CHRD 2021: Abstract & Poster Submission Form

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Research Category:

- ◉ Basic Science
- ○ Clinical
- ○ Community Health / Policy

What was your role in the project?

- ☑ Design
- ☑ Perform Experiments
- ☑ Analyze Data
- ☑ Write Abstract
- ☐

Presenter Status:

- ○ Undergraduate Students
- ◉ Masters Student
- ○ PhD Student
- ○ Post-Doctoral Fellows
- ○ Residents
- ○ Non-Trainee

Title

Inhibition of Adenylyl Cyclase Isoform 6 by Cysteine Nitrosylation in Hypoxic Pulmonary Hypertension of the Newborn

Background

Persistent pulmonary hypertension of the newborn (PPHN) is a disastrous failure of the neonatal pulmonary vascular relaxation causing 10-25% mortality. It features hypoxemia and pulmonary vasoconstriction; first-line therapies include inhaled nitric oxide (NO). The adenylyl cyclase signaling pathway is a crucial therapeutic target in PPHN, causing vascular relaxation. We previously reported that activity of the dominant pulmonary arterial AC isoform, AC6, is persistently inhibited after neonatal hypoxia in vivo and in vitro.

Objective

In this study, we hypothesized that both hypoxia and NO can promote cysteine thiol nitrosylation of AC6, decreasing its activity; and that the allosteric AC activator forskolin can reverse this inhibition.

Methods

HEK cells stably overexpressing individual AC isoforms (AC 3, 5, 6, 7, 9) were cultured in a normoxic (21% O2, 5% CO2) or hypoxic (10% O2, 5% CO2) incubator for 72 hours. Normoxic cells were also treated with nitroso-donor S-nitrosocysteine (CSNO, 250μM) for 30 mins. Cells from all treatment groups were lysed for measuring AC activity (as loss of terbium(III)-norfloxacin fluorescence due to ATP catalysis; and by live-cell cAMP assay) and detection of protein nitrosylation (biotin-switch assay kit, to label nitrosylated thiols). The forskolin dose-response of hypoxic and normoxic cells was calculated to determine biochemical reactivation of AC6 function.

Results

Among the five AC isoforms studied, only AC6 activity was significantly inhibited by hypoxia and CSNO treatment, resulting in decreased ATP catalysis and impaired cAMP generation. This inhibition correlated with increased nitrosylation of AC6 protein. The inhibited AC6 could be activated by forskolin.

Conclusion

We conclude that protein nitrosylation is a critical modification occurring during hypoxia which inhibits AC6 activity, impairing cAMP production. Forskolin partially reactivated AC6 activity and restored cAMP generation. These findings may lead the way to design novel forskolin derivatives that selectively activate AC6 to provide new treatments for hypoxic PPHN.

Authors

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