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Title: Acetylcholine leads to STAT-1 mediated oxidative/nitrosative stress in human bronchial epithelial cells

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Body: The induction of nitric oxide synthase (iNOS) expression via the signal transducer and activator of transcription 1 (STAT-1) is involved in the mechanism of oxidative/nitrosative stress. Oxidative/nitrosative stress and Acetylcholine (ACh) are implicated in the activation of the bronchial epithelial cells in COPD. We aimed to investigate whether ACh generates oxidative/nitrosative stress in bronchial epithelial cells during airway inflammation of COPD and to evaluate the effects of anticholinergic drugs and long-acting β2-agonists in this mechanism. Human bronchial epithelial cells (16HBE) were stimulated (4 hrs, 37°C) with induced sputum supernatants (ISSs) from healthy controls (HC) (n=6), healthy smokers (HS) (n=6) or COPD patients (n=6), respectively, as well as with ACh (10 μM). STAT-1 pathway activation (Ser727 and Tyr701) and iNOS were evaluated in the cell lysates using Western blot analysis, while reactive oxygen species (ROS) in the cells and nitrotyrosine in the supernatants were evaluated by flowcytometry and by ELISA, respectively. The effect of Tiotropium (Spiriva®) (20nM), alone or in combination with Olodaterol (1nM), was tested. ISSs from COPD patients and ACh significantly increased the phosphorylation of STAT-1Ser727 and STAT-1Tyr701, the expression of iNOS and the production of ROS/nitrotyrosine in stimulated 16HBE when compared with ISSs from HC or HS subjects. The use of Tiotropium and Olodaterol alone well controlled these events. These results support the use of Tiotropium and Olodaterol to reduce the oxidative/nitrosative stress generated by ACh during airway inflammation of COPD via the STAT-1 pathway activation in human bronchial epithelial cells.