Severe asthma manifests as airway remodeling and irreversible airway obstruction, in part because of the proliferation and migration of human airway smooth muscle (HASM) cells. We previously reported that cyclic adenosine monophosphate–mobilizing agents, including β2-adrenergic receptor (β2AR) agonists, which are mainstay of asthma therapy, and prostaglandin E2 (PGE2), inhibit the migration of HASM cells, although the mechanism for this migration remains unknown. Vasodilator-stimulated phosphoprotein (VASP), an anticaclping protein, modulates the formation of actin stress fibers during cell motility, and is negatively regulated by protein kinase A (PKA)–specific inhibitory phosphorylation at serine 157 (Ser157). Here, we show that treatment with β2AR agonists and PGE2 induces the PKA-dependent phosphorylation of VASP and inhibits the migration of HASM cells. The stable expression of PKA inhibitory peptide and the small interfering (si) RNA-induced depletion of VASP abolish the inhibitory effects of albuterol and PGE2 on the migration of HASM cells. Importantly, prolonged treatment with albuterol prevents the agonist-induced phosphorylation of VASP at Ser157, and reverses the inhibitory effects of albuterol and formoterol, but not PGE2, on the basal and PDGF-induced migration of HASM cells. Collectively, our data demonstrate that β2AR agonists selectively inhibit the migration of HASM cells via a β2AR/PKA/VASP signaling pathway, and that prolonged treatment with albuterol abolishes the inhibitory effect of β2-agonists on the phosphorylation of VASP and migration of HASM cells because of β2AR desensitization.

**Keywords:** airway hyperresponsiveness; β2-adrenergic receptor desensitization; protein kinase A; albuterol; formoterol

Human airway smooth muscle (HASM) hyperplasia and remodeling are characteristic features of airways that contribute to their irreversible obstruction in patients with severe asthma. Accumulating evidence suggests that cell migration contributes to airway smooth muscle hyperplasia and remodeling (1, 2). Recently, the migration of airway smooth muscle (ASM) cells toward the epithelium and lumen of the airway was reported as a potential contributor to the increased smooth muscle mass in patients with asthma (3, 4). Further, cell-culture studies show that mitogens and inflammatory mediators involved in asthma pathogenesis and pharmacological agents in current used for the treatment of asthma modulate the migration of ASM cells (1, 5, 6). Taken together, these studies suggest that the migration of ASM cells contributes to ASM cell hyperplasia under asthma-related conditions (1). Although the role of HASM hyperplasia in asthma is well established, the molecular mechanisms regulating the migration of HASM cells under asthma-related conditions are poorly understood (1).

Evidence demonstrates that cyclic adenosine monophosphate (cAMP)–inducing agents, including the long-acting β2-adrenergic receptor (β2AR) agonist formoterol, inhibit the agonist-dependent migration of ASM cells (5, 7). We previously reported that the cAMP-inducing agents salmeterol, cilomilast, and prostaglandin E2 (PGE2) attenuate the migration of HASM cells (6) and induce actin depolymerization in HASM cells (8). However, the mechanisms by which β2AR agonists modulate the actin cytoskeleton and inhibit cell migration remain unknown.

Cell migration requires actin cytoskeleton remodeling, including the disassembly of actin stress fibers and the formation of actin branching at the lamellipodia protrusion at the leading edge of migrating ASM cells. Vasodilator-stimulated phosphoprotein (VASP) belongs to a conserved family of actin-regulatory proteins that regulate actin stress fiber assembly during cell migration (9). VASP facilitates the assembly of the actin filaments by serving as an anticaclping protein (9). Agents that elevate cellular concentrations of cAMP and cyclic guanosine monophosphate promote the inhibitory phosphorylation of VASP and attenuate the anticaclping activity of VASP (9). The activity of VASP is negatively regulated by the inhibitory phosphorylation of serine 157 (Ser157) by protein kinase A (PKA)
Although the importance of protein kinase A in cell migration is supported by several reports, published data indicate that PKA may serve to either promote or inhibit cell motility (10–12), suggesting that the role of PKA in cell migration is highly cell type–dependent and context-dependent. β2AR agonists modulate the activity of PKA via the production of cAMP, but the role of PKA/VASP signaling in the β2AR agonist-dependent migration of HASM cells remains unclear.

In this study, we demonstrate that β2AR agonists modulate the migration of HASM cells through a β2AR/PKA/VASP signaling pathway. We also show that the prolonged exposure of HASM cells to albuterol abrogates the PKA-specific phosphorylation of VASP, and prevents the β2AR agonist-dependent inhibition of HASM cell migration, suggesting the potential clinical relevance of our findings.

MATERIALS AND METHODS

Cell Culture

HASM cells were dissociated from human tracheas obtained from human lung transplant donors, in accordance with procedures approved by the Committee on Studies Involving Human Beings at the University of Pennsylvania, as described previously (13). Cells were cultured in Ham’s F12 media (Life Technologies, Grand Island, NY), supplemented with 10% FBS (HyClone Laboratories, Logan, UT), 100 U/ml penicillin, and 0.1 mg/ml streptomycin. Before the experiments, HASM cells were maintained for 48 hours in serum-free Ham’s F12 media, supplemented with 0.1% BSA.

Immunoblot Analysis

Growth-arrested cells were transfected with siRNA VASP or control small interfering (si) RNA, or treated with different concentrations of racemic, (R)-albuterol (where “R” stands for rectus) or (S)-albuterol (where “S” stands for sinistre), formoterol, PGE2, or propranolol in the presence or absence of preincubation with albuterol, followed by immunoblot analysis with anti-phospho VASP Ser157 or anti-VASP antibodies (Cell Signaling Technology, Inc., Danvers, MA).

Immunocytochemical Analysis

Cells grown-arrested for 48 hours were incubated with 0.01, 0.1, 1, or 10 μM formoterol or diluent for 5, 15, or 30 minutes, or with 10 μM (R)-albuterol, (S)-albuterol, or diluent for 5, 15, and 30 minutes, followed by immunocytochemical analysis with anti-phospho VASP Ser157 antibody, as previously described (14). A detailed explanation of the technique is provided in Figure E1 in the online supplement.

Transient Transfection

Cells were transfected with 100 or 250 nM siRNA VASP or control siGLO RISC-Free (GLO) siRNA for 48 hours with RNAiFect transfection reagent (Qiagen, Valencia, CA), according to the manufacturer’s protocol, as described elsewhere (15). The siRNA-induced depletion of VASP was confirmed by immunoblot analysis with anti-VASP antibody.

Cell Migration Assay

Cell migration was examined using a Boyden chamber apparatus, as we described previously (6, 16). Briefly, HASM cells were growth-arrested for 48 hours, transfected with siRNA VASP or control siRNA GLO, or incubated with albuterol. HASM cells were then briefly trypsinized by 0.05% trypsin/0.53 mM EDTA, centrifuged at 900 rpm for 10 minutes, and resuspended in serum-free media supplemented with BSA. Cells (5 x 10^4) were then placed into the upper wells of the Boyden chamber, fitted with an 8-μm pore membrane coated with Vitrogen (Advanced BioMatrix, San Diego, CA) (100 μg/ml). Agonists or vehicle in serum-free media supplemented with BSA were added to the lower chambers. Cells in the Boyden chamber were incubated for 4 hours at 37°C in a 5% CO2 incubator. Nonmigrated cells were scraped off. The membrane was fixed with methanol, stained with a Hemacolor stain set (EM Industries, Inc., Gibbstown, NJ), and scanned. Cell migration was analyzed using the Gel-Pro analyzer program (Media Cybernetics, Silver Spring, MD).

Statistical Analysis

Data points from individual assays represent mean values ± SE. Statistically significant differences among groups were assessed according to ANOVA (Bonferroni-Dunn test), with values of P < 0.05 considered sufficient to reject the null hypothesis for all analyses. All experiments were designed with matched control conditions within each experiment, to enable statistical comparisons as paired samples. All experiments were performed with a minimum of three different HASM cell cultures.

RESULTS

Short-Term Treatment with β2AR Agonists and PGE2 Promotes the Phosphorylation of VASP in HASM Cells

Because cAMP-mobilizing agents induce the activation of PKA and the inhibitory phosphorylation of VASP in different types of cells (9), we examined whether the β2AR agonists albuterol and formoterol modulate the PKA-dependent activity of VASP in HASM cells by using two different techniques, that is, immunoblot and immunocytochemical analyses with antibodies specifically recognizing VASP phosphorylated at Ser157, a major site of PKA phosphorylation.

As shown in Figure 1, a 30-minute incubation with either albuterol or formoterol induced the dose-dependent phosphorylation of VASP, compared with diluent-treated cells at concentrations comparable to those for PGE2 (Figures 1A and 1B, respectively). Notably, formoterol induced the phosphorylation of VASP at 10–100 times lower doses compared with albuterol, which is consistent with the systemic dose potency of formoterol, which is 10–100 times higher than that of albuterol (17). Immunocytochemical analysis demonstrated that formoterol increased the phosphorylation of VASP at Ser157 in a concentration-dependent and time-dependent manner (Figure E1). Similarly, lower doses of formoterol were required to inhibit the migration of HASM cells, compared with albuterol (the half maximal inhibitory concentration was approximately 0.1 μM and 1 μM for formoterol and albuterol, respectively) (Figure E2),

Figure 1. Short-term incubation with β2-adrenergic receptor (β2AR) agonists and prostaglandin E2 (PGE2) promotes the phosphorylation of vasodilator-stimulated phosphoprotein (VASP) in human airway smooth muscle (HASM) cells. HASM cells were growth-arrested for 48 hours and incubated with different concentrations of albuterol (A), formoterol (B), PGE2, or diluent (D) for 30 minutes, followed by immunoblot analysis with anti-phospho VASP serine 157 (Ser157) or anti-total VASP antibodies. Images are representative of three independent experiments. P, phosphorylated.
suggesting that a negative correlation exists between the phosphorylation levels of VASP and the migration of HASM cells.

Collectively, these data show that the β2AR agonists albuterol and formoterol induce the PKA-dependent phosphorylation of VASP at Ser157 in HASM cells, and inhibit the migration of HASM cells.

**Binding with β2AR Is Required for the Albuterol-Dependent Phosphorylation of VASP and Inhibition of HASM Cell Migration**

To determine whether binding with β2AR is required for the β2AR agonist–induced phosphorylation of VASP, we examined whether the nonselective β2AR inhibitor propranolol affects the albuterol-dependent phosphorylation of VASP. As shown in Figure 2, cotreatment with propranolol markedly inhibited the albuterol-induced phosphorylation of VASP at Ser157, depending on the concentration of propranolol. In contrast, even the maximal concentration of propranolol exerted little effect on the PGE2-induced phosphorylation of VASP, demonstrating that propranolol specifically blocks the β2AR-dependent phosphorylation of VASP (Figure 2).

To confirm our findings, we used (R)-albuterol and (S)-albuterol enantiomers, which possess differential affinity to β2AR. (R)-albuterol binds to β2AR with high affinity and promotes the formation of cAMP, whereas (S)-albuterol shows weak β2AR affinity (18). We found that a 10-minute treatment with 0.05–50 μM (R)-albuterol markedly increased the phosphorylation of VASP at Ser157 in a concentration-dependent manner. In contrast, (S)-albuterol exerted little effect on the phosphorylation of VASP, and only high doses (50 μM) of (S)-albuterol induced a modest increase in VASP phosphorylation (Figures 3A and 3B). Immunochemical analysis also demonstrated that (R)-albuterol but not (S)-albuterol promoted the time-dependent phosphorylation of VASP in HASM cells (Figure E3). Taken together, these data demonstrate that binding with β2AR is required for the albuterol-dependent phosphorylation of VASP at Ser157.

Because our previous study demonstrates that β2AR agonists inhibit the migration of HASM cells via an unknown mechanism (6), we examined whether the affinity of albuterol to β2AR is sufficient for the ability of albuterol to inhibit the migration of HASM cells. We found that (R)-albuterol, which demonstrates high affinity to β2AR (18) but not (S)-albuterol (which has weak β2AR binding properties) (18), inhibited the basal or PDGF-induced migration of HASM cells (Figure 3C). These data suggest that binding with β2AR is required for the albuterol-dependent inhibition of HASM cell migration.

**PKA Modulates the Albuterol-Dependent Inhibition of HASM Cell Migration**

Because PKA is a downstream effector of β2AR signaling, we next determined whether PKA is required for the albuterol-dependent inhibition of HASM cell migration. We used HASM cells stably expressing green fluorescent protein (GFP)-tagged protein kinase inhibitor peptide (PKI-GFP) or the dominant negative mutant of regulatory PKA subunit (RevAB) (RevAB-GFP). HASM cells stably expressing GFP were used as a control. As we reported previously, both cell lines display a marked...
inhibition of PKA activity and phosphorylation of VASP at Ser157 (14). As shown in Figure 4A, albuterol markedly inhibited the basal and platelet derived growth factor (PDGF)-induced migration of GFP-expressing cells. In contrast, albuterol exerted little effect on the migration of cells stably expressing PKI-GFP or RevAB-GFP (Figure 4A), demonstrating that PKA is required for the albuterol-dependent inhibition of HASM cell migration.

VASP Is Required for the cAMP-Dependent Inhibition of HASM Cell Migration

Because PKA inhibits VASP via phosphorylation at Ser157 in HASM cells (14), we next determined the role of VASP in the cAMP-dependent inhibition of HASM cell migration. We depleted endogenous VASP protein concentrations with a specific siRNA (Figure 4B), and examined the migration of HASM cell in the presence of a high dose (10 μM) of the potent cAMP-mobilizing agent PGE2 (6). As seen in Figure 4B, control siRNA GLO exerted little effect on both total VASP protein concentrations and the phosphorylation of VASP at Ser157 induced by PGE2. In contrast, siRNA VASP specifically attenuated total VASP concentrations, and completely suppressed the PGE2-dependent phosphorylation of VASP.

Consistent with our previous data (6), PGE2 markedly inhibited basal and PDGF-induced migration in control siRNA-transfected cells (Figure 4C). Importantly, siRNA VASP attenuated the PGE2-induced inhibition of basal and PDGF-induced HASM cell migration in a concentration-dependent manner (Figure 4C, gray bars), suggesting that the phosphorylation of VASP is required for the cAMP-dependent inhibition of HASM cell migration.

Collectively, our data demonstrate that the albuterol-dependent inhibition of HASM cell migration is attributable to the β2AR-dependent and PKA-dependent phosphorylation of VASP.

Prolonged Exposure to Albuterol Abolishes the β2AR Agonist-Dependent Phosphorylation of VASP

To determine whether prolonged treatment with β2AR agonists modulates the phosphorylation of VASP, we incubated growth-arrested HASM cells with albuterol or formoterol for up to 18 hours, followed by immunoblot analysis with phosphoSer157 VASP antibodies. As shown in Figure 5, both albuterol and formoterol induced a transient increase in the phosphorylation of VASP that decreased by 3 hours of treatment.

To examine further the effects of prolonged exposure to β2AR agonists on the inhibition of VASP, HASM cells were preincubated with 3 μM albuterol or diluent for 24 hours and maintained for 2 hours in albuterol-free media, and then short-term treatment with albuterol was performed, followed by immunoblot analysis to detect the phosphorylation of VASP at Ser157 (see Figure E4 for a schematic representation). The dose and kinetics of β2AR tolerance were characterized as we previously described (19). We found that 0.1–1 μM albuterol induced a marked phosphorylation of VASP at Ser157 in diluent-pretreated cells (Figure 6A), but failed to induce the phosphorylation of VASP at Ser157 in cells pretreated with albuterol for 24 hours (Figure 6A). Interestingly, the PGE2-dependent phosphorylation of VASP was not suppressed by preincubation with albuterol (Figure 6A), demonstrating that desensitization is attributable to alterations in the function of β2AR.

To determine the time required for the desensitization of β2AR, HASM cells were preincubated for 30–120 minutes with 1 μM albuterol, followed by the removal of albuterol and short-term treatment with 1 μM albuterol (a schematic representation of this experiment is provided in Figure E5). As shown in Figure 6B, the level of desensitization was dependent on preincubation time. Preincubation with 1 μM albuterol for 30–120 minutes led to the time-dependent inhibition of VASP phosphorylation, and the suppression of VASP phosphorylation was detected after 120 minutes of preincubation (Figure 6B). Thus, prolonged incubation with albuterol inhibits the albuterol-dependent phosphorylation of VASP in a time-dependent and concentration-dependent manner, potentially because of alterations in the responsiveness of β2AR.

Chronic Exposure to Albuterol Abolishes Albuterol-Dependent and Formoterol-Dependent Inhibition of Migration of HASM Cells, and Further Increases Migratory Potential of HASM Cells

Because our data demonstrate that albuterol inhibits the migration of HASM cell attributable to the phosphorylation of VASP,
we examined whether prolonged exposure to albuterol modulates the β2AR agonist–dependent inhibition of HASM cell migration. As shown in Figures 7A and 7B (solid bars), albuterol, formoterol, and PGE2 significantly inhibited the basal and PDGF-induced migration of HASM cells preincubated with diluent. However, preincubation with 1 μM albuterol for 24 hours attenuated the inhibitory effects of albuterol and formoterol on the basal and PDGF-induced migration of HASM cells, compared with diluent-pretreated cells (Figures 7A and 7B, gray bars). Importantly, pretreatment with albuterol exerted little effect on the ability of PGE2 to inhibit either the basal or PDGF-induced migration of HASM cells (Figure 7B), thus confirming our previous observation that chronic exposure to albuterol desensitizes cells at the β2AR level. Interestingly, chronic exposure to albuterol significantly promoted the PDGF-induced migration of HASM cells, compared with cells preincubated with diluent (Figure 7B). Collectively, these data demonstrate that the chronic exposure of HASM cells to albuterol specifically desensitizes cells to the inhibitory effects of β2-agonists on cell migration because of the suppression of β2AR-dependent VASP phosphorylation.

**DISCUSSION**

Anticapping VASP proteins play a critical role in actin cytoskeleton rearrangements during cell motility by directly modulating the formation of actin stress fibers at the leading edge of migrating cells. Our study shows that β2AR agonists inhibit the migration of HASM cells through the PKA-dependent phosphorylation of VASP. Importantly, prolonged exposure of HASM cells to the β2AR agonist albuterol prevented the β2AR-dependent phosphorylation of VASP as well as the inhibition of HASM cell migration, suggesting that albuterol-induced β2AR receptor tolerance abolishes the β2AR agonist-dependent inhibition of HASM cell migration through VASP.

Our data and those of others show that cAMP-mobilizing agents and glucocorticoids modulate the basal and mitogen-induced migration of HASM cells (5, 6). We demonstrated that cAMP-inducing agents promote the depolymerization of actin in HASM cells (8). PKA is activated by increases in cAMP concentrations, and inhibits the lysophosphatidic acid–induced migration of bovine ASM cells (7). The molecular mechanisms regulating these effects, however, are not well understood. Evidence suggests that cAMP-inducing agents may modulate the actin cytoskeleton during cell motility through an activation of

**Figure 5.** β2AR receptor agonists induce the transient phosphorylation of VASP in HASM cells. HASM cells were growth-arrested for 48 hours and incubated with different concentrations of albuterol (A), formoterol (B), PGE2, or diluent (0) for indicated times, followed by immunoblot analysis with anti-phospho VASP Ser157 or anti-total VASP antibodies. Images are representative of three independent experiments.

**Figure 6.** (A) Prolonged treatment with albuterol desensitizes HASM cells to the albuterol-dependent phosphorylation of VASP. Cells were growth-arrested for 48 hours, incubated with 3 μM albuterol or diluent for 24 hours, washed twice with PBS, and maintained for 2 hours in albuterol-free media. Cells were then stimulated with 0.1, 0.3, and 1 μM albuterol, 10 μM PGE2, or diluent for 30 minutes, followed by immunoblot analysis with anti-phospho VASP Ser157 and anti-total VASP antibodies (a schematic representation of the experiment is provided in Figure E5 of the online supplement). Representative images (top) and statistical analysis (bottom) of three independent experiments are shown. The data represent mean values ± SE according to ANOVA (Bonferroni-Dunn test). *P < 0.01 for albuterol-treated cells versus diluent-treated cells, and for PGE2-treated cells versus diluent-treated cells. **P < 0.01 for cells preincubated with albuterol versus cells preincubated with diluent. (B) The albuterol-induced desensitization of HASM cells to the albuterol-dependent phosphorylation of VASP is time-dependent. Growth-arrested HASM cells were incubated with 1 μM albuterol for 0, 30, 60, 90, and 120 minutes, followed by washing with PBS and incubation in media with 0.1% BSA for 2 hours. Afterward, cells were treated with 1 μM albuterol, 1 μM PGE2, or diluent for 30 minutes, followed by immunoblot analysis with anti-total VASP and anti-phospho VASP Ser157 antibodies (see schematic representation in Figure E5 of the online supplement). Top: Images are representative of three independent experiments. Bottom: Data represent mean values ± SE from three separate experiments. *P < 0.05 for cells treated with albuterol versus diluent-treated cells. **P < 0.05 for cells pretreated with albuterol for 30 minutes and then treated with albuterol, versus cells pretreated with diluent for 30 minutes and then treated with albuterol. ***P < 0.01 for cells preincubated with albuterol for 30 minutes and then treated with albuterol, versus cells preincubated with diluent for 30 minutes and then treated with albuterol. All P values were determined according to ANOVA (Bonferroni-Dunn test).
Our data show that phosphorylation of VASP to the inhibition of HASM cell migration. Interestingly, a previous report by Hastie et al. demonstrated in rat aortic vascular smooth muscle cells (20). The stimulation of human neutrophils by a chemoattractant induces phosphorylation of VASP at Ser157. The PKA-dependent phosphorylation of VASP was demonstrated in HASM cells. We found that the desensitization of VASP to the inhibition of HASM cell migration was also suggested (23).

Here, we provide evidence linking the β2AR agonist–dependent phosphorylation of VASP to the inhibition of HASM cell migration induced by cAMP-mobilizing agents. Our data show that short-term treatment with β2AR agonists induces the β2AR-dependent and PKA-dependent phosphorylation of VASP required for the β2AR agonist–induced inhibition of HASM cell migration.

Importantly, the stimulation of HASM cells with albuterol or formoterol induced a transient phosphorylation of VASP that was completely abolished after 3 hours of treatment with albuterol and 18 hours of treatment with formoterol. Because β2AR agonists are currently used as asthma therapeutics, we investigated whether HASM cells chronically treated with β2AR agonists will maintain their responsiveness to the albuterol-dependent and formoterol-dependent phosphorylation of VASP and their inhibition of cell migration. Interestingly, a previous report by Hastie and colleagues demonstrated a decreased phosphorylation of VASP in epithelial cells derived from patients with asthma who had been chronically treated with β2AR agonists (24). We report that prolonged treatment with albuterol decreases the responsiveness of HASM cells to β2AR agonists. Thus, albuterol failed to induce the phosphorylation of VASP in HASM cells treated with albuterol for 24 hours. Further, preincubation with albuterol for 24 hours reversed the ability of albuterol and formoterol to inhibit the migration of HASM cells. Our data demonstrate that prolonged treatment with β2AR agonists desensitizes HASM to β2AR agonists, and suggest that the phosphorylation level of VASP may serve as a potential biomarker of airway responsiveness to β2AR agonists and β2AR HASM desensitization.

Interestingly, pretreatment with albuterol did not affect the inhibitory effects of PGE2 on the phosphorylation of VASP and of HASM cells, suggesting a differential mechanism of the level of receptor or downstream signaling. Our previously published studies demonstrated that PGE2 promotes the cAMP-dependent activation of PKA and phosphorylation of VASP in HASM cells via the prostaglandin E2 (EP2) receptor (25, 26), which, in contrast to β2AR, is relatively resistant to agonist-specific desensitization. Indeed, the accumulation of cAMP and of PKA during both acute and chronic with agonists were markedly greater with PGE2 compared with β2AR agonists (14, 25–28) with regard to greater efficiency in the inhibition of HASM cell growth (26, 29) and migration (6). Indeed, our published data show that, in contrast to β2AR agonists that transiently increase the phosphorylation of VASP but decrease in effectiveness by 3 hours, PGE2 promotes a PKA-dependent phosphorylation of VASP that is sustained for up to 18 hours, suggesting that prolonged exposure to PGE2 does not desensitize EP2 receptors (25). Such differences between β-agonists and PGE2 may be explained by differential mechanisms of β2AR and EP2 receptor desensitization in HASM cells. Our previous data demonstrate that, in HASM cells, the desensitization of the β2AR receptor is selectively mediated by G protein-coupled receptor kinases 2/3 (GRK2/3) that exert little effect on EP2 receptor functions (26).

Our data demonstrate that the desensitization of VASP to β2AR agonists impairs the inhibitory effects of β2-agonists and promotes the migration of HASM cells. In terms of clinical significance, our findings suggest that the chronic use of β2-agonists could enhance the migration of ASM cells or prime them for an enhanced mitogenic response to growth factors. Ultimately, the enhanced migration of HASM cells and mitogenic responses may promote an asthma phenotype manifested by irreversible airflow obstruction. Although our data suggest that such mechanisms may be operative in chronic severe asthma, further clinical studies are needed to characterize the role of VASP definitively in the migration and proliferation of HASM cells in mediating the irreversible airflow obstruction of chronic asthma.

Understanding the cellular and molecular mechanisms of HASM remodeling is critically important, not only for advancing our knowledge of disease pathobiology, but also because such an understanding may lead to indentifying novel molecular targets for therapeutic intervention. This study identifies a mechanism of the inhibitory effects of β2AR agonists on the migration of HASM cells. We found that the β2AR agonist–induced activation of PKA modulates the phosphorylation of VASP that inhibits the migration of HASM cells. Furthermore, we found that the prolonged treatment of HASM with β2AR agonists desensitizes airways to further treatment with β2AR agonists. Our data suggest that a prolonged use of β2AR agonists in asthma as single therapy may promote the development of tolerance to β2AR agonists and the migration of ASM cells.

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