Oxidized Phosphatidylcholine Reduces Agonist-Induced β_2Adrenergic Receptor (β_2AR) Internalization: A Mechanism for β_2AR Signaling Inhibition?

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INTRODUCTION

- Asthma, the number one cause of children visiting the ER in Canada, affecting 12% of Canadian children. There's a significant number of patients whose asthma remains uncontrolled.
- β_2AR agonists are the front-line reliever therapy, as they bronchodilate airways for temporary relief from asthmatic symptoms.
- However, insensitivity to β_2AR agonists can develop in some patients, making symptoms difficult to control. We showed that asthma-associated oxidized phosphatidylcholine (OxPAPC) exposure leads to β_2AR desensitization in mouse airway tissue, but the mechanism for this effect is unknown.

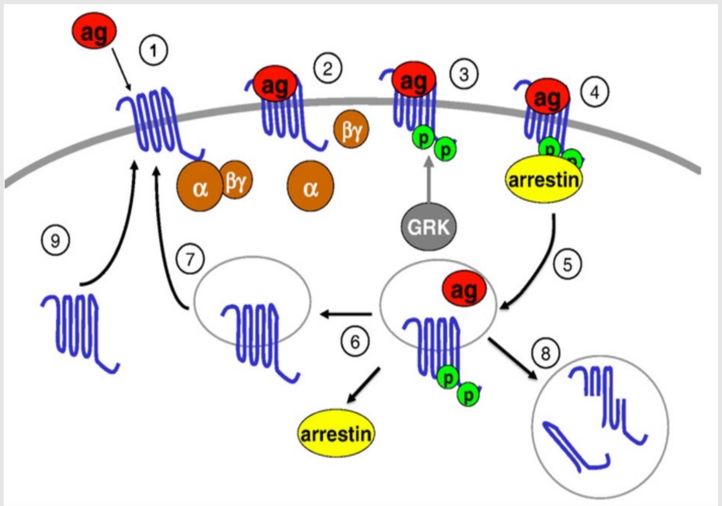


Figure 1. Beta-agonist induced β_2AR internalization via β-arrestin pathway.

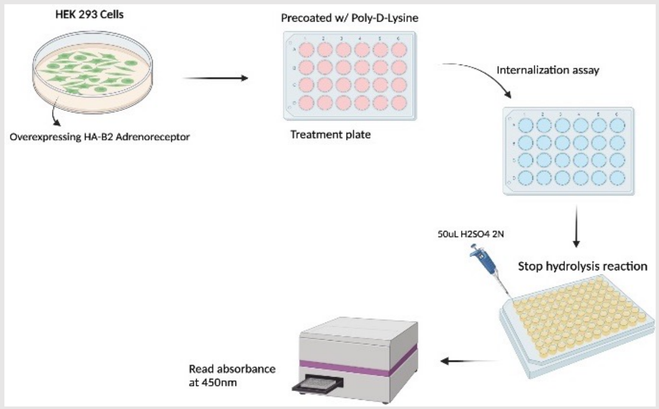
HYPOTHESIS

Oxidized phosphatidylcholine ***increases*** *β_2AR agonist-induced receptor internalization to inhibit receptor signaling.*

METHODS

Treatments:

- OxPC treatment (10-80 ug/mL).
- Different isoproterenol (iso) concentrations (0.1-10uM). Incubate for 20 minutes.
- HRP-conjugated chicken polyclonal anti-HA-tag antibody/1hr.
- TMB (ELISA) used as substrate.



RESULTS

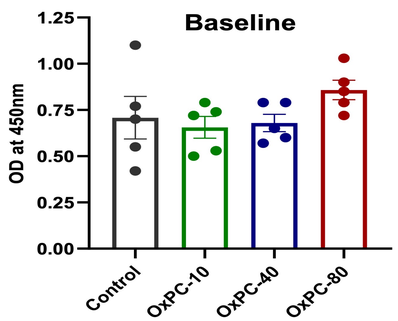


Figure 2. Cell surface abundance of β_2AR was not affected by pre-exposure with concentration of oxidized phosphatidylcholine at 10-80 µg/mL (n=5).

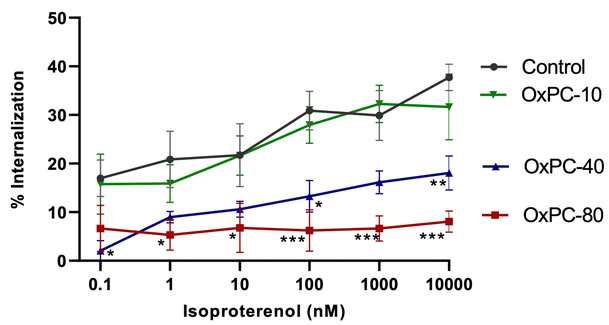


Figure 3. Isoproterenol treatment induced significant receptor internalization with a maximum internalization of 37.7% observed at 10 µM Iso. OxPAPC (80 µg/mL) reduced agonist-induced internalization at all Iso concentrations, including a 75% less maximum internalization in response to 10 µM Iso. OxPAPC (40 µg/mL) decreased maximum Iso-induced β_2AR internalization by 50%, and 10 µg/mL OxPAPC pre-treatment was sufficient to reduce maximum Iso-induced β_2AR internalization by 16%. n=5.

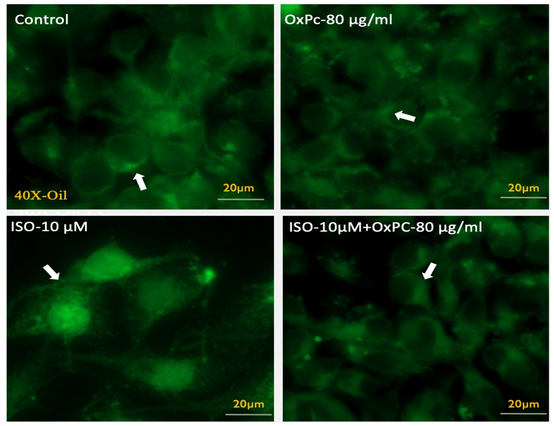


Figure 4. Immunocytochemistry was performed using HEK cells and FITC rabbit polyclonal anti-HA tag/IgG . The figure above depicts a similar pattern of receptor expression in control, OxPC-80, and Iso-10uM with OxPC groups.

CONCLUSION

- Oxidized phosphatidylcholine impairs β_2AR-agonist mediated receptor internalization, but not baseline cell surface receptor availability.
- Implicates OxPCs as regulators of β_2AR sensitivity and the response to bronchodilator therapies.

REFERENCES

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