CHRD 2022: Abstract & Poster Submission Form

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Presenter Status

- ◉ Undergraduate Students
- ○ Masters Student
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- ○ 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 Induced-β2 Adrenergic Receptor Desensitization Requires Protein Kinase C Activation in Airway Smooth Muscle Cells

Background

Asthma affects 1 in 10 Canadian children and is characterized by persistent airway inflammation and airway hyperresponsiveness. A significant number of asthmatics are refractory to bronchodilator therapies targeting β2 adrenergic receptors (β2AR) in the airways, but the mechanism remains unclear. In cultured human airway smooth muscle (HASM) cells, we showed that oxidized phosphatidylcholines (OxPAPC) induce inflammatory mediator release via pathways involving protein kinase C (PKC) and cyclooxygenase-2 (COX2). OxPAPC also inhibits β2AR-agonist mediated airway relaxation.

Objective

We hypothesized that PKC, COX2, or both pathways are required for OxPAPC-induced β2AR desensitization.

Methods

Human-telomerase immortalized HASM cells from 5 independent donors were used for experiments. β2AR-agonist induced cAMP signalling was assessed by tracking phosphorylation of the protein kinase A substrate, VASP, using immunoblotting. Serum starved cells were pre-incubated for 2 hours with PKC inhibitor (GF-109203x, 10 μM) or COX2 inhibitor (indomethacin, 10 μM) then treated with OxPAPC (80 μg/mL) for 24 hours. Controls included: control (media), vehicle (DMSO), inhibitor alone, or OxPAPC alone. Cells were stimulated with isoproterenol (1 nM), a β2AR agonist, for 7 minutes and cell lysates were obtained for immunoblotting. Using densitometry, band signal was quantified to calculate the % p-VASP. Data was analyzed by one-way ANOVA with Tukey's post-hoc test.

Results

Stimulation with isoproterenol caused a significant increase in cAMP signaling (58.5% p-VASP) compared to unstimulated cells (10.9% p-VASP). OxPAPC pre-exposure significantly reduced cAMP signaling in response to isoproterenol (17.0% p-VASP). Indomethacin did not prevent OxPAPC effects on cAMP signaling (13.5% p-VASP) whereas PKC inhibitor treatment significantly inhibits the suppressive effects of OxPAPC on intracellular cAMP signaling (43.3% p-VASP). All independent controls (media, vehicle, inhibitor alone) showed comparable responses to stimulated cells.

Conclusion

Oxidized phosphatidylcholine requires PKC activation to inhibit β2AR function in HASM cells. This finding reveals a contributing molecule in the desensitization of β2AR, furthering our understanding of the mechanism.

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