How a novel lipid relaxes lung smooth muscle

Understanding the mechanism underlying 19-HETE's bronchodilating effect

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INTRODUCTION

Asthma is the most common chronic disease in Canadian children. Airway narrowing, caused by smooth muscle (ASM) contraction is a hallmark of the disease. Bronchodilators are used to relax ASM and ease breathing, but these drugs are rendered ineffective in severe disease. Therefore, alternative bronchodilators are needed.19-HETE is an abundant lipid signalling molecule in the lungs. We have shown that **19-HETE relaxes ASM. However, the mechanism by which 19-HETE induces ASM relaxation is unclear.**

AIM

To establish an understanding of the signalling network leveraged by 19-HETE to promote ASM relaxation.

METHODS

- Experiments were conducted on immortalized human airway smooth muscle cells *in vitro*.
- Cells were exposed to 19-HETE (1μM) and inhibitors of key signaling proteins.
- Changes in cAMP production, vasodilator stimulated phosphoprotein (VASP) phosphorylation, p21-activated kinase (PAK1) phosphorylation, and myosin light chain (MLC) phosphorylation were measured as indicators of specific signaling pathways.
- cAMP was measured using an ELISA and protein phosphorylation using Western Blotting.
- Significance threshold set at $p<0.05$

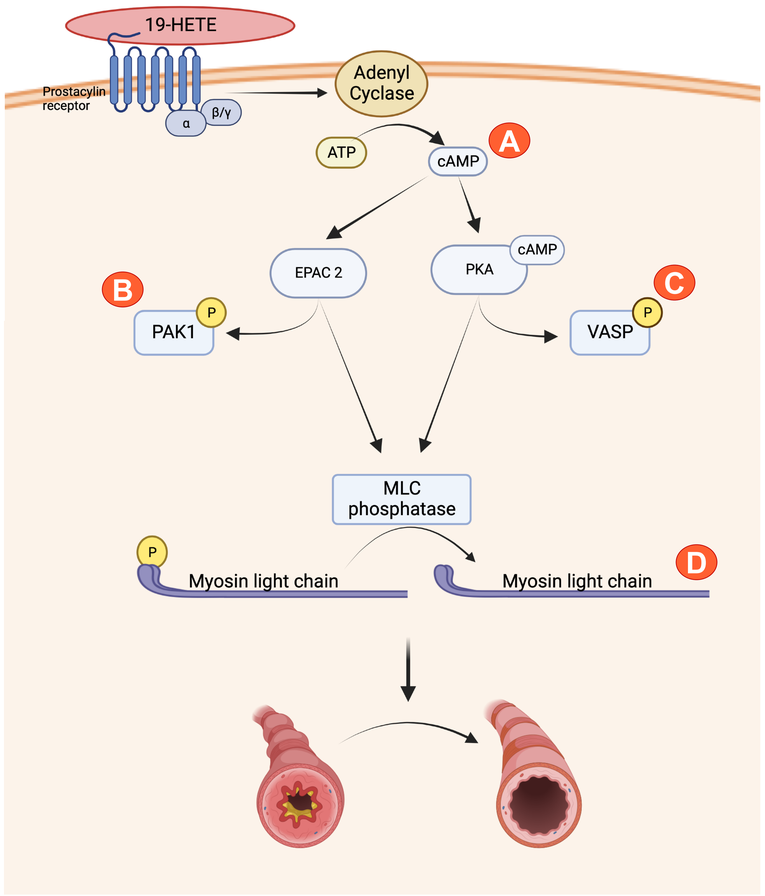

Figure 1. Proposed signalling pathway of 19-HETE in human airway smooth muscle

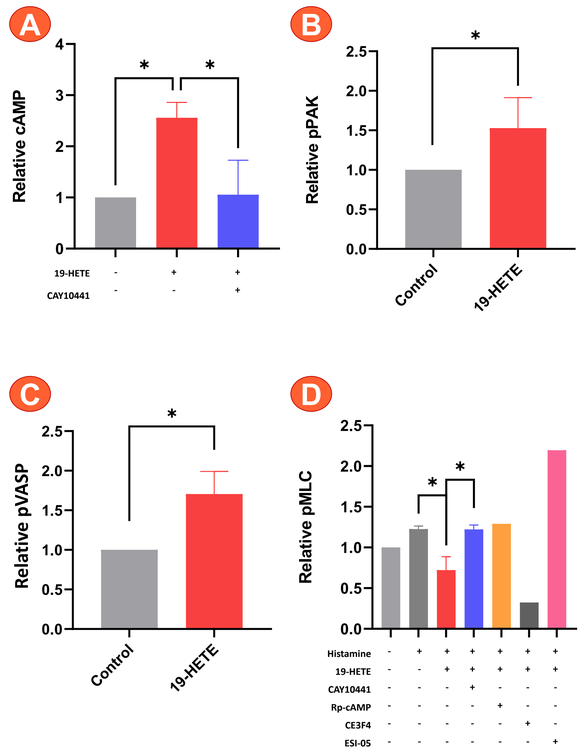

*Figure 2. A) cAMP is elevated in response to 1 μM 19-HETE. Signal ablated with 10 μM CAY10441, a prostacyclin inhibitor (n=4) B) PAK phosphorylation increased in response to 1 μM 19-HETE (n=6, 3 cell lines) C) VASP phosphorylation increased in response to 1 μM 19-HETE (n=3) D) 19-HETE reverses histamine induced MLC phosphorylation (n=3). The reversal is inhibited by CAY10441, prostacyclin receptor inhibitor (n=2), Rp-cAMP, PKA inhibitor (n=1), and ESI-05, EPAC2 inhibitor n=1). *, p<0.05.*

RESULTS

- 19-HETE significantly increased cAMP abundance relative to control (+156%), which was significantly reversed with the prostacyclin receptor antagonist. This suggests **19-HETE binds to the prostacyclin receptor**. (Figure 2A)
- 19-HETE increases PAK1 phosphorylation, which may be an indicator of EPAC activation downstream of cAMP (+49%). This suggests **EPAC may be important in 19-HETE induced relaxation.** (Figure 2B)
- 19-HETE significantly increased VASP phosphorylation (+70%), an indicator of PKA activation downstream of cAMP, relative to control. This supports the importance of **PKA in the signalling pathway.** (Figure 2C)
- 19-HETE significantly decreases histamine induced myosin light chain phosphorylation, a key molecular indicator of ASM contraction (-39%). This decrease is ablated by incubation with inhibitors for prostacyclin receptor, PKA, and EPAC2 inhibitor. This suggests **19-HETE promotes relaxation through cAMP effectors by decreased MLC phosphorylation.** (Figure 2D)

CONCLUSION

- 19-HETE promotes ASM relaxation through the prostacyclin receptor and a signaling network that depends on PKA and EPAC2.
- Understanding the mechanism for 19-HETE's bronchodilatory effect provides a foundation for research into the role this molecule has in asthma and future potential therapies for asthma.

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