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Reactive Oxygen Species Behaving Badly: Oxidized Phosphatidylcholines Corrupt Ca2+ Signaling in Airway Smooth Muscle

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Increasing evidence points to oxidative stress affecting multiple cell types as an important driver of obstructive lung diseases. Reactive oxygen species and reactive nitrogen species damage lipid and protein components of extracellular fluids, cell membranes, organelles, and the cell cytosol. Oxidized phosphatidylcholines (OxPCs) are among the bioactive agents generated by oxidation of the unsaturated fatty acyl chain, leading to hundreds of potential fragmented and cyclized variants and breakdown products such as malondialdehyde (1). Pascoe and colleagues previously reported that OxPCs disrupt mitochondrial metabolic activity in human airway epithelial cells to promote reactive oxygen species generation, reduce cell viability, and impair epithelial barrier function and barrier recovery after a wound (2). More recently, the same group identified specific OxPCs that associate with airway hyperresponsiveness in BAL samples from asthmatic cohorts, thus establishing OxPCs to be effectors of oxidative stress in asthma (3). Moreover, treatment of human airway smooth muscle (ASM) cells with a subset of OxPCs (oxidized 1-palmitoyl-2-arachidonoyl-sn-glycero-3-phosphocholines [OxPAPCs]) induced the expression of multiple inflammatory cytokines and oxylipins, suggesting a proinflammatory role of OxPCs in asthma. In addition, OxPAPCs caused airway narrowing in murine precision-cut lung slices, consistent with the correlation of OxPAPC expression and airway hyperresponsiveness in human subjects.

In this issue of the *Journal*, Vaghasiya and colleagues (pp. 649–665) provide mechanistic insight into the procontractile effect of OxPCs on ASM (4). Employing human ASM cultures, murine lung slices, and human lung tissue sections, they dissect the sources of intracellular calcium mobilization by OxPCs in ASM and causally link them to ASM contraction (Figure 1). Specifically, they demonstrate that, in ASM cells, OxPAPCs induce an immediate and sustained Ca^{2+} flux. The former is independent of extracellular Ca^{2+} and does not involve inositol 1,4,5-trisphosphate (IP_3) receptors (IP_3Rs), but does involve ryanodine receptors (RyRs) and cyclic ADP ribose. The latter requires extracellular Ca^{2+} as well as TRPA1 (transient receptor potential ankyrin 1), whose expression was verified in human ASM.

From a functional perspective, this study describes the interesting (and somewhat surprising) discovery that inhibition of peak or sustained Ca^{2+} by RyR or TRPA1 inhibition, respectively, was sufficient to inhibit OxPC-induced ASM 20-kD regulatory myosin light chain phosphorylation and murine precision-cut lung slice contraction. Further experiments revealed that peak and sustained Ca^{2+} induction were not interdependent, as approaches that inhibit each fail to significantly affect the other. The unique observation that inhibition of peak or sustained Ca^{2+} alone is sufficient to inhibit OxPC-stimulated contraction demonstrates that these phases of mobilized Ca^{2+} are cooperative and are both required to induce ASM contraction. Interestingly, features of Ca^{2+} induction stimulated by OxPCs are qualitatively distinct from those promoted by the Gq-coupled M3 muscarinic acetylcholine receptor. For example, IP_3R inhibition prevents Ca^{2+} flux stimulated by acetylcholine but not by OxPCs, whereas RyR inhibition affects peak Ca^{2+} by OxPCs but not by acetylcholine. TRPA1 activity induced by OxPAPCs is not a result of the release of RyR-regulated intracellular Ca^{2+} pools.

A wide variety of molecules that are spatially and temporally distinct elicit Ca^{2+} signals in airway cells (5). For example, localized Ca^{2+} increases remain confined whereas other Ca^{2+} signals gradually diffuse to form Ca^{2+} waves or oscillations. Each of these patterns involves distinct cellular Ca^{2+} sources and downstream effectors to effect specific cellular functions. Previous studies in ASM cells have demonstrated distinct patterns of Ca^{2+} increases (oscillations, transient, biphasic, sparks), subcellular localizations (mitochondria, cytosolic), and sources of Ca^{2+} (IP_3R, RyR, VDCC, and SOCE), albeit using ionotropic and metabotropic ligands (6–9). Vaghasiya and colleagues demonstrate that oxidized lipids, OxPCs, induce Ca^{2+} signals via distinct sources of Ca^{2+} in ASM cells. These findings further underscore the complex nature by which the spatial and temporal features of intracellular Ca^{2+} affect the ASM contractile state.

Classical pharmacomechanical coupling by which procontractile G protein–coupled receptors (GPCRs) cause ASM contraction involves increases of Ca^{2+} and phosphorylation of myosin light chain. Most importantly, Gq-coupled GPCR-mediated Ca^{2+} increase is initiated with the production of IP_3 and the release of Ca^{2+} via IP_3R on sarcoplasmic reticulum, with a subsequent activation of RyR channels mediated via Ca^{2+} or cyclic ADP ribose (6, 10). Uniquely, OxPC-induced Ca^{2+} increases do not involve IP_3Rs. Moreover, studies during the past decade note diverse patterns of Ca^{2+} increases that can be coupled or decoupled from ASM contraction and, in some instances (e.g., activation of bitter tastant–stimulated receptors), cause ASM relaxation (11, 12). Although the details of which spatiotemporal features of intracellular Ca^{2+} regulate effectors of ASM contraction remain poorly understood, advances in microscopic/analytical tools and the development of genetically engineered calcium indicators (13) will likely enable mechanistic insight in the near future. Genetic approaches involving deletion of Ca^{2+} sensing proteins and channels (TRP/STIM1) and molecular description of posttranslational modifications of Ca^{2+} channels and transporters by OxPCs are critical.

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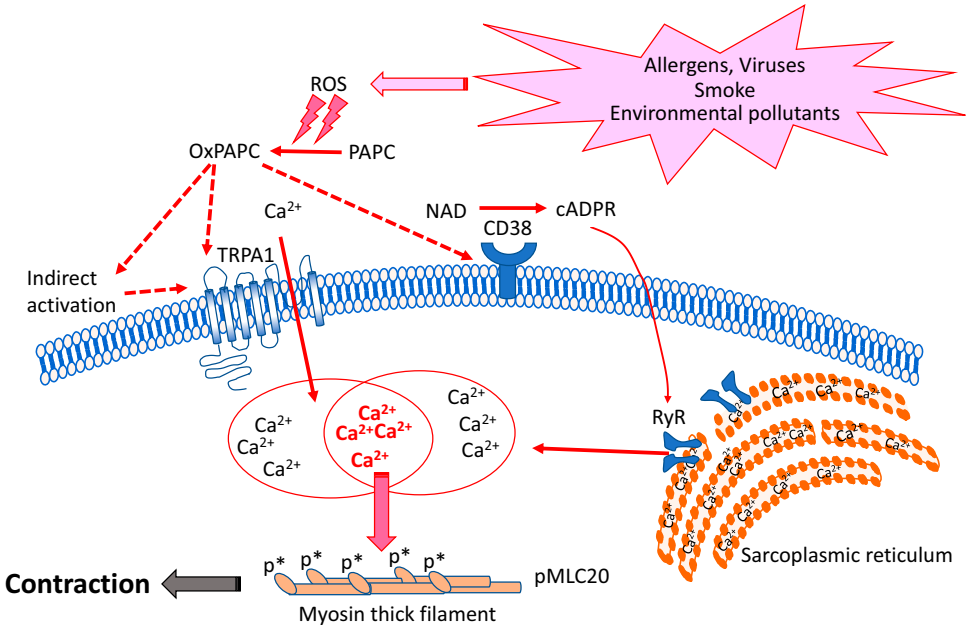

Figure 1. Intracellular signaling mechanisms for oxidized phosphatidylcholine–induced airway smooth muscle (ASM) contraction. Inhaled biological and chemical environmental factors can overwhelm intrinsic antioxidant pathways, resulting in accumulation of ROS, which oxidize biomolecules. This includes phosphatidylcholine (e.g., 1-palmitoyl-2-arachidonoyl-sn-glycero-3-phosphocholine [PAPC]), which is abundant in cell membranes and extracellular fluid such as lung surfactant. Oxidation generates myriad oxidized PAPC (OxPAPC) variants, which induce bronchial narrowing. In human ASM cells, OxPAPC triggers concomitant, but mutually exclusive, activation of the ryanodine receptor (RyR) and transient receptor potential ankyrin 1 (TRPA1) that mediates flux of Ca^{2+} from sarcoplasmic reticulum and extracellular stores, respectively, resulting in an acute and sustained increase in intracellular Ca^{2+}. OxPAPC may activate TRPA1directly or indirectly, e.g., by altering cell membrane properties. RyR activation is dependent on cADPR, a product derived from NAD by cyclase activity of the ectoenzyme CD38, which can be induced by OxPAPC. These phases of mobilized Ca^{2+} work cooperatively, as both are required to induce ASM contraction. Inhibition of either pool is sufficient to prevent pMLC20, which is essential for activation of actomyosin cross-bridge cycling, after OxPAPC exposure, as well as for airway contraction in murine thin-cut lung slices. cADPR = cyclic ADP ribose; NAD = nicotinamide adenine dinucleotide; pMLC20 = phosphorylation of 20 kDa myosin light chain; ROS = reactive oxygen species.

Given the dependence on reductionist cell-based models to explore the mechanisms mediating ASM contraction by OxPCs, insight into the effect of OxPCs under integrative (and chronic) conditions will require future studies. OxPCs *in vivo* will undoubtedly affect multiple resident airway cells, as well as the interactions among resident and infiltrating cells, in the context of allergic inflammation. Moreover, it is likely that OxPCs will influence airway remodeling effected by allergic inflammation. Finally, whether OxPCs affect receptor-mediated ASM contraction (e.g., M3 muscarinic acetylcholine receptor) or relaxation (e.g., β-2 adrenoceptor) is another important question; it is likely that various GPCRs, and possibly their downstream signaling partners, are directly modified by OxPCs, similar to modifications such as protonation and nitrosylation that occur during inflammation and are believed to influence airway function and disease (14–16).

In summary, Vaghasiya and colleagues contribute to a growing body of evidence indicating that molecules generated as the result of oxidative stress in the lung play a role in asthma. Thus, OxPCs could represent a component of persistent inflammation that prevents optimized control in many patients. In this light, a focus on fully extrapolating the pathobiological effects of OxPCs and other oxidative stress mediators in the lung may yield some unexpected new avenues for future therapeutic approaches. Oral antioxidants such as N-acetylcysteine have not proven to be effective in clinical trials, but the data generated here indicate that a more targeted delivery of specific inhibitors of the receptors and pathways activated by mediators such as OxPCs may hold future promise. One of the most telling observations of the study by Vaghasiya and colleagues is the finding that TRPA1 is activated by OxPCs. Notably, prior preclinical studies have identified TRPA1 as a target to suppress allergic inflammation and bronchospasm, and this has led to the development of a TRPA1 inhibitor that is already being tested in phase I human trials (17). Thus, the work by Vaghasiya and colleagues has identified a mechanism that is consistent with the therapeutic potential of TRPA1 inhibition for asthma. ■

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