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OXIDIZED PHOSPHATIDYLCHOLINE INDUCES RELEASE OF PRO-CONTRACTILE INTRACELLULAR CA2+ IN HUMAN AIRWAY SMOOTH MUSCLE CELLS.

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Background:
Oxidative stress develops in lungs of asthmatics, leading to oxidation of phosphatidylcholine (PC) in lung cell membranes and extracellular space, and the formation of bioactive variants that sustain inflammation. Oxidized phosphatidylcholine (OxPC) accumulates in the lungs of mice and humans after inhaled allergen challenge, and in parallel with the emergence of a hallmark symptom of asthma, airway hyperresponsiveness. We have observed that OxPC induces inflammatory mediator release by cultured human airway smooth muscle cells (HASM).

Objective:
We test the hypothesis that OxPC increases cytosolic concentration of Ca^{2+} ($[Ca^{2+}]_i$) in HASM, a key trigger for cell contraction.

Methods:
Serum starved HASM were used to assess real-time changes in $[Ca^{2+}]_i$ using the Ca^{2+} sensitive dye, Fura-2, and fluorescent microscopy. In the presence and absence of extracellular Ca^{2+}, we evaluated the effects of acute OxPC exposure (10-80 mg/mL) on $[Ca^{2+}]_i$. To decipher the source of $[Ca^{2+}]_i$ release, studies also included pre-exposure to xestospongine (5 μM, IP-3 channel antagonist), or the ryanodine channel modulators, ryanodine (100 μM) and caffeine (25 mM).

Results:
In presence or absence of extracellular Ca^{2+}, OxPC induced a dose-dependent increase in peak $[Ca^{2+}]_i$ (85.3 ± 23.5nM with 10 mg/mL; 200.8 ± 28.7nM at 80 mg/mL; n=4). In the presence, but not in the absence of extracellular Ca^{2+}, OxPC induced concentration-dependent repeated waves of $[Ca^{2+}]_i$ in some cells (35% of cells at 10 mg/mL, and 81% of cells at 80 mg/mL OxPC). Ryanodine receptor inhibition with ryanodine and caffeine significantly inhibited OxPC-induced $[Ca^{2+}]_i$ flux ($P<0.01$), whereas IP3 receptor inhibition with xestospongine was without effect.

Conclusion:
These findings are the first to demonstrate that OxPC induces intracellular calcium flux in HASM, likely via pathways involving membrane ion channels and ryanodine receptor-sensitive stores of the sarcoplasmic reticulum. This suggests OxPCs may modulate contractile activity of airway smooth muscle and regulate airway responsiveness.