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OXIDIZED PHOSPHATIDYLCHOLINE MODULATES CAVEOLAE AND CAVEOLIN-1 EXPRESSION IN HUMAN AIRWAY SMOOTH MUSCLE CELLS

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Background:
Asthma pathobiology is associated with oxidative stress that leads to oxidation of phosphatidylcholine, a major phospholipid in lung cells. We showed that allergen challenge of the lung leads to oxidized phosphatidylcholine (OxPC) formation, and prolonged OxPC exposure impairs receptor-mediated contractile activation in human airway smooth muscle cells (HASM). Caveolin-1 (Cav-1) is a principal component of specialized plasma membrane lipid rafts called caveolae, which are focal points for regulation of receptor mediated signaling.

Objective:
We hypothesize that OxPC exposure disrupts caveolae and spatial distribution of Cav-1 in HASM.

Methods:
Confluent cultures of HASM were serum starved for 7 days and incubated with OxPC (20mg/mL) for 24 hours. Cells were lysed with carbonate buffer and fractionated using sucrose gradient ultracentrifugation to isolate caveolae-enriched membranes. Individual fractions and total protein lysates were subjected to SDS-electrophoresis (15% polyacrylamide) and western blotting for the Cav-1, and $G_{q\alpha}$, a G-protein subunit associated with M3 receptors for the contractile agonist, acetylcholine. In other studies, we performed fluorescent immunocytochemistry for Cav-1 in HASM .

Results:
OxPC downregulated Cav-1 by 53% in total protein lysates ($p<0.01$), whereas, $G_{q\alpha}$ protein abundance was not affected compared to control conditions. OxPC exposure resulted in redistribution of $G_{q\alpha}$ from the Cav-1 enriched caveolae fractions; accumulating in cell fractions that include cytosol and lipid-raft deficient membranes. Immunocytochemistry revealed that OxPC treatment disrupted spatial organization of caveolae, causing a dramatic loss of longitudinal linear arrays of Cav-1 that are evident on HASM under control conditions.

Conclusion:
Our results reveal that OxPC may integrate with the plasma membrane of HASM, leading to disruption of caveolae distribution and the association of key signaling proteins, such as $G_{q\alpha}$. This suggests that OxPC exposure has significant effects on receptor mediated airway biology that could be important in understanding responses to asthma pharmaco-therapeutics.