Tachykinin-1 receptor stimulates proinflammatory gene expression in lung epithelial cells through activation of NF-κB via a Gq-dependent pathway

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Williams R, Zou X, Hoyle GW. Tachykinin-1 receptor stimulates proinflammatory gene expression in lung epithelial cells through activation of NF-κB via a Gq-dependent pathway. Am J Physiol Lung Cell Mol Physiol 292: L430–L437, 2007. First published October 13, 2006; doi:10.1152/ajplung.00475.2005.—The respiratory tract is innervated by irritant-responsive sensory nerves, which, on stimulation, release tachykinin neuropeptides in the lung. Tachykinins modulate inflammatory responses to injury by binding to tachykinin (neurokinin) receptors present on various pulmonary cell types. In a previous study, the activation of the proinflammatory transcription factor NF-κB in lung epithelial cells was investigated as a mechanism by which tachykinins stimulate inflammatory processes. In A549 human lung epithelial cells transfected with the tachykinin-1 receptor (Tacr1), treatment with the Tacr1 ligand substance P (SP) resulted in NF-κB activation, as judged by transcription of an NF-κB-luciferase reporter gene and production of interleukin-8, a chemokine whose expression is upregulated by NF-κB. SP caused a dose-dependent activation of NF-κB that was inhibited by the selective Tacr1 antagonist RP67580. Tacr1 is a G protein-coupled receptor capable of activating both the Gi and Gq families of G proteins. Expression of inhibitory peptides and constitutively active G protein mutants revealed that Gq signaling was both necessary for Tacr1-induced NF-κB activation and sufficient for NF-κB activation in the absence of any other treatment. Treatment with pharmacological inhibitors to investigate events downstream of Gq revealed that Tacr1-induced NF-κB activation proceeded through an intracellular signaling pathway that was dependent on phospholipase C, calcium, Ras, Raf-1, MEK, Erk, and proteasome function. These results identify intracellular signaling mechanisms that underlie the proinflammatory effects of tachykinins, which previously have been implicated in lung injury and disease.

MATERIALS AND METHODS

Materials. A549 human adenocarcinoma cells, MLE-12 mouse adenocarcinoma cells, and BEAS-2B human bronchial epithelial cells were purchased from American Type Culture Collection (ATCC; Manassas, VA). The C10 mouse lung epithelial cell line was obtained from Dr. Randall Ruch, Medical College of Ohio (Toledo, OH). Plasmids encoding NF-κB-luciferase and pRL-TK-luciferase reporters were purchased from Promega (Madison, WI). Murine Tacr1 cDNA was purchased from ATCC and cloned into the pCAGGS expression vector provided by Jun-ichi Miyazaki, Osaka University Medical School (Osaka, Japan). Plasmids encoding constitutively active Gq and Gq and the pcDNA3.1+ vector were purchased from the University of Missouri-Rolla cDNA Resource Center (Rolla, Missouri). Plasmids encoding Gq and Gq inhibitory peptides and the Gq, nonfunctional control peptide were purchased from Cue BioTech (Evaston, IL). Antibodies were purchased from Cell Signaling Technology (Beverly, MA). The IL-8 ELISA Kit was purchased from Pierce Biotechnology (Rockford, IL). The enhanced chemiluminescence (ECL) Western Blot Detection Kit and the ECL Advance Western Blot Detection Kit were obtained from Amersham Biosciences (Buckinghamshire, UK). Protease inhibitor cocktail was purchased from Sigma-Aldrich (St. Louis, MO); U0126 was purchased from Promega. RP67580 and SP were purchased from Tocris Cookson (Ellisville, MO). U73122, forskolin, Go6850, MDL-12,330, MG-132, and BAPTA-AM were purchased from Calbiochem (San Diego, CA). TNF was obtained from PeproTech (Rocky Hill, NJ).

Cell culture, transfection, and treatment. A549 cells were cultured in DMEM containing 10% FBS, 100 units/ml penicillin, 100 μg/ml streptomycin, and 2 mM glutamine. BEAS-2B cells were cultured in DMEM containing 10% FBS, 100 units/ml penicillin, and 2 mM glutamine. The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

The resin particles used were purchased from Sigma-Aldrich (St. Louis, MO). The resin particles were washed, and the particles were packed into a chromatography column. The column was then connected to a pump, which delivered a solution of acetic acid to the column. The solution was then passed through the column, and the protein was eluted from the column.

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keratinocyte growth medium (Cambrex Bioproducts, Rockland, ME). C10 epithelial cells were cultured in CMRL-1066 media containing 10% FBS, 0.5 mM glutamine, and 50 μg/ml gentamycin sulfate. MLE-12 cells were cultured in DMEM/F-12 media containing 2% FBS, 5 μg/ml insulin, 5 μg/ml transferrin, 5 ng/ml selenium, 10 nM hydrocortisone, 10 nM β-estradiol, and 10 mM HEPES. For NF-κB-luciferase experiments, cells were seeded into 12-well plates, grown to ~50% confluence (24-48 h), and transfected with NF-κB-luciferase (firefly luciferase) in conjunction with pRL-TK (Renilla luciferase) as a transfection control and Tacr1, G protein, or control plasmids using FuGENE-6 according to the manufacturer’s instructions (Roche Applied Science, Indianapolis, IN). Cells were incubated at 37°C for 4 h, at which point the transfection medium was replaced with DMEM growth medium. Cells were incubated at 37°C overnight before treatment. Cells were treated with SP for 4 h, after which cells were collected for luciferase assay. Except when investi-

![Fig. 1](image1.png)

**Fig. 1.** Activation of NF-κB-luciferase in tachykinin-1 receptor (Tacr1)-transfected cells. A549, BEAS-2B, C10, and MLE-12 cells were transfected with cDNA for Tacr1 (or empty vector as a control), a firefly NF-κB-luciferase reporter gene, and the Renilla luciferase plasmid pRL-TK as a transfection efficiency control. Cells were treated with substance P (SP) 24 h after transfection, and cells were collected for luciferase assay 4 h following treatment. Luciferase activity was normalized to transfected cells not treated with SP. Values are means ± SE for 3 wells/group. A: A549. B: BEAS-2B. C: C10. D: MLE-12. *P < 0.0001 for A549 and C10, P < 0.01 for BEAS-2B, and P < 0.02 for MLE-12 vs. untreated.

![Fig. 2](image2.png)

**Fig. 2.** Tacr1 expression and comparison of NF-κB activation in response to SP and tumor necrosis factor (TNF). A: immunoblot of Tacr1 expression in A549 cells transfected with Tacr1 (lane 1) or control vector (lane 2). The mobility of molecular mass standards, in kDa, is indicated at left. The band running between 250 and 148 kDa in both lanes is nonspecific. B: comparison of the magnitude of NF-κB activation by SP and TNF. Tacr1-transfected cells were treated with SP, and NF-κB-luciferase activity was measured (left). A549 cells were treated with TNF, and NF-κB-luciferase activity was measured (right). Values are means ± SE for 3 wells/group. *P = 0.001 vs. untreated.
gating dose response, SP was used at a concentration of \(10^{-7}\) M. TNF was used at a concentration of 10 ng/ml. When used, inhibitors were added to cultures 40 min before SP treatment, except for experiments with constitutively active \(G_{q}\), for which the inhibitors were added when the transfection medium was replaced with growth medium.

For immunoblotting experiments, cells were seeded into six-well plates, grown to ~90% confluence (24-48 h), and transfected with Tacr1, G protein, or control plasmids using Lipofectamine/Plus reagent according to the manufacturer’s instructions (Invitrogen, Carlsbad, CA). Cells were then incubated at 37°C for 3 h, at which point the transfection medium was replaced with DMEM containing 20% FBS, 100 units/ml penicillin, 100 µg/ml streptomycin, and 2 mM glutamine. Cells were incubated at 37°C overnight before treatment.

Luciferase assay. Cells were agitated in Passive Lysis buffer (Promega) for 10 min. Wells were then scraped gently, and the lysates were stored at −70°C until assayed. Lysates were thawed and centrifuged at 16,000 g for 10 s to remove insoluble material. Firefly and Renilla luciferase activities were measured using the Dual-Luciferase Reporter Assay (Promega) according to the manufacturer’s instructions. NF-κB-luciferase activity was normalized to that of the pRL-TK-luciferase.

Immunoblotting. Cells were lysed with chilled RIPA buffer (50 mM Tris, pH 8.0, 150 mM NaCl, 0.05% sodium deoxycholate, 1% Igepal CA-630, 0.1% SDS) containing protease inhibitors [1 mM 4-(2-aminoethyl)benzenesulfonyl fluoride, 0.8 µM aprotinin, 20 µM leupeptin, 40 µM bestatin, 15 µM pepstatin A, and 14 µM E-64] and passed through a 27-gauge needle six to eight times. The solution was then transferred to individual containers for storage at −70°C. Cell lysates were subjected to SDS-PAGE, and immunoblotting was performed as previously described (1). Tacr1 (neurokinin-1 receptor) antibody (Abcam, Cambridge, MA) and Erk and phosphoErk antibodies (Cell Signaling, Danvers, MA) were used at a 1:1,000 dilution with the ECL Western Blot Detection Kit (Amersham).

IL-8 assay. IL-8 was measured in tissue culture supernatants by ELISA, according to the manufacturer’s instructions.

Data analysis. Data are presented as group means ± SE. Group means were compared by ANOVA. The criterion for significance was set at \(P < 0.05\). All data presented are representative of at least two independent experiments that were performed on cells of different passage numbers.

RESULTS

Activation of NF-κB by Tacr1. To determine whether Tacr1 stimulation resulted in NF-κB activation, lung epithelial cell lines of human and mouse origin were transfected with Tacr1 and treated with SP. Treatment of Tacr1-expressing cells with SP resulted in a significant increase in NF-κB-luciferase activity (Fig. 1). This effect was observed in the four cell lines tested, suggesting that it may be a general property of lung epithelial cells. Subsequent studies were carried out in A549 cells because of the robust response observed in this cell line.

To document expression of Tacr1, A549 cells were transfected with the Tacr1-expressing plasmid, and cell lysates were analyzed by immunoblotting with Tacr1 antibody (Fig. 2A). Receptor expression was detected in Tacr1-transfected cells as a broad, high-molecular-weight species near the top of the resolving gel. This behavior of Tacr1 has been observed previously and is thought to result from difficulty in solubilizing the receptor (10, 30). Tacr1 was not detected by this analysis in cells transfected with vector, indicating that Tacr1 expression in A549 cells is low or absent. This observation is consistent with the results of the NF-κB-luciferase analysis, which showed activation of the reporter gene in Tacr1-transfected but not in vector-transfected cells.

The relative magnitude of SP-induced NF-κB activation was assessed by comparison with the effect of TNF treatment, a known inducer of NF-κB activation (Fig. 2B). NF-κB-luciferase activation caused by SP treatment of Tacr1-transfected cells and TNF treatment of A549 cells was measured in parallel. The extent of NF-κB-luciferase activation was roughly equivalent in both treatments. This result suggested that the magnitude of the effect initiated by Tacr1 signaling was sufficient as to be physiologically relevant.
NF-κB activation induced by SP in A549 cells exhibited a dose-response relationship consistent with a receptor-mediated effect (Fig. 3A; EC_{50} = 9 \times 10^{-11} M). Activation of NF-κB by SP in Tacr1-expressing A549 cells was inhibited in a dose-dependent manner by the selective Tacr1 antagonist RP67580 (Fig. 3B). To confirm that Tacr1 could induce the expression of an endogenous NF-κB-responsive gene, IL-8 was measured in tissue culture supernatants. Treatment of Tacr1-transfected cells with SP resulted in a significant increase in IL-8 secretion, whereas cotransfection with a mutant “superrepressor” IκB lacking Ser^{32} and Ser^{36} phosphorylation sites, which is known to prevent activation of NF-κB (20), inhibited the SP-induced increase in IL-8 secretion (Fig. 3C). These results confirmed that the observed effects in A549 cells are caused by activation of NF-κB via the stimulation of Tacr1.

**Activation of NF-κB via G_q-dependent signaling.** The intracellular signaling pathways associated with the G protein-coupled receptor Tacr1 were investigated. Tacr1 is coupled to intracellular effectors through both G_q and G_s (14, 23). A549 cells were cotransfected with plasmids expressing Tacr1 and COOH-terminal Go peptides that specifically inhibit G protein function (8, 9). Expression of G_q and G_s inhibitory peptides, but not a control peptide, inhibited SP-induced NF-κB activation (Fig. 4A). This result indicates that both G_q and G_s function are required for NF-κB activation. To confirm this result pharmacologically, cells were treated with inhibitors of phospholipase C, which is activated by G_q, or adenylyl cyclase, which is activated by G_s. Both U73122, a phospholipase C inhibitor (Fig. 4B), and MDL-12,330, an adenylyl cyclase inhibitor (Fig. 4C), resulted in a dose-dependent inhibition of NF-κB activation, which was consistent with the results of the Go inhibitory experiments.

To determine whether stimulation of individual G proteins was sufficient for NF-κB activation, plasmids encoding constitutively active G protein mutants (32, 34) were cotransfected with the NF-κB-luciferase reporter gene. Expression of the constitutively active G_q (Q209L) and the related G_s (Q227L), but not G_s (Q227L), resulted in significant NF-κB activation in the absence of any other treatment (Fig. 5A). To confirm that

**Fig. 4.** G_q and G_s signaling are required for NF-κB activation. A: A549 cells were transfected with Tacr1 and NF-κB-luciferase reporter genes and treated with SP. Cells were cotransfected with plasmids encoding peptides specifically inhibiting G_q (inh-Gq) or G_s (inh-Gs), or a random peptide. Inactivation of G_q or G_s suppressed SP-induced NF-κB activation. *P < 0.0001 vs. no inhibitory peptide and vs. the random peptide. B: treatment with the phospholipase C inhibitor U73122 reduced NF-κB activation. *P < 0.0001 vs. untreated. †P < 0.0001 vs. SP treated without inhibitor. C: treatment with the adenylyl cyclase inhibitor MDL-12,330 (MDL) blocked NF-κB activation. *P < 0.0001 vs. untreated (Unt.). †P < 0.01 vs. SP treated without inhibitor. A–C: luciferase activity was normalized to transfected cells not treated with SP. Values are means ± SE for 3 wells/group.

**Fig. 5.** G_q, but not G_s, stimulation is sufficient for NF-κB activation. Values are means ± SE for 3 wells/group. A: constitutively active G_q subunits and NF-κB-luciferase were transfected into A549 cells. *P < 0.0001 vs. vector control. B: A549 cells were transfected with NF-κB-luciferase and treated with 100 μM forskolin. Stimulation of adenylyl cyclase by forskolin did not activate NF-κB. Cells transfected in parallel with Tacr1 and treated with SP exhibited NF-κB activation. *P < 0.0001 vs. untreated.
signaling pathways activated by Gs are not sufficient to activate NF-κB, cells were transfected with NF-κB-luciferase and treated with forskolin, an activator of adenylate cyclase. Forskolin failed to activate NF-κB, whereas Tacr1-transfected cells treated with SP in parallel as a positive control exhibited significant NF-κB activation (Fig. 5B). This result, in conjunction with the Gs inhibitory peptide experiment, suggests that there is basal Gs activity in unstimulated cells that is required for NF-κB activation, but that increased Gs stimulation alone does not activate NF-κB. In contrast, Gq activity is required for Tacr1-induced NF-κB activation and is sufficient to activate NF-κB in the absence of other stimuli. Therefore, Gq appears to be the major G protein activator in this system.

Suppressing events downstream of Gq inhibits NF-κB activation. Activation of Gq is known to stimulate phospholipase C, which in turn can promote intracellular calcium release and protein kinase C (PKC) activation. Pharmacological inhibitors of calcium signaling and PKC were used to examine the role of these molecules in the activation of NF-κB by SP. The calcium chelator BAPTA-AM (Fig. 6A) and the PKC inhibitor Go6850 (Fig. 6B) significantly inhibited SP-induced NF-κB activation. The small GTPases Ras and Raf are also known to be activated downstream of phospholipase C. Treatment of cells with the Ras inhibitor manumycin A (Fig. 6C) and the Raf-1 inhibitor GW5074 (Fig. 6D) resulted in significant inhibition of NF-κB activation caused by SP stimulation. The MAP kinases Erk1 and Erk2, which are known to be activated by the Ras/Raf pathway, have been shown to activate NF-κB in some experimental systems (6, 31). The potential involvement of Erk in mediating SP-induced NF-κB activation was investigated. Treatment of Tacr1-transfected cells with SP resulted in activation of Erk, as detected by immunoblotting for phosphorylated Erk (Fig. 7A). Phosphorylation of Erk was inhibited in cells treated with the MEK inhibitor U0126. To investigate a functional link between MEK/Erk and NF-κB activation, Tacr1-transfected A549 cells were treated with U0126 (Fig. 7B). In cells treated with U0126, a dose-dependent inhibition of NF-κB activation was observed. U0126 also blocked NF-κB activation induced by transfection of constitutively active Gq into A549 cells (Fig. 7C). These results demonstrate that MEK/Erk signaling is involved in NF-κB activation following SP binding to Tacr1 and subsequent Gq activation.

The most well-characterized mechanism of NF-κB activation involves proteasomal degradation of the NF-κB inhibitor IκB. Tacr1-transfected cells were treated with the proteasome inhibitor MG-132 to determine whether this mechanism was involved in NF-κB activation induced by SP. For a positive control, TNF treatment was used as a stimulus known to activate NF-κB through proteasomal degradation of IκB. MG-132 completely inhibited NF-κB activation induced by SP or TNF (Fig. 8). This effect of MG-132 suggests that Tacr1 stimulation activates NF-κB through the classical mechanism of proteasomal degradation of IκB.

![Fig. 6. Inhibition of NF-κB activation by mediators downstream of Gq, A549 cells were transfected with Tacr1 and NF-κB-luciferase reporter genes and treated with SP plus the indicated concentration of inhibitors. Values are means ± SE for 3 wells/group. A: calcium chelator BAPTA-AM. *P < 0.0001 vs. untreated. †P < 0.0001 vs. SP treated without inhibitor. B: protein kinase C inhibitor Go6850. *P < 0.0001 vs. untreated. †P < 0.001 vs. SP treated without inhibitor. C: Ras inhibitor manumycin A. *P < 0.0001 vs. untreated. †P < 0.0001 vs. SP treated without inhibitor. D: Raf-1 inhibitor GW5074. *P < 0.0001 vs. untreated. †P < 0.001 vs. SP treated without inhibitor.](http://ajplung.physiology.org/)

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modulating inflammatory processes in the lung through binding to Tacr1. Increased pulmonary expression of Tacr1 is associated with pathological states in which lung inflammation is observed. Asthmatic patients were observed to have increased epithelial expression of Tacr1, particularly in ciliated and goblet cells, compared with healthy controls, and this was associated with Mycoplasma infection (7). In patients with sarcoidosis, Tacr1 expression was upregulated in both bronchial and alveolar epithelial cells as well as in inflammatory cells (24). Rats infected with respiratory syncytial virus exhibited increased Tacr1 expression and increased SP binding sites in bronchial epithelium compared with uninfected animals (16). Lung inflammation in rats infected with respiratory syncytial virus was diminished by a Tacr1 inhibitor, demonstrating a functional role for Tacr1 in promoting inflammation following infection. Tacr1 has been shown in multiple contexts to be upregulated by noxious or stressful stimuli (2, 33, 35). Potential mechanisms underlying this regulation include NF-κB and the neuropeptide calcitonin gene-related peptide (29, 33). In the current study, Tacr1 expression was increased in cultured lung epithelial cells by transfection, so that signaling pathways specifically activated by Tacr1 could be dissected in the absence of additional intracellular events triggered by injury to the cells. Our results identify signaling pathways activated by Tacr1 that may stimulate proinflammatory gene expression in lung epithelial cells, thereby promoting inflammatory processes in the lung.

Tacr1 is a G protein-coupled receptor that is known to activate intracellular effectors through both the Gq and Gs families of G proteins (14, 23). Our results demonstrate by multiple lines of evidence that NF-κB activation induced by Tacr1 occurs via an intracellular signaling cascade primarily activated by Gq. Expression of a Gq inhibitory peptide blocked SP-induced NF-κB activation, and expression of constitutively active Gq was sufficient to activate NF-κB. SP-induced NF-κB activation was inhibited by pharmacological agents that interfere with the function of signaling molecules known to be downstream of Gq, e.g., phospholipase C, calcium, PKC, and Ras/Raf/Erk. In contrast, although an inhibitory Gs peptide blocked NF-κB activation, expression of constitutively active Gs alone did not activate NF-κB. Similarly, pharmacological inhibition of adenylate cyclase, an immediate downstream effector of Gs, blocked SP-induced NF-κB activation, but stimulation of adenylate cyclase with forskolin did not result in activation of NF-κB. Thus the Gq cascade appears to be the primary mechanism by which NF-κB is activated through Tacr1. Our results suggested that, although basal Gs activity is inhibited increased Tacr1 expression and increased SP binding sites in bronchial epithelium compared with uninfected animals (16).

**DISCUSSION**

The results of the present study demonstrate that SP activates NF-κB in lung epithelial cells expressing Tacr1. Tachykinin neuropeptides, including SP, have been implicated in

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**Fig. 7.** Erk mediates Tacr1- and Gq-induced NF-κB activation. A: treatment of Tacr1-transfected cells with SP (lane 3) stimulated Erk phosphorylation, which was inhibited by U0126 (MEK1 inhibitor, 10 μM; lane 5). Lane 1, Tacr1; lane 2, vector; lane 3, Tacr1 + SP; lane 4, vector + SP; lane 5, Tacr1 + SP + U0126; lane 6, vector + SP + U0126. B: treatment of Tacr1-transfected cells with U0126 inhibited SP-induced NF-κB activation. Luciferase activity was normalized to transfected cells not treated with SP. *P < 0.0001 vs. untreated. †P < 0.001 vs. SP treated without inhibitor. C: treatment with U0126 inhibited NF-κB activation induced by constitutively active Gq. Luciferase activity was normalized to cells transfected with vector (vec). *P < 0.0001 vs. untreated. †P < 0.0001 vs. constitutively active Gq without inhibitor. B and C: values are means ± SE for 3 wells/group.

![Image](http://ajplung.physiology.org/)

**Fig. 8.** Inhibition of NF-κB activation by the proteasome inhibitor MG-132. A549 cells were transfected with Tacr1 and luciferase reporter genes and treated with SP plus 100 μM MG-132 (left). A549 cells were transfected with luciferase reporter genes and treated with TNF plus 100 μM MG-132 (right). Values are means ± SE for 3 wells/group. *P < 0.0001 vs. untreated. †P < 0.0001 vs. no inhibitor.
required, additional stimulation did not result in NF-κB activation.

Our results have identified a Ras/Raf/Erk pathway as a downstream target of the Gq cascade that is involved in the activation of NF-κB. Inhibition of Ras or Raf-1 significantly reduced NF-κB activation. SP treatment of Tacr1-expressing cells increased the phosphorylation of Erk p42/p44. Inhibition of MEK1, an immediate upstream activator of Erk, blocked this phosphorylation and inhibited SP-induced NF-κB activation. MEK1 inhibition also blocked NF-κB activation induced by constitutively active Gq. Activation of NF-κB by a Ras/Raf-1/Erk-dependent pathway has been demonstrated previously in response to inflammatory stimuli, but the intermediate events between Erk phosphorylation and NF-κB activation have not been fully characterized. Experimental evidence has been obtained for multiple mechanisms underlying Erk-dependent NF-κB activation, including activation of IκB kinase (IKK) by Erk (presumably through intermediate kinases) (6), stimulation of kinases that induce NF-κB activation independently of IKK activity and IκB degradation (3), direct interaction of Erk and the NF-κB subunit p65 (31), and stimulation of the transactivation activity of p65 (15). In our system, NF-κB activation appeared to proceed through the classical pathway involving the proteasomal degradation of IκB. Further studies will be required to determine the exact mechanisms by which Erk may trigger IκB degradation in response to Tacr1 stimulation.

Tachykinins have been shown to activate NF-κB and stimulate production of proinflammatory cytokines in a variety of cell types, including colonic epithelial cells (17), macrophages (22), mast cells (1), T lymphocytes (12), and astroc-toma cells (18). However, the mechanisms underlying these processes have not been fully elucidated. Activation of NF-κB by SP was dependent on calcium in astrocytoma cells (18) but not in macrophages (22) or colonic epithelial cells (17). In colonic epithelial cells transfected with Tacr1, SP-induced NF-κB activation and IL-8 production were dependent on the activity of Rho GTPases and PKCα but were not dependent on calcium release or Erk activation (17, 36). This is in contrast to the results of the present study, which demonstrated a dependence on calcium and Erk for NF-κB activation through Tacr1 in A549 cells. Thus tachykinins appear to have the ability to activate NF-κB by multiple mechanisms in different epithelial cell types.

In conclusion, Tacr1 signaling initiated by SP binding leads to NF-κB activation and proinflammatory cytokine expression in human lung epithelial cells. NF-κB activation in this system is Gq dependent and involves phospholipase C, calcium, PKC, Ras/Raf/Erk, and proteasomal degradation of IκB. Lung epithelial cells are known to be a major site of cytokine gene expression in animal models following direct injury from noxious stimuli (5, 13, 19) and from global ischemia (13). Tachykinins may therefore promote inflammation via NF-κB activation in lung epithelial cells in vivo. Better understanding of the signal transduction pathways involved in this phenomenon may reveal clinically relevant targets for inhibition and better control of inflammation-associated lung injury.

DISCLOSURES
This publication was developed under Science To Achieve Results (STAR) Research Assistance Agreement No. R-83068501 awarded by the United States Environmental Protection Agency (EPA) to G. W. Hoyle. It has not been formally reviewed by the EPA. The views expressed in this document are solely those of the recipient and the EPA does not endorse any products or commercial services mentioned in this publication.

REFERENCES


