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Effects of hexarelin on the modulation of apoptosis pathway induced by hydrogen peroxide

Ramona Meanti[1,2], Laura Rizzi[1], Laura Molteni[1], Elena Bresciani[1], Vittorio Locatelli[1], Antonio Torsello[1]

[1]School of Medicine and Surgery, University of Milano-Bicocca, Monza, Italy
[2]PhD student in Neuroscience, University of Milano-Bicocca, Monza, Italy

Amyotrophic Lateral Sclerosis

Amyotrophic Lateral Sclerosis (ALS) is a fatal motor neuron disease characterized by degeneration of upper and lower motor neurons.
The underlying causes of the onset of ALS are not yet completely clarified but on one side, oxidative stress, mitochondrial damage and protein aggregation are considered as causative of the disease.
Reportedly, an increased production of reactive oxygen species (ROS) leads to the dysregulation of mitochondrial dynamics and activates apoptosis mechanisms.

Hexarelin

Hexarelin is a synthetic hexapeptide, analogue of ghrelin, that binds the growth hormone secretagogue receptor-1a (GHSR-1a) and exerts several endocrine and extraendocrine properties.
Recently, some studies have demonstrated the ability of hexarelin to modulate the activation of different pathways that could be relevant in neurodegenerative diseases.
In particular, hexarelin reduced the expression of caspase-3, caspase-9 and the ratio of pro-apoptotic protina Bax to anti-apoptotic protein Bcl-2 in different cell lines.

Aim

In this study, we have investigated the protective effects of hexarelin against oxidative stress induced by H_2O_2 in mouse neuroblastoma Neuro2A cells.

Methods

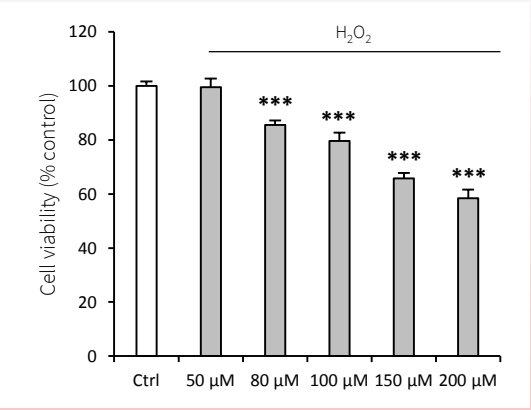
- Neuro2A cells were incubated with increasing concentrations of H_2O_2 (50 μM- 200 μM); H_2O_2 100 μM was choosen for the experimental treatments.

- Neuro2A cells were treated with H_2O_2 100 μM and hexarelin 10^{-6} M for 24h, alone or in association.

The effects of H_2O_2 and hexarelin were evaluated with MTT assay and by semi-quantitative real-time polymerase chain reaction (q-PCR).

Contacts:
Ramona Meanti, PhD student
r.meanti@campus.unimib.it
02-6448-8225

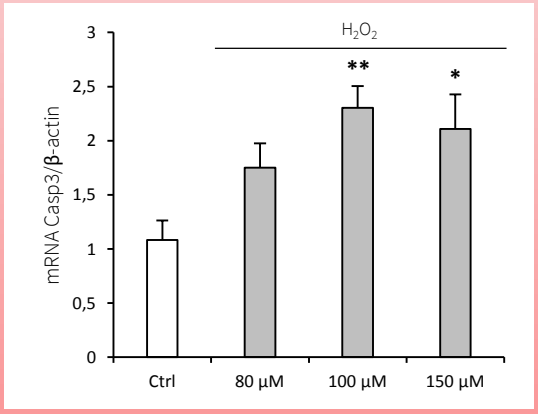
Results

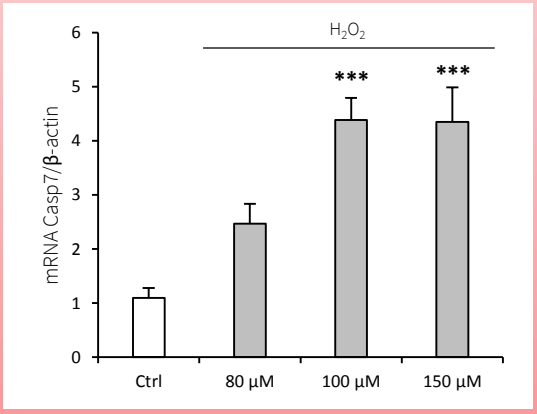


Effect of H_2O_2 on cell viability in Neuro2A cells.

Neuro2A cells were incubated with increasing concentrations of H_2O_2 (50-200 µM) for 24 h. MTT results show a concentration-dependent decrease in cell viability.

***p<0.001 vs Ctrl.

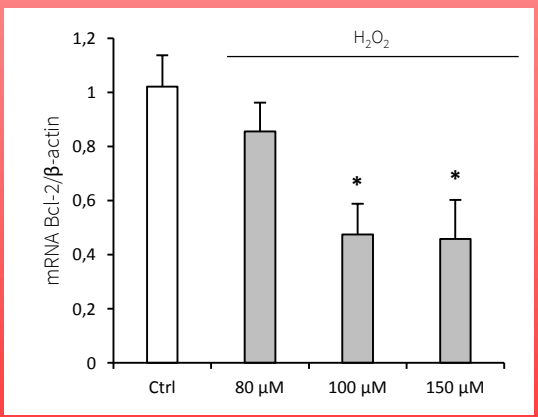


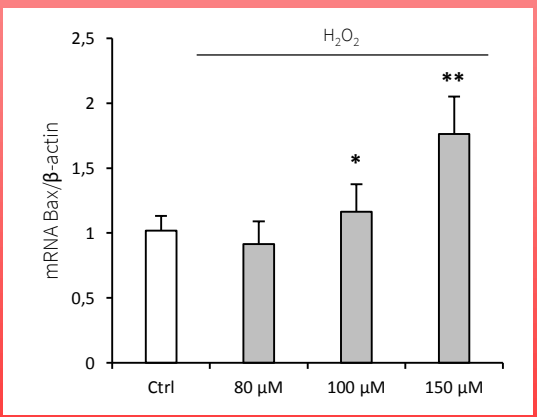


H_2O_2 increases caspase-3 and caspase-7 mRNA expression in Neuro2A.

To assess whether H_2O_2 activated the apoptosis pathway, Neuro2A cells were treated with H_2O_2 (80-150 µM). The mRNA levels of caspase-3 and caspase-7 were significantly increased after H_2O_2 treatment compared to Ctrl group.

*p<0.05, **p<0.01, ***p<0.001 vs Ctrl.





H_2O_2 activates apoptosis pathway in Neuro2A.

We investigated the mRNA expression levels of pro-apoptotic Bax and anti-apoptotic Bcl-2. H_2O_2 treatment (80-150 µM) up-regulated the expression of Bax but decreased Bcl-2 mRNA level compared to Ctrl group.

*p<0.05, **p<0.01 vs Ctrl.

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r.meanti@campus.unimib.it
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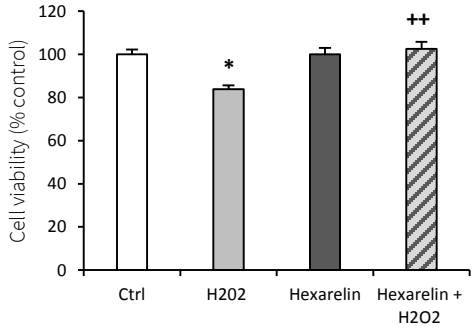
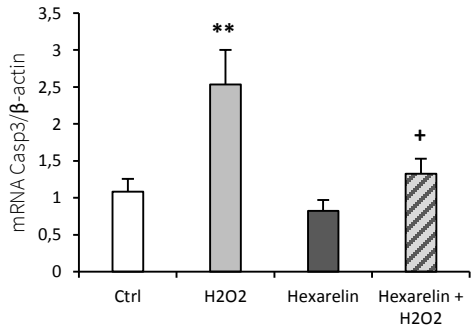
Results

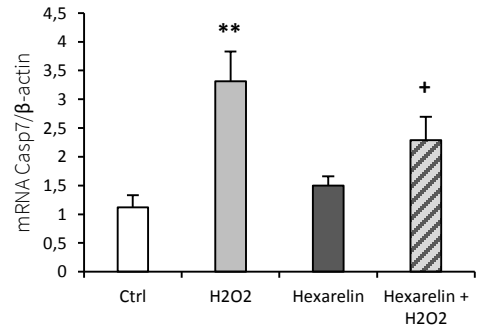


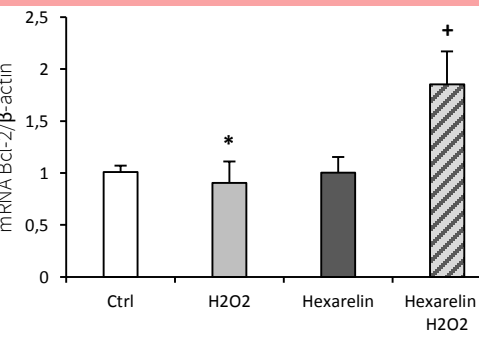
Effects of hexarelin on cell viability in Neuro2A cells treated with H_2O_2.

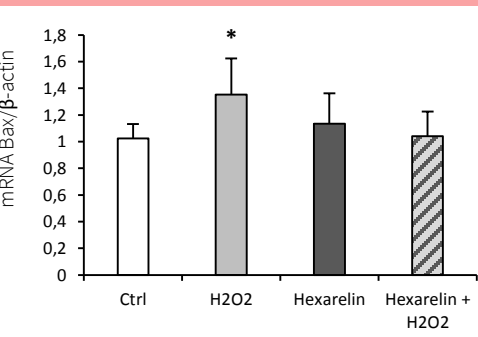
Neuro2A cells were treated for 24 h with H_2O_2 100 µM and hexarelin 10^{-6} M. MTT assay show that hexarelin protected the cells from H_2O_2 toxicity.

*p<0.05 vs Ctrl.
++p<0.01 vs H_2O_2.









Hexarelin reduces the expression of caspase-3 and caspase-7 and the apoptosis pathway in H_2O_2-stimulated Neuro2A cells.

Neuro2A cells were treated with H_2O_2 100 µM and hexarelin 10^{-6} M alone or in association, for 24 h. Hexarelin reduced the mRNA levels of caspase-3 and caspase-7 and modulated the mRNA level of Bax and Bcl-2, induced by H_2O_2.

*p<0.05, **p<0.01 vs Ctrl. +p<0.05 vs H_2O_2.

Conclusion

Our results demonstrate that hexarelin is capable to antagonize H_2O_2-toxicity in Neuro2A cells. In particular, H_2O_2 significantly reduced cell survival and activated the apoptosis mechanism. Hexarelin antagonized H_2O_2 effects, both on cell viability and mRNA expression levels. Further expreriments are needed to identify the molecular mechanisms underlying the neuroprotective activity of hexarelin.

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