Pharmacological Effects of Urocortin (Ucn) on Nicotine-Induced Oxidative Stress to Cardiomyocytes

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OBJECTIVES

Background:
One of the cytotoxic actions of nicotine is oxidative stress[1].
Recently, it is repoted that corticotropin-releasing hormone (CRH) related peptide, urocortin (Ucn) I, protects cultured hippocampal neurons and human umbilical endothelial cells against oxidative stress[2, 3].

The aim(s) of this present study:
To clarify the effects of Ucn I against nicotine-induced oxidative stress in cardiomyocytes.
Through which mechanism Ucn I exerts anti-oxidative stress in cardiomyocytes.

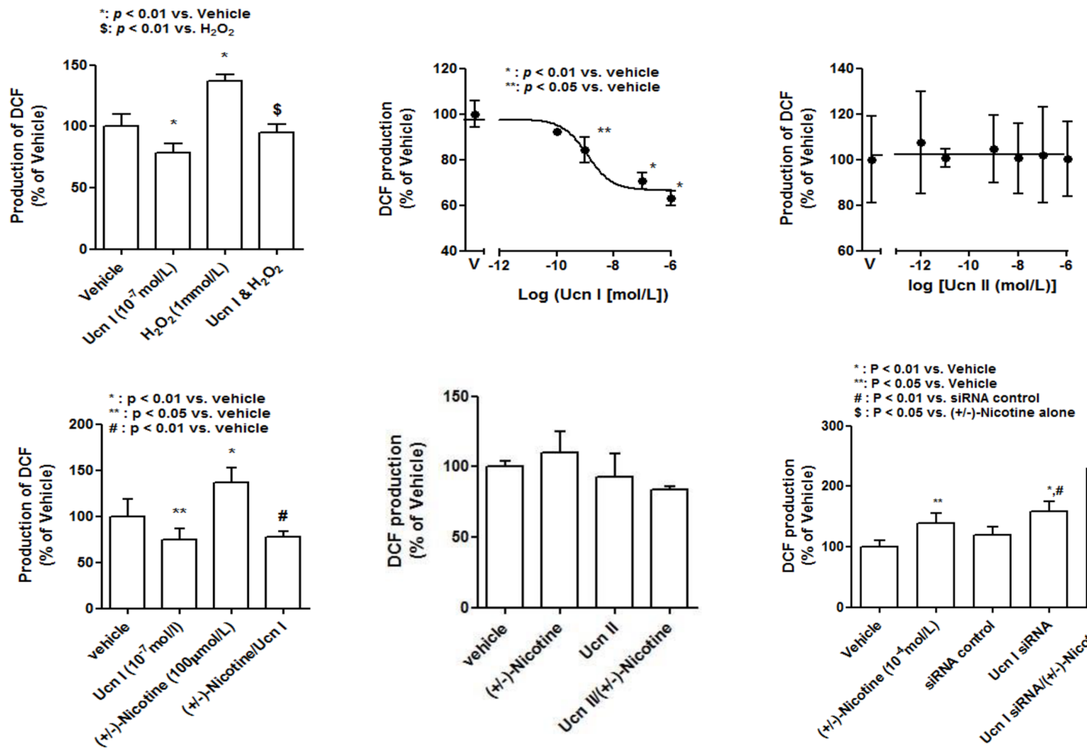
METHODS

- HL-1 caridomyocytes (mouse atrial cardiomyocyte cell line, gift from Prof. William C Claycomb, LSU Health Sciences Center, New Orleans, LA, USA)
- ROS assay (quatification & imaging)
- Agents: urocortin I/II, (+/-)-nicotine, H_2O_2
- Ucn I siRNA: siRNA designed by BLOCK-iT™ RNAi Designer (Thermofischer Scientific, Inc.)

ROS iquantification/maging:
1. Cells are plated in 96-well plate.
2. Culture with Claycomb medium containing 10% fetal bovine serum (FBS) for 48 hours.
3. FBS starvation for 24 hours.
4. Quantification :Loading of 2', 7'-Dichlorodihydrofluorescin diacetate (DCFH-DA) to the cells for 1 hour prior to stiumulation.
5. Stimulation with or without above mentioned agents.
6. Quantification: 12 hour after stimulation, conversion of DCFH-DA to 2', 7'-dichlorodihydrofluorescein (DCF) was measured.
7. Imaging: Dihydroethidium was loaded to the cardiomyocytes 30min prior to complete 24 hour incubation.

SUMMARY OF RESULTS

1.Ucn I exerted antioxidative actions against H_2O_2-/(+/-)-nicotine-induced oxidative stress.
2.Ucn I, but not Ucn II, exerted antioxidative actions in dose-dependent manner in the stimulant-free culture condition.
3. Knockdown of Ucn I mRNA resulted in increase in nicotine-induced oxidative stress.
4.Ucn II may reduce (+/-)-nicotine-induced anti-oxidative stress.



CONCLUSIONS

1.Ucn I may have anti-oxidative stress against denovo-synthesized and oxidant-induced oxidative stress.
2.Knockdown of Ucn I mRNA resulted in increase in oxidative stress, indicating that Ucn I may play essential roles on cell protection in HL-1 cardiomyocytes.
3.Ucn II did not exert antioxidative stress in stimulant-free HL-1 cardiomyocyte culture, whereas Ucn II may exert antioxidative stress against nicotine-induced oxidative stress, indicating that the mechanisms of anti-oxidative actions by these peptides may be defferent in spite of same receptor agonists.

References

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3.Honjo T, Inoue N, Shiraki R, et al. Endothelial urocortin has antioxidative properties and is upregulated by inflammatory cytokines and pitavastatin. J Vasc Res 2006; 43: 131 – 138

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