Oxidized Phosphatidylcholine Induces Release of Intracellular Ca\textsuperscript{2+} in Human Airway Smooth Muscle Cells


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Rationale: Asthma pathobiology is associated with oxidative stress in the lung. Phosphatidylcholine (PC), a major phospholipid in lung cells and extracellular fluids, can be oxidized into bioactive pro-inflammatory variants. We have shown that a profile of oxidized phosphatidylcholine (OxPC) accumulates in the lungs of mice and humans after inhaled allergen challenge, in parallel with the emergence of a hallmark asthma symptom of asthma, airway hyperresponsiveness. Exposure to OxPC also induces inflammatory mediator release by cultured human airway smooth muscle cells (HASM). Here we test the hypothesis that OxPC increases intracellular free Ca\textsuperscript{2+} concentration ([Ca\textsuperscript{2+}]\textsubscript{i}) in HASM, a key trigger of contraction. Methods: Confluent cultures of human telomerase immortalized HASM were serum starved for 7 days. Real-time changes of [Ca\textsuperscript{2+}]\textsubscript{i} were recorded in cells loaded with the Ca\textsuperscript{2+} sensitive dye, Fura-2, using a fluorescent microscope. The maximum change in [Ca\textsuperscript{2+}]\textsubscript{i} from baseline was measured. In the presence and absence of extracellular Ca\textsuperscript{2+}, we evaluated change in [Ca\textsuperscript{2+}]\textsubscript{i} upon acute exposure to the OxPC, oxidized products of 1-palmitoyl-2-arachidonoyl-sn-glycerol-3-phosphatidylcholine (OxPAPC, 10-80 µg/mL). To decipher the source of [Ca\textsuperscript{2+}]\textsubscript{i} release, some studies also included xestospongine (5 µM, IP-3 channel blocker), or ryanodine channel modulators, ryanodine (100 µM) or caffeine (25 mM). Additionally, in HASM pre-exposed to OxPAPC (80 µg/mL) for 30 minutes, we evaluated changes in [Ca\textsuperscript{2+}]\textsubscript{i} flux induced by contractile agonist, acetylcholine (0.1 µM). Results: In presence or absence of extracellular Ca\textsuperscript{2+}, OxPAPC induced a dose-dependent increase in peak [Ca\textsuperscript{2+}]\textsubscript{i} (85.3 ± 23.5nM with 10 µg/mL; 200.8 ± 28.7nM at 80 µg/mL; n=4). OxPC also induced a sustained elevation of [Ca\textsuperscript{2+}]\textsubscript{i} in 35%-to-81% of cells (10 µg/mL and 80 µg/mL OxPAPC, respectively). This phenomenon was abolished by removal of extracellular calcium (Figure 1). Ryanodine receptor inhibition with higher concentrations of ryanodine and caffeine significantly inhibited OxPC-induced [Ca\textsuperscript{2+}]\textsubscript{i} flux (P<0.01), whereas IP3 receptor inhibition with xestospongine was without any significant effect. OxPAPC (80 µg/mL) pre-exposure augmented peak [Ca\textsuperscript{2+}]\textsubscript{i} flux by 3-fold in response to acetylcholine (1µM; P<0.001). Conclusion: These findings demonstrate that OxPC induce release of intracellular calcium in HASM via pathways involving membrane-associated cation channels and ryanodine receptor-sensitive stores of the sarcoplasmic reticulum. This suggests OxPC may modulate contractile activity of airway smooth muscle and regulate airway responsiveness.

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