Oxidized Phosphatidylcholine Induced-β2 Adrenergic Receptor Desensitization Requires Protein Kinase C Activation in Airway Smooth Muscle Cells

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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.

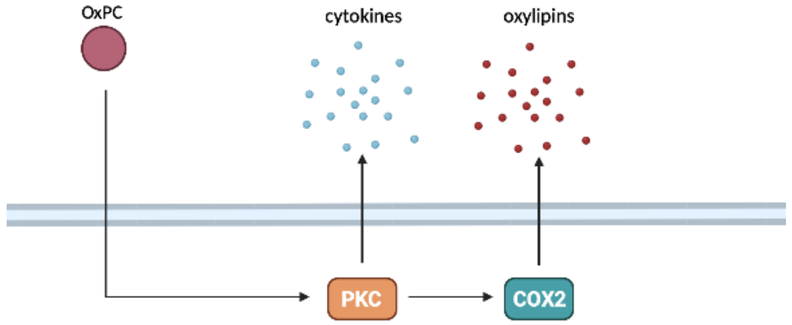

Figure 1. Schematic pathway of OxPC induced inflammatory mediator release via activation of PKC and COX-2.

Hypothesis

- 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.

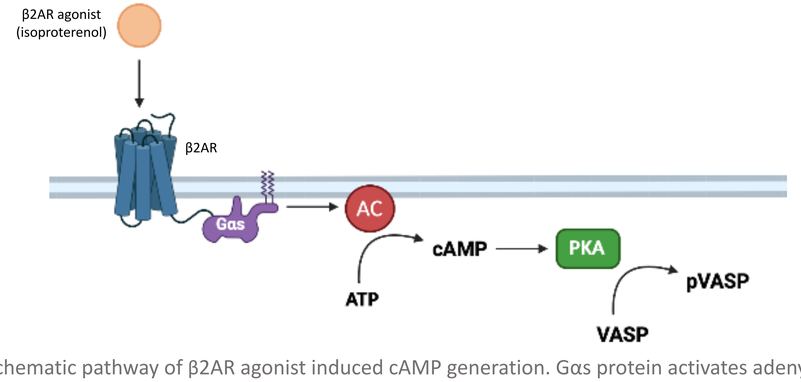

Figure 2. Schematic pathway of β2AR agonist induced cAMP generation. Gαs protein activates adenylyl cyclase (AC), causing the conversion of ATP to cAMP. cAMP activates protein kinase A (PKA), causing the phosphorylation of VASP.

Results

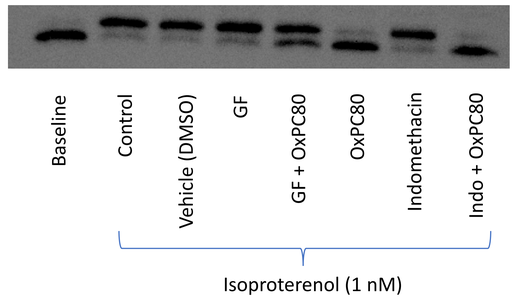

Figure 3. Imaged membrane showing P-VASP (top band) and VASP (bottom band) for all 8 conditions. Cells stimulated with isoproterenol (1 nM).

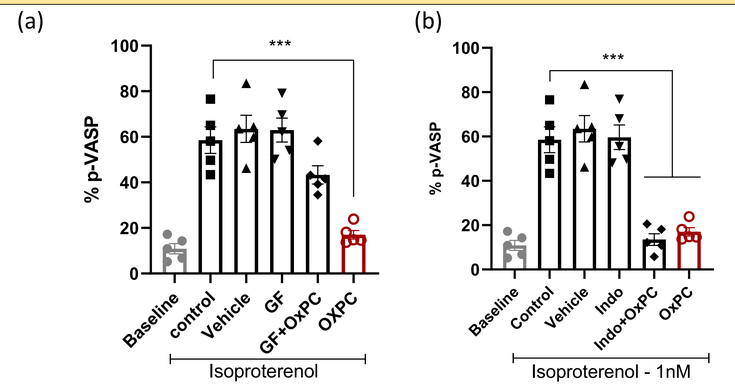

Figure 4. Densitometric analysis of PKC Inhibition (a) and COX-2 inhibition (b). Isoproterenol (1 nM) stimulated cells show 58.5% p-VASP compared to PKC inhibited cells treated with OxPCs (43.3% p-VASP) and COX-2 inhibited cells treated with OxPCs (13.5% p-VASP).

Conclusions

- OxPC induced desensitization of beta-2-adrenergic receptors does not involve the activation of COX-2
- OxPC induced desensitization of beta-2-adrenergic receptors requires PKC activation

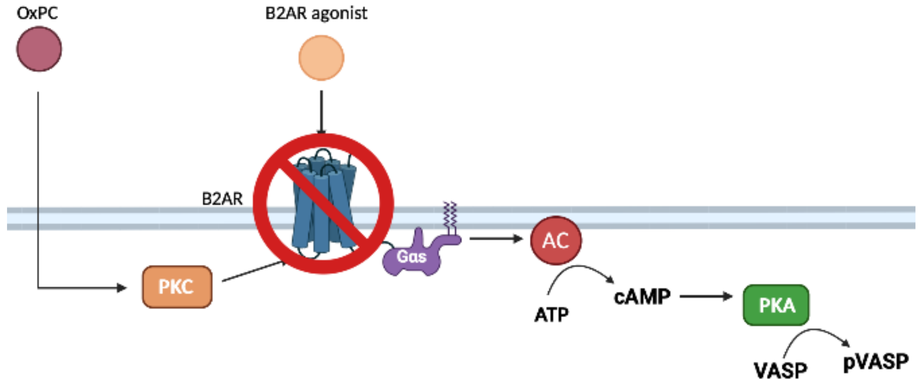

Figure 5. Proposed pathway of OxPC-induced beta-2-adrenergic receptor desensitization via PKC activation.

References

1. Pascoe CD, Jha A, Ryu MH, et al. Allergen inhalation generates pro-inflammatory oxidised phosphatidylcholine associated with airway dysfunction. Eur Respir J 2021; 57: 2000839 [https://doi.org/10.1183/13993003.00839-2020].
2. Vaghasiya et al. Oxidized Phosphatidylcholine Impairs B2-Adrenoceptor Mediated Relaxation of Murine Airways. Am J Respir Crit Care Med 2022;205:A3275

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