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Basic Science

Abstract Title
Arachidonic acid metabolite 19-HETE evokes a robust airway smooth muscle relaxation response by activating both PKA and EPAC

Background
Hydroxyeicosatetraenoic acids (HETEs) are a class of oxylipins widely abundant throughout the lung, with 19-HETE being the most abundant. Yet direct functional studies on the role of 19-HETE in the lung are lacking. We have previously shown that 19-HETE promotes airway smooth muscle (ASM) relaxation through the prostacyclin receptor (IP) and cyclic-AMP production

Objective
The objective of this study was to identify the specific intracellular pathways that 19-HETE activates in ASM.

Methods
Human ASM cells were exposed to 19-HETE (1μM) to measure markers of intracellular signaling events by Western blotting. Activation of protein kinase A (PKA) and exchange protein activated by cAMP (EPAC1/2) pathways were measured through phosphorylation of vasodilator-stimulated phosphoprotein (pVASP) or GTP bound Rap1 respectively. Traction force microscopy (TFM) was used to monitor 19-HETE induced cellular relaxation when exposed to specific inhibitors. One-way ANOVA with Dunnet's post-test was used for analysis.

Results
19-HETE significantly increased the abundance of pVASP by 50% relative to control, an indicator of PKA activation. 19-HETE stimulation also enhanced PAK1 phosphorylation (49%) and increased active, GTP-RAP1, both downstream of EPAC. 19-HETE significantly reduced contractile force of ASMC by 40%, an effect that was reversed by inhibiting PKA, or EPAC1/EPAC2. 19-HETE reduced histamine (10μM) induced phosphorylation of myosin light chain (pMLC, biomarker of contraction) and inhibiting IP or EPAC2 mitigated this response. However, pre-treatment with inhibitors for PKA or EPAC1 did not prevent reductions in pMLC2 by 19-HETE.

Conclusion
19-HETE promotes ASM relaxation by activating both PKA and EPAC dependent pathways intracellularly. Our results show that PKA and EPAC lead to relaxation through different mechanisms. EPAC2 promotes dephosphorylation of MLC, a key step in canonical cross-bridge cycling. PKA promotes pVASP formation, which is involved in changing actin tethering for cell shortening. Understanding the mechanism for 19-HETE's bronchodilatory effect provides a crucial foundation for its therapeutic potential.

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