Endothelin-1 induces hypertrophy and inhibits apoptosis in human airway smooth muscle cells

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McWhinnie R, Pechkovsky DV, Zhou D, Lane D, Halayko AJ, Knight DA, Bai TR. Endothelin-1 induces hypertrophy and inhibits apoptosis in human airway smooth muscle cells. Am J Physiol Lung Cell Mol Physiol 292: L278–L286, 2007. First published August 18, 2006; doi:10.1152/ajplung.00111.2006.—Endothelin-1 (ET-1), a G protein-coupled receptor-activating peptide, is increased in airway epithelium, plasma, and bronchoalveolar lavage fluid of asthmatic patients. We hypothesized that ET-1 may contribute to the increased airway smooth muscle mass found in severe asthma by inducing hypertrophy and inhibiting apoptosis of smooth muscle cells. To investigate this hypothesis, we determined that treatment of primary human bronchial smooth muscle cells with ET-1 dose dependently [10^{-11}–10^{-7} M] inhibited the apoptosis induced by serum withdrawal. ET-1 treatment also resulted in a significant increase in total protein synthesis, mediated through both ET_{A} and ET_{B} receptors, cell size, as well as increased expression of myosin heavy chain, α-smooth muscle actin, and calponin. ET-1-induced hypertrophy was accompanied by activation of JAK1/STAT-3 and MAPK1/2 (ERK1/2) cell signaling pathways. Inhibition of JAK1/STAT-3 pathways by piceatinol or ERK1/2 by the MAPK/ERK kinase 1/2 inhibitor U0126 blunted the increase in total protein synthesis. The hypertrophic effect of ET-1 was equivalent to that of the gp130 cytokine oncostatin M and greater than that induced by cardiotoxin-1. ET-1 induced release of IL-6 but not IL-11, leukemia inhibitory factor, oncostatin M, or cardiotoxin-1, although treatment of cells with IL-6 alone did not induce hypertrophy. These results suggest that ET-1 is a candidate mediator for the induction of increased smooth muscle mass in asthma and identify signaling pathways activated by this mediator.

interleukin-6; oncostatin M; signal transducer and activator of transcription 3; extracellular signal-regulated kinase 1/2; asthma

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Asthma, particularly if severe and/or of long duration, is accompanied by increased airway smooth muscle (ASM) mass (1). There is evidence that the increase in ASM mass seen in asthmatic airways occurs as a result of both hyperplasia and hypertrophy (2, 6, 33). Benayoun and colleagues (2) have shown that increases in muscle cell size may be related to the severity of disease, and Ebina et al. (6) suggested that there are two strikingly different patterns of smooth muscle increase: one predominantly of central (large) airway hyperplasia and another of predominantly diffuse hypertrophy. However, the mechanisms and pathways involved in smooth muscle hypertrophy have been less well studied than those of proliferation. Our laboratory (35) and others (19) have suggested that regulation of apoptosis may be a primary process by which ASM cell number is determined, as the threshold for change in rates of apoptosis may be lower than for proliferation, and reduction in rates of apoptosis may contribute to hypertrophy. Thus mediators that induce hypertrophy or decrease the rate of apoptosis of ASM cells require investigation.

The endothelins (ETs) are a family of three isopeptides, acting through two G protein-coupled receptors, ET_{A} and ET_{B}. ET-1 in particular elevates smooth muscle tone (4) and causes a marked potentiation of cholinergic nerve-evoked contraction of ASM (13). ET-1 is released early in eosinophilic inflammatory cascades and causes an increase in airway microvascular leakage (11, 16) and extravascular sequestration of granulocytes (17). Asthma is associated with elevated expression and release of ET-1, which is thought to be primarily released from the bronchial epithelium (22, 23, 28). On the surface of human bronchial smooth muscle cells, the ET_{B} receptor predominates, representing ~82–88% of the total ET receptor population (14). Stimulation of the ET_{B} receptor causes airway contraction, whereas stimulation of the ET_{A} receptor has been associated with effects such as mediator release and recruitment of inflammatory cells, as well as contraction. Some actions involve the release of secondary mediators (12, 14). ET-1 may be an essential synergistic factor in human bronchial fibroblasts, along with growth factors, to induce collagen production (5). Tschumperlin et al. (31, 32) showed that airway epithelial cells submitted to increased pressure gradients increase production of ET-1. The airway wall in severe or uncontrolled asthma is likely subject to increased mechanical strain, and thus structural changes may be induced directly from the mucosa via ET-1 release.

ET-1 has only a small, direct mitogenic effect on human ASM cells in culture (7). It is anti-apoptotic and able to induce hypertrophy in human vascular smooth muscle cells (20, 26). However, there are no reports of whether ET-1 induces similar effects in human ASM. In this paper, we report our findings on hypertrophic and anti-apoptotic effects of ET-1 and compare these results with the effect of specific members of the gp130 cytokine family, which our laboratory has previously shown to have these actions (34, 35). Moreover, as ET-1 induction of atrial natriuretic peptide and β-myosin heavy chain (MHC) expression and ET-1-stimulated DNA synthesis are dependent on gp130 signaling and activation of signal transducer and activator of transcription 3 (STAT-3) in cardiac fibroblasts (20, 24), we
also investigated whether members of the IL-6 cytokine family were involved in responses to ET-1.

MATERIALS AND METHODS

Reagents

Recombinant human oncostatin M (OSM) and recombinant human cardiostatin-1 (CT-1) were obtained from R&D Systems (Minneapolis, MN). [3H]Thymidine was purchased from Amersham Pharmacia Biotech (Piscataway, NJ), and [3H]Leucine was purchased from Perkin Elmer Life Sciences (Boston, MA). Antibodies against phosphorylated and nonphosphorylated JAK1, ERK, and STAT-3 were purchased from Cell Signaling Technology (Danvers, MA). Human ET-1, the MAPK/ERK kinase (MEK) 1/2 inhibitor U0126, and the JAK1/STAT-3 inhibitor piceatannol were purchased from Sigma (St. Louis, MO) and added to experiments 30 min before addition of ET-1. The ET\(_A\) and ET\(_B\) receptor antagonists BQ-123 and BQ-788 were purchased from Elmer Life Sciences (Boston, MA). Antibodies against phosphorylated JAK1, STAT-3, and ERK1/2 were involved in responses to ET-1.

**Human Bronchial Smooth Muscle Cell Culture**

Adult human primary bronchial smooth muscle cells (HBSMC) were isolated as previously described and used between passages 3 and 7 (15). Cells were seeded at 2,500 cells/cm\(^2\) and grown to ~80% confluence in DMEM medium containing 10% FBS and 100 \(\mu\)M nonessential amino acid solution. Cells were then grown arrested for 48 h in serum-free F-12 medium containing insulin (10 \(\mu\)g/ml), transferrin (5.5 \(\mu\)g/ml), and selenium A (6.7 ng/ml).

**Apoptosis Assay**

Apoptosis was measured as described previously (35). Briefly, cells were grown in 24-well plates, serum starved for 24 h in culture with insulin-transferrin-selenium (ITS) medium, and treated with ET-1 for 72 h. Histone-associated DNA fragments were measured using a commercially available Cell Death Detection ELISA kit (Roche Bioscience, Indianapolis, IN), according to the manufacturer’s instruction.

**Cell Size and Protein-to-DNA Ratio**

As a measure of a potential hypertrophic effect of ET-1, cell size was determined by fluorescence-activated cell sorting, as described (16). Cells were sorted by cell size: high forward scatter vs. side scatter. In addition, protein-to-DNA ratio in ET-1-stimulated cells was determined. HBSMC were grown in six-well plates and incubated for 72 or 144 h in ITS medium, with or without ET-1, OSM, and CT-1.

**[3H]Leucine Incorporation Assay**

Cells were grown in 24-well plates and then serum starved in F-12 medium supplemented with ITS. Medium was then replaced with fresh medium containing 1 \(\mu\)Ci/ml [3H]Leucine, together with drugs or vehicle. When inhibitors of signaling pathways were used, they were added 30 min before treatment with ET-1. After 48 h, cells were washed twice with PBS and incubated with 5% TCA for 20 min at 4\(^\circ\)C, washed again with 5% TCA, then harvested in 0.5 M NaOH containing 1% Triton X-100. Lysates were then added to five volumes of scintillation fluid and counted in a scintillation counter.

**ELISA for IL-6, IL-11, OSM, CT-1, and Leukemia Inhibitory Factor**

IL-6, IL-11, OSM, CT-1, and leukemia inhibitory factor were quantified using commercially available ELISA Kits from R&D Systems (Minneapolis, MN) using the protocols as suggested by the supplier.

**Immunoblotting**

**Contractile proteins.** As described previously (27), cellular proteins were resolved by SDS-PAGE, transferred to nitrocellulose membranes, and probed with mouse monoclonal antibody against \(\alpha\)-smooth muscle actin (SMA) (clone IA4, 1:1,000), calponin (clone hCP, 1:2,000), smooth muscle MHC (sm-MHC) (clone hSM-V, 1:500), and vimentin (clone V9, 1:1,000) (Sigma) overnight at 4\(^\circ\)C in Tris-buffered saline with Tween 20. Following successive washes, an anti-mouse or anti-rabbit horseradish peroxidase-conjugated secondary antibody (1:3,000) was added and incubated for 1 h at room temperature. This was followed by repeated washes in Tris-buffered saline with Tween 20. Blots were visualized by enhanced chemiluminescence (Amersham).

**JAK1, STAT-3, and ERK1/2 phosphorylation.** Cellular proteins were resolved by SDS-PAGE, transferred to polyvinylidene fluoride membranes, and probed with a cocktail of antibodies (monoclonal mouse antibodies against phosphorylated proteins and rabbit polyclonal antibodies against nonphosphorylated proteins). To control for equal protein loading, membranes were probed with anti-\(\beta\)-tubulin mouse monoclonal antibodies (Upstate, Lake Placid, NY). Detection was performed with IR700 and IR800 anti-mouse and anti-rabbit antibodies (Cell Signaling Technology) and the Odyssey Infrared Imaging System (LI-COR Biotechnology, Lincoln, NE) using a protocol as suggested by the supplier. Density of the bands was analyzed with Odyssey software 1.1 (LI-COR Biotechnology) using two infrared channels independently. The results are expressed as a phosphorylated protein-to-nonphosphorylated protein density ratio.

**Data Analysis**

Mean values were compared by one-way ANOVA or a repeated-measures ANOVA; Bonferroni’s correction for multiple comparisons was performed post hoc. A \(P\) value of <0.05 was considered statistically significant. Mean ± SE values are presented in the text and figures.

**RESULTS**

**ET-1 Inhibits Apoptosis of HBSMC Induced by Serum Withdrawal**

The control level of apoptosis induced by serum withdrawal at 72 h was 17.9 ± 5.1%. Treatment of cells with ET-1 dose dependently inhibited apoptosis of HBSMC with a maximal effect of 67.6 ± 10.1% reduction compared with control (Fig. 1A, \(P < 0.05\)). In comparison, the highest dose of CT-1 used (10 ng/ml) produced a 37 ± 5.9% reduction in apoptosis (Fig. 1B). Another gp130 cytokine, OSM, inhibited apoptosis to similar levels as ET-1 (Fig. 1B).

**ET-1 Induces Hypertrophy of HBSMC**

ET-1 significantly increased protein synthesis in HBSMC in a concentration-dependent manner (Fig. 2A), as assessed by [3H]leucine incorporation. The [3H]leucine incorporation into HBSMC increased to a maximum of 63% above control (Fig. 2A) after treatment with ET-1 at a concentration range from 0.1 nM and higher. Figure 2B shows that the ET-1-induced increase in [3H]leucine incorporation was not a result of enhanced proliferation of HBSMC. Protein-to-DNA ratios significantly increased after treatment with ET-1, reflecting increased protein synthesis.
ET-1 on protein-to-DNA ratio was comparable with OSM but was greater than that seen with CT-1 (Fig. 2B). Treatment with the selective ETA receptor antagonist BQ-123 (20 μM) markedly diminished the effects of ET-1 on protein synthesis (Fig. 2C). Blockade of ETB receptor by BQ-788 also significantly inhibited [3H]leucine incorporation in HBSMC induced by treatment with ET-1 (Fig. 2C). The additive effect of both ET receptor antagonists was not greater than that of either inhibitor alone (data not shown). In parallel with its effect on protein synthesis, ET-1 induced a significant change in cell morphology and cell size (Fig. 3). After 72-h stimulation with 10 nM of ET-1, HBSMC acquired a hypertrophic phenotype of enlarged cells under phase contrast (Fig. 3A, top) and immunofluorescence (Fig. 3A, bottom). This was confirmed by flow cytometric analysis of cell size (Fig. 3, B and C).

**ET-1 Treatment Increases Contractile Protein Expression**

The effects of 48 h serum deprivation followed by 48 h with and without ET-1 on the expression of contractile proteins in primary human ASM are summarized in Fig. 4. Exposure of HBSMC to ET-1 induced or increased the expression of sm-MHC, calponin, and α-SMA. In contrast, ET-1 treatment did not influence the expression of vimentin.

The Hypertrophic Effects of ET-1 Are Dependent on JAK1/STAT-3 and ERK Signaling

Treatment with ET-1 induced a small, transient inhibition of STAT-3 phosphorylation during 24 h of culture, which was followed by restoration of steady-state levels of STAT-3 acti-
vation (Fig. 5A), and after 48–72 h induced a more sustained STAT-3 phosphorylation, which was repeatedly observed in five independent experiments performed (Fig. 5A). The pSTAT-3-to-STAT-3 ratio was 48 ± 14% above control values at 48 h (P < 0.05) and 25 ± 6% at 72 h (P = 0.08). In contrast, exposure of HBSMC to OSM induced a robust phosphorylation of STAT-3 that was maximal at 10 min and remained elevated above baseline for the duration of exposure (Fig. 5B). Phosphorylation of ERK1/2 in response to ET-1 was rapid but transient, with a maximal activation at 10 min but returned to basal phosphorylation after 1–3 h (Fig. 5A), and it was comparable with that induced by OSM (Fig. 5B). Densitometry data from five independent experiments showed a significant increase in ERK1/2 phosphorylation after 10 min (fivefold) and 30 min (twofold) of ET-1 stimulation compared with non-stimulated control cells (Fig. 5A).

To demonstrate that both cell signaling pathways are involved in the observed effects of ET-1 on HBSMC hypertrophy, we used a pharmacological approach to inhibit JAK1/STAT-3 and MAPK/ERK activation using piceatannol and U0126, respectively. The specific MEK1/2 inhibitor U0126 (10) efficiently prevented ERK1/2 activation induced by ET-1.

Fig. 3. ET-1 induces cell hypertrophy in primary HBSMC. A: cell hypertrophy of HBSMC treated with 10 nM ET-1 for 72 h [top: phase contrast microscopy of cells in a primary culture, original magnification ×200; bottom: immunostaining for α-smooth muscle actin (α-SMA), α-SMA-indocarbocyanine (Cy3), and nuclei (Hoescht 33342), original magnification ×400]. B: HBSMC were treated with 10 nM ET-1, CT-1 (10 ng/ml), or OSM (2 ng/ml) for 144 h, and cell size differences were estimated by flow cytometry, as described in MATERIALS AND METHODS. Top: forward scatter (FS) histogram from one representative experiment. Bottom: changes in cell size after cytokine treatment, and the means ± SE of three independent experiments. *P < 0.05 vs. control. #P < 0.05 vs. CT-1. C: HBSMC size is increased after treatment with 10 nM ET-1 for 72 and 144 h, as assessed by flow cytometry. **P < 0.05 vs. ET-1-stimulated cells for 72 h.

Fig. 4. Effect of ET-1 on expression of proteins associated with hypertrophy and contractility. Immunoblots were performed on lysates of HBSMC stimulated with ET-1 (10 nM) for 48 h using specific antibody to smooth muscle myosin heavy chain (sm-MHC) (A), α-SMA (B), calponin (C), and vimentin (D), as described in MATERIALS AND METHODS.
but did not influence ET-1-induced JAK1 (Fig. 6A) at 30 or 60 min, although there was a blunted response at 10 min. In contrast, piceatannol (3 μg/ml) (8) strongly abrogated immediate JAK1 activation (Fig. 6A) induced by ET-1 and blunted STAT-3 phosphorylation induced by ET-1 or OSM at 48 and 72 h (Fig. 6B). U0126, while depressing ERK1/2 activation induced by ET-1 at earlier time points, also attenuated ET-1-induced STAT-3 inhibition (Fig. 6A). At the same time, piceatannol did not display any inhibitory effects on ERK1/2 phosphorylation induced by ET-1 (Fig. 6A, immunoblots).

These data suggest that both the MEK/ERK and JAK1/STAT-3 pathways are activated by ET-1 and may be involved in mediating the hypertrophic effects of ET-1 on HBSMC, although the activation of STAT-3 is delayed, and thus pathways other than STAT-3 that follow JAK1 activation may be responsible for the effects observed. Additionally, these data indicate that ET-1 actually can induce STAT-3 tyrosine phosphorylation at early time points, but the MEK/ERK pathway simultaneously activated by ET-1 inhibits this STAT-3 activation. We then exposed serum-starved HBSMC to U0126 (10 μM) and piceatannol (3 μg/ml) and subsequently stimulated cells with ET-1. The results of this experiment demonstrate that both inhibitors U0126 and piceatannol significantly inhibited [3H]leucine incorporation induced by ET-1 (Fig. 6C). Neither U0126 nor piceatannol added alone without ET-1 altered leucine incorporation over 48 h.

**ET-1 Induces Release of IL-6 From HBSMC**

Because delayed STAT-3 activation may play a role in mediating the hypertrophic effects of ET-1, we evaluated if the action of ET-1 involved members of the IL-6/gp130 cytokine family, which potently activate this transcription factor. To determine this, we measured levels of these cytokines in the cell culture supernatants following exposure of HBSMC to ET-1. Levels of OSM, CT-1, IL-11, and leukemia inhibitory factor were below detection limits of ELISAs, and CT-1 levels in the whole cell lysates were not influenced by ET-1 treatment. In contrast, exposure of HBSMC to ET-1 resulted in a
dramatic increase in the amount of IL-6 released during 48 h of culture (Fig. 7A). Subsequent experiments showed that ET-1 induced production of IL-6 in a dose-dependent manner (Fig. 7B). Importantly, ET$_A$ antagonist BQ-123 (20 μM) significantly reduced IL-6 release in response to ET-1, and ET$_B$ antagonist BQ-788 (20 μM) completely abolished the release of IL-6 induced by ET-1 (Fig. 7C). Having established that ET-1 induces IL-6 release from HBSMC, we next determined whether IL-6 was capable of inducing hypertrophy or influencing apoptosis in our HBSMC cultures. Stimulation of HBSMC with IL-6 did not significantly change the level of protein synthesis compared with ET-1 (Fig. 8A). Furthermore, while exposure to IL-6 inhibited serum withdrawal-induced apoptosis, the magnitude of this effect was considerably less than the anti-apoptotic effect of ET-1 (Fig. 8B).

**DISCUSSION**

Although ASM hypertrophy has been documented in asthma, little is known about the mediators and pathways regulating cell size or expression of contractile proteins. In this study, ET-1 was shown to be a potent inducer of hypertrophy and survival of human ASM cells and at the same time increased the contractile potential of these cells by increasing expression of sm-MHC, calponin, and α-SMA. The hypertrophic and anti-apoptotic effects of ET-1 were mediated by both ET$_A$ and ET$_B$ receptors and activation of the ERK1/2, JAK-1, and to a lesser extent STAT-3, pathways. The magnitude of the hypertrophic/anti-apoptotic effects approximated that of OSM, which is the most potent cytokine in this regard that we have tested to date. Despite the observation that ET-1 activated STAT-3 and induced the release of IL-6, we could find no evidence for an interaction between these two mediators. As an increase in both hypertrophy and survival will increase muscle mass (19), the results of this study have identified ET-1 as a potentially important mediator in the regulation of ASM mass in asthma.

Responses to ET-1 usually correlate with receptor density. Although ET$_A$ receptors appear to predominate on ASM, both receptor subtypes have been implicated in ET-1-induced responses. For example, ET$_A$ receptors appear to mediate the mitogenic and some of the synthetic responses of human ASM to this mediator, whereas ET$_B$ receptors mediate the contractile responses. We found that both receptor subtypes appear to mediate the hypertrophic effects to this mediator. No evidence for an additive effect of the receptor antagonists was seen, possibly because of the marked effect of BQ-123 alone; thus a small additive effect cannot be excluded.

Both STAT-3 and ERK kinase activation have been linked to hypertrophy, particularly involving cardiomyocytes (24, 25). Although we observed that ET-1 activated JAK1/STAT-3 and the MEK/ERK pathway, the patterns of activation were different. Phosphorylation of ERK was transient following exposure
to ET-1, peaking at 10 min and returning to basal levels around 1 h. It would seem that this level of ERK activation is required to induce hypertrophic effects, since pharmacological inhibition of this pathway significantly blunted the activity of ET-1.

In contrast, exposure to ET-1 induced a delayed effect on STAT-3 phosphorylation, with an initial rapid but minor inhibitory effect, followed by a second wave of phosphorylation that was maintained for up to 72 h. Booz and coworkers (3), using rat cardiomyocytes, showed somewhat similar findings to the current study in that ET-1 induced a biphasic effect on STAT-3 phosphorylation, an initial inhibitory effect that was followed by a rebound increase in phosphorylation after 90 min of exposure. However, the authors only commented on the initial inhibitory response, suggesting that ET-1 attenuated STAT-3 signaling. They did not speculate on the mechanisms behind the latter stimulatory effect. We also note that the degree of STAT-3 phosphorylation induced by ET-1 was small (1.5-fold) compared with the degree of activation induced by OSM.

The STAT-3 inhibitor (8) piceatannol is a kinase inhibitor with activity toward JAK1 and Syk/70-kDa zeta-associated protein (ZAP70) kinases, which leads to inhibition of STAT-3 phosphorylation induced by these pathways. It is thus possible that pathways other than STAT-3 are, in part, mediating the hypertrophic effect of ET-1 that is inhibited by piceatannol. Moreover, the delayed phosphorylation of STAT-3 is consistent with activation by a downstream intermediate released as a consequence of ET-1 exposure. Su and David (29) showed that ZAP70-deficient Jurkat cells, which do not express detectable levels of Syk kinase, were still able to support STAT-3 tyrosine phosphorylation, and piceatannol inhibition of this effect was observed. Thus piceatannol can inhibit STAT-3 activation independently of its effects on Syk/ZAP70.

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JAK 1 was rapidly activated by ET-1, and this activation coincided with inhibition of STAT-3 at early time points. It has been shown that members of the MAPK family, including ERK, elicit both positive and negative effects on JAK/STAT signaling, which are dependent on cells and receptors activated (30). We extended the data of Booz and coworkers (3), showing that ET-1-mediated inhibition of STAT-3 tyrosine phosphorylation at the initial exposure is MEK/ERK dependent. Pretreatment of HBSMC with the MEK1/2 inhibitor U0126 completely abrogated the early ET-1-induced inhibition of STAT-3 activation, and this effect correlated with the U0126-induced decrease in the level of ERK1/2 activation. Additionally, we observed a transient inhibitory effect of U0126 on JAK1 activation. The inhibitory effect of ERK is mainly associated with repression of JAK phosphorylation and consequent STAT inhibition; it has also been shown that ERK inhibits STAT-3 by direct association. Our findings that ET-1 induces STAT-3 activation, but ERK1/2, which is induced in parallel, represses this effect, suggest that STAT-3 regulation by ET-1 is a complex process. It has been demonstrated that Rho family GTPases are required for activation of JAK/STAT signaling by G protein-coupled receptors (21), and Rac1 may serve as an alternate mechanism for targeting STAT-3 to tyrosine kinase signaling complexes (27). It is noteworthy that Rac1 mediates STAT-3 activation via autocrine IL-6 release (9), and constitutively active Gα16 induce STAT-3 activation via c-Src/JAK- and ERK-dependent mechanisms (18). In the present study, we demonstrated that ET-1 induced IL-6 release in HBSMC, and this effect was completely abrogated by ET-1 receptor antagonists. Thus it is tempting to speculate that the delayed STAT-3 activation, which was correlated with a peak of IL-6 release in HBSMC induced by ET-1 after 48 h of culture, may be partly associated with Rac1 and Src family protein tyrosine kinase activation pathways, as described in the experimental models referenced above. This possibility needs to be addressed in future studies.

The major activators of STAT-3 are members of the gp130 cytokine family, which we and others have previously implicated in ASM hypertrophy. In the present study, we demonstrated that exogenously added OSM produced a comparable effect to ET-1 on HBSMC hypertrophy and strongly activated STAT-3 and MEK/ERK pathways, which were almost completely blocked by piceatannol and U0126, respectively. However, IL-6 was the only member of this cytokine family that we could detect following ET-1 exposure. Surprisingly, addition of the cytokine itself had no appreciable effect on HBSM size. Similarly, we have previously shown that IL-6 does not have a major anti-apoptotic effect on this cell type (35). These data contrast with previous studies that have shown that guinea pig tracheal smooth muscle cells undergo hypertrophy in response to IL-6. The reasons behind this discrepancy are unknown, but it is likely that species differences may be contributory. Other molecules, such as EGF and IL-10, also activate STAT-3, but these also had minimal effects on ASM size in our studies.

In summary, we have shown that ET-1 inhibits apoptosis, upregulates contractile protein expression, and increases the size and synthetic activity of human bronchial smooth muscle cells in primary cultures. These effects are mediated through both ETA and ETB receptors and involve the activation of JAK-1, downstream activation of the latent transcription factor STAT-3, and activation of the MEK/ERK pathway. Under continuous compressive stress, epithelial cells upregulate gene expression and secretion of ET-1 (32). Thus ET release from the airway epithelium may contribute to the increase in ASM mass via mechanical forces that accompany airway narrowing. Given that the frequency and magnitude of airway narrowing are greater in more severe or uncontrolled disease, this may be why hypertrophy has been associated with severe (2, 6), rather than mild (33), disease.

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