Role of cyclooxygenase-2 in modulating gastric acid secretion in the normal and inflamed rat stomach

KIRSTY BARNETT, CAMERON J. BELL, WEBB MCKNIGHT, MICHAEL DICAY, KEITH A. SHARKEY, AND JOHN L. WALLACE

1Mucosal Inflammation Research Group and 2Neuroscience Research Group, Faculty of Medicine, University of Calgary, Calgary, Alberta, T2N 4N1 Canada; and 2Department of Gastroenterology, Royal North Shore Hospital, St. Leonards, New South Wales, 2065 Australia

Received 30 March 2000; accepted in final form 10 July 2000

Barnett, Kirsty, Cameron J. Bell, Webb McKnight, Michael Dicy, Keith A. Sharkey, and John L. Wallace. Role of cyclooxygenase-2 in modulating gastric acid secretion in the normal and inflamed rat stomach. Am J Physiol Gastrointest Liver Physiol 279: G1292–G1297, 2000.—Nonsteroidal anti-inflammatory drugs (NSAIDs) exert both their therapeutic and toxic effects mainly through inhibiting prostaglandin (PG) synthesis (32). PGs have long been known to be protective to the gastric mucosa (23) and to have inhibitory effects on gastric acid secretion. Indeed, it is possible that these effects contribute to the ability of NSAIDs to cause gastric ulceration and to exacerbate preexisting ulcers (34).

The primary enzyme responsible for PG synthesis, cyclooxygenase, exists in at least two isoforms. Cyclooxygenase-1 (COX-1) is constitutively expressed in the gastrointestinal tract and most other tissues, whereas COX-2 is expressed at very low levels in most tissues but has been shown to be induced at sites of inflammation (15, 33). In animal models of gastrointestinal ulceration and inflammation there is strong expression of COX-2, and this isoform makes an important contribution to the generation of PGs in these situations (21, 22). There are also recent human data demonstrating expression of COX-2 in the inflamed/ulcerated stomach and colon (10, 20). The discovery of COX-2 has led to considerable efforts to develop highly selective inhibitors of COX-2 in the hope that they will retain the anti-inflammatory and analgesic effects of conventional NSAIDs but will exhibit greatly reduced gastrointestinal toxicity. Studies in animal models (3, 19) and humans (16, 27) have provided data that support this theory. However, investigations by Reuter et al. (22) and Mizuno et al. (21) revealed that inhibition of COX-2 activity resulted in exacerbation of experimental colitis and delayed healing of experimental gastric ulcers, respectively. The latter finding, together with evidence that COX-2 can be very rapidly induced in the stomach (4) and that PGS derived from COX-2 are important in normal mucosal defense (12, 38), emphasize the need for a better understanding of the role of COX-2 in modulating gastric function.

Although it is clear that NSAIDs can increase acid secretion by virtue of their ability to inhibit PG synthesis (14, 17, 18), it is not known whether this effect is mediated by suppression of COX-1, COX-2, or both. In the normal stomach, where there is very little expression of COX-2, it is likely that COX-1 is the principal source of PGS affecting acid secretion. However, in the inflamed stomach it is entirely possible that COX-2 makes an important contribution to production of the PGs that modulate gastric acid secretion. Given that a significant percentage of the human population has ongoing gastric inflammation as a consequence of infection with Helicobacter pylori, it is important to determine the effects of selective COX-2 inhibition on gastric acid secretion in a setting of gastric inflammati-
tion. Thus in the present study we have examined the effects of indomethacin, a nonselective COX inhibitor, and DuP-697, a highly selective COX-2 inhibitor, on basal and pentagastrin-stimulated acid secretion by both the normal and inflamed rat stomach.

**MATERIALS AND METHODS**

**Animals.** Male Wistar rats weighing 175–225 g were obtained from Charles River Breeding Farms (Montreal, QC, Canada) and housed in polycarbonate cages. Rats had free access to standard pellet chow and tap water (modified as described in *Induction of gastritis*) throughout the experiment. All experimental protocols were approved by the Animal Care Committee at the University of Calgary, and the experiments were performed in accordance with the guidelines of the Canadian Council on Animal Care.

*Induction of gastritis.* Gastric inflammation was induced by the addition of iodoacetamide (0.1%) and sucrose (1.0%) to the drinking water, based on the model described by Szabo et al. (30). Controls were given drinking water supplemented only with sucrose (1%). All experiments were performed on the 5th day after the rats were given the modified drinking water. At that time, groups of rats (n = 5/group) were euthanized, and samples of the corpus region of the stomach were excised for measurement of myeloperoxidase (MPO) activity (2) and PGE$_2$ synthesis (35). MPO is an enzyme found primarily in the azurophilic granules of neutrophils and therefore has been used extensively as a biochemical marker of granulocyte infiltration into various tissues, including the gastrointestinal tract. For measurement of gastric PGE$_2$ synthesis, tissue samples (~100 mg) were incubated in 1 ml of 10 mM sodium phosphate buffer (pH 7.4) for 20 min at 37°C. Following centrifugation (9,000 g), the supernatants were frozen at −20°C, and subsequently the concentrations of PGE$_2$ were measured with a specific enzyme-linked immunosorbent assay (35). Additional samples of the corpus region of the stomach were fixed in Zamboni’s fixative and processed, as described in detail previously (4), for immunohistochemical detection of COX-2.

Effects of iodoacetamide on gastric histamine content were examined in a separate series of experiments. Rats were treated with iodoacetamide-supplemented water or the control solution, as described in *Induction of gastritis*. The rats (n = 5/group) were then euthanized, and samples of the corpus region were excised and immediately frozen on dry ice, then stored at −70°C. The samples were deproteinized by homogenization with 5 volumes of ice-cold 0.1 M perchloric acid. Following centrifugation (5,000 g, 15 min, 10°C), the samples were diluted with water and the content of histamine was determined with an enzyme-linked immunosorbent assay, according to the manufacturer’s instructions (ICN, Costa Mesa, CA).

*Gastric acid secretion.* Rats were fasted for 18 h before surgery, with free access to drinking water (supplemented as described in *Induction of gastritis*). Anesthesia was induced by an intraperitoneal injection of urethane (6.0 ml/kg of a 20% solution prepared in 0.9% sterile saline). During the entire experiment, the rats were kept on homeothermic blankets and the rectal temperature was maintained at 37°C. A tracheostomy was performed, and a jugular vein was cannulated to allow for infusion of pentagastrin. A laparotomy was then performed, and a duodenogastric cannula was inserted and secured. An orogastric cannula was inserted and secured with a suture. The stomach was gently flushed with 20–30 ml of 0.9% saline at 37°C and then perfused with 0.9% saline at 37°C at a rate of 3.0 ml/h. After a 15-min period for stabilization, perfusates were collected for three 30-min periods. The first period represented basal acid secretion. At the beginning of the second period, a 20 μg/kg bolus of pentagastrin was injected intravenously followed by a continuous intravenous infusion at 20 μg·kg$^{-1}$·h$^{-1}$. This procedure has previously been shown to reproducibly elicit a significant increase in gastric acid secretion (37). The concentration of acid in each sample was determined by titration to pH 7.0 using a Metrohm automated titration system (Brinkmann Instruments, Rexdale, ON, Canada).

To determine whether significant changes in fluid secretion or absorption during the perfusion of the stomach might impede the correct determination of the amount of acid secretion, a series of experiments was performed in which rats were treated with vehicle or indomethacin, as described in *Test drugs*, and the perfusion was performed with a modified perfusate. In these experiments, the nonabsorbable marker [3H]polyethylene glycol 400 was added to the perfusate and an aliquot of each sample collected over the course of the experiment was subjected to liquid scintillation counting to determine whether dilution or concentration of the polyethylene glycol 400 had occurred. The amounts of recovered [3H]polyethylene glycol (expressed as a percentage of the concentration in the perfusate) in rats treated with indomethacin did not differ from those in controls, either in the basal period (133 ± 9% vs. 117 ± 21%, respectively) or after pentagastrin administration (100 ± 22% vs. 120 ± 31%, respectively).

*Test drugs.* One hour before beginning the perfusion of the stomach, the rats were given vehicle, indomethacin (5 mg/kg), or DuP-697 (1 mg/kg) intraperitoneally (n = 6–8/group). The vehicle for indomethacin was 5% (wt/vol) sodium bicarbonate, whereas that for DuP-697 was 0.5% carboxymethylcellulose. The effectiveness and selectivity of the doses of DuP-697 and indomethacin that were selected was confirmed using the carrageenan-airpouch model of inflammation in the rat, as described in detail previously (36). In this model, inflammatory PG synthesis is derived almost exclusively from COX-2. Rats were given vehicle, DuP-697 (1 mg/kg), or indomethacin (5 mg/kg). Samples of the exudate were collected from the airpouch 6 h later for measurement of PGE$_2$ concentrations, as was a sample of blood for determination of thromboxane B$_2$ production (as an index of COX-1 activity).

Additional studies were performed in which rats that had received iodoacetamide in their drinking water for 4 days were given one of the cyclooxygenase inhibitors or vehicle intraperitoneally, as above. One hour later, the rats were euthanized and samples of the corpus region of the stomach were excised for measurement of PGE$_2$ synthesis, as described in *Induction of gastritis*.

**Statistical analysis.** All data are expressed as means ± SE. Comparisons among groups of data were made using a one-way analysis of variance and the Dunnett’s multiple-comparisons test. An associated probability of <5% was considered significant.

**Materials.** Pentagastrin, indomethacin, and urethan were obtained from Sigma Chemical (St. Louis, MO). DuP-697 was obtained from DuPont-Merck (Wilmington, DE). The kits for measurement of PGE$_2$ were obtained from Cayman Chemical (Ann Arbor, MI). The antibody to COX-2 was kindly provided (Ann Arbor, MI). The antibody to COX-2 was kindly provided (Ann Arbor, MI).

**RESULTS**

*Iodoacetamide-induced gastritis.* Iodoacetamide treatment resulted in an approximately threefold increase in
gastric MPO activity (Fig. 1), confirming a previous study that showed that this agent causes significant increases in the number of neutrophils in the mucosa (30). Histologically, infiltration of granulocytes (mainly neutrophils) was evident in the mucosa and submucosa. Despite this gastric inflammation, there was no discernible effect of iodoacetamide treatment on gastric PGE2 synthesis (Fig. 1), nor was there any change in gastric tissue histamine content (28.8 ± 5.6 ng/mg in iodoacetamide-treated vs. 34.9 ± 9.9 ng/mg in controls). Nevertheless, immunohistochemistry confirmed a marked increase in COX-2 expression in the gastric mucosa of rats treated with iodoacetamide versus the vehicle-treated controls (Fig. 2). The expression of COX-2 appeared to be most marked in cells in the middle and lower parts of the gastric glands, with many of the COX-2-positive cells being histomorphologically identified as parietal cells. COX-2 was also evident in infiltrating neutrophils.

Administration of indomethacin to rats with gastritis resulted in a significant reduction in gastric PG synthesis (Fig. 3). However, DuP-697 did not significantly affect the capacity of the inflamed gastric tissue to synthesize PGE2. The ability of indomethacin to inhibit both COX-1 and COX-2 was confirmed by the marked suppression of whole blood thromboxane synthesis and inflammatory PG synthesis (carrageenan-airpouch model), respectively, that was seen with this drug (Fig. 3). In contrast, DuP-697 markedly suppressed COX-2, but had no effect on COX-1.

**Basal acid secretion.** Basal acid secretion in the rats receiving the vehicle for iodoacetamide averaged ~6 meq/30 min period (Fig. 4). Pretreatment with indomethacin or DuP-697 did not significantly affect basal acid secretion in the normal rats. In the rats in which gastritis had been induced with iodoacetamide, basal acid secretion was similar to that in the normal rats. Indomethacin administration to rats with gastritis resulted in a significant increase in acid secretion (117%), whereas DuP-697 had no effect.

**Pentagastrin-stimulated acid secretion.** In normal rats, intravenous administration of pentagastrin resulted in a significant increase (two- to fourfold) in acid secretion compared with basal levels (Fig. 4). Similar increases in acid secretion were observed in the rats pretreated with indomethacin or DuP-697. In the rats with iodoacetamide-induced gastritis, pentagastrin caused significant increases in acid secretion in all three groups compared with their respective basal secretory rates. Moreover, the rats with gastritis (pretreated with vehicle) exhibited significantly higher rates of pentagastrin-induced acid secretion than the normal rats. Pretreatment with indomethacin resulted
in rates of acid secretion that were significantly greater (twofold) than those in the corresponding vehicle-treated rats. DuP-697 did not significantly affect acid secretion relative to the vehicle-treated controls.

**DISCUSSION**

Several studies have shown that NSAIDs can potentiate secretagogue-stimulated acid secretion in vivo and in vitro (17, 18, 26). This effect is attributable to inhibition of PG synthesis, since PGs inhibit acid secretion by reducing adenylate cyclase activity in the parietal cell (28). In the present study, we sought to determine whether selective inhibition of COX-2 would produce a similar increase in acid secretion, as can be observed with a conventional NSAID that blocks both COX-1 and COX-2. In normal rats, the conventional NSAID (indomethacin) did not significantly affect basal or pentagastrin-stimulated acid secretion. These results are consistent with the findings of Holm and Jagare (14) but contrast with several previous studies of the effects of indomethacin on gastric acid secretion in humans and rhesus monkeys (5, 8, 17). We also observed that treatment with a selective COX-2 inhibitor did not affect acid secretion in the normal rats. This is perhaps not surprising given the confirmation, by immunohistochemistry, that COX-2 is expressed only at very low levels in the normal stomach. On the other hand, COX-2 has recently been shown to make an important contribution to mucosal defense in the normal stomach (12, 38).

In contrast to the results in normal rats, significant effects of indomethacin on acid secretion were apparent in the rats with gastritis. Indomethacin pretreatment caused a doubling of both basal and pentagastrin-stimulated acid secretion relative to the vehicle-treated control group. It is noteworthy that inflammation of the stomach was associated with a significant increase in pentagastrin-stimulated acid secretion (but no effect on basal secretion). The observation that pretreatment with the selective COX-2 inhibitor DuP-697 did not significantly affect basal or pentagastrin-stimulated acid secretion in the rat with gastritis suggests that the increase in acid secretion observed in rats treated with indomethacin was attributable to suppression of COX-1 activity.

The observed increase in gastric acid secretion in the rats with gastritis is consistent with studies of humans infected with *H. pylori*. Although acute infection with *H. pylori* can result in hypochlorhydria, chronic non-atrophic *H. pylori*-induced gastritis has been shown to be associated with an increase in acid secretion (6, 7). The hypersecretion of acid is thought to be due to two main factors: 1) increased gastrin release (due at least in part to decreased somatostatin production in the

![Fig. 3. Effects of indomethacin (COX-1 and COX-2 inhibitor; 5 mg/kg) and DuP-697 (selective COX-2 inhibitor; 1 mg/kg) on inflammatory PG synthesis (COX-2 activity), whole blood thromboxane synthesis (COX-1), and gastric PGE2 synthesis in rats with iodoacetamide-induced gastritis. Top: % inhibition (relative to the vehicle-treated group) of COX-2 and COX-1. Bottom: effects of the test drugs on gastric PG synthesis. Data are means ± SE; n = 5 rats/group. *P < 0.05 and **P < 0.01 vs. vehicle-treated group.](http://ajpgi.physiology.org/)

![Fig. 4. Effects of indomethacin and DuP-697 on basal (A) and pentagastrin-stimulated (B) gastric acid secretion in normal rats and in rats with iodoacetamide-induced gastritis. Pentagastrin caused a significant increase in acid secretion in all groups relative to the basal period (P < 0.05). Data are means ± SE; n = 6–8 rats/group. *P < 0.05 and **P < 0.01 vs. corresponding normal rats. #P < 0.05 vs. vehicle-treated, inflamed group.](http://ajpgi.physiology.org/)
gastric antrum) and 2) increased responsiveness of parietal cells to the stimulatory effects of gastrin, at least in *H. pylori*-positive patients with duodenal ulcer (6, 7). That these changes are a consequence of *H. pylori* infection is supported by the observation that acid secretion normalizes after eradication of the infection (6, 7, 10, 13). In the present study, we determined that induction of gastritis did not significantly affect the gastric tissue histamine content.

Induction of gastritis was associated with a marked increase in COX-2 expression. The cells expressing COX-2 were concentrated mainly in the midgland region, where there is the highest density of parietal cells. Indeed, many of the COX-2-positive cells were histomorphologically identified as parietal cells. Another possible source of the increased COX-2 expression is infiltrating leukocytes. We have previously observed that infiltrating neutrophils were a major source of COX-2 in the inflamed paw (35) and colon (22). Despite the clear induction of COX-2 in the inflamed stomach, we could not detect any increase in PG synthesis. It is possible that this is due to limitations of the assay we used to assess gastric PG synthesis, which is a measure of PG synthetic capacity. We and others have previously observed significant effects of selective COX-2 inhibitors on gastric function, despite the fact that a reduction of gastric PG synthesis could not be detected (12, 38).

Although a significant inhibition of gastric PG synthesis with DuP-697 was not observed, the ability of this drug to selectively inhibit COX-2 at the dose used was confirmed in the carrageenan-airpouch model. DuP-697 inhibited inflammatory PG synthesis (COX-2) by >80% but did not have any significant effect on platelet thromboxane synthesis (COX-1).

The ability of NSAIDs to interfere with ulcer healing is well established (1, 29). Recent evidence suggests that coadministration of agents that markedly suppress acid secretion is sufficient to overcome the delaying effects of NSAIDs on ulcer healing (31). Studies using experimental models of gastric ulcer suggest that it is the suppression of COX-2 by NSAIDs that causes the delay in ulcer healing (21, 24). Together, therefore, these results are consistent with the notion that acid secretion may be elevated as a consequence of NSAID ingestion, possibly due to the suppression of COX-2, and that this contributes to the impairment of healing. The results of the present study clearly demonstrate that COX-2 does not play a major role in regulating acid secretion in the rat stomach, even in the presence of inflammation. We cannot rule out the possibility that in a setting of more severe gastric inflammation and/or ulceration, there would be a stronger induction of COX-2 and that COX-2-derived PGs would exert an influence on acid secretion.

This work was supported by grants from the Medical Research Council of Canada (MRC). J. L. Wallace is an MRC Senior Scientist and an Alberta Heritage Foundation for Medical Research (AHFMR) Senior Scientist. K. A. Sharkey is an AHFMR Senior Scholar.

REFERENCES


