Mitogen-activated protein kinases regulate COX-2 and mucosal recovery in ischemic-injured porcine ileum

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Shifflett, Donnie E., Samuel L. Jones, Adam J. Moeser, and Anthony T. Blikslager. Mitogen-activated protein kinases regulate COX-2 and mucosal recovery in ischemic-injured porcine ileum. Am J Physiol Gastrointest Liver Physiol 286: G906–G913, 2004. First published February 5, 2004; 10.1152/ajpgi.00478.2003.—Mitogen-activated protein kinase (MAPK) pathways transduce signals from a diverse array of extracellular stimuli. The three primary MAPK-signaling pathways are the extracellular regulated kinases (ERK1/2), p38 MAPK, and c-Jun NH2-terminal kinase (JNK). Previous research in our laboratory has shown that COX-2-elaborated prostanoids participate in recovery of mucosal barrier function in ischemic-injured porcine ileum. Because COX-2 expression is regulated in part by MAPKs, we postulated that MAPK pathways would play an integral role in recovery of injured mucosa. Porcine mucosa was subjected to 45 min of ischemia, after which tissues were mounted in Ussing chambers, and transepithelial electrical resistance (TER) was monitored as an index of recovery of barrier function. Treatment of tissues with the p38 MAPK inhibitor SB-203580 (0.1 mM) or the ERK1/2 inhibitor PD-98059 (0.1 mM) abolished recovery. Western blot analysis revealed that SB-203580 inhibited upregulation of COX-2 that was observed in untreated ischemic-injured mucosa, whereas PD-98059 had no effect on COX-2 expression. Inhibition of TER recovery by SB-203580 or PD-98059 was overcome by administration of exogenous prostaglandin E2 (1 μM). The JNK inhibitor SP-600125 (0.1 mM) significantly increased TER and resulted in COX-2 upregulation. COX-2 expression appears to be positively and negatively regulated by the p38 MAPK and the JNK pathways, respectively. Alternatively, ERK1/2 appear to be involved in COX-2-independent reparative events that remain to be defined.

INTRODUCTION

The mitogen-activated protein kinases (MAPKs) are an evolutionarily conserved family of enzymes that form a highly integrated network to regulate cellular functions such as differentiation, proliferation, and cell death. Thus MAPKs transduce extracellular signals into intracellular responses. At least three distinct MAPK groups have been identified: extracellular regulated kinases 1 and 2 (ERK1/2), p38 MAPK, and c-Jun NH2-terminal kinase (JNK). MAPK signaling cascades are viewed as parallel pathways, although cross talk may exist (18, 34). Upon phosphorylation and subsequent activation of an MAPK, the MAPK can enter the cell nucleus and activate transcription factors. This ultimately results in expression of target genes and a biological response (15).

One biologically responsive gene is COX-2 (15, 26, 33). There are three known isoforms of cyclooxygenase (COX): COX-1, COX-2, and COX-3 (10, 28). Although COX-1 is constitutively expressed in most tissues, COX-2 is inducible under a variety of conditions, including exposure to select cytokines and growth factors (16, 27), experimental colitis (1) and ischemia (8). Both COX-1 and COX-2 appear to play a role in recovery of ischemic-injured intestinal mucosa (8, 23) and in colonic mucosal defense (25, 30). Furthermore, it has been shown that COX-2 is expressed in the epithelium at the margins of experimentally induced gastric ulcers in rats and that selective inhibition of COX-2 retards epithelial repair (23). Alternatively, although inhibition of COX-2 did not interrupt recovery of barrier function in ischemic-injured mucosa in previous studies from our laboratory, COX-2 prostanoids were able to stimulate recovery in the absence of COX-1 prostanoids (8). In additional studies from our laboratory (unpublished observations), we noted that IL-1β was able to enhance recovery of ischemic-injured ileal mucosal in a COX-2-dependent fashion.

The purpose of the present study was to investigate the role of the MAPK pathways in regulating COX-2 has yielded conflicting results. It has been shown in various intestinal epithelial cell lines, including I407, Caco-2, and HT-29 cells, that basic fibroblast growth factor (bFGF) increases COX-2 expression via p38 MAPK (32). However, in porcine aortic smooth muscle cells, bFGF-induced COX-2 expression was inhibited by PD-98059, an ERK pathway inhibitor, indicating an important role for ERK1/2 (19). In rat mesangial cells, JNK and p38 MAPK mediate IL-1β-stimulated COX-2 expression (13, 14), whereas ERK and p38 MAPK regulate COX-2 expression induced by transforming growth factor (TGF)-α and interferon-γ in human epidermal keratinocytes and squamous carcinoma cells (21). In NIH-3T3 cells, regulation of COX-2 by platelet-derived growth factor is mediated through the ERK and JNK pathways (35). Thus it is apparent that the MAPK pathways are critical in regulating COX-2 expression, but there is considerable variability in this regulation depending on the cell type and stimulus.

MATERIALS AND METHODS

Experimental surgery. All procedures were approved by the North Carolina State University Institutional Animal Care and Use Com-
mittee and have been previously described in detail (5, 6, 7). Briefly, 6- to 8-wk-old Yorkshire-cross pigs of either sex were anesthetized using a combination of xylazine (1.5 mg/kg) and ketamine (11 mg/kg). Pigs were intubated via a tracheostomy, placed on a heating pad, and ventilated with 100% \( \text{O}_2 \) via a tracheotomy using a time-cycled ventilator. Anesthesia was maintained using periodic intravenous administration of a 5% thiopental sodium solution via a jugular catheter. Maintenance fluids were administered intravenously at a rate of 15 ml·kg\(^{-1} \)·h\(^{-1} \) throughout the surgery. The ileum was located via a midline incision, after which 10-cm ileal segments were ligated and subjected to ischemia by ligating the local mesenteric blood supply. Additional 10-cm ileal loops not subjected to ischemia were utilized as control tissues. After 45 min of ischemia, pigs were euthanized with an overdose of pentobarbital sodium, and the 10-cm ileal loops were promptly removed and placed in oxygenated Ringer solution (95% \( \text{O}_2 \)-5% \( \text{CO}_2 \)).

**Ussing chamber studies.** The mucosa was stripped from the serosal surface and mounted in Ussing chambers. The tissues were bathed on both serosal and mucosal sides with 10 ml of oxygenated (95% \( \text{O}_2 \)-5% \( \text{CO}_2 \)) Ringer solution. In addition, the serosal bathing solution contained 10 mM glucose, which was osmotically balanced by 10 mM mannitol on the mucosal side. Bathing solutions were circulated in water-jacketed reservoirs and maintained at 37°C. The transmucosal potential difference (PD) was measured using Ringer-agar bridges connected to calomel electrodes, and the PD was short-circuited through Ag-AgCl electrodes by means of a voltage clamp that corrected for fluid resistance. Resistance (\( \Omega \)-cm\(^2 \)) was calculated from the spontaneous PD and short-circuit current (\( I_s \)). If the spontaneous PD was between 1.0 and 1.0 mV, tissues were current clamped at \( \pm 100 \mu \text{A} \) for 5 s and the PD recorded. Short-circuit current and PD were recorded every 15 min for 180 min.

**Experimental treatments.** The JNK inhibitor SP-600125 was purchased from Sigma (St. Louis, MO).

**Histology.** Tissues were taken immediately following ischemia and following the 180-min recovery period for histological evaluation. Tissues were sectioned (5 μm) and stained with hematoxylin and eosin. For each tissue, an investigator blinded to the treatment group evaluated three sections. The height of the villus and the width at the midpoint of the villus were obtained using a light microscope with an ocular micrometer. For height measurements, the base of the villus was defined as the intersection between adjacent villi at the opening of the crypt. For villi in which the height of one side of the villus was disparate from that of the other side the average height was recorded. In addition, the height of the epithelial covered portion of each villus was measured. The surface area of the villus was calculated using the formula for the surface area of a cylinder. The formula was modified by subtracting the area of the base of the villus and multiplying by a factor accounting for the variable position at which each villus was cross-sectioned. In addition, the formula was modified by a factor that accounted for the hemispherical shape of the upper portion of the villus (3). The percentage of the villous surface area that remained denuded was calculated from the total surface area of the villus and the surface area of the villus covered by epithelium. The percentage of denuded villous surface area was used as an index of epithelial restitution.

**Gel electrophoresis and Western blotting.** Tissues for total MAPK and phospho-MAPK Western blots were taken immediately after ischemia and before mounting in the Ussing chambers. Tissues mounted in the Ussing chambers were removed upon completion of the collection of electrical data (180 min) for COX-2 Western blots. Mucosal scraping was performed to remove additional connective tissue, after which tissue was snap-frozen in liquid nitrogen and stored at \(-70^\circ \text{C} \) until analysis for COX-2 protein. In preparation for SDS-PAGE, tissues were thawed to \( 4^\circ \text{C} \). Tissue portions were added to 1 ml of chilled RIPA buffer (0.15 M NaCl, 50 mM Tris, pH 7.2, 1% deoxycholic acid, 1% Triton X-100, 0.1% SDS), including protease inhibitors (PMSE and aprotinin). The mixture was homogenized on ice and then centrifuged twice at 10,000 g for 10 min at \( 4^\circ \text{C} \) and the supernatant saved. Protein analysis of extract aliquots was performed (DC protein assay; Bio-Rad, Hercules, CA). Tissue extracts (amounts equalized by protein concentration) were mixed with an equal volume of 2× SDS-PAGE sample buffer and boiled for 4 min at \( 100^\circ \text{C} \). Lysates were loaded on a 10% SDS-polyacrylamide gel, and electrophoresis was carried out according to standard protocols. Proteins were transferred to a nitrocellulose membrane (Hybond ECL; Amersham Life Science, Birmingham, UK) by use of an electrophoretic minitransfer apparatus according to the manufacturer’s protocol. Membranes were blocked in Tris-buffered saline (TBS) containing 0.05% (TweeTBS and TBS) and 5% dry powdered milk. Membranes were washed three times for 5 min each with TBS and incubated for 2 h at room temperature in primary antibody. After being washed three times for 5 min each with TBS-T, the membranes were incubated for 1 h with horseradish peroxidase-conjugated secondary antibody. After being washed twice for 5 min each with TBS-T and once with TBS for 15 min, the membranes were developed for visualization of protein by the addition of enhanced chemiluminescence reagent (Amersham, Princeton, NJ).

**Data analysis.** All data were analyzed using a statistical software package (Sigmasoft; Jandel Scientific, San Rafael, CA). Data were reported as means \( \pm \) SE for a given number (n) of animals for each experiment. Results were analyzed by two-way ANOVA on repeated measures. For analyses where significance was detected by ANOVA, Tukey’s test was utilized for post hoc pairwise multiple comparisons. The \( \alpha \)-level for statistical significance was set at \( P < 0.05 \). Where a significant treatment interaction was noted, a one-way ANOVA was utilized to differentiate individual treatment effects.

**RESULTS**

**Effect of ischemia on activation of MAPKs.** To clarify the role of the MAPKs in regulating recovery from ischemia, we first sought to determine whether subjecting ileal mucosa to ischemic injury resulted in MAPK activation. Accordingly, mucosa scraped from normal ileum (control) and ileum exposed to 45 min of ischemia was analyzed by Western blot for the total levels of each of the three primary MAPKs and the phosphorylated (activated) forms of MAPKs (Fig. 1). Total protein levels for each of the MAPKs were unchanged in ischemic-injured tissue, whereas ischemia resulted in marked
increases in the phosphorylated forms of p38 MAPK, ERK1/2, and JNK compared with control mucosa (Fig. 1).

In vitro recovery of ischemic-injured ileal mucosa. Porcine ileum was subjected to 45 min of ischemia in vivo, after which normal (control) and ischemic-injured mucosa was removed and monitored over 180 min in Ussing chambers. Histological analyses revealed a loss of ~20% of the apical villous epithelium following ischemia, and restitution was complete within 60 min (data not shown), as reported in previous publications from our laboratory (5, 7). Ischemic-injured mucosa had transepithelial electrical resistance (TER) values that were significantly lower than control values and mucosal-to-serosal fluxes of mannitol that were significantly higher than in control tissues during the early part of the recovery period (Fig. 2). Ischemic-injured tissues recovered control levels of these indexes of barrier function within 120–135 min. The latter phases of recovery were presumed to result from recovery of paracellular resistance because of the early completion of restitution, as shown in previous studies (5, 7).

Effect of ERK1/2 inhibition on TER. In further studies, we sought to determine the role of MAPKs in recovery of TER in vitro. A range of doses (0.1 mM to 1 μM) of the ERK1/2 inhibitor PD-98059 was applied to ischemic-injured tissues, resulting in dose-dependent inhibition of recovery of TER (Fig. 3A). PD-98059 significantly (P < 0.05) inhibited recovery at a dose of 0.1 mM, whereas lower doses (0.01 mM and 1 μM) had no effect on recovery of ischemic-injured mucosa. Uninjured tissue was also exposed to 0.1 mM PD-98059, which had no effect (data not shown). Histological analysis revealed that ERK1/2 inhibition had no effect on histological indexes of restitution compared with untreated ischemic-injured mucosa.

Because we have previously shown that recovery of TER in this tissue is regulated largely by prostaglandins (PGs) (5, 6, 7), we next wanted to determine whether we could reverse the effect of PD-98059 by addition of exogenous 16,16-dimethyl prostaglandin E2 (PGE2). PGE2 at a dose of 1 μM caused a significant (P < 0.05) increase in TER of ischemic-injured ileum when added alone, and PGE2 also overcame inhibition of TER by 0.1 mM PD-98059 (Fig. 3B). However, tissues treated with PGE2 and PD-98059 did not reach the same levels as tissues treated with PGE2 alone, suggesting a continued partial inhibitory effect of PD-98059 on mucosal recovery.

In previous experiments on porcine ileal mucosa (8), we have shown upregulation of COX-2 following ischemic injury, and we have shown that COX-2 participates in recovery of TER. Therefore, we wanted to examine the possibility that ERK inhibition decreased COX-2 expression. However, there was no effect of PD-98059 on COX-2 protein expression levels (Fig. 3C). Thus blockade of TER recovery with ERK1/2 inhibition appeared to be independent of COX-2 expression.

Effect of p38 MAPK inhibition on TER. To determine whether other signaling kinases played a role in recovery of ischemic-injured mucosa, the p38 MAPK inhibitor SB-203580 was placed on ischemic-injured porcine ileum. Mucosal recovery was inhibited in a dose-dependent fashion with SB-203580 (Fig. 4A). Similarly to ERK1/2 inhibition, less concentrated doses of the p38 MAPK inhibitor (0.01 mM and 1 μM) had no effect on TER compared with untreated ischemic-injured porcine ileum, whereas 0.1 mM SB-203580 significantly inhibited recovery of TER. Uninjured tissue was also exposed to 0.1 mM SB-203580, but there were no observable effects (data not shown). Additionally, histological analysis revealed that p38 inhibition had no significant effect on restitution compared with untreated ischemic-injured mucosa.

Addition of 1 μM PGE2 to ischemic-injured tissue significantly (P < 0.05) reversed the inhibitory effect of 0.1 mM SB-230580 (Fig. 4B). Western blot analysis was also performed for COX-2 protein expression in untreated ischemic-injured ileum and in tissue treated with SB-203580. In contrast to our experiments with the ERK inhibitor PD-98059, 0.1 mM SB-203580 decreased COX-2 protein expression compared with untreated ischemic-injured tissue (Fig. 4C).

Effect of JNK inhibition on TER. Because we had noted inhibition of COX-2 expression and recovery of TER with an inhibitor of p38 MAPK, we also wanted to assess the other stress-activated protein kinase pathway, JNK. Low doses (0.01 mM and 1 μM) of the JNK inhibitor SP-600125 had no effect

Fig. 2. Recovery of barrier function of ischemic-injured porcine ileal mucosa as assessed by changes in transepithelial electrical resistance (TER) and mucosal-to-serosal fluxes (Jms) of mannitol. A: at the beginning of the recovery period, TER of ischemic-injured mucosa was significantly below that of uninjured control tissues (*P < 0.05). Ischemic-injured mucosa reached TER values not significantly different from those of control mucosa by 135 min. B: corresponding Jms of [3H]mannitol revealed significant increases in flux of this paracellular probe by 60 min of the recovery period, whereas fluxes reached levels not significantly different from those of control by 120 min (*P < 0.05). Values represent means ± SE; n = 8.
on recovering ischemic-injured ileum, similar to the responses we observed with low doses of ERK1/2 and p38 inhibitors. Conversely, we observed a significant ($P < 0.05$) increase in recovery of TER over time with a more concentrated dose (0.1 mM) of SP-600125 (Fig. 5A). SP-600125 (0.1 mM) was also

Fig. 3. Response of ischemic-injured (Isch) porcine ileal mucosa to addition of the ERK inhibitor PD-98059 (PD). A: there was significant inhibition of recovery of TER in the presence of 0.1 mM PD-98059 by the end of the recovery period ($*P < 0.05$), whereas less concentrated doses of PD-98059 had no effect on TER compared with untreated Isch mucosa (CONT). Values represent means ± SE; $n = 8$. B: recovery of TER in tissue treated with 0.1 mM PD-98059 was significantly suppressed (*$P < 0.05$) compared with untreated Isch mucosa, whereas Isch tissue treated with prostaglandin (PGE$_2$) had a significant increase (+$P < 0.05$) in TER. Tissue treated with 1 μM PGE$_2$ in the presence of PD-98059 had a significantly ($#P < 0.05$) increased TER compared with untreated Isch tissue but did not attain TER values of PGE$_2$ alone. Values represent means ± SE; $n = 8$. C: Western analysis for cyclooxygenase (COX)-2 protein expression indicated that COX-2 was upregulated in Isch mucosa compared with control mucosa. However, COX-2 protein expression appeared unchanged in tissue treated with 0.1 mM PD-98059 compared with untreated Isch tissue. Blot is representative of 4 experiments.

Fig. 4. Response of Isch porcine ileal mucosa to addition of the p38 MAPK inhibitor SB-203580 (SB). A: addition of 0.1 mM SB-203580 to Isch mucosa significantly suppressed recovery of TER (*$P < 0.05$), whereas less concentrated doses of SB-203580 had no effect on TER compared with untreated Isch mucosa. Values represent means ± SE; $n = 8$. B: recovery of TER in tissue treated with 0.1 mM SB-203580 was significantly suppressed (*$P < 0.05$) compared with untreated Isch mucosa, whereas Isch tissue treated with PGE$_2$ had a significant increase (+$P < 0.05$) in TER. Tissue treated with 1 μM PGE$_2$ in the presence of SB-203580 had a significantly ($#P < 0.05$) increased TER compared with untreated Isch tissue but did not attain TER values of PGE$_2$ alone. Values represent means ± SE; $n = 8$. C: Western analyses for COX-2 protein expression indicated that COX-2 protein expression was reduced in tissue treated with 0.1 mM SB-203580 compared with untreated Isch tissue. Blot is representative of 4 experiments.
placed on uninjured tissue and had no effects (data not shown). Additionally, histological analysis revealed that JNK inhibition had no significant effect on restitution compared with untreated ischemic-injured mucosa.

PGE$_2$ (1 µM) was utilized in combination with SP-600125 to determine whether there would be an additive increase in TER. The combination of 0.1 mM SP-600125 and 1 µM PGE$_2$ caused a significant ($P < 0.05$) increase in TER compared with either treatment alone (Fig. 5B). Western blot analysis for COX-2 protein expression in untreated ischemic-injured porcine ileum and in ischemic-injured ileum treated with 0.1 mM SP-600125 showed that the increase in TER observed with 0.1 mM SP-600125 correlated with increased expression of COX-2 (Fig. 5C). Furthermore, the increase in TER observed with 0.1 mM SP-600125 was blocked by the addition of a COX-2 selective inhibitor, NS-398 (5 µM; Fig. 6).

**Effect of dual inhibition of JNK and p38 MAPK on TER.** Our data showed that JNK inhibition increases TER, whereas p38 MAPK inhibition resulted in blockade of TER compared with untreated ischemic-injured mucosa. Thus we next sought to determine whether one of these pathways had a more dominant role in recovering ischemic-injured ileum. Accordingly, p38 MAPK inhibition with SB-203580 (0.1 mM) abolished recovery of TER, and application of the JNK inhibitor SP-600125 (0.1 mM) significantly increased peak TER, whereas a combination of SP-600125 and SB-203580 resulted in an inhibitory response similar to that of SB-203580 treatment alone (Fig. 7A). Furthermore, dual inhibition of p38 MAPK and JNK resulted in reduced COX-2 protein expression (Fig. 7B).

**DISCUSSION**

MAPKs form a highly integrated network to regulate various cellular processes. Using a porcine model of intestinal ischemia we have shown that 1) ERK1/2 and p38 MAPK positively regulate recovery from ischemia, whereas JNK negatively regulates recovery from ischemia; 2) ERK1/2 do not regulate COX-2 expression, whereas p38 MAPK and JNK positively and negatively regulate COX-2 protein expression, respectively; and 3) p38 MAPK appears to have a dominant role over
JNK in regulating recovery from ischemia. There has been recent, intensive clinical interest in inhibition of p38 MAPK for a variety of clinical conditions. For example, there have been more than 48 patent applications from more than 15 pharmaceutical companies for inhibitors of p38 MAPK. Inhibitors of p38 MAPK are being developed for various conditions, including rheumatoid arthritis, psoriasis, and inflammatory lung diseases (9). Furthermore, p38 MAPK and JNK inhibition have been evaluated for treatment of Crohn’s disease. CNI-1493, an inhibitor of both p38 MAPK and JNK, resulted in endoscopic healing and significant clinical improvement in patients suffering from Crohn’s disease (17). Despite the fact that CNI-1493 purportedly inhibits p38 MAPK and JNK, the in vivo effects of CNI-1493 appeared to be attributable to a reduction in JNK activity. This would lend support to our study, which showed that inhibition of JNK results in enhanced recovery barrier function, whereas inhibition of p38 MAPK had a deleterious effect on mucosal recovery from ischemia.

Mechanisms of mucosal recovery in our model of porcine ischemic injury appear to relate predominantly to recovery of paracellular resistance. For instance, there was no effect of any of the treatments in the present study on epithelial restitution, despite significantly differing effects on TER. Furthermore, restitution is complete by 60 min, before full recovery of TER. In addition, we (5, 7) have previously shown morphological evidence of tight junction closure in response to PG administration, and recovery of TER is correlated with reductions of mucosal-to-serosal flux of the paracellular probe mannitol. The nature of mucosal recovery in vitro, as studied in the present series of experiments, may differ substantially from recovery noted in vivo. For example, in vitro recovery is a rapid process completed within 180 min, whereas in vivo recovery is not as rapid, requiring 4–6 h to complete, most likely because of the in vivo effects of neutrophils (12). Thus the in vitro model abolishes any effects of the classic pathway of reperfusion injury, which involves infiltration of neutrophils. However, it must be acknowledged that in vitro recovery does constitute reoxygenation of tissues by using solutions gassed with a 95% O2-5% CO2 mixture. This reoxygenation may provide the beneficial effects of reperfusion (return of an O2 source) without the deleterious effects of mucosal inflammation.

The present study suggests a role for COX-2 in mucosal recovery from ischemic injury. Previous studies have indicated that COX-2 is important for mucosal recovery in porcine ischemic-injured intestinal mucosa (8) and acetic acid-induced gastric ulcers in rodents (23). Evidence in support of the importance of COX-elaborated prostanoids in mucosal recovery from ischemic injury from our previous studies includes inhibition of recovery by treatment with the nonselective COX inhibitor indomethacin or treatment with the COX-1 and COX-2 inhibitors SC-560 and NS-398, respectively, and restoration of recovery following these treatments with addition of PGE2. More specifically, PGE2 appears to act via cAMP, a second messenger that has previously been shown to augment paracellular resistance (11). For example, administration of PGE2 resulted in an approximately twofold increase in tissue cAMP levels (7), and selective PGE receptor agonists and antagonists suggested a role for Gs-linked prostanoid receptors (4). Furthermore, addition of the cAMP agonist vasoactive intestinal polypeptide or 8-bromo-cAMP simulated the PGE2-induced recovery response (7). However, not all of the effects of the MAPKs shown in the present study necessarily have a direct link to prostanoid effects on mucosal recovery despite demonstrated effects on COX-2 expression. For example, PGE2 administration restored baseline levels of mucosal recovery in the presence of p38 MAPK inhibition but did not raise TER to levels in the presence of PGE2 alone by the end of the recovery period. Thus PGE2 and p38 MAPK may be operating in parallel, rather than sequential, pathways. The evidence in support of a direct role of JNK in prostanoid pathways is stronger, because inhibition of JNK enhanced COX-2 expression and recovery of TER, and JNK-enhanced mucosal recovery was inhibited by a selective COX-2 inhibitor. On the other hand, ERK pathways appear to regulate mucosal recovery without affecting COX-2 expression.

Regardless of the exact role of COX-2 in mucosal recovery, the present data provide insight into upstream regulators of COX-2 expression. It has been proposed that p38 MAPK regulates COX-2 expression at the posttranscriptional level by increasing mRNA stability in bFGF-stimulated human intestinal epithelial cells (32) and IL-1-stimulated intestinal myofibroblasts (22). In contrast, Guo et al. (15) have shown that bombesin-induced p38 MAPK activation regulates COX-2 transcription rather than mRNA stability. In agreement with our work, Tessner et al. (32) reported that p38 MAPK posi-
tively regulates COX-2 protein expression. In our model of intestinal ischemia, we have shown that p38 MAPK inhibition blocks upregulation of COX-2 protein expression, which appears to be critical for recovery.

Recent work also suggests that ERK1/2 can regulate COX-2 expression. For example, ERK1/2 inhibition suppresses COX-2 protein expression by ~76% in a human colonic subepithelial myofibroblast cell line stimulated with IL-1 (22) and inhibits proinflammatory cytokine-induced COX-2 mRNA levels in rat intestinal epithelial cells (15). In contrast, ERK1/2 inhibition has no effect on COX-2 protein expression in Caco-2 cells (2), and previous reports have demonstrated no effect of ERK1/2 inhibition on COX-2 mRNA stability (22, 24). Data presented herein demonstrate that ERK1/2 inhibition has no effect on COX-2 protein expression in ischemic-injured porcine ileal mucosa, yet is capable of suppressing recovery. Previous research from our laboratory has shown that recovery of TER is largely correlated with recovery of paracellular resistance and that prostanoids have no perceptible effect on epithelial restitution (5, 6, 7). Similarly, in the present report, ERK inhibition had no effect on early reparative events of villous contraction and restitution while markedly inhibiting recovery of TER. Thus it can be speculated that ERK1/2 is regulating some component of tight junction reassembly or closure. As an example of this potential mechanism, it has been shown that inhibition of ERK1/2 inhibits claudin-2 mRNA and protein expression in T84 cells exposed to IL-17 (20). Inhibition of ERK1/2 appears to be critical for recovery.

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As an example of this potential mechanism, it has been shown that inhibition of ERK1/2 inhibits claudin-2 mRNA and protein expression in T84 cells exposed to IL-17 (20). It should be noted that, in the present study, PGE2 was able to partially reverse the inhibitory effects of the ERK inhibitor PD-98059. However, tissues treated with PGE2 and PD-98059 failed to reach levels of TER seen in tissues exposed to PGE2 alone, suggesting a continued PG effect independent of the effect of the ERK inhibitor on mucosal recovery.

Studies examining the regulation of COX-2 via the JNK pathway are limited. Moon and Pestka (24) have shown that impairment of JNK function through a dominant negative JNK vector has no effect on COX-2 protein expression. It has also been shown that JNK has no effect on COX-2 mRNA stability in cells stimulated with endogenous promoters of gastrointestinal cancer (36). We report here that pharmacological inhibition of JNK increases COX-2 protein expression in ischemic-injured ileal mucosa. From this finding it can be speculated that JNK has a downregulatory effect on COX-2 protein expression. The fact that the beneficial effect of SP-600125 on TER was blocked by the selective COX-2 inhibitor NS-398 further supported a role for JNK in the regulation of COX-2. In addition, the effects of SP-600125 on TER and the effect of exogenous PGE2 on TER were approximately equal, whereas PGE2 in combination with SP-600125 had an additive beneficial effect on TER.

Finally, we report that p38 MAPK appears to have a more dominant role in regulating recovery from intestinal ischemia than JNK. The increase in recovery of TER observed upon inhibition of JNK was abolished when the p38 MAPK inhibitor SB-203580 was added in combination with the JNK inhibitor SP-600125. The concept of cross communication among the three primary MAPK pathways has been previously reported. For example, in corneal epithelial cells, evidence exists that cross-talk activation of p38 MAPK and ERK1/2 occurs during corneal wound healing (31). Inhibition of either p38 MAPK or ERK1/2 induced cross-activation of the other. Furthermore, inhibition of JNK suppressed TGF-β activation of p38 MAPK in FaO rat hepatoma cells (29). On the basis of the data presented herein, we speculate that there is cross talk between JNK and p38 MAPK in the regulation of COX-2, with p38 MAPK activation resulting in enhanced expression of COX-2, possibly by inhibiting the JNK pathway.

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