ALPHA-SYNuclein phosphorylation at serine 129 is induced by oxidative stress and influences its secretion in a Parkinson’s disease cellular model

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Alpha-synuclein (alpha-syn) phosphorylation at serine (Ser) 129 is a post-translational modification characteristic of Parkinson’s disease (PD). However, its role in neurotoxicity and degeneration is still elusive. Furthermore, mitochondrial dysfunction and oxidative stress constitute key pathogenic events of this disorder. The aim of this work was to analyse the role of iron and rotenone (complex I inhibitor)-induced formation of reactive oxygen species (ROS) on alpha-syn phosphorylation, and its correlation with cell death, protein aggregation and extracellular alpha-syn. We used a human neuroblastoma cell line (SH-SY5Y) transiently transfected with wild type (WT) or mutant A53T alpha-syn, linked to eGFP. Using the fluorescent probe dichlorodihydrofluorescein-diacetate we show that prolonged and short exposures to iron or rotenone increase the formation of endogenous peroxides in cells overexpressing WT or A53T alpha-synGFP. Results also show that prolonged incubations with iron or rotenone increase phosphorylation at Ser129, particularly of mutant A53T alpha-syn. Despite a decrease in the fluorescence of MitoTracker Red upon exposure to iron or rotenone, suggesting mitochondrial depolarization, only a small percentage of cells exhibited cell death by necrosis. Indeed, increased alpha-syn phosphorylation induced by iron was correlated with a higher percentage of cell death. No protein aggregates were detected. Nonetheless, extracellular levels of A53T alpha-synGFP were decreased in the presence of iron, but increased in cells treated with rotenone. The data suggest that early ROS formation induced by iron or mitochondrial impairment may be important for defining subsequent alpha-syn phosphorylation and release processes, which may precede massive cell death in PD.