Free Fatty Acids Receptor 1/4 (FFAR1/4) Agonists, GW9508, and TAK875 Attenuate Agonist-Induced Shortening in Human Airway Smooth Muscle (HASM) Cells

Author Block: S. Xu, A. Schwab, N. Karmacharya, G. Cao, R. Panettieri, and J. Jude. *Rutgers Institute for Translational Medicine and Science, New Brunswick, NJ.*

Asthma is an airway disease characterized by airway hyper-responsiveness, inflammation and remodeling. Enhanced contractile phenotype of airway smooth muscle (ASM) cells mediates bronchoconstriction in asthma. Obesity is associated with asthma development, severity and response to treatments. The mechanisms linking obesity and asthma are yet to be determined. Free fatty acids receptors (FFARs) are G-Protein Coupled Receptors (GPCRs) and are emerging as crucial signaling molecules with importance in metabolic and inflammatory diseases like asthma. Therefore, we hypothesized that the free fatty acid receptors FFAR1 and FFAR4 modulate cell shortening in HASM cells. In our experiments, HASM cells were treated with vehicle (DMSO) or synthetic FFAR1/4 agonists, GW9508 or TAK875 (0.1 - 10 μM), for a short duration (5, 10, 20, or 30 min), followed by stimulation with contractile agonists carbachol (CCh, 10 μM) or histamine (2.5 μM). Myosin light Chain (MLC) phosphorylation and agonist-induced cytosolic Ca^{2+} ($[Ca^{2+}]_i$) were determined as a surrogate measure of ASM cell shortening. In parallel experiments, histamine-induced cellular stiffness was measured in HASM cells by magnetic twisting cytometry (MTC). To determine whether ASM relaxation signaling is altered by FFAR1/4 agonists, isoprenaline-induced cyclic AMP (cAMP) levels were measured in HASM cells in the presence of vehicle, GW9508 or TAK875. Both GW9508 and TAK875 significantly attenuated agonist-induced MLC phosphorylation in HASM cells in a concentration and time-dependent manner. Histamine-induced HASM cell stiffening was similarly reduced by GW9508 and TAK875, although the decrease was not statistically significant. FFAR1/4 agonists had little effect on agonist-induced $[Ca^{2+}]_i$ or isoprenaline-induced cAMP levels in HASM cells. Our findings show that FFAR1/4 agonists, GW9508 and TAK875, attenuate agonist-induced HASM cell shortening by inhibiting MLC phosphorylation. FFAR1/4 may be novel therapeutic targets to broncho-protect human airways in airway diseases such as asthma and COPD.