PROLACTIN LEADS TO A DECREASE IN AUTOPHAGY LEVELS IN CYTOKINES AND ER STRESSORS-INDUCED BETA-CELLS

Terra LF\(^1\); dos Santos AF\(^1\); Wailemann RAM\(^1\); Oliveira TC\(^1\); Sogayar MC\(^{1,2}\); Labriola L\(^1\)

\(^1\) Instituto de Química, Departamento de Bioquímica – Universidade de São Paulo (USP), São Paulo, Brasil
\(^2\) Nucleo de Terapia Celular e Molecular (NUCEL-NETCEM), Faculdade de Medicina – USP, São Paulo, Brasil

Autophagy role in diabetes (DM1) is still obscure, but recent observations suggest that it may have important roles in DM1 development and prevention. Previous results showed a correlation between cytokines, known to have an important action in the development of DM1, and endoplasmic reticulum (ER) stressors, with autophagy induction in beta-cells. We also showed that prolactin (PRL) promotes significant cytoprotection against cytokines- and serum starvation (SS)- induced beta-cells apoptosis. In order to analyze the cytoprotective capacity of PRL after other cell death-inducing mechanisms, we set out to investigate whether the cotreatment with PRL would lead to a restoration of the expression of autophagy markers after different combinations of cytokines and ER stressors, as well as after SS. Viability was analyzed through immunofluorescence, autophagy was inhibited by knockdown of the ATG5 gene, autophagy markers expression and/or phosphorylation were accessed by Western Blotting and acidic vesicular formation was analyzed by acidine orange staining in INS-1E beta-cell cultures or primary cultures of human islets treated under different conditions. PRL treatment was able to inhibit cell death after cytokine treatment, as expected. While no alteration in beta-cell apoptosis was seen upon rapamycin treatment, we observed an increase in apoptosis in ATG5 silenced cells. PRL treatment restored p-mTOR levels after treatment with different cytokines combination (IL-1β+IFN-γ, TNF-α+IFN-γ) or different ER stressors (Thapsigargin, Tunicamycin) as well as decreased acidic vesicles formation. Moreover, LC3-II/LC3-I ratio was also decreased in primary cultures of human islets treated with PRL after exposure to either cytokines (IFN-γ+IL-1β+TNF-α) or SS. These findings support the hypothesis that autophagy plays a protective role in beta-cells and provide a deeper characterization of the PRL prosurvival mechanisms. We hypothesize that the reason PRL is able to restore autophagy markers in beta-cells is because it prevents general cell damage.

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