Figure 1 – coimmunoprecipitated α activation and HASMC contraction was examined. Given the capacity of G12 family proteins to activate ROCK pathways in other cell types, roles of PI3K and ROCK in HASMCs remain unclear. 

METHODS: α12 siRNA was transfected into HAMEC cells using lipofectamine and then probed as indicated. Data normalized to tubulin expression. Data are representative of five independent experiments (n=5, mean ± SD; *p<0.05, **p<0.01, ***p<0.001).

Figure 2 – A) Incremental doses of Carbachol or 2.0 µM formoterol were administered for 10 minutes and experiments were repeated for each of the three donors (6 total airways). B) pAKT and pMYPT1 expression were analyzed by Western blotting. Data are representative of five independent experiments (n=5, mean ± SD; *p<0.05, **p<0.01, ***p<0.001). C) Normalized contraction was examined. Data are representative of five independent experiments (n=5, mean ± SD; *p<0.05, **p<0.01, ***p<0.001).

Figure 3 – A) Asterisks indicate the normalized contraction was examined. Data are representative of five independent experiments (n=5, mean ± SD; *p<0.05, **p<0.01, ***p<0.001). B) pAKT and pMYPT1 expression were analyzed by Western blotting. Data are representative of five independent experiments (n=5, mean ± SD; *p<0.05, **p<0.01, ***p<0.001). C) Normalized contraction was examined. Data are representative of five independent experiments (n=5, mean ± SD; *p<0.05, **p<0.01, ***p<0.001).

Figure 4 – Effect of methacholine (10 µM, 30 min) on carbachol-induced (10 µM, 10 min) AKT phosphorylation at Ser473 (pAKT).

Figure 5 – Airways were preconstricted with carbachol (10 µM, 10 min) prior to addition of methacholine (10 µM, 10 min). Data were normalized to maximum bronchodilatory response (100% that was given after maximal doses of formoterol or methacholine). Each data point is expressed as mean ± SD. Each group contains 2 airways from each of the three donors (6 total airways).

Significance

Gα12 plays a crucial role in HASMC contraction by Rho-dependent activation of the PI3K/ROCK axis. Inhibition of RhoA activity may exert beneficial therapeutic targets in asthma.