

Effect of Sphingosine 1-Phosphate on Cyclo-Oxygenase-2 Expression, Prostaglandin E₂ Secretion, and β_2 -Adrenergic Receptor Desensitization

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Abstract

Tachyphylaxis of the β_2 -adrenergic receptor limits the efficacy of bronchodilatory β_2 -agonists in respiratory disease. Cellular studies in airway smooth muscle (ASM) have shown that inflammatory mediators and infectious stimuli reduce β_2 -adrenergic responsiveness in a cyclo-oxygenase (COX)-2-mediated, prostaglandin E₂ (PGE₂)-dependant manner. Herein, we show that sphingosine 1-phosphate (S1P), a bioactive sphingolipid that plays an important role in pathophysiology of asthma, also induces β_2 -adrenergic receptor desensitization in bronchial ASM cells and exerts hyporesponsiveness to β_2 -agonists. We treated ASM cells with S1P (1 μ M) for up to 24 hours and then examined the temporal kinetics of COX-2 mRNA expression, protein up-regulation, and PGE₂ secretion. S1P significantly enhanced COX-2 expression and PGE₂ secretion, and this was repressed by the selective COX-2 inhibitor celecoxib, the corticosteroid dexamethasone, or small interfering RNA (siRNA) knockdown of COX-2 expression. In combination with another proinflammatory mediator found elevated in asthmatic airways, the cytokine TNF- α , we observed that S1P-induced COX-2 mRNA expression and protein up-regulation and PGE₂ secretion from ASM cells were significantly enhanced. Notably, S1P induced heterologous β_2 -adrenergic desensitization, as measured by inhibition of cyclic adenosine monophosphate production in response to the short-acting β_2 -agonist,

salbutamol, and the long-acting β_2 -agonist, formoterol. Taken together, these data indicate that S1P represses β_2 -adrenergic activity in ASM cells by increasing COX-2-mediated PGE₂ production, and suggest that this bioactive sphingolipid found elevated in asthma may contribute to β_2 -adrenergic desensitization.

Keywords: sphingosine 1-phosphate; cyclo-oxygenase 2; prostaglandin E₂; β_2 -adrenergic receptor desensitization; cyclic adenosine monophosphate

Clinical Relevance

With this study, we have highlighted the potential impact of sphingosine 1-phosphate (S1P) on β_2 -adrenergic receptor (β_2 -AR) receptor function. We have revealed that S1P can increase cyclo-oxygenase-2 mRNA expression, which, in turn, leads to enhanced secretion of prostaglandin E₂ from airway smooth muscle cells *in vitro*, and suggest that S1P may contribute to β_2 -AR desensitization in patients with asthma. This new knowledge may be exploited to discover novel targets for restoring the efficacy of bronchodilatory β_2 -agonists in the future.

Bronchodilatory β_2 -agonists are used in first-line therapy to relieve acute bronchoconstriction in people with asthma or chronic obstructive pulmonary disease.

β_2 -agonists exert their effects by binding with the β_2 -adrenergic receptor (β_2 -AR), a seven-transmembrane G protein-coupled receptor and ultimately relax the airways by

rapidly increasing cyclic adenosine monophosphate (cAMP) levels in airway smooth muscle (ASM) cells. However, heterologous and homologous β_2 -AR

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desensitization can occur in a diverse range of clinically relevant contexts, including β_2 -agonist overuse, inflammation, and bacterial and viral infectious exacerbation (1–3). As this can result in hyporesponsiveness to β_2 -agonists, β_2 -AR desensitization is an important issue limiting effective treatment of chronic respiratory disease; thus, further investigation into the causes and underlying molecular mechanisms are warranted.

Accumulating evidence has demonstrated the key role played by the enzyme, cyclo-oxygenase (COX)-2 and its prostanoid products (prostaglandin E_2 [PGE $_2$] in particular) in mediating β_2 -AR desensitization. A number of proinflammatory cytokines elevated in bronchoalveolar lavage (BAL) from people with asthma have been shown to induce COX-2 expression and result in PGE $_2$ secretion from ASM cells *in vitro*. Cytokines investigated include TNF- α , IL-1 β , and IFN- γ , added alone or in combination (cytomix) (4–7). PGE $_2$ is known to exert heterologous desensitization of β_2 -AR (8), and we have recently confirmed COX-2-dependent/PGE $_2$ -mediated hyporesponsiveness to β_2 -agonists in models of viral- and bacterial-induced exacerbation (9–11).

Sphingosine 1-phosphate (S1P) is a bioactive sphingolipid found elevated in BAL from individuals with allergic asthma, and has been associated with inflammatory cell influx into the airways (12). S1P exerts potent effects in *in vitro* and *in vivo* models of asthmatic inflammation and airway remodeling (12–16). S1P has been shown to induce β_2 -AR desensitization in a guinea pig model of bronchoconstriction (17), and was recently shown to increase COX-2/PGE $_2$ in tracheal ASM cells *in vitro* (18). Moreover, Fuerst and colleagues (19) reported that S1P potently regulated calcium mobilization and gene expression (including COX-2) in ASM grown from bronchial biopsies from healthy individuals and subjects with asthma. Notably, an S1P analog (FTY720; fingolimod) was recently shown to be linked to exacerbation of asthma symptoms (20). Thus, although COX-2/PGE $_2$ expression has been linked to S1P, its role in hyporesponsiveness of the β_2 -AR in ASM cells has not been examined. Herein, we investigate the effect of S1P on COX-2

up-regulation alone and in combination with TNF- α in bronchial ASM cells, and provide the first demonstration of β_2 -AR desensitization by S1P *in vitro*. We show the bioactive sphingolipid S1P induces hyporesponsiveness to the β_2 -agonists, salbutamol and formoterol, and demonstrate that S1P augments COX-2

expression and PGE $_2$ secretion induced by TNF- α in an additive manner. Notably, S1P-induced effects are repressed by the COX-2 selective inhibitors, celecoxib and dexamethasone, thus, representing feasible pharmacotherapeutic strategies for restoring β_2 -AR sensitivity.

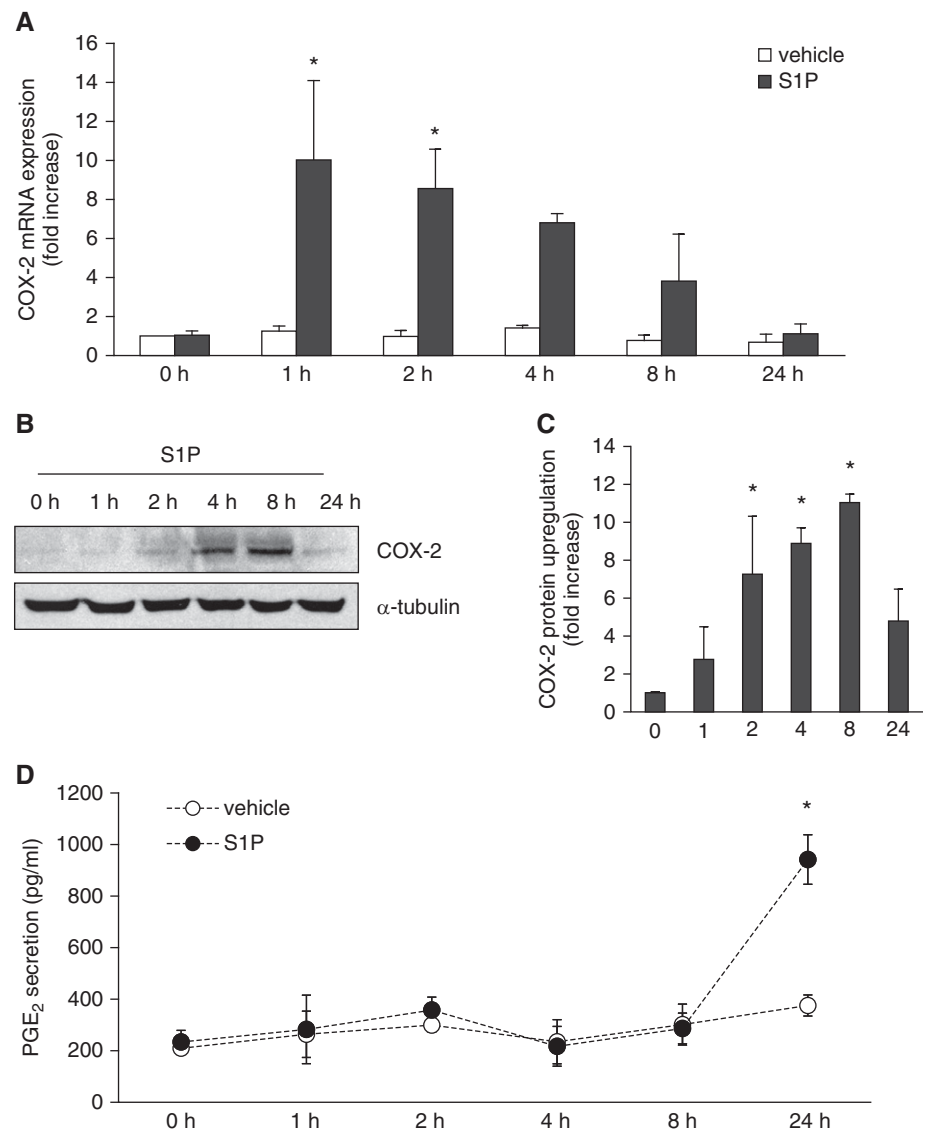


Figure 1. Sphingosine 1-phosphate (S1P) up-regulates cyclo-oxygenase (COX)-2 mRNA expression and protein up-regulation to increase prostaglandin E_2 (PGE $_2$) secretion from airway smooth muscle (ASM) cells. Growth-arrested ASM cells were treated with vehicle or S1P (1 μ M) for 0, 1, 2, 4, 8, and 24 hours. (A) COX-2 mRNA expression was quantified by real-time RT-PCR (results expressed as fold increase compared with vehicle-treated cells at 0 h). (B and C) COX-2 protein was detected by Western blotting (with α -tubulin as the loading control), where results are representative Western blots (B), whereas (C) demonstrates densitometric analysis (results expressed as fold increase compared with vehicle-treated cells at 0 hours). (D) PGE $_2$ secretion was measured by enzyme immunoassay (EIA). Statistical analysis was performed using one-way or two-way ANOVA, then Bonferroni's post-test (*significant effect of S1P, $P < 0.05$). Data are mean $\pm$ SEM values from $n = 3$ primary ASM cell cultures.

Materials and Methods

ASM Cell Culture

Human bronchi were obtained from patients undergoing surgical resection for carcinoma, or from lung transplant donors, in accordance with procedures approved by the Sydney South West Area Health Service and the Human Research Ethics Committee of the University of Sydney. ASM cells were dissected, purified, and cultured as previously described by Johnson and colleagues (21). A minimum of three different ASM primary cell lines were used for each experiment.

Chemicals

TNF- α was purchased from R&D Systems (Minneapolis, MN) and celecoxib from Cayman Chemical Co. (Ann Arbor, MI). S1P (Biomol) was purchased from Enzo Life Sciences (Farmingdale, NY). Unless otherwise specified, all chemicals were from Sigma-Aldrich (St. Louis, MO).

Real-Time RT-PCR

Total RNA was extracted using the RNeasy Mini Kit (Qiagen Australia, Doncaster, VIC, Australia) and reverse transcribed using the RevertAid First strand cDNA Synthesis Kit (Fermentas Life Sciences, Hanover, MD). Real-time RT-PCR was performed on an ABI Prism 7500 with COX-2 (Hs0015133_m1) TaqMan Gene Expression Assays and the eukaryotic 18S rRNA endogenous control probe (Applied Biosystems, Foster City, CA) subjected to the following cycle parameters: 50°C for 2 minutes, 1 cycle; 95°C for 10 minutes, 1 cycle; 95°C for 15 second, 60°C for 1 minute, 40 cycles, and mRNA expression (fold increase) quantified by $\Delta\Delta C_t$ calculations.

PGE₂ Assay

PGE₂ was measured by enzyme immunoassay (Prostaglandin E₂ EIA 514010; Cayman Chemical Co.) according to the manufacturer's instructions.

Western Blotting

Western blotting was performed using mouse monoclonal antibodies against COX-2 Antibody (29) (Santa Cruz Biotechnology, Santa Cruz, CA), compared with α -tubulin as the loading control (clone DM 1A; Santa Cruz Biotechnology). Primary antibodies were detected with goat anti-mouse horseradish peroxidase-conjugated

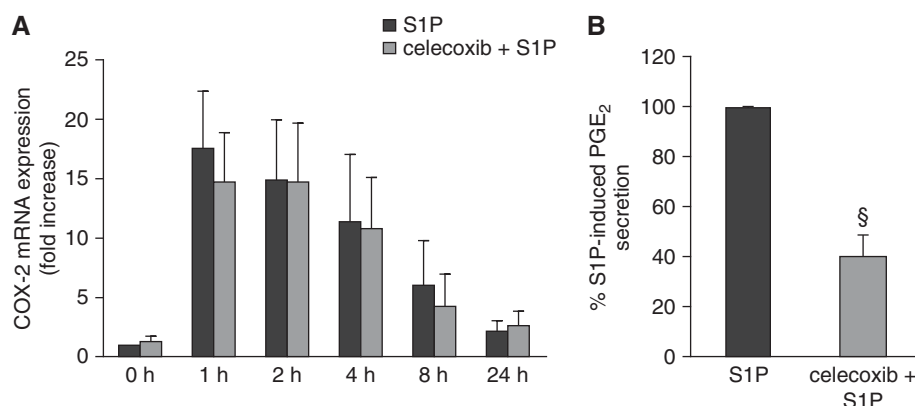


Figure 2. Celecoxib had no effect on S1P-induced COX-2 mRNA but significantly inhibited S1P-induced PGE₂ secretion. Growth-arrested ASM cells were pretreated with vehicle or 10 μ M celecoxib for 1 hour before treatment with S1P (1 μ M) for 0, 1, 2, 4, 8, and 24 hours. (A) COX-2 mRNA expression was quantified by real-time RT-PCR (results expressed as fold increase compared with vehicle-treated cells at 0 hour). (B) Repression of S1P-induced PGE₂ secretion (results expressed as a percentage of S1P-induced PGE₂ secretion at 24 hours). Statistical analysis was performed using Student's unpaired *t* test ([§]significant repression by celecoxib). Data are mean $\pm$ SEM values from *n* = 3 primary ASM cell cultures.

secondary antibodies (Cell Signaling Technology, Danvers, MA) and visualized by enhanced chemiluminescence (PerkinElmer, Wellesley, MA).

COX-2 Small Interfering RNA

ASM cells were transiently transfected using nucleofection with 1 μ g COX-2-specific ON-Target SMART pool small interfering RNA (siRNA), consisting of a pool of four individual siRNAs (Dharmacon; Thermo Fisher Scientific, Waltham, MA) or a scrambled siRNA control (ON-Target plus Control Non-targeting siRNA; Dharmacon), using methods established in our earlier publication (22).

ASM cells were plated for 16 hours after transfection before being growth arrested for a further 24 hours. Cells were then stimulated with S1P (1 μ M) before COX-2 mRNA was measured by RT-PCR at 1 hour, COX-2 protein detected by Western blotting at 24 hours, and secreted PGE₂ at 24 hours measured by enzyme immunoassay.

cAMP Assay

Desensitization of the β_2 -AR was assessed by measuring production of cAMP in response to stimulation with β_2 -agonists (11). To exclude the involvement of adenylate cyclase, cells were treated with

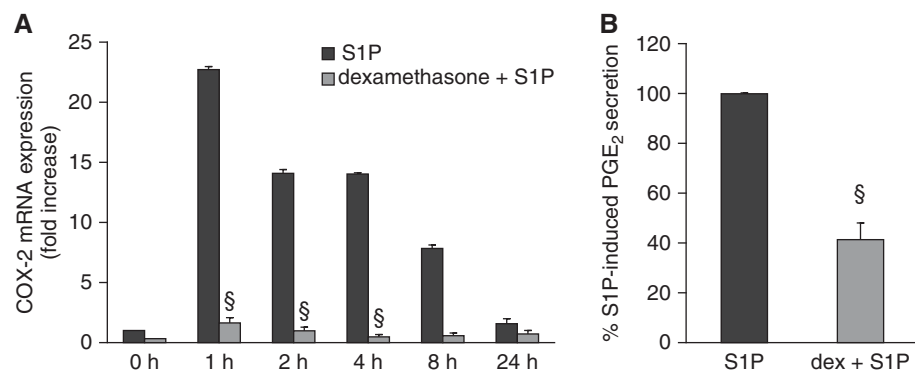


Figure 3. Dexamethasone (dex) represses S1P-induced COX-2 mRNA expression and PGE₂ secretion. Growth-arrested ASM cells were pretreated with vehicle or 100 nM dexamethasone for 30 minutes before treatment with S1P (1 μ M) for 0, 1, 2, 4, 8, and 24 hours. (A) COX-2 mRNA expression was quantified by real-time RT-PCR (results expressed as fold increase compared with vehicle-treated cells at 0 hour). (B) Repression of S1P-induced PGE₂ secretion (results expressed as a percentage of S1P-induced PGE₂ secretion at 24 hours). Statistical analysis was performed using one-way or two-way ANOVA, then Bonferroni's post-test ([§]significant repression by dexamethasone). Data are mean $\pm$ SEM values from *n* $\geq$ 3 primary ASM cell cultures.

the adenylate cyclase activator, forskolin (10 μ M), for 15 minutes in the presence of 3-isobutyl-1-methylxanthine (IBMX). Intracellular cAMP was measured by enzyme immunoassay (cAMP EIA 581001; Cayman Chemical Co.) according to the manufacturer's instructions.

Statistical Analysis

Statistical analysis was performed using Student's unpaired *t* test, one-way ANOVA, then Fisher's *post hoc* multiple comparison test, or two-way ANOVA then Bonferroni's post-test. *P* values less than 0.05 were sufficient to reject the null hypothesis for all analyses.

Results

S1P Up-Regulates COX-2 mRNA Expression and Protein Up-Regulation to Increase PGE₂ Secretion from ASM Cells

To explore the role of S1P in β_2 -AR desensitization, we first treated ASM cells with S1P (1 μ M) over 24 hours and measured the temporal kinetics of COX-2 mRNA expression, protein up-regulation and PGE₂ secretion. Stimulation of ASM cells with S1P significantly up-regulated COX-2 mRNA expression at 1 and 2 hours (Figure 1A). COX-2 protein up-regulation followed with a peak at 8 hours (Figures 1B and 1C), and resulted in increased levels of PGE₂ by 24 hours (Figure 1B; *P* < 0.05).

Celecoxib Has No Effect on S1P-Induced COX-2 mRNA, but Significantly Inhibited S1P-Induced PGE₂ Secretion

We then treated cells with the selective COX-2 inhibitor, celecoxib, and examined its effect on COX-2 mRNA expression and PGE₂ secretion. As expected, celecoxib had no effect on S1P-induced COX-2 mRNA expression (Figure 2A). In contrast, celecoxib pretreatment significantly repressed S1P-induced PGE₂ secretion (Figure 2B; *P* < 0.05). These results confirm that celecoxib inhibits COX-2 enzymatic activity, but not its mRNA expression, and demonstrate that S1P-induced PGE₂ secretion occurs in a COX-2-dependent manner.

Dexamethasone Represses S1P-Induced COX-2 mRNA Expression and PGE₂ Secretion

Corticosteroids are used as first-line antiinflammatory therapies in asthma, and

we have already shown that they have repressive effects on S1P-induced cytokine secretion (15, 16). Earlier publications have shown that cytokine-induced COX-2 expression and PGE₂ secretion can be repressed by the corticosteroid, dexamethasone (4, 5). To assess whether corticosteroids repress S1P-induced effects, ASM cells were pretreated with 100 nM dexamethasone for 30 minutes before stimulation. As shown in Figure 3A, dexamethasone abolished S1P-induced COX-2 mRNA expression, with significant repression observed at the 1-, 2-, and 4-hour time points (*P* < 0.05). Moreover,

we observed that pretreatment with dexamethasone significantly inhibited S1P-induced PGE₂ secretion at 24 hours (Figure 3B; *P* < 0.05).

COX-2 Knockdown by siRNA Represses S1P-Induced COX-2 mRNA Expression, Protein Up-Regulation, and PGE₂ Secretion

To further support the role of S1P-induced COX-2 expression on PGE₂ secretion, we examined the impact of specifically knocking down COX-2 with siRNA on S1P-induced COX-2 mRNA expression, protein up-regulation, and PGE₂ secretion

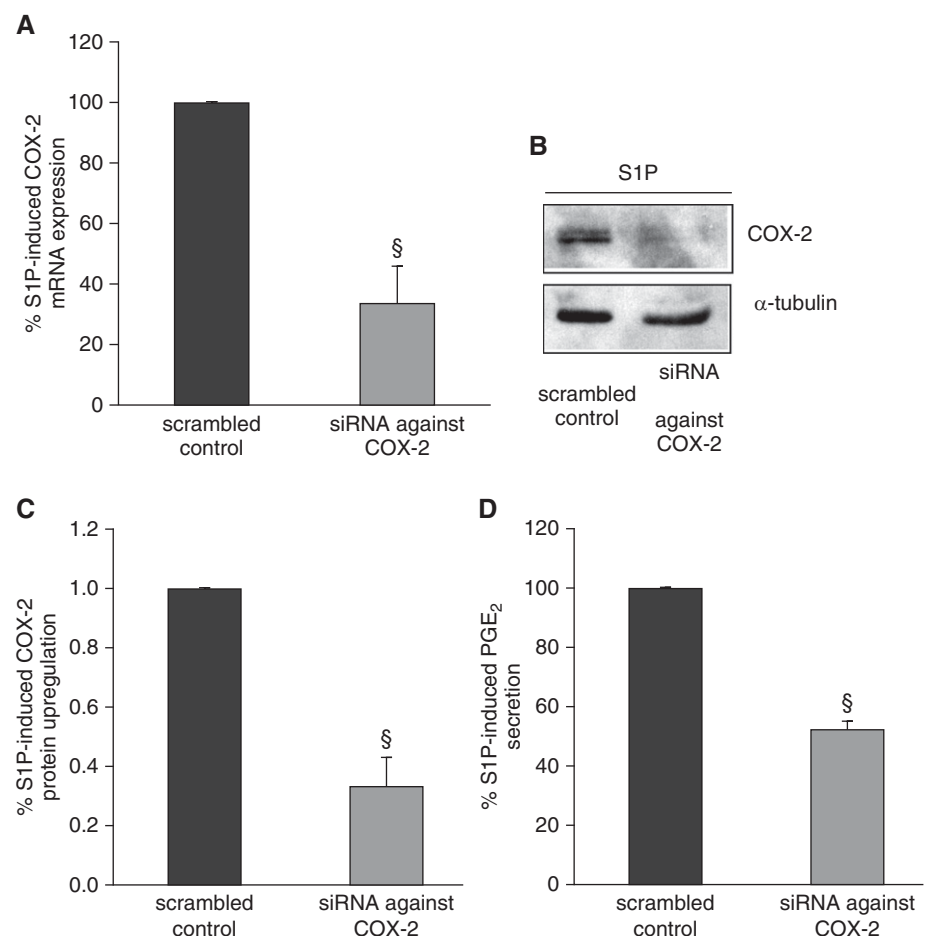


Figure 4. COX-2 knockdown by small interfering RNA (siRNA) represses S1P-induced COX-2 mRNA expression, protein up-regulation, and PGE₂ secretion. ASM cells were transiently transfected using nucleofection with scrambled control or COX-2 siRNA, growth arrested, and then stimulated with S1P (1 μ M). (A) COX-2 mRNA was measured by RT-PCR at 1 hour (results expressed as percentage of S1P-induced COX-2 mRNA expression, scrambled control). (B and C) COX-2 protein was detected by Western blotting (with α -tubulin as the loading control) at 24 hours (results are representative Western blots [B], whereas [C] demonstrates densitometric analysis). (D) Secreted PGE₂ at 24 hours was measured by enzyme immunoassay (results expressed as percentage of S1P-induced PGE₂ secretion, scrambled control). Statistical analysis was performed using Student's unpaired *t* test (§significant effect of siRNA against COX-2, compared with scrambled control). Data are mean $\pm$ SEM values from *n* = 3 primary ASM cell cultures.

(Figure 4). ASM cells were transiently transfected using nucleofection with scrambled control or COX-2 siRNA, growth arrested, then stimulated with S1P (1 μ M). As shown in Figure 4A, the peak of COX-2 mRNA expression at 1 hour was significantly reduced by COX-2 siRNA ($P < 0.05$). S1P-induced COX-2 protein expression was significantly reduced by siRNA knockdown (Figure 4B), with densitometric analysis revealing a significant ($66.9 \pm 9.8\%$) reduction of COX-2 protein expression at 24 hours in cells transfected with siRNA against COX-2 compared with scrambled control (Figure 4C; $P < 0.05$). This resulted in corresponding decrease in the amount of PGE₂ secreted (Figure 4D; $P < 0.05$). Collectively, these results confirm that S1P significantly enhances COX-2 expression and PGE₂ secretion, as this was repressed by the selective COX-2 inhibitor, celecoxib, the corticosteroid dexamethasone, or siRNA knockdown of COX-2 expression.

TNF- α Enhances S1P-Induced COX-2 mRNA Expression and Protein Up-Regulation, and Increases PGE₂ Secretion

We (11) and others (4, 5) have shown that the proinflammatory cytokine, TNF- α , found elevated in BAL, increases COX-2 mRNA expression and induces PGE₂ secretion from ASM cells. Comparatively, TNF- α alone is a relatively modest inducer of COX-2 mRNA expression and PGE₂ secretion (4, 5, 23), but robust up-regulation can be observed in combination with other cytokines, IL-1 β in particular (4), or as cytomix (4, 7, 23). We proposed that the effects of S1P would be enhanced in the presence of TNF- α , and, to test this, we treated growth-arrested ASM cells with S1P (1 μ M) or TNF- α (10 ng/ml), alone or in combination. As shown in Figure 5A, the temporal kinetics of COX-2 mRNA expression induced by TNF- α is similar to that observed when ASM cells are stimulated with S1P alone. However, when cells are stimulated with both mediators together, there is an additive effect, with TNF- α significantly enhancing S1P-induced COX-2 mRNA expression at 4 and 8 hours (Figure 5A; $P < 0.05$). COX-2 protein up-regulation at 24 hours (Figure 5B) aligned with the mRNA data, and densitometric analysis confirmed that TNF- α significantly

enhanced S1P-induced COX-2 protein up-regulation (Figure 5C; $P < 0.05$). These data concur with the temporal kinetics of PGE₂ secretion. As shown in Figure 5D, when added in combination, we observed that TNF- α significantly increased S1P-induced PGE₂ secretion at 4, 8, and 24 hours ($P < 0.05$).

S1P Induces Heterologous β_2 -Adrenergic Desensitization, as Measured by Inhibition of β_2 -Agonist-Induced cAMP Production, in a Manner Independent of Adenylate Cyclase

Taken together, our data thus far shows that S1P increases COX-2 up-regulation and

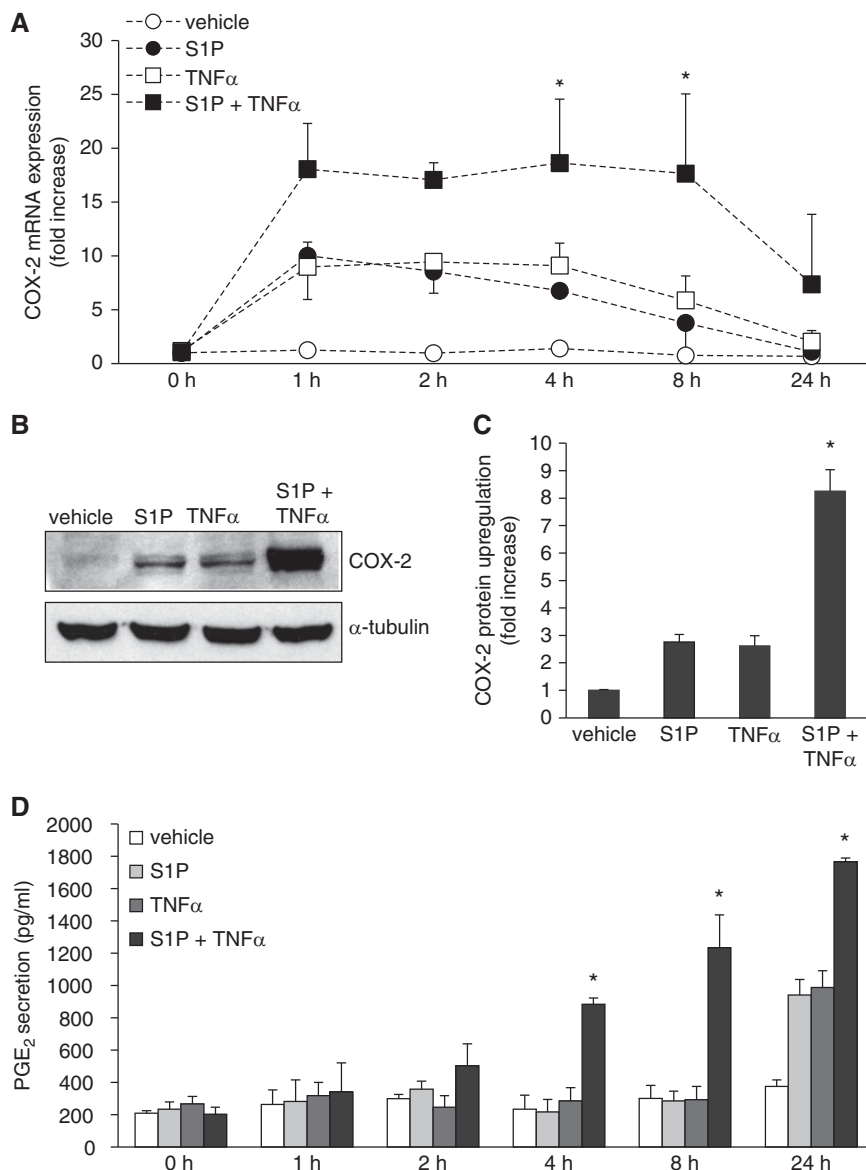


Figure 5. TNF- α enhances S1P-induced COX-2 mRNA expression and protein up-regulation and increases PGE₂ secretion. Growth-arrested ASM cells were treated with vehicle, S1P (1 μ M), TNF- α (10 ng/ml), or S1P + TNF- α , for 0, 1, 2, 4, 8, and 24 hours. (A) COX-2 mRNA expression was quantified by real-time RT-PCR (results expressed as fold increase compared with vehicle-treated cells at 0 hour). (B and C) COX-2 protein was detected by Western blotting at 24 hours (compared with α -tubulin as a loading control), where (B) is a representative blot and (C) is densitometric analysis of COX-2 protein up-regulation (results normalized with α -tubulin expressed as fold increase compared with vehicle-treated cells). (D) PGE₂ secretion was measured by EIA. Statistical analysis was performed with two-way ANOVA and then Bonferroni's post-test or Student's unpaired *t* test (*significant effect of TNF- α on S1P-induced effects, $P < 0.05$). Data are mean $\pm$ SEM values from $n = 3$ primary ASM cell cultures.

significantly enhances PGE₂ secretion from ASM cells. Moreover, when added in combination with TNF- α , COX-2 expression and PGE₂ secretion is substantially increased in an additive manner. PGE₂ is a known inducer of β_2 -AR desensitization, and so we now wished to demonstrate that S1P, alone and in combination with TNF- α , caused β_2 -AR desensitization and resulted in β_2 -AR

hyporesponsiveness to two widely used β_2 -agonists in asthma and chronic obstructive pulmonary disease: short-acting salbutamol and long-acting formoterol. ASM cells were exposed to a concentration of PGE₂ known to induce heterologous desensitization (i.e., 100 nM) (11), in parallel with S1P, or S1P plus TNF- α , compared with vehicle. We then stimulated cells for

15 minutes with either salbutamol (Figure 6A) or formoterol (Figure 6B), and measured the cAMP produced in response to β_2 -agonists as a measure of β_2 -AR desensitization. As shown in Figure 6A, salbutamol significantly increased cAMP production in ASM cells, and this was significantly repressed by pretreatment with PGE₂. These data concur with our previous study (11).

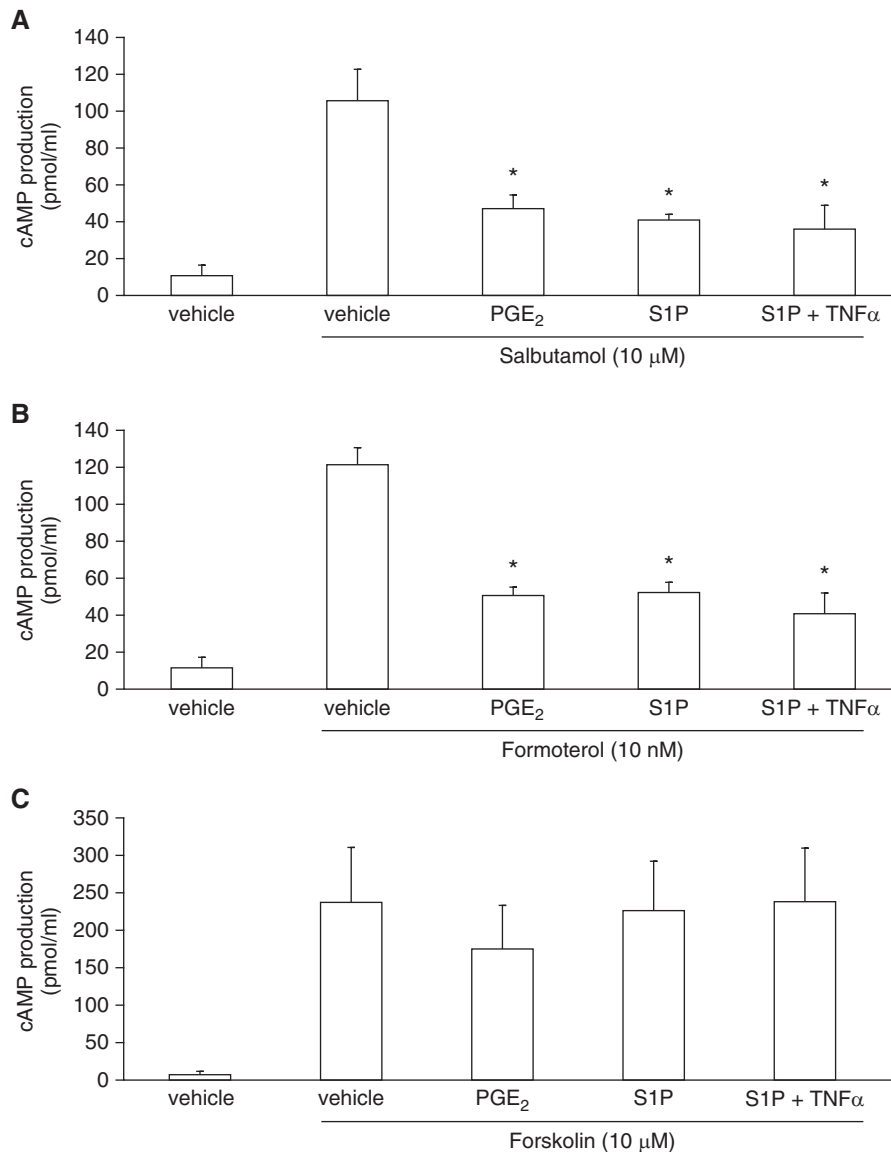


Figure 6. S1P induces heterologous β_2 -adrenergic desensitization as measured by inhibition of β_2 -agonist-induced cyclic adenosine monophosphate (cAMP) production in a manner independent of adenylylase. Growth-arrested ASM cells were pretreated for 24 hours with vehicle, 100 nM PGE₂, vehicle, S1P (1 μ M), S1P (1 μ M) plus TNF- α (10 ng/ml). (A and B) Desensitization of the β_2 -adrenergic receptor was assessed by measuring production of cAMP (pmol/ml) in response to stimulation with (A) 10 μ M salbutamol or (B) 10 nM formoterol for 15 minutes, compared with vehicle, in the presence of the pan-phosphodiesterase inhibitor, 3-isobutyl-1-methylxanthine (IBMX). (C) To exclude the involvement of adenylylase, we measured production of cAMP (pmol/ml) in response to stimulation with the adenylylase activator forskolin (10 μ M) for 15 minutes compared with vehicle, in the presence of IBMX. Statistical analysis was performed using one-way ANOVA and then Bonferroni's post-test (*significant effect on β_2 -agonist-induced cAMP production, $P < 0.05$). Data are mean+SEM values from $n = 3$ (A and B) and $n = 4$ (C) primary ASM cell cultures.

Notably, pretreating cells with S1P, or S1P plus TNF- α , also resulted in significantly less cAMP, indicative of β_2 -AR desensitization ($P < 0.05$). There was also evidence of hyporesponsiveness to the β_2 -agonist, formoterol, as shown in Figure 6B, where formoterol induced 121.0 (± 9.1) pmol cAMP/ml that was significantly repressed by PGE₂, S1P, or S1P plus TNF- α ($P < 0.05$). This is independent of adenylate cyclase involvement, as shown in Figure 6C, where the adenylate cyclase activator, forskolin, significantly increased cAMP secretion from ASM cells, and this was unaffected by PGE₂ alone, S1P, or S1P in combination with TNF- α . These data support the assertion that S1P induces heterologous β_2 -adrenergic desensitization via PGE₂, in a manner that may be upstream of adenylate cyclase. Collectively, these studies provide the first evidence that, like other inflammatory mediators found elevated in asthma, the bioactive sphingolipid, S1P, induces β_2 -adrenergic desensitization in ASM.

Discussion

S1P is a bioactive sphingolipid found elevated in the BAL from people with allergic asthma. It has potent effects on airway inflammation and, herein, we show that S1P robustly up-regulates COX-2 expression to result in PGE₂ production in bronchial ASM cells. We also provide the first evidence to demonstrate that S1P induces β_2 -AR desensitization to result in reduced cAMP production to clinically used β_2 -agonists, salbutamol and formoterol. Moreover, S1P acts in an additive manner to enhance the COX-2/PGE₂-mediated up-regulation in response to the proinflammatory cytokine, TNF- α . Thus, with this study, we are the first to implicate this mediator in β_2 -AR desensitization.

β_2 -AR desensitization is an important issue in respiratory medicine. Mechanisms have been elucidated in part, and are clearly linked to COX-2

up-regulation in ASM and the subsequent autocrine effects of PGE₂. A range of proinflammatory cytokines has been shown to increase COX-2/PGE₂ (see review in Ref. 2). Collectively, these studies reinforce the notion that a key characteristic of proinflammatory cytokines in airway inflammation, in addition to their established ability to initiate, potentiate, and amplify the proasthmatic cascade, is their capacity to dampen the ability of β_2 -agonists to exert their bronchodilatory effects. Moreover, cytokine responses can be robustly potentiated by activation of pattern-recognition receptors on ASM cells, with our recent studies showing that activation of Toll-like receptors for viral and bacterial products augments COX-2 expression/PGE₂ secretion and amplifies β_2 -AR desensitization (9–11). These studies may provide a mechanistic basis for clinically significant hyporesponsiveness to β_2 -agonists observed in infectious exacerbation (24).

Our study is the first to examine the impact of S1P on β_2 -AR desensitization in ASM cells. S1P is a bioactive sphingolipid found in increased levels in the BAL of people with allergic asthma (12), with effects on asthmatic inflammation and bronchoconstriction *in vitro* (15, 16) and *in vivo* (13, 14, 19). It is a legitimate target in asthma (25), and our current study reveals that, in addition to its known proinflammatory and bronchoconstrictive functions, S1P can also induce β_2 -AR desensitization. In this way, S1P can accelerate the proasthmatic phenotype, while disabling the brake.

COX-2 is an inducible gene and is known to be rapidly expressed in ASM cells in response to a diverse range of mediators, *viz*, cytokines (including TNF- α , IL-1 β , and IFN- γ , added alone or in combination as cytomix) (4–7), Toll-like receptor activators (9–11), and now S1P (Ref. 18 and this study). Thus, the COX-2/PGE₂ pathway has emerged as a legitimate therapeutic target pathway to restore β_2 -AR responsiveness. Understanding the molecular mechanisms

responsible will reveal future pharmacotherapeutic strategies. COX-2 mRNA expression can be repressed by corticosteroids, as we have shown for S1P, and in confirmation of studies with COX-2 induced by other stimuli (4, 5, 7). Targeting COX-2 more specifically offers great promise, and, in a notable recent study, Comer and colleagues (23) demonstrate that cytokine stimulation of ASM cells from people with asthma has enhanced COX-2 mRNA and protein, and increased secretion of PGE₂, compared with nonasthmatic controls, and this is due to epigenetic regulation involving miR-155, a microRNA found elevated in asthma. Prostanoid products of COX-2 enzymatic action can also be blocked with the use of nonselective (4, 5) and selective COX-2 inhibitors, such as celecoxib (10, 11, 23). We could also target the specific receptors for PGE₂, and this may also be relevant, given that increased E-prostanoid receptor surface expression has been reported in ASM cells from people with asthma (26). Further studies are warranted.

With this study, we have highlighted the potential impact of S1P on β_2 -AR receptor function. We have revealed that S1P can increase COX-2 mRNA expression, which, in turn, leads to enhanced secretion of PGE₂ from ASM cells *in vitro* and suggests that S1P may contribute to β_2 -AR desensitization in patients with asthma. This new knowledge may be exploited to discover novel targets for restoring the efficacy of bronchodilatory β_2 -agonists in the future. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

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