Regulation and function of COX-2 gene expression in isolated gastric parietal cells

Nonthalee Pausawasdi, Saravanan Ramamoorthy, Leslie J. Crofford, Frederick K. Askari, and Andrea Todisco

Department of Internal Medicine, University of Michigan Medical Center, Ann Arbor, Michigan 48709-0682

Received 24 April 2001; accepted in final form 24 January 2002

Pausawasdi, Nonthalee, Saravanan Ramamoorthy, Leslie J. Crofford, Frederick K. Askari, and Andrea Todisco. Regulation and function of COX-2 gene expression in isolated gastric parietal cells. Am J Physiol Gastrointest Liver Physiol 282: G1069–G1078, 2002; 10.1152/ajpgi.00164.2001.—We examined expression, function, and regulation of the cyclooxygenase (COX)-2 gene in gastric parietal cells. COX-2-specific mRNA was isolated from purified (>95%) canine gastric parietal cells in primary culture and measured by Northern blots using a human COX-2 cDNA probe. Carbachol was the most potent inducer of COX-2 gene expression. Gastrin and histamine exhibited minor stimulatory effects. Carbachol-stimulated expression was inhibited by intracellular \( \text{Ca}^{2+} \) chelator 1,2-bis(2-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid-AM (90%), protein kinase C (PKC) inhibitor GF-109203X (48%), and p38 kinase inhibitor SB-203580 (48%). Nuclear factor (NF)-\( \kappa \)B inhibitor 1-pyrrolidinecarbodithioic acid inhibited carbachol-stimulated expression by 80%. Similar results were observed in the presence of adenoviral vector Ad.dom.neg.I\( \kappa \)B, which expresses a repressor of NF-\( \kappa \)B. Addition of SB-203580 with Ad.dom.neg.I\( \kappa \)B almost completely blocked carbachol stimulation of COX-2 gene expression. We examined the effect of carbachol on PGE\(_2\) release by enzyme-linked immunoassay. Carbachol induced PGE\(_2\) release. Ad.dom.neg.I\( \kappa \)B, alone or with SB-203580, produced, respectively, partial (70%) and almost complete (\( \geq 80\% \)) inhibition of carbachol-stimulated PGE\(_2\) production. Selective COX-2 inhibitor NS-398 blocked carbachol-stimulated PGE\(_2\) release without affecting basal PGE\(_2\) production. In contrast, indo-methacin inhibited both basal and carbachol-stimulated PGE\(_2\) release. Carbachol induces COX-2 gene expression in the parietal cells through signaling pathways that involve intracellular \( \text{Ca}^{2+} \), PKC, p38 kinase, and activation of NF-\( \kappa \)B. The functional significance of these effects seems to be stimulation of PGE\(_2\) release.

The cyclooxygenase isozymes COX-1 and COX-2 catalyze the key step in the biochemical reaction that leads to the generation of prostaglandins, agents known to regulate a broad array of physiological functions (43). In the gastrointestinal tract, prostaglandins promote mucosal growth defense and repair (52). In addition, prostaglandins have been shown to inhibit gastric acid secretion and to regulate gastrointestinal mucosal blood flow (19, 45, 52). The importance of prostaglandins in gastrointestinal mucosal homeostasis has been underscored by the observation that inhibition of prostaglandin generation by the use of nonsteroidal anti-inflammatory drugs (NSAIDs) leads to the development of significant gastrointestinal mucosal damage (19, 52).

The genes that encode COX-1 and COX-2 exhibit considerable differences in regard to their regulation and biological functions (43). Whereas COX-1 appears to be constitutively expressed, COX-2 is rapidly induced in response to cytokines and growth factors through the coordinated activation of numerous intracellular signal transduction pathways (43).

COX-2 expression has been documented in several pathological conditions of the gastrointestinal tract. In particular, COX-2 has been shown to regulate colon cancer cell growth and apoptosis (39) and to be expressed in a significant number of human gastric carcinomas (41). Furthermore, selective inhibition of COX-2 appears to delay the healing of gastric ulcers and to exacerbate inflammation-associated colonic injury in rats (32, 33, 40, 46).

The gastric parietal cells are highly specialized gastric epithelial cells that secrete gastric acid in response to stimulation with histamine, gastrin, and acetylcholine (19, 44, 45). Recent reports have indicated that, in addition to this well-established function, the parietal cells are also the major site of production of growth factors in the gastric epithelium (3, 9, 10). In particular, transforming growth factor (TGF)-\( \alpha \), a polypeptide growth factor known to regulate important biological processes such as secretion, growth, and differentiation (20), is expressed and released by the parietal cells (3, 9, 25). These studies have suggested that production of TGF-\( \alpha \) by the parietal cells might be an important mechanism for the paracrine regulation of gastric epithelial cell growth and differentiation (3, 9, 10, 25). Accordingly, the parietal cells appear to exert important regulatory actions in the stomach through their...
ability to produce and secrete polypeptide growth factors.

A recent study has demonstrated that *Helicobacter pylori*-induced acute and chronic antral inflammation in humans is associated with immunohistochemical detection of COX-2 in the parietal cells, suggesting that these cells might be an important source of prostaglandins during the onset and development of gastric inflammation (31).

We hypothesize that, in addition to their ability to express and secrete TGF-α, the parietal cells might also produce other agents, such as prostaglandins, that are known to exert multiple important cytoprotective actions in the gastric mucosa. Accordingly, we undertook studies to examine the expression and regulation of the COX-2 gene in isolated gastric parietal cells in primary culture. In particular, we investigated the intracellular mechanisms that regulate COX-2 gene expression in the parietal cells and the linkage of these events to the production of gastric prostaglandins.

**MATERIALS AND METHODS**

**Adenoviral vectors.** The replication-defective adenoviral vector expressing a hemagglutinin (HA)-tagged mutated IκB (Ad.dom.neg.IκB), under the control of the cytomegalovirus (CMV) promoter, was a gift of D. Brenner (Chapel Hill, NC) (23, 24). The adenoviral vector expressing the β-galactosidase enzyme under the control of the CMV promoter (Ad.CMV-β-gal) was previously described (1).

**Primary parietal cell preparation and culture.** For preparation of primary parietal cells, we used a modification of the method of Soll and colleagues (8, 12, 34, 37, 44). The mucosal layer of freshly obtained canine gastric fundus was bluntly separated from the submucosa and rinsed in Hanks’ balanced salt solution containing 0.1% BSA. The cells were then dispersed by sequential exposure to collagenase (0.35 mg/ml) and 1 mM EDTA, and parietal cells were enriched by centrifugation using a Beckman JE-6B ultracentrifugation rotor. Our best preparations contained 70% parietal cells as determined by staining with a specific monoclonal antibody against the hog H+K+-ATPase α-subunit (a gift of A. Smolka, Charleston, SC) (47). The isolated parietal cells (2 × 10^6 cells/well) were cultured according to the method of Chen et al. (9) with some modifications (12, 34, 47). Briefly, the cells were cultured in Ham’s F-12 DMEM (1:1) containing 0.1 mg/ml gentamicin, 48 U/ml penicillin G, and 2% DMSO (Sigma, St. Louis, MO) on six-well culture dishes (Costar) coated with 150 μg of H2O-diluted (1:5) growth factor-reduced Matrigel (Becton Dickenson, Bedford, MA). For our studies, the cells were incubated with carbachol (100 μM; Sigma), histamine (100 μM; Sigma), and gastrin (10 nM; Bachem, Torrance, CA) for various time periods. In some experiments, bisindolylmaleimide I (GP-109203X), bisindolylmaleimide V (3.5 μM; Calbiochem, La Jolla, CA), 1,2-bis(2-aminoxy ethanol)-N,N,N',N'-tetraacetic acid (BAPTA)-AM (100 μM; Calbiochem), PD-98059 (50 μM; New England Biolabs, Beverly, MA), SB-203580 (10 μM; Calbiochem), and 1-pyrrolidinecarboxibothioic acid (PDTC, 1 mM; Calbiochem) were added before the addition of the secretagogue. BAPTA-AM, GP-109203X, bisindolylmaleimide V, PD-98059, and SB-203580 were dissolved in DMSO. All other test substances were dissolved in either water or culture medium. The parietal cells were transduced, when indicated, with 100 multiplicity of infection (moi) of either Ad.dom.neg.IκB, or Ad.CMV-β-gal, for 16–18 h.

**Northern blot analysis.** The parietal cells were lysed with TRIZol (GIBCO-BRL, Grand Island, NY) according to the manufacturer’s instructions. Northern blot hybridization assays were performed as previously described (35). Equal amounts of each RNA sample, with ethidium bromide (10 mg/ml) in a final volume of 20 μl, were electrophoresed on a 1.25% agarose gel containing formaldehyde, and the RNA was transferred from the gel to nitrocellulose filters. The ethidium-stained ribosomal RNA bands in the gel were photographed before and after transfer to ensure that equivalent amounts of RNA were loaded onto each lane and that no residual RNA was left on the gel. The human COX-1 and -2 cDNA probes were generated as previously described (16, 17). The human glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA probe was obtained from Clontech (Palo Alto, CA). The cDNAs were labeled with [32P]dCTP by the random priming procedure, and the nitrocellulose filters were hybridized to the 32P-labeled cDNA probes as previously described (35). For analysis of c-jun mRNA, we used a 48-base single-stranded c-jun synthetic oligonucleotide probe that was obtained from Calbiochem (Cambridge, MA). Labeling and hybridizations were carried out according to the manufacturer’s instructions as previously described (35).

**Western blots.** Parietal cell lysates (80 μg) were loaded on 10% SDS-polyacrylamide minigels and run at 200 V for 1 h. Protein concentrations were measured by the Bradford method (7). The gels were transferred onto Immobilon-P transfer membranes (Millipore, Bedford, MA) in 25 mM Tris, 150 mM glycine, and 20% methanol. After transfer, the membranes were blocked in 10 ml of TBST (20 mM Tris, 0.15 M NaCl, and 0.3% Tween) and 5% dry milk for 1 h and then incubated for 16–18 h at 4°C in 10 ml of TBST and 5% dry milk, containing either a specific mouse monoclonal anti-HA antibody (1:1,000; Babco Berkeley Antibody, Richmond, CA) or a rabbit polyclonal anti-COX-2 antibody (1:1,000; Cayman Chemical, Ann Arbor, MI). At the end of the incubation period, the membranes were washed in TBST for 30 min at room temperature and then incubated for 1 h in TBST and 5% dry milk, containing either protein A, directly conjugated to horseradish peroxidase (1:2,500; Amersham Life Science, Arlington Heights, IL) for the COX-2 Western blots or a horseradish peroxidase-conjugated anti-mouse secondary antibody (1:2,000; Transduction Laboratories, Lexington, KY) for the HA blots. The membranes were washed in TBST for 30 min at room temperature and then exposed to the Amersham enhanced chemiluminescence detection system according to the manufacturer’s instructions.

**Immunohistochemistry.** The parietal cells were transduced with Ad.dom.neg.IκB and cultured on slides for 16–18 h. At the end of the incubation period the cells were fixed in 4% formalin-PBS. The slides were blocked for 30 min with 20% donkey serum and incubated for 2 h with the anti-HA antibody. The slides were rinsed with PBS, and a 1:150 dilution of a FITC-conjugated donkey anti-mouse IgG secondary antibody (Jackson Immunoresearch Laboratories, West Grove, PA) was added for 1 h. After a wash with PBS, the slides were mounted in Vectashield (Vector Laboratories, Burlingame, CA) containing 5 μg/ml propidium iodide and were visualized by fluorescence microscopy.
Amplification and purification of adenoviral vectors. Briefly, the recombinant adenoviruses were amplified as previously described using 293 cells (1). The viruses were subsequently concentrated and purified on a cesium chloride gradient (1). The concentration of the recombinant adenoviruses was assessed on the basis of the absorbance at 260 nm and on a limiting dilution plaque assay (1).

Detection of adenoviral-delivered β-galactosidase. For identification of parietal cells transduced with the adenoviral vector expressing β-galactosidase, the cells were cultured on slides for 16–18 h and stained with 5-bromo-4-chloro-3-indolyl β-d-galactopyranoside (X-gal) after 24 h of infection. The cells were washed with PBS and then fixed in 0.5% glutaraldehyde at room temperature for 10 min. After two washes with 1 mM MgCl₂ in PBS, the cells were incubated in 0.1% X-gal. At the end of the incubation, the cells were rinsed with PBS and observed with a light transmission microscope.

RESULTS

COX-2 plays an important role in gastric pathophysiology. Because the gastric parietal cells express receptors for carbachol, gastrin, and histamine, agents known to stimulate gastric acid secretion (19, 44, 45), we investigated the regulation of COX-2 gene expression in response to the gastric acid secretagogues. As depicted in Fig. 1, stimulation of the parietal cells with carbachol (100 μM) for 2 h induced COX-2 gene expression >10-fold (12.52 ± 1.95-fold induction over control, mean ± SE, n = 3). In contrast, stimulation of the parietal cells for 2 h with either gastrin (10 nM) or histamine (100 μM) induced COX-2 gene expression by only 2- and 1.7-fold, respectively (2.41 ± 0.65-fold over control for gastrin, n = 9, and 1.73 ± 0.13-fold over control for histamine, n = 3). Moreover, induction of COX-2 gene expression in response to carbachol in combination with either gastrin or histamine, or in the presence of both agents combined, was not statistically different from that observed in the presence of carbachol alone. Similarly, exposure of the parietal cells to a combination of gastrin and histamine for 2 h did not significantly affect COX-2 gene expression compared with the effects observed in the presence of either gastrin or histamine alone. Accordingly, exposure of the parietal cells to different combinations of gastric acid secretagogues failed to exhibit any significant synergistic effects.

Since carbachol was the most potent inducer of COX-2 gene expression in the parietal cells, we focused our efforts primarily on the understanding of the intracellular pathways that target the COX-2 gene in response to carbachol stimulation and to analyze the functional relevance of this phenomenon.

We investigated the kinetics of carbachol induction of COX-2 gene expression. In these studies, we observed that the action of carbachol (100 μM) was time dependent, with a maximal stimulatory effect detected between 30 min and 6 h of incubation (Fig. 2, A and B). In addition, as shown in Fig. 2C, carbachol stimulated the expression of the COX-2 protein after 6 h of incubation. We confirmed the specificity of the stimulatory action of carbachol on COX-2 gene expression. As indicated in Fig. 2D, although carbachol (100 μM) potently induced the COX-2 gene, it failed to affect the expression of the COX-1 gene.

Carbachol is known to induce multiple signal transduction pathways in the gastric parietal cells (19, 37, 47, 48, 50). Accordingly, we examined the intracellular mechanisms that mediate carbachol induction of COX-2 gene expression. Carbachol-stimulated COX-2 gene expression was inhibited by the intracellular Ca²⁺ chelator BAPTA-AM (100 μM; 95%; Fig. 3A), the
PKC inhibitor GF-109203X (3.5 μM; 50%; Fig. 3B), and the p38 kinase inhibitor SB-203580 (10 μM; 50%; Fig. 3C). Addition of the specific mitogen-activated protein kinase (MAPK)-extracellular signal-regulated kinase (ERK) kinase 1 inhibitor PD-98059 (50 μM) led to a modest (30%) inhibitory effect (data not shown). The specificity of the effect of GF-109203X was confirmed by the observation that the inactive PKC inhibitor bisindolylmaleimide V had no effect on carbachol-stimulated COX-2 gene expression (data not shown). Since carbachol induces NF-κB activation (50), we examined the role of the NF-κB inhibitor PDTC (1 mM) on COX-2 gene expression. As shown in Fig. 3D, PDTC inhibited carbachol-stimulated COX-2 gene expression by 80%.

To confirm the involvement of NF-κB in carbachol induction of COX-2 gene expression, we transduced the gastric parietal cells with 100 moi of the adenoviral vector Ad.dom.neg.IκB, which overexpresses a HA-tagged IκBα in which serines 32 and 36 were substituted with alanines. Because of this mutation, IκBαS32A/S36A acts as a repressor of NF-κB (24). Ad.dom.neg.IκB expression was monitored by Western blots with an anti-HA antibody (data not shown). Immunohistochemical staining of Ad.dom.neg.IκB-transduced parietal cells with an anti-HA antibody demonstrated expression of Ad.dom.neg.IκB in the parietal cells (Fig. 4C). The specificity of the immunohistochemistry was demonstrated by incubating the transduced parietal cell with the secondary antibody without the primary antibody (Fig. 4D). Additional experiments were performed by transducing the parietal cells with 100 moi of the control adenoviral vector Ad.CMV-β-gal, which expresses β-galactosidase. As shown in Fig. 4B, histochemical staining for β-galactosidase demonstrated that >80% of the parietal cells were transduced with Ad.CMV-β-gal. Control experiments were conducted in noninfected cells, in which no cells stained with X-gal (Fig. 4A). To demonstrate that transduction of the parietal cells with adenoviral vectors does not affect their function, we conducted studies in which we compared the effect of carbachol on [14C]aminopyrine uptake between transduced and nontransduced parietal cells. In these studies, we observed that Ad.CMV-β-gal did not alter the function of the parietal cells because carbachol-stimulated uptake of [14C]aminopyrine was similar in both infected and noninfected cells (data not shown; see Ref. 49).

Transduction of the parietal cells with Ad.dom.neg.IκB produced a partial (70%) inhibition of carbachol stimulation of COX-2 gene expression (Fig. 5, A and B). In contrast, this inhibition was almost complete (90%) in the presence of Ad.dom.neg.IκB in combination with SB-203580, suggesting that p38 kinase and NF-κB act in concert to regulate COX-2 gene expression in the gastric parietal cells (Fig. 5, A and B). To confirm the specificity of the inhibitory action of Ad.dom.neg.IκB on COX-2 gene expression, we examined the effect of this construct on the expression of the highly inducible early response gene c-jun in response to carbachol stimulation. As shown in Fig. 5C, transduction of the parietal cells with Ad.dom.neg.IκB significantly inhibited carbachol-stimulated COX-2 gene expression but failed to affect the expression of c-jun. Thus Ad.dom.neg.IκB exerts specific inhibitory effects on COX-2 gene expression in the gastric parietal cells.

Because activation of COX-2 leads to prostaglandin production (43), we examined the effect of both Ad.dom.neg.IκB and SB-203580 on PGE2 release in response to carbachol stimulation. Carbachol induced a statistically significant increase in PGE2 release in Ad.CMV-β-gal-transduced parietal cells (620 ± 190
Fig. 3. Signal transduction pathways regulating carbachol stimulation of COX-2 gene expression in isolated gastric parietal cells. Aliquots of total RNA extracted following exposure of the parietal cells to carbachol (100 μM), either alone or in combination with the intracellular Ca²⁺ chelator 1,2-bis(2-aminophenoxy)ethane-N,N',N''-tetraacetic acid (BAPTA)-AM (100 μM; A), the protein kinase C (PKC) inhibitor GF-109203X (3.5 μM; B), the p38 kinase inhibitor SB-203580 (10 μM; C) and the nuclear factor (NF)-κB inhibitor 1-pyrrolidinecarboxothioic acid (PDTC; 1 mM; D), were examined by Northern blot analysis using a ³²P-labeled human cDNA probe for COX-2. Bar graphs represent results obtained from densitometric analysis of blots from several parietal cell preparations. Data are expressed as percentage of carbachol-stimulated stimulated COX-2 gene expression in the absence of the inhibitors (means ± SE).
Fig. 4. Infection of isolated gastric parietal cells. The parietal cells were infected with either Ad.CMV-β-gal (100 multiplicity of infection (moi)) or with Ad.dom.neg.IκB (100 moi). A: histochemical stain for β-galactosidase of control, nontransduced parietal cells. B: histochemical stain for β-galactosidase of Ad.CMV-β-gal-transduced parietal cells. C: immunohistochemical stain of Ad.dom.neg.IκB-transduced parietal cells with the anti-hemagglutinin (HA) antibody and a donkey antimouse FITC-conjugated secondary antibody. D: Ad.dom.neg.IκB-transduced parietal cells stained with the antihemagglutinin FITC-conjugated secondary antibody without the primary antibody. Parietal cell nuclei were stained in red with propidium iodide. Magnification, ×40 in A and B, ×60 in C and D. Identical results were obtained in experiments with 1 other separate parietal cell preparation.

pg/ml in untreated cells vs. 2,050 ± 581 pg/ml in cells incubated for 2 h in the presence of 100 μM carbachol, n = 6). As shown in Fig. 6, either transduction of the parietal cells with Ad.dom.neg.IκB or addition of SB-203580 led to 70 and 60% inhibition, respectively, of carbachol-stimulated PGE2 release. In addition, treatment of the parietal cells with SB-203580 in combination with Ad.dom.neg.IκB induced a >80% inhibition of

Fig. 5. Effect of Ad.dom.neg.IκB and SB-203580 on carbachol-stimulated COX-2 and c-jun gene expression in isolated gastric parietal cells. A: aliquots of total RNA extracted following exposure of either Ad.dom.neg.IκB- or Ad.CMV-β-gal-transduced parietal cells to carbachol (100 μM), alone and in combination with the p38 kinase inhibitor SB-203580 (10 μM), were examined by Northern blot analysis using a 32P-labeled human cDNA probe for COX-2, as shown in the representative blots obtained with a single parietal cell preparation. B: results obtained from densitometric analysis of blots from several parietal cell preparations. Data are expressed as percentage of carbachol-stimulated COX-2 gene expression in the absence of the inhibitors (means ± SE); *P < 0.05. C: aliquots of total RNA extracted following exposure of either Ad.dom.neg.IκB- or Ad.CMV-β-gal-transduced parietal cells to carbachol (100 μM) were examined by Northern blot analysis using the 32P-labeled human cDNA probe c-jun. Data are results from a single parietal cell preparation.
carbachol-stimulated PGE2 release. Accordingly, carbachol activates both NF-κB and p38 kinase to regulate prostaglandin release.

To examine in more detail the role of COX-2 in carbachol-stimulated PGE2 production, we examined the effects of both NS-398 (10 μM), a selective COX-2 inhibitor (43), and indomethacin (10 μM), an agent known to inhibit both COX-1 and COX-2 (43), on PGE2 release from the isolated parietal cells. Carbachol induced a statistically significant increase in PGE2 release in nontransduced parietal cells (688 ± 203 pg/ml in untreated cells vs. 2,601 ± 874 pg/ml in cells incubated for 4 h in the presence of 100 μM carbachol, n = 6). In contrast, no significant increase in PGE2 release was observed in response to either gastrin or histamine (data not shown). As indicated in Fig. 7, treatment of the parietal cells with NS-398 completely blocked carbachol stimulation of PGE2 production without inhibiting basal PGE2 release. In contrast, indomethacin led to a complete inhibition of carbachol-stimulated PGE2 release and to a 50% reduction in basal PGE2 production. Thus, in the gastric parietal cells, COX-2 regulates PGE2 production in response to carbachol stimulation.

DISCUSSION

The gastric parietal cells are complex biological structures whose behavior is regulated by a broad array of hormones, neurotransmitters, and growth factors (19, 35, 44, 45). In particular, the parietal cells express receptors for gastrin, acetylcholine, and histamine agents known to stimulate gastric acid secretion (19, 44, 45).

The cholinergic agonist carbachol is one of the most potent activators of the canine gastric parietal cells (19). Carbachol is known to interact with specific M3 muscarinic receptors present on the parietal cell surface to stimulate gastric acid secretion and PGE2 production (19, 38, 44, 45). In this study, we dissected the mechanisms and the intracellular signal transduction pathways that mediate carbachol stimulation of PGE2 production. In particular, we have demonstrated that carbachol-stimulated PGE2 generation is mediated by the ability of this agent to rapidly induce COX-2 gene expression. In contrast, we have reported that gastrin and histamine have extremely weak stimulatory actions on COX-2 gene expression and that both agents fail to regulate PGE2 production. Accordingly, induction of COX-2 is a signaling event that is specifically activated by carbachol but not by the other gastric acid secretagogues.

Previous work from our laboratory has indicated that activation of the parietal cell M3 muscarinic receptor leads to the induction of numerous protein kinases. In particular, we and others have shown that carbachol induces the ERKs (36, 47, 48), the c-Jun NH2-terminal kinases (JNKs) (35), and p38 kinase (37), molecules known to regulate multiple important physiological functions in the parietal cells. Whereas the ERKs and p38 kinase appear to be involved in the process of gastric acid production (37, 48), activation of the JNKs seems to be an important step in the response of the parietal cells to stress and inflammation (35). Here, we demonstrate that p38 kinase, and to a
lesser extent MAPK, mediate, at least in part, the stimulatory action of carbachol on COX-2 gene expression, suggesting a novel role for these kinases in the regulation of the COX-2 gene in the stomach. These findings are in agreement with observations accumulated in other cellular systems in which both p38 kinase and MAPK have been shown to regulate COX-2 gene expression (6, 18, 21, 26, 42). Since the JNKs are known to contribute to the regulation of COX-2 (24), it is possible that these kinases might also be involved in the modulation of the COX-2 gene in the parietal cells. However, because of the lack of specific inhibitors, we were unable to establish a specific link between activation of the JNKs and induction of COX-2 gene expression.

We previously reported that in the gastric parietal cells, carbachol induces the NF-κB signal transduction pathway through intracellular Ca²⁺ and PKC-dependent mechanisms that do not require the activation of both p38 kinase and MAPK (data not shown; see Ref. 50). NF-κB plays a crucial role in the regulation of numerous genes that are involved in the inflammatory response because it modulates the expression of proinflammatory cytokines, chemokines, immune receptors, adhesion molecules, and enzymes that generate mediators of inflammation (2). In unstimulated cells, NF-κB is present in the cytoplasm complexed with a repressor protein known as IκB, which prevents it from moving into the nucleus to regulate gene transcription (2). On cell stimulation, IκB is rapidly phosphorylated by the IκB kinases, which are responsible for the specific phosphorylation of IκB on serine residues (27). Phosphorylated IκB is then rapidly degraded by the proteasome, leading to the movement of NF-κB to the nucleus, where it binds to specific DNA sequences present in the promoters of numerous target genes (2, 27).

Activation of NF-κB appears to be an important event in numerous inflammatory processes of the gastrointestinal tract. Induction of this transcription factor has been reported in H. pylori gastritis (28), inflammatory bowel disease (51), and pancreatitis (22). Accordingly, we have investigated the role of NF-κB in carbachol induction of COX-2 gene expression. For these experiments, we have taken advantage of both pharmacological and biochemical tools. In particular, we have transduced the parietal cells with an adenoviral vector overexpressing an IκB molecule that acts as an inhibitor of NF-κB. Through these studies, we have demonstrated that PKC, intracellular Ca²⁺, and activation of NF-κB are crucial for the induction of the COX-2 gene in the parietal cells in response to carbachol stimulation. In fact, the PKC inhibitor GF-109203X, the intracellular Ca²⁺ chelator BAPTA-AM, the NF-κB inhibitor PDTC, and dominant-negative IκB all blocked carbachol stimulation of COX-2 gene expression. Our observations confirm those of other studies, which have indicated that activation of NF-κB is an important mechanism for the regulation of COX-2 gene expression. In particular, induction of NF-κB appears to modulate COX-2 gene expression in intestinal epithelial cells and in rheumatoid synovocytes treated with tumor necrosis factor (TNF)-α (16, 23) and in human myometrial cells stimulated with interleukin-1β (4).

Another important observation of our study is that, in the gastric parietal cells, blockade of either p38 kinase or NF-κB leads to only partial inhibition of carbachol induction of COX-2 gene expression. In contrast, exposure of the parietal cells to both SB-203580 and dominant-negative IκB leads to an almost complete inhibitory effect. These data suggest that both the p38 kinase signal transduction pathway and an IκB-dependent mechanism of NF-κB activation act in concert to regulate COX-2 gene expression in response to carbachol stimulation. These findings confirm earlier reports from our laboratory in which we observed that, in the gastric parietal cells, inhibition of p38 kinase by SB-203580 does not affect carbachol stimulation of the IκB kinases (data not shown). Similarly, we demonstrated that BAPTA-AM, which potently blocks carbachol induction of the IκB kinases, fails to inhibit the stimulatory effect of carbachol on p38 kinase activation (37).

The functional relevance of these findings was underscored by the observation that inhibition of carbachol-stimulated COX-2 gene expression by SB-203580 and dominant-negative IκB correlates with inhibition of PGE₂ production. Thus NF-κB and p38 kinase are important mechanisms employed by carbachol to regulate prostaglandin production in the stomach.

Although one report has suggested that macrophages and capillary endothelial cells might be the major producers of PGE₂ (11), our study clearly indicates that the canine parietal cells can express COX-2 and release prostaglandins in response to carbachol stimulation.

Since cholinergic neurotransmitters are released in the inflamed gastrointestinal mucosa (14, 15), carbachol induction of COX-2 gene expression in the parietal cells might be one of the events that are activated in the gastric epithelium during the onset and development of inflammation. Indeed, several reports have demonstrated that activation of cholinergic efferent pathways might play an important role in the regulation of the inflammatory response. Cholinergic agonists have been shown to inhibit lipopolysaccharide-induced TNF-α release from human macrophages and to block TNF-α synthesis in the liver during lethal endotoxemia in rats (5). Moreover, the COX-2 gene appears to be potently induced in the gastric mucosa of rats during the recovery from cold-restraint-induced gastritis (30), a pathophysiological condition mediated by the activation of efferent cholinergic mechanisms (29).

Prostaglandins regulate important functions such as secretion, growth, and differentiation through the activation of both autocrine and paracrine mechanisms (19, 45, 52). Accordingly, we speculate that COX-2-mediated PGE₂ production by the parietal cells might represent an important step for the activation of cytoprotective pathways in the stomach.

Previous studies conducted in isolated canine parietal cells (45) have shown that PGE₂ has no effect on
carbachol-stimulated aminopyrine uptake. In contrast, PGE₂ is known to inhibit histamine-stimulated gastric acid secretion. Since the parietal cells are regulated by the coordinated action of carbachol, histamine, and gastrin (19, 44, 45), it is possible that carbachol induces the release of PGE₂ to modulate the secretory function of the gastric parietal cells. Thus activation of the parietal cell M₃ muscarinic receptor appears to lead to the simultaneous induction of gastric acid secretion and of cytoprotective mechanisms such as stimulation of COX-2 gene expression and of PGE₂ production.

In conclusion, we have reported that carbachol induces COX-2 gene expression in the parietal cells through signaling pathways that involve intracellular Ca²⁺ concentration, PKC, p38 kinase, and activation of NF-κB. The functional significance of this effect appears to be the stimulation of PGE₂ release.

We thank Jung Park for preparing the parietal cells, Thomas Witham, Daniel Miller, Jace Nilsen, and Huimin Bian for technical assistance, Dr. James Scheiman for critical review of the manuscript, and Judy Poore for assistance with the immunohistochemical studies.

This work was supported by National Institute of Diabetes and Digestive and Kidney Diseases Grants 3R01-DK-58312 (to A. Todisco) and P30-DK-34833 (to the University of Michigan Gastrointestinal Peptide Research Center). A. Todisco is a recipient of an American Gastroenterological Association Industry Research Scholar Award, a Clinical Investigator Award from the National Institute of Diabetes and Digestive and Kidney Diseases (K08-DK-02336), and a grant from the Charles E. Culpeper Foundation Health Program.

REFERENCES


COX-2 GENE EXPRESSION IN GASTRIC PARIELT CELLS


