A narrow spectrum kinase inhibitor, RV1729, induces bronchodilation of human small airways and rescues agonist-induced desensitization of the β_2 adrenoreceptor (β_2AR)

Cynthia J. Koziol-White[1], Jie Zhang[1], Edwin Yoo[1], Gaoyuan Cao[1], Catherine E. Charron[2], Christopher S. Stevenson[2,3], and Reynold A. Panettieri Jr.[1]

[1]Rutgers Institute for Translational Medicine and Science, Rutgers University, New Brunswick, NJ; [2]RespiVert Ltd., LBIC, London; [3]Respiratory Discovery, Immunology Therapeutic Area, Janssen Pharmaceuticals, London

Abstract

RATIONALE: Asthma manifests as a heterogeneous syndrome characterized by airflow obstruction, inflammation, and hyperreactivity (AHR). Phosphoinositide 3-kinase (PI3K) p110 δ and γ isoforms mediate immune cell activation, migration and AHR in murine models of allergic airways disease. However, the molecular mechanisms by which PI3K modulates airway smooth muscle function remains unknown. We hypothesize that PI3K p110 δ and γ reverses agonist-induced bronchoconstriction and β_2AR tachyphylaxis.

METHODS: Human precision cut lung slices (PCLS), each containing a small airway, were preconstricted to carbachol, then dilated to formoterol , RV1729 (courtesy of RespiVert Ltd.), LY294002, or CAL-101 (10^{-11} – 10^{-4} M). Bronchodilation was normalized to that induced by forskolin (10^{-5} M). Additionally, bronchodilation to formoterol alone was used as an industry standard. In parallel, PCLS were preincubated with RV1729 LY294002, CAL-101, or dexamethasone for 1 hr prior to treatment with 100 nM formoterol overnight (β_2AR desensitization). Slices were contracted to carbachol (10^{-8} – 10^{-4} M) and then bronchodilated to formoterol (10^{-8} – 10^{-4} M).

RESULTS: Carbachol-induced bronchoconstriction was reversed by LY294002, CAL-101, RV1729 and formoterol with no significant difference in efficacy among the agents (Emax 86±4%, n=8 donors) Formoterol treatment overnight markedly decreased agonist-induced bronchodilation by 47±7% as compared with diluent treated slices. Although dexamethasone rescued desensitization (Emax 83±13%, p=0.05 vs formoterol alone), LY294002 and CAL-101 partially rescued tachyphylaxis (66±10% and 61±9%, respectively; p=NS vs formoterol desensitization alone). However, RV1729 (100 nM) was more effective in rescuing tachyphylaxis than LY294002 or CAL-101, reversing formoterol-induced tachyphylaxis (Emax 76±12%; p=0.05 vs formoterol desensitization).

CONCLUSIONS: These data suggest that PI3K p110 δ and γ isoforms modulate bronchomotor tone of human small airways by acting directly as a bronchodilator and by reversing β_2AR tachyphylaxis. These data further suggest that PI3K inhibition serves as a unique therapeutic target in airways diseases that can attenuate airway inflammation, promote bronchodilation and reverse β2AR tachyphylaxis.

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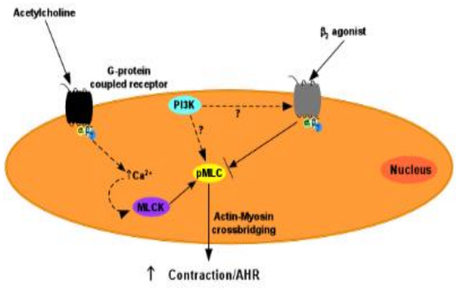
Roles for PI3K in allergic airways disease and β_2 adrenoreceptor function

- Modulates mucus production, inflammatory cell influx to the lungs, degranulation of mast cells, and remodeling of the airways in a murine model of allergic airways disease.
- Inhibition of PI3K p110 δ and γ attenuates allergen-induced airway hyperresponsiveness.
- PI3K p110 isoforms modulate vascular smooth muscle contractile signaling and shortening.
- RV1729 is a PI3K p110 δ and γ inhibitor.



Hypothesis

Selective inhibition of PI3K p110 δ and γ reverses agonist-mediated bronchoconstriction and modulates tachyphylaxis of the β_2AR.

A model of airway dilation in human precision cut lung slices (hPCLS)

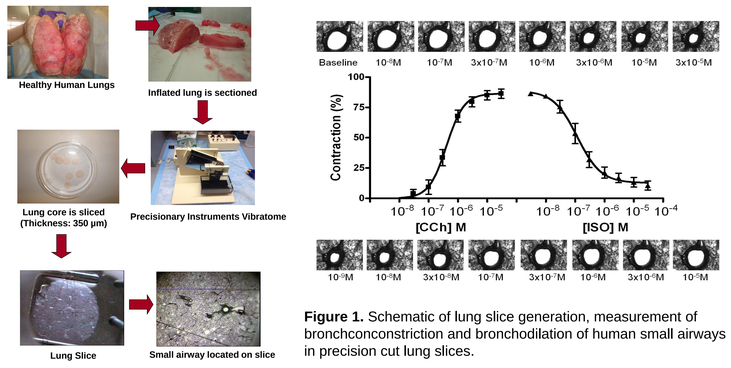


Figure 1. Schematic of lung slice generation, measurement of bronchconconstriction and bronchodilation of human small airways in precision cut lung slices.

PI3K inhibition induces dilation of carbachol-constricted airways to a level similar to that induced by formoterol

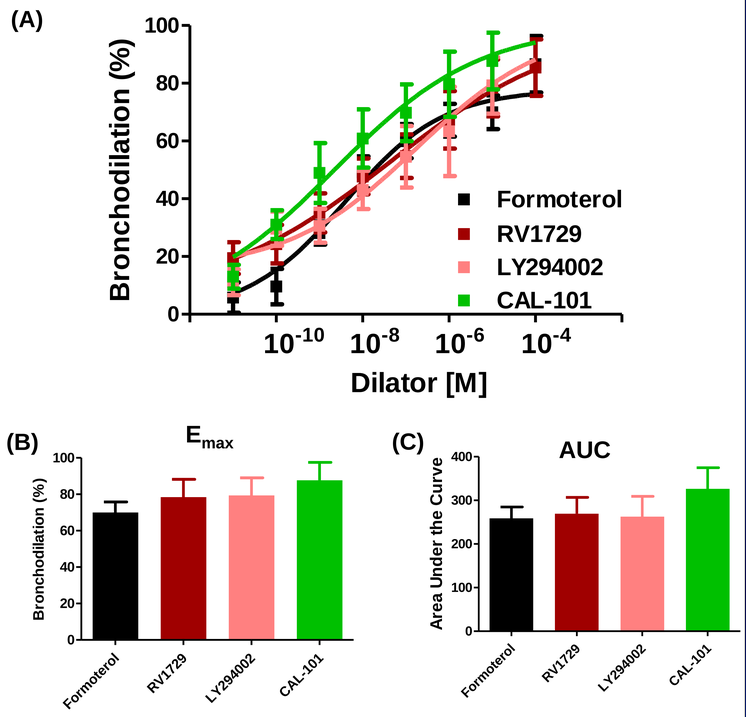


Figure 2. Reversal of carbachol-induced bronchoconstriction by PI3K inhibitors. Concentration response curves for formoterol, LY294002, CAL-101, and RV1729 (A), maximal bronchodilation (E_{max}) (B), and area under the curve (C). Values are representative of n≥7 donors. Bars represent mean ± SEM.

PI3K inhibition partially rescues formoterol-mediated hyporesponsiveness of human small airways to β_2AR agonist-induced dilation

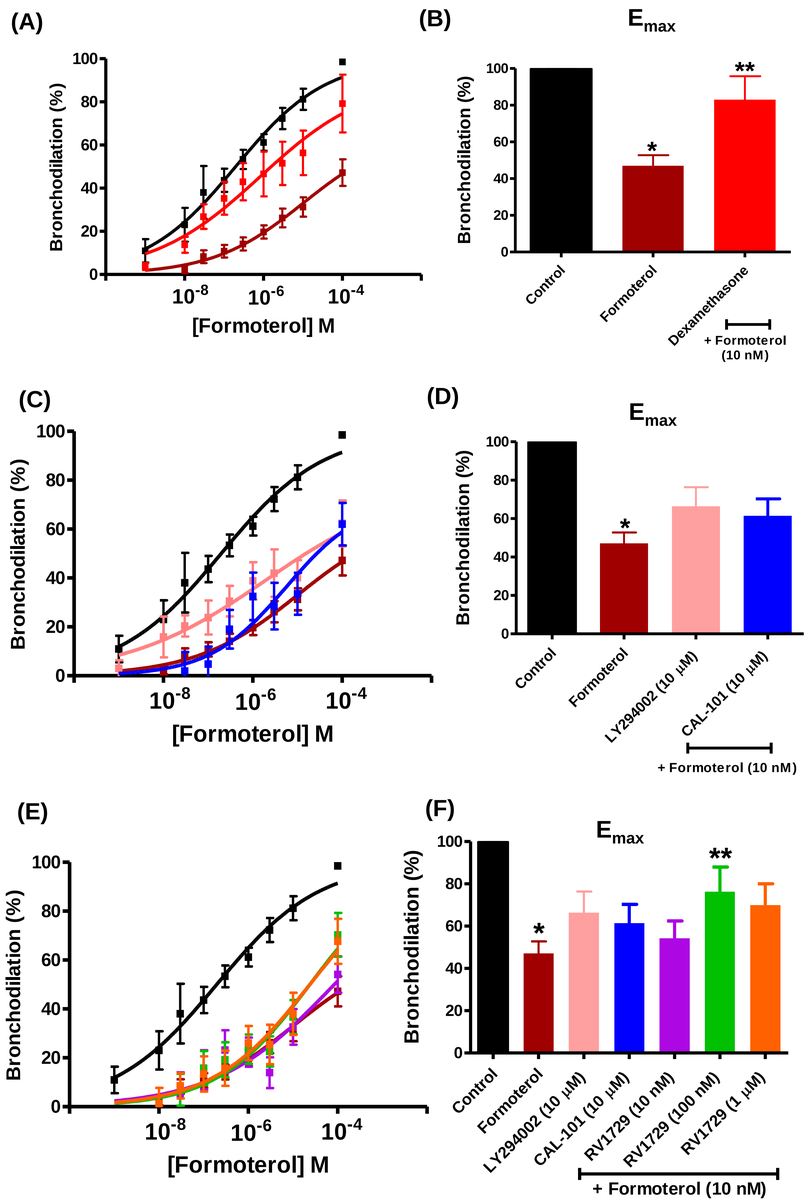


Figure 3: Effect of PI3K on formoterol-induced $\beta_2$ar tachylphylaxis. Dose response curves were generated (A,C,E) with maximal bronchodilation (E_{max}) plotted (B,D,F). Values are representative of n≥8 donors. Bars represent mean ± SEM. *p<0.05 vs control, **p<0.05 vs formoterol.

Summary

- Inhibition of PI3K p110 isoforms (all isoforms using LY294002) as well as selective inhibition of γ and δ isoforms induces dose-dependent bronchodilation.
- Dilation induced by PI3K inhibitors is similar to that induced by the long acting β_2AR agonist formoterol.
- Formoterol pretreatment (18 hr) significantly reduces agonist-mediated bronchodilation.
- Dexamethasone pretreatment prior to desensitization of the β_2AR rescues receptor tachyphylaxis.
- Inhibition of PI3K p110 isoforms partially rescues formoterol-induced β_2AR desensitization.

Significance

Defining molecular mechanisms that mediate airway relaxation and reverse tachyphylaxis to β_2AR agonists will provide novel therapeutic targets for treatment of asthma and COPD.

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