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**Presenting Author Category**

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**Research Category**

Basic Science

**Abstract Title**

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

## Authors

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**Background**

Hydroxyeicosatetraenoic acids (HETEs) are an abundant class of oxylipins in the lung, with 19-HETE being the most prevalent in mouse lung and human airway smooth muscle (ASM). 19-HETE exhibits vasodilatory effects mediated by the prostacyclin (IP) receptor and cyclic adenosine monophosphate (cAMP) production, yet no studies explored whether it has similar relaxant potential in the lung.

**Objective**

This study aims to delineate the specific intracellular pathways that 19-HETE utilizes to promote ASM relaxation.

**Methods**

Murine tracheae and human ASM cells were exposed to 19-HETE (0.1–10  $\mu$ M). Tracheal relaxation was assessed by myography, and cAMP levels were measured by ELISA. Western blotting assessed phosphorylated vasodilator-stimulated phosphoprotein (pVASP) and phosphorylated myosin light chain 2 (pMLC2). PKA and EPAC signaling were evaluated using pVASP and active GTP-bound Rap1, respectively. Traction force microscopy assessed ASM contractile force following treatment with 19-HETE and pathway-specific inhibitors. Data were analyzed using one-way ANOVA with Dunnett's post hoc test.

**Results**

19-HETE induced dose-dependent tracheal relaxation (max 90%). In human ASM cells, 19-HETE increased cAMP levels by 3-fold, this response was reversed by IP inhibition. 19-HETE increased pVASP abundance by 50% and increased active Rap1, indicating activation of PKA and EPAC pathways, respectively. 19-HETE reduced ASM contractile force by 40%, this effect was reversed by IP, PKA, or EPAC1/2 inhibition, suggesting involvement of both PKA and EPAC pathways. Histamine-induced pMLC2 upregulation was reduced two-fold by 19-HETE (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 activates IP-dependent cAMP signaling to promote ASM relaxation through PKA and EPAC mediated pathways. EPAC2 modulates MLC phosphorylation, whereas PKA likely alters cytoskeletal dynamics. These findings identify 19-HETE as a potent endogenous bronchodilator with therapeutic potential for bronchodilator-unresponsive patients.