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

Keiichi Ikeda1, 2, Yoshinobu Manome1, and Katsuyoshi Tojo2, 3
Core Research Facilities for Basic Science 1(Division of Molecular Cell Biology), Research Center for Medical Sciences
2Institute of Clinical Medicine and Research
3Division of Diabetes and Endocrinology, Department of Internal Medicine
The Jikei University School of Medicine

OBJECTIVES

Background:
One of the cytotoxic actions of nicotine is oxidative stress1. Recently, it is reported that corticotropin-releasing hormone (CRH) related peptide, urocortin (Ucn) I, protects cultured hippocampal neurons and human umbilical endothelial cells against oxidative stress2, 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 cardiomyocytes (mouse atrial cardiomyocyte cell line, gift from Prof. William C Claycomb, LSU Health Sciences Center, New Orleans, LA, USA)
• ROS assay (qualification & imaging)
• Agents: urocortin I/II, (+/-)-nicotine, H2O2
• Ucn I siRNA: siRNA designed by BLOCK-it™ RNAi Designer (Thermofisher Scientific, Inc.)

ROS quantification/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 stimulation.
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.

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 anti-oxidative stress in stimulant-free HL-1 cardiomyocyte culture, whereas Ucn II may exert anti-oxidative stress against nicotine-induced oxidative stress, indicating that the mechanisms of anti-oxidative actions by these peptides may be different in spite of same receptor agonists.

SUMMARY OF RESULTS

1. Ucn I exerted antioxidative actions against H2O2-/(+/-)-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.

References


The present study was supported by the grant from Smoking Research Foundation.