Cholesterol: A Novel Regulator of Vasoreactivity in Pulmonary Arteries

Chronic hypoxia (CH) causes Ca^{2+} sensitization in pulmonary artery smooth muscle cells (PASMCs), thus augmenting pulmonary artery vasoconstrictor responses (1). Although increased vasoreactivity is considered to contribute to the development of pulmonary hypertension (PH), the exact mechanism of Ca^{2+} sensitization remains unresolved. In this issue of the *Journal*, Norton and colleagues (pp. 709–718) report on studies testing whether alterations in lipid domains of smooth muscle cells may contribute to increased pulmonary artery vasoreactivity in CH (2). This hypothesis was fueled by the authors' previous finding that increased Ca^{2+} sensitization in CH is mediated via EGFR (epidermal growth factor receptor)-dependent NOX2 (NADPH oxidase 2) activation and subsequent superoxide-regulated ROCK (Rho-associated protein kinase) activation (3). Importantly, both EGFR and NOX2 can be regulated by Cav-1 (caveolin-1) (4, 5), the eponymous structural protein of the 50- to 80-nm Ω-shaped cholesterol-rich invaginations of the plasma membrane termed caveolae. Cav-1 also controls local cholesterol content and acts as regulatory scaffold protein for various signaling molecules, resulting in the compartmentalization of vasoregulatory signaling molecules and ion channels, including eNOS (endothelial nitric oxide synthase), TRPC6 (transient receptor potential channel 6), and transient receptor potential vanilloid 4 in caveolae (6, 7). Consistent with an important role for Cav-1 in PH, preclinical studies showed increased pulmonary vascular resistance in *Cav1*-deficient mice (8), and whole-exome sequencing identified an association of *CAV1* with human PH disease (9).

In intrapulmonary arteries isolated from CH and control rats, Norton and colleagues did not detect any significant changes in overall Cav-1 content or number of caveolae per cell membrane length of PASMCs. Interestingly, however, the authors detected a distinct loss of ∼50% in membrane cholesterol in the medial layer of intrapulmonary arteries in CH rats compared with control rats. This finding is in line with previous reports documenting reduced cholesterol levels in hypoxia due to a degradation of the rate-controlling enzyme of cholesterol synthesis, 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase (10).

To test for the functional relevance of cholesterol depletion, the authors repleted cholesterol in isolated pressurized arteries, which normalized the vasoconstrictive response to depolarization in pulmonary arteries of CH rats but had no effect in control rats. These experiments were conducted in Ca^{2+}-permeabilized and endothelium-disrupted arteries, thus excluding changes in Ca^{2+} influx or release, or endothelial-mediated tone regulation. Rather, these findings identify changes in cholesterol as a cause of Ca^{2+} sensitization in CH pulmonary arteries. Conversely, however, cholesterol depletion of control pulmonary arteries by methyl-β-cyclodextrin to a similar extent as in CH pulmonary arteries did not increase vasoreactivity, indicating that cholesterol loss is required but by itself is not sufficient for Ca^{2+} sensitization. Consistent with the previously reported role for NOX2 in Ca^{2+} sensitization, depolarization caused an increase in superoxide in PASMCs of CH rats but not control rats, which was again inhibited by cholesterol repletion, whereas cholesterol depletion had no effect in PASMCs of control rats.

Although total Cav-1 levels were unchanged in CH, the authors next tested for effects of the membrane-permeable Cav-1 scaffolding domain peptide AP-Cav, which was previously shown to prevent the development of experimental PH in rats after administration of monocrotaline (11). Similar to what was observed with cholesterol repletion, AP-Cav normalized depolarization-induced vasoreactivity and superoxide production in pulmonary arteries of CH but not control rats. The exact mechanism of action of AP-Cav remains unclear; however, the authors show that AP-Cav increased membrane cholesterol content in PASMCs from CH rats (albeit not from controls). The authors propose that this finding may be attributable to AP-Cav promoting cholesterol trafficking, as Cav-1 has been shown to facilitate cholesterol transport from the endoplasmic reticulum to the plasma membrane (12). However, because AP-Cav contains a cholesterol recognition amino-acid consensus motif (13), which promotes cholesterol segregation, AP-Cav may also redistribute and sequester cholesterol into lipid domains, or it may interact directly with signaling molecules relevant for Ca^{2+} sensitization.

Finally, the authors link their findings to the previously documented role of EGFR in Ca^{2+} sensitization (3, 14). Although EGF induced vasoconstriction only in intrapulmonary arteries of CH rats, neither EGFR expression nor EGF-induced EGFR phosphorylation differed between the arteries of CH and control rats. Conversely, although cholesterol reduced the vasoconstriction response to EGF, it did not affect EGFR phosphorylation, suggesting that the role of cholesterol as a critical "switch" between control and CH vasoreactivity is distal to EGFR signaling.

Taken together, the findings by Norton and colleagues identify a novel role of cholesterol in the regulation of Ca^{2+} sensitization of pulmonary arterial vasoreactivity in CH. Specifically, the authors propose the novel concept that CH modulates EGFR-NOX2-ROCK signaling in response to membrane depolarization via reduced plasmalemmal cholesterol contents, which affects the signaling cascade distal of EGFR activation (Figure 1). Whether cholesterol acts only proximal or also distal of NOX2 in this cascade remains to be determined. Although a position of cholesterol upstream of NOX2 can be deduced from the fact that cholesterol repletion attenuated superoxide production in response to membrane depolarization, a reverse effect may also exist, as NOX2 may also promote cholesterol oxidization and thus

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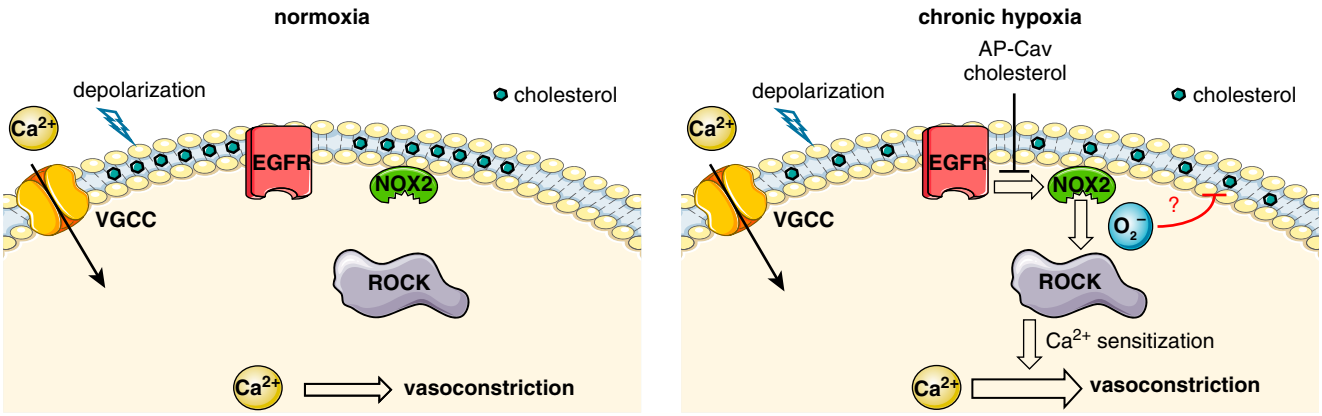

Figure 1. Under baseline (normoxic) conditions, depolarization of the cell membrane causes vasoconstriction by triggering Ca^{2+} influx via voltage-gated calcium channels (VGCC). In addition, chronic hypoxia causes Ca^{2+} sensitization via EGFR (epidermal growth factor receptor)-dependent activation of NOX2 (NADPH oxidase 2) and subsequent superoxide (O_2^-)-mediated activation of ROCK (Rho-associated protein kinase), resulting in increased vasoreactivity. Loss of membrane cholesterol in chronic hypoxia facilitates the crosstalk between EGFR and NOX2, which is prevented by exogenous cholesterol or the Cav-1 (caveolin-1) scaffolding peptide AP-Cav. NOX2-derived superoxide may further aggravate cholesterol loss by oxidation, establishing a detrimental feedback loop. Elements of the illustrations were provided by Servier Medical Art and modified under a Creative Common Attribution 3.0 Generic License.

deficiency. The latter scenario would establish a detrimental feedback loop with cholesterol loss, NOX2 activation, and superoxide production mutually amplifying each other.

The exact mechanism by which cholesterol regulates the interplay between EGFR and NOX2 remains to be elucidated. To this end, it seems key to obtain deeper insights into the effects of CH on the composition of caveolae, i.e., the "caveosome." Although the authors did not detect changes in total Cav-1 expression or number of caveolae per membrane surface, this does not preclude local changes in Cav-1 abundance in the lipid domain fraction, as previously demonstrated for endothelial cells (15). As a scaffolding protein, Cav-1 may directly regulate the abundance and/or activity of EGFR and/or NOX2 in caveolae (4, 16), thereby facilitating their interaction. Local changes in Cav-1 may also explain changes in cholesterol given the established role of Cav-1 in facilitating cholesterol trafficking (12). This notion would be in line with the beneficial effects of AP-Cav on Ca^{2+} sensitization and cholesterol levels. As such, the effects of CH (or AP-Cav) on the caveolar abundance of Cav-1, EGFR, or NOX2 would be of critical interest for further studies. Redistribution of Cav-1 may also explain in part why some studies have reported disease-promoting versus-attenuating effects for Cav-1 and caveolae in PH (8, 17).

Finally, the emerging role of cholesterol in pulmonary artery vasoreactivity raises the question of whether cholesterol-lowering strategies may have detrimental effects in CH or PH disease. An extensive body of literature has demonstrated the beneficial effects of statins in animal models of PH; however, these therapeutic effects could not be confirmed in clinical trials (18, 19). In contrast, in the ASA-STAT (Randomized Clinical Trial of Aspirin and Simvastatin for Pulmonary Arterial Hypertension) trial, patients who were randomized to simvastatin as compared with placebo tended to have a lower 6-minute-walk distance at 6 months that was associated with a marked reduction in serum total cholesterol levels (18). The latter result could potentially be explained by the findings of Norton and colleagues, which would enhance our mechanistic understanding of the seemingly double-edged effect of statins in PH disease. ■

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