Cross-talk between Bradykinin and Epidermal Growth Factor in Regulating IL-6 Production in Human Airway Smooth Muscle Cells

Po-Hao Feng, MD; Te-Chih Hsiung[1], MD; Han-Pin Kuo, MD, PhD; Chien-Da Huang, MD

Background: Bradykinin (BK), a G-protein-coupled-receptor (GPCR) agonist via the B2 receptor induces interleukin (IL)-6 expression in airway smooth muscle (ASM) cells by involving the extracellular signal-regulated kinase 1/2 (ERK1/2) signaling pathway. In some cell species, GPCR agonists have been shown to activate the ERK 1/2 pathway via transactivation of epidermal growth factor (EGF) receptor (EGFR). In this study, we tested whether there is cross-talk between BK and EGF in the regulation of IL-6 gene expression in ASM cells.

Methods: ASM cells were treated with BK, EGF, AG-1478 and genistein. IL-6 production was analyzed by enzyme-linked immunosorbent assay (ELISA). Immunoblot study was used for detection of ERK1/2 activation. Transactivation of EGFR phosphorylation was detected by immunoprecipitation.

Results: ELISA showed that EGF (10 ng/ml, 18 hr) increased IL-6 secretion (from 234 ± 35 to 923 ± 494 pg/ml, n = 5, $p > 0.05$), and significantly enhanced BK-induced IL-6 secretion (from 4383 ± 296 to 8312 ± 1267 pg/ml, n = 5, $p < 0.05$) in ASM. Moreover, AG-1478 (2 μM), reduced BK-induced IL-6 secretion by 28% and abrogated the synergic induction of IL-6 induced by BK plus EGF (from 8312 ± 1267 to 3229 ± 597 pg/ml, n = 5, $p < 0.05$). AG-1478 dual effects on IL-6 secretion induced by BK alone or BK plus EGF were also observed in cells treated with genistein, a tyrosine kinase inhibitor, and AG-825, an ErbB-2 inhibitor. Immunoblot analysis demonstrated that AG-1478 had no effect on ERK1/2 activation by BK (1 μM, 10 min). Immunoprecipitation studies showed that BK (1 μM for 2, 5 and 10 min) did not directly transactivate EGFR phosphorylation.

Conclusion: These data show that BK and EGF act in concert to regulate the expression of IL-6 in ASM cells possibly via transcriptional mechanisms involving EGFR-associated key signaling molecules.

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Key words: bradykinin, EGF, EGF receptor, IL-6, airway smooth muscle

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Bradykinin (BK), an inflammatory nonapeptide generated from kininogens, regulates a variety of biological responses in airways, including microvascular leakage, smooth muscle contraction and mucus secretion.[1] Most biological effects of BK are mediated by B_2 receptors, a kind of G protein-coupled receptor (GPCR), subsequently activating a variety of downstream signaling which include activating phospholipases,[2] increasing cyclic adenosine monophosphate by activating cyclooxygenase -2 expression[3] and even activating extracellular signal-regulated protein kinase 1/2 (ERK 1/2) and mitogen-activated protein kinase (MAPK) pathways.[4] Previous reports also showed that the downstream signaling of BK, via the B2 receptor, induces interleukin (IL)-6 expression in human airway smooth muscle (ASM) cells by involving the ERK1/2 signaling pathway.[5]

ASM hypertrophy and hyperplasia as well as epithelial damage are major pathophysiologic features of airway remodeling in chronic asthma. Restitution of a damaged epithelium is modulated by growth factors and the extracellular matrix.[6] In considering ASM hypertrophy and hyperplasia, evidence shows that GPCR agonists have mitogenic effects in cultured airway smooth muscle cells and augment growth factor-induced proliferation.[7] Among the growth factors, epidermal growth factor (EGF) plays an important role in morphogenesis and repair. The clear immunoreactivities of EGF receptors (EFGR) observed on bronchial epithelium, submucosal glands, airway smooth muscle, and the basement membrane of asthmatic airways suggest a possible contribution of EGF to the pathophysiology of bronchial asthma with airway remodeling.[8]

The synergic effects of GPCRs and receptor tyrosine kinases on human ASM proliferation has been demonstrated.[9] However, whether there is cross-talk between BK and EGF in the regulation of synthetic gene expression, such as IL-6, in ASM cells had not been mentioned before. In this study, we investigated whether there is cross-talk between BK and EGF in the regulation of IL-6 gene expression in ASM cells.

METHODS

Human ASM cell culture and characterization

Human trachea was obtained from lung transplant donors, in accordance with procedures approved by the University of Pennsylvania Committee on Studies Involving Human Beings. A segment of trachea just proximal to the carina was removed under sterile conditions and the tracheal muscle was isolated. The muscle was then centrifuged and resuspended in 10 ml of buffer containing 0.2 mM $CaCl_2$, 640 U/ml collagenase, 1 mg/ml soybean trypsin inhibitor and 10 U/ml elastase. Enzymatic dissociation of the tissue was performed for 90 min in a shaking water bath at 37°C. The cell suspension was filtered through 105 μm Nitex mesh (Tetco, Depew, NY), and the filtrate was washed with equal volumes of cold Ham's F12 medium (Gibco BRL Life Technologies, Grand Island, NY, U.S.A.) supplemented with 10% FBS (HyClone, Logan, UT) 100 U/ml penicillin (Gibco), 0.1 mg/ml streptomycin (Gibco), and 2.5 μg/ml fungizone (Gibco). Aliquots of the cell suspension were plated at a density of 1.0×10^4 cells/cm^2. The cells were cultured in Ham's F12 media supplemented with 10% FBS, 100 U/ml penicillin, and 0.1 mg/ml streptomycin and this was replaced every 72 h. Human ASM cells in subculture during the second through the fifth cell passages were used, because during these cell passages, the cells retain native contractile protein expression, as demonstrated by immunocytochemical staining for smooth muscle actin and myosin.[10] AG-1478, genistein, and AG-825 were purchased from Calbiochem (San Diego, CA, U.S.A.). Unless otherwise specified, all chemicals used in this study were purchased from Sigma/Aldrich (St. Louis, MO, U.S.A.).

Measurement of IL-6 secretion by ASM cells

Confluent ASM cells were growth-arrested by incubating the monolayers in Ham's F12 with 0.1% bovine serum albumin (BSA) for 24 h. The growth-arrested human ASM cells were treated with 1 μM BK, 10 ng/ml EGF, and both BK and EGF in the presence or the absence of AG-1478 (2 μM) genistein (20 μM) and AG-825 (5 μM), (Calbiochem), added 30 min before. After 18 h, the concentrations of IL-6 in the culture medium were determined by enzyme-linked immunosorbent assay (ELISA) (R & D Systems, Minneapolis, MN, U.S.A.) as described previously.[11] When organic vehicles were used to dissolve inhibitors (e.g. dimethyl sulfoxide), control cells were treated with similar concentrations

of the vehicle alone.

Sodium Dodecyl Sulfate-Polyacrylamide (SDS) Gel Electrophoresis and Western Blot Analysis of ERK 1/2 (p42/p44) MAPK Phosphorylation

Human ASM cells were pretreated with 2 μM AG-1478 for 30 minutes, then treated with BK 1 μM thrombin 1 U/mol or EGF 10 ng/ml for another 10 minutes. Human ASM cells were washed with cold phosphate-buffered saline and resuspended in lysis buffer containing 10 mM Tris-HCl, pH 7.4, 0.5% sodium deoxycholate, 1 mM EDTA, 0.5% Nonidet P-40, 1 mM phenylmethylsulfonyl fluoride, 1 mM Na_3VO_4, and 10 μg/ml aprotinin and leupeptin. Proteins were analyzed on a 12.5% SDS-polyacrylamide gel electrophoresis and blotted onto a nitrocellulose membrane. The membranes were blocked in 3% BSA in Tris-buffered saline then incubated with a rabbit monoclonal IgG against the phosphorylated form of p42/44 (Cell Signaling, Beverly, MA, U.S.A.). After incubation with the appropriate peroxidase-conjugated secondary antibody (Roche Molecular Biochemicals, Minneapolis, MN, U.S.A.), the bands were visualized by an enhanced chemiluminescence system (Amersham Pharmacia Biotech, Piscataway, NJ, U.S.A.) and autoradiographed. To ensure equal loading, the membranes were stripped and reprobed with anti-ERK 1/2 antibodies.

Immunopreciptation studies for EGFR

Immunoprecipitation using sheep polyclonal IgG against EGFR (Upstate, Lake Placid, NY, U.S.A.) was performed as indicated by the manufacturer's instructions. Equal amounts of proteins were analyzed on 4-12% SDS-polyacrylamide gel electrophoresis and blotted onto a nitrocellulose membrane. The membranes were blocked in 5% BSA in Tris-buffered saline, then incubated with a mouse monoclonal IgG against the phosphorylated form of EGFR (Upstate). After incubation with the appropriate peroxidase-conjugated secondary antibody (Roche Molecular Biochemicals), the bands were visualized by an enhanced chemiluminescence system (Amersham Pharmacia Biotech), and autoradiographed.

Statistics

Data are expressed as mean ± SD. One-way analysis of variance (ANOVA) was used to compare mean values of more than two experimental groups. If variance among groups was noted, a Bonferroni test was used to examine significant differences between groups. Some data were also analyzed by Student's *t*-test for paired or unpaired data. A *p*-value of less than 0.05 is considered statistically significant.

RESULTS

EGF (10 ng/ml, 18 hr) increased IL-6 secretion (from 234 ± 35 to 923 ± 494 pg/ml, n = 5, $p > 0.05$), and significantly enhanced BK-induced IL-6 secretion (from 4383 ± 296 to 8312 ± 1267 pg/ml, n = 5, $p < 0.05$) in ASM cells. Moreover, AG-1478 (2 μM), a specific EGFR inhibitor, reduced BK-induced IL-6 secretion by 28% and abrogated the synergic induction of IL-6 induced by BK plus EGF (from 8312 ± 1267 to 3229 ± 597 pg/ml, n = 5, $p < 0.05$) (Fig. 1).

AG-1478 dual effects on IL-6 secretion induced by BK alone or BK plus EGF were also observed in cells treated with both genistein and AG-825. Both genistein (20 μM) and AG-825 (5 μM) reduced BK-

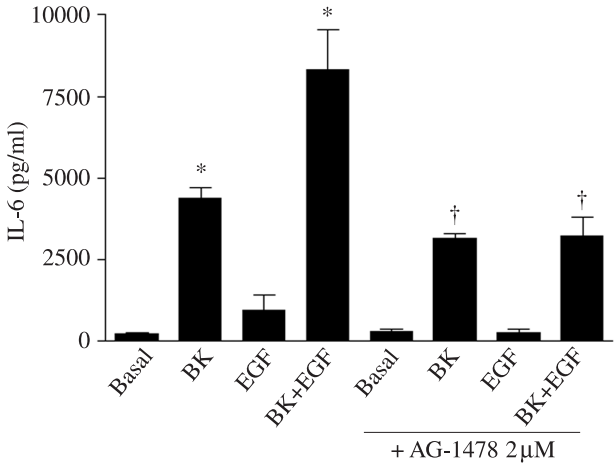

Fig. 1 EGF significantly enhanced BK-induced IL-6 secretion in ASM cells. AG-1478, a specific EGFR inhibitor, reduced BK-induced IL-6 secretion and abrogated the synergic induction of IL-6 induced by BK plus EGF. ASM cells were stimulated with 1 μM BK, 10 ng/ml EGF, and both BK and EGF for 18 hrs in the presence or absence of 2 μM AG-1478 added 30 min before. IL-6 secretion in the supernatant was then assessed by ELISA. Values shown are mean ± SD, (n = 5). *: $p < 0.05$ compared to untreated cells and †: $p < 0.05$ compared to cells with the corresponding conditions.

induced IL-6 secretion (genistein, from 3661 ± 50 to 1728 ± 45 pg/ml; AG-825, from 3661 ± 50 to 2439 ± 81 pg/ml, n = 2, $p < 0.05$ in each group) and the synergy of IL-6 secretion induced by BK and EGF (genistein, from 7375 ± 303 to 2861 ± 313 pg/ml; AG-825, from 7375 ± 303 to 4439 ± 151 pg/ml, n = 2, $p < 0.05$ in each group) after 18 hrs of ASM culture (Fig. 2).

To test whether tyrosine kinase inhibitors affect BK-induced IL-6 secretion, we pretreated with different concentrations of AG-1478 ASM cells for 30 min. Subsequently, ASM cells were treated with 1 μM BK for 18 h and IL-6 secretion was measured. The results showed that AG-1478 dose-dependently inhibited BK-induced IL-6 secretion (from 3483 ± 95 to 2436 ± 170 with 1 μM AG-1478, and to 2145 ± 65 with 5 μM AG-1478, n = 3, $p < 0.05$ in each group) (Fig. 3).

We performed immunoblot analysis to test whether the ERK1/2 pathway induced by BK or EGF is suppressed by AG-1478. ASM cells were pretreated with 2 μM AG-1478 for 30 minutes, then treated with BK 1 μM, thrombin 1 U/ml or EGF 10 ng/ml for another 10 minutes. Since AG-1478 is an inhibitor of EGFR tyroine kinase, immunoblot analysis demonstrated that the ERK1/2 pathway activated by EGF was inhibited by AG-1478. However, 2 μM AG-1478 had no effect on ERK1/2 activation by BK (1 μM, 10 min) (Fig. 4).

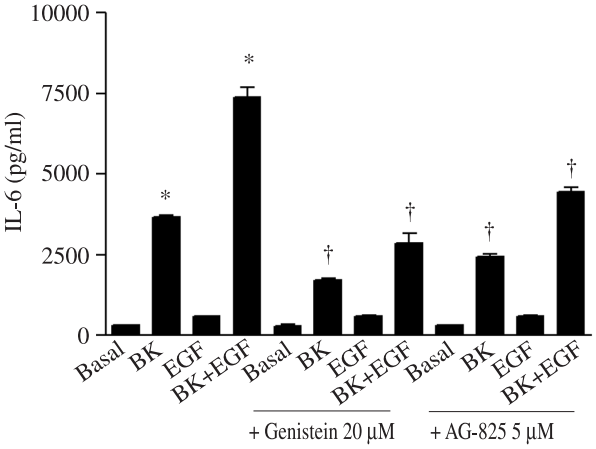

Fig. 2 IL-6 secretion induced by BK alone or BK plus EGF was also observed in cells treated with genistein, a tyrosine kinase inhibitor, or AG-825, an ErbB-2 inhibitor. ASM cells were stimulated with 1 μM BK, 10 ng/ml EGF, and both BK and EGF for 18 hrs in the presence or the absence of genistein (20 μM) and AG-825 (5 μM) added 30 min before. IL-6 secretion in the supernatant was then assessed by ELISA. Values shown are mean ± SD, (n = 2). *: $p < 0.05$ compared to untreated cells and †: $p < 0.05$ compared to cells with the corresponding conditions.

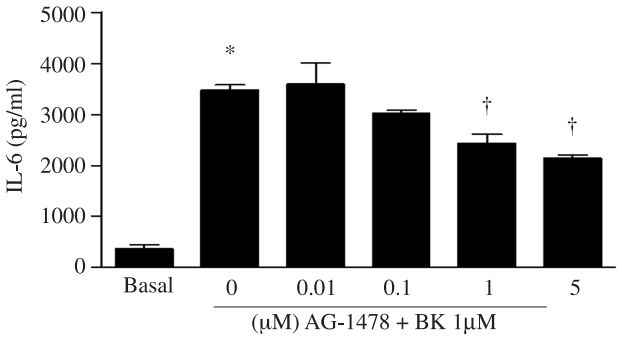

Fig. 3 AG-1478 dose-dependently inhibited BK-induced IL-6 secretion. ASM cells were stimulated with 1 μM BK for 18 hrs in the presence of different concentrations of AG-1478 added 30 min before. IL-6 was measured by ELISA. Values shown are mean ± SD, (n = 3), *: $p < 0.05$ significant from untreated cells and †: $p < 0.05$ significant compared with cells treated with 1 μM BK.

GPCR agonists, such as thrombin and lysophosphotidic acid (LPA), activate ERK 1/2 pathways via the transactivation of EGFRs in a variety of cell species.[12] To investigate the possibility of direct tansactivation of EGFR phosphorylation by BK, immunoprecipitation studies were performed. BK (1 μM for 2, 5 and 10 min) did not directly transactivate EGFR phosphorylation (Fig. 5).

DISCUSSION

Our results showed that BK and EGF act in concert to regulate the expression of IL-6 in ASM cells. AG-1478, a specific EGFR inhibitor, reduced BK-induced IL-6 secretion and abrogated the synergic induction of IL-6 induced by BK plus EGF. These dual effects were also observed in cells treated with genistein, a tyrosine kinase inhibitor, and AG-825, an ErbB-2 inhibitor. AG-1478 dose-dependently inhibited BK-induced IL-6 secretion. However, AG-1478 had no effect on ERK1/2 activation by BK, and BK did not directly transactivate EGFR phosphorylation.

BK, an inflammatory nonapeptide, plays an important role in regulating airway inflammation. In human ASM cells, BK, via the B2 receptor, induces IL-6 expression by involving the ERK1/2 and p38

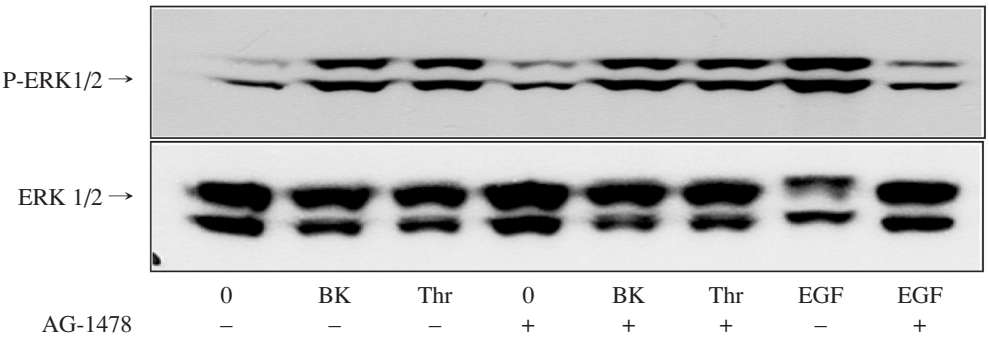

Fig. 4 AG-1478 had no effect on ERK1/2 activation by BK. The ASM cells were pretreated with 2 μM AG-1478 for 30 minutes, and then BK 1 μM, Thr 1 U/ml or EGF 10 ng/ml was added for 10 minutes. Immunoblot analysis of ERK1/2 is described in the Methods section.

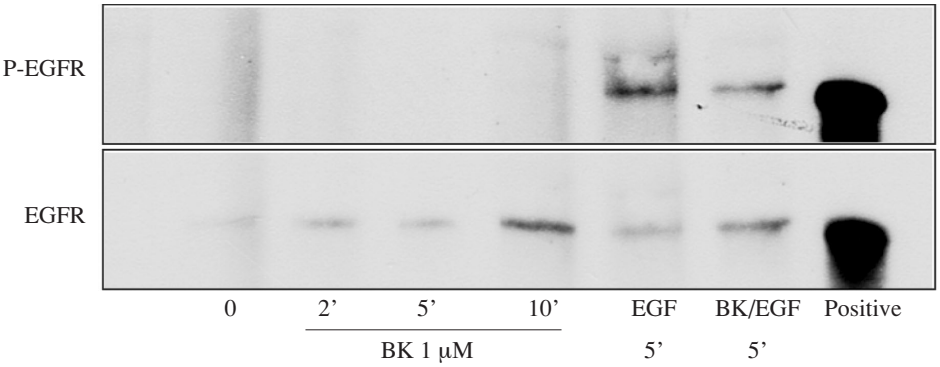

Fig. 5 BK did not directly transactivate EGFR phosphorylation. ASM cells were stimulated with 1 μM BK for 2, 5, or 10 minutes. EGFR and EGFR phosphorylation were analyzed by immunoprecipitation and immunoblot. ASM cells were stimulated with 10 ng/ml EGF alone and EGF plus BK for 5 minutes as control groups.

MAPK signaling pathways.[5] IL-6 has a pleiotropic effect in B-cell differentiation, T-cell activation and acute-phase protein induction.[13] Circulating IL-6 levels significantly increase in asthmatic patients after inhalation of allergens.[14] ASM cells treated with cytokines secrete IL-6.[15-17] Evidence also shows that activation of ERK 1/2 and p38 MAPK regulates expression of proinflammatory genes in ASM cells, including IL-6, in response to growth factors such as EGF.[18]

The synergic effect of BK and EGF in IL-6 secretion is regulated by EGFR tyrosine kinase, since AG-1478, a specific EGFR inhibitor, abrogates the synergic induction of IL-6 induced by BK plus EGF. One potential mechanism is the enhanced activation of the EGFR by GPCR-mediated transactivation. Daub and coworkers have shown that in Rat-1 fibroblasts and COS-7 cells, GPCR activation can induce the transphosphorylation of the EGFR.[12,19] The ability of bradykinin B_2 receptors to induce tyrosine phosphorylation of EGFR has been described in COS-7 cells transiently transfected with bradykinin B_2 receptor. In these cells, BK used two independent pathways to activate ERK1/2: a protein kinase C (PKC) -dependent pathway and EGFR transactivation, although the mechanism of the latter was not elucidated.[20] BK-induced trans-inactivation of EGFR by stimulation of protein-tyrosine phosphatase was shown in epidermoid carcinoma A431 cells. Interestingly, in these cells, BK simultaneously activated ERK1/2 independent of EGFR.[21] Recent accumulating evidence has also suggested that a disintegrin and metalloproteases (ADAMs) are the key metalloproteases activated by several GPCR agonists to produce a mature EGFR ligand leading to EGFR transactivation.[22]

In human ASM, Krymskaya et al. found that EGF, but not histamine, carbachol, or thrombin, promotes EGFR tyrosine phosphorylation.[9] In the presence of AG1478, the level of $^{[3H]}$thymidine incorpo-

ration in human ASM costimulated with EGF and thrombin is reduced to levels observed in human ASM treated with thrombin alone, and AG1478 has no effect on thrombin-mediated mitogenesis.[9] Our results are compatible with these findings. AG-1478 dose-dependently inhibits BK-induced IL-6 secretion. However, AG-1478 has no effect on ERK1/2 activation by BK and BK does not directly transactivate EGFR phosphorylation. A similar observation has been reported for A431 cells where BK independently activates MAPK via a pathway that is sensitive towards inhibitors of phosphoinositide 3-kinase and PKC, but not to AG1478, corroborating that EGFR transactivation is not necessarily a prerequisite for GPCR-induced activation of MAPK.[21,23]

EGFR activation may involve two different pathways, ligand-dependent and ligand-independent EGFR tyrosine phosphorylation. In ligand-dependent EGFR tyrosine phosphorylation, EGFR ligands bind to EGF receptors in the extracellular domain and activate them. Alternatively, activation of EGFR may occur in the absence of ligand binding (ligand-independent activation) by phosphorylation of tyrosine residues in the intracellular domain directly in response to stimuli.[24] In contrast to that observed in Rat-1 fibroblasts and COS-7 cells,[12,19] our study showed that BK did not appear to transactivate EGFR tyrosine kinase activity and BK plus EGF did not synergically transactivate EGFR phosphorylation in human ASM cells. It may be unnecessary to investigate whether BK could up-regulate EGFR expression on the cell surface at this time. However, this deserves further study.

In this study, BK did not directly transactivate EGFR phosphorylation in human ASM cells, but AG-1478 dose-dependently inhibited BK-induced IL-6 secretion when ASM cells were stimulated with 1 μM BK for 18 hrs in the presence of different concentrations of AG-1478 added 30 min before. Our previous data have shown that the BK, via the B2 receptor, induced IL-6 expression in human ASM cells by involving the ERK1/2 signaling pathway. The amount of IL-6 secretion in response to BK increased in a time-dependent manner, with a significant increase noted at 2 h; maximum levels were reached at 16 h. Since BK does not directly transactivate EGFR phosphorylation in human ASM cells, it is suggested that there are possible transcriptional mechanisms involving EGFR-associated key signaling molecules rather than a change in gene expression of BK-induced IL-6 by AG-1478.

Inflammatory and contractile agents that activate GPCRs can significantly modulate RTK-mediated ASM growth through a p70 S6 kinase–dependent, p42/p44-independent mechanism.[9] GPCR-mediated ASM growth potentiation is consistently associated with sustained activation of p70 S6 kinase for several hours after the initial early phase of activation. Recently, the synergic effect of BK and EGF in augmenting ASM proliferation was also mentioned.[25] In this study, we demonstrated the synergic effect of BK and EGF on airway inflammation with IL-6 secretion. We tried to illuminate the possible mechanism of inhibitory effect of tyrosine inhibitors on BK-induced IL-6 secretion and BK/EGF induced IL-6 secretion. However, in this study, we focused on the initial early phase (within 10 min) of activation by GPCRs. We did not examine whether augmented activation of p70 S6 kinase might serve as a potential mediator of the synergistic effects of BK and EGF agonists. Nevertheless, further study is needed to clarify the mechanisms.

In conclusion, the results suggest that there is cross-talk between BK and EGF to regulate the expression of IL-6 in ASM cells. AG-1478, a specific EGFR inhibitor, abrogates the synergic induction of IL-6 induced by BK plus EGF. However, BK does not directly transactivate EGFR phosphorylation in human ASM cells, suggesting possible transcriptional mechanisms involving EGFR-associated key signaling molecules rather than direct EGFR transactivation by BK.

Acknowledgements

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REFERENCES

1. Barnes PJ, Chung KF, Page CP. Inflammatory mediators

of asthma: an update. Pharmacol Rev 1998;50:515-96.
2. Farmer SG, Burch RM. Biochemical and molecular pharmacology of kinin receptors. Annu Rev Pharmacol Toxicol 1992;32:511-36.
3. Pyne NJ, Tolan D, Pyne S. Bradykinin stimulates cAMP synthesis via mitogen-activated protein kinase-dependent regulation of cytosolic phospholipase A2 and prostaglandin E2 release in airway smooth muscle. Biochem J 1997;328 Pt 2:689-94.
4. Hayashi R, Yamashita N, Matsui S, Fujita T, Araya J, Sassa K, Arai N, Yoshida Y, Kashii T, Maruyama M, Sugiyama E, Kobayashi M. Bradykinin stimulates IL-6 and IL-8 production by human lung fibroblasts through ERK- and p38 MAPK-dependent mechanisms. Eur Respir J 2000;16:452-8.
5. Huang CD, Tliba O, Panettieri RA Jr, Amrani Y. Bradykinin induces interleukin-6 production in human airway smooth muscle cells: modulation by Th2 cytokines and dexamethasone. Am J Respir Cell Mol Biol 2003;28:330-8.
6. Kirsner RS, Eaglstein WH. The wound healing process. Dermatol Clin 1993;11:629-40.
7. Ediger TL, Toews ML. Synergistic stimulation of airway smooth muscle cell mitogenesis. J Pharmacol Exp Ther 2000;294:1076-82.
8. Amishima M, Munakata M, Nasuhara Y, Sato A, Takahashi T, Homma Y, Kawakami Y. Expression of epidermal growth factor and epidermal growth factor receptor immunoreactivity in the asthmatic human airway. Am J Respir Crit Care Med 1998;157:1907-12.
9. Krymskaya VP, Orsini MJ, Eszterhas AJ, Brodbeck KC, Benovic JL, Panettieri RA Jr, Penn RB. Mechanisms of proliferation synergy by receptor tyrosine kinase and G protein-coupled receptor activation in human airway smooth muscle. Am J Respir Cell Mol Biol 2000;23:546-54.
10. Panettieri RA, Murray RK, DePalo LR, Yadvish PA, Kotlikoff MI. A human airway smooth muscle cell line that retains physiological responsiveness. Am J Physiol 1989;256:C329-35.
11. Huang CD, Ammit AJ, Tliba O, Kuo HP, Penn RB, Panettieri RA Jr, Amrani Y. G-protein-coupled receptor agonists differentially regulate basal or tumor necrosis factor-alpha-stimulated activation of interleukin-6 and RANTES in human airway smooth muscle cells. J Biomed Sci 2005;12:763-76.
12. Daub H, Wallasch C, Lankenau A, Herrlich A, Ullrich A. Signal characteristics of G protein-transactivated EGF receptor. EMBO J 1997;16:7032-44.
13. Kishimoto T. The biology of interleukin-6. Blood 1989;74:1-10.
14. Yokoyama A, Kohno N, Fujino S, Hamada H, Inoue Y, Fujioka S, Ishida S, Hiwada K. Circulating interleukin-6 levels in patients with bronchial asthma. Am J Respir Crit Care Med 1995;151:1354-8.
15. Ammit AJ, Hoffman RK, Amrani Y, Lazaar AL, Hay DW, Torphy TJ, Penn RB, Panettieri RA Jr. Tumor necrosis factor-alpha-induced secretion of RANTES and interleukin-6 from human airway smoothmuscle cells. Modulation by cyclic adenosine monophosphate. Am J Respir Cell Mol Biol 2000;23:794-802.
16. Amrani Y, Ammit AJ, Panettieri RA Jr. Tumor necrosis factor receptor (TNFR) 1, but not TNFR2, mediates tumor necrosis factor-alpha-induced interleukin-6 and RANTES in human airway smooth muscle cells: role of p38 and p42/44 mitogen-activated protein kinases. Mol Pharmacol 2001;60:646-55.
17. McKay S, Hirst SJ, Haas MB, de Jongste JC, Hoogsteden HC, Saxena PR, Sharma HS. Tumor necrosis factor-alpha enhances mRNA expression and secretion of interleukin-6 in cultured human airway smooth muscle cells. Am J Respir Cell Mol Biol 2000;23:103-11.
18. Pascual RM, Billington CK, Hall IP, Panettieri RA Jr, Fish JE, Peters SP, Penn RB. Mechanisms of cytokine effects on G protein-coupled receptor-mediated signaling in airway smooth muscle. Am J Physiol Lung Cell Mol Physiol 2001;281:L1425-35.
19. Daub H, Weiss FU, Wallasch C, Ullrich A. Role of transactivation of the EGF receptor in signalling by G-protein-coupled receptors. Nature 1996;379:557-60.
20. Adomeit A, Graness A, Gross S, Seedorf K, Wetzker R, Liebmann C. Bradykinin B(2) receptor-mediated mitogen-activated protein kinase activation in COS-7 cells requires dual signaling via both protein kinase C pathway and epidermal growth factor receptor transactivation. Mol Cell Biol 1999;19:5289-97.
21. Graness A, Hanke S, Boehmer FD, Presek P, Liebmann C. Protein-tyrosine-phosphatase-mediated epidermal growth factor (EGF) receptor transinactivation and EGF receptor-independent stimulation of mitogen-activated protein kinase by bradykinin in A431 cells. Biochem J 2000;347:441-7.
22. Ohtsu H, Dempsey PJ, Eguchi S. ADAMs as mediators of EGF receptor transactivation by G protein-coupled receptors. Am J Physiol Cell Physiol 2006;291:C1-10.
23. Vidal MA, Astroza A, Matus CE, Ehrenfeld P, Pavicic F, Sanchez T, Salem C, Figueroa J, Concha M, Gonzalez CB, Figueroa CD. Kinin B2 receptor-coupled signal transduction in human cultured keratinocytes. J Invest Dermatol 2005;124:178-86.
24. Burgel PR, Nadel JA. Roles of epidermal growth factor receptor activation in epithelial cell repair and mucin production in airway epithelium. Thorax 2004;59:992-6.
25. Gosens R, Grootte Bromhaar MM, Maarsingh H, ten Damme A, Meurs H, Zaagsma J, Nelemans SA. Bradykinin augments EGF-induced airway smooth muscle proliferation by activation of conventional protein kinase C isoenzymes. Eur J Pharmacol 2006;535:253-62.

緩激肽與上皮生長激素調控人類呼吸道平滑肌介白質 6 分泌的交互作用

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背 景： 緩激肽 (Bradykinin) 是一種胜肽，當作用在呼吸道平滑肌細胞時，可經 B2 受器，藉由活化 G 蛋白偶聯受體 (G-coupled protein receptor, GPCR)，同時透過 ERK1/2 途徑，使介白質 6 (Interleukin-6, IL-6) 分泌增加。在某些細胞，G-蛋白偶聯受體促進劑 (GPCR agonist)，可以藉由轉活化 (transactivate) 上皮生長因子受器 (Epidermal growth factor receptor, EGFR) 進而活化 ERK1/2 途徑。本實驗目的，在證實緩激肽和上皮生長因子是否在調控呼吸道平滑肌細胞分泌介白質 6 上有交互作用。

方 法： 將呼吸道平滑肌細胞分別加入緩激肽、上皮生長因子以及上皮生長因子抑制劑 AG-1478 及 Genistein，一種酪胺酸酶抑制劑，以 ELISA 方式測量介白質 6 的分泌。免疫染色法用以分析 ERK1/2 路徑的活化。免疫沉澱分析用以分析緩激肽是否有轉活化上皮生長因子受器。

結 果： 結果顯示上皮生長因子 (10 ng/ml, 18 hr) 增加了呼吸道平滑肌細胞介白質 6 的分泌 (from 234 ± 35 to 923 ± 494 pg/ml, n = 5, $p > 0.05$)，而上皮生長因子加入緩激肽時，比單獨緩激肽刺激時更顯著增加了介白素 6 的分泌 (from 4383 ± 296 to 8312 ± 1267 pg/ml, n = 5, $p < 0.05$)。而 AG-1478 (2 μM) 降低了緩激肽刺激的介白質 6 分泌達 28%，同時也降低了緩激肽與上皮生長因子作用於介白素 6 的協同作用 (from 8312 ± 1267 to 3229 ± 597 pg/ml, n = 5, $p < 0.05$)。AG-1478 作用於緩激肽與上皮生長因子的雙重作用也可見於 Genistein (一種酪胺酸酶抑制劑)，以及 AG-825 (ErbB-2 抑制劑)。免疫染色分析發現 AG-1478 的效果並非經由抑制緩激肽活化 ERK1/2 的路徑。而免疫沉澱分析反映也顯示出緩激肽 (1 μM for 2, 5 and 10 min) 並未直接轉活化上皮生長因子受器。

結 論： 本實驗證實，緩激肽與上皮生長因子刺激呼吸道平滑肌細胞分泌介白質 6，會有協同反應。而此反應可能藉由轉錄的機轉，產生上皮生長因子受器相關的重要分子，而非直接轉活化上皮生長因子受器。

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關鍵詞： 緩激肽，上皮生長因子，上皮生長因子受器，介白質 6，呼吸道平滑肌細胞

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