CHRD 2022: Abstract & Poster Submission Form

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Presenter Status

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

Research Category

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

Role in the project

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

Title

Oxidized Phosphatidylcholine Reduces-Agonist Induced β2Adrenergic Receptor (β2AR) Internalization: A Mechanism for β2AR Signaling Inhibition?

Background

Asthma, the number one cause of children visiting the ER in Canada, affecting 12% of Canadian children. β2AR agonists are the front-line reliever therapy, as they bronchodilate airways for temporary relief from asthmatic symptoms. However, insensitivity to β2AR agonists can develop in some patients, making symptoms difficult to control. We showed that asthma-associated oxidized phosphatidylcholine (OxPAPC) exposure leads to β2AR desensitization in mouse airway tissue, but the mechanism for this effect is unknown.

Objective

We hypothesized that OxPAPC increases β2AR agonist-induced receptor internalization to inhibit receptor signalling.

Methods

Human Embryonic Kidney (HEK) 293 cells expressing HA-tagged β2AR were grown to confluence, then treated with OxPAPC (10-80 μg/mL) or regular medium (control) for 24 hours. Cells were stimulated with isoproterenol (Iso) (0.1 pM to 10 μM) for 20 minutes to induce β2AR internalization, compared to baseline cell surface receptor abundance. The surface abundance of β2AR was determined by colorimetry, using HRP-conjugated chicken polyclonal anti-HA-tag antibody with TMB substrate. Data were analyzed by one-way or two-way ANOVA with relevant post-hoc test.

Results

Cell surface abundance of β2AR was not affected by pre-exposure with any concentration of OxPAPC (10-80 μg/mL). Isoproterenol treatment induced significant receptor internalization in a concentration-dependent fashion, with a maximum internalization of 37.7% observed at 10 μM Iso. Of note, we measure a concentration dependent effect of OxPAPC pre-exposure on Iso-induced β2AR internalization: OxPAPC (80 μg/mL) reduced agonist-induced internalization at all Iso concentrations, including a 75% less maximum internalization (8%) in response to 10 μM Iso. OxPAPC (40 μg/mL) decreased maximum Iso-induced β2AR internalization by 50% (18% internalization), and 10 μg/mL OxPAPC pre-treatment was sufficient to reduce maximum Iso-induced β2AR internalization by 16% (31.6% internalization).

Conclusion

Oxidized phosphatidylcholine impairs β2AR-agonist mediated receptor internalization, but not baseline cell surface receptor availability. This implicates OxPCs as regulators of β2AR sensitivity and the response to bronchodilator therapies.

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Authors

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