EFFECTS OF HEXARELIN ON THE MODULATION OF APOPTOSIS PATHWAY INDUCED BY HYDROGEN PEROXIDE

Ramona Meanti[1], Laura Molteni[1], Laura Rizzi[1], Elena Bresciani[1], Vittorio Locatelli[1], Antonio Torsello[1]
[1]School of Medicine and Surgery, University of Milan-Bicocca, Monza - Italy

Introduction: Intracellular hydrogen peroxide (H_2O_2) generated through oxidative stress is involved in necrosis and apoptosis, ultimately resulting in aging, cancer and several neurodegenerative diseases, including Amyotrophic Lateral Sclerosis (ALS). In particular reactive oxygen species (ROS) modulate the extracellular signal-regulated kinase (ERK) and phosphatidylinositol 3-kinase (PI3K/Akt) pathways, and the activation of pro-apoptotic protein Bax. Evidences demonstrate that neurotrophic factors, including insulin-like growth factor 1(IGF-1), regulate survival and differentiation of nerve cells and maintain neuronal structural integrity. Experimental data showed that growth hormone secretagogues (GHS) not only inhibit cytotoxic effect of β-amyloid in N9cells and counteract atrophy in cachectic rats, but can stimulate the release of IGF-1. Hexarelin is a well-known GHS endowed with growth hormone-releasing effects and neuroprotective activities, such as prevention of status epilepticus and promotion of neurogenesis. Moreover, hexarelin reduces the activation of caspase-3and caspase-7involved in apoptosis pathway, caused by brain hypoxia-ischemia in neonatal rats. In this study, we explored the protective effects of hexarelin against oxidative stress and its anti-apoptotic mechanisms to attenuate H_2O_2-induced neurotoxicity in mouse neuroblastoma Neuro2A cells.

Materials and methods: Neuro2A cells were incubated with increasing concentration of $H_2O_2$for 24h. After determining the optimal concentration of H_2O_2, Neuro2A cells were treated with H_2O_2in absence or presence of hexarelin (24h). Cell viability was evaluated using MTT assay, and mRNA levels of Bax, Bcl-2, caspase-3and caspase-7were obtained by Real Time PCR. Western blot analysis was used to measure ERK1/2, Akt and NF- κB proteins expression.

Results: $H_2O_2$100 μM was selected as the lower concentration that significantly induced oxidative stress and a reduction of cells viability. MTT assay demonstrated that hexarelin increased cells survival. H_2O_2induced the activation of apoptosis pathway: the mRNA level of pro-apoptotic protein Bax was significantly increased as well as the levels of caspase-3and caspase-7, while Bcl-2mRNA levels were reduced. Hexarelin also reduced the apoptosis activation: Bax, caspase-3and caspase-7mRNA levels were reduced while anti-apoptotic Bcl-2mRNA levels were increased. Western blotting analysis confirmed the role of hexarelin via the anti-apoptotic Akt and ERK1/2pathways and the reduction of NF-κB translocation to the nucleus.

Discussion and conclusion: In conclusion, our results suggest that hexarelin exerts neuroprotective activities against H_2O_2-induced toxicity in Neuro2A cells. Further experiments are needed to identify the molecular mechanisms underlying this neuroprotective effects.