MAPK superfamily activation in human airway smooth muscle: mitogenesis requires prolonged p42/p44 activation

MICHAEL J. ORSINI,1 VERA P. KRYMSKAYA,2 ANDREW J. ESZTERHAS,2 JEFFREY L. BENOVIC,1 REYNOLD A. PANETTIERI, J.R.,2 AND RAYMOND B. PENN1
1Department of Microbiology and Immunology, Kimmel Cancer Institute, Thomas Jefferson University, Philadelphia 19107; and 2Division of Pulmonary and Critical Care, Department of Medicine, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania 19104

Orsini, Michael J., Vera P. Krymskaya, Andrew J. Eszterhas, Jeffrey L. Benovic, Reynold A. Panettieri, J. R., and Raymond B. Penn. MAPK superfamily activation in human airway smooth muscle: mitogenesis requires prolonged p42/p44 activation. Am. J. Physiol. 277 (Lung Cell. Mol. Physiol. 21): L479–L488, 1999.—Asthma is frequently associated with abnormal airway smooth muscle (ASM) growth that may contribute to airway narrowing and hyperresponsiveness to contractile agents. Although numerous hormones and cytokines have been shown to induce human ASM (HASM) proliferation, the cellular and molecular mechanisms underlying HASM hyperplasia are largely unknown. Here we characterize the roles of the mitogen-activated protein kinase (MAPK) superfamily [p42/p44 MAPK, c-jun amino-terminal kinase/stress-activated protein kinase (JNK/SAPK), and p38] in mediating hormone- and cytokine-induced HASM proliferation. Significant enhancement of [3H]thymidine incorporation in HASM cultures was observed on stimulation with interleukin (IL)-1β and tumor necrosis factor-α, agents that did not appreciably stimulate HASM proliferation. Two different inhibitors of MAPK/extracellular signal-regulated kinase (MEK), PD-98059 and U-0126, inhibited mitogen-induced [3H]thymidine incorporation in a manner consistent with their ability to inhibit p42/p44 activation. Elk-1 and activator protein-1 reporter activation by mitogens was similarly inhibited by inhibition of MEK, suggesting a linkage between p42/p44 activation, transcription factor activation, and HASM proliferation. These findings establish a fundamental role for p42/p44 activity in regulating HASM proliferation and provide insight into species-specific differences observed among studies in ASM mitogenesis.

asthma; mitogen-activated protein kinase; inflammation; G protein-coupled receptor; receptor tyrosine kinase

INCREASED AIRWAY SMOOTH MUSCLE (ASM) mass consequent to cellular hypertrophy or hyperplasia has long been implicated in the pathophysiology and pathogenesis of airway diseases such as asthma (14, 20). Although numerous factors are known to contribute to excessive airway narrowing, an increase in ASM mass has been asserted to be the most important abnormality responsible for the increased airway resistance observed in response to bronchoconstricting agents in both asthma and chronic obstructive pulmonary disease (16). Thus considerable interest lies in the characterization of stimuli and associated intracellular mechanisms that regulate ASM proliferation.

The mitogen-activated protein kinases (MAPKs) constitute a family of serine/threonine kinases that mediate the transduction of external stimuli (typically via receptor tyrosine kinase (RTK) or G protein-coupled receptor (GPCR) activation) into intracellular signals that regulate cell growth and differentiation. Among the best-characterized mammalian MAPKs are 1) the 42- and 44-kDa extracellular signal-regulated kinases (ERKs) ERK2 and ERK1, also collectively referred to as p42/p44 MAPK; 2) the c-jun amino-terminal kinase (JNK) or stress-activated protein kinase (SAPK); and 3) p38 MAPK. Each is activated by a signaling cascade composed of a series of sequentially activated protein kinases acting downstream from small G proteins representative of the Ras superfamily. These highly homologous MAPK cascades, strongly conserved through evolution, are subject to regulation at numerous junctures and exhibit significant cross talk among themselves and other pathways for the purpose of integrating various intercellular signals into discrete physiological responses (for reviews of MAPK signaling, see van Biessen et al. (33), Gutkind (9), and Hershenson et al. (11)).

To date, studies of ASM mitogenesis have focused primarily on the identification of agents capable of inducing ASM proliferation. Unfortunately, little is known regarding the intracellular signaling pathways in ASM involved in transducing signals from external mitogenic stimuli. Although a handful of studies have asserted the role of p42/p44 MAPK in promoting nonhuman ASM proliferation, similar studies in human ASM (HASM) are lacking. Moreover, the roles of other MAPK homologs (J NK/SAPK, p38) and other signaling intermediates associated with cell cycle regulation have remained largely uncharacterized in ASM.

In the present report, we characterize the relationship between HASM proliferation and activation of the p42/44, J NK/SAPK, and p38 MAPKs by numerous physiologically relevant agents. A fundamental role of p42/p44 activation in regulating HASM proliferation is revealed by data that demonstrate its requirement for and correlation with mitogen-activated growth. In addition, comparison of these data with those observed from other species suggests possible explanations for the species-specific differences among studies in ASM mito-
genesis and offers future directions for studies in this field.

METHODS

Materials. p20-5XGal4-Luc (35) and MLV.Gal4-EIk-1 (19) were provided by Channing Der (Univ. of North Carolina, Chapel Hill, NC). ΔFosbΔ6AP-1-Luc (8) was provided by Craig Hauser (The Burnham Institute, La Jolla, CA). [Methyl-3H]thymidine (1 µCi/ml) and enhanced chemiluminescence reagents were purchased from Amersham (Arlington Heights, IL). Phosphorylation state-specific and phosphorylation state-independent antibodies against p42/p44 MAPK, J NK/SAPK, and p38 were purchased from New England Biolabs (Beverly, MA). Luciferase assay reagent was purchased from Promega (Madison, WI). PD-98059 was purchased from Calbiochem (La Jolla, CA). U-0126 was a gift from DuPont Pharmaceuticals (Wilmington, DE). All other reagents were purchased from Sigma (St. Louis, MO) or from previously identified sources (21).

HASM cell culture. HASM cultures were established as described by Panettieri et al. (24) from human tracheae obtained from lung transplant donors in accordance with procedures approved by the University of Pennsylvania Committee on Studies Involving Human Beings. Briefly, a segment of trachea superior to the carina was dissected under sterile conditions, and the trachealis muscle was isolated. Approximately 1 g of wet tissue was obtained, minced, centrifuged, and resuspended in 10 ml of buffer containing 0.2 mM CaCl2, 640 U/ml of collagenase, 10 mg of soya bean trypsin inhibitor, and 10 U/ml of elastase. Tissue was digested for 90 min in a shaking water bath at 37°C. The cell suspension was filtered through 105-µm Nytex mesh, and the filtrate was washed with equal volumes of cold Ham's F-12 medium supplemented with 10% fetal bovine serum (FBS). Aliquots of cell suspension were plated at a density of 1.0 × 104 cells/cm2 in Ham's F-12 medium supplemented with 10% FBS, 100 U/ml of penicillin, 100 µg/ml of streptomycin, and 100 µg/ml of amphotericin B. Characterization of this cell line with regard to immunofluorescence of smooth muscle actin and agonist-induced changes in cytosolic calcium has been previously reported (24).

For experimentation, third- to fourth-passage cells were plated at a density of 1.0 × 104 cells/cm2 in Ham's F-12 medium supplemented with 10% FBS. Eight hours later, the medium was aspirated onto filter paper and counted in scintillation vials. Cells were pretreated for 30 min with either vehicle or 10 µM PD-98059, or 10 µM U-0126. Standard concentrations of agents, unless otherwise noted, were as follows: epidermal growth factor (EGF), 0.01–10 ng/ml; platelet-derived growth factor (PDGF)-BB, 10 ng/ml; thrombin, 1 U/ml; interleukin (IL)-1β, 20 U/ml; tumor necrosis factor (TNF)-α, 10 ng/ml; histamine, 10 µM; carbocyclic 1,10-methyl-3-acetate (PMA), 100 µM. After 16 h of stimulation, cells were labeled with 3.0 µCi [methyl-3H]thymidine (1 µCi/ml) and incubated at 37°C for 24 h. Cells were then washed with PBS, harvested with 0.05% trypsin-0.53 mM EDTA, and lysed with 20% trichloroacetic acid. The precipitate was aspirated onto filter paper and counted in scintillation vials. Data points from individual proliferation experiments represent the mean values derived from six wells.
RESULTS

Activation of p42/p44 MAPK and cell proliferation. HASM cells were cultured such that experiments examining the effects of various agents on both cell proliferation and MAPK superfamily activation could be performed simultaneously. Stimuli included activators of RTKs (EGF and PDGF) and GPCRs (thrombin, histamine, and carbachol), the cytokines IL-1β and TNF-α, and the protein kinase C activator PMA. As shown in Fig. 1A, EGF, PDGF, thrombin, and PMA strongly induced [3H]thymidine incorporation in HASM, whereas histamine, carbachol, IL-1β, and TNF-α produced little or no effect. We have previously demonstrated that EGF, thrombin, and PMA-induced increases in [3H]thymidine incorporation in HASM are associated with significant increases in cell number, whereas small increases in DNA synthesis elicited by numerous other agents are not associated with proliferation (4, 22, 23, 25). In parallel cultures, these agents were also tested for their capacity to activate p42/p44 MAPK in a time-dependent manner. EGF, PDGF, thrombin, and PMA induced a strong and sustained activation of p42/p44 MAPK for up to 3 h after cell stimulation (Fig. 1, B and C), after which activation declined toward basal levels by 12 h (see legend to Fig. 1 and below). In contrast, the cytokines IL-1β and TNF-α each activated p42/p44 MAPK more transiently, inducing maximal activation of p42/p44 at 30 min, with activated p42/p44 levels rapidly returning to basal levels thereafter. Histamine- and carbachol-stimulated p42/p44 activation was also transient (peak activation at 10 min) and decidedly less robust (approximately one-third of that induced by EGF at 10 min). When the integrated p42/p44 response was calculated over the first 3 h of stimulation, responses determined for EGF, thrombin, and PMA stimulation were significantly greater (2.5- to 10-fold) than those determined for IL-1β, TNF-α, histamine, and carbachol (Fig. 1D). This disparity in integrated activity was even greater (5- to 20-fold) when values were calculated from experiments examining p42/p44 activation from 0 to 12 h (data not shown). These data suggest that the ability of a given agonist to induce HASM cell proliferation is dependent on its capacity to provoke a strong and sustained activation of p42/p44 MAPK.

Activation of JNK/SAPK and p38 kinases. The activation of JNK and p38 kinases has been shown in several cell types to influence cell proliferation and differentiation (27). Examination of p38 (Fig. 2) and JNK/SAPK (Fig. 3) activation in HASM cultures revealed that IL-1β and TNF-α were the only agents capable of significantly activating these kinases. With respect to p38, EGF, thrombin, and histamine weakly and transiently activated p38 (up to 4-fold over basal levels over the first 30 min), whereas PMA and carbachol had virtually no effect (Fig. 2A). In separate experiments, PDGF also exhibited a weak and transient activation of p38 (-3-fold of basal at 30 min; data not shown). IL-1β and TNF-α activated p38 maximally at 10–30 min (both ~25-fold), with levels diminishing significantly by 3 h (Fig. 2B). As noted, however, IL-1β and TNF-α were not able to induce significant [3H]thymidine incorporation (Fig. 1A), suggesting that activation of the p38 kinase pathway does not significantly stimulate HASM proliferation.

The activation of JNK was examined in a similar manner. Because of cross-reactivity between the JNK and p42/p44 phospho-specific antibodies, we could not sufficiently resolve the p46 isoform of JNK from the strong phospho-p44 signal. Thus activation of the p54 isoform, which is also recognized by the phospho-specific antibody, was used to examine and quantify JNK activation. As with p38 activation, only IL-1β and TNF-α activated JNK to an appreciable extent (Fig. 3A). In contrast, EGF, PDGF, thrombin, histamine, and carbachol induced little or no activation of JNK (Fig. 3A and data not shown). The kinetics of JNK activation by IL-1β and TNF-α were similar to those of p38 activation, peaking at 30 min and returning toward basal levels between 1 and 3 h (Fig. 3, B and C). Extended time courses of up to 12 h did not reveal further activation of either p38 or JNK by any agonist (Fig. 3B and data not shown). These data suggest that JNK activation, like activation of p38, is not sufficient to promote HASM mitogenesis.

Effect of MEK1 and -2 inhibition on mitogenesis and p42/p44 activation. To further explore the relationship between p42/p44 MAPK activation and HASM proliferation, we tested the effects of PD-98059 and U-0126, both specific inhibitors of the p42/p44 activators MEK1 and MEK2 (1, 7). Thirty minutes of pretreatment with 30 µM PD-98059 or 10 µM U-0126 inhibited PMA-, thrombin-, EGF-, and PDGF-mediated activation of p42/p44 (Fig. 4A). Interestingly, U-0126 was significantly more effective than PD-98059 in inhibiting mitogen-induced phosphorylation of p42/p44. We attribute this effect largely to the disparity in apparent IC50 values between the two compounds (~1 µM and 10–50 µM for U-0126 and PD-98059, respectively) and to the limitation of solubility of PD-98059, which renders it difficult to achieve effective concentrations in intact cells. PD-98059 and U-0126 also had similar effects on mitogen-induced HASM proliferation, demonstrating inhibition of [3H]thymidine incorporation commensurate with their respective abilities to inhibit p42/p44 MAPK activation (Fig. 4B). Interestingly, 90% of EGF-stimulated [3H]thymidine incorporation could still be inhibited when U-0126 was added up to 6 h after stimulation with EGF (Fig. 4C). When added 12 h after stimulation, a significant mitogenic effect of EGF was retained, although U-0126 exposure throughout the remaining 28 h of the experiment was still able to inhibit ~50% of EGF-stimulated [3H]thymidine incorporation. These data suggest that late activation of p42/p44 influences the magnitude of EGF-stimulated DNA synthesis. Alternatively, the capacity of U-0126 to reduce p42/p44 activity significantly below basal levels may influence these results if a minimal level of p42/p44 activity is required during the period of [3H]thymidine incorporation.

Dose-dependent effect of EGF on HASM proliferation and p42/p44 activation. To further examine the relationship between the magnitude and duration of p42/
p44 activation and HASM proliferation, we assessed agonist-induced [3H]thymidine incorporation and the time-dependent activation of p42/p44 in parallel cultures stimulated by EGF in a dose-dependent manner. As shown in Fig. 5, A and B, stimulation with increasing concentrations of EGF (0.01–10 ng/ml) resulted in a progressive and sustained activation of p42/p44 (EC50 of the integrated response ~0.5 ng/ml). A concentration of 0.01 ng/ml of EGF produced a very weak activation of p42/p44. A significantly greater and more sustained
Activation of p42/p44 was observed after stimulation with 0.1 ng/ml of EGF. Progressively greater p42/p44 activation by 1.0 and 10 ng/ml of EGF was observed over the first 3 h, whereas at later time points, activation of p42/p44 was attenuated but relatively sustained. The dose-dependent effect of EGF on HASM [3H]thymidine incorporation was similar, displaying an EC50 of 0.5 ng/ml, consistent with previous observations (21). When values of [3H]thymidine incorporation are plotted as a function of the integrated p42/p44 activation, a relationship is revealed in which EGF-stimulated p42/p44 activation correlates (r = 0.96) with proliferation (data not shown).

Transcriptional activation of Elk-1 and activator protein-1. HASM cultures were transfected with luciferase reporter constructs responsive to intracellular p38 activation. A: HASM cultures were treated with 10 ng/ml of EGF, 1 U/ml of THR, 20 U/ml of IL-1β, 10 ng/ml of TNF-α, 10 µM HIST, 1 mM CARB, 100 µM PMA, or 10 µM anisomycin (ANI) for either 10 or 30 min. Lysates were harvested and analyzed by immunoblotting with use of a rabbit polyclonal antibody specific to phosphorylated form of p38. BAS, basal. B: time course of IL-1β- and TNF-α-stimulated p38 activation. C: activation of p38 by EGF, IL-1β, and TNF-α was quantitated by densitometric analysis of autoradiographs representing 0–180 min of activation by each agent. Data are means ± SE from 5 experiments.

Activation of p42/p44 was observed after stimulation with 0.1 ng/ml of EGF. Progressively greater p42/p44 activation by 1.0 and 10 ng/ml of EGF was observed over the first 3 h, whereas at later time points, activation of p42/p44 was attenuated but relatively sustained. The dose-dependent effect of EGF on HASM [3H]thymidine incorporation was similar, displaying an EC50 of ~0.5 ng/ml, consistent with previous observations (21). When values of [3H]thymidine incorporation are plotted as a function of the integrated p42/p44 activation, a relationship is revealed in which EGF-stimulated p42/p44 activation correlates (r = 0.96) with proliferation (data not shown).

Transcriptional activation of Elk-1 and activator protein-1. HASM cultures were transfected with luciferase reporter constructs responsive to intracellular p38 activation. A: HASM cultures were treated with indicated agents as described in Fig. 2. Lysates were harvested and analyzed by immunoblotting with use of a rabbit polyclonal antibody specific to phosphorylated form of p38. BAS, basal. B: time course of IL-1β- and TNF-α-stimulated p38 activation. C: activation of p38 by EGF, IL-1β, and TNF-α was quantitated by densitometric analysis of autoradiographs representing 0–180 min of activation by each agent. Data are means ± SE from 5 experiments.
Phosphorylation/activation of the transcription factors Elk-1 and activator protein (AP)-1. As shown in Fig. 6, each of the mitogens, EGF, PDGF, thrombin, and PMA, stimulated Elk-1 and AP-1 activation approximately two- to fourfold that of basal activity. None of the other agents tested (histamine, carbachol, IL-1β, and TNF-α) appreciably induced Elk-1 luciferase activity, although stimulation with histamine and IL-1β did significantly increase AP-1 luciferase activity to ~1.5-fold of basal levels (data not shown). Pretreatment with 10 µM U-0126 before stimulation with mitogens essentially eliminated the mitogen-induced increases in both Elk-1 and AP-1 luciferase activity. These findings suggest a requirement for p42/p44 activation for Elk-1 and AP-1 activation in HASM as well as linkage between p42/p44 MAPK activation, Elk-1/AP-1 activation, and HASM proliferation.

**DISCUSSION**

In this study we demonstrate that potent HASM mitogens such as EGF, PDGF, thrombin, and phorbol esters produce a strong and sustained activation of p42/p44 MAPK. In contrast, agents that do not significantly promote HASM proliferation, such as histamine, carbachol, IL-1β, and TNF-α, produced only a transient...

Fig. 4. Effect of PD-98059 and U-0126 on p42/p44 activation and HASM mitogenesis. Growth-arrested HASM cells were pretreated with either vehicle (0.1% dimethyl sulfoxide; CON), 30 µM PD-98059, or 10 µM U-0126 for 30 min, followed by stimulation with 10 ng/ml of EGF, 1 U/ml of THR, 100 nM PMA, or 10 ng/ml of PDGF. Values for p42/p44 activation (A) and [3H]thymidine incorporation (B) were determined as described in Fig. 1. Data are means ± SE from 4–6 experiments for CON and PD-98059 group values and 3 experiments for U-0126 group values. For A and B: *P < 0.05 (t-test for paired samples), CON vs. PD-98059- and CON vs. U-0126-pretreated groups. C: cells were treated with vehicle or 10 µM U-0126 at indicated times before and after stimulation by 10 ng/ml of EGF. [3H]thymidine incorporation was subsequently determined as described in METHODS. Data are means ± SE from 4 experiments.
increase in p42/p44 MAPK activation. PD-98059 and U-0126, both specific inhibitors of p42/p44 MAPK activation, inhibited mitogen-induced [3H]thymidine incorporation in a manner consistent with their ability to inhibit p42/p44 activation. The apparent requirement of sustained p42/p44 MAPK activation for promoting HASM mitogenesis is further suggested by studies examining the dose-dependent effects of EGF. Low

**Fig. 5.** Relationship between EGF-mediated p42/p44 activation and mitogenesis in HASM. A: autoradiographs depicting dose-dependent effect of EGF (0.01–10 ng/ml) on p42/p44 activation. B: time course of EGF-stimulated p42/p44 activation. Data are means ± SE from 6 experiments.

**Fig. 6.** Elk-1 and activator protein (AP)-1 activation in HASM. HASM cultures were transfected as previously described (see Ref. 28) with either MLV.Gal4-Elk-1 and p20-5XGal4-Luc (A) or ΔFosΔE6AP-1-Luc (B). Twelve hours after transfection, cells were passaged into 12-well dishes at a density of 2.0 × 10⁴ cells/cm² in Ham's F-12 medium supplemented with 10% fetal bovine serum. Eight hours later, medium was switched to insulin-transferrin medium, and cells were maintained for 48 h. Cells were then pretreated with either vehicle (0.1% dimethyl sulfoxide; CON) or 10 µM U-0126 for 30 min and then stimulated for 15 h with indicated agonists. Triton-soluble extracts were prepared and assayed for luciferase activity as described in METHODS. Mean ± SE EGF-stimulated luciferase activity was 17.4 ± 4.4 × 10³ relative light units (RLU)·well⁻¹·s⁻¹ for cells transfected with MLV.Gal4-Elk-1/p20-5XGal4-Luc and 160 ± 64 × 10³ RLU·well⁻¹·s⁻¹ for cells transfected with ΔFosΔE6AP-1-Luc. Mean ± SE values depicted were normalized to basal (vehicle-stimulated) luciferase activity (n = 4). *P < 0.05 (t-test for paired samples) for U-0126- vs. CON-pretreated cells.
The activation of MAPKs in human airway smooth muscle (HASM) is crucial for understanding their regulatory roles in proliferation. Agents with respect to their induction of proliferation have been studied extensively. Thrombin, and TNF-α (32) identified JNK activation by endothelin, thrombin, and TNF-α in rat ASM, and Pyne et al. (31) observed JNK activation in guinea pig ASM treated with sphingosine and ceramides. Larsen et al. (17) identified p38 activity in canine ASM stimulated with carbachol, and Pyne and Pyne (29) found evidence of PDGF-stimulated p38 activity in guinea pig ASM. However, the sum of information to date has provided little insight into the roles that JNK/SAPK or p38 might play in regulating ASM mitogenesis. Studies in other cell types have attributed growth (2, 5, 15, 40), but more frequently anti-proliferative or apoptotic effects (3, 36, 38, 39), to JNK/SAPK and p38. Although our findings suggest that activation of p38 or JNK/SAPK is not sufficient to stimulate growth, an inhibitory or facilitatory role of these MAPKs in HASM proliferation cannot be excluded. Of note, pretreatment of HASM cells with the p38 inhibitor SB-203580 (6) did result in a small (~20–30%) but significant increase in both basal and mitogen-induced [3H]thymidine incorporation (data not shown). However, this effect was partially mimicked by the analogous control compound SB-202474, which lacks the ability to inhibit p38. Thus any effect of p38 on growth in HASM would appear to be minimal, and further insight into the roles of JNK/SAPK and p38 in modulating HASM mitogenesis will likely depend on future development of specific pharmacological inhibitors or overexpression/dominant-negative strategies, the application of which in ASM cultures to date has been problematic (28).

Although p42/p44 activation appears required for HASM proliferation and modulation of its activity by a given mitogen appears to correlate with growth, the level of p42/p44 activation observed among the different HASM mitogens is not a precise predictor of an agent’s relative mitogenicity. For example, in the present study, thrombin exhibited one of the strongest effects in stimulating [3H]thymidine incorporation but was the least efficacious among the mitogens in activating p42/p44. Conversely, EGF exhibited the most robust activation of p42/p44 but stimulated [3H]thymidine incorporation to a lesser extent than did thrombin and PDGF. This disparity between the rank order of agents with respect to their induction of proliferation and p42/p44 activation suggests that other signaling pathways are important in modulating HASM growth.

Interestingly, the profile of agents that promote proliferation of HASM differs significantly from that observed for nonhuman ASM. EGF, a potent mitogen in HASM, is a weak mitogen in bovine ASM (13). Conversely, insulin-derived growth factor I is mitogenic in bovine ASM but is a relatively weak mitogen in HASM (Panettieri, unpublished observations). IL-1β and IL-6 can be shown to induce guinea pig ASM proliferation through secondary activation of the PDGF receptor (29), although this does not appear to occur in HASM. Histamine appears to induce a greater mitogenic response in canine ASM (26) than in HASM. In addition, histamine has been shown to inhibit both GPCR- and RTK-activated mitogenesis in bovine ASM (10) in contrast to preliminary data from our laboratory that suggest that histamine potentiates EGF-stimulated mitogenesis in HASM (Penn, unpublished data).

What accounts for the differences in the capacity of various agents to induce proliferation among different species of ASM? As mentioned, data from the present study suggest that sustained p42/p44 activation is required for significant enhancement of HASM proliferation, a finding consistent with previous studies of bovine (12, 13, 18), rat (32, 37), and guinea pig ASM (30). For example, in bovine ASM, activation of p42/p44 by PDGF and insulin-like growth factor I is strong and sustained (12, 13) compared with that of EGF, which is relatively weak and transient (13) (similar to that observed for histamine- and carbachol-stimulated p42/p44 in HASM). Thus the cumulative evidence leads us to suggest (as previously postulated by others (11) that species-specific responses to potential mitogens may not reflect differences in the fundamental mitogenic-signaling pathway mechanisms. Instead, a more likely explanation may be that differences among species with respect to expression level, coupling, and/or regulation of various cell surface receptors dictate the relative mitogenic effect of a given agent. In HASM, high levels of EGF receptor expression are suggested by the low EC₅₀ values (0.5 ng/ml) for both EGF-stimulated p42/p44 activation and proliferation and by the observation that EGF receptor autophosphorylation can be significantly inhibited without diminishing either p42/p44 activation or the mitogenic response to 10 ng/ml of EGF (i.e., evidence of spare receptors) (Krymskaya, Panettieri, and Penn, unpublished observations). Conversely, EGF receptors in bovine ASM may have significantly lower expression or be poorly coupled as suggested by the weak and transient p42/p44 response to EGF stimulation and the relatively higher EC₅₀ (~1.0 ng/ml) for EGF-stimulated [3H]thymidine incorporation (13). The effect of histamine as an inhibitor of RTK-mediated mitogenesis in bovine ASM can be explained by differential expression of histamine receptors linked to different heterotrimeric G protein-signaling pathways. In bovine ASM, activation of H₁ histamine receptors coupled to G₅ protein, cAMP production, and protein kinase A activation mediates the inhibition of HASM proliferation via inhibition of...
raf-1 (10). In HASM, histamine does not appreciably stimulate CAMP production (Penn and Benovic, unpublished observations), suggesting that histamine effects in HASM are mediated primarily through the activation of the H1-histamine receptor coupled to G蛋白质. Thus, in two specific instances, the expression or complement of receptors expressed in a given ASM culture appears important in mediating the mitogenic response to agonists. A more extensive characterization of RTK and GPCR signaling in ASM should help to elucidate the mechanisms underlying species-specific differences in the response to mitogens.

In summary, the present study establishes a fundamental role of p42/p44 MAPK in regulating HASM proliferation and suggests that activation of JNK/SAPK and p38 pathways has little effect on HASM growth. Small differences in the relationship between p42/p44 activation and HASM proliferation among mitogens suggest that input from other mitogenic pathways serves to modulate the proliferative response to different agents. Future studies examining the interplay between MAPK and other putative mitogenic pathways (34) will help to establish further the Cellular play between MAPK and other putative mitogenic pathways serves to modulate the proliferative response to mitogens.

We thank Kristin Brodebeck and Sybil Kane for technical assistance, Channing Der and Craig Hauser for providing reporter constructs, and James Trzaskos for advice concerning the use of U-0126.

This work was supported in part by National Heart, Lung, and Blood Institute Grants HL-58906 and HL-55301 and National Institute of General Medical Sciences Grant GM-44994.

L. Benovic is a recipient of the American Heart Association Established Investigator Award. R. A. Panettieri, Jr., is a recipient of the Career Investigator Award from the American Lung Association.

Address for reprint requests and other correspondence: R. B. Penn, Kimmel Cancer Institute, Thomas Jefferson Univ., Rm. 930 Bluemle Life Sciences Bldg., 233 S. 10th St., Philadelphia, PA 19107 (E-mail: rpen@acji.tju.edu).

Received 15 December 1998; accepted in final form 14 April 1999.

REFERENCES


