurons enriched or not with microglial cells and injured with mitochondrial toxins.

Methods

Culture of rat mesencephalic dopaminergic neurons: Neurons were cultured as described by Visanji et al., 2008 (no microglia) or according to Zhang et al., 2005 (microglia). Briefly, the midbrains obtained from 14 or 15-day old rat embryos were dissected. Six wells/conditions. Pharmacological treatments: On day 6, fresh medium was added, without or with 6 hydroxyl-dopamine (6-OHDA), 1-methyl-4-phenyl pyridinium (MPP⁺) or Rotenone or aggregated α-synuclein. Survival of TH positive neurons: After 24 and 48 hours of intoxication, cells were fixed by a solution of 4% paraformaldehyde. The cells were incubated with Monoclonal Anti-Tyrosine Hydroxylase (TH) that was revealed with Alexa Fluor 488 goat anti-mouse IgG. The immuno-labelled cultures were examined with MetaXpress (Molecular Devices, USA) at X 10 magnification. α-syn evaluation (Western-blotting): After 24h of toxin treatment, cells were lysed with celllytic and immediately frozen at -80°C. All reagents were prepared and used according to manufacturer’s recommendations (Simon™ - ProteinSimple - www.proteinsimple.com). Anti-α-syn, primary antibody was used for WB analysis. ATP evaluation: 4 and 8 after injuries, ATP level in culture wells was measured using Celltiter-Glo luminescent assay kit (Promega).

Results

Fig. 1: What is known about PD physiopathology

Fig. 2: Time/dose effect of MPP⁺, 6-OHDA and Rotenone on TH positive neuron survival. The 3 toxins induced TH neuron cell death. This effect was depending of time of application and dose.

Fig. 3: Effect of MPP⁺, 6-OHDA and Rotenone on the ATP pools in mesencephalic cells. Only rotenone (10 nM) induced a large decrease of ATP pools. This major depletion started 4h after application.

Fig. 4: Effect of respiratory impairment on α-syn protein level. In presence of toxin, a large and significant α-syn protein expression was observed (~12 kDa).

Fig. 5: Effect of α-syn on a primary mesencephalic culture in presence or absence of microglia. TH positive neuron toxicity induced with α-syn was observed only in presence of microglia in the culture.

Fig. 6: Intracellular (white arrow) and extracellular (arrowhead) aggregates of α-syn

Hypothesis

Respiratory chain impairments induce an over production of reactive oxygen species (ROS) and decrease production of ATP inducing the aggregation of α-syn and LB production and neuron death. This results in release of α-syn aggregated that activates microglial cells and leads to local inflammation.

Endless circle:

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