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Cigarette smoke and calcium conspire to impair CFTR function in airway epithelia

Andrew P Braun*
University of Calgary; Calgary, AB Canada

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*Correspondence to: Andrew P Braun; Email: abraun@ucalgary.ca

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Rasmussen JE, Sheridan JT, Polk W, Davies CM, Tarran R. Cigarette smoke-induced Ca2+ release lead to cystic fibrosis transmembrane conductance regulator (CFTR) dysfunction. J Biol Chem 2014; 289:7671-81; PMID:24448802; http://dx.doi.org/10.1074/jbc.M113.545137

To maintain health and function in response to inhaled environmental irritants and toxins, the lungs and airways depend upon an innate defense system that involves the secretion of mucus (i.e., mucin, salts, and water) by airway epithelium onto the apical surface to trap foreign particles. Airway mucus is then transported in an oral direction via ciliary beating and coughing, which helps to keep the airways clear. CFTR (cystic fibrosis transmembrane conductance regulator) is a cAMP-regulated Cl^- channel in the apical membrane of epithelium that contributes to salt and water secretion onto the luminal surface of airways, thereby ensuring that secreted mucus is sufficiently hydrated for movement along the epithelial surface. Dehydration of airway mucus, as occurs in cystic fibrosis, results in a more viscous, less mobile secretion that compromises the lung's innate defense system by facilitating a build-up of foreign particles and bacterial growth. Related to this situation is chronic obstructive pulmonary disease (COPD), which is a leading cause of death globally. A major cause of COPD is cigarette smoking, which has been reported to decrease the cellular levels of CFTR in airway epithelia. In their recent article, Rasmussen and coworkers now report that exposure to cigarette smoke elevates cytosolic free Ca^{2+} in airway epithelium, leading to decreased surface localization and cellular expression of CFTR and reduced levels of secreted airway surface liquid. Blocking this increase in cytosolic Ca^{2+} largely prevented CFTR loss in airway epithelium and surprisingly, cellular lysosomes appear to be a major source for smoke-induced Ca^{2+} elevation.

Experimentally, the authors tracked native CFTR expression/localization in human bronchial airway cells or recombinant CFTR expressed in established cell lines (i.e., BHK, CALU3, and HEK293T) either by western blot analysis or immunocytochemistry. Cytosolic free Ca^{2+} was monitored either by Fura-2 imaging or GFP-based Ca^{2+} indicators targeted to select sub-cellular organelles (i.e., mitochondria, lysosomes). Exposure of cultured cells to the vapor phase of cigarette smoke was used to mimic that experienced by a typical smoker (i.e., 1 puff per min for 10 min (acute exposure) or 10 puffs per 2 h for 8 h (chronic exposure)).

Using a variety of pharmacologic inhibitors to block major signal transduction pathways, the authors discovered that cigarette smoke-induced decrease in cellular CFTR was insensitive to agents that interfered with protein phosphorylation (i.e., the cAMP/PKA inhibitor H89, the broad spectrum kinase inhibitor staurosporine, the phosphatase 1/2A inhibitor okadaic acid and the PI-3 kinase inhibitors LY294002 and wortmannin). In contrast, chelation of intracellular free Ca^{2+} by BAPTA-AM largely prevented the smoke-mediated CFTR reduction, indicating an essential role for cytosolic free Ca^{2+} in this response. Interestingly, cigarette smoke exposure did not disrupt the cellular expression of the Ca^{2+}-sensitive Cl^- channel Ano1 (aka TMEM16A), suggesting that cigarette smoke may not cause broad impairment of membrane ion channels.

Whereas BAPTA-AM prevented CFTR downregulation in response to cigarette smoke exposure, treatment of cells with the Ca^{2+} ionophore ionomycin mimicked the decrease in CFTR expression, suggesting that elevated cytosolic Ca^{2+} per se was an important condition. Monitoring intracellular Ca^{2+} dynamics in airway epithelial cells further revealed that cigarette smoke exposure evoked a slow rising and prolonged increase in cytosolic free Ca^{2+} that reach its peak within 5–10 min and occurred with a delay of 1–2 min following smoke exposure. This unusual response profile contrasted that observed following the UTP/ATP-dependent activation of endogenous G-protein-coupled purinergic receptors, which evoked a rapid and transient increase in cytosolic Ca^{2+} that was not associated with the loss of CFTR. Whereas this latter response was typical of Ins(1,4,5)P_3-mediated Ca^{2+} release from the endoplasmic reticulum and involved STIM1 activation, the authors could find no evidence that cigarette smoke evoked a similar clustering of activated STIM1 or an increase in cellular second messengers known to elevate cytosolic free Ca^{2+} (i.e., Ins(1,4,5)P_3, cyclic ADP-ribose or NAADP). The smoke-induced Ca^{2+} elevation was also insensitive to the established SERCA inhibitor thapsigargin, whereas this agent reduced/prevented Ca^{2+} elevations in response to UTP.

Although it was evident that cigarette smoke exposure could elevate cytosolic free Ca^{2+} in airway epithelium, the source of the Ca^{2+} remained a puzzle. Acute removal of external Ca^{2+} did not influence either the amplitude or kinetics of the smoke-mediated increase, suggesting release from an internal pool. Mitochondria are known to contain millimolar levels of Ca^{2+}, but treatment of cells with CCCP, an agent that decreases mitochondrial Ca^{2+} uptake by uncoupling the electron transport chain, did not interfere with or desensitize cigarette smoke-induced Ca^{2+} elevation or CFTR loss.

In contrast to the above manipulations, the authors observed that pre-treatment of cells with the lysosomal inhibitor bafilomycin A reduced the cigarette smoke-mediated elevation in cytosolic free Ca^{2+}, along with the loss of CFTR, both at the cell surface and whole cell levels. By blocking the vacuolar H^+ ATPase, bafilomycin A reduces acidification of lysosomes, leading to a loss of internal Ca^{2+}. Using a FRET-based, genetically-encoded Ca^{2+} indicator coupled to the lysosomal protein LAMP1, the authors further noted that cigarette smoke exposure evoked Ca^{2+} elevations in the proximity of lysosomes, consistent with the possible release of Ca^{2+} from this organelle upon smoke exposure. It was further observed that bafilomycin A treatment could prevent the reduction of airway surface liquid secretion evoked by cigarette smoke exposure; this result thus provides an important functional correlate for the preceding molecular and cell biological data describing changes in CFTR protein levels.

Although the exact mechanisms by which cigarette smoke exposure and lysosomal release of Ca^{2+} lead to loss of epithelial CFTR remain unclear, the authors speculate that cigarette smoke may compromise the integrity of CFTR structure and/or the cellular protein quality control machinery regulating CFTR levels, leading to CFTR internalization and aggregation in a detergent-insoluble cellular compartment. Based on the results of the study, it also remains unclear how elevated cytosolic Ca^{2+} contributes to CFTR loss, as the authors did not elaborate a clear temporal relation or mechanistic link between intracellular Ca^{2+} mobilization and CFTR expression. Identifying cellular events/processes induced by prolonged vs. transient Ca^{2+} elevations may provide important insights into this issue. It is also suggested that cigarette smoke-induced elevations in cytosolic Ca^{2+} may be involved in long-term events (e.g., gene transcription) that could promote epithelial cell survival in response to the toxic insults associated with smoking. Although the connection between cigarette smoking and COPD is well-established from a health care perspective, the results of this study increase our knowledge of the underlying molecular pathology evoked by chronic cigarette smoke exposure and point to additional targets/strategies that may mitigate the airway dysfunction associated with this disease.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.