Upregulation of RGS4 expression by IL-1β in colonic smooth muscle is enhanced by ERK1/2 and p38 MAPK and inhibited by the PI3K/Akt/GSK3β pathway

Wenhui Hu, Fang Li, Sunila Mahavadi, and Karnam S. Murthy

1Department of Physiology and Biophysics, Medical College of Virginia Campus, Virginia Commonwealth University, Richmond, Virginia; and 2Department of Neuroscience, Temple University School of Medicine, Philadelphia, Pennsylvania

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Hu W, Li F, Mahavadi S, Murthy KS. Upregulation of RGS4 expression by IL-1β in colonic smooth muscle is enhanced by ERK1/2 and p38 MAPK and inhibited by the PI3K/Akt/GSK3β pathway. Am J Physiol Cell Physiol 296: C1310–C1320, 2009; doi:10.1152/ajpcell.00573.2008.—Initial Ca2+−dependent contraction of intestinal smooth muscle is inhibited upon IL-1β treatment. The decrease in contraction reflects the upregulation of regulator of G protein signaling-4 (RGS4) via the canonical inhibitor of NF-κB kinase-2 (IKK2/IKb-α/NF-κB pathway. Here, we show that the activation of various protein kinases, including ERK1/2, p38 MAPK, and phosphoinositide 3-kinase (PI3K), differentially modulates IL-1β-induced upregulation of RGS4 in rabbit colonic muscle cells. IL-1β treatment caused a transient phosphorylation of ERK1/2 and p38 MAPK. It also caused the phosphorylation of Akt and glycogen synthase kinase-3β (GSK3β), sequential downstream effectors of PI3K. Pretreatment with PD-98059 (an ERK inhibitor) and SB-203580 (a p38 MAPK inhibitor) significantly inhibited IL-1β-induced RGS4 expression. In contrast, LY-294002 (a PI3K inhibitor) augmented, whereas GSK3β inhibitors inhibited, IL-1β-induced RGS4 expression. PD-98059 blocked IL-1β-induced phosphorylation of IKK2, degradation of IkB-α, and phosphorylation and nuclear translocation of NF-κB subunit p65, whereas SB-203580 had a marginal effect, implying that the effect of ERK1/2 is exerted on the canonical IKK2/IκBα/NF-κB pathway of NF-κB activation but that the effect of p38 MAPK may not predominantly involve NF-κB signaling. The increase in RGS4 expression enhanced by LY-294002 was accompanied by an increase in the phosphorylation of IKK2/IκBα/p65 and blocked by pretreatment with inhibitors of IKK2 (IKK2-IV) and IκB-α (MG-132). Inhibition of GSK3β abolished IL-1β-induced phosphorylation of IKK2/p65. These findings suggest that ERK1/2 and p38 MAPK enhance IL-1β-induced upregulation of RGS4; the effect of ERK1/2 reflects its ability to promote IKK2 phosphorylation and increase NF-κB activity. GSK3β acts normally to augment the activation of the canonical NF-κB signaling. The PI3K/Akt/GSK3β pathway attenuates IL-1β-induced upregulation of RGS4 expression by inhibiting NF-κB activation.

INFLAMMATORY MEDIATORS, including cytokines, chemokines, growth factors, and cell adhesion molecules, contribute to the maintenance and resolution of the inflammatory responses in patients with either inflammatory bowel diseases (IBD) or irritable bowel syndrome (IBS). Different patterns of inflammatory cytokines have been identified that distinctly regulate the motility of gastrointestinal smooth muscle (51, 69, 81). A pattern involving the time-dependent release of IL-1β, TNF-α, IL-6, and IL-8 is accompanied by decreases in the response of smooth muscle to excitatory neurotransmitters (acetylcholine, neurokinin A, etc.) (70, 77, 81, 85), whereas the pattern of T helper cell (Th)2 cytokines observed with helminth infection involves the transient activation of IL-4 and IL-13, resulting in initial hypercontractility followed by sustained expression of transforming growth factor-β1 and cyclooxygenase (COX)-2, leading to persistent hypercontractility (2, 5, 51, 106). Recently, an additional pattern of Th17 cytokines, including IL-17 and IL-23, has been shown to play an important role in the pathophysiology of IBD and IBS (84, 86, 100, 105). The specific steps in the signaling pathways mediating the contraction or relaxation of smooth muscle that are affected by these cytokine patterns have not been identified.

IL-1β has been well known to inhibit the contractile response of intestinal smooth muscle (8, 9, 15, 18, 31, 48, 49, 62, 67, 95). The mechanisms for such inhibition may involve inhibitory neural regulation on the release of excitatory neurotransmitters (8, 9, 14, 67) and/or a reduction in muscular contractile responses (15, 18, 70, 85). The cellular mechanisms are becoming increasingly identified. H2O2, formed in colonic and esophageal sphincter smooth muscle in response to IL-1β (16, 18), inhibits contraction by interfering with Ca2+ mobilization. IL-1β treatment of rat ileal smooth muscle strip decreases the phosphorylation of myosin light chain phosphatase-targeting subunit 1 and protein expression of 17-kDa PKC-potentiated myosin phosphatase inhibitor (CPI-17), the two key signaling components in mediating agonist-induced sustained (tonic) contraction of smooth muscle (70). By screening the signaling targets mediating IL-1β-induced inhibition on acetylcholine-stimulated initial and sustained contraction in isolated or cultured colonic smooth muscle cells (SMCs), we have previously demonstrated that IL-1β upregulates regulator of G protein signaling-4 (RGS4) expression, which contributes to the inhibitory effect of IL-1β on the initial contraction, and confirmed that IL-1β downregulates CPI-17 expression, which is associated with IL-1β-induced inhibition on the sustained contraction (37).

RGS4 is one of seven members of the classic R4 RGS protein family that accelerates the intrinsic GTPase activity of Gαq/o and Gαq/11 family members. RGS4 is well known to regulate the strength and duration of Gαq signaling and plays an important role in regulating smooth muscle contraction, cardiac myocyte development, neural plasticity, and psychiatric disorders (36, 37, 59, 99). However, the regulatory mecha-
nisms of RGS4 expression remain elusive. At the protein level, RGS4 is regulated by the NH2-end rule pathway (10). At the mRNA level, RGS4 is regulated by the neural type-specific transcription factor Phox2b (28). Our recent study (36) provided the first evidence that IL-1β-induced upregulation of RGS4 is transcription dependent and mediated by the canonical inhibitor of NF-κB kinase-2 (IKK2)/ICB-α pathway of NF-κB activation (36).

MAPks are a family of serine/threonine kinases and are activated upon dual phosphorylation at threonine and tyrosine by upstream kinases in response to diverse extracellular stimuli. Recent evidence has suggested that both ERK1/2 and p38 MAPK are implicated in the Ca2+ sensitization (39) and PKC-dependent contraction of gastrointestinal smooth muscle (17, 39). However, the mechanisms by which these MAPks modulate smooth muscle contraction are not well understood. Phosphorylation of caldesmon and/or calponin may contribute to the effect of ERK1/2 (41, 54), whereas p38 MAPK may modulate smooth muscle contraction through the sequential phosphorylation and activation of MAPK-activated protein kinase-2 (94) and heat shock protein 27 (102).

Phosphoinositide 3-kinases (PI3K) are heterodimeric proteins and linked to an extraordinarily diverse group of cellular functions. The product of PI3K, phosphotidylinositol-3,4,5-triphosphate, triggers the accumulation of pleckstrin homology domain-containing proteins such as the serine-threonine kinase Akt. Activated Akt inactivates glycogen synthase kinase 3 (GSK3) through phosphorylation (55, 61). IL-1β is known to activate the PI3K/Akt/GSK3β pathway in several types of cells, such as epithelia cells, hepatocytes, sertoli cells, and airway SMCs (23). Our previous study (38) has shown that Gi-coupled receptors activate PI3K in gut SMCs. However, the effect of the PI3K/Akt/GSK3β pathway on gut smooth muscle contraction remains to be elucidated.

Here, we show that IL-1β-induced activation of either ERK1/2 or p38 MAPK enhances the upregulation of RGS4 expression, which reflects the ability to inhibit initial contraction, whereas the PI3K/Akt/GSK3β pathway attenuates IL-1β-induced upregulation of RGS4 expression. The effect of the ERK1/2 and PI3K pathway is dependent on the signaling of NF-κB activation, whereas p38 MAPK exerts its role independently of NF-κB signaling.

MATERIALS AND METHODS

Reagents and antibodies. IL-1β was obtained from Alexis Biochemicals (San Diego, CA). 2′-Amino-3′- methoxyflavone (PD-98059), 4-(4-fluorophenyl)-2-(4-methylsulfonylphenyl)-5-(4-pyridyl)-1H-imidazole (SB-203580), 2′-(4-morpholinyl)-4-fluor-1H-benzo[4-one (LY-294002), triciribine [Akt/PKB signaling inhibitor-2 (API-2)], 3-[1-(3-hydroxypropyl)-1H-pyrrrole-2-(3-bipyrindin-3-yl)-4-pyrizin-2-yl-pyrrrole-2,5-dione [a specific GSK3β inhibitor (GSK3β-XI)], 4-(4′-phenoxyanilino)-6,7-dimethoxyquinazoline (Src kinase inhibitor I), [5-(p-fluorophenyl)-2-ureido]thiophene-3-carboxamide [IKK2 inhibitor for IV (IKK2-IV)], and carbobenzyoxyl-ε-leucyl-ε-leucyl-ε-leucinal Z-L-LLL-CHO (MG-132) were from EMD Chemicals (San Diego, CA) and dissolved in DMSO. Antibodies against p65, IKK2, IκB-α, GAPDH, and β-actin were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). Affinity-purified anti-RGS4 antibody was kindly provided by Dr. Susanne M. Mumbey (University of Texas Southwest Medical Center). Antibodies against ERK1/2, phospho-ERK1/2 (Thr202/Tyr204), phospho-p38 MAPK (Thr180/Tyr182), phospho-Akt (Ser473), phospho-GSK3β (Ser9), and phospho-IκB-α (Ser27/32) were from Cell Signal Technology (Danvers, MA). All other reagents were from Sigma (St. Louis, MO).

Isolation and culture of SMCs. Institutional Animal Care and Use Committee (IACUC) approval was obtained from Virginia Commonwealth University. Rabbit colonic circular muscle cells were isolated and cultured as previously described (37, 66). Briefly, the distal colon from euthanized New Zealand White rabbits (2–2.5 kg) was placed in HEPES-buffered smooth muscle media. The circular smooth muscle layer was dissected from the mucosa and the longitudinal muscle layer under a stereomicroscope and treated with 0.1% collagenase (type II) and 0.1% soybean trypsin inhibitor for 30 min at 31°C. The isolated single muscle cells after several rounds of spontaneous dispersion were harvested by filtration through 500-μm Nitex and centrifuged twice at 350 g for 10 min. Aliquots of freshly isolated SMCs in HEPES-buffered smooth muscle media without serum and antibiotics were placed in six-well plates and incubated at 37°C for 30 min before treatment with various inhibitors and cytokines. For cultures, isolated SMCs were placed in a 100-mm dish with DMEM containing 10% FBS and 1% antibiotics and antimycotics. After 10–14 days, the SMCs attained confluence and were then passaged once for use in various experiments. Full confluent muscle cells were deprived of serum for 24 h before experiments.

Conventional and real-time RT-PCR. Freshly dispersed or cultured colonic SMCs were treated with the TRIZol reagent (Invitrogen, Carlsbad, CA) for total RNA extraction. The potentially contaminated genomic DNA was removed by treating 10 μg of the RNA sample at 37°C for 30 min with 1 μl of TURBO DNase (Ambion, Austin, TX) followed by an extraction with phenol-chloroform-isooamylalcohol (25:24:1). RNA (2 μg) was used to synthesize cDNA using SuperScript II reverse transcriptase (Invitrogen) with random hexanucleotide primers. Conventional PCR was performed on cDNA using the HotMaster Taq DNA polymerase kit (Eppendorf). The primer sequences for rabbit RGS4 (GenBank Accession No. DQ120011) were forward 5′-ATGTGCCAAAGGACTTGCAGTGC-3′ and reverse 5′-GTGA-GAATTGACACACTGGG-3′, generating a fragment of 624 bp. The primer sequences for rabbit GAPDH (GenBank Accession No. DQ403051) were forward 5′-TCCATCTTTCACAGGAAGCGCA-3′ and reverse 5′-CACATCAGGACGTGTCCT-3′, generating a fragment of 292 bp. The PCR product was purified and cloned into the T-A vector for confirmation by sequencing.

Real-time PCR analysis was carried out on the ABI Prism 7300 Sequence Detection System (Applied Biosystems, Foster, CA). Expression of RGS4 was analyzed using the TaqMan PCR Master Mix Reagent Kit (Applied Biosystems). The TaqMan probe and primers for rabbit RGS4 designed using Primer Express (version 2.0) were as follows: forward (nucleotides 232–252, exon 2) 5′-TCCACAG-CAGGAAGACAAA-3′; reverse (nucleotides 303–284, exon 3) 5′-TTCCGCCCCATTCTGACCTT-3′; and probe (nucleotides 254–279, across exons 2 and 3 with 321 bp of intron 2) 5′-TGGACTCAC-CCTCTGGCACCACACACCA-3′. cDNA was synthesized from 500 ng RNA using the TaqMan RT Reagent Kit (Applied Biosystems). The optimized concentrations for real-time PCR were 0.4 μM for both primers and 0.2 μM for probe and 5 ng cDNA in a 20-μl reaction volume. Rabbit GAPDH primers (forward 5′-CGCTTGAGAAG- GCTGCTA-3′ and reverse 5′-CGACCTGTCCTCCTGTTAG-3′) were used as internal controls. Each sample was tested in triplicate. Cycle threshold (Ct) values were obtained graphically for RGS4 and GAPDH. The difference in Ct values between GAPDH and RGS4 were represented as ΔCt values. ΔΔCt values were obtained by subtracting ΔCt values of control samples from those of treated samples. The relative fold change in gene expression was calculated as 2^-ΔΔCt.

Immunofluorescent cytochemistry and semiquantitative analysis. SMCs were seeded on eight-well glass chamber slides (Nalge Nunc, Lab-Tek, Rochester, NY) and cultured until full confluence. After 24 h of serum starvation, cells were treated with IL-1β for different conditions.
MAPK/PI3K MODULATE RGS4 UPREGULATION IN SMOOTH MUSCLE

Activation of MAPK by IL-1β in rabbit colonic SMCs. The activation of ERK1/2 and p38 MAPK in rabbit colonic SMCs was examined by determining the level of phosphorylation of ERK1/2 at Thr202/Tyr204 and p38 MAPK at Thr180/Tyr182. As shown in Fig. 1, exposure of cultured and serum-starved rabbit colonic smooth muscle cells to IL-1β (10 ng/ml) led to a rapid and transient phosphorylation of ERK1/2 and p38 MAPK. Parallel experiments using freshly isolated colonic SMCs were performed. As shown in Fig. 1B, IL-1β treatment of freshly isolated SMCs induced a similar pattern of activation to p38 MAPK. In addition, the constitutive phosphorylation of MAPK was detectable in freshly isolated muscle cells, which may result from residual serum. A similar pattern of activation occurred with ERK1/2 and PI3K (data not shown).

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and specificity of the inhibitors were validated by Western blot analysis in cultured SMCs after IL-1β exposure for 15 min using anti-phospho-specific antibody against the corresponding MAPK (Fig. 2A). Treatment with either inhibitor alone for 4 and 24 h did not affect the basal expression of RGS4 in serum-starved colonic SMCs (data not shown). Pretreatment with either PD-98059 (20 μM) or SB-203580 (1 μM) for 1 h before IL-1β exposure for 3 h significantly inhibited IL-1β-induced increases in the expression of RGS4 mRNA and protein, as determined by real-time RT-PCR (Fig. 2B) and Western blot analysis (Fig. 2, C and D), respectively. These data suggest that ERK1/2 and p38 MAPK are stimulatory for IL-1β-induced upregulation of RGS4 expression.

**ERK1/2 enhances RGS4 upregulation via NF-κB activation, whereas p38 MAPK enhances RGS4 expression independently of NF-κB signaling.** The canonical IKK2/IκB-α pathway of NF-κB activation mediates IL-1β-induced upregulation of RGS4 (36). To address the interaction between the ERK1/2 and/or p38 MAPK pathways and the NF-κB signaling pathway, we performed Western blot analysis and immunocytochemistry in cells treated with selected MAPK inhibitors. The MEK inhibitor (PD-98059, 20 μM) almost completely blocked (from 26- to 3-fold) IL-1β-induced phosphorylation of p65 (Ser536; Fig. 3A), implying that the effect of ERK1/2 on RGS4 expression was dependent on NF-κB signaling, predominantly at the level of p65. The MEK inhibitor also inhibited the phosphorylation of IKK2 (Ser177/Ser181, from 112- to 13-fold) and the degradation of IκB-α (from 99% to 63%), suggesting that ERK1/2 also exerted its effect at least partially on IκB-α and IKK2 or its upstream signaling components. On the other hand, the p38 MAPK inhibitor (SB-203580, 1 μM) had a marginal effect on IκB-α degradation and p65 phosphorylation (Fig. 3A), implying that NF-κB activation may not be the predominant target for the effect of p38 MAPK on RGS4 expression. However, SB-203580 partially inhibited the phosphorylation of IKK2 (from 112- to 55-fold), implying that p38 MAPK may regulate IKK2 activity that involves other signaling pathways in addition to the canonical IKK2/IκB-α/NF-κB signaling pathway.

To further corroborate the effect of the selected MAPK inhibitors on NF-κB activation, we determined the nuclear translocation of p65 in serum-starved SMCs by immunofluorescent cytochemistry and semiquantitative analysis. As shown in Fig. 3, B and D, pretreatment with PD-98059 prevented IL-1β-induced p65 nuclear translocation, whereas SB-203580 induced a partial inhibition of IL-1β-induced p65 nuclear translocation. Treatment with PD-98059 or SB-203580 alone did not affect the constitutive cellular distribution of p65 (Fig. 3B).

**IL-1β activates PI3K in rabbit colonic circular SMCs.** In multiple cell types, IL-1β has been shown to activate PI3K (33). To address whether IL-1β activates PI3K in rabbit colonic SMCs, we examined the phosphorylation of Akt at Ser473 and GSK3β at Ser9, two sequential downstream effectors of PI3K, by performing Western blot analysis with phospho-specific antibodies. As shown in Fig. 4A, IL-1β treatment caused the phosphorylation of Akt and GSK3β, implying that IL-1β activates PI3K.

**PI3K inhibits IL-1β-induced upregulation of RGS4.** As previously shown (37), IL-1β treatment of colonic SMCs induced an acute and long-term effect on RGS4 expression (Fig. 4, B and C). Pretreatment with the selective PI3K inhibitor (LY-294002, 10 μM) enhanced IL-1β-induced upregulation of RGS4 mRNA expression, whereas Src inhibitor I (10 μM) had no effect on RGS4 expression (Fig. 4, B and C). This was confirmed by Western blot analysis of RGS4 protein expression (Fig. 4D). The affinity-purified anti-RGS4 antibody recognizes one band of endogenous RGS4 protein but two bands of overexpressed HA-RGS4 fusion protein, consistent with a previous report (36). LY-294002 treatment alone had a marginal effect on the constitutive expression of RGS4 mRNA and protein (Fig. 4, B and D). These data suggest that the activation of PI3K by IL-1β plays an inhibitory role in regulating IL-1β-induced upregulation of RGS4 expression.
PI3K inhibits RGS4 upregulation via inactivation of GSK3β signaling. PI3K phosphorylates Akt, which further induces the phosphorylation and thus inactivation of GSK3β in distinct cell types. This concept was corroborated in rabbit colonic SMCs. As shown in Fig. 5A, IL-1β-induced phosphorylation of GSK3β in rabbit SMCs was blocked by either the PI3K inhibitor (LY-294002) or Akt inhibitor (API-2), implying that the PI3K/Akt pathway mediates IL-1β-induced GSK3β phosphorylation. The effectiveness of LY-294002 to inhibit PI3K was verified by Western blot analysis by examining the phosphorylation of Akt at Ser473 (Fig. 5A). GSK3β is inactive upon phosphorylation. As predicted, IL-1β-induced GSK3β phosphorylation was augmented by LiCl (Fig. 5B). As shown above, the activation of endogenous PI3K/Akt signaling by IL-1β inhibits RGS4 expression through inactivating GSK3β signaling by increasing the phosphorylation of GSK3β. Exogenous addition of GSK3β inhibitors also inactivate GSK3β signaling by increasing the phosphorylation of GSK3β and ultimately generate a synergistic role with IL-1β-initiated activation of PI3K/Akt signaling (see the signaling model in Fig. 7).

GSK3β stimulates the canonical IKK2/IκB-α/NF-κB pathway. Phosphorylation of IKK2, IκB-α, and p65 induced by IL-1β treatment for 15 min was augmented by pretreatment with the PI3K inhibitor LY-294002 (10 μM). In addition, LY-294002 treatment alone induced the constitutive phosphorylation of IκB-α and p65 (Fig. 6A), leading to a marginal increase in the constitutive expression of RGS4 (Fig. 4, B and D). Therefore, endogenous PI3K retains a tonic inhibition on IKK2-mediated NF-κB signaling in colonic SMCs. To further confirm the role of the canonical IKK2/IκB-α pathway of NF-κB activation in mediating the enhancing effect of PI3K inhibition, the selective inhibitors for IKK2 and IκB-α were
used to pretreat the cells before treatment with LY-294002 and IL-1β. Indeed, either the IKK2 inhibitor IKK2-IV or IκB-α degradation inhibitor MG-132 significantly blocked the LY-294002-induced enhancement of IL-1β-stimulated upregulation of RGS4 expression (Fig. 6B).

Interestingly, the GSK3β inhibitor LiCl abolished the IL-1β-induced phosphorylation of IKK2 and p65 (Fig. 6C), implying that GSK3β by itself is capable of activating IKK2 through increasing the phosphorylation of the IKK2 activation loop. The inactivation of GSK3β via inhibitory phosphorylation by PI3K/Akt (Fig. 5A) leads to the inhibition of IKK2 and p65 phosphorylation. Therefore, the IL-1β-induced activation of PI3K/Akt negatively modulates IL-1β-induced NF-κB activity and subsequent RGS4 upregulation through inhibiting GSK3β activity.

**DISCUSSION**

RGS4 is a member of the R4 family of RGS proteins, which has been best investigated at the structural, biochemical, and functional levels (53, 59, 79, 80, 98, 99). However, the regulatory (molecular) mechanism of RGS4 expression has not been fully understood. In our previous study (36), we demonstrated that the proinflammatory cytokine IL-1β transcriptionally upregulates RGS4 expression through the canonical IKK2/IκB-α pathway of NF-κB activation in rabbit colonic SMCs. Upregulated RGS4 has been implicated in IL-1β-induced inhibition of acetylcholine-stimulated initial contraction of colonic SMCs (37). Here, we show that the ERK1/2 and p38 MAPK pathways enhance, whereas the PI3K/Akt/GSK3β pathway inhibits, IL-1β-induced upregulation of RGS4 expression.
The effects of the ERK1/2 and PI3K/Akt/GSK3β pathways are exerted through the IKK2/NF-κB pathway, whereas the effect of the p38 MAPK pathway may not involve NF-κB signaling. These data are summarized in a proposed model showing the signaling pathway and regulatory mechanism for RGS4 induction by IL-1β in colonic SMCs (Fig. 7).

IL-1β is well known to stimulate all of the three MAPKs in SMCs of the vasculature, airway, and intestine. However, the role and outcome of these MAPK pathways are different. In airway SMCs, IL-1β-induced upregulation of COX-2 and eotaxin is inhibited by either MEK1 inhibitors or p38 MAPK inhibitors (34, 87, 103), whereas IL-1β-induced release of the protein RANTES (regulated upon activation, normal T cell expressed, and secreted) is sensitive to inhibition of MEK1 (32) or JNK (71) but not to inhibition of p38 MAPK (32). IL-1β-induced upregulation of matrix metalloproteinase-9 (60) and TNF-α-induced expression of VCAM-1 (57) are sensitive to the inhibition of all the three MAPK pathways. In vascular SMCs, IL-1β-stimulated inducible nitric oxide synthase (iNOS) expression is prevented by MEK1 inhibition but potentiated by p38 MAPK inhibition (27, 30). Inhibition of MEK1 or p38 MAPK, but not PI3K, reduced IL-1β-stimulated expression of LIM domain kinase 2 and cofilin (11). However, in human vascular SMCs, IL-1β activates only p38 MAPK, which mediates IL-1β-induced IL-8 and VEGF expression (46, 47). In colonic SMCs, IL-1β-induced H2O2 production is inhibited by MEK inhibitor but not p38 MAPK inhibitor (18), whereas IL-1β-induced upregulation of IL-6, IL-8, and COX-2 is reduced by p38 MAPK inhibitor but not MEK-1 inhibitor (81). Sphingosylphosphorylcholine-induced contraction of ileal SMCs is blocked by MEK-1 inhibitor but not p38 MAPK inhibitor (58). In animal colitis induced by 2,4,6-trinitrobenzene sulfonic acid, ERK1/2 mediates the restoration of reduced muscle contractility by meloxicam, a COX-2 inhibitor (50). In the present study, IL-1β-induced upregulation of RGS4 mRNA is sensitive to inhibition of both MEK1 and p38 MAPK. Therefore, the functional significance of MAPK activation in SMCs relies on the stimuli, target genes, and cell resources.

The cross-talk between the MAPK and NF-κB signaling pathways is not well understood. The MEK/ERK pathway has been widely shown to affect IL-1β-induced NF-κB activation. In cultured rat vascular SMCs, ERK is required for IL-1β-induced persistent NF-κB activation, whereas IL-1β induces...
only acute and transient NF-κB activation without ERK activation (42, 43). Inhibition of ERK does not affect IL-1β-induced phosphorylation and degradation of IkB-α but attenuates the degradation of IkB-β and, therefore, inhibits the expression of iNOS and COX-2 but has no effect on the expression of VCAM-1 and Mn-SOD (44). In the present study, we demonstrate, for the first time, that ERK activation enhances IL-1β-induced NF-κB activation and RGS4 upregulation at the levels of p65, IkB-α, and IKK2. We also show that compared with the ERK pathway, the p38 MAPK pathway has less effect on NF-κB activation in rabbit colonic SMCs. This concurs with previous reports in airway SMCs (40, 87, 96) and vascular SMCs (92, 101). In contrast, p38 MAPK has been shown preferentially to regulate the mRNA stability of IL-1β target genes such as COX-2, iNOS, IL-6, etc. (56, 76, 87, 97). In conclusion, we demonstrate, for the first time, that IL-1β-induced upregulation of RGS4 expression by IL-1β-converting enzyme (ICE), p65 and subsequent upregulation of RGS4 mRNA expression in rabbit colonic SMCs. The mechanisms for the PI3K-negative modulation on IL-1β-induced NF-κB activation and target gene expression are not well understood. Akt, the downstream substrate of PI3K, has been shown to increase p65 phosphorylation and NF-κB activation (7, 24). For example, GSK3β, a serine/threonine kinase, attenuates the degradation of IκBα and, therefore, inhibits the activation of NF-κB DNA-binding activity. In monocytes or peripheral blood mononuclear cells, GSK3β regulates the association of p65 with the nuclear coactivator cAMP response element-binding protein (CREB) and, therefore, inhibits the DNA-binding activity of NF-κB (64).

On the other hand, several studies have suggested a negative regulation of GSK3β on NF-κB signaling in primary astrocytes (52, 82), renal medullary interstitial cells (78), the rat pheochromocytoma PC12 cell line (12), and vascular SMCs (65). Therefore, the modulatory effects and specific levels of GSK3β on NF-κB signaling depend on the cell types, stimuli, and target genes.

In conclusion, we demonstrate, for the first time, that IL-1β-induced upregulation of RGS4 expression is differentially modulated by MAPKs and PI3K/Akt/GSK3β signaling, which directly or indirectly influence the activation of NF-κB signaling. Increased RGS4 expression by IL-1β contributes to the deactivation of Gαq signaling and inhibition of initial muscle contraction during inflammatory responses of the gut. Intervention of these signaling pathways may be a potential target for the pharmaceutical industry as well as clinical therapy.

**REFERENCES**

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